

# Ventilation with high tidal volume induces inflammatory lung injury

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## Abstract

Mechanical ventilation with high tidal volumes ( $V_T$ ) has been shown to induce lung injury. We examined the hypothesis that this procedure induces lung injury with inflammatory features. Anesthetized male Wistar rats were randomized into three groups: group 1 (N = 12):  $V_T$  = 7 ml/kg, respiratory rate (RR) = 50 breaths/min; group 2 (N = 10):  $V_T$  = 21 ml/kg, RR = 16 breaths/min; group 3 (N = 11):  $V_T$  = 42 ml/kg, RR = 8 breaths/min. The animals were ventilated with fraction of inspired oxygen of 1 and positive end-expiratory pressure of 2 cmH<sub>2</sub>O. After 4 h of ventilation, group 3, compared to groups 1 and 2, had lower PaO<sub>2</sub> [280 (range 73-458) vs 517 (range 307-596), and 547 mmHg (range 330-662), respectively, P<0.05], higher wet lung weight [ $3.62 \pm 0.91$  vs  $1.69 \pm 0.48$  and  $1.44 \pm 0.20$  g, respectively, P<0.05], and higher wet lung weight/dry lung weight ratio [18.14 (range 11.55-26.31) vs 7.80 (range 4.79-12.18), and 6.34 (range 5.92-7.04), respectively, P<0.05]. Total cell and neutrophil counts were higher in group 3 compared to groups 1 and 2 (P<0.05), as were baseline TNF- $\alpha$  concentrations [134 (range <10-386) vs 16 (range <10-24), and 17 pg/ml (range <10-23), respectively, P<0.05]. Serum TNF- $\alpha$  concentrations reached a higher level in group 3, but without statistical significance. These results suggest that mechanical ventilation with high  $V_T$  induces lung injury with inflammatory characteristics. This ventilatory strategy can affect the release of TNF- $\alpha$  in the lungs and can reach the systemic circulation, a finding that may have relevance for the development of a systemic inflammatory response.

## Key words

- Lung injury
- Mechanical ventilation
- Inflammation
- TNF- $\alpha$

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## Introduction

Mechanical ventilation is an important therapy in patients with acute respiratory failure, providing adequate gas exchange and rest to respiratory muscles. For this reason, it is widely used in intensive care units (1). In spite of its great importance, mechanical ventilatory support has its own risks. Since its introduction, the association between high

airway pressures and barotrauma (pneumothorax, pneumomediastinum, pulmonary interstitial emphysema, subcutaneous emphysema, pneumoperitoneum, or pneumopericardium) has been demonstrated (2). Many studies have shown that mechanical ventilation can increase microvascular permeability and edema formation (3-6). These problems raised the question that mechanical ventilation may increase lung injury and

hamper recovery, especially in patients with acute respiratory distress syndrome (ARDS), contributing to the development of infection, multisystem organ damage, and increased mortality (7).

In the early stages of ARDS there is a small number of working lung units, sometimes as little as 25% of normal (referred to some authors as “baby lung”), which receive all the adjusted tidal volume ( $V_T$ ), resulting in high ventilation pressures (8). These high ventilation pressures, achieved with conventional  $V_T$  (e.g., 10 to 12 ml/kg), have been related to pulmonary injury in different experimental models (9,10). Despite the wide variations among different animal species, these results can be, at least in part, extrapolated to clinical practice, as shown by some studies that achieved good results with protective strategies during mechanical ventilation (11-13).

The ventilator-induced lung injury was initially attributed to the overdistension and repetitive opening and collapse of alveolar units. More recently, experimental studies suggested that mechanical ventilation increases pulmonary levels of inflammatory mediators and induces neutrophil accumulation (14-16). These studies suggest the hypothesis that ventilator-induced lung injury has an inflammatory component. The aim of the present study was to verify if mechanical ventilation with high  $V_T$  induces lung injury with inflammatory characteristics.

## Material and Methods

Animal care was provided according to the Principles of Laboratory Animal Care published by the National Institutes of Health (Guide for the Care and Use of Experimental Animals, NIH Publication No. 86-23, 1985).

### Experimental preparations

Male Wistar rats (Escola Paulista de Medicina, Universidade Federal de São Paulo,

São Paulo, SP, Brazil), weighing 300 to 350 g were anesthetized by intraperitoneal (*ip*) injection of 50 mg/kg thiobarbital and placed in dorsal decubitus throughout the experiment. After tracheostomy a 14-gauge cannula was inserted into the trachea. The rats were ventilated for 20 min at a  $V_T$  of 7 ml/kg, respiratory rate (RR) of 50 breaths/min, positive end-expiratory pressure (PEEP) of 2 cmH<sub>2</sub>O, and fraction of inspired oxygen ( $F_I O_2$ ) of 1, with a ventilator for small animals (Inter-3, Intermed, São Paulo, SP, Brazil). The animals were kept paralyzed with 1 mg/kg pancuronium bromide *ip* throughout the experiments. A 24-gauge catheter was inserted into the left carotid artery for arterial blood sampling.

### Experimental protocol

After 20 min, the baseline values were measured and the animals were randomly assigned to one of three groups. Group 1 (N = 12): ventilated at the same setting used during the baseline period for a total of 4 h. Group 2 (N = 10): ventilated at a  $V_T$  of 21 ml/kg, RR of 16 breaths/min, PEEP of 2 cmH<sub>2</sub>O, and  $F_I O_2$  of 1, for a total of 4 h. Group 3 (N = 11): ventilated at a  $V_T$  of 42 ml/kg, RR of 8 breaths/min, PEEP of 2 cmH<sub>2</sub>O, and  $F_I O_2$  of 1, for a total of 4 h.

After 4 h, while the rat was still being ventilated, the abdomen was opened and a blood sample was obtained from the inferior vena cava for later cytokine analysis, before exsanguinating the animal by aortic section.

### Measurements

Arterial blood gases were measured using an automatic analyzer (Radiometer ABL, 330, Copenhagen, Denmark) at baseline, and after 2 and 4 h. Airway pressure was measured through the tracheostomy tube using a transducer (Pneumotach, Hans Rudolph, Kansas City, KS, USA) and was continuously recorded. After the animal was sacri-

ficed, a pressure-volume curve was determined by the stepwise injection of 1 ml room air every 2 min, measuring the pressures with a water column. The lungs were inflated until the measured pressure reached 35 cmH<sub>2</sub>O, and then deflated in the same way, with measurements of the corresponding pressures.

The thorax of the animals was opened, and the lungs were removed and carefully dissected from mediastinal tissue. The wet weight of the left lung (WLW) was obtained (Marte, A200, São Paulo, SP, Brazil), the lung was then heated at 90°C in a gravity convection oven (Fanem, 315SG, São Paulo, SP, Brazil) for 72 h, and the residue was weighed (dry lung weight, DLW). The right lung was washed three times with 28 ml/kg physiological saline. A small aliquot of the combined lavage was used for total cell count in a hemocytometer (Neubauer chamber), and the remaining washings were centrifuged at 1,500 *g* for 20 min to separate cellular from noncellular elements. The supernatant was separated, frozen at -80°C, and subsequently used for protein concentration and TNF- $\alpha$  analysis. The cell pellet was suspended in 1 ml physiological saline, cytocentrifuged (LABHO, CT12, São Paulo, SP, Brazil), air dried and stained with May-Grünwald-Giemsa. A differential cell count was performed on a minimum of 200 cells.

Concentrations of TNF- $\alpha$  at baseline and in serum were measured in duplicate by ELISA (Factor-test-x/RAT TNF- $\alpha$ , Genzyme Diagnostics, Cambridge, MA, USA). Total baseline protein concentrations were determined spectrophotometrically in duplicate by the method of Lowry et al. (17).

### Statistical methods

Data are reported as mean  $\pm$  SEM or median when appropriate. One-way analysis of variance was used for WLW and DLW. For all other variables, one-way rank analysis of variance was used. Scheffé's correction was used for multiple comparisons. A *P* value <0.05 was considered to be statistically significant.

### Results

Values of arterial blood gases (PaO<sub>2</sub> and PaCO<sub>2</sub>) and pH for the three groups are listed in Table 1 and illustrated in Figure 1. During the baseline period and after 2 h of mechanical ventilation, all variables were similar among groups. After 4 h, PaO<sub>2</sub> was lower in group 3 compared with groups 1 and 2 (*P*<0.05). PaCO<sub>2</sub> and pH continued to be similar among groups after 4 h of experiment.

Table 2 lists the lung volumes (ml) at

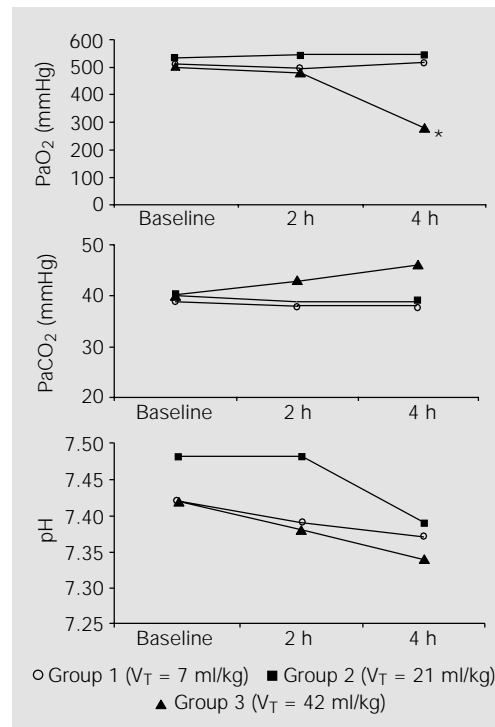
Table 1. PaO<sub>2</sub>, PaCO<sub>2</sub> and pH in the three groups at baseline and after 2 and 4 h of mechanical ventilation.

	Baseline			2 h			4 h		
	G1	G2	G3	G1	G2	G3	G1	G2	G3
PaO <sub>2</sub> (mmHg)	508 (434-592)	530 (477-598)	501 (431-597)	496 (429-604)	541 (257-669)	482 (128-635)	517 (307-596)	547 (330-662)	280* (73-458)
PaCO <sub>2</sub> (mmHg)	39 (32-49)	40 (29-60)	40 (28-54)	39 (31-49)	39 (22-63)	43 (31-62)	39 (23-48)	39 (33-46)	46 (11-87)
pH	7.42 (7.34-7.50)	7.48 (7.30-7.58)	7.42 (7.30-7.55)	7.39 (7.30-7.45)	7.48 (7.33-7.54)	7.38 (7.17-7.51)	7.37 (7.29-7.46)	7.39 (7.26-7.51)	7.34 (7.18-7.59)

G1 = group 1 (7 ml/kg, N = 12), G2 = group 2 (21 ml/kg, N = 10), and G3 = group 3 (42 ml/kg, N = 11). Values are reported as median and range.

\**P*<0.05 for G3 compared with G2 and G1 at 4 h (one-way rank analysis of variance).

Figure 1. PaO<sub>2</sub>, PaCO<sub>2</sub>, and pH in the three groups at baseline and after 2 and 4 h of mechanical ventilation. After 4 h, PaO<sub>2</sub> was lower in group 3 (V<sub>T</sub> = 42 ml/kg). PaCO<sub>2</sub> and pH were similar for all groups throughout the experiment. \*P<0.05 compared to groups 1 and 2 (one-way rank analysis of variance). V<sub>T</sub> = tidal volume.



pulmonary pressures of 10, 20, and 35 cmH<sub>2</sub>O, achieved during inflation. The volumes were lower in group 3 compared with groups 1 and 2 at pulmonary pressures of 10 and 20 cmH<sub>2</sub>O (P<0.05).

The WLW and the WLW/DLW ratio were higher in group 3 (P<0.05). The baseline protein contents and the baseline proteins/DLW ratio were also higher in group 3 (P<0.05). These results are listed in Table 3.

The total cell and neutrophil counts were higher in group 3 compared with groups 1 and 2 (P<0.05) (Figure 2). Baseline TNF- $\alpha$  was higher in group 3 compared with group 1 (P<0.05) (Figure 3). Although serum TNF- $\alpha$  reached a higher level in group 3, the difference was not statistically significant (Figure 4).

## Discussion

The results of this study showed that mechanical ventilation with high V<sub>T</sub> (42 ml/kg) induced lung injury in rats after 4 h compared to mechanical ventilation with a lower V<sub>T</sub> (7 and 21 ml/kg). The lung injury was demonstrated by the decrease in PaO<sub>2</sub> and the increase in WLW, WLW/DLW ratio, baseline protein contents, and baseline protein contents/DLW ratio, and by the worsening of the pulmonary compliance measured at pulmonary pressures of 10 and 20 cmH<sub>2</sub>O. The rats were ventilated with a V<sub>T</sub> of 42 ml/kg because in ARDS, a clinical condition where lung injury induced by mechanical ventilation is critical, sometimes even less than 25% of the lungs are ventilated. So, in these cases, traditional adjustments of V<sub>T</sub> to 10 to 12 ml/kg may correspond to a V<sub>T</sub> as high as 42 ml/kg in a previously normal lung. In the groups ventilated with lower V<sub>T</sub>, the RR were increased in order to keep the same minute volume, as can be seen by the same pH and PaCO<sub>2</sub> levels at baseline.

Research in different species has shown that mechanical ventilation with high V<sub>T</sub> can induce lung injury similar to that seen in

Table 2. Lung volumes (ml) at pulmonary pressures of 10, 20, and 35 cmH<sub>2</sub>O.

	Group 1	Group 2	Group 3
Lung volume (10 cmH <sub>2</sub> O)	5.0 (3.2-7.8)	5.3 (1.9-8.5)	2.5 (1.1-6.0)*
Lung volume (20 cmH <sub>2</sub> O)	11.9 (7.2-17.0)	12.0 (5.9-19.0)	7.8 (3.5-13.5)*
Lung volume (35 cmH <sub>2</sub> O)	19.7 (13.5-24.0)	19.3 (14.6-30.2)	19.1 (14.1-22.3)

Group 1, V<sub>T</sub> = 7 ml/kg (N = 12); group 2, V<sub>T</sub> = 21 ml/kg (N = 10); group 3, V<sub>T</sub> = 42 ml/kg (N = 11). Values are reported as median and range.

\*P<0.05 for group 3 compared with groups 2 and 1 (one-way rank analysis of variance).

Table 3. Wet lung weight (WLW), dry lung weight (DLW), WLW/DLW ratio, baseline protein contents, and baseline protein contents/DLW ratio.

	Group 1	Group 2	Group 3
WLW (g)	1.69 ± 0.48	1.44 ± 0.20	3.62 ± 0.91*
DLW (g)	0.22 ± 0.07	0.23 ± 0.04	0.20 ± 0.03
WLW/DLW	7.80 (4.79-12.18)	6.34 (5.92-7.04)	18.14 (11.55-26.31)*
Baseline proteins	16.47 (8.31-29.56)	11.32 (5.41-15.51)	42.81 (16.32-67.91)*
Baseline proteins/DLW (mg/g)	76.06 (31.60-150.81)	50.51 (23.00-67.89)	211.46 (142.32-304.77)*

Group 1, V<sub>T</sub> = 7 ml/kg (N = 12); group 2, V<sub>T</sub> = 21 ml/kg (N = 10); group 3, V<sub>T</sub> = 42 ml/kg (N = 11). Values are reported as median and range for WLW/DLW ratio, baseline proteins, and baseline proteins/DLW ratio, and as the mean ± SEM for WLW and DLW.

\*P<0.05 for group 3 compared with groups 2 and 1 (one-way rank analysis of variance for WLW/DLW ratio, baseline proteins, and baseline proteins/DLW ratio, and one-way analysis of variance for WLW and DLW).

ARDS. Webb and Tierney in 1974 (18) demonstrated that rats ventilated for 1 h with high inspiratory pressures (45 cmH<sub>2</sub>O) developed hypoxemia, increased WLW, and histological findings of alveolar edema. Kolobow et al. (19), studying sheep ventilated for 4 h either with low inspiratory pressures (15 to 20 cmH<sub>2</sub>O) or high inspiratory pressures (50 cmH<sub>2</sub>O), found in the latter group development of hypoxemia, worsening of respiratory compliance, and histological findings indistinguishable from those seen in ARDS.

Lung injury induced by mechanical ventilation is multifactorial and includes the structural disruption generated by lung overdistension and by the shear forces created during repetitive opening and closing of atelectatic regions. Mechanical ventilation has also deleterious effects on the surfactant function, increasing the tendency of distal airways and alveoli to collapse, and increasing the pressure necessary to open the lung (20,21). Although the higher airway pressures achieved may result in increased transmural capillary pressure, facilitating the development of hydrostatic edema, the lung injury induced by high V<sub>T</sub> includes alterations in the pulmonary capillary permeability and alveolar epithelium leaks (22). The increase in baseline protein contents and in the baseline protein contents/DLW ratio seen in this study excludes hydrostatic edema as the only hypothesis. DLW, another argument for a high protein content edema, did not differ among the three groups. This might have occurred because, during the experiments, the rats ventilated with 42 ml/kg presented with lung fluid that had to be aspirated from the trachea by a catheter. The loss of protein with this fluid aspiration may have avoided the increase in DLW. Other authors have demonstrated, also by experimental studies, defects in the blood-air barrier induced by mechanical ventilation with high V<sub>T</sub> and inspiratory pressures. Egan et al. (22) demonstrated in sheep that epithelial pore

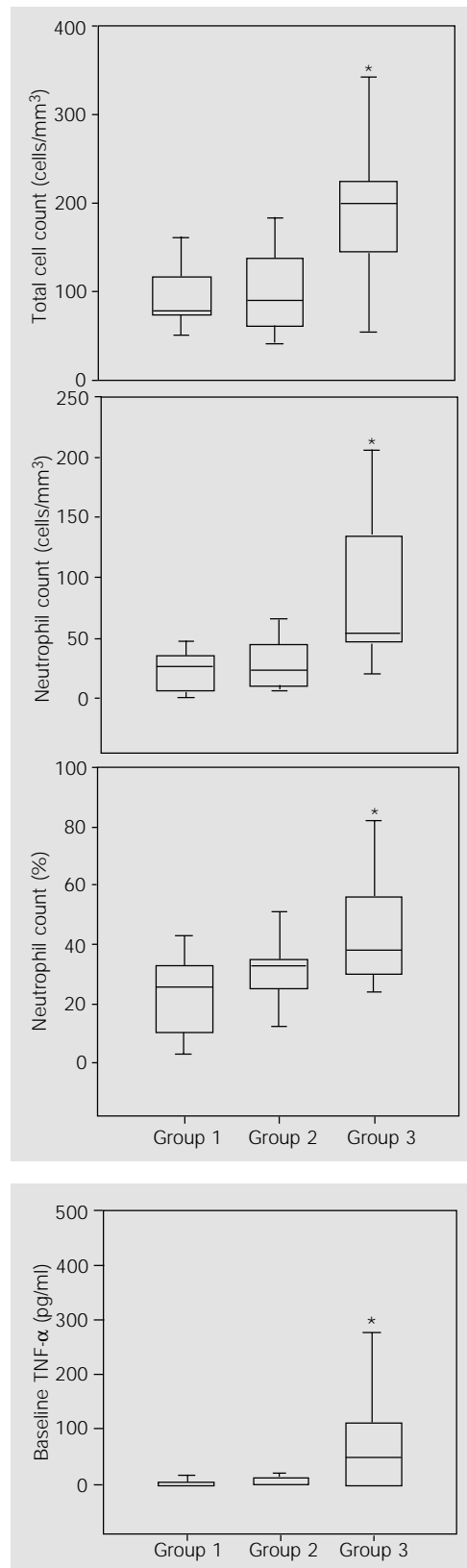
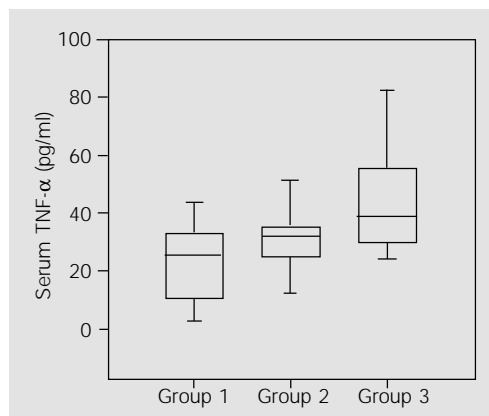


Figure 2. Total cell and neutrophil counts and the percentage of baseline in the three groups. After 4 h, total cell count, neutrophil count and its percentage of baseline were higher in group 3 compared with groups 1 and 2. \*P<0.05 (one-way rank analysis of variance).

Figure 3. Baseline TNF- $\alpha$  levels in the three groups. After 4 h, the baseline TNF- $\alpha$  was higher in group 3 (V<sub>T</sub> = 42 ml/kg) compared with group 1 (V<sub>T</sub> = 21 ml/kg) and group 2 (V<sub>T</sub> = 7 ml/kg). \*P<0.05 (one-way rank analysis of variance).

Figure 4. Serum TNF- $\alpha$  levels in the three groups. After 4 h, although serum TNF- $\alpha$  was higher in group 3 ( $V_T = 42$  ml/kg) compared with group 1 ( $V_T = 21$  ml/kg) and group 2 ( $V_T = 42$  ml/kg), it did not reach statistical significance (one-way rank analysis of variance).



radii increased, and leaks developed at static inflation pressures greater than 35 cmH<sub>2</sub>O. Parker et al. (23) examined the effects of ventilation of open-chest dogs with high peak airway pressures (>60 cmH<sub>2</sub>O), and showed a higher lung lymph protein clearance and higher lymph/plasma protein ratio, which indicate increases in microvascular permeability. Dreyfuss et al. (5) found that high positive pressure ventilation resulted in a dramatic increase in pulmonary microvascular permeability associated with parenchymal ultrastructural lesions. They showed an increase in DLW and fractional <sup>125</sup>I-labeled albumin uptake by the lungs in the group ventilated at 45 cmH<sub>2</sub>O peak inspiratory pressure compared with those ventilated at 7 cmH<sub>2</sub>O, and ultrastructural alterations such as damage of type I cells, denuding of the epithelial basement membrane, interstitial and alveolar edema and hyaline membranes. West et al. (24), using electron microscopy, demonstrated microvascular injury induced by high distending pressures. These authors detected a large number of endothelial and epithelial breaks, which they called stress fractures, at high lung volumes compared with low lung volumes.

Many studies are producing evidence that mechanical ventilation has significant effects on lung levels of inflammatory cells and mediators. Valenza et al. (25) have shown that mechanical ventilation at 15 ml/kg and

low levels of PEEP increased the levels of interleukin-1 $\beta$  compared with ventilation at 7 ml/kg. Tremblay et al. (26) have also shown that mechanical ventilation with excessive end-inspiratory lung volume and without PEEP increased the concentration of lung lavage cytokines. The results of our study are in agreement with this evidence. The group ventilated at 42 ml/kg had a larger number of cells and neutrophils at baseline compared with the other groups (Figure 2). Also, baseline TNF- $\alpha$  levels were higher in these animals (Figure 3). We hypothesize that ventilating the rats with high  $V_T$  induced an increase in the production and release of TNF- $\alpha$  by the lung macrophages and that TNF- $\alpha$  induced neutrophil accumulation and activation in the lungs, contributing to their injury.

More recently, some studies have shown results suggesting that lung injury induced by mechanical ventilation may initiate and propagate a systemic inflammatory response that may play an important role in the development of multiple system organ failure in critically ill patients. von Bethman et al. (27) reported, in an isolated perfused lung model, that ventilation with high transpulmonary pressures leads to a significant increase in concentrations of TNF- $\alpha$  and interleukin-6 in the perfusate, indicating the loss of compartmentalization of the inflammatory process within the lungs. Chiumello et al. (28) demonstrated in rats that ventilation at high  $V_T$  and without PEEP for 4 h increased the release of inflammatory mediators into the systemic circulation in a lung injury model using hydrochloric acid instillation. In the present study, the serum TNF- $\alpha$  levels in the group ventilated at 42 ml/kg were higher compared with the other two groups, although without statistical significance (Figure 4). This could be explained by the fact that even ventilation with lower  $V_T$  can release some amount of mediators. We sampled serum from unventilated rats and no TNF- $\alpha$  was detected (data not shown).

In conclusion, the results of the present study provide further evidence that strategies of mechanical ventilation at high  $V_T$  lead to lung injury at least in part by an inflammatory mechanism. We speculate that

this inflammatory response may not be compartmentalized within the lungs because of the epithelial and endothelial damage, and may propagate a systemic inflammatory response.

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