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Quantifying the Roles of Tidal Volume and PEEP in the Pathogenesis of Ventilator-Induced Lung Injury

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Abstract—Management of patients with acute lung injury (ALI) rests on achieving a balance between the gas exchanging benefits of mechanical ventilation and the exacerbation of tissue damage in the form of ventilator-induced lung injury (VILI). Optimizing this balance requires an injury cost function relating injury progression to the measurable pressures, flows, and volumes delivered during mechanical ventilation. With this in mind, we mechanically ventilated naive, anesthetized, paralyzed mice for 4 h using either a low or high tidal volume (Vt) with either moderate or zero positive end-expiratory pressure (PEEP). The derecruitability of the lung was assessed every 15 min in terms of the degree of increase in lung elastance occurring over 3 min following a recruitment maneuver. Mice could be safely ventilated for 4 h with either a high Vt or zero PEEP, but when both conditions were applied simultaneously the lung became increasingly unstable, demonstrating worsening injury. We were able to mimic these data using a computational model of dynamic recruitment and derecruitment that simulates the effects of progressively increasing surface tension at the air–liquid interface, suggesting that the VILI in our animal model progressed via a vicious cycle of alveolar leak, degradation of surfactant function, and increasing tissue stress. We thus propose that the task of ventilating the injured lung is usefully understood in terms of the Vt–PEEP plane. Within this plane, non-injurious combinations of Vt and PEEP lie within a ''safe region'', the boundaries of which shrink as VILI develops.

Keywords—Acute lung injury/ARDS, Lung derecruitment, Mechanical ventilation, Mouse model, Computational model.

INTRODUCTION

Acute lung injury (ALI) is a common entity in the intensive care unit,[24] and is managed primarily through supportive mechanical ventilation. However, mechanical ventilation can itself further damage the lung in a process known as ventilator-induced lung injury (VILI),[30] which is thought to occur via two distinct biophysical mechanisms. One mechanism is the over-distention of the lung during inspiration, termed *volutrauma*.[10] Even normal lung tissue has a limit to how much stretch it can sustain without damage, and when the tissue is already injured the threshold for further damage is reduced substantially.[11] Indeed, the current standard of care for ARDS involves the use of low tidal volumes (6 mL/kg),[5,6] the proven efficacy of which is presumably a consequence of reduced tissue stresses. The second biophysical mechanism postulated to cause VILI is referred to as *atelectrauma*.[26] Here, damage is caused by the repetitive re-opening of lung regions that become derecruited (atelectatic) with each breath. Conventionally it has been thought that atelectrauma is caused by the shear forces associated with alveolar and airway re-opening early in inspiration, but recent data suggest that the normal forces generated by surface tension are the most damaging to the pulmonary epithelium.[9,32] Both volutrauma and atelectrauma are thought to lead to systemic inflammatory consequences referred to as *biotrauma*,[27,29] which appears to be responsible for the multi-organ failure behind most ALI-related fatalities.[28]

Ventilatory management of patients with ALI thus rests on achieving a balance between adequate ventilation and minimal progression of volutrauma and atelectrauma. At present, we have very little idea how to achieve this balance, which almost certainly has a strong dependence on the nature and degree of lung

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injury. Put in engineering terms, we have yet to determine the VILI cost function that describes the quantitative relationship between progression of lung injury and the measurable stresses and strains (pressures and volumes) applied to the lung by mechanical ventilation. The optimal ventilatory regimen would then be that which minimizes the VILI cost function. The purpose of this study was to begin the process of defining the VILI cost function by studying the conditions that lead to the onset and progression of VILI in initially normal mice. In particular, we characterize the extent of VILI in terms of the dynamic derecruitability of the lung, and capture these dynamics in a computational model of time-dependent opening and closing of small airways. Such a model has the potential to serve as a virtual laboratory for exploring modes of mechanical ventilation that minimize the progression of VILI.

METHODS

Animal Procedures

This study was approved by the Institutional Animal Care and Use Committee of the University of Vermont. Animal treatment was in compliance with the Animal Welfare Act.

All experiments were performed on healthy 8–10 week-old BALB/c mice. (Jackson Laboratories, Bar Harbor, ME). Weights ranged from 19 to 26 g with the average weight being 22.3 ± 2.0 g. Acclamation period was not less than 3 days from arrival.

Anesthesia was induced with 90 mg/kg of intraperitoneal (IP) sodium pentobarbital, after which a modified 18 gauge PrecisionGlide needle (Becton Dickson & Co., NJ) was surgically placed into the trachea. Animals were then connected to a computer-driven small-animal ventilator (FlexiVent, SCIREQ, Montreal, QC, Canada) at baseline ventilatory settings of 200 breaths/min, a tidal volume (Vt) of 0.25 mL (piston volume). A positive end-expiratory pressure (PEEP) of 3 cmH_2O was maintained by submerging the expiratory vent under water. Taking gas compression in the ventilator cylinder into account, Vt delivered to the animal was about 0.2 mL and the minute ventilation approximated 40 mL/min. After observing bilateral chest rise and confirming proper ventilator settings, IP pancuronium bromide was administered (0.8 mg/kg) to ensure that subsequent measurements of lung mechanics were collected during purely passive mechanical ventilation. To maintain anesthesia, sodium pentobarbital (approximately 5 μg/kg) was given IP every 30 min. Continuous electrocardiogram monitoring was used to assess animal viability and adequacy of anesthesia during the experimental protocol (below).

Prior to the start of each experiment, the animals were given approximately 5 min to normalize to baseline ventilation settings. At the conclusion of the experiment, animals were euthanized with an overdose of pentobarbital (150 mg/kg) and thoracotomy. Immediately after euthanasia, bronchoalveolar lavage fluid (BALF) was collected by instilling 1 mL of phosphate buffered-saline into the lungs via the tracheostomy and then using gentle suction for a return of approximately 0.8 mL.

Study Protocol

We applied a number of different ventilatory regimens to separate groups of mice, as follows.

Control Group

Mice ($n = 6$) were subjected to baseline mechanical ventilation (Vt 0.2 mL, PEEP 3 cmH_2O, 200 breaths/min) for 4 h. At the beginning of the experiment, the lungs were sighed to a pressure of 25 cmH_2O and then the respiratory system impedance was measured by applying a 2 s broad-band (1–20 Hz) oscillating volume signal (amplitude 0.17 mL) to the lungs as previously described.[2,25] The constant phase model of lung impedance[15] was fit to each measurement. The impedance stiffness parameter (H) was taken as a measure of baseline lung elastance.[2] Every 18 min thereafter, for the duration of the experiment, we assessed the derecruitability of the lungs, as follows. This began with the lungs being given 10 deep breaths (Vt 0.8 mL, 50 breaths/min). Then, PEEP was reduced to 0 cmH_2O, ventilation was returned to baseline (Vt 0.2 mL, 200 breaths/min), and H was determined every 15 s for the following 3 min. We have shown in a number of prior studies that this produces a 3-min sequence of H values that increase progressively as the lung derecruits with time. The increase is typically modest in a normal lung, but may become greatly amplified in both rate and magnitude when the lung is injured.[2–4]

High Vt Group

After 5 min of baseline ventilation and baseline measurements of H, these mice ($n = 6$) were subjected to 4 h of high tidal volume mechanical ventilation with a Vt delivered by the ventilator piston of 1.0 mL (Vt reaching the animal being about 0.8 mL taking into account gas compression within the ventilator cylinder). PEEP was set to 3 cmH_2O and the breathing frequency was 50 breaths/min. Derecruitability was assessed every 18 min as for the control group above,

except that this time no prior deep inflation was required because the animals had already been over-ventilated with every breath prior to each sequence of H measurements.

Zero PEEP Group

Mice ($n = 6$) were ventilated with normal Vt at a low PEEP level (Vt 0.2 mL, PEEP 0 cmH_2O, 200 breaths/min). Also, because they were not receiving high Vt, the lungs were recruited with 10 deep slow breaths (Vt 0.8 mL, 50 breaths/min) prior to each assessment of derecruitability, which was performed every 18 min.

High Vt/Zero PEEP Group

Mice (n = 6) were ventilated with both a high Vt and a low PEEP level (Vt 0.8 mL, PEEP 0 cmH_2O, 50 breaths/min). Derecruitability was assessed every 18 min, and no prior deep inflation was required because the animals had already been over-ventilated with every breath prior to each sequence of H measurements.

PEEP Rescue Groups

Mice ($n = 6$ each group) were ventilated with both high volume ventilation and low PEEP (Vt 0.8 mL, PEEP 0 cmH_2O, 50 breaths/min) for either 2 h (*early PEEP rescue group*) or 3 h (*late PEEP rescue group*), after which PEEP was raised to the normal level of 3 cmH_2O and ventilation was continued for a total of 4 h. Derecruitability was assessed every 18 min throughout the entire 4 h period.

Vt Rescue Groups

Mice ($n = 6$ each group) were ventilated with both high volume ventilation and low PEEP (Vt 0.8 mL, PEEP 0 cmH_2O, 50 breaths/min) for either 2 h (*early Vt rescue group*) or 3 h (*late Vt rescue group*), after which Vt was lowered to the normal level of 0.2 mL and ventilation was continued for a total of 4 h. Derecruitability was assessed every 18 min throughout the entire 4 h period.

The Vt and PEEP values applied to each group are summarized in Table 1.

TABLE 1. Summary of the ventilatory parameters of the various experimental groups (see text for additional details).

Group name	Vt (mL)	PEEP (cmH_2O)
Control	0.2	3
High Vt	0.8	3
High Vt/zero PEEP	0.8	0
Early PEEP rescue	0.8	0 until 2 h, then 3
Late PEEP rescue	0.8	0 until 3 h, then 3
Early Vt rescue	0.8 until 2 h, then 0.2	3
Late Vt rescue	0.8 until 3 h, then 0.2	3

Note that 0.2 mL in these mice is equivalent to approximately 9 mL/kg, while 0.8 mL is 36 mL/kg.

BALF Analysis

BALF specimens were centrifuged at 450g for 10 min, the supernatant removed, and the cell pellets re-suspended in 1% BSA. Cell counts were determined manually in triplicate using a hemacytometer, and cell differentials were calculated from fixed cytospun slides stained with hematoxylin and eosin. Total protein was quantified in the BALF supernatants using a Bradford (Hercules, CA) colorimetric assay standardized to graded concentrations of bovine serum albumin. Both standards and samples were run in duplicate. Samples that exceeded the 595 nm absorbance were diluted 1:5 and re-run. A custom Millapore Milliplex^{TM} kit using standard procedure was used to quantify G-CSF, IL-1β, IL-6, KC, MCP-1, MIP-1α, MIP-1β, and TNFα. Controls and samples were run in duplicate.

Statistical Analysis

One-way ANOVA was used to compare cytokine and protein contents of the BALF between groups. For cytokines in which Levene's test demonstrated significant differences in population variance between groups, values were logarithmically transformed prior to ANOVA. Results were considered significant for $p < 0.05$. *Post hoc* means comparisons between groups were made using the Bonferroni correction.

Computational Modeling

We have previously developed a computational model that mimics the time dependence of recruitment and derecruitment seen during mechanical ventilation of the injured lung.[7] This model consists of a parallel collection of identical elastic lung units each served by a single airway that can exist in two states, open or closed. The state in which an individual airway finds itself is determined by its pressure history according to movement along a virtual trajectory. The trajectory variable, x, takes values between 0 and 1 and does not correspond to anything physical in particular. The virtual trajectory is merely an empirical mechanism that imbues the airways with hysteretic behavior, as follows. Each airway has a critical opening pressure, P_o, and a critical closing pressure, P_c. Movement to the right along the virtual trajectory (i.e., toward increasing values of x) occurs when the pressure, P, applied to the airway exceeds P_o, with the speed of movement

being proportional to the pressure difference ($P - P_o$). The constant of proportionality is s_o. Similarly, movement to the left (i.e., toward decreasing values of x) occurs at a rate $s_c(P - P_c)$, where s_c is another constant. x does not change for $P_c < P < P_o$. If an airway is open it only closes if its value of x reaches 0. It stays closed thereafter until such time as x reaches 1. Figure 1 provides a flowchart of the decisions made at each time step for each airway in the model. The values of P_o, P_c, s_o, and s_c for each airway are chosen randomly from designated probability distribution functions so that the recruitment and derecruitment behavior of the whole model is essentially continuous when the number of parallel lung units is large.

We found that this model is able to accurately mimic time courses of H measured at different levels of PEEP following recruitment maneuvers in both normal mice and in mice injured by intra-tracheal instillation of hydrochloric acid.[17] We found that good fits to these data were obtained when the values of P_o and P_c were drawn from Gaussian distributions having equal variance, with the mean value of P_o being about 4 cmH_2O above mean P_c. The values of s_o and s_c were drawn from hyperbolic distributions. The only significant effect of acid injury on these parameter values was a parallel shift upwards in the Gaussian distributions defining P_o and P_c, indicating an increase in the surface tension of the fluid in the lungs.[22,23]

We used this model to mimic the progression of VILI by simulating the maneuver used to assess lung derecruitability. Specifically, the lung was divided into 500 identical parallel units, each served by an airway, such that total lung elastance was 20 cmH_2O/mL and total lung resistance was 2 cmH_2O s/mL. The model included a representation of the Flexivent ventilator as described in Massa *et al.*,[17] which ventilated the lung with a frequency of 200 breaths/min and a piston displacement tidal volume of 0.2 mL. The model was initialized with all lung units open and all associated values of $x = 1$. A run in time of 200 s mechanical ventilation was then simulated, followed by 10 breaths of 1 mL at a frequency of 60 breaths/min. This was followed immediately by 3 min of regular ventilation during which lung stiffness was tracked as the product of the initial stiffness (20 cmH_2O/mL) and the fraction of lung units that were open. The latter gradually decreased with time as units closed, yielding a progressively increasing apparent lung elastance.

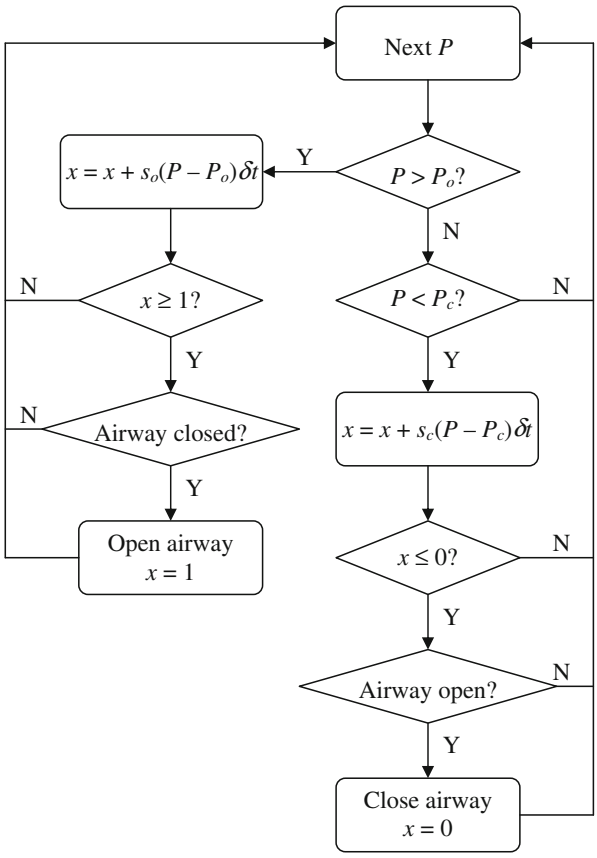

FIGURE 1. Flowchart of the decisions made concerning each airway in the computational model at each time step. δt is the time step used to integrate the model equations.

RESULTS

Figure 2 shows the 3 min time courses of H that were measured every 18 min during periods of mechanical ventilation in the various groups of mice that were studied. When the mice were ventilated under baseline conditions (control group—Fig. 2, top left) these time courses remained unaltered over 4 h, demonstrating that the propensity of the lung to derecruit was modest and constant. The same was true both when Vt was increased (high Vt group—Fig. 2, bottom left) and when PEEP was reduced (zero PEEP group—Fig. 2, top right). However, in the high Vt/zero PEEP group (Fig. 2, bottom right), the upward sweeps in H became progressively more pronounced over time. This indicates that increasing Vt and removing PEEP together caused VILI to develop over the 4 h ventilation period, whereas either intervention alone did not.

Figure 3 shows the evolution of the peak airway pressures produced by over-ventilating mice at 3 and 0 cmH_2O PEEP. Peak pressure started off lower in the zero PEEP group, but increased progressively and passed the pressure in the 3 cmH_2O PEEP group at about 2 h into mechanical ventilation. This further confirms that the combination of high Vt and zero PEEP was injurious in these animals (as in Fig. 2d). By contrast, peak pressure in the 3 cmH_2O PEEP group remained stable over the 4 h of mechanical ventilation.

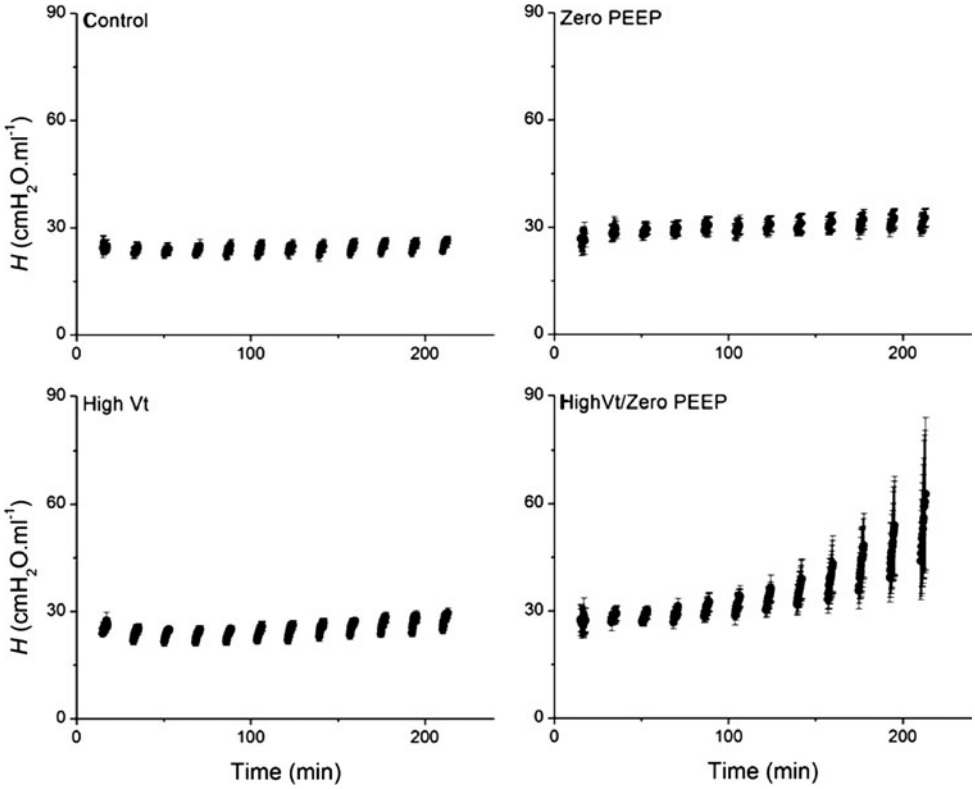

FIGURE 2. Derecruitability measured every 18 min during 4 h periods of mechanical ventilation under control conditions (*top left*), with high Vt (*bottom left*), with zero PEEP (*top right*), and with both high Vt and zero PEEP (*bottom right*). Each collection of data points (mean ± SE from the mice in each group) represents the sequential elastance measurements made during a single assessment of derecruitability. Note that the nature of these time course remains fairly stable except in the case of the high Vt/zero PEEP group where the rate and magnitude of rise in *H* increase progressively with time.

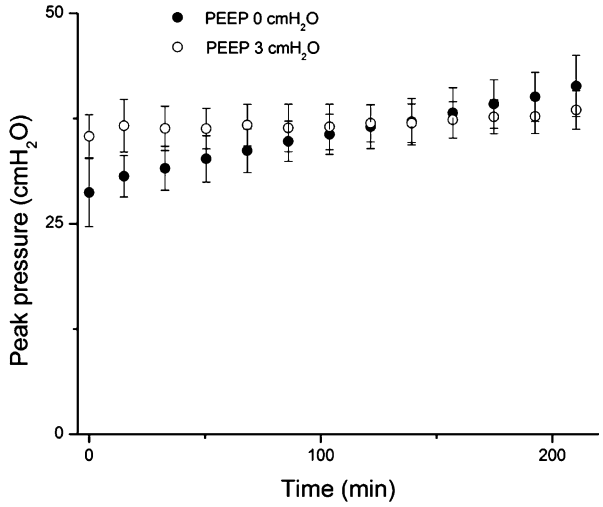

FIGURE 3. Peak airway pressures vs. time during 4 h of mechanical ventilation in mice ventilated with 0.8 mL Vt at a PEEP of 3 cmH_2O (*open symbols*) and 0 cmH_2O (*closed symbols*).

These results together demonstrate that peak pressure alone was not responsible for the progression of VILI, and that the injury in the zero PEEP group resulted from some synergistic interaction between atelectrauma and volutrauma.

The effects of altering the pattern of ventilation on the progression of VILI are shown in Fig. 4. Figure 4a shows sequential assessments of derecruitability obtained in the early PEEP rescue group in which PEEP was raised from 0 to 3 cmH_2O at 2 h (top panel), and in the early Vt rescue group in which Vt was decreased from 0.8 to 0.2 mL at 2 h (bottom panel). Figure 4b shows the final 2 h of data from the two groups with their respective linear regression lines. Progression of injury beyond the 2 h point was evident in both groups, but reducing Vt had more of an ameliorating effect than did increasing PEEP. The mean slope of *H* following Vt rescue was 0.045 cmH_2O/mL/min for the early Vt rescue group, a 58% reduction in slope compared to that over the three tests of derecruitability prior to rescue. For the early PEEP rescue group the slope in *H* post-increase in PEEP was 0.086 cmH_2O/mL/min, a 40% reduction from its value prior to rescue. Both post-rescue slopes are significantly different from zero.

Figure 5 shows the BALF protein levels measured in the various experimental groups. The protein levels

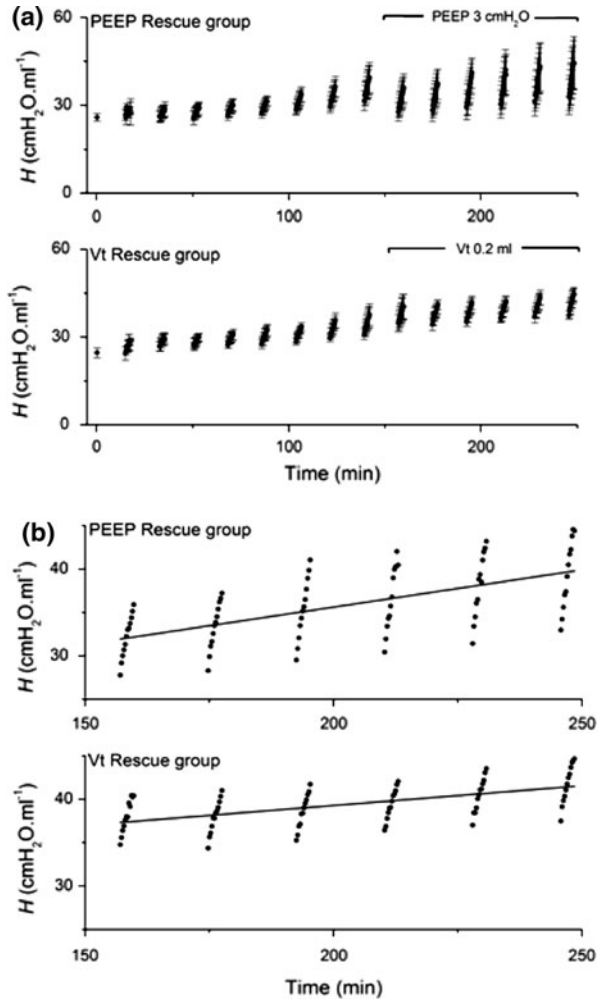

FIGURE 4. (a) Derecruitability measured every 18 min for 4 h in the early PEEP rescue and early Vt rescue groups of mice. Injurious ventilation was applied in both groups for 2 h, after which either PEEP was raised to 3 cmH_2O or Vt was reduced to 0.2 mL. (b) Derecruitability in the two groups after the changes in ventilation pattern, together with the regression lines fit to *H* vs. time.

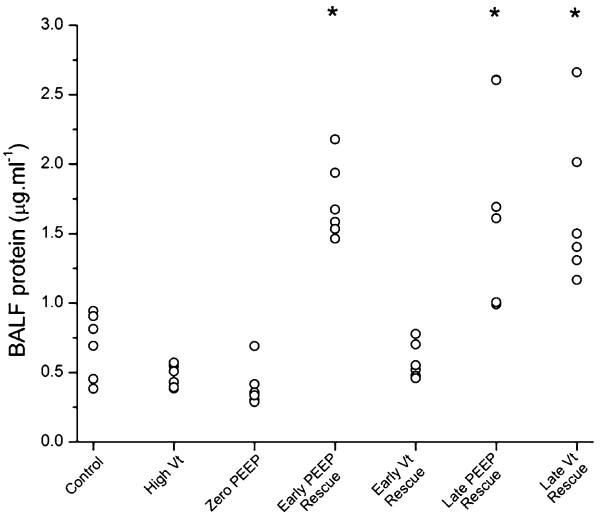

FIGURE 5. BALF protein levels measured after 4 h of mechanical ventilation in the various groups of mice studied. *Significant difference from control.

in the control, high Vt, and zero PEEP groups are all similar, corresponding to their associated lack of obvious VILI. The remaining data come from the four rescue groups that received either 2 or 3 h of combined high Vt/zero PEEP ventilation. When PEEP was normalized to 3 cmH_2O after 2 h (early PEEP rescue group) the final BALF protein levels were significantly elevated relative to control. By contrast, normalizing Vt to 0.2 mL after 2 h but maintaining PEEP at zero (early Vt rescue group) prevented BALF protein from rising above normal. Allowing injurious ventilation to continue for 3 h led to elevated BALF protein regardless of whether Vt or PEEP was subsequently normalized (late Vt rescue and late PEEP rescue groups, respectively). Figure 6 shows a variety of pro-inflammatory cytokines measured in the various experimental groups. Differences between values from each study group and those of the non-injurious high Vt and the zero PEEP groups did not reach the same degrees of statistical significance for each of the cytokines, which is likely a reflection of inconsistent degrees of variance among the different cytokines. However, the overall pattern of cytokine differences between experimental groups was similar to that of the BALF protein levels (Fig. 5). That is, ventilating with a combination of high Vt and zero PEEP for 3 h led to an increase in inflammatory cytokines regardless of whether this was followed by an hour of reduced Vt (late Vt rescue group) or elevated PEEP (late PEEP rescue group). By contrast, when Vt was reduced at 2 h (early Vt rescue group) the cytokines were less elevated relative to controls compared to when PEEP was elevated after 2 h (early PEEP rescue group).

Figure 7 shows simulated time courses of lung elastance (corresponding to the experimentally measured values of *H*) produced by the computational model. We obtained these times courses by adjusting the model parameters, using trial and error, until they closely matched the measured time courses. This was achieved when the means of the Gaussian distributions for P_c and P_o were 0 and 4 cmH_2O, respectively, both with standard deviations of 3.5 cmH_2O. These values are similar to those found experimentally for a normal lung by Massa *et al.*[17] The velocity parameters s_o and s_c were drawn from probability distributions defined as the inverse of a uniformly distributed random number on the interval [0,1] multiplied by 0.006 and 0.0008, respectively. These values are a factor of 5 less than those found by Massa *et al.*[17] for lung injured by hydrochloric acid. To simulate the progression of VILI

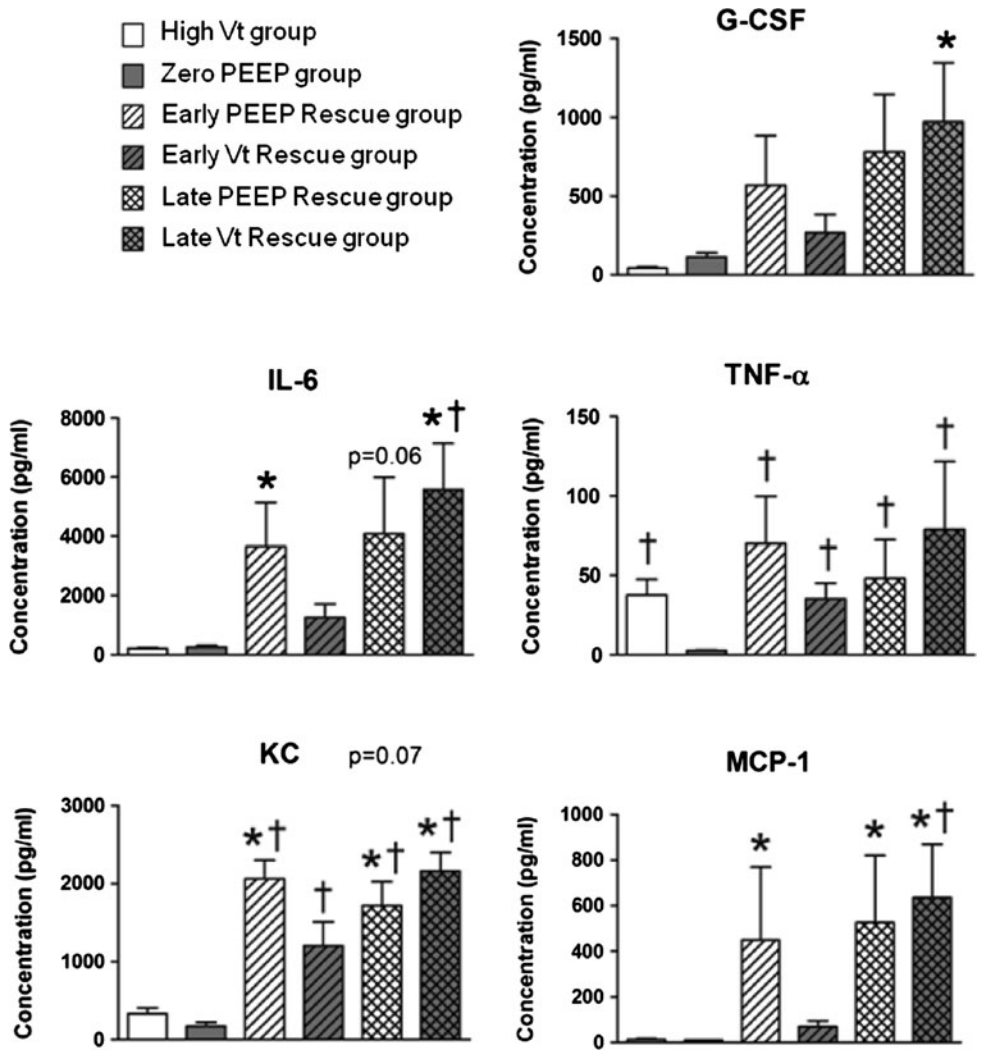

FIGURE 6. Inflammatory cytokines measured in the various treatment groups (*G-CSF* Granulocyte Colony Stimulating Factor, *TNF-α* Tumor Necrosis Factor-alpha, *KC* murine Keratinocyte Chemoattractant, *IL-6* Interleukin six, *MCP-1* Monocyte Chemotactic Protein-1). *Significant difference compared to the high Vt group, while †a significant difference compared to the zero PEEP group.

due to surfactant dysfunction (increased surface tension) in this model, we repeated the simulation of derecruitability 12 times, each time increasing mean P_c and P_o both by 1 cmH_2O. Of note is the fact that the model reproduces both the progressive increase in initial elastance and the increase in its rise throughout each 3 min test of derecruitability, as observed in the experimental data (Fig. 2). The model thus mimics the progressively reduced capacity of a large inflation to fully recruit the lung, as well as the increased propensity for the lung to become derecruited as injury progresses.

DISCUSSION

Mechanical ventilation of patients with ALI/ARDS is a double-edged sword; it provides essential life support yet at the same time may cause fatal injury. Indeed, balancing these two outcomes is the essence of management, there being little else that can be done in the way of treatment. It is thus crucial that we understand as much as possible about what causes VILI and how it progresses so that ventilatory management can be optimized. With this in mind, we have focused in this study on how VILI develops in a lung that is initially normal. In particular, we have examined the relative roles of volutrauma and atelectrauma, the two putative mechanisms by which VILI is thought to occur. Our results show that the normal mouse lung is rather resistant to the development of VILI. When the mice were ventilated at a normal level of PEEP for 4 h using a Vt of 0.8 mL, their mechanical signature of derecruitability (Fig. 2, bottom left) was essentially stable over time, and was very similar to mice receiving baseline non-injurious ventilation (Fig. 2, top left).

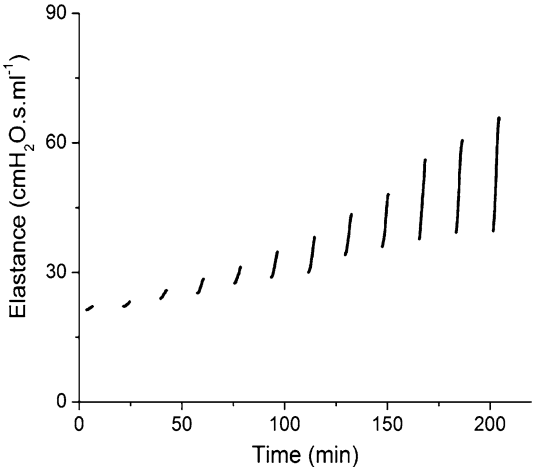

FIGURE 7. Simulations of measurements of derecruitability provided by the computational model. The left-hand most rise in Elastance (equivalent to experimental *H*) was produced using model parameter values corresponding to a control lung. The remaining 11 traces were produced by progressively increasing the mean critical open and closing pressures in the model by 1 cmH_2O, corresponding to a progressively increasing surface tension.

The high Vt group also showed no elevation in BALF protein (Fig. 5), although it must be noted that we allowed the lungs of the high Vt group to rest periodically by reducing Vt for 3 out of every 18 min in order to perform derecruitability tests. This may have been sufficient to allow for the repair of subtle early damage[12] that might otherwise have developed had the high Vt been continuous throughout the 4 h period of mechanical ventilation. Nevertheless, our results show that ventilation for most of the 4 h period with a Vt that was four times the usual Vt for a mouse did not lead to obvious lung injury. Similarly, when PEEP was zero in the presence of a normal Vt, the lung was again able to undergo extended mechanical ventilation with no obvious damage (top right panel of Figs. 2, 5).

Clear evidence of VILI was produced, however, when we combined high Vt with zero PEEP. Here, the *H* time courses showed an accelerating upward trend that resulted in lung stiffness nearly tripling by the end of the 4 h ventilation period (Fig. 2, bottom right), indicative of a dramatic increase in the derecruitability of the lung. This implies that high Vt and zero PEEP acted synergistically in these animals, which raises the intriguing question of how such synergy might arise. One possibility is that lowering the PEEP to zero caused a certain fraction of the lung to derecruit permanently, so that the application of a high Vt caused the remaining open fraction of lung to become over inflated to an injurious degree. We were able to refute this possibility, however, by comparing the time courses of peak airway pressure in mice ventilated with high Vt at PEEP levels of zero vs. 3 cmH_2O (Fig. 3). We found that peak pressure was initially lower in the zero PEEP group, which means that any lung regions that remained open in these animals were not being over inflated compared to the 3 cmH_2O PEEP group. Nevertheless, as the experiment proceeded, the peak pressures in the zero PEEP group rose to exceed the stable peak pressures of the 3 cmH_2O PEEP group, indicating that the former group experienced developing injury while the latter did not (Fig. 3).

Another possible explanation for the synergy between high Vt and zero PEEP is that more atelectrauma occurs with a high Vt than with a normal Vt. We cannot eliminate this possibility on the basis of our present results. On the other hand, one would expect that most of the re-opening of lung units taking place with zero PEEP would occur as lung volume is raised through the normal Vt, with limited extra re-opening occurring thereafter. Of course, the re-opening of lung units would occur more rapidly when a high Vt is used (inspiratory duration remaining fixed), but it has recently been shown that epithelial damage is inversely related to the speed of opening.[9,32] It seems, therefore, that the injurious effects of the atelectrauma that occurs at low lung volumes are somehow potentiated by the large tissue stresses produced by a high Vt, or vice versa. We do not know the mechanistic basis of this potentiation, but it seems clear that the effects of high Vt and zero PEEP are more than additive.

The situation gets more complicated when we attempt to halt or reverse the progression of VILI. In the case of our mouse model of VILI, there are two obvious interventions—either reduce Vt or increase PEEP. Figure 4 shows that reducing Vt after 2 h of injurious ventilation was more effective than increasing PEEP at suppressing the subsequent rate of progression of VILI. Reducing Vt was also more effective than increasing PEEP at preventing BALF protein (Fig. 5) and pro-inflammatory cytokines (Fig. 6) from becoming elevated. These observations are reminiscent of recent clinical findings that low Vt ventilation in ARDS patients has proven to be highly efficacious,[5,6] while increasing PEEP has yielded equivocal benefits in patients[8,19,20] despite the fact that it is known to improve alveolar stability[14] and prevent atelectrauma[31] in animal models. Nevertheless, Fig. 4 shows that although the progression of VILI after 2 h of injurious ventilation in our mice was more limited following reduction of Vt compared to increasing PEEP, it still continued to progress in both cases. Thus, even though ventilation had been returned to ventilatory regimens that were non-injurious in the healthy lung (Fig. 2, top right and bottom left), they were injurious in the injured lung. This demonstrates that the progression of VILI depends on the injury history of the lung, consistent with previous findings.[11]

Another notable feature of the data shown in the bottom right panel of Fig. 2 is an accelerating course for VILI once it starts. Specifically, while derecruitability of the lung remained relatively stable for the first hour or so of mechanical ventilation with combined high Vt and zero PEEP, once the upward sweeps in *H* started to increase in magnitude, they continued to do so at an ever increasing rate. This suggests the onset of a positive feedback mechanism, or "vicious cycle". A possible mechanism that might cause this effect is fluid and/or protein leak from the vasculature into the airspaces of the lung. Both volutrauma and atelectrauma are known to cause increased leak of this kind, which will interfere with the function of the pulmonary surfactant[16] and cause an increase in surface tension at the air–liquid interface. This would, in turn, increase the tissue stresses associated with mechanical ventilation, thereby causing more injury, more leak, and worsening lung mechanics. This view of the situation suggests that the onset of VILI might be defined by those conditions of mechanical ventilation under which the intrinsic alveolar fluid clearance mechanisms of the lung are unable to keep up with the increased leak of fluid and protein caused by the ventilation. Furthermore, one would expect this balance to be compromised when the pulmonary epithelium and endothelium are already damaged, which would explain why normally safe ventilatory regimens (e.g., Fig. 2, top right and bottom left) can still be further injurious in the already injured lung (Fig. 4).

As a further test of the altered surface tension theory, we used our previously developed computational model of lung recruitment and derecruitment[1,7,17] to simulate the accelerating time courses of derecruitability shown in the bottom right panel of Fig. 2. This model was shown in a previous study from our laboratory to be able to mimic the time courses of *H* following a deep lung inflation in both control and acid-injured mice[17] when the parameters of the model are drawn from appropriate probability distributions. Importantly, the only effect of acid injury on the best-fit model parameters in this previous study was a parallel upward movement of the means of the Gaussian distributions determining the critical opening and closing pressures. Although the mechanism of delayed recruitment and derecruitment in the model was originally designed to be purely empirical,[7] it actually bears a striking resemblance to processes that have been identified in previous in vitro studies from other laboratories on the opening and closing of collapsible tubing lined with fluid; it seems that an increase in opening and closing pressures are most likely attributable to increased surface tension in the fluid lining.[22,23] This does not, of course, rule out other possibilities that may have occurred in our mice, such as a change in fluid viscosity, or non-equal increases in critical opening and closing pressures. Nevertheless, when we start the computational model off with the mean values we found previously for the opening and closing pressures in normal mice,[17] and then increase these means linearly with time, we predict *H* time courses (Fig. 7) that are very similar to those observed experimentally (Fig. 2d).

These model simulations thus support the notion that continued leakage of fluid and protein into the airspaces of the lung over the course of mechanical ventilation caused a progressive increase in surface tension, leading to the vicious cycle of damage outlined in the preceding paragraph. This might then be further exacerbated by feedback from other developing pathologic processes related to biotrauma coming from locally elaborated inflammatory cytokines.[18] Although these ideas remain to be fully developed, by incorporating them into a computational model such as the one we present here, we are beginning to develop a framework for the *in silico* assessment of the injury potential of arbitrary ventilatory regimens, something that can be done much more rapidly and efficiently than in the animal or human laboratory.

The results of this study thus show that the development of VILI depends on Vt and PEEP, both individually and in combination, and that these contributions change with the state of injury of the lung. In many ways this is not a new concept, as it is well recognized that the combination of high PEEP and low Vt improves stabilization of lung units and reduces lung injury.[14] On the other hand, we have little idea at present about how to determine the optimum combination of Vt and PEEP for a given lung. The general problem can be understood by considering the Vt–PEEP plane, shown in Fig. 8, in which any given point corresponds to a particular combination of Vt and PEEP. We can imagine the Vt–PEEP plane to be composed of two distinct regions, a region that is damaging to the lung (leads to the development of VILI) and a region that is "safe". The borders of the safe region define a closed curve in the middle of the plane inside which Vt is neither too high (thereby avoiding volutrauma) nor too low (thereby providing sufficient ventilation). Likewise, PEEP in the safe region is neither too high (again avoiding volutrauma) nor too low (avoiding atelectrauma). The safe region is also asymmetric, reflecting the synergistic interactions between high Vt and Low PEEP. Importantly, the area of the safe region decreases as lung injury develops because points close to its borders for a normal lung may represent combinations of Vt and PEEP that can cause VILI in an injured lung, as our results have shown. Finally, the zones we have begun to identify here (Fig. 8) apply to the situation of particular values

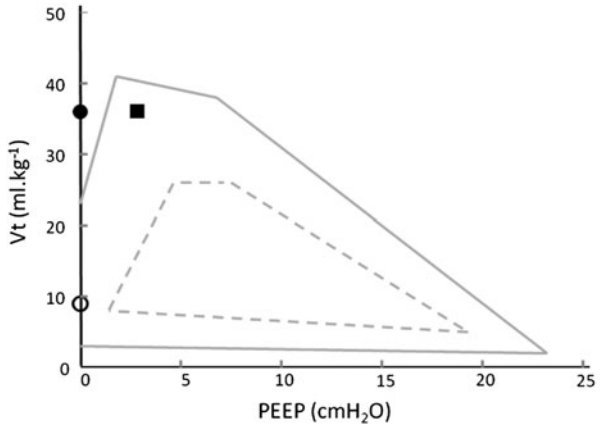

FIGURE 8. The Vt–PEEP plane defining arbitrary combinations of Vt and PEEP for mechanical ventilation. Within the center of this plane lies a "safe region", represented schematically by the *solid boundary*, within which the normal lung can be ventilated without fear of generating VILI. This region contains the ventilator settings for the high Vt group (*filled square*) and the zero PEEP group (*open circle*) but not the high Vt/zero PEEP group (*filled circle*). Figure 3 shows that the borders of this safe region have moved inwards after 3 h of injurious ventilation (*dashed boundary*) such that it no longer includes either of the previously safe points corresponding to the high Vt and zero PEEP groups. It is important to note that the borders of these regions are, for the most part, highly speculative. We have evidence from this study that these borders are constrained to be as shown either side of the three data points shown, and they must obviously lie some physiologically reasonable distance above Vt = 0. Apart from that, however, their positions are at present unknown.

of Vt and PEEP applied for 15 min intervals interspersed with 3 min of low Vt (as required to assess derecruitability). These low Vt segments may have provided important rest periods without which the lung might have succumbed more quickly. The size and shape of the safe zone in the Vt–PEEP plane is thus likely to depend on if and how the lung is periodically allowed to rest.

Thus, when treating the injured lung, there is a shrinking window of opportunity to intervene with a mode of mechanical ventilation that resides within the safe region of the Vt–PEEP plane. If VILI progresses too far, then the safe region may disappear altogether, highlighting the need for early recognition and treatment of ALI/ARDS, which may be the first step in reducing its morbidity and mortality.[13] The challenge of ventilating the injured lung then reduces to one of identifying the safe region in the Vt–PEEP plane. Our results suggest that ongoing assessment of lung mechanics, and derecruitability in particular, may provide a means for achieving this goal. Nevertheless, the safe region illustrated in Fig. 8 is still speculative; defining it accurately and completely for any given lung remains a daunting challenge.

We must also be cognizant of the limitations of this study upon which this notion rests. Our data come from mice that were mechanically ventilated for only a few hours, while patients with ALI/ARDS are often ventilated for days or weeks. Consequently, we have no intermediate or long term data about recovery from or progression of VILI following changes in the pattern of ventilation. We also only studied mice with initially normal lungs, so we do not yet know how the notion of the safe region carries over to lungs that have already been injured by clinically relevant events such as trauma, aspiration or sepsis. Also, the mice in this study were paralyzed throughout mechanical ventilation, a condition not always met in patients with injured lungs. However, although neuromuscular blockage was shown to improve survival in severe acute respiratory distress syndrome, it did not significantly affect the pressures experienced by the lungs,[21] so it is difficult to say if paralysis might have had any effect on the degree of injury in this study. There is thus clearly much more to be done both in terms of elucidating the mechanisms of the synergy between high Vt and low PEEP, and in understanding how to define the safe region in the Vt–PEEP plane and how it evolves with developing injury.

In conclusion, we have shown that VILI develops when initially normal mice are ventilated for 4 h with a combination of high Vt and zero PEEP, while 4 h of either setting on its own is not measurably injurious. Furthermore, high Vt and zero PEEP appear to act synergistically. Our data also show that VILI progresses inexorably once started, suggesting the involvement of a vicious cycle in which continued leak, increasing surface tension, and further tissue stress conspire to drive the lung toward complete failure. Consequently, combinations of Vt and PEEP that are safe in a normal lung may no longer be safe once the lung becomes injured. The non-injurious combinations of Vt and PEEP thus define a safe region within the Vt–PEEP plane, the area of which tends to shrink as injury develops. The challenge of ventilating the injured lung can thus be seen as one of defining the changing borders of the safe region in the Vt–PEEP plane, and ventilating within it.

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