Mechanical Ventilation in Healthy Mice Induces Reversible Pulmonary and Systemic Cytokine Elevation with Preserved Alveolar Integrity

An In Vivo Model Using Clinical Relevant Ventilation Settings

Michiel Vaneker, M.D.,* Feico J. Halbertsma, M.D., † Jan van Egmond, Ph.D., † Mihai G. Netea, M.D., Ph.D., §
Henry B. Dijkman, Ph.D., ‡ Dirk G. Snijderlaar, M.D., Ph.D., ‡ Leo A. Joosten, Ph.D., **
Johannes G. van der Hoeven, M.D., Ph.D., †† Gert Jan Scheffer, M.D., Ph.D. †††

Background: Mechanical ventilation (MV) may activate the innate immune system, causing the release of cytokines. The resulting proinflammatory state is a risk factor for ventilator-induced lung injury. Cytokine increase results from direct cellular injury but may also result from cyclic stretch alone as demonstrated in vitro: mechanotransduction. To study mechanotransduction in vivo, the authors used an animal MV model with clinically relevant ventilation settings, avoiding alveolar damage.

Methods: Healthy C57BL6 mice (n = 82) were ventilated (tidal volume, 8 ml/kg; positive end-expiratory pressure, 4 cm H2O; fraction of inspired oxygen, 0.4) for 30, 60, 120, and 240 min. Assigned animals were allowed to recover for 2 days after MV. Both pulmonary tissue and plasma interleukin (IL)-1α, IL-1β, tumor necrosis factor α, IL-6, IL-10, and keratinocyte-derived chemokine levels were measured. Histopathologic appearance of lung tissue was analyzed by light microscopy and electron microscopy.

Results: In lung tissue, all measured cytokines and keratinocyte-derived chemokine levels increased progressively with MV duration. Light microscopy showed increased leukocyte influx but no signs of alveolar leakage or alveolar protein deposition. Electron microscopy revealed intact epithelial cell and basement membranes with sporadically minimal signs of partial endothelial detachment. In plasma, increased levels of IL-1α, tumor necrosis factor α, IL-6, and keratinocyte-derived chemokine were measured after MV. In the recovery animals, cytokine levels had normalized and no histologic alterations could be found.

Conclusions: Mechanical ventilation induces reversible cytokine increase and leukocyte influx with preserved tissue integrity. This model offers opportunities to study the pathophysiologic mechanisms behind ventilator-induced lung injury and the contribution of MV to the “multiple-hit” concept.

MECHANICAL ventilation (MV) is widely used in general anesthesia and is a lifesaving intervention in critically ill patients. It can, however, induce lung injury in the healthy lung or exacerbate damage in the already injured lung. This has been termed ventilator-induced lung injury (VILI).1,2 Clinical studies show that the use of large tidal volumes (VT ≥ 12–15 ml/kg) is associated with a poor prognosis; however, a “lung-protective ventilation strategy” (low tidal volumes [VT < 10–12 ml/kg], optimizing positive end-expiratory pressure [PEEP]) reduces but cannot prevent VILI.1,3–8

Ventilator-induced lung injury is characterized by the release of inflammatory mediators (especially cytokines), infiltration of leukocytes, alveolar and interstitial edema, alveolar protein deposition, cellular necrosis, and tissue disruption.9,10 It is now commonly accepted that increased production of cytokines, especially interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α, plays a key role in initiating or perpetuating lung injury.11–17

The clinical relevance of cytokine up-regulation by MV is the resulting proinflammatory state, because this makes the host more vulnerable to a “second hit” (e.g., major surgery).18 To note, MV itself can be the “second hit” where an already comprised host exists (e.g., MV in the critically ill patient).3,4,19

Two mechanisms are believed to be responsible for MV-induced cytokine release. The first is direct trauma to the cell with disruption of the membranes, resulting in translocation of cytokines into both the alveolar space and the systemic circulation. This “decompartmentalization” has been demonstrated in vivo.15,20,21 The second has been termed mechanotransduction. In vitro studies show that most pulmonary cells, such as alveolar macrophages, epithelial cells, and endothelial cells, can produce cytokines in response to cyclic stretch.22–24 However, the sensing mechanism of these physical forces and the translation into intracellular signals is largely unknown.25

In many of the currently available experimental VILI models, injurious MV settings (e.g., VT > 25 ml/kg or peak pressures > 20–40 cm H2O) have been used in healthy animals,11,12,14,26,27 or the “multiple-hit” model was used by applying MV in already injured animals.13,15,16,28,29 From these study designs, it is not pos-
sible to differentiate whether the observed increase in cytokine levels is the result of decompartmentalization, mechanotransduction, or both.

For a better understanding of the relevant pathophysiologic mechanisms leading to VILI, it is important to study in vivo the effects of ventilation in the healthy lung, using ventilatory protocols analogous to those currently used during general anesthesia and in the intensive care unit patient. We studied the effects of MV in healthy mice, carefully searched for pulmonary cell or tissue disruption, counted leukocyte numbers, and measured cytokine production in lung tissue and plasma.

Materials and Methods

All experiments were approved by the Regional Animal Ethics Committee in Nijmegen, The Netherlands, and performed under the guidelines of the Dutch Council for Animal Care and the National Institutes of Health.

Animals

Experiments were performed in male C57BL6 mice (n = 82; Charles River, Sulzfeld, Germany) aged 10–12 weeks, with weights ranging from 23 to 28 g.

Mechanical Ventilation in Mice

Mice were anesthetized with an intraperitoneal injection of a combination of ketamine, medetomidine, and atropine (KMA): 7.5 μl per gram of body weight of induction KMA mix (consisting of 1.26 ml ketamine, 100 mg/ml; 0.2 ml medetomidine, 1 mg/ml; 1 ml atropine, 0.5 mg/ml; and 5 ml NaCl, 0.9%) was given just before intubation. Animals were orally intubated under direct vision with an endotracheal tube (0.82 mm ID, 1.1 mm OD, length 25 mm). Endotracheal tube position was confirmed by end-tidal carbon dioxide analysis, using mass spectrometry. Subsequently animals were connected to the ventilator (MiniVent®; Hugo Sachs Electronik-Harvard Apparatus, March-Hugstetten, Germany). Vt was set at 8 ml/kg and frequency was set at 150/min, which is well within the range of measured Vt and respiratory rate during spontaneous ventilation in C57BL6 mice. All animals received 4 cm H2O PEEP. To avoid direct oxygen toxicity, as reported by several authors, the fraction of inspired oxygen (Fio2) was set at 0.4.

To maintain anesthesia, 5.0 μl per gram of body weight boluses of maintenance KMA mix (consisting of 0.72 ml ketamine, 100 mg/ml; 0.08 ml medetomidine, 1 mg/ml; 0.3 ml atropine, 0.5 mg/ml; and 18.9 ml NaCl, 0.9%) were given, via an intraperitoneally placed catheter, every 30 min. Throughout the experiment, rectal temperature was monitored and maintained between 36° and 37.5°C using a heating pad.

Study Groups

Animals were divided into seven groups. Group C (n = 9) served as control group: After induction of anesthesia, these mice were killed immediately, without being ventilated. Animals in groups 30 (n = 6), 60 (n = 6), 120 (n = 9), and 240 (n = 9) were ventilated for 30, 60, 120, and 240 min, respectively, and were killed immediately thereafter. In group R (recovery group), animals (n = 6) were extubated after being ventilated for 240 min and were killed after 2 days of recovery. Anesthesia was discontinued in the group R animals 1 h before extubation. Group D (depleted group) animals (n = 6) were first leukocyte depleted by administering cyclophosphamide as described previously. These animals were then ventilated for 240 min and killed immediately thereafter.

A separate set of experiments (intraarterial blood pressure [IABP] group, n = 15) was conducted to assess whether the chosen anesthetic and ventilation regime resulted in a stable and reproducible cardiorespiratory condition. In these animals, continuous intraarterial carotid blood pressure was measured. Arterial blood gas analysis was performed after 120 min (n = 6) and 240 min (n = 9). The same ventilator settings were used as for the mice in the aforementioned groups. We decided not to include the animals from the IABP group for the cytokine or histopathologic analysis to avoid possible interference with cytokine response resulting from instrumentation induced tissue damage.

In addition, two control experiments were performed. In the first control experiment, animals (n = 12) received the standard ventilation strategy (Vt, 8 ml/kg; PEEP, 4 cm H2O; Fio2, 0.4). The lungs were removed after 0 min (n = 4, control), 120 min (n = 4), and 240 min (n = 4) of MV to measure wet/dry ratios. In the second control experiment, animals (n = 4) were ventilated with a Vt of 16 ml/kg, PEEP of 4 cm H2O, and Fio2 of 0.4 for 240 min. The lungs of these mice were used to histopathologically assess the effects of high Vt ventilation in our model.

Material Harvesting

After the animals were killed, blood was collected by exsanguination and centrifuged at 14,000 rpm (13,000g) (Eppendorf 5415 C; Nethler-Hinz GmbH, Hamburg, Germany) for 2 min, and plasma was stored at −80°C. Immediately after exsanguination, the heart and lungs were carefully removed en block via midline sternotomy. The right middle lobe was fixed for light microscopy (LM) and electron microscopy (EM), except in animals analyzed for wet/dry ratio. The remaining lung tissue was homogenized for the determination of cytokine concentrations.

Preparation and Analysis of Lung Tissue

For LM, the material was fixed in 4% buffered formalin solution overnight at room temperature, dehydrated,
Table 1. Intraarterial Blood Pressure and Arterial Blood Gas Analysis during Mechanical Ventilation

<table>
<thead>
<tr>
<th>Duration of MV, min</th>
<th>MAP, mmHg</th>
<th>pH</th>
<th>PaO2, mmHg</th>
<th>PaCO2, mmHg</th>
<th>HCO3, mm</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>102 (10)</td>
<td>7.36 (0.06)</td>
<td>229 (50)</td>
<td>38 (7)</td>
<td>20.4 (1.2)</td>
<td>-4.4 (1.5)</td>
</tr>
<tr>
<td>60</td>
<td>89 (12)</td>
<td>7.35 (0.07)</td>
<td>194 (50)</td>
<td>36 (8)</td>
<td>19.6 (3.1)</td>
<td>-6.3 (2.0)</td>
</tr>
<tr>
<td>120</td>
<td>83 (14)</td>
<td>7.38 (0.06)</td>
<td>229 (50)</td>
<td>38 (7)</td>
<td>20.4 (1.2)</td>
<td>-4.4 (1.5)</td>
</tr>
<tr>
<td>180</td>
<td>78 (6)</td>
<td>7.35 (0.07)</td>
<td>194 (50)</td>
<td>36 (8)</td>
<td>19.6 (3.1)</td>
<td>-6.3 (2.0)</td>
</tr>
<tr>
<td>240</td>
<td>79 (8)</td>
<td>7.38 (0.06)</td>
<td>229 (50)</td>
<td>38 (7)</td>
<td>20.4 (1.2)</td>
<td>-4.4 (1.5)</td>
</tr>
</tbody>
</table>

Values are mean (SD).

BE = base excess; MAP = mean arterial pressure; MV = mechanical ventilation; PaCO2 = arterial carbon dioxide tension; PaO2 = arterial oxygen tension.

and embedded in paraplast (Amstelstad, Amsterdam, The Netherlands). Sections of 4-μm thickness were used for further analysis. The enzyme activity of leukocytes was visualized by enzyme histochemistry using chlororacetateesterase staining (Leder). Periodic acid–Schiff staining was performed to analyze for alveolar albumin presence. Leukocytes were counted manually (20 fields per mouse), and after automated correction for air/tissue ratio, leukocytes per μm² were calculated.

For EM, the material was fixed in 2.5% glutaraldehyde dissolved in 0.1 M sodium cacodylate buffer, pH 7.4, overnight at 4°C and washed in the same buffer. The tissue fragments were postfixed in cacodylate-buffered 1% OsO₄ for 120 min, dehydrated, and embedded in Epon 812 (Merck, Darmstadt, Germany). Ultrathin sections were cut on an Ultratome (Leica, Reichert Ultra, Vienna, Austria), and contrasted with 4% uranyl acetate for 45 min and subsequently with lead citrate for 4 min at room temperature. Sections were examined in a Jeol 1200 EX2 electron microscope (JEOL, Tokyo, Japan). The evaluating pathologist was blinded to the group and ventilation protocol to which the animal had been assigned.

For wet/dry ratios, both lungs were used; ratios were calculated by measuring lung weight before and after heating for 24 h in a stove at 50°C.

**Laboratory Tests**

Interleukin-1α and IL-1β were assessed using specific radioimmunoassays, as described previously. Levels of TNF-α, IL-6, IL-10, and keratinocyte-derived chemokine (KC) in lung homogenate and plasma were measured using enzyme-linked immunosorbent assay (for TNF-α, IL-6, and IL-10: CytoSet, BioSource, Camarillo, CA; for KC: ELISA-Kit, R&D Systems, Minneapolis, MN). Lower detection limits were as follows: IL-1α and IL-1β: 40 pg/ml; TNF-α: 32 pg/ml; IL-6: 160 pg/ml; IL-10: 16 pg/ml; and KC: 160 pg/ml. For the assessment of KC in plasma in group 60, insufficient plasma was available; the plasma had to be diluted for analysis, which increased the detection limit to 1,600 pg/ml. To investigate whether lipopolysaccharide contamination was present in our experimental setting, we measured lipopolysaccharide in air, tubing, and the ventilator by Limulus Amebocyte Lysate testing (Cambrex Bio Science, Walkersville, MD; detection limit: 0.06 U/ml).

**Statistical Analysis**

Data are expressed as mean (SD) when distributed normally (leukocyte counts and wet/dry ratios) and expressed as median (range) otherwise (cytokine concentrations). Statistical analysis was performed with SAS (SAS Institute Inc., Cary, NC) statistical procedures. Because cytokine concentrations are not normally distributed, Kruskal-Wallis procedures were used, with post hoc comparisons of subgroups (Duncan). Data of a particular cytokine concentration variable were ranked, followed by analysis of variance in the General Linear Models procedure using the MEANS procedure with the Duncan option and Bonferroni correction for multiple comparisons. For the analysis of leukocyte counts and wet/dry ratios, analysis of variance was used on non-ranked data with post hoc comparison of group means (Duncan). The level of significance was set at $P < 0.05$.

**Results**

**Cardiorespiratory Parameters**

The animals with an intraarterial canula (IABP group) exhibited stable hemodynamic parameters throughout the experiments. Mean arterial pressure was within normal limits and remained above 65 mmHg in all animals. Blood gas analysis showed normal pH, arterial carbon dioxide tension (PaCO₂), and arterial oxygen tension (PaO₂) levels with a small decrease in base excess (table 1). Two of six animals in group R (recovery group) died directly after extubation. Before extubation, these animals did not differ from surviving subjects in cardiorespiratory parameters. The remaining four animals were stable during the ventilation-free interval, with normal activity and behavior and no respiratory distress or weight loss.

**Histologic Examination**

Electron microscopy examination of the lung tissue from animals in groups 30, 60, 120, and 240 revealed intact basement membranes and no signs of alveolar.
flooding. Type I pneumocytes sporadically showed signs of minimal membrane disruption and small partial detachment of endothelium (figs. 1A and B). Animals that were allowed to recover (group R) and unventilated healthy control animals (group C) showed no signs of membrane disruption or detachment of endothelium (fig. 1C). The four animals in the control experiment that were ventilated with a VT of 16 ml/kg showed extensive damage with lungs appearing overinflated with loss of septal walls and injury of type I pneumocyte (fig. 1D).

Light microscopy examination using periodic acid-Schiff staining showed no intraalveolar albumin. Leder staining revealed a substantially higher number of pulmonary leukocytes after 120 and 240 min of MV (fig. 2). No differences in leukocyte counts were found in the animals that were allowed to recover (group R) compared with the unventilated animals (group C).

Wet/dry ratios showed increased ratios only after 240 min of MV. Data are presented in table 2.

Cytokine Concentration Induced by Mechanical Ventilation

Mechanical ventilation with a VT of 8 ml/kg, PEEP of 4 cm H₂O, and FIO₂ of 0.4 resulted in a significant increase in IL-1α, IL-1β, TNF-α, IL-6, IL-10, and KC in lung tissue homogenate when compared with unventilated animals (group C). These cytokine concentrations increased with the duration of MV, with KC being the first to increase from 30 min of MV onward. IL-1β, TNF-α, IL-6, and IL-10 levels increased after 60 min, whereas IL-1α increased after 240 min compared with group C animals (fig. 3).

In plasma, TNF-α, IL-6, and KC levels were elevated from 120 min onward compared with group C animals, and IL-1α after 240 min of MV. IL-1β and IL-10 levels were not different from those of the group C animals (fig. 4).

When animals were allowed to recover for 2 days (group R), after being ventilated for 240 min, all lung tissue cytokine levels were lower compared with levels found in animals killed immediately after 240 min of ventilation (group 240). IL-1α and KC levels in group R animals were higher compared with group C animals. Plasma levels of TNF-α, IL-6, IL-10, and KC were found to be lower when compared with group 240.

The Effect of Leukocyte Depletion on the Release of Cytokines

In leukocyte-depleted animals (group D), MV resulted in significantly higher levels of lung tissue IL-1α, IL-6, IL-10, and KC compared with group C animals. IL-1β, TNF-α, and KC levels were lower compared with the levels found after 240 min of MV in healthy (non-leukocyte-depleted) animals (group 240). MV resulted in higher plasma levels of KC compared with group C animals. TNF-α, IL-6, and IL-10 levels in plasma were lower compared with the levels found in group 240.

No lipopolysaccharide could be detected in our experimental setting.

Discussion

The current study demonstrates that MV in healthy mice using clinically relevant ventilator settings with low
VT preserves alveolar integrity but induces reversible cytokine increase and leukocyte influx. This rapid increase in cytokine levels and leukocyte influx, however, does not result in persistent inflammation and VILI. Our findings suggest that “noninjurious” or “lung-protective” ventilation does not exist and that even this careful mode of ventilation strategy leads to a reversible inflammatory response. Fortunately, MV in elective, healthy patients rarely leads to clinical significant injury. Apparently, in most circumstances, the lung is able to cope with the MV-induced inflammatory reaction. This is demonstrated in a clinical study by Plotz et al.,36 which showed that 2 h of MV (VT 10 ml/kg) in healthy children, anesthetized for cardiac catheterization, resulted in elevated alveolar IL-6 and TNF-α concentrations without clinical signs of pulmonary dysfunction. In contrast with this are the findings in the clinical study of Wrigge et al.,37 who found no ventilation-induced increase in cytokines. However, in this study, the ventilation duration was limited to 1 h, and cytokines were only measured in plasma and not in the lung.

Fig. 2. Light microscopy. Light microscopy examination of lung tissue after Leder staining revealed a significantly higher number of pulmonary leukocytes in healthy animals after 120 (group 120, D) and 240 min (group 240, E) of mechanical ventilation compared with the unventilated control animals (group C, A). Significantly lower numbers of pulmonary leukocytes were found in the animals that were allowed to recover (group R, F) compared with animals ventilated for 240 min (group 240, E). No differences were found between unventilated controls (group C, A) and the animals that were allowed to recover (group R, F). For the results of leukocyte counts, see table 2. (A) Unventilated control animals. (B–E) Healthy animals receiving mechanical ventilation for 30, 60, 120, and 240 min. (F) Animals that were allowed to recover for 2 days after being ventilated for 240 min (group R). Magnification: 750×.

Table 2. Leukocyte Counts and Wet/Dry Ratios

<table>
<thead>
<tr>
<th>Group</th>
<th>Leukocytes × 10^{-3}/μm²</th>
<th>P Value</th>
<th>Wet/Dry Ratio, Mean (SD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.1 (1.3)</td>
<td></td>
<td>4.68 (0.014)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2.4 (1.6)</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>2.2 (2.2)</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>9.7 (5.0)</td>
<td>&lt; 0.05</td>
<td>4.81 (0.13)</td>
<td>NS</td>
</tr>
<tr>
<td>240</td>
<td>5.7 (3.1)</td>
<td>&lt; 0.05</td>
<td>5.01 (0.06)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Recovery</td>
<td>1.2 (0.9)</td>
<td>&lt; 0.05*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SD).

P values compared with control group (unventilated animals). * P value compared with group 240 (240 min of ventilation).

NS = not significant.

Anesthesiology, V 107, No 3, Sep 2007
In this study, EM analysis revealed that our MV mode almost completely retained histologic integrity, with only sporadically minimal changes in a few samples. Most importantly, basement membranes were not disrupted, signifying alveolar integrity. Although LM has been used for quantitative analysis of leukocytes and can require EM.41 The effects of large V\textsubscript{T} in the animals served increased wet/dry ratios in animals after 240 min of MV. However, we conclude that this increased wet/dry ratio (without any other signs of possible lung damage as shown by EM and LM examination) is not of clinical significance. This is supported by the finding of the complete recovery of the animals who were allowed to recover after 240 min of ventilation (group R).

Factors affecting cytokine response other than MV were carefully avoided. The possibility of triggering an inflammatory response by invasive procedures, (i.e., insertion of an intrararterial line)\textsuperscript{18} was eliminated by performing our experiments in noninvasively monitored
animals, after having documented cardiorespiratory stability in invasively monitored animals (IABP group). A
limulus amebocyte lysate test excluded possible aero-
genic lipopolysaccharide contamination during our ex-
periments. Cardiorespiratory parameters and the choice
of the anesthetics are known to influence the cytokine
profile. In the current study, mean arterial pressure was
maintained above 65 mmHg, and blood gas analysis
showed normal pH, PaCO2, and PaO2 levels. Only a small
decrease in base excess after 120 and 240 min of MV was
observed, comparable with other studies.11,12 The slight
decrease in base excess in the presence of a normal
mean arterial pressure unlikely interferes with our ob-
servations. The effect of anesthetics on hemodynamic
stability in mice has been studied extensively by Zuur-
bier et al.,42 who found KMA mix superior compared
with other regimens (e.g., fentanyl–fluanisone–midazo-
lam mix or isoflurane). Some anesthetics, e.g., propo-
fol,43 volatile anesthetics,14,45 and ketamine,46,47 are
known to influence cytokine profiles. Ketamine is
known to have an inhibitory effect on lipopolysaccha-
ride-induced cytokine production.46,48–50 In the current
study, all animals received the KMA mix. Ideally, an
additional control group of spontaneously breathing an-
imals under KMA anesthesia is needed. However, this
will result in hypventilation with severe respiratory
acidosis and hemodynamic instability. Two mice in
this study could not be intubated; the cause of death was due to difficulty breathing and closely related to
residual effects of the anesthesia. The other mice in group R made uneventful recoveries. By excluding
these confounding factors, we attribute the increase in
cytokine levels to MV.

Therefore, even low-Vt MV induces an inflammatory
response and, in a “multiple-hit” situation, might be the
additional “proinflammatory hit” resulting in lung injury.
Modulation of the inflammatory response may offer stra-
tegies to reduce VILI. In this respect, anesthetics may
play a role because volatile anesthetics have been shown
to exhibit antiinflammatory effects in different organ
systems and might be able to modulate the release of
cytokines.51 The influence of different anesthetics on
the inflammatory response in our model needs further inves-
tigation. Recently, Jiang et al.52 discovered that Toll-like
receptor 4–mediated inflammation by endogenous com-
 pounds might also be important in the development of
VILI. Further study of the Toll-like receptors and the
molecules with which they interact may reveal more insight
into the molecular mechanisms of VILI.

Conclusion

The current study shows that in healthy male mice, a
short period of “noninjurious” ventilation induces a re-
versible inflammatory reaction, while preserving tissue
integrity. This model offers opportunities to study the
pathophysiologic mechanisms of VILI and the contribu-
tion of MV to the “multiple-hit” concept.

The authors thank Francien van de Pol, Ing., and Ilona van den Brink, Ing. (Lab
Technicians, Department of Anesthesiology, Radboud University Nijmegen Med-
cal Centre, Nijmegen, The Netherlands), for their expertise in performing the
animal experiments and Ineke Verschueren, Ing. (Lab Technician, Nijmegen
Centre for Infectious Diseases, Radboud University Nijmegen Medical Centre,
Nijmegen, The Netherlands), for her help with cytokine assays. The authors also
thank Eric N. Robertson, M.D., Ph.D., Staff Anesthesiologist, Kris C. Versier,
M.D., Ph.D., Professor, Department of Anesthesiology (both Radboud University
Nijmegen Medical Centre), and J. Han J. M. van Krieken (Professor, Department
of Pathology, Radboud University Nijmegen Medical Centre) for their helpful
comments on the manuscript.

References

1. Ventilation with lower tidal volumes as compared with traditional tidal


6. Parsons PE, Eisner MD, Thompson BT, Mathaty MA, Ancukiewicz M, Ber-

7. Ranieri VM, Suter PM, Tortorella C, De Tullio, R, Dayer JM, Brienza A, Bruno


10. Tremblay LN, Slutsky AS: Ventilator-induced lung injury: From barotrauma to


12. Wilson MR, Chourdhury S, Takata M: Pulmonary inflammation induced by high

Ventilator-induced lung injury leads to loss of alveolar and systemic compartmen-


15. Gurkan OU, O'Donnell C, Brower R, Ruckdeschel E, Becker PM: Differen-
tial effects of mechanical ventilatory strategy on lung injury and systemic organ

16. Chiumento D, Pristine G, Slutsky AS: Mechanical ventilation affects local and systemic cytokines in an animal model of acute respiratory distress syn-

BV, Beppu OS: Ventilation with high tidal volume induces inflammatory lung

ventilation of healthy lungs induces pro-inflammatory cytokine gene transcrip-

19. Slutsky AS, Tremblay LN: Multiple system organ failure: Is mechanical

20. Chung KB, Slutsky AS, van Vught AJ, Heijnen CJ: Ventilator-induced lung
injury and multiple system organ failure: A critical review of facts and hypothe-

Anesthesiology, V 107, No 3, Sep 2007
33. van’t Wout JW, Linde I, Leijh PC, van FR: Effect of irradiation, cyclophosphamide, and etoposide (VP-16) on number of peripheral blood and peritoneal leukocytes in mice under normal conditions and during acute inflammatory reaction. Inflammation 1989; 13:1–14
34. Netea MG, Kullberg BJ, Blok WL, Netea RT, Van der Meer JW: The role of hyperthermia in the increased cytokine production after lipopolysaccharide challenge in neutropenic mice. Blood 1997; 89:577–82