Effects of ventilation on the surfactant system in sepsis-induced lung injury

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Malloy, Jaret L., Ruud A. W. Veldhuizen, and James F. Lewis. Effects of ventilation on the surfactant system in sepsis-induced lung injury. J. Appl. Physiol. 88: 401–408, 2000.—The present study examined the effects of mechanical ventilation, with or without positive end-expiratory pressure (PEEP), on the alveolar surfactant system in an animal model of sepsis-induced lung injury. Septic animals ventilated without PEEP had a significant deterioration in oxygenation compared with ventilated animals (arterial PO2/inspired O2 fraction 316 ± 16 vs. 151 ± 14 Torr; P < 0.05). This was associated with a significantly lower percentage of the functional large aggregates (59 ± 3 vs. 72 ± 4%) along with a significantly reduced function (minimum surface tension 17.7 ± 1.8 vs. 11.8 ± 3.8 mN/m) compared with nonventilated septic animals (P < 0.05). Sham animals similarly ventilated without PEEP maintained oxygenation, percent large aggregates and surfactant function. With the addition of PEEP, the deterioration in oxygenation was not observed in the septic animals and was associated with no alterations in the surfactant system. We conclude that animals with sepsis-induced lung injury are more susceptible to the harmful effects of mechanical ventilation, specifically lung collapse and reopening, and that alterations in alveolar surfactant may contribute to the development of lung dysfunction.

ventilation-induced lung injury; lung collapse; positive-end expiratory pressure; aggregates

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THE ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) is an acute lung injury resulting in respiratory failure and is defined by hypoxemia, nonhydrostatic pulmonary edema, and decreased lung compliance. This syndrome can arise from either direct or indirect pulmonary insults, ultimately resulting in diffuse alveolar damage (2). Although the reported survival of patients with ARDS has varied over the last several years (2), some studies have documented mortality as high as 70% (20). These reports most often include patients with systemic sepsis, which is also the most common predisposing condition leading to ARDS (3).

Studies investigating ventilation-induced lung injury (VILI) observed that some of the alterations of the endogenous surfactant system were similar to those reported for patients with ARDS (12, 26). Specifically, a decrease in the proportion of the superior functioning large surfactant aggregates relative to the functionally inferior small surfactant aggregates has been documented in both situations (13, 15, 27). Moreover, ventilation strategies utilizing relatively low tidal volumes resulted in less in vivo conversion of large aggregates into small aggregates compared with strategies that used higher tidal volumes (12). This decreased conversion was associated with superior oxygenation responses compared with the larger tidal volume strategies. In general, these findings have led to the hypothesis that there is an important relationship between the ventilatory effects on the surfactant system and the progression of lung injury in patients with severe respiratory failure due to ARDS.

Despite the growing concern that mechanical ventilation may contribute to the pathophysiology of acute lung injury (ALI), ventilation remains an important therapeutic intervention for patients with ARDS. It is, therefore, imperative that one attempt to minimize the harmful effects of ventilation while optimizing its beneficial effects. This can only be accomplished with a greater understanding of the mechanisms responsible for the lung damage induced by mechanical ventilation over the course of this disease.

Recently, we characterized an adult rat model of systemic sepsis induced by cecal ligation and perforation (CLP) (17). The relatively mild lung injury observed in these spontaneously breathing septic animals was associated with significant alterations in the endogenous surfactant system. The objective of the present study was to examine the effects of mechanical ventilation on lung function and the endogenous alveolar surfactant system in this clinically relevant model of lung injury. This information will provide insight into mechanisms contributing to the development of ARDS, which will ultimately lead to superior therapeutic strategies for these patients.

METHODS

Animal preparation. Adult male Sprague-Dawley rats weighing 350–450 g were acclimatized to the laboratory environment for 1 wk before surgery with free access to food and water. Rats were anesthetized with 4% halothane in oxygen in an anesthetizing box. Once sedation was induced, rats were transferred to a surgical table with a nose cone setup to maintain anesthesia. Two PE-50 monitoring catheters were inserted in the right external jugular vein and right carotid artery, respectively. These lines were routed subcutaneously to the back of the neck and attached to a three-fluid-channel (22-gauge) swivel system. The incision...
made to insert the catheters was closed with 3-0 silk suture. To induce sepsis and subsequent lung injury, the cecal ligation and perforation (CLP) technique was performed immediately after catheter placement as previously described (17). Briefly, this procedure involved performing a laparotomy, exposing the entire cecum with ligation of the distal one-third of the cecum, and then puncturing the ligated section twice with a 16-gauge needle. Sham control groups consisted of animals undergoing identical anaesthetic procedures and catheter placements for subsequent monitoring, but the laparotomy and CLP procedures were not performed.

After the animals’ harnesses were attached to secure the swivel system, individual rats were placed in plastic cages to recover. The swivel device allowed rats unlimited movement within the cage and free access to rat chow and water. Postoperatively, all animals received a continuous infusion of sterile saline at 7.5 ml·kg$^{-1}$·h$^{-1}$ containing 2 µg/ml of fentanyl for analgesia via the venous catheter. The arterial catheter was continually flushed with sterile heparinized saline (1 U/ml) at 1 ml/h to maintain patency. Measurements of arterial blood-gas values were performed by using an ABL 500 blood-gas analyzer (Radiometer, Copenhagen, Denmark). Arterial lactate levels were measured by using a YSI 2300 STAT Plus glucose/ lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH). Mean arterial blood pressure (MABP) and heart rate (HR) were measured via a pressure transducer attached to the arterial line. Respiratory rate (RR) was also recorded. All of these parameters were measured 5 h after surgery to confirm adequate recovery of each animal and to establish baseline parameters for subsequent measurements. The University of Western Ontario Animal Care Committee in conformity with the guidelines set by the Canadian Council of Animal Care approved the animal protocol.

Experimental groups. Both sham and septic animals were randomly assigned after surgery to one of six experimental groups. Two experimental groups consisted of both sham and septic animals that remained spontaneously breathing for 24 h after surgery and then were killed. These groups were designated Sham-Spon and Septic-Spon, respectively. The other four experimental groups consisted of sham and septic animals that were mechanically ventilated with or without positive end-expiratory pressure (PEEP) for 90 min starting at the 22.5-h time point after surgery and then were killed at the 24-h time point. As noted, two different ventilation strategies were utilized in these latter four groups. The Sham-Vent and Septic-Vent groups were ventilated by using a tidal volume of 9 ml/kg body wt, a RR of 62 breaths/min, and 0 cmH$_2$O of PEEP. The other two groups were ventilated by using identical parameters but with 5 cmH$_2$O of PEEP. These groups were designated Sham-PEEP and Septic-PEEP.

Mechanical ventilation. A volume-cycled rodent ventilator (Harvard Instruments, St. Laurent, PQ, Canada) was used to ventilate animals involved in this study. PEEP was added to the ventilation system by submerging the expiratory tube in water to a depth of 5 cm. The tidal volume and RR were chosen on the basis of preliminary studies involving normal rats in which arterial $\text{PCO}_2$ ($\text{P}_{\text{A}}\text{CO}_2$) values were maintained between 35 and 45 Torr over a 90-min time period. An inspired oxygen fraction (FiO$$_{2}$) of 50% was delivered to all ventilated animals to maintain adequate arterial $\text{PO}_2$ ($\text{P}_{\text{A}}\text{O}_2$) values in the septic groups. To determine the specific effects of the increased FiO$$_{2}$ in these animals, separate groups of spontaneously breathing sham and septic animals were placed in an airtight chamber at the 22.5-h time point after surgery and exposed to 50% oxygen for 90 min.

Experimental protocol. For all ventilated animals, a sample of arterial blood was obtained for measurements of $\text{PA}_{\text{O}_2}$, $\text{PA}_{\text{CO}_2}$, and lactate levels ~22.5 h after surgery. At the same time, MABP, HR, and RR were recorded. For these ventilated groups, animals were anesthetized in their cage via a slow intravenous injection of pentobarbital sodium until the toe pinch reflex was absent. The rats were then transferred to a surgical table where a tracheotomy was performed. Lidocaine (1%) was administered subcutaneously as local anesthetic, and the trachea was exposed via a midline incision. A 14-gauge angiocatheter was inserted into the trachea and used as an endotracheal tube for ventilation. Animals were then connected to the ventilator. 10.2 ml pancreatic bromide was administered intravenously to eliminate spontaneous breathing, and 0.2 ml buprenorphin was administered intramuscularly for further analgesia. Additional boluses of both pancromunium bromide and pentobarbital sodium were administered intravenously to maintain paralysis and adequate depth of anesthesia, respectively. At 10, 20, 30, 60, and 90 min after initiation of ventilation, arterial blood samples were obtained, and similar physiological parameters as noted previously were recorded. All mechanically ventilated animals were killed after 90 min of ventilation via an intravenous bolus of pentobarbital sodium and transection of the abdominal descending aorta.

An arterial blood sample was obtained from all spontaneously breathing animals 24 h after surgery, with identical physiological parameters recorded as in the ventilated animals at this time point. The spontaneously breathing animals were subsequently killed in the same manner as were the ventilated animals.

Lung lavage analysis. Immediately after being killed, all animals underwent a whole lung lavage as previously described (13, 17). Briefly, the lungs were inflated with sterile 0.15 M saline until fully distended, and the saline was withdrawn and reinfused two more times. This procedure was repeated a total of four times, and the combined volume of the total lavage was recorded. There were no differences in the total volume of saline infused or recovered after the lavage procedure among the six experimental groups.

A 10-ml aliquot of the total lavage was used for analysis of total protein and surfactant-associated protein levels. The remainder of the lavage was centrifuged at 150 g for 10 min to yield a pellet containing cellular debris. The 150-g supernatant was then spun at 40,000 g for 15 min, yielding a supernatant that represented the small surfactant aggregate fraction. The 40,000-g pellet was suspended in 2 ml of 0.15 M saline and represented the large surfactant aggregate fraction (13).

Phospholipid and protein measurements. To measure the total phospholipid pool size and individual surfactant aggregate phospholipid phospholipid sizes, aliquots from the total lavage, 150-g pellet, large-aggregate, and small-aggregate fractions were extracted by using the method of Bligh and Dyer (4). Phospholipid-phosphorous levels in each of these extracts were determined by using the Duck-Chong phosphorous assay (7). Briefly, 100 µl of 10% magnesium nitrate in methanol were added to the extracted lipids. After being dried, the samples were ashed in a fume hood on an electric rack for ~1 min. After 1 ml of 1 M HCl was added, the samples were rewarmed on a heating block while covered for 15 min at 95°C. After cooling, a 66-µl aliquot of each sample was added to individual wells of a 96-well plate along with 134 µl of a dye consisting of 4.2% ammonium molybdate in 4.5 M HCl with 0.3% malachite green (1:3 vol:vol). The absorbency of each sample was read at 650 nm by using a MKII
Titretek Multiskan ELISA plate reader and compared with reference standards on the same plate. The protein content of the total lavage was determined by the method of Lowry and colleagues (16) by using bovine serum albumin as a standard.

Phospholipid compositional analysis. Lipids from the total lavage were extracted by using the method of Bligh and Dyer (4), and a cold acetone precipitation was performed to remove neutral lipids. Phospholipids were then separated by thin-layer chromatography as described previously (25). The individual phospholipids were then measured by using the previously described Duck-Chong assay (7).

Surfactant protein analysis. Surfactant-associated proteins A (SP-A) and B (SP-B) were measured in the total lavage obtained from the experimental groups by using ELISAs as previously described (25).

Surface activity analysis. In vitro surface tension measurements of unextracted large aggregate samples recovered from animals in all groups were performed by using a pulsating bubble surfactometer (Electronetics, Amherst, NY) as described by Enhorning (8). Aliquots of the large-aggregate fractions were prepared to obtain a final concentration of 1 mg phospholipid/ml in 0.1 M NaCl and 1.5 mM CaCl_2. The samples were incubated for 90 min at 37°C before analysis. Briefly, a bubble was created in the suspension containing the large surfactant aggregate fraction. After 10 s of adsorption, the bubble was pulsated for a period of 5 min between a maximum radius of 0.55 mm and a minimum radius of 0.44 mm at a rate of 20 pulsations/min and a temperature of 37°C. Pressure was monitored across the air-liquid interface by using a pressure transducer, and surface tension was calculated at the minimum and maximum bubble radii.

Calculations and statistics. Data are expressed as means ± SE. Values between groups were compared using a two-way ANOVA followed by the Student-Newman-Keuls test for multiple comparison. A repeated-measures ANOVA was used to compare measurements within groups over time. A probability level of P < 0.05 was considered statistically significant.

RESULTS

Physiological parameters. Table 1 shows the total number of animals, the mean body weights, and baseline physiological measurements of both sham and septic animals 5 h after recovery from the initial surgery. There were no significant differences in PaO_2/FIO_2 values or in arterial lactate levels between these two groups at the 5-h time point. Although there were statistical differences noted in MABP, HR, and RR values between the two groups, all values were within the normal physiological range. In general, these data indicate that all animals adequately recovered from the surgical procedures within 5 h.

Table 2. Physiological parameters for spontaneously breathing animals

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<th>Sham</th>
<th>Septic</th>
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<tr>
<td></td>
<td>Preventilation (22.5 h)</td>
<td>Death (24 h)</td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>PaO_2/FIO_2, Torr</td>
<td>438 ± 14</td>
<td>441 ± 29</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>121 ± 2</td>
<td>117 ± 6</td>
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<tr>
<td>HR, beats/min</td>
<td>326 ± 9</td>
<td>324 ± 8</td>
</tr>
<tr>
<td>RR, breaths/min</td>
<td>94 ± 2</td>
<td>96 ± 2</td>
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<tr>
<td>Lactate, mmol/l</td>
<td>0.65 ± 0.04</td>
<td>0.39 ± 0.07</td>
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*P < 0.05 vs. respective Sham group.

Table 2 shows the physiological parameters of all animals while they were spontaneously breathing at either 22.5 or 24 h after surgery. Values at the 22.5-h time point correspond to data recorded immediately before the 90-min period of ventilation for sham and septic animals (preventilation). Values at the 24-h time point represent data collected immediately before we killed the sham and septic animals that were allowed to spontaneously breathe for the entire 24 h (time of death). There were no significant differences in the measured physiological parameters between the 22.5-h (preventilation) and 24-h time points (death) within the sham groups. There were also no significant differences in any of these physiological parameters between the different septic groups at these two time points. These comparisons reveal that, within the sham and septic groups, there were no significant differences in physiology over this 1.5 h.

There were significant differences, however, in all parameters measured when the sham and septic animals were compared at each of these time points. Systemic sepsis in the CLP animals was confirmed by documenting a significantly lower MABP and significantly higher HR, RR, and arterial lactate values compared with the sham animals (P < 0.05). These physiological changes were similar to those reported previously in adult rats undergoing the CLP procedure (17). In the animals used in this study, as well as in other studies utilizing this model, sepsis was confirmed by culturing gram-negative bacteria from the blood, whereas cultures from sham animals were negative. Septic animals also had evidence of lung injury, albeit relatively mild, as reflected by significantly lower PaO_2/FIO_2 ratios compared with those of the sham control animals (P < 0.05) while they maintained normal PaCO_2 values.

Mean PaO_2/FIO_2 values for the four groups that underwent 90 min of mechanical ventilation are shown in Fig. 1. Oxygenation values at 0 min represent the mean preventilation values recorded at the 22.5-h time point after surgery for these groups. There were no significant changes in oxygenation in the Sham-Vent group over the 90-min period of mechanical ventilation. Of
note, the mean final $\text{PaCO}_2$ value for this group was 42.5 ± 2.0 Torr. In the Septic-Vent group, $\text{PaO}_2$/FiO$_2$ values significantly decreased within 10 min of initiation of ventilation and remained significantly lower than the preventilation value of this group over the remaining period of ventilation ($P < 0.05$). The mean final $\text{PaCO}_2$ value for these animals was 47.0 ± 3.7 Torr. In addition, $\text{PaO}_2$/FiO$_2$ values for the Septic-Vent group were significantly lower than those for the Sham-Vent group at all time points during mechanical ventilation ($P < 0.05$). With the addition of PEEP to the ventilation strategy, there were no significant differences in oxygenation values between the two Sham groups over the 90 min of mechanical ventilation (Sham-Vent vs. Sham-PEEP). The mean final $\text{PaCO}_2$ value for the Sham-PEEP group was 37.0 ± 1.8 Torr, which was not significantly different from that of the Sham-Vent group. In contrast, PEEP improved $\text{PaO}_2$/FiO$_2$ values in the septic animals to levels not significantly different from those of the two Sham groups. The mean final $\text{PaCO}_2$ value for the Septic-PEEP group was 36.5 ± 1.7 Torr.

Peak inspiratory pressure (PIP) values at the end of the ventilation period in the Sham-Vent group (11.8 ± 0.5 cmH$_2$O) were significantly lower than those in the Sham animals ventilated with PEEP (14.6 ± 0.4 cmH$_2$O; $P < 0.05$). In contrast, PIP values for the Septic-Vent group (17.3 ± 0.5 cmH$_2$O) were significantly higher than those for the Septic-PEEP group (15.6 ± 0.4 cmH$_2$O; $P < 0.05$) and the Sham-Vent group ($P < 0.05$) at the end of the ventilation period.

Lung lavage and surfactant analysis. Total protein recovered from the lung lavage in the six experimental groups is shown in Fig. 2. There were no significant differences between the two spontaneously breathing groups (Sham-Spon vs. Septic-Spon). Similarly, protein levels among the four ventilated groups were not significantly different; however, they were significantly higher than the spontaneously breathing groups ($P < 0.05$).

Total surfactant phospholipid pool sizes recovered from the six experimental groups are shown in Fig. 3. Although the phospholipid pools in the septic animals tended to be lower than in the sham animals, there were no significant differences in total pool sizes between the two spontaneously breathing groups (Sham-Spon vs. Septic-Spon) or among any of the ventilated groups.

Animals in all groups had normal surfactant phospholipid composition, with no significant differences observed among the groups (data not shown). Similarly, measurements of SP-A and SP-B also revealed no significant differences between the different experimental groups (data not shown).
Figure 4 shows the percentage of large aggregates recovered relative to the total quantity of surfactant isolated from the different experimental groups. In the Septic-Spon group, there was a significantly higher percent large-aggregate recovery compared with the Sham-Spon group (P < 0.05). Interestingly, ventilation of these animals without PEEP resulted in marked changes in the relative recovery of the large aggregates. Although the Sham-Vent group had a greater percent large-aggregate recovery compared with the Sham-Spon group (P < 0.05), the Septic-Vent group had a significantly lower percent large-aggregate recovery compared with the Septic-Spon group (P < 0.05). In addition, ventilating septic animals with PEEP (Septic-PEEP) resulted in a significantly greater percent large-aggregate recovery compared with the Septic-Spon and Septic-Vent groups (P < 0.05).

Functional analyses of recovered large-aggregate fractions isolated from the six experimental groups are shown in Table 3. There were no significant differences in the minimum surface tension values measured at adsorption (0 pulsations) among all six experimental groups. After 100 pulsations, the Septic-Vent group had significantly higher minimum surface tension values compared with the other groups (P < 0.05).

The separate groups of spontaneously breathing sham and septic animals exposed to 50% oxygen for 90 min underwent identical measurements as those performed on the experimental groups shown above. Results of these experiments revealed no significant differences in total phospholipid pool sizes, percent large-aggregate recovery, functional activity of these large aggregates, and surfactant-associated proteins levels compared with the animals exposed to room air for the entire 24-h period. These findings were similar within both the sham and septic groups (data not shown). These results confirm that the higher FIO₂ administered to the ventilated animals was, by itself, not responsible for the observed differences between the various groups. Furthermore, a separate group of sham animals were ventilated by using an FIO₂ of 30% to obtain comparable PaO₂ values as those recorded in the septic animals at the start of ventilation. This group of sham animals did not have significant changes in oxygenation over 90 min of ventilation, confirming that the initial PaO₂ value was also not responsible for the decline in oxygenation in the ventilated septic animals.

### DISCUSSION

Mechanical ventilation is utilized to maintain adequate oxygenation in patients with respiratory failure. In fact, this intervention is one of the few supportive therapies available for patients with respiratory failure severe enough to meet the criteria for ALI and ARDS (2). Unfortunately, mechanical ventilation itself has been shown to have deleterious effects on lung function as reviewed by Dreyfuss and Saumon (5). These observations have led to several studies evaluating potential factors contributing to the lung damage induced by this intervention. The mechanical forces attributable to ventilation have been thought to be responsible for the damage incurred. These forces include alveolar overdistension or “volutrauma” as well as repetitive opening and collapse of both small airways and distal lung units that create shear forces (21, 30). The present study evaluated the effects of this latter phenomenon by utilizing ventilation strategies with and without PEEP.

Mechanisms by which alveolar overdistension and airflow collapse result in lung dysfunction include increased microvascular permeability, initiation, and/or perpetuation of an inflammatory response within the lung due to physical stretch and alterations of the endogenous surfactant system (5, 12, 23). Although these particular issues have been addressed in previous studies, most were conducted in normal lungs by using relatively nonphysiological ventilation parameters to induce the injury (6, 24, 29, 30). We studied the effects of more conventional ventilatory strategies in a
clinically relevant animal model of lung injury, namely systemic sepsis-induced lung injury.

A 90-min period of mechanical ventilation indicated that septic animals associated with a relatively mild lung injury experienced increased susceptibility to the harmful effects of this intervention compared with sham control animals. This was only evident, however, when repeated collapse and reopening of lung units were allowed to occur when PEEP was not utilized (Septic-Vent group). These animals exhibited a marked deterioration in lung function, which was associated with significant alterations of the endogenous surfactant system. These alterations included a shift in the proportion of functionally superior large-aggregate forms to inferior functioning small aggregates, as well as significantly impaired function of the remaining large aggregates compared with large aggregates isolated from septic animals ventilated with PEEP (Septic-PEEP).

Alveolar surfactant can be separated into a surface-active large-aggregate fraction and a less-surface-active small-aggregate fraction. Large aggregates are secreted from alveolar type II cells and are believed to represent a surfactant reservoir for the surface film located at the air-liquid interface of the alveoli. Pulse-chase studies, as well as in vitro surface area cycling experiments, have shown that these large surfactant aggregates are converted into small surfactant aggregates. This conversion process is predominately dependent on two factors: a change in the alveolar surface area and protease activity (10, 13, 22, 28). Increases in either of these two variables can result in an increase in large-aggregate conversion (10, 28). Increases in alveolar surface area occur when patients are mechanically ventilated, because the administered tidal volumes during ventilation are usually greater than those generated during spontaneous breathing during respiratory failure. This factor, together with the inflammatory changes generally observed in injured lungs, can ultimately result in an increased conversion of large aggregates. As the injury progresses, this increased conversion results in changes in aggregate pools that are thought to impact lung function and thus represent an important mechanism contributing to the lung dysfunction observed in severe ARDS (11, 27).

Surfactant changes observed in the sham animals revealed that ventilation without PEEP resulted in an increase in percent large aggregates compared with spontaneously breathing sham animals that was associated with the maintenance of PaO2/F1O2 levels and low airway pressures. These observations are similar to those described by Webb and Tierney (30), where no lung damage was evident in normal, control rats when ventilated for 1 h with inspiratory pressures of 14 and 0 cmH2O PEEP. The observed increase in percent large aggregates in these lungs was presumably due to an increase in freshly secreted surfactant, because alveolar stretch has been shown to induce surfactant secretion in normal lungs (18). A relatively normal rate of conversion of these large aggregates would also be expected within the air space of normal lungs. However, in a situation of lung injury, as observed in the septic animals in the present study, there was a significant decrease in the percent large-aggregate fraction compared with that of spontaneously breathing septic animals. There was an opposite response compared with sham animals ventilated with the identical strategy. This observation is consistent with previous studies that have implicated a decrease in percent large aggregates as a contributor to lung dysfunction (14, 15, 27). However, these alterations in aggregate forms were not likely fully responsible for the lung impairment observed in these septic animals (Septic-Vent), because the quantity of large aggregates remaining in the lung at death were similar to the ventilated sham group (Sham-Vent). Other factors may, therefore, have contributed to the lung dysfunction observed in the Septic-Vent group. Analysis of the surface tension-reducing function of the remaining large-aggregate forms in the experimental groups revealed that the surface tension values of the large aggregates isolated from the Septic-Vent group was markedly higher compared with those from the other groups. Large aggregates from all sham groups reduced surface tension values below 10 mN/m, indicating excellent physiological activity, which is presumably adequate for maintaining alveolar stability at end expiration in these animals, even in the absence of extrinsic PEEP. In contrast, large aggregates from the Septic-Vent group had surface tension values >17 mN/m after 100 pulsations. Therefore, the relative change in large-aggregate pools combined with the impairment of large aggregate functional activity over the period of ventilation would contribute to alveolar instability and consequently impact lung compliance (increased PIP values) and oxygenation in the septic group ventilated without PEEP. The mechanism responsible for the inferior surface activity of the isolated large aggregates from this group is unknown at the present time and was surprising considering the similar lipid and protein composition. We speculate that, although the quantitative analysis of SP-A as determined by the ELISA technique revealed no significant difference among the experimental groups, there may well have been conformational and/or monomer or multimer changes that occurred in this protein that would not be reflected by the ELISA measurement. Such functional vs. quantitative abnormalities in SP-A and/or the other surfactant-associated proteins would potentially make these large aggregates more susceptible to conversion and/or protein inhibition within the air space of the injured lung.

The addition of PEEP to the ventilation strategy of septic animals mitigated both the physiological impairment and alterations of surfactant. PEEP prevents the repetitive opening and collapse of alveolar lung units, thereby decreasing shear stresses within the lung, which can damage lung units (30). In the present study, PEEP mitigated both the conversion of large aggregates and the decreased function of these large aggregates in the lungs of septic animals compared with those of similar animals ventilated without PEEP. Similar findings were recently reported by Michna et
al. (19) because the addition of increasing amounts of PEEP to surfactant-treated, ventilated preterm lambs resulted in superior lung function as well as preservation of the administered surfactant in large aggregate forms.

Surprisingly, both phospholipid and surfactant-associated protein composition were not altered in either the spontaneously breathing or mechanically ventilated septic animals in the present study. Although previous studies have demonstrated significant alterations in these parameters in patients with severe ARDS (9, 27), the lung injury induced by the CLP procedure in the present study was relatively mild and represented an early stage of lung injury not well characterized in the clinical setting to date. We conclude that the changes in alveolar surfactant may represent an initial response to mechanical ventilation. Furthermore, we suggest that the rapid onset of deleterious alterations of alveolar surfactant that occur in the present study can, during extended ventilation, contribute to the progression to severe lung dysfunction. Thus it is imperative that an optimal ventilation strategy be employed at the start of this intervention.

In summary, we have shown that animals with a systemic sepsis-induced lung injury were more susceptible to the deleterious effects of mechanical ventilation than noninjured animals. Although several mechanisms may contribute to ventilation-induced lung injury, the present study has implicated the repetitive opening and collapse of lung units resulting in alterations of the endogenous surfactant system as at least one potential mechanism. The importance of utilizing adequate levels of PEEP in patients with ARDS is underscored by our results, as is the necessity to implement optimal ventilatory strategies at the onset of this intervention. Because ventilation strategies utilizing smaller tidal volumes are being implemented in patients with ARDS (1), it is important to understand the mechanisms responsible for the physiological differences observed in these studies so that the deleterious effects of mechanical ventilation are minimal.

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