Pulmonary inflammation induced by high-stretch ventilation is mediated by tumor necrosis factor signaling in mice

Michael R. Wilson, Sharmila Choudhury, and Masao Takata

Department of Anaesthetics and Intensive Care, Faculty of Medicine, Imperial College London, Chelsea and Westminster Hospital, London, United Kingdom

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Wilson, Michael R., Sharmila Choudhury, and Masao Takata. Pulmonary inflammation induced by high-stretch ventilation is mediated by tumor necrosis factor signaling in mice. Am J Physiol Lung Cell Mol Physiol 288: L599–L607, 2005. First published October 15, 2004; doi:10.1152/ajplung.00304.2004.—Although high-stretch mechanical ventilation has been demonstrated to induce lung inflammation, the roles of soluble mediators, in particular TNF, remain controversial. We have previously shown in mice that high-stretch ventilation, in the absence of preceding lung injury, induces expression of proinflammatory cytokine TNF, which mediates CXC chemokine expression in models of lung inflammation (4, 12, 56), remains controversial. In the presence of underlying lung injury, a number of animal studies of VILI have reported upregulation of TNF within the lung (2, 7, 25, 31, 45), and increased TNF levels have been observed in bronchoalveolar lavage fluid from ARDS patients receiving conventional nonprotective ventilation (37, 44). We previously found in rabbits that lung injury induced by mechanical ventilation following saline lung lavage was attenuated by anti-TNF antibody (25). However, with such a “two-hit” approach, there are inherent difficulties in distinguishing the component of inflammation due to the preexisting lung injury from that due to ventilation per se (14). Our saline-lavaged rabbit study could not precisely determine to what extent anti-TNF treatment attenuated the injury induced by the lavage procedure and the injury induced by ventilation. In addition, two-hit models may sometimes be associated with complicated, potentially model-specific interactions between preinjury and ventilation, making the interpretation of results difficult (15, 54). In the absence of predisposing lung injury, there has been considerable debate in the literature as to whether high-stretch ventilation alone can initiate TNF-mediated pulmonary inflammation (14, 50). Studies of such “one-hit” animal VILI models, which should provide a clearer answer regarding the effect of mechanical ventilation per se, have in fact produced conflicting and inconclusive results regarding upregulation of TNF within the lung (11, 20, 27, 38, 47, 48, 51). We have recently addressed this controversy in a mouse model of VILI by demonstrating that TNF protein upregulation is highly transient in response to mechanical lung stretch in vivo and therefore often difficult to detect during the course of experiments (55). However, demonstrating the presence of TNF during VILI does not necessarily indicate a significant biological role, and the in vivo physiological impact of TNF signaling in mediating stretch-induced pulmonary inflammation has yet to be determined.

Although mechanical ventilation is an indispensable tool in the treatment of critically ill patients, high stretch/high tidal volume ventilation has been demonstrated to result in lung damage (16, 41). Clinically, ventilation of acute respiratory distress syndrome (ARDS) patients with high tidal volumes is associated with increased mortality (1, 3). The pathophysiology of ventilator-induced lung injury (VILI) is still unclear, but animal models have demonstrated development of pulmonary inflammation in response to excessive lung stretch, resulting in recruitment of neutrophils and tissue damage (27, 28, 36, 49). High-stretch ventilation promotes the release of various inflammatory mediators into the alveolar space (26, 36, 47, 55), although the roles and relative involvement of these are not well understood. CXC chemokines have been demonstrated to play a role in VILI (5, 36), but the involvement of the proinflammatory cytokine TNF, which mediates CXC chemokine expression in models of lung inflammation (4, 12, 56), remains controversial (14).

In the presence of underlying lung injury, a number of animal studies of VILI have reported upregulation of TNF within the lung (2, 7, 25, 31, 45), and increased TNF levels have been observed in bronchoalveolar lavage fluid from ARDS patients receiving conventional nonprotective ventilation (37, 44). We previously found in rabbits that lung injury induced by mechanical ventilation following saline lung lavage was attenuated by anti-TNF antibody (25). However, with such a “two-hit” approach, there are inherent difficulties in distinguishing the component of inflammation due to the preexisting lung injury from that due to ventilation per se (14). Our saline-lavaged rabbit study could not precisely determine to what extent anti-TNF treatment attenuated the injury induced by the lavage procedure and the injury induced by ventilation. In addition, two-hit models may sometimes be associated with complicated, potentially model-specific interactions between preinjury and ventilation, making the interpretation of results difficult (15, 54). In the absence of predisposing lung injury, there has been considerable debate in the literature as to whether high-stretch ventilation alone can initiate TNF-mediated pulmonary inflammation (14, 50). Studies of such “one-hit” animal VILI models, which should provide a clearer answer regarding the effect of mechanical ventilation per se, have in fact produced conflicting and inconclusive results regarding upregulation of TNF within the lung (11, 20, 27, 38, 47, 48, 51). We have recently addressed this controversy in a mouse model of VILI by demonstrating that TNF protein upregulation is highly transient in response to mechanical lung stretch in vivo and therefore often difficult to detect during the course of experiments (55). However, demonstrating the presence of TNF during VILI does not necessarily indicate a significant biological role, and the in vivo physiological impact of TNF signaling in mediating stretch-induced pulmonary inflammation has yet to be determined.

Address for reprint requests and other correspondence: M. Takata, Dept. of Anaesthetics and Intensive Care, Imperial College London, Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9NH, United Kingdom (E-mail: m.takata@imperial.ac.uk).
In this study, we therefore investigated the role of TNF in the inflammatory response to high-stretch mechanical ventilation in the absence of underlying injury in mice. The involvement of TNF signaling was assessed using genetically modified mice lacking TNF receptors and wild-type (WT) mice treated with anti-TNF antibody. Mice were ventilated by high-stretch ventilation until lung injury started to become apparent, and then ventilation was continued using a noninjurious strategy. This protocol allowed the animal to survive long enough after the onset of injury to develop detectable intra-alveolar neutrophil infiltration in response to a highly standardized, short-duration mechanical stretch. The results demonstrated that pulmonary inflammation induced by high-stretch ventilation without underlying injury has a substantial TNF-mediated component.

METHODS

Animal Preparation

Experiments were carried out under the guidelines of the Animals (Scientific Procedures) Act 1986, United Kingdom, using WT male C57BL6 mice (Charles River) aged 9–13 wk (22–28 g) or age-matched male TNF receptor double knockout (DKO) mice (p55−/− p75−/−) (32) backcrossed onto their WT C57BL6 strain for five generations (generous gift from Amgen, Thousand Oaks, CA). To investigate the effects of high-stretch ventilation on pulmonary inflammation, we used a modification of our previous mouse model of VILI (10, 55). Mice were anesthetized by intraperitoneal injection of 2.5 ml/kg of Hypnorm (0.8 mg/kg of fentanyl and 25 mg/kg of fluanisone) and 2.5 ml/kg of midazolam (12.5 mg/kg) and acutely instrumented as described in detail previously (55). In brief, animals were ventilated via endotracheal tube by a custom-made, flow-regulated mouse ventilator-pulmonary function testing system using O2 supplemented with 0–4% CO² as required to avoid hypocapnia. Cannulae were placed in the left carotid artery for monitoring blood pressure (BP), blood gas analysis, and fluid infusion (0.9% NaCl containing 10 U/ml of heparin at 0.4 ml/h) and in the right jugular vein for antibody administration. During surgery and stabilization, animals were ventilated with a tidal volume (VT) of 43–44 ml/kg, zero PEEP, and a respiratory rate of 90 breaths/min using O2 supplemented with 2% CO². Inspiratory-expiratory ratio was kept at 1:2 throughout the experiment. After instrumentation, sustained inflation of 35 cmH²O for 5 s was given two times to standardize volume history of the lungs.

Chronic Inhibition of TNF Signaling

After baseline physiological measurements, WT and TNF receptor DKO mice were randomly allocated to receive either low- or high-stretch ventilation protocols. 1) Low stretch group: mice continued to be ventilated with the baseline low-stretch ventilation used during surgery (described above) for a further 240 min using O₂ with 2% CO₂. Sustained inflation of 35 cmH²O for 5 s was performed every 30 min throughout the experiments to prevent atelectasis. 2) High-stretch group: mice were ventilated with the same settings as the low-stretch group except for a short period of high-stretch ventilation (Fig. 1). Mice were first ventilated with a VT of 43–44 ml/kg, zero PEEP, and RR of 90 breaths/min using O₂ supplemented with 2% CO₂. Initial peak inspiratory pressure (PIP) attained with these settings was 48.8 ± 0.8 (means ± SD) cmH²O. At the start of high stretch, animals received an intra-arterial bolus of 200 μl of saline. High-stretch ventilation was terminated when PIP started to increase by 5–10% (average duration 67 ± 18 min). We have previously shown that high-stretch ventilation of this level, if maintained longer (>2 h), produces progressive VILI in C57BL6 mice with a substantial pulmonary edema, decrease in lung compliance, and lung pathology characterized by airway epithelial damage and hyaline membrane formation (55). After the high-stretch period, animals were returned to baseline low-stretch ventilation (VT of 9–10 ml/kg) for a further 180 min to allow the animals to survive long enough to develop intra-alveolar neutrophil infiltration, since continuing the high-stretch ventilation leads to very rapidly progressing pulmonary edema and death in rodents (13, 16, 55). During this period, animals were ventilated using O₂ with 0% CO₂, since impaired gas exchange induced by the high-stretch ventilation meant that use of additional CO₂ was not required. The level of mechanical lung injury induced by high stretch was standardized by ensuring that on return to low stretch, PIP was 40–50% increased compared with pre-high-stretch values. Animals that showed <30% (4/22 mice) or >70% (3/22 mice) increases in the post-high-stretch PIP values were excluded from the study.

Acute Inhibition of TNF Signaling

WT mice were ventilated with high-stretch ventilation and then received anti-TNF antibody either intratracheally or intravenously. 1) Intratracheal antibody group: mice were ventilated using the same high-stretch protocol described above. Immediately after ventilation was returned from the high-stretch to low-stretch settings, 50 μg of anti-TNF-α antibody (hamster anti-mouse TNF-α monoclonal antibody, TN3-19.12; BD Pharmingen, Oxford, UK) or isotype control antibody (hamster IgG, BD Pharmingen) were administered via the endotracheal tube in a total volume of 100 μl. Antibody administrations were carried out only after the high-stretch period to ensure a consistent degree of stretch-induced mechanical lung injury, since intratracheal administration of any fluid before high stretch will have somewhat unpredictable effects on lung mechanics, making it more difficult to standardize lung injury by monitoring PIP. Pilot studies indicated that intratracheal fluid administration produced severe hypoxemia (presumably due to airway obstruction and resultant ventilation/perfusion mismatch) after the return to low-stretch ventilation if the animals were ventilated with 2.5 cmH²O of PEEP. To counteract...
this, low-stretch ventilation after the high-stretch period was carried out with 5 cmH2O of PEEP. 2) Intravenous antibody group: WT mice were ventilated using the same high-stretch protocol described above. Immediately after ventilation was returned to the low-stretch settings, 50 µg of anti-TNF-α antibody or isotype control were administered in a volume of 200 µl via the right jugular vein over 10 min.

As in the chronic TNF inhibition experiments, animals that showed <30% (1/20 mice) or >70% (2/20 mice) increases in PIP immediately after high stretch (before the administration of antibody) were excluded from the study. The TN3-19.12 antibody has been shown to inhibit TNF-mediated cell cytotoxic activity >95% at a dose of 5–10 ng/pg of mouse TNF (40). Assuming ~2 ml of circulating blood volume for a 25-g mouse, the dose of the antibody used in this study (50 µg) should neutralize 2,500–5,000 pg/ml of mouse TNF activity in blood.

### Physiological Measurements

Airway pressure, airway flow, and mean BP were monitored continuously, and blood gas analyses and determination of respiratory system compliance using the end-inflation occlusion technique (18) were carried out at intervals throughout the experiments. No animals died during any of the protocols used.

### Lung Lavage

At the end of the protocols, mice were euthanized by anesthetic overdose and subjected to lung lavage as described previously (55). Lavage fluids recovered were centrifuged (5 min, 1,500 rpm, 4°C) and supernatants were stored at −80°C. Cell pellets were used for cell counting by hemocytometer, and differential cell analysis by Diff-Quik staining of samples was prepared by Cytospin (Shandon, Cornwall, UK).

### Protein and Chemokine Measurements

Protein concentration in lavage fluid was determined by the Bradford method (6) using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hemel Hempstead, UK) with BSA (Sigma-Aldrich, Gillingham, UK) as a standard. Macrophage inflammatory protein-2 (MIP-2) and keratinocyte-derived chemokine (KC) protein levels were measured using commercially available sandwich ELISA kits (R&D Systems, Abingdon, UK).

### Data Analysis

Data are expressed as means ± SD. Statistical analysis was carried out by t-tests or ANOVA with Scheffé’s tests. A P value of <0.05 was considered significant.

### RESULTS

#### Chronic Inhibition of TNF Signaling

**Physiological measurements.** In the low-stretch group, PIP was not affected by ventilation in either WT or DKO mice, remaining at baseline levels throughout (data not shown). In the high-stretch group, the period of high-stretch ventilation led to an increase in PIP in both WT and DKO animals (Fig. 1), and when this increased by 5–10% of the starting value, the ventilator settings were returned to the baseline low-stretch ventilation. The total length of experiments in the high-stretch group varied depending on the time required to reach this predetermined level of mechanical lung injury and was not different between the WT and DKO mice (255 ± 15 min for WT vs. 237 ± 18 min for DKO). On return to low-stretch ventilation, PIP was significantly increased compared with pre-high-stretch values (P < 0.01), with decreases in respiratory system compliance of −46 ± 4% for WT (n = 8) and −52 ± 11% for DKO (n = 7). There were no differences in these parameters between DKO and WT animals, demonstrating that a standardized level of mechanical lung injury was successfully achieved in all animals in the high-stretch group.

Mean BP was well maintained during both the low- and high-stretch protocols in both WT and DKO animals (Table 1) within the range expected for C57BL6 mice anesthetized with Hypnorm and midazolam (57), consistent with our previous

### Table 1. Blood pressure and blood gas variables in WT and TNF receptor DKO animals ventilated with high- and low-stretch protocols

<table>
<thead>
<tr>
<th></th>
<th>Baseline (Low Stretch)</th>
<th>High/Low Stretch Period</th>
<th>Return to Low Stretch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+60 min</td>
<td>+120 min</td>
</tr>
<tr>
<td><strong>WT low stretch</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>61 ± 5</td>
<td>58 ± 5</td>
<td>65 ± 4</td>
</tr>
<tr>
<td>pH</td>
<td>7.49 ± 0.08</td>
<td></td>
<td>7.44 ± 0.08</td>
</tr>
<tr>
<td>PCO2, mmHg</td>
<td>35.5 ± 6.2</td>
<td></td>
<td>36.8 ± 5.5</td>
</tr>
<tr>
<td>PO2, mmHg</td>
<td>369 ± 43</td>
<td></td>
<td>400 ± 41</td>
</tr>
<tr>
<td><strong>WT high stretch</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>63 ± 5</td>
<td>57 ± 9</td>
<td>59 ± 6</td>
</tr>
<tr>
<td>pH</td>
<td>7.49 ± 0.04</td>
<td></td>
<td>7.30 ± 0.03†</td>
</tr>
<tr>
<td>PCO2, mmHg</td>
<td>36.7 ± 5.2</td>
<td></td>
<td>56.5 ± 5.8†</td>
</tr>
<tr>
<td>PO2, mmHg</td>
<td>381 ± 38</td>
<td></td>
<td>158 ± 41†</td>
</tr>
<tr>
<td><strong>DKO low stretch</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>70 ± 9</td>
<td>65 ± 8</td>
<td>70 ± 6</td>
</tr>
<tr>
<td>pH</td>
<td>7.47 ± 0.03</td>
<td></td>
<td>7.44 ± 0.02</td>
</tr>
<tr>
<td>PCO2, mmHg</td>
<td>41.2 ± 3.4</td>
<td></td>
<td>40.8 ± 1.6</td>
</tr>
<tr>
<td>PO2, mmHg</td>
<td>382 ± 54</td>
<td></td>
<td>408 ± 59</td>
</tr>
<tr>
<td><strong>DKO high stretch</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>63 ± 6</td>
<td>54 ± 4</td>
<td>58 ± 9</td>
</tr>
<tr>
<td>pH</td>
<td>7.52 ± 0.06</td>
<td></td>
<td>7.32 ± 0.05†</td>
</tr>
<tr>
<td>PCO2, mmHg</td>
<td>34.3 ± 6.0</td>
<td></td>
<td>53.9 ± 6.7†</td>
</tr>
<tr>
<td>PO2, mmHg</td>
<td>364 ± 47</td>
<td></td>
<td>132 ± 49†</td>
</tr>
</tbody>
</table>

Values are means ± SD. N = 7–8 for all observations. Measurements show data at baseline (low stretch), at the end of the high-flow-stretch period, and 60, 120, and 180 min after return to low-stretch ventilation. *P < 0.05, †P < 0.01 vs. same mouse strain low-stretch group. BP, mean arterial blood pressure; WT, wild-type animals; DKO, double knockout animals.
observations in similar high V<sub>T</sub>-induced VILI models in mice (10, 55). Blood gas parameters were also maintained within a physiological range and were not different between WT and DKO mice throughout the experiment. The high-stretch group displayed relative hypoxemia on return to baseline low-stretch ventilation.

**Neutrophil infiltration.** The high-stretch protocol provoked a significant increase in percentage of polymorphonuclear leukocytes (PMN) as well as total number of PMN recovered in lavage fluid in WT animals compared with low-stretch ventilation (Fig. 2). However, these stretch-induced increases in both the percentage and total number of PMN were significantly attenuated in DKO compared with WT animals.

**Protein and chemokine measurements.** The high-stretch protocol led to similar increases in the lavage fluid protein concentration in both WT and DKO animals (Fig. 3A), indicating a comparable degree of lung injury and pulmonary edema. Concentrations of MIP-2 (Fig. 3B) and KC (Fig. 3C) in lavage fluid were increased by high-stretch ventilation in WT animals. DKO animals displayed similar levels of these chemokines to WT animals after high-stretch ventilation but had somewhat higher levels of KC with low-stretch ventilation. However, two-way ANOVA indicated that high-stretch ventilation did not produce statistically different chemokine responses between the two mouse strains.

**Acute Inhibition of TNF Signaling by Intratracheal Antibody**

**Physiological measurements.** The high-stretch protocol produced the expected changes in PIP and blood gases, which were not different between animals receiving intratracheal anti-TNF or isotype control antibodies, confirming a similar degree of mechanical lung injury in all animals (Table 2). Total length of ventilation was not different between the animals receiving either antibody (264 ± 20 min for isotype vs. 266 ± 15 min for anti-TNF).
Neutrophil infiltration. Both percentage and number of PMN recovered in lavage fluid at the end of the ventilatory protocol were significantly reduced in animals receiving anti-TNF antibody compared with those receiving isotype control (Fig. 4). Circulating PMN numbers in arterial blood were not reduced by the antibody administration (1.4 ± 0.0.3 × 10⁶ vs. 1.2 ± 0.6 × 10⁶ PMN/ml at the end of ventilation in isotype control (n = 4) and anti-TNF (n = 5) groups, respectively), confirming that the observed attenuation of PMN infiltration was due to a specific effect of intra-alveolar inhibition of TNF signaling on PMN migration.

Chemokine measurements. There was no difference in either MIP-2 or KC values in lavage fluid between animals receiving anti-TNF and isotype control antibodies (Fig. 5).

Acute Inhibition of TNF Signaling by Intravenous Antibody

The high-stretch protocol produced the anticipated changes in physiological parameters, as in the animals receiving intratracheal antibodies (data not shown). Intravenous administration of anti-TNF antibody had no effect on lavage PMN percentage or number recovered compared with animals receiving isotype control (Fig. 6).

DISCUSSION

In the current study, we examined the involvement of TNF in the pulmonary inflammation in response to high-stretch ventilation in the absence of underlying injury in mice. Using chronic (TNF receptor knockout animals) as well as acute (administration of anti-TNF antibodies) inhibition of TNF signaling, we demonstrated directly for the first time that neutrophil recruitment induced by pure mechanical lung stretch has a significant TNF-dependent component. Furthermore, the difference in the responses to the intratracheal vs. intravenous antibody suggests that decompartmentalization of TNF from the alveolar compartment into the circulation is not required for this neutrophil recruitment.

### Table 2. Physiological variables in animals ventilated with high lung stretch receiving intratracheal antibody

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (Pre-High Stretch)</th>
<th>Return to Low Stretch</th>
<th>Isotype control</th>
<th>Anti-TNF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+60 min</td>
<td>+120 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIP, cmH₂O</td>
<td>10.7±0.1</td>
<td>21.3±0.8</td>
<td>20.8±1.3</td>
<td>20.5±2.0</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>58±4</td>
<td>56±4</td>
<td>57±2</td>
<td>56±1</td>
</tr>
<tr>
<td>pH</td>
<td>7.45±0.02</td>
<td>7.30±0.05</td>
<td>7.32±0.05</td>
<td>7.33±0.05</td>
</tr>
<tr>
<td>P CO₂, mmHg</td>
<td>39.1±1.3</td>
<td>52.7±7.5</td>
<td>50.9±7.7</td>
<td>47.1±7.7</td>
</tr>
<tr>
<td>P O₂, mmHg</td>
<td>351±34</td>
<td>298±75</td>
<td>274±51</td>
<td>261±36</td>
</tr>
<tr>
<td>Isotype control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIP, cmH₂O</td>
<td>11.0±0.8</td>
<td>21.9±1.6</td>
<td>21.9±1.3</td>
<td>21.6±1.4</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>68±7</td>
<td>63±4</td>
<td>59±4</td>
<td>58±7</td>
</tr>
<tr>
<td>pH</td>
<td>7.44±0.02</td>
<td>7.29±0.02</td>
<td>7.30±0.03</td>
<td>7.31±0.03</td>
</tr>
<tr>
<td>P CO₂, mmHg</td>
<td>39.9±1.4</td>
<td>52.0±4.2</td>
<td>49.9±4.2</td>
<td>48.1±4.2</td>
</tr>
<tr>
<td>P O₂, mmHg</td>
<td>374±48</td>
<td>288±34</td>
<td>296±37</td>
<td>299±28</td>
</tr>
</tbody>
</table>

Values are means ± SD. N = 4–5 for all observations. PIP, peak inspiratory pressure.

Fig. 4. Percentage (A) and total number (B) of PMN recovered in lavage fluid from WT animals ventilated with high-stretch protocol and exposed to intratracheal anti-TNF or isotype control antibodies. Both PMN number and %PMN were significantly reduced by anti-TNF antibody compared with isotype control. N = 4–5 for each group. *P < 0.05, †P < 0.01 vs. isotype control.

Fig. 5. Lavage fluid concentration of MIP-2 (A) and KC (B) in WT animals ventilated with high-stretch protocol and exposed to intratracheal anti-TNF or isotype control antibodies. Anti-TNF antibody had no impact on either MIP-2 or KC concentrations. N = 5 for each observation.
ventilated with high VT for a short period of time until PEEP induces a highly reproducible lung injury. Mice were potential collapse and reopening of small airways due to zero stretch-induced pulmonary inflammation, we modified the protocol also allowed us to create a highly standardized stretch-induced pulmonary inflammation (8, 9) and a crucial step in the progression of inflammatory injury during VILI (28, 37). The protocol enabled the animals to survive long enough after this starting point of high stretch-induced lung injury, expression of bioactive TNF (which is transient and becomes undetectable in the later stages) was present in the lung vasculature was commencing (10). The protocol therefore suggests that TNF and CXC chemokines may play at least partly independent roles in mediating high stretch-induced pulmonary neutrophil recruitment, although the data taken at a single time point may not reflect overall kinetics of chemokine expression across the course of the experiment (55). It is not clear precisely what mechanisms may be involved in modulation of neutrophil recruitment by TNF, but these could include the stimulation of adhesion molecule expression on endothelial cells via NF-κB activation (19) or the actions of neutrophil attractants other than MIP-2 and KC.

In contrast to intratracheal administration, intravenous administration of the same anti-TNF antibody at the same saturation dose had no impact on alveolar neutrophil recruitment. Previous in vivo studies using preinjured lungs (7, 23) and studies using isolated perfused lungs (24, 53) have suggested that injurious ventilation promotes the release of soluble me-
diators, including TNF into the circulation. If such decompartmentalization of TNF occurs in vivo in response to high-stretch ventilation in healthy lungs, and if this plays a role in neutrophil recruitment and migration, then intravenous anti-TNF administration would have attenuated recruitment in the current study. It is still possible that intravenous antibody could work at higher doses or with different timing, as with any negative experiments using antibodies. However, our results strongly suggest that TNF signaling in response to pure mechanical stretch is largely localized within the alveolar space. The mechanism by which this localized TNF signaling transmits to the circulation, ultimately leading to substantial neutrophil sequestration, adhesion, and migration, remains to be further investigated, although enhancement of calcium influx via arachidonate has been postulated to mediate TNF signaling between the alveolar epithelium and endothelial cells (29). Our results do not exclude the possibility that decompartmentalization of TNF may play some role during mechanical ventilation of preinjured lungs, mediating systemic propagation of lung inflammation leading to multiple system organ failure (42).

The current study was designed to investigate the impact of TNF signaling in VILI by evaluating an in vivo “impulse” response of pulmonary inflammation to a highly standardized, short-duration mechanical lung injury. At the end of the protocol, few differences were observed in physiological indexes of lung injury (PIP, blood gas, protein levels in lavage fluid etc.) between WT and DKO mice or between the animals with/without anti-TNF treatment. At first glance, this observation may be interpreted to mean that TNF signaling has little clinical relevance in the development of VILI. However, this is because in acute one-hit animal VILI models, these physiological parameters are predominantly a reflection of rapidly developing, mechanically induced pulmonary edema (13, 16). We intentionally controlled the degree of initial mechanical lung injury in all animals, and once a standardized injury was produced, no further deterioration of the physiological parameters occurred with 3 h of additional low-stretch ventilation, consistent with previous reports on the reversibility of stretch-induced pulmonary edema (21). Despite the similarity in physiological indexes of injury, we found a marked difference in intra-alveolar neutrophil infiltration, an inflammatory index of lung injury, between animals with/without TNF inhibition. This implies that mechanical lung injury induced by high stretch results in a more substantial pulmonary inflammatory response in the presence of TNF signaling. The ongoing neutrophil recruitment did not lead to further deterioration of lung physiological function within the time frame of our acute model, but previous studies have demonstrated the crucial importance of neutrophil-mediated inflammation in the progression of VILI in more prolonged models (5, 28, 39). Thus it is reasonable to assume that attenuation of neutrophil recruitment due to inhibition of TNF signaling would eventually lead to improved physiological outcome of VILI in clinically relevant conditions.

To reproducibly create high stretch-induced pulmonary inflammation and VILI in mice within a relatively short experimental period, we used a VT (43–44 ml/kg) much greater than would be used in the clinical setting in humans. However, the VT employed is similar to those used in previous in vivo and ex vivo animal studies in the literature (13, 27, 38, 47) that have substantially contributed to our understanding of the mechanisms of VILI. It has also been suggested that such very high VT in healthy lungs may produce a similar degree of alveolar stretch to that observed regionally in heterogeneously injured lungs of ARDS patients receiving much lower VT (42, 47). Moreover, Soutiere and Mitzner (43) have recently demonstrated that intact mouse lungs can be temporarily inflated to pressures >60 cmH2O, relating to a VT of 60–70 ml/kg, without reaching a traditionally defined total lung capacity (i.e., plateau of the pressure volume curve) or producing apparent morphological damage. Thus attempts to directly compare the absolute values of “injurious” VT between mice and other species, particularly humans, may be misleading due to the much larger compliance of the mouse respiratory system at high lung volumes. However, principles derived should give important insight into the pathophysiology of VILI in humans.

In conclusion, we have demonstrated directly for the first time that pulmonary neutrophil recruitment in response to high-stretch ventilation in the absence of underlying injury involves a significant TNF-mediated component. Present strategies aimed at minimizing VILI in the intensive care unit consist of avoiding high VT and pressures while maintaining the lung in an open state (1). However, the optimal ventilator settings are often controversial (17, 34), and because most patients with ARDS have highly heterogeneous lung injury, overstretching and/or repetitive collapse of the alveoli are inevitable in some regions of their lungs (22, 33, 42). Alternative therapies are therefore required, and strategies to attenuate pulmonary inflammation may help to reduce VILI and improve the outcome of acute lung injury. We found in the current study that local administration of anti-TNF antibodies directly into the alveolar space attenuated high stretch-induced pulmonary inflammation, with the intravenous route apparently ineffective. Our results are consistent with the hypothesis that local, as opposed to systemic, blockade of TNF signaling may have therapeutic potential to reduce pulmonary inflammation in ventilated patients.

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REFERENCES


