Atelectasis Causes Alveolar Injury in Nonatelectatic Lung Regions

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Rationale: Many authors have suggested that the mechanism by which atelectasis contributes to injury is through the repetitive opening and closing of distal airways in lung regions that are atelectatic. However, neither the topographic nor mechanistic relationships between atelectasis and distribution of lung injury are known.

Objectives: To investigate how atelectasis contributes to ventilator-induced lung injury.

Methods: Surfactant depletion was performed in anesthetized rats that were then allocated to noninjurious or injurious ventilation for 90 min.

Measurements: Lung injury was quantified by gas exchange, compliance, histology, wet-to-dry weight, and cytokine expression, and its distribution by histology, stereology, cytokine mRNA expression, in situ hybridization, and immunohistochemistry. Functional residual capacity, percent atelectasis, and injury-induced lung water accumulation were measured using gravimetric and volumetric techniques.

Main Results: Atelectasis occurred in the dependent lung regions. Injurious ventilation was associated with alveolar and distal airway injury, while noninjurious ventilation was not. With injurious ventilation, alveolar injury (i.e., histology, myeloperoxidase protein expression, quantification, and localization of cytokine mRNA expression) was maximal in nondependent regions, whereas distal airway injury was equivalent in atelectatic and nonatelectatic regions.

Conclusions: These data support the notion that lung injury associated with atelectasis involves trauma to the distal airways. We provide topographic and biochemical evidence that such distal airway injury is not localized solely to atelectatic areas, but is instead generalized in both atelectatic and nonatelectatic lung regions. In contrast, alveolar injury associated with atelectasis does not occur in those areas that are atelectatic but occurs instead in remote nonatelectatic alveoli.

Keywords: atelectasis; distribution; ventilator-induced lung injury

Lung aeration has a heterogeneous distribution in the setting of acute injury, with a continuum ranging from some degree of atelectasis to some degree of overdistention (1–3). Two contrasting compartments may be considered in the acutely injured lung, with a range of intermediate states. One such compartment is atelectatic (i.e., deaerated, compressed, or fluid filled), less ventilated, and characteristically distributed in the dependent lung regions; the other is overdistended and more ventilated, and is termed the “baby lung” (4). Ventilation of the “baby lung” is based on the principle that smaller tidal volumes will prevent undue tidal overdistension of the small volume of available aerated lung (4, 5). The study by the Acute Respiratory Distress Syndrome (ARDS) Network investigators validated this concept beyond the issue of lung injury by demonstrating a relative mortality benefit associated with a lower tidal volume compared with a higher one where levels of positive end-expiratory pressure (PEEP) were similar (6). Our understanding of the atelectatic part of the lung is limited. Ventilator-induced lung injury is worsened by atelectasis, and injury caused by repetitive opening and closing of distal airways in atelectatic areas has been suggested to explain this phenomenon (7–11). However, the coexistence of regional atelectasis and local lung injury has not been established.

To directly address the distribution of injury associated with atelectasis, we used an in vivo model of surfactant depletion and allocated animals to one of two ventilator strategies: (1) high tidal volume and low PEEP, which causes ventilator-induced lung injury and regional (i.e., dependent) atelectasis; and (2) low tidal volume and higher PEEP, which does not cause ventilator-induced lung injury or atelectasis. We used this model to test the hypothesis that in the presence of extensive atelectasis, airway and alveolar injury associated with high tidal volumes would be predominantly localized to the atelectatic regions.

METHODS

After institutional ethics approval (conforming to the guidelines of the Canadian Council for Animal Care), male Sprague-Dawley rats (300–400 g) were used in all experiments. For complete details of the experimental protocol, see the online supplement. A tracheostomy was performed, arterial and venous catheters inserted, arterial blood pressure continuously monitored, and anesthesia maintained with infusion of intravenous ketamine and xylazine.

Experimental Outline

Surfactant depletion was induced by repetitive saline lavage. Warmed saline was instilled into the lungs and gently retrieved; this was repeated until PaO2 fell below 125 mm Hg with 100% inspired oxygen. After surfactant depletion, the animals were allocated to either a noninjurious or an injurious ventilation strategy, and mechanically ventilated for 90 min. In the noninjurious group, lungs were recruited and maintained with tidal volume 8 ml/kg and PEEP of 14 cm H2O throughout. In the injurious group, ventilation was with tidal volume of 25 ml/kg and PEEP of 4–7 cm H2O. Five series of experiments were performed (see Figure E1 of the online supplement) as follows.

Series I—Histopathology, Stereology, and Pathophysiology

Histopathologic evaluation was performed on the dependent and nondependent tissues of animals in both groups (n = 8 in each group),

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TABLE 1. SYSTEMIC ARTERIAL PRESSURE AND ARTERIAL BLOOD GAS DATA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Postlavage</th>
<th>Recruited</th>
<th>5 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noninjurious</td>
<td>118 ± 30</td>
<td>122 ± 36</td>
<td>102 ± 36</td>
<td>111 ± 38</td>
<td>123 ± 38</td>
<td>126 ± 37</td>
<td>125 ± 42</td>
</tr>
<tr>
<td>Injurious</td>
<td>128 ± 23</td>
<td>111 ± 24</td>
<td>112 ± 36</td>
<td>105 ± 34</td>
<td>102 ± 38</td>
<td>75 ± 30*</td>
<td>81 ± 29*</td>
</tr>
<tr>
<td>PIP, cm H₂O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noninjurious</td>
<td>8.8 ± 1.5</td>
<td>23.0 ± 3.1</td>
<td>33.2 ± 2.3</td>
<td>33.3 ± 2.2</td>
<td>33.0 ± 2.2</td>
<td>33.0 ± 2.3</td>
<td>33.2 ± 2.2</td>
</tr>
<tr>
<td>Injurious</td>
<td>8.7 ± 1.8</td>
<td>22.2 ± 2.2</td>
<td>32.6 ± 1.9</td>
<td>38.1 ± 2.1*</td>
<td>42.2 ± 2.2*</td>
<td>42.7 ± 4.8*</td>
<td>43.0 ± 3.3*</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noninjurious</td>
<td>7.37 ± 0.05</td>
<td>7.25 ± 0.06</td>
<td>7.21 ± 0.06</td>
<td>7.20 ± 0.02</td>
<td>7.18 ± 0.05</td>
<td>7.17 ± 0.02</td>
<td>7.17 ± 0.02</td>
</tr>
<tr>
<td>Injurious</td>
<td>7.38 ± 0.06</td>
<td>7.25 ± 0.03</td>
<td>7.20 ± 0.05</td>
<td>7.26 ± 0.10</td>
<td>7.20 ± 0.06</td>
<td>7.12 ± 0.07</td>
<td>7.07 ± 0.05*</td>
</tr>
<tr>
<td>PaO₂, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noninjurious</td>
<td>489 ± 23</td>
<td>84 ± 15</td>
<td>426 ± 26</td>
<td>440 ± 35</td>
<td>399 ± 37</td>
<td>396 ± 35</td>
<td>387 ± 44</td>
</tr>
<tr>
<td>Injurious</td>
<td>512 ± 30</td>
<td>92 ± 23</td>
<td>434 ± 52</td>
<td>92 ± 20*</td>
<td>71 ± 15*</td>
<td>66 ± 9*</td>
<td>61 ± 12*</td>
</tr>
<tr>
<td>HCO₃⁻, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noninjurious</td>
<td>42.9 ± 7.6</td>
<td>50.9 ± 6.6</td>
<td>58.4 ± 9.4</td>
<td>53.8 ± 6.4</td>
<td>55.8 ± 7.3</td>
<td>60.6 ± 3.8</td>
<td>59.1 ± 4.0</td>
</tr>
<tr>
<td>Injurious</td>
<td>38.1 ± 7.5</td>
<td>49.5 ± 7.1</td>
<td>57.3 ± 8.8</td>
<td>45.4 ± 9.3</td>
<td>50.3 ± 11.2</td>
<td>58.6 ± 7.4</td>
<td>60.2 ± 9.6</td>
</tr>
<tr>
<td>Base excess, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noninjurious</td>
<td>23.6 ± 2.4</td>
<td>20.6 ± 1.6</td>
<td>21.8 ± 1.3</td>
<td>20.0 ± 1.9</td>
<td>20.0 ± 0.9</td>
<td>20.9 ± 1.0</td>
<td>20.8 ± 1.2</td>
</tr>
<tr>
<td>Injurious</td>
<td>22.0 ± 1.4</td>
<td>20.9 ± 2.1</td>
<td>21.8 ± 2.0</td>
<td>19.2 ± 2.2</td>
<td>18.6 ± 2.7</td>
<td>18.5 ± 2.5*</td>
<td>17.0 ± 2.7*</td>
</tr>
<tr>
<td>Pa₅₀, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noninjurious</td>
<td>−0.9 ± 1.9</td>
<td>−6.9 ± 2.3</td>
<td>−6.9 ± 1.5</td>
<td>−8.4 ± 1.6</td>
<td>−8.9 ± 1.3</td>
<td>−8.6 ± 1.1</td>
<td>−8.5 ± 1.2</td>
</tr>
<tr>
<td>Injurious</td>
<td>−2.4 ± 1.4</td>
<td>−6.3 ± 1.7</td>
<td>−6.7 ± 2.0</td>
<td>−7.6 ± 3.0</td>
<td>−9.5 ± 2.2</td>
<td>−11.7 ± 3.5*</td>
<td>−14.1 ± 3.2*</td>
</tr>
</tbody>
</table>

Definition of abbreviations: ABP = mean arterial blood pressure; PIP = peak inspiratory pressure.

Data are mean ± SD.

*p < 0.05 versus noninjurious.

as previously described (12). All histopathologic examinations were conducted by investigators blinded as to experimental group allocation and anatomic site of the tissue sample. The same lung tissues embedded in paraffin were utilized for the stereologic analysis, and mean alveolar volume of each tissue was calculated (Figure E2) (13, 14).

Airspace and airway injury were based on a quantitative method as previously described (8, 12). Airspace injury was expressed in terms of Hyaline Membrane Score: the total number of alveoli, alveolar ducts, and alveolar sacs with (any) hyaline membrane present in eight randomly selected fields per lung section, divided by the total number of alveoli, alveolar ducts, and alveolar sacs in the same fields, then multiplied by 100. All airways, which include intrapulmonary bronchi and terminal bronchioles, were evaluated and scored in each lung section. Each airway was evaluated by the total range of epithelial desquamation injury (bubble formation, cleft formation, sloughing, and denudation), scored from 0–3, summed per lung section. Entirely flattened epithelium without desquamation injury was scored as 1. Airway Injury Score was calculated by dividing this sum by the number of all airways examined. A composite lung injury score (comprising airspace and alveolar injury) was calculated. Scores were normalized as described in the complete methodology (see online supplement).

Finally, a semiquantitative analysis of inflammatory cell infiltration was assessed by the following three parameters: presence of inflammatory cells (1) in interalveolar septa and (2) within alveoli, both representing airspace inflammation; and (3) peribronchiolar infiltration of inflammatory cells, representing airway inflammation. Each parameter was evaluated semiquantitatively, using a five-grade scale: normal = 0, questionable change = 1, minimal change = 2, moderate change = 3, and marked change = 4.

Series II—Gravimetric and Volumetric Analysis and Real-Time Polymerase Chain Reaction

Animals were allocated to nonlavaged control, lavaged control, noninjurious strategy, or injurious strategy groups as before (n = 6 in each group). At the conclusion of the protocol, the trachea was occluded at end-expiratory lung volume and the animals exsanguinated. The functional residual capacity (FRC), degree of atelectasis, and lung wet-to-dry weight ratio were measured (15, 16). The dependent and nondependent tissues of the left lung were assessed for cytokine mRNA expression using real-time polymerase chain reaction (PCR) (17).

Series III—Computed Tomography Scan

To exhibit the representative computed tomography (CT) scan images of lung injury, animals were ventilated with either noninjurious or injurious strategy as before (n = 2 in each group). The trachea was occluded at end-expiratory lung volume, and CT scanning performed (18).

Series IV—Cytokine mRNA and Myeloperoxidase Protein Expression

To localize the expression of cytokine mRNA and myeloperoxidase protein, in situ hybridization and immunohistochemistry was performed as previously described (19) on lungs from animals ventilated with either noninjurious or injurious strategy (n = 1 in each group).

TABLE 2. STATIC COMPLIANCE OF RESPIRATORY SYSTEM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lavaged Noninjurious</th>
<th>Lavaged Injurious</th>
<th>Difference between Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>328 ± 20</td>
<td>348 ± 18</td>
<td>N.S.</td>
</tr>
<tr>
<td>Prelavage compliance, ml/cm H₂O/kg</td>
<td>1.282 ± 0.172</td>
<td>1.202 ± 0.291</td>
<td>N.S.</td>
</tr>
<tr>
<td>Postlavage compliance, ml/cm H₂O/kg</td>
<td>0.654 ± 0.109</td>
<td>0.613 ± 0.061</td>
<td>N.S.</td>
</tr>
<tr>
<td>Final compliance, ml/cm H₂O/kg</td>
<td>0.655 ± 0.110</td>
<td>0.499 ± 0.044*</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

Definition of abbreviation: N.S.— no significance.

The static compliance of respiratory system decreased in the lavaged injurious. All data are mean ± SD.

*p < 0.05 final versus postlavage.
Series V—Pressure–Volume Characteristics

The pressure–volume (P-V) curves were constructed on animals after ventilation with either a noninjurious or an injurious strategy (n = 4 in each group).

Statistics

Statistical analysis was as previously recommended (20). Data are expressed as mean ± SD, or median ± quartiles (nonparametric data).

### Table 3. Stereologic Analysis

<table>
<thead>
<tr>
<th></th>
<th>Lavaged Noninjurious</th>
<th>Lavaged Injurious</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nondependent</td>
<td>Dependent</td>
</tr>
<tr>
<td><strong>Cavalieri volume, mm³</strong></td>
<td>48.3 ± 21.5</td>
<td>72.5 ± 20.3</td>
</tr>
<tr>
<td><strong>VdAs, block</strong></td>
<td>0.595 ± 0.046</td>
<td>0.572 ± 0.061</td>
</tr>
<tr>
<td><strong>VdAl wall, block</strong></td>
<td>0.341 ± 0.047</td>
<td>0.365 ± 0.035</td>
</tr>
<tr>
<td><strong>VdEx.ac, block</strong></td>
<td>0.045 ± 0.054</td>
<td>0.036 ± 0.034</td>
</tr>
</tbody>
</table>

*Definition of abbreviations: VdAs, block = the volume of intraacinar air space per unit volume of lung tissue; VdAl wall, block = the volume of alveolar wall per unit volume of lung tissue; VdEx.ac, block = the volume of all extracellular structures (including the contained air space) per unit volume of lung tissue.*

*VdAs, block was greater in lavaged noninjurious than in lavaged injurious (p < 0.05).*

†VdAl wall, block was greater in lavaged injurious versus lavaged noninjurious (p < 0.05).
number of alveoli per unit volume) in tissue sampled from dependent (74.1 ± 18.8 \times 10^3/\text{cm}^3) versus nondependent (51.9 ± 12.8 \times 10^3/\text{cm}^3) regions (p < 0.05; Figure 1A). There were no regional differences (i.e., dependent vs. nondependent) in alveolar density in animals ventilated noninjurally.

Additional stereologic data, including Cavalieri volume (13), and the proportion of lung tissue composed of intraacinar tissue (i.e., airspace, alveolar wall) and extraacinar structures are presented (Table 3). Quantitative stereologic analysis showed that lungs ventilated with injurious ventilation demonstrated a greater volume density of alveolar wall and a smaller volume density of airspace compared with lungs of the noninjurious group (p < 0.05, two-way ANOVA; Table 3), lacking regional differences in either group. Because we believed that the alveolar wall would directly reflect the regional differences in alveolar recruitment, we compared the mean alveolar volume between the dependent and nondependent regions. The estimated mean regional alveolar volume was less in the dependent versus nondependent regions of the injuriously ventilated lungs (p < 0.05; Figure 1B), but regional differences in alveolar volume were not observed in the noninjurally ventilated lungs (Figure 1B). There was a significant interaction between ventilatory strategy and vertical region, indicating that ventilation strategy (noninjurious vs. injurious) with position in the lung (dependent vs. nondependent) determined mean regional alveolar volume (p < 0.05, two-way ANOVA; Figure 2).

Analysis of lung injury. The composite lung injury histopathologic score was greater with injurious than with noninjurious ventilation (6.55 ± 3.06 vs. 0.56 ± 0.41, p < 0.05). There were also significant regional differences in the composite lung injury score after injurious (but not noninjurious) ventilation, with the following rank order: injurious/nondependent > injurious/dependent > noninjurious/dependent = noninjurious/nondependent (p < 0.05; Figure 3A).

Alveolar injury (i.e., hyaline membrane score) was minimal—and without regional differences—in noninjurious ventilation (Figure 3B, Figures E3A and E3B). In contrast, injurious ventilation caused severe alveolar-associated injury, which was greatest in the nondependent regions (p < 0.05; Figure 3B, Figures E3C and E3D). In addition, alveolar injury in both dependent and nondependent regions was significantly greater after injurious ventilation than in either region after noninjurious ventilation (p < 0.05; Figure 3B).

Airway-associated injury (i.e., epithelial lesions) was also minimal after noninjurious ventilation (Figure 3C, Figures E3E and E3F). However, there was marked airway epithelial injury in both dependent and nondependent regions after injurious ventilation that was greater than in either region after noninjurious ventilation (p < 0.05; Figure 3C, Figures E3G and E3H). Furthermore, there were no differences in the degree of airway

**Table 4. Lung Injury—Inflammatory Cells**

<table>
<thead>
<tr>
<th></th>
<th>Lavaged Noninjurious</th>
<th>Lavaged Injurious</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nondependent</td>
<td>Dependent</td>
</tr>
<tr>
<td>Airspace score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septal cells</td>
<td>2.0 ± 0.0</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td>Alveolar cells</td>
<td>2.0 ± 0.0</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td>Airway score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peribronchial cells</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Data are median ± quartiles.

*p < 0.05 versus both regions of noninjurally ventilated lungs.

†p < 0.05 versus dependent regions of injuriously ventilated lungs, and both regions of noninjurally ventilated lungs.
epithelial injury between dependent and nondependent regions after injurious ventilation (Figure 3C).

Finally, a semiquantitative analysis of inflammatory cell infiltration paralleled the above findings (Table 4). Alveolar infiltration of inflammatory cells ( airspace inflammation) was greater in the nondependent than in the dependent regions after the injurious ventilation, and the rank order of inflammatory cells in the interalveolar septa, as well as within alveoli, was as follows: injurious/nondependent > injurious/dependent > noninjurious/dependent ≈ noninjurious/nondependent (Table 4). In contrast, peribronchial infiltration of inflammatory cells ( airway inflammation) did not show regional differences in either group, with the following rank order: injurious/nondependent = injurious/dependent > noninjurious/dependent = noninjurious/nondependent (Table 4).

Series II—Gravimetric and Volumetric Analysis and Real-Time PCR

Baseline comparisons. The entire protocol was completed on six animals in each group. All parameters were comparable among the groups at baseline, and changes in $P_{aO_2}$ and static compliance reflected the initial experimental series (above).

FRC and atelectasis. The effects of the two ventilation strategies on atelectasis formation (i.e., percent atelectasis) and the FRC were measured at end-expiration. The FRC and calculated percent atelectasis were similar in the lavaged control and lavaged noninjurious groups, but the FRC was significantly less ($p < 0.05$; Figure 4A), and atelectasis significantly greater ($p < 0.05$; Figure 5) in the lavaged injurious group.

Global and regional lung water. The rank order of overall wet-to-dry weight ratio was as follows: injurious > noninjurious = lavaged control > nonlavaged control ($p < 0.05$; Figure 4B). The rank order of the whole-lung wet weight corrected for body weight was the same (Table 5). However, there are two sources of lung water in the context of the current experiment: exogenous (i.e., derived from the saline lavage process) and endogenous (i.e., derived from pulmonary edema resulting from acute lung injury). The quantity of retained water (i.e., saline) was similar in each group ( Table 5), but the injury-associated edema was as follows: injurious > noninjurious = lavaged control ($p < 0.05$; Table 5).

The regional wet-to-dry weight ratio was the same in all four groups: nonlavaged control, lavaged control, lavaged noninjurious, and lavaged injurious (Figure 4B). The ratio was not different—in total or by region—between the lavaged control and lavaged noninjurious, but the ratio was significantly greater in the lavaged injurious than in the other three groups, regardless of the lung region ($p < 0.05$; Figure 4B).

Pulmonary cytokine mRNA expression. The regional cytokine mRNA expression was examined in the noninjurious and injurious groups, as well as in an additional control group (lavaged, not ventilated), and was expressed as fold-change relative to the normal control group (not lavaged, not ventilated). The profile of interleukin (IL)-1β, IL-6, and macrophage-inflammatory protein (MIP)-2 was similar, with minimal mRNA expression in the lungs of lavaged control (nonventilated), and the following rank order:
TABLE 5. MEASUREMENT OF LUNG WATER

<table>
<thead>
<tr>
<th></th>
<th>Nonlavaged Control</th>
<th>Lavaged Control</th>
<th>Lavaged Noninjurious</th>
<th>Lavaged Injurious</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>338 ± 5</td>
<td>352 ± 17</td>
<td>362 ± 23</td>
<td>352 ± 22</td>
</tr>
<tr>
<td>Instilled saline, g</td>
<td>—</td>
<td>44.81 ± 7.56</td>
<td>43.71 ± 4.47</td>
<td>46.71 ± 6.04</td>
</tr>
<tr>
<td>Withdrawn saline, g</td>
<td>—</td>
<td>41.42 ± 7.74</td>
<td>40.30 ± 4.25</td>
<td>43.03 ± 6.02</td>
</tr>
<tr>
<td>Retained saline, g</td>
<td>—</td>
<td>3.38 ± 0.57</td>
<td>3.41 ± 0.49</td>
<td>3.69 ± 0.82</td>
</tr>
<tr>
<td>Lung tissue weight, g/kg</td>
<td>3.33 ± 0.30</td>
<td>7.98 ± 1.41*</td>
<td>9.11 ± 1.58*</td>
<td>23.07 ± 3.18*</td>
</tr>
<tr>
<td>Injury-associated edema, ml</td>
<td>—</td>
<td>1.65 ± 0.55</td>
<td>2.11 ± 0.64</td>
<td>9.74 ± 1.08*</td>
</tr>
</tbody>
</table>

For definition of abbreviation, see Table 2.
* Lung tissue weight was greater in lavaged injurious, lavaged noninjurious, and lavaged control versus nonlavaged control.
† Lung tissue weight and injury-associated edema were greater in lavaged injurious versus lavaged control and lavaged noninjurious.
All data are mean ± SD.

injurious/nondependent > injurious/dependent = noninjurious/nondependent = noninjurious/dependent > control/nondependent = control/dependent (p < 0.05; Figures 6A–6C). The tumor necrosis factor-α mRNA expression was minimal in the lavaged control, and was greater after noninjurious or injurious ventilation (p < 0.05), without regional differences in either group (Figure 6D).

Series III—CT Scan
Six animals (i.e., two animals in each of three groups) were studied and CT performed at end-expiration. In the nonlavaged control group, the lungs were homogeneously aerated and no significant densities noted (Figures 7A and 7B). The lungs ventilated by the noninjurious strategy after saline lavage are similar to the normal control lungs, and were homogeneously aerated with minimal densities (Figures 7C and 7D). The lungs ventilated by the injurious strategy after saline lavage showed heterogeneous distribution of densities that were preferentially located in the dependent lung regions, with aeration predominantly in the nondependent regions (Figures 7E and 7F).

![Figure 6](image_url)

**Figure 6.** Effects of saline lavage and two ventilatory strategies on the expression of cytokine mRNA measured by the real-time polymerase chain reaction. (A) Expression of inteleukin (IL)-1β mRNA was maximal in the injurious/nondependent group (*p < 0.05 vs. injurious/dependent, both regions of noninjurious and lavaged control), and minimal in both regions of control, with values for injurious/dependent and both regions of noninjurious at intermediate levels (*p < 0.05 smaller than injurious/nondependent and greater than both regions of lavaged control). The profile of mRNA expression was similar for IL-1β, IL-6 (B), and macrophage-inflammatory protein (MIP)-2 (C). (D) Expression of tumor necrosis factor (TNF)-α mRNA was minimal in the lavaged control group, and was greater after noninjurious or injurious ventilation (*p < 0.05 vs. both regions of lavaged control), without regional differences in either group. All data are mean ± SD. D = dependent region; ND = nondependent region.
Remote Injury

A striking finding in the current study is the relative absence of alveolar injury in the dependent (i.e., atelectatic) regions in the injuriously ventilated lungs and the presence of injury in the nondependent (i.e., nonatelectatic) regions. This is consistent with the “baby lung” concept proposed byGattinoni and colleagues (4, 5). The pattern of the injury is schematically illustrated in Figure 10.

Distribution of lung injury has been described in different settings. Extremely high tidal volumes in a canine model resulted in dependent injury (28, 29) whether the lungs were preinjured (28) or not (29). In clinical ARDS, overinflation occurs in aerated areas and bronchial distention in nonaerated regions (30), and similar patterns were produced in a porcine model of multifocal
pneumonia (31). The contribution of atelectasis may extend beyond ventilator-induced lung injury as recent work from our group suggests that, without supplemental oxygen, atelectasis per se can cause pulmonary vascular injury (16, 18).

In the current study, the key markers in the evaluation of lung injury—and of its distribution—were histology, cytokine mRNAs, and MPO expression. Hyaline membranes and the infiltration of polymorphonuclear cells are well-established indicators of acute lung injury (32, 33). Because rodents have very short or absent respiratory bronchioles, formation of hyaline membranes confined to the alveolar ducts, sacs, and alveoli is therefore representative of the alveolar injury. MPO reflects neutrophil and monocyte extravasation (34), and together with the increased polymorphonuclear cell infiltration, the increased number of MPO-positive inflammatory cells confirmed increased inflammatory cell activity in the nondependent versus dependent regions after injurious ventilation.

Proinflammatory cytokine mRNA expression (IL-1β, IL-6, and MIP-2) evaluated by real-time PCR also supports the concept of regional distribution of lung injury. Both IL-6 and IL-1β mRNAs followed the same pattern of expression depending on the ventilatory strategies and vertical topography. The expression of IL-6 and IL-1β mRNAs was weak in the noninjurally ventilated lung, with no differences between dependent and nondependent regions. In the injuriously ventilated lung, the strongest positive signals for IL-1β and IL-6 mRNA were detected predominantly at the alveolar epithelium in the nondependent versus dependent regions.

To our knowledge, this is the first study quantitatively demonstrating the regional differences in the expression of cytokine mRNA in an in vivo model. In the injuriously ventilated lungs, the tidal volume would be shifted by the dependent atelectasis toward the nondependent regions, in which alveolar overdistention would occur, resulting in increased mRNA expression of proinflammatory cytokines.

**Atelectasis and Distal Airway Injury**

The classic paradigm of atelectasis causing local repetitive opening and closing of the airways was initially proposed more than 20 yr ago (7) and has been supported by several elegant studies (8, 9), but not directly proved (35). This hypothesis predicts that both airway and alveolar injury will be most severe in the
albeit not the sole, focus in the pathogenesis of ventilator-induced lung injury (19). Taken together with the current data this suggests that ventilation with high tidal volume induces significant airway injury through the entire lung. Such a concept does not refute repetitive airway open and closing as a pathologic process associated with atelectasis, but strongly suggests that associated airway injury is generalized and not localized to atelectatic regions.

The potential mechanisms whereby atelectasis accentuates injury induced by high tidal volume are illustrated in Figure 10. In the current experiments, airway injury was generalized in all lung regions (i.e., atelectatic and inflated regions), and although the mechanism of such injury is unclear, it likely involves local shear stress that might be related to the movement of foam (i.e., air and fluid) with tidal ventilation (36).

**Study Limitations**

There are several limitations to extrapolation of the current data. First, the atelectasis clinically develops secondarily to lung injury over several days, and the occurrence of atelectasis and lung injury in our model was not identical to the clinical experiences. However, we believe that the surfactant depleted model is useful for our purpose because we focused on the role of atelectasis in the ongoing lung injury process. Second, we considered testing low tidal volume and low PEEP to evaluate the effects of derecruitment per se, but animals were not able to tolerate such a strategy in the current pilot studies, or in other models (16, 18, 37). Third, we could not actually observe the opening and closing of distal airways, and although dynamic CT could have been illustrative as in larger animal models (38–40), it has not been reported in small rodents. Although histology may be a gold standard for demonstration of lung injury, epithelial lesions are optimally seen with electron microscopy.

Fixation, dehydration, and embedding will obviously alter alveolar dimensions from those that existed in vivo due to a number of reasons. For example, lungs were fixed at an airway pressure that produces inflation to total lung capacity in a normal lung and served as a useful standard across all experimental groups, allowing comparison under uniform inflation conditions. The alveolar volumes in any region thus reflected regional compliance. However, this standard fixation pressure was clearly different from the continuously varying pressures that occurred throughout the respiratory cycle in vivo. The further steps in the preparation of histologic sections including dehydration and embedding also alter tissue volumes. Other approaches have been used previously to try to avoid artifacts due to section preparation including frozen sections. However, these are also subject to problems such as the marked changes in gas and fluid volumes that can take place during freezing. Although no method of slide preparation is without artifact, the method of fixation that we used ensured that the postfixation alveolar volume was determined by local regional compliance and thus reflected differences throughout the lung. This view is corroborated by the CT images, which were obtained at the values of PEEP used in vivo. Thus, the absence of regional variability on CT parallels the absence of regional variability in alveolar volume on histology in the noninjuriously ventilated lungs. Conversely, the heterogeneity of regional injury observed in the CT images of the injuriously ventilated lungs parallels that observed in the histology of these lungs and strongly supports our finding that the injurious ventilation strategy produced both regional atelectasis and hyperinflation in the injured lung.

Finally, it is important to consider the saline lavage process. The relative protection of the dependent lung tissue could have occurred as a result of retained saline perhaps forming an impermeable layer of fluid that protected the alveoli from injurious inflation pressure. It is unlikely that such protection represents

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**Figure 9.** The static pressure–volume (P-V) curve after the two ventilations. The P-V curves were constructed from the end-expiratory pressure up to the airway pressure of 25 cm H2O above the end-expiratory pressure. The lung P-V curves showed that the static compliance of lung was more impaired in the lavaged injurious than in the lavaged noninjurious group.

**Figure 10.** A schematic explanation of regional lung injury associated with dependent atelectasis. End-expiratory alveolar size is smaller in the dependent than in the nondependent region. At end-inspiration, alveolar overdistention occurs in the nondependent region, resulting in marked alveolar injury therein. In contrast, distal airway injury is equally distributed between the dependent and nondependent regions. It is not clear whether repetitive opening and closing actually takes place, or if it does, that it is a direct cause of injury. However, tidal foam movement has been suggested as a possible mechanism of distal airway injury.
an artifact of the experimental model because the retained vol-
ume of saline from the lavage process was not different in any of
the experimental groups (noninjurious vs. injurious ventilation),
and in context of injurious ventilation (where the dependent
protection was observed) the retained saline was far exceeded by
lung water produced endogenously from the ongoing lung
injury process.

CONCLUSIONS

We demonstrate that atelectasis in this model is distributed pre-
dominantly in the dependent regions. The alveolar injury was
maximal in the nondependent (i.e., nonatelectatic) regions of
the injuriously ventilated lungs, consistent with redistribution
of ventilation from atelectatic to nonatelectatic areas resulting in
overinflation injury. In contrast, distal airway injury was homoge-
neously distributed. Although we could not actually observe the
repetitive opening and closing of distal airways, the current data
suggest that the hypothesis of distal airway injury associated
with the cyclical phenomenon occurring exclusively in atelectatic
regions should be broadened to include distal airways through-
out the entire lung. Taken together, these findings suggest that
we might revise our current views on the role of atelectasis in
the pathogenesis of ventilator-induced lung injury.

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