

Bactericidal Function of Alveolar Macrophages in Mechanically Ventilated Rabbits

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Protective ventilation strategies have been universally embraced because of reduced mortality. We tested the hypothesis that tidal volume (V_T) in an *in vivo* model of mechanical ventilation would modulate bactericidal function of alveolar macrophages (AMs). Adult New Zealand White rabbits were mechanically ventilated for 4 h with a V_T of 6 ml/kg (low) or a V_T of 12 ml/kg (traditional), with each group receiving 3 cm H_2O positive end-expiratory pressure with and without intratracheal lipopolysaccharide (LPS) instillation (20 mg/kg). AMs were isolated from bronchoalveolar lavage fluid taken from the whole left lung and used for bacterial killing assays. There were no significant differences in steady-state levels of nitrite or AM phagocytosis and killing of *Klebsiella pneumoniae*, although these values trended to be slightly higher in the traditional V_T group. However, bronchoalveolar lavage fluid protein concentrations were significantly increased in traditional V_T groups receiving LPS compared with animals ventilated with a low V_T (1,407.8 \pm 121.4 versus 934.7 \pm 118.2; $P < 0.001$). Lung wet:dry weight ratio in the traditional V_T group was increased when compared with the low V_T group without LPS (7.3 \pm 0.4 versus 6.1 \pm 0.3, respectively; $P < 0.05$). Additionally, IL-8 expression was significantly greater under conditions of LPS treatment and mechanical ventilation at V_T of 12 ml/kg. These results suggest that the traditional ventilator approach (12 ml/kg V_T) in a model of *in vivo* mechanical ventilation results in lung pathology without affecting AM antibacterial function.

Keywords: acute lung injury; acute respiratory distress syndrome; alveolar macrophages; lipopolysaccharide; protective ventilation

The basic purpose of mechanical ventilation is to support patients whose respiratory systems have failed until adequate function returns. Mechanical ventilation can also relieve respiratory distress in patients for whom the work of breathing has increased. However, traditional approaches to mechanical ventilation have been demonstrated to contribute to lung injury via upregulation of local pulmonary inflammatory responses and worsened increased alveolar capillary permeability (1–5). The release of inflammatory mediators can increase lung inflammation and contribute to injury to other vital organ systems, thus contributing to or sustaining the multiple organ dysfunction syndrome (4, 6). Mechanical ventilation also induces stress by overstretching small airways and alveoli due to increased volumes in some

regions of the lung, the so-called “volu-trauma.” This continuous cycle of opening and closing of alveolar groups in other regions also causes severe wall stress and surfactant depletion, leading to recruitment-derecruitment injury (7, 8). Thus, lung injury, or worsened lung injury during mechanical ventilation, is a matter of major concern.

Animal studies show that ventilation using large tidal volumes (V_T) and low positive end-expiratory pressure causes the disruption of pulmonary epithelium and endothelium, lung inflammation, atelectasis, hypoxemia, and the release of inflammatory mediators (4, 9–11). However, a large prospective study showed that mortality was reduced from 40 to 31% in patients with acute respiratory distress syndrome (ARDS) with the use of a ventilatory strategy that used low V_T and limited plateau pressure to < 30 cm H_2O (12). Although it is not known why ventilation at lower V_T results in an improved outcome, possibilities include a reduction in injurious lung stretch (13, 14), less barotrauma (15), and diminished inflammatory cytokine production (4, 16, 17).

Likewise, persons subjected to mechanical ventilation are susceptible to bacterial infections (18). Because intubation and mechanical ventilation alter first-line patient defenses, they greatly increase the risk for nosocomial bacterial pneumonia. Lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, is the causative agent of endotoxin shock. Severe lung injury caused by endotoxin is challenging to control or treat and often causes death. It is a major complication in the control of infections in many patients. Exposure of the lower respiratory tract to LPS by intratracheal installation of LPS is a common model of acute lung inflammation and ARDS (19–22). LPS activates alveolar macrophages (AMs) and causes neutrophils to infiltrate and damage the lungs (23). AMs are thought to be an important clearance vehicle due to their ability to phagocytize, transport, and digest inhaled particles. AMs also play a central and functional role in a variety of acute lung injury models via secretion of oxygen and nitrogen-derived free radicals and proinflammatory cytokines, such as TNF- α , IL-6, and IL-8 (24–26). Thus, it is imperative to understand the pathogenic mechanisms involved, especially as they relate to the AM role in ventilator–lung interactions. Innate immunity involving AMs is of major importance in the early response of the lung to air–space pathogens; thus, a reduction in this population with traditional ventilation strategies may increase susceptibility to infection. In fact, one study showed that the AM population and the early innate immune response of the lung were profoundly affected by mechanical ventilation (27). It is possible that mechanical ventilation initiates or enhances an inflammatory response, with release of proinflammatory cytokines, which may better activate AMs. However, O’Reilly and colleagues (28) showed that oxidative stress produced by increased levels of reactive oxygen nitrogen species impaired AM antibacterial function.

The objective of this study was to compare bactericidal function of AMs at low (6 ml/kg) and traditional (12 ml/kg) V_T in *in vivo* models of mechanical ventilation. To better mimic the

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in vivo situation, we repeated these measurements after infusion of LPS, which resulted in significant levels of acute lung injury. Our results indicate that animals treated with LPS and ventilated at traditional V_T exhibit the greatest degree of lung injury; however, the AM seems to be a noncontributory participant in provoking injury.

MATERIALS AND METHODS

All experimental work conformed to the National Institutes of Health guidelines and was approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham.

Animal Model

New Zealand white rabbits weighing 2.5–3.0 kg were used. Rabbits were anesthetized with intravenous ketamine (10 mg/kg) administered via a marginal ear vein. Anesthesia was maintained with ketamine (10 mg/kg/h) and xylazine (3 mg/kg/h). Intermittent neuromuscular blockade was maintained with intravenous administration of pancuronium bromide (0.5 mg/kg/h). A 3.5-mm endotracheal tube was placed via tracheostomy. Ventilation was initiated with V_T at 6 ml/kg (a physiologic tidal volume) or 12 ml/kg, positive end-expiratory pressure at 3 cm H_2O , with a respiratory rate to maintain a P_{aCO_2} values between 35–45 mm Hg and $FiO_2 = 0.4$ for 4 h. Mean arterial pressure (mm Hg), heart rate (beats per minute), and esophageal temperature ($^{\circ}C$) were measured throughout the study protocol and recorded at 30-min intervals. In one group of rabbits, lung injury was induced by a single intratracheal instillation of LPS (20 mg/kg) (*Escherichia coli*, serotype 055:B5; Sigma Chemical, St. Louis, MO), which was administered ~15 min after the initiation of mechanical ventilation.

Isolation of AMs

Resident AMs were obtained by bronchoalveolar lavage (BAL) of the left lung with five aliquots of 30 ml 0.9% NaCl. Cells in the BAL fluid (BALF) were pelleted (800 \times g, 10 min, $4^{\circ}C$), washed in ammonium chloride lysing buffer to lyse red blood cells, and resuspended in Dulbecco's modified Eagle's medium (DMEM). Cell numbers were counted with a hemacytometer, and the number of different cells was assessed via cytospin preparations stained with Diff-Quik (Dade AG, Duding, Switzerland).

Bacterial Killing Assay

We used *Klebsiella pneumoniae* (American Type Culture Collection 43816, type 2). Bacterial stocks were thawed, inoculated into broth, and grown to log phase. All bacteria were washed free of growth media before infection. Internalization and intracellular killing of *K. pneumoniae* was monitored using a modification of the assay by Bidani and colleagues (29). In brief, 5×10^5 AMs were suspended in DMEM and infected with *K. pneumoniae* in a concentration ratio of 100:1 to 200:1 bacteria per cell on a shaker platform at $37^{\circ}C$ for 1 h. The cell suspensions were chilled on ice and centrifuged (100 \times g, 7 min, $4^{\circ}C$). The supernatants were collected, and cells were washed twice in DMEM and lysed. Quantitative bacterial culture was performed on the supernatants and cell lysates to obtain CFU in the extracellular and intracellular cell environments, respectively. CFUs were determined by enumeration after serial dilution and inoculation onto agar plates. *K. pneumoniae* was grown in BBL brain heart infusion broth and nutrient agar (DIFCO; Becton Dickinson) and incubated in room air at $37^{\circ}C$ for 18–24 h. Phagocytosis was quantified as the difference in the CFUs between paired studies with and without cells. Intracellular killing of the phagocytosed bacteria was calculated as the difference in the CFUs of phagocytosed bacteria and that of cell lysate in paired studies.

Phagocytosis Assay

K. pneumoniae was labeled with 5- (and 6-) Carboxyfluorescein succinimidyl ester (Molecular Probes, Eugene, OR) as described (30) and stored at $-20^{\circ}C$. AMs (5×10^6) were incubated with fluorescent bacteria in 0.5 ml serum-free DMEM on a shaker at $37^{\circ}C$ for 1 h, pelleted by centrifugation at 180 \times g, and washed in ice-cold PBS with 0.02% EDTA to stop phagocytosis and remove extracellular bacteria. Cytospin preparations were made from cells, fixed with 3% formaldehyde, perme-

abilized with 0.1% Triton X-100 in PBS, and stained with AlexaFluor 594 phalloidin (Molecular Probes) diluted 1:40 in 1% BSA. Sections were mounted in VectaShield containing 4,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA). Imaging was performed on a Leica DMIRBE inverted epifluorescence/Nomarski microscope outfitted with Leica TCS NT Laser Confocal optics (Leica Inc., Exton, PA). This method allowed us to distinguish intracellular from outer-membrane-adhered pathogens.

Lung Injury Indices

The right lower lobe was used for determination of lung wet-to-dry weight ratio. Lung segments were weighed before and after drying in an oven at $120^{\circ}C$ for 2 wk to calculate the wet-to-dry weight ratios. Nitric oxide (NO) concentration in the BAL was quantified by measuring nitrite (NO_2^-), a stable oxidation product of NO, as previously described (30). Nitrate (NO_3^-) was converted to NO_2^- with *Escherichia coli* reductase, and 100 μ l of sample was incubated with 25 μ l of freshly prepared 2,3-diaminonaphthalene (0.05 mg/ml in 0.62 M HCl) and incubated for 10 min. The reaction was stopped by the addition of 25 μ l of 5.6 N NaOH, and the signal was measured using a fluorescent plate reader with excitation at 360 nm and emission at 450 nm. NO_2^- concentrations were determined using a $NaNO_2$ standard. Total protein concentration in the BAL was measured using the bicinchoninic acid (BCA) assay (Pierce Biotechnology, Inc., Rockford, IL).

Measurement of TNF- α

A 100-mg section of the upper right lung lobe was homogenized in Hanks' balanced salt solution. Total protein concentration was measured using the BCA protein assay. TNF- α expression was measured using a sandwich ELISA. Each well of a 96-well plate was coated with purified polyclonal goat anti-rabbit TNF- α (1:250 dilution; BD Biosciences, Mountain View, CA). Protein from homogenized lungs (1 mg/ml) was added into antibody-coated wells. Wells were washed, and biotinylated monoclonal mouse anti-rabbit TNF- α (1:5,000 dilution; Amersham Biosciences, Piscataway, NJ) was added. After extensive washing, avidin-HRP was added to detect the "antibody-antigen-antibody sandwich." Absorbance was read at 450 nm, and results are expressed as percentage of change.

Immunohistology

Paraffin-embedded lung tissue sections 5- μ m in thickness were stained for IL-8 as previously described (31). In preparation for immunohistochemistry, sections were dewaxed and rehydrated through graded alcohols to a final wash in Tris-buffered saline (TBS). Sections were treated with 0.3% hydrogen peroxide for 10 min to block endogenous peroxidase activity and washed with TBS containing 0.1% Triton X-100 and TBS. Nonspecific protein binding was blocked with 5% normal donkey serum for 2 h at room temperature. Sections were incubated with a goat polyclonal IL-8 antibody (1:40 dilution) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) overnight at $4^{\circ}C$. Control sections were treated in parallel but incubated with normal goat serum (as a negative control) instead of the primary antibody. All sections were incubated in a moist chamber. Sections were incubated with biotin-conjugated donkey anti-goat IgG (1:200 dilution) (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 30 min. After 30 min of incubation in an avidin-biotin complex, the reaction product was visualized by 3,3'-diaminobenzidine (DAKO Corp., Carpinteria, CA). Finally, sections were dehydrated and cleared in ethanol and xylene and mounted in Permount (Fisher Scientific, Pittsburgh, PA). Representative photomicrographs were acquired via a Nikon Diaphot microscope with bright-field optics and a Nikon Coolpix 950 digital color camera (Nikon, Tokyo, Japan).

ELISA Measurement of IL-8

Concentrations of IL-8 in the BALF were measured using ELISA. Total protein concentration of BALF was measured using the BCA protein assay. Each well of a 96-well plate was coated with 1 ml diluted BALF fluid, which is 1 mg/ml. Blocking buffer (PBS containing 1% BSA and 0.02% azide) was added to block nonspecific protein binding. These wells were incubated with goat anti-human IL-8 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The concentrations of IL-8 were visualized with a horseradish peroxidase-conjugated secondary antibody

followed by the addition of 3,3',5,5'-tetramethylbenzidine peroxidase substrate. The acid stop solution was added after incubated for 15 min at room temperature, and the expression was evaluated by the reading at 450 nm.

Statistical Analysis

The data are presented as means \pm SEM. Data were analyzed by one-way ANOVA followed by Tukey-Kramer's multiple group comparison of the means for parametric data and by the Kruskal-Wallis ANOVA and Dunn's multiple group comparison of the means for nonparametric data. A P value of ≤ 0.05 was considered statistically significant.

RESULTS

Indices of Lung Injury

The ratio between P_{aO_2} and F_{iO_2} was used as a measure of lung injury. A decrease in this ratio to < 300 signified the onset of lung injury (Figure 1). Lung wet-to-dry weight ratios, BALF protein, and steady-state levels of nitrite were used to express indices of inflammatory-mediated lung injury. Lung wet-to-dry weight ratio in the traditional V_T group was increased when compared with the low V_T group without LPS (7.3 ± 0.4 versus 6.1 ± 0.3 , respectively; $P < 0.05$) (Figure 2A). LPS administration significantly increased lung wet-to-dry weight ratios in the low V_T and traditional V_T groups compared with untreated lungs (9.1 ± 0.9 versus 6.1 ± 0.3 and 10.1 ± 0.9 versus 7.3 ± 0.4 , respectively; $P < 0.05$) (Figure 2A). Protein concentration was significantly greater in the LPS-treated low V_T and traditional V_T groups (934.7 ± 118.2 versus 227.1 ± 45.4 and $1,407.8 \pm 121.4$ versus 199.8 ± 28.8 , respectively; $P < 0.001$) (Figure 2B). There were no differences in protein concentration between low and traditional V_T groups without LPS; however, there was a significant increase in the LPS-treated traditional V_T group compared with the LPS-treated low V_T group ($1,407.8 \pm 121.4$ versus 934.7 ± 118.2 ; $P < 0.05$) (Figure 2B). Steady-state levels of nitrate were significantly increased in the traditional V_T group after LPS treatment when compared with the untreated group (13.5 ± 2.5 versus 0.9 ± 0.3 ; $P < 0.001$) (Figure 2C). There was also a significant difference in steady-state levels of nitrate between LPS-treated traditional and low V_T groups (13.5 ± 2.5 versus 6.6 ± 1.1 ; $P < 0.05$) (Figure 2C).

Phagocytosis and Intracellular Killing of *K. pneumoniae*

Phagocytosis and intracellular killing of *K. pneumoniae* was observed by use of quantitative bacterial culture as previously

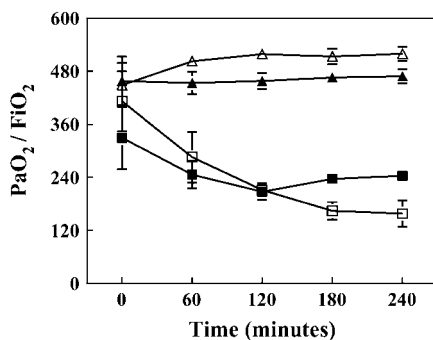


Figure 1. P_{aO_2}/F_{iO_2} values for mechanically ventilated rabbits. Mean arterial pressure (mm Hg) was measured and recorded at 30-min intervals. Data from each treatment group represents the average of five experiments \pm SEM. $V_T 6$ = tidal volume 6 ml/kg; $V_T 12$ = tidal volume 12 ml/kg. Solid triangles, $V_T 6$; open triangles, $V_T 12$; solid squares, $V_T 6 + LPS$; open squares, $V_T 12 + LPS$.

described (5). There were no significant differences in phagocytosis and killing between low and traditional V_T groups (Figures 3A and 3B). Phagocytosis was significantly increased only in the LPS-treated low V_T group ($P < 0.001$) (Figure 3A); however, killing was significantly increased in the low and high V_T LPS-treated groups ($P < 0.01$ and $P < 0.001$) (Figure 3B). LPS treatment resulted in increased percentage of killing in low and traditional V_T groups when compared with untreated controls (73.5% versus 33.2% and 70.8% versus 43.7%, respectively). Treatment with LPS resulted in an influx of neutrophils into the alveolar space, thereby increasing the BALF neutrophil cell count. We assessed intracellular bacterial killing in AM and neutrophil cell populations isolated from BALF of LPS-treated animals. There was no significant difference in percentage killing between these two cell types (Figure 4).

Immunofluorescent detection of bacterial phagocytosis corroborated our findings in the quantitative bacterial culture

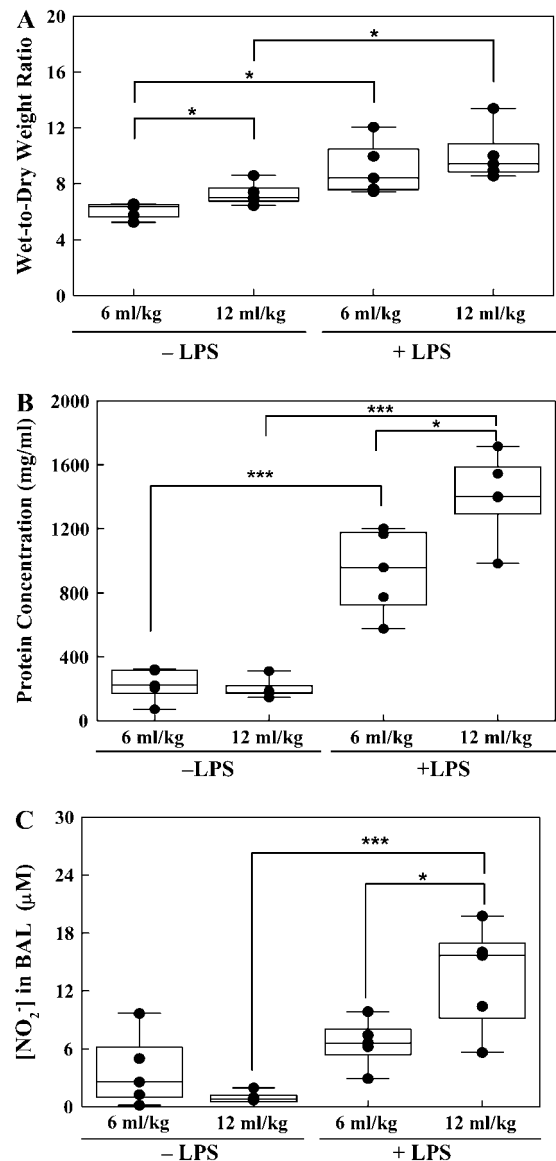


Figure 2. Lung wet-to-dry weight ratios (A), BALF protein (B), and nitric oxide (C) content from ventilated rabbits untreated or treated with LPS. Data are mean values \pm SEM. For each condition, $n = 5$. * $P < 0.05$; *** $P < 0.001$.

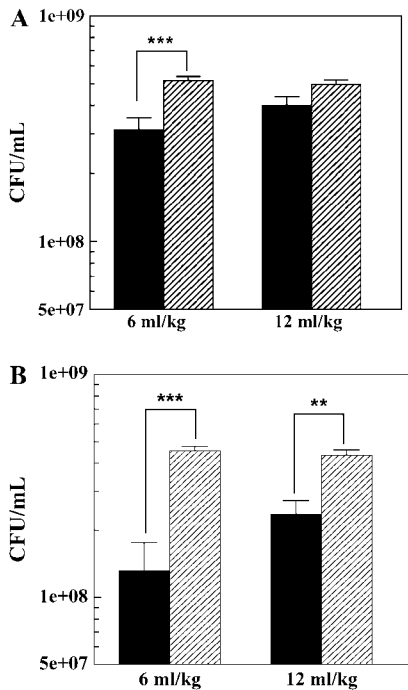


Figure 3. Effects of mechanical ventilation on phagocytosis (A) and intracellular killing (B) of *K. pneumoniae* by AMs over 1 h. Data are mean values \pm SEM. Data represent the average of five experiments. ** $p < 0.01$; *** $p < 0.001$. Solid bars, no LPS; shaded bars, 20 mg/kg LPS.

assays. Cells were infected with fluorescein-labeled *Klebsiella* in the presence or absence of 10 μ M cytochalasin D. LPS treatment increased phagocytosis of fluorescein-labeled bacteria compared with controls (Figures 5A and 5B), whereas cytochalasin D treatment decreased phagocytosis (data not shown).

ELISA for TNF- α

TNF- α is one of several inflammatory cytokines that have been shown to be crucial to antibacterial host defense in gram-negative infection. There were no significant differences between ventilatory strategies with regard to TNF- α expression. However, significantly higher TNF- α levels were seen after intratracheal instillation of LPS ($P < 0.001$) (Figure 6). In both V_T groups, ventilation after intratracheal LPS was associated with TNF- α levels five times greater than those seen in the untreated control groups.

IL-8 Immunohistochemistry

Lungs were analyzed by immunohistochemistry for expression of IL-8. IL-8 staining of bronchoalveolar segments (Figure 7)

was noticeably increased in animals receiving LPS. Staining was greatest in animals ventilated with traditional V_T when compared with low V_T .

IL-8 ELISA

Lung BALF was analyzed for IL-8 expression after exposing the animals to 6 h of the experimental conditions described. IL-8 expression was significantly increased under stimulation of LPS treatment but further increased when exposed to V_T of 12 ml/kg compared with 6 ml/kg ($1,200 \pm 130$ versus 980 ± 20 , respectively; $P < 0.05$) (Figure 8). Both increases in V_T and stimulation with LPS increased IL-8 expression.

DISCUSSION

The major goal of this study was to determine whether “protective” (6 ml/kg V_T) and “injurious” (12 ml/kg V_T) ventilation strategies would differ in their impact on bactericidal function of AMs. Studies show that ventilation with tidal volumes lower than those used conventionally reduces the relative risk of mortality (12, 14, 32). However, the mechanisms associated with the protective effect are not fully understood; thus, information gained shedding light on contributing variables or noncontributors are important to the critical care physician. Potential mechanisms include decreased production of proinflammatory cytokines (1, 3, 4, 12), decreased disruption of the alveolar-capillary barrier (11, 33, 34), and reduction in injurious lung stretch (13, 14). The results of our study show that animals treated with LPS and ventilated with traditional V_T displayed the greatest degree of lung injury regarding increased wet-to-dry weight ratios, BALF protein, and steady-state levels of nitrite. Likewise, other studies show that LPS treatment causes an increase in lung permeability (35–38), BALF protein (4, 17, 36, 37, 39), and NO concentration (35, 36, 39). In our study, although the wet-to-dry weight ratios were greatest in the “injurious” V_T group receiving LPS, they were not significantly greater than the “protective” group treated with LPS, probably due to the variability, the low number of animals studied, and the short duration of ventilation. A study by Peng and colleagues (40) showed that inducible nitric oxide synthase gene expression and activity are significantly upregulated by high V_T ventilation, are accompanied by nitrotyrosine deposition (mainly in capillary endothelial cells), and correlate with increased lung capillary permeability. However, the animals used in this study were not treated with LPS. Because NO (the product of inducible nitric oxide synthase induction) is involved in host defense, mechanical ventilation may stimulate or decrease innate immunity.

Effective host defense against lung bacterial infection is primarily dependent on the rapid clearance of the organism from the respiratory tract. Early clearance is mediated by neutrophils and macrophages, which must be recruited and activated at the site of infection (41). Functional impairment or reduction in this population of cells increases host susceptibility to lung infection (42, 43). Several studies have shown that injurious ventilation results in a marked reduction in immune functioning (44–49). In our study, there were no significant differences in bacterial phagocytosis and killing between the low and traditional V_T groups, although both parameters were slightly increased in the traditional V_T group. In fact, although LPS treatment significantly increased phagocytosis in the low V_T group and killing in low and high V_T groups, there was no difference between low and traditional V_T . Other studies involving *ex vivo* phagocytosis after LPS treatment show significant increases in AM uptake of degraded lipids (50) and apoptotic neutrophils (51). In our study, it is possible that the increased phagocytosis due to LPS treatment is a consequence of ventilation alone because previous

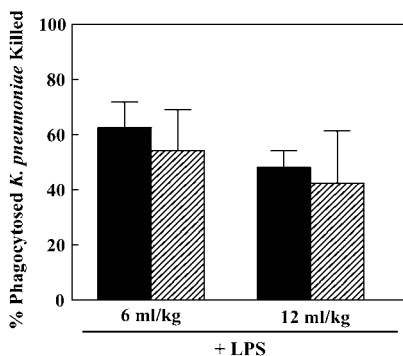


Figure 4. Bacterial killing in AMs (solid bars) and neutrophils (shaded bars) isolated from LPS-treated mechanically ventilated rabbits. AMs and neutrophils were isolated from the BALF, and cell populations were separated using FicolI-Paque PLUS (Amersham Biosciences) according to the manufacturer’s protocol. Bacterial phagocytosis and killing were assessed in each population.

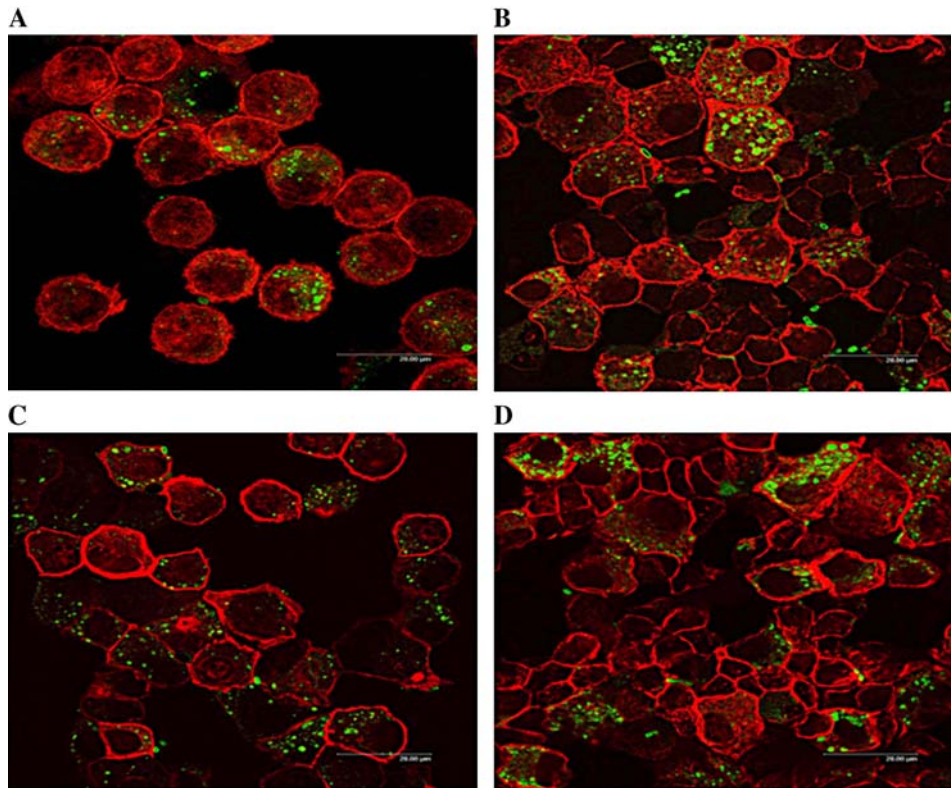


Figure 5. Immunofluorescent analysis of phagocytosis of *Klebsiella* in cells isolated from mechanically ventilated rabbits. Cells were infected with fluorescein-labeled *K. pneumoniae* in the presence or absence of 10 μ M cytochalasin D for 1 h at 37°C with gentle shaking. Original magnification: $\times 100$. Cells are from rabbits ventilated at 6 ml/kg (A, B) or 12 ml/kg (C, D). B and D represent LPS treatment.

studies reported that LPS had no effect on phagocytosis by AM (52, 53). Moreover, Brackenbury and colleagues (54) recently demonstrated that “low-stretch” mechanical ventilation alone did not significantly alter the pulmonary response to a bacterial challenge within a normal host over a 4 h time course.

Evidence suggests that mechanical ventilation, especially of injured lungs, causes the release of inflammatory mediators, which may pass into the circulation (4, 55, 56). Leukocyte emigration and AM-derived cytokines may contribute to lung microvascular injury associated with ARDS. Pugin and colleagues (26), using an *in vitro* model of mechanical ventilation of cell monolayers, identified the lung macrophage as the likely source for the secretion of proinflammatory mediators (e.g., TNF- α , IL-8, IL-6, and MMP-9) in response to mechanical ventilation. We measured TNF- α and IL-8 levels because of published data suggesting an association between mechanical stretch and sepsis

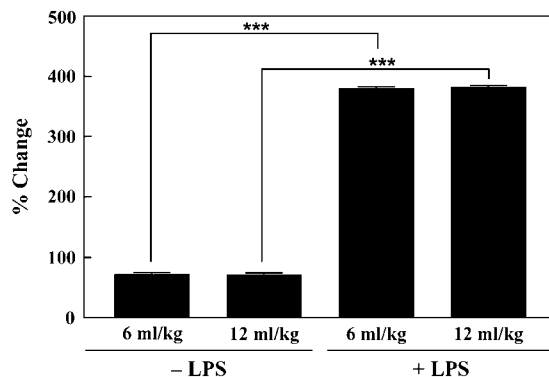


Figure 6. TNF- α levels in lung tissue of mechanically ventilated rabbits. For each condition, $n = 5$. *** $P < 0.001$.

(4, 26). TNF- α plays an important role in innate immunity. It is believed to enhance the release of chemokines and cytokines, increase lung vascular permeability, and modulate the eventual recruitment and activation of neutrophils (57, 58). IL-8, a potent neutrophil chemoattractant, was chosen as a relevant mediator because a sign of ventilator-induced lung injury is the lung recruitment of neutrophils and neutrophil-mediated tissue injury (59, 60). In our study, TNF- α and IL-8 expression measured in lung BALF and tissues, respectively, were significantly increased in animals treated with LPS, and increases in animals mechanically ventilated with “traditional” Vt were observed. It is possible that conventional or higher-volume mechanical ventilation does not cause a significant release of proinflammatory cytokines. Numerous studies involving proinflammatory cytokine production after high-volume mechanical ventilation have been conducted. Our results, like the results of these studies, are contradictory but continue to add to the knowledge base of this complex subject matter. Some investigators found increased concentrations of proinflammatory cytokines in lungs subjected to moderate to high-volume ventilation (1, 4, 26, 56, 61, 62), whereas others saw no effect on TNF- α production (17, 26, 62, 63). Clinical studies show that the lower tidal volume ventilation strategy is associated with a greater decrease in plasma cytokine levels and morbidity and mortality in patients with acute lung injury and ARDS (3, 12, 16, 32).

There are a few limitations associated with the present study. In our rabbit model of acute lung injury, LPS may already be a maximum stimulus for the AMs; therefore, the addition of mechanical ventilation may not result in any additional effect on bactericidal activity. Because the administration of LPS alone does not completely mimic the systemic or pulmonary effects of bacteremia or endotoxemia, use of another model of acute lung injury that is not dependent on LPS, such as cecal ligation and puncture (64), may show differences in AM bactericidal

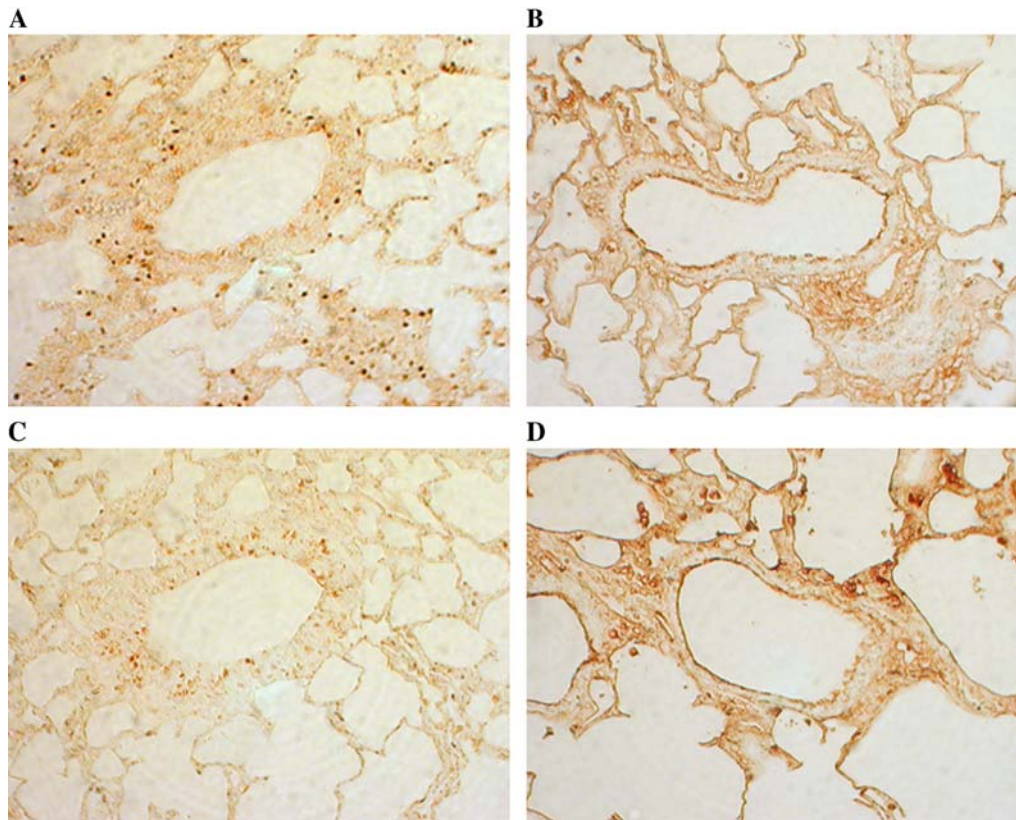


Figure 7. Immunohistochemistry of IL-8 in bronchopulmonary segments of mechanically ventilated rabbits. Paraffin-embedded lung sections from rabbits ventilated at 6 ml/kg (A, B) or 12 ml/kg (C, D) were stained for IL-8. Lungs were untreated (A, C) or treated (B, D) with LPS. Original magnification: $\times 20$.

activity. Previously, Cardozo and colleagues (65) demonstrated increased phagocytosis by AMs 12 h after intravenous injection of LPS. No change was observed at 4 h. However, they used intravenous instead of intratracheal LPS and used a different animal species (rats). Because the rabbits in our study were ventilated for only 4 h, it may be possible that any effect of ventilation on phagocytosis did not manifest. However, intratracheal delivery of LPS generally upregulates pulmonary inflammatory responses in a more timely manner.

In conclusion, in this animal model of LPS-induced lung injury, animals ventilated with traditional V_T exhibited the greatest degree of lung injury. Although the addition of LPS enhanced injury between the tidal volumes prospectively tested, the AM seemed to be a noncontributory participant in provoking injury. Future investigations should be encouraged to reconfirm this

observation and to elucidate additional possible mechanisms by which lower tidal volumes lead to reduced patient mortality.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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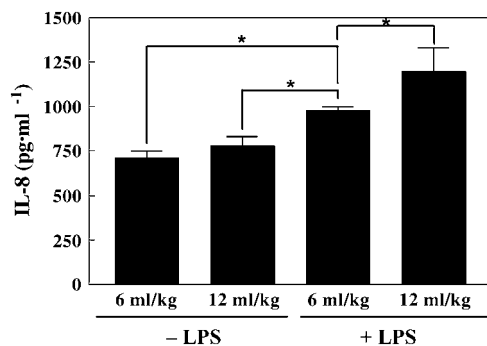


Figure 8. ELISA of IL-8 in the BALF of mechanically ventilated rabbits. BALF collected from rabbits ventilated under the conditions described. Data are means \pm SEM ($n = 5$). $*P < 0.05$.

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