Mechanical Ventilation Affects Lung Function and Cytokine Production in an Experimental Model of Endotoxemia

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Background: Mechanical ventilation using tidal volumes around 10 ml/kg and zero positive end-expiratory pressure is still commonly used in anesthesia. This strategy has been shown to aggravate lung injury and inflammation in preinjured lungs but not in healthy lungs. In this study, the authors investigated whether this strategy would result in lung injury during transient endotoxemia in the lungs of healthy animals.

Methods: Volume-controlled ventilation with a tidal volume of 10 ml/kg and zero positive end-expiratory pressure was applied in two groups of anesthetized–paralyzed rabbits receiving either intravenous injection of 5 μg/kg Escherichia coli lipopolysaccharide (n = 10) or saline (n = 10) 2 h after the start of mechanical ventilation. The third group consisted of 10 spontaneously breathing anesthetized animals receiving lipopolysaccharide. Anesthesia was then continued for 4 h in the three groups while the ventilatory modes were maintained unchanged. Lung injury was studied using blood gases, respiratory physiologic variables, analysis of the bronchoalveolar lavage cell counts, and cytokine concentrations and lung pathologic examination.

Results: Significant histologic lung alterations, hypoxemia, and altered lung mechanics were observed in rabbits treated with mechanical ventilation and intravenous lipopolysaccharide but not in the mechanically ventilated animals injected with saline or in spontaneously breathing animals treated with lipopolysaccharide. Endotoxicemic ventilated animals also had significantly more lung inflammation as assessed by the alveolar concentration of neutrophils, and the concentrations of the chemokines interleukin 8 and growth-related oncogene α.

Conclusions: These results showed that positive-pressure mechanical ventilation using a tidal volume of 10 ml/kg and zero positive end-expiratory pressure was harmful in the setting of endotoxemia, suggesting that the use of this ventilator strategy in the operating room may predispose to lung injury when endotoxemia occurs.

There is some evidence from experimental studies of intratracheally instilled bacteria indicating that ventilatory strategies using Vt around 15 ml/kg enhance lung injury, and that this may be related to enhanced bacterial translocation from the lung to the systemic circulation, although this was not seen in all studies. Synergism between very high doses of lipopolysaccharide and moderately high Vt (15 ml/kg) on cytokine concentrations has been reported both ex vivo and in vitro. This could be related to the synergistic effect of lipopolysaccharide and MV on transcriptional factors such as c-fos, nuclear factor κB, or c-jun (AP-1). An important remaining question is whether a commonly used ventilatory strategy could predispose to lung injury in healthy subjects in whom endotoxemia develops, particularly at much lower concentrations of circulating lipopolysaccharide.

We hypothesized that positive-pressure mechanical ventilation (PPMV) using conventional Vt of 10 ml/kg and zero positive end-expiratory pressure (ZEEP) could predispose to lung injury during transient nonlethal endotoxemia, which may occur during or after major surgery.

Materials and Methods

Animal Preparation

Thirty pathogen-free New Zealand rabbits weighing 2.56 ± 0.02 kg (Charles River Laboratories, L’Aresle, France) were studied. The protocol conformed to the guidelines in the Guide for the Care and Use of Laboratory Animals and was approved by the animal research committee (Co-
The fraction of inspired oxygen (FIO2) was measured in the samples (ABL 330; Radiometer, Copenhagen, Denmark).

The MV animals that received lipopolysaccharide (MV-LPS) or saline (MV-saline) were paralysed with 0.2 mg/kg vecuronium bromide followed by hourly injections of 0.2 mg/kg and were connected to a constant-volume ventilator pump that delivered airflow in a sinusoidal wave form (Beaudoin, Paris, France). $V_T$ was set at 10 ml/kg. Only six respiratory rates were available with this device: 14, 28, 32, 40, 64, and 80 cycles/min. A respiratory rate of 32 breaths/min was the most compatible with a physiologic arterial carbon dioxide tension (PaCO2), and that did not produce hypercapnia (hypercapnia was unacceptable because of its possible effect on cytokine production\textsuperscript{14,15}). This setting was also used in our previously published work. The inspiratory:expiratory ratio was 50\%, and no inspiratory plateau was applied.

Continuous infusion of saline (initially set at 5 ml · kg$^{-1}$ · h$^{-1}$) was administered 	extit{via} a ear vein catheter. When mean arterial pressure decreased below 65 mmHg, the infusion flow rate was increased progressively to a maximum of 20 ml · kg$^{-1}$ · h$^{-1}$.

**Physiologic Variables**

Systemic arterial blood pressure was measured continuously with a calibrated pressure transducer and recorded on a polygraph. Arterial oxygen tension ($P_{\text{aO}_2}$), $P_{\text{aCO}_2}$, and arterial pH ($p\text{H}$) were measured hourly from 0.3-ml blood samples (ABL 330; Radiometer, Copenhagen, Denmark). The fraction of inspired oxygen ($F_{\text{IO}_2}$) was measured in the inspiratory tube coming from the mixing chamber with a Beckman OM11 analyzer (Fullerton, CA).

Airflow was measured with a Fleisch pneumotachograph attached to the tracheal cannula and connected to a differential electromanometer. $V_T$ was obtained through electronic integration of the flow signal. Tracheal pressure (Ptr) was measured from a lateral outlet of the tracheal cannula. Maximum tracheal pressure (peak inspiratory pressure) was measured, and $V_T$, airflow, Ptr, and arterial pressure were continuously recorded on a Gould ES1000 polygraph. Respiratory rate and minute ventilation were calculated from $V_T$ recordings.

**Measurements of Pulmonary Mechanics**

The total resistance of the respiratory system was calculated, according to the method of Amdur and Mead\textsuperscript{16} from the changes in Ptr and flow rate measured between two successive isovolumetric levels (1/2 $V_T$).

A pressure-volume curve was performed at reference time and at the end of the experiment after a short inhalation of pure oxygen, using a 100-ml syringe filled with pure oxygen to successively fill the lungs step by step (5–10 ml) until Ptr reached 30 cm H$_2$O and emptying them until Ptr had returned to the atmospheric pressure. For each volume level, Ptr values were determined from the plateau value measured 2 s after the initial peak. To do this in the spontaneously breathing group, transient muscle relaxation was induced using succinylcholine chloride (1 mg/kg intravenous). Static compliance was measured from the static volume-pressure relation.

**Experimental Design.** Animals were randomized into three groups: In one group, animals were allowed to breathe spontaneously and received intravenous lipopolysaccharide after an initial 2-h steady state period (SB-LPS; $n = 10$), one group was mechanically ventilated and treated with 0.9% intravenous NaCl (MV-saline; $n = 10$), and one group was mechanically ventilated and treated with intravenous lipopolysaccharide (MV-LPS; $n = 10$). The intravenous injection was of 5 µg/kg lipopolysaccharide (0.1 ml/kg of a solution of 5 µg/0.1 ml lipopolysaccharide, Escherichia coli serotype O111: B4; Sigma Chemical, St. Louis, MO) dissolved in 0.9% NaCl. This dose was chosen from data from literature\textsuperscript{17} showing that intravenous administration of a single bolus of 5 µg/kg lipopolysaccharide resulted in a systemic cytokine response but not lung injury in the absence of an additional stimulation. In initial experiments, we tested several doses of lipopolysaccharide and found that at substantially higher doses of lipopolysaccharide, the animals were not hemodynamically stable. All animals were monitored continuously for 4 h after the lipopolysaccharide injections and then euthanized.

**Lung Processing**

After a midline sternotomy, a lethal cardiac injection was administered, and the lungs were clamped at the hilum at end inspiration. The left lung was lavaged with 6 ml/kg sterile saline. The right lung was filled with 10% buffered formalin at a transpulmonary pressure of 15 cm H$_2$O \textit{via} a 3-French catheter.

Cell analysis was performed on 2 ml fluid recovered from lavage. The remaining fluid was spun to pellet the cells, and the supernatant was stored at $-80^\circ$C for further cytokine determination. The pelleted cells were homogenized in Trizol reagent (Gibco-BRL; Life Technologies, Eragny, France) and stored at $-80^\circ$C until tumor necrosis factor $\alpha$ (TNF-$\alpha$) messenger RNA (mRNA) analysis was performed.
Cytokine Determination

Concentrations of TNF-α, interleukin 8 (IL-8), and growth-related oncogene α (GRO-α)/CXCL1 were measured in plasma and bronchoalveolar lavage (BAL) supernatant using rabbit-specific immunoassay as previously described. For TNF-α, monoclonal anti-rabbit TNF antibodies were used. IL-8 was measured by immunoassay using two different murine monoclonal antibodies raised against recombinant rabbit IL-8. This assay was modified from our previously published work. Assay lower limits were 0.1, 0.1, and 0.01 ng/ml for GRO-α, TNF-α, and IL-8, respectively.

Real-time polymerase chain reaction was performed using oligonucleotide polymerase chain reaction primers synthesized according to the published complementary DNA sequences of rabbit TNF-α and 18S ribosomal RNA.

Histologic Analyses

In fixed lungs, the cranial, middle, and caudal lobes were sampled from 3-mm horizontal sections including the whole circumference of each lobe. All slides were examined by two pathologists blinded to the group assignment of the lungs. The following histologic criteria were assessed: edema (alveolar and interstitial congestion), hemorrhage, interstitial cell infiltration, and intraalveolar cell accumulation. Their respective intensities were semiquantitatively scored as 0 (absent), + (mild), ++ (moderate), or ++++ (severe). Intravascular aggregation of neutrophils on the vessel walls and the existence of thrombi were recorded. When disagreements occurred between the observers, the pathologists were asked to rescore the sections and to give a final consensus result. The lungs of six additional control animals that had not been ventilated and were killed after a lethal injection of anesthetic were also analyzed for comparison. For each criterion, a final score was calculated from the unique final decision of the two pathologists by dividing the sum of individual results by the number of animals (minimum, 0; maximum, 3).

Statistical Analysis

All data were expressed as the mean ± SEM. The Sigmastat 2.03 program was used for statistics (Sigma Company, Erkrath, Germany). Data were tested for normal distribution with the Kolmogorov–Smirnov test. The differences in the values among the three study groups were compared using parametric one-way analysis of variance for normally distributed data. When the data were not normally distributed (cytokine concentrations), differences between the times were identified using either the Student–Newman–Keuls comparison test after repeated-measures analysis of variance or the Tukey multiple comparison test after the nonparametric Friedman test.

When a parameter was measured twice only (i.e., pulmonary mechanics), comparisons over time were assessed by a paired t test. A P value of less than 0.05 was used to determine statistical significance. Agreement between the two pathologists was assessed by the κ coefficient calculation (κ = observed agreement – expected agreement/1 – expected agreement). A κ statistic of less than 0.4 is considered to show a weak correlation, whereas values from 0.4 to 1 are interpreted as moderate, good, and very good.

Results

Preinjection Characteristics

In the three groups, arterial blood gases and pH remained stable throughout the 2-h period preceding injection of lipopolysaccharide or saline (fig. 1). Table 1 shows the mean values of physiologic variables measured at baseline, i.e., just before injection of lipopolysaccharide or saline. There was no significant difference between the two MV groups (MV-LPS and MV-saline) for any of the baseline parameters. Spontaneously breathing animals had higher PaCO₂, lower V̇E, and higher respiratory rate values at baseline than MV animals. However, their minute ventilation, resistance of the respiratory system, and static compliance were similar to those measured in MV animals.

Lipopolysaccharide- and/or MV-induced Changes in Physiologic Variables

In the three groups, the mean arterial pressure decreased significantly at the end of the experiment, but despite the fact that the MV-saline group tended to have higher mean arterial pressure values, no significant difference between groups was observed at any time during the study. Heart rate did not differ among any of the groups at any time. Therefore, there was no evidence of a significant deleterious effect of our dose of lipopolysaccharide on cardiovascular function. At the end of the experiment, values were 72 ± 8, 73 ± 7, and 66 ± 8 mmHg in the SB-LPS, MV-saline, and MV-LPS groups, respectively.

No modification of arterial blood gases and pH (fig. 1) were observed after saline injection in the MV-saline group. In spontaneously breathing animals (SB-LPS group), lipopolysaccharide injection did not induce a PaO₂ change, but hypocapnia developed in the first hour after injection, because of hyperventilation. In this group, V̇E increased.
PaCO₂ increased significantly in the MV-LPS group (increase in PaCO₂ and a decrease in pH in MV-LPS and in MV-LPS with LPS, both p < 0.05). Despite the decrease in pH, the difference in pH with the other groups did not reach significance at any time. In contrast, PaCO₂ remained significantly increased above baseline at the end of the experiment (P < 0.05), although there was a trend toward further decline after the early lipopolysaccharide-induced increase. Static compliance was significantly lower at the end of the experiment than at baseline (decreasing to 0.87 ml · cmH₂O⁻¹ · kg⁻¹; P < 0.05 vs. baseline), whereas it remained stable in the two other groups. In addition, in MV-LPS rabbits, peak inspiratory pressure increased up to 14.5 ± 1.1 cmH₂O (P = 0.08). The resistance of the respiratory system did not change significantly in any of the groups.

**Lipopolysaccharide- and/or MV-induced Inflammatory Response**

All groups had similar baseline concentrations of blood leukocytes. In the two groups challenged with lipopolysaccharide, the blood leukocyte count, particularly the neutrophil count, decreased significantly and with the same magnitude. The peripheral blood leukocyte count did not change in the MV-saline group (fig. 2A).

Lipopolysaccharide injection induced an increase in the BAL total leukocyte count of both ventilated and nonventilated animals. The BAL leukocyte counts were 525 ± 51 and 457 ± 38 × 10⁶/ml in MV-LPS and SB-LPS groups, respectively (not significant), versus 316 ± 27 × 10⁶/ml in the MV-saline group (P < 0.01 vs. MV-LPS and SB-LPS). In endotoxemic animals, this increase was due mainly to an increase in neutrophils and macrophages. Because of the significant increase in neutrophils in the BAL of MV-LPS animals in comparison with the two other groups (fig. 2B), the percentage of macrophages was lower in the MV-LPS group than in the SB-LPS and MV-saline animals (80.8, 88.6, and 97.2%, respectively; P < 0.05). All groups had similar baseline plasma concentrations of cytokines (fig. 3). The changes in plasma concentration of cytokines due to lipopolysaccharide injection were sim-
ilar at the different times (baseline and 1 and 4 h after lipopolysaccharide injection) in the MV-LPS and SB-LPS groups. The plasma IL-8 concentration markedly increased after lipopolysaccharide injection, and high concentrations were detectable at 4 h. TNF-α increased early (1 h) and then decreased to relatively low concentrations at 4 h. GRO-α increased slightly in the groups challenged with lipopolysaccharide but remained at concentrations less than 1 ng/ml of plasma. Cytokine concentrations remained low in the MV-saline group at all times.

The BAL concentrations of IL-8 and GRO-α were both significantly higher in MV-LPS group than in the two other groups (fig. 4). TNF-α was not detected in the BAL of SB-LPS animals but was detected in some MV-saline and MV-LPS animals; however, the median value was at zero in all groups. Because these negative results differed from what we expected, we performed mRNA analysis in all of the animals. Animals treated with MV only (MV-saline group) had higher expression of TNF-α mRNA than spontaneously breathing endotoxemic animals, and there was nonsignificant further increase with the combination of lipopolysaccharide and MV (9-fold and 11-fold, respectively). The total protein content of the BAL was not different among the three groups.

**Histopathology**

In the histologic evaluation, disagreement between the two pathologists was never noted for more than two of the histologic criteria for each animal. The disagreements concerned the presence or absence of interstitial edema. For this parameter, the κ coefficient was 0.37 in the SB-LPS group, 0.58 in the MV-LPS group, and 0.8 in the MV-saline group. There was 100% agreement in all cases after reevaluation of the coded samples.

Light microscopic lung examination mainly showed interstitial cell infiltrates in the MV-LPS, SB-LPS, and MV-saline groups. These changes were least noticeable in the MV-saline group and most apparent in the MV-LPS group. In this latter group, an increase in the alveolar wall thickness was noted. Figure 5A shows four representative sections of control, MV-saline, MV-LPS, and SB-LPS groups. In some sections from MV-LPS and MV-saline animals, leukocytes were present in the alveolar lumen. Neutrophil adherence to extraalveolar vessel walls was observed in 50% of the MV-LPS animals, in one animal of the SB-LPS group, and also in one animal of the MV-saline group but in none of the controls (fig. 5B,
Discussion

The major goal of this study was to determine whether an MV strategy commonly used in major surgical procedures enhances the effects of transient endotoxemia on the lungs. The results show that injection of 5 \( \mu \)g/kg endotoxemia by itself caused only minor changes in the lungs of spontaneously breathing animals, whereas endotoxemia had a marked effect in ventilated lungs in vivo, reflected by impaired function and increases in lung inflammatory cells and chemotactic cytokines. These results are important because they suggest that conventional MV is a risk factor for inflammatory lung injury when endotoxemia occurs, as during or after major surgery. These observations add to a growing body of experimental work suggesting that PPMV can be harmful even when moderate pressures and volumes are applied to the lungs. The novelty of our approach consists of testing a ventilatory strategy that reflects current practice in healthy humans, i.e., using moderate volume and moderate pressure levels in previously healthy lungs.

Experimental Model

Positive-pressure mechanical ventilation using noninjurious \( V_T \) and ZEEP does not usually induce significant lung injury \textit{per se} in animals with healthy lungs, in contrast to what is observed when lungs are injured. Some experimental observations suggest that PPMV may also sensitize the lung to a secondary injury. PPMV is also associated with qualitative or quantitative changes in surfactant. However, whether a ventilatory strategy using 10 ml/kg \( V_T \) in ZEEP could favor lung injury in healthy lungs in the presence of a further inflammatory stimulation had not been shown before this study. The MV protocol that we used was based on recent reports in clinical practice. In humans, Wrigge et al. found that a potentially injurious ventilatory strategy using 10–15 ml/kg \( V_T \) and ZEEP did not enhance systemic or lung inflammatory responses after 3 h in the operating room. Recently, Altemeier et al. found that a moderately high \( V_T \) (\( V_T = 15 \) ml/kg) had a synergistic effect with high concentrations of lipopolysaccharide (5 mg/kg), significantly higher than we used in this study. In contrast with that study, our experimental approach using a \( V_T \) of 10 ml/kg and lipopolysaccharide at 5 μg/ml more closely simulates the MV strategy commonly used in the operating room and should more closely reflect the concentration range of circulating lipopolysaccharide associated with the perioperative periods of major surgery. At high doses of lipopolysaccharide, plasma endotoxin concentration reaches levels thousands-fold higher than that documented in humans during the operative period or during septic shock.

Depending on the animal species, experimental conditions, and the dose of lipopolysaccharide, the lung response to endotoxemia ranges from leukocyte accumulation in lung capillaries without changes in vascular permeability to severe lung damage. Our protocol of intravenous administration of 5 μg/kg was based on the study of Schimke et al., which showed no marked lung injury at this dose of lipopolysaccharide in the absence of an additional stimulus. As expected, we observed only minor changes in spontaneously breathing endotoxemic animals, according to previous reports with doses of lipopolysaccharide varying from 0.1 to 3 mg/kg. Because a 2-h period has been sufficient to observe ventilation-induced lung inflammation \textit{ex vivo} and in vivo, we chose a 2-h delay before lipopolysaccharide injection to provide a chance for PPMV to prime the lung for an accentuated response to the second stimulus.

Lung Inflammation

We measured IL-8 and TNF-\( \alpha \) because of published data suggesting synergy between mechanical stretch and sepsis. In addition, the CXC chemokine, GRO-\( \alpha \), appeared interesting to investigate acute inflammation in rabbits. Named CXCL1 in the novel nomenclature, GRO-\( \alpha \) is produced by humans and mouse cells and is homologous in rats to the cytokine-induced neutrophil chemoattractant protein. Like other \( \alpha \) chemokines, GRO-\( \alpha \) is a potent neutrophil attractant and activator

![Fig. 4. Concentration of the cytokines interleukin 8 and growth-related oncogene \( \alpha \) (GRO-\( \alpha \)) in the bronchoalveolar lavage (BAL) performed at the end of the experiment in animals injected with lipopolysaccharide while mechanically ventilated (closed bars) or breathing spontaneously (open bars) and in mechanically ventilated animals injected with saline (dashed bars). Nonparametric analysis of variance on rank test (Kruskal–Wallis test) showed significant differences among the three groups for both cytokines (\( P < 0.05 \)). Asterisks indicate significant post hoc test results. ** \( P < 0.01 \), *** \( P < 0.001 \) versus the two other groups. Tumor necrosis factor \( \alpha \) is not represented because its median was at zero value in all groups.](image-url)
that may play an important role in the pathogeneses of ventilator-induced lung injury.\textsuperscript{19,39}

Neutrophils were moderately increased in the BAL of spontaneously breathing animals, in accord with previous reports showing that in the absence of another lung stimulus, neutrophils remain sequestered in capillaries.\textsuperscript{40,41} The number of sequestered cells is both dose and time dependent\textsuperscript{33}; however, transmigration to the alveolar surface requires chemotactic factors\textsuperscript{32} such as IL-8 and GRO-\(\alpha\). We report lower concentration of cytokines in the BAL fluid than in plasma on crude measurement. However, performing BAL causes dilution of \textit{in situ} molecules of as much as 50- to 100-fold, depending on the method used. Here, we instilled approximately 15–20 ml saline in a left lung of approximately 50 ml total lung capacity in the open thorax. Because the BAL was standardized, we believe the dilution factor was similar in all animals, so comparison should be accurate between groups assessed with the same methodology. It may not be the same when cytokine concentrations from different studies are compared: Lavage procedure and assays for cytokine measurement may differ.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure5}
\caption{(A) Four representative hematoxylin and eosin \(\times100\) microphotographs (light microscopy) of lungs fixed at the same transpulmonary pressure mechanically in ventilated animals receiving lipopolysaccharide (MV-LPS) or saline (MV-saline) or in spontaneously breathing animals receiving lipopolysaccharide (SB-LPS) and control rabbits. Note interalveolar septa thickened by an interstitial cell infiltrate and several alveoli collapsed in the MV-LPS lung section. (B) (Top) hematoxylin and eosin stain \(\times400\) showing polymorphonuclear aggregates (arrow) in the lumen of small lung vessel. (Bottom) Hematoxylin and eosin stain \(\times25\) of an MV-LPS subject showing perivascular edema (stars) and lymphatic dilatation (arrows).}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure6}
\caption{Histopathologic scores independently grading congestion, interstitial inflammation, and the presence of intraalveolar leukocytes in animals injected with lipopolysaccharide while mechanically ventilated (MV-LPS) or breathing spontaneously (SB-LPS) and in mechanically ventilated animals injected with saline (MV-saline). All score criteria showed statistical differences among the three groups as assessed by analysis of variance. Asterisks indicate the groups that differed from the others using the \textit{post hoc} analysis of variance test. * \(P < 0.05\), ** \(P < 0.01\), *** \(P < 0.001\). ns = not significant.}
\end{figure}
generating discrepancies between measured concentration in different studies.  

*In vitro*, the potentiating effect of a mechanical stretch and lipopolysaccharide on IL-8 production by lung cells has been reported.  

The kinetics of release of TNF-α, with elevation at the early stage of exposure to PPMV and then decline in the late stage, does not necessarily explain these discrepancies. Interestingly, we found that TNF-α mRNA production was markedly increased in the RNA samples of the alveolar cell pellets of the ventilated animals. Because most TNF-α is secreted after transcription, this suggests that TNF-α was present in the lower airways of the ventilated animals. However, the role of TNF-α in ventilation-induced inflammation remains unclear and is debated in the literature.  

In some animal models of ventilator-induced lung injury, alveolar concentrations of TNF-α are increased, whereas in other models they are not. Even in acute respiratory distress syndrome patients, TNF-α is present in BAL fluids but is largely inactivated.

**Lung Injury**

A striking difference in the response to lipopolysaccharide between ventilated and spontaneously breathing animals was the early alteration in gas exchanges at 1 h. This was not related to significant hemodynamic impairment in the MV-LPS group, because the systemic arterial pressure and heart rate remained in normal range. However, the occurrence of a lipopolysaccharide-induced hyperkinesia could not be fully ruled out in the absence of intrapulmonary shunt measurement. The increase in PaCO₂ in MV-LPS animals suggests an increase in physiologic dead space as a marker of lung physiologic alteration.

The exact cause of the significant differences between the spontaneously breathing and the mechanically ventilated animals in the response to lipopolysaccharide is not clear from this study. In mechanically ventilated animals, V̅f was nearly twice that of spontaneously breathing animals (1.7-fold). Compared with negative-pressure spontaneous breathing, PPMV causes differences in the pattern of applied forces across the lungs. As another possible mechanism, there may also have been an increase in capillary transmural pressure, either because of changes in intrathoracic pressure on the pulmonary vasculature or because of the interdependence phenomenon. Muscle paralysis may also have a role. In a similar rabbit model of MV in ZEEP, avoiding muscle paralysis resulted in fewer lung macroscopic abnormalities and decreased inflammatory response (including cytokines) while the V̅f was unchanged. Therefore, we asked whether the suppression of diaphragmatic activity could have played a role in the enhancement of lung injury in mechanically ventilated endotoxemic animals. The regional distribution of ventilation, especially in prediaphragmatic locations, may differ between mechanically ventilated paralyzed animals as compared with spontaneously breathing animals. This might favor a harmful effect of MV. In addition, it is not known whether cellular hypoxia develops in microatelectatic areas (that are known to develop during MV in anesthetized and paralyzed subjects) and acts as a trigger for inflammatory responses. The role of the absence of positive end-expiratory pressure in our model should also be questioned because, by decreasing microatelectatic areas, positive end-expiratory pressure may minimize the injury, possibly because of atelectotrauma (biotrauma due to opening and closing unstable alveoli). Major differences in hemodynamic, anesthetic/muscle paralysis management and ventilatory strategy between the current study and the study of Altemeier et al. may have influenced the above-cited mechanisms (even hypothetical) and might explain some differences in lung physiologic response between the two studies.

**Limitations**

The experimental design comparing ventilated with spontaneously breathing animals resulted in baseline differences between groups in the airway pressure regimen and several physiologic parameters. For example, the spontaneous breathing animals developed marked hypocapnia, which could influence the lung injury, the cytokine response, or both. As demonstrated by some authors, hypocapnia could accentuate both the lung injury and the inflammatory response. However, our spontaneously breathing animals had fewer lung alterations than the ventilated animals, so hypocapnia cannot explain the differences that we observed between the experimental groups.

In sum, this study shows that MV and circulating lipopolysaccharide have important interactions that could be relevant during and after surgery. MV with routinely used V̅f and ZEEP enhanced responses to circulating lipopolysaccharide. The degree of hypoxemia correlated with the alteration of the pulmonary mechanics and with increased alveolar neutrophils, IL-8 and GRO-α, suggesting a possible role for the inflammatory response in lung functional abnormalities. These results show that further information is needed about more effective ventilatory strategies that could be used in healthy patients undergoing major surgery, particularly those at risk for intraoperative or postoperative endotoxemia or sepsis.
VENTILATOR-INDUCED LUNG INJURY IN HEALTHY LUNGS

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