Acute ventilator-induced vascular permeability and cytokine responses in isolated and in situ mouse lungs

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Yoshikawa, S., J. A. King, R. N. Lausch, A. M. Penton, F. G. Eyal, and J. C. Parker. Acute ventilator-induced vascular permeability and cytokine responses in isolated and in situ mouse lungs. J Appl Physiol 97: 2190–2199, 2004; doi:10.1152/japplphysiol.00324.2004.—To determine the influence of experimental model and strain differences on the relationship of vascular permeability to inflammatory cytokine production after high peak inflation pressure (PIP) ventilation, we used isolated perfused mouse lung and intact mouse preparations of Balb/c and B6/129 mice ventilated at high and low PIP. Filtration coefficients in isolated lungs and bronchoalveolar lavage (BAL) albumin in intact mice increased within 20–30 min after initiation of high PIP in isolated Balb/c lungs and intact Balb/c, B6/129 wild-type, and p55 and p75 tumor necrosis factor (TNF) dual-receptor null mice. In contrast, the cytokine response was delayed and variable compared with the permeability response. In isolated Balb/c lungs ventilated with 25–27 cmH2O PIP, TNF-α, interleukin (IL)-1β, IL-1α, macrophage inflammatory protein (MIP)-2, and IL-6 concentrations in perfusate were markedly increased in perfusate at 2 and 4 h, but only MIP-2 was detectable in intact Balb/c mice using the same PIP. In intact wild-type and TNF dual-receptor null mice with ventilation at 45 cmH2O PIP, the MIP-2 and IL-6 levels in BAL were significantly increased after 2 h in both groups, but there were no differences between groups in the BAL albumin and cytokine concentrations or in lung wet-to-dry weight ratios. TNF-α was not detected in BAL fluids in any group of intact mice. These results suggest that the alveolar hyperpermeability induced by high PIP ventilation occurs very rapidly and is initially independent of TNF-α participation and unlikely to depend on MIP-2 or IL-6.

METHODS

Isolated perfused lung preparation. Balb/c male mice weighing 19.2–25.3 g (n = 14; 22.5 ± 0.5 g) were anesthetized with an intraperitoneal injection of ketamine and xylazine. The trachea was cannulated, and the lungs were ventilated with a gas mixture of 20% O2–5% CO2–75% N2 using a piston-type respirator (model 683 rodent ventilator, Harvard, South Natick, MA). The tidal volume was adjusted to obtain a PIP of ∼7 cmH2O at a respiratory rate of 85 breaths/min, with a positive end-expiratory pressure (PEEP) of ∼3 cmH2O. The chest was opened by a midline incision, heparin (100 IU) was injected into the right ventricle, and sutures were placed around the pulmonary artery and aorta. Cannulas were placed in the pulmonary artery and left atrium, and pulmonary arterial, venous, and airway pressures were continuously monitored using pressure transducers (Cobe, Lakewood, CO) zeroed at the mid-lung level with
pressures recorded on a Grass model 7D polygraph. The initial 1–2 ml of perfusate, which contained residual blood cells and plasma, were discarded and not recirculated. Lungs were then perfused in situ with 4% bovine albumin in RPMI 1640 cell culture medium using a roller pump (Minipuls 2, Gilson, Middleton, WI) at a flow rate of 0.5 ml/min in a recirculating system that had a nominal system volume of 7 ml. Temperature was maintained at 37°C using a heating pad and heat lamp. These animal protocols were approved by the College of Medicine Animal Care and Use Committee and met all National Institutes of Health guidelines for care and handling of animals.

\[ K_{fc} = \frac{(dW/dt)}{dPpc} \]  

All \( K_{fc} \) values were normalized to 100 g predicted lung weight (PLW) on the basis of the ratio of lung weight to body weight (BW) in six control mice according to

\[ \text{PLW} = 0.00452 \pm 0.0003 \text{ BW} \]  

**Isolated lung protocols.** \( K_{fc} \) measurements were performed in two groups of Balb/c mice (n = 5). After a baseline isogravimetric state was obtained during ventilation at 85 breaths/min with a PIP of 10 cmH₂O, a \( K_{fc} \) measurement was performed. Mice were ventilated with a PIP of 10 cmH₂O (low-PIP group) or a PIP of 25–28 cmH₂O (high-PIP group). In the high-PIP group, ventilation rate was reduced to 65 breaths/min to prevent automatic PEEP.

The time course for cytokine production was also measured in isolated perfused lungs of Balb/c mice. After a baseline state was attained, the mice were separated into two groups of seven mice each: a high-PIP group where PIP was increased to 25–26 cmH₂O and a low-PIP group where PIP was maintained at the baseline level of 7–8 cmH₂O. Lungs were perfused for 4 h, and 0.5-ml perfusate samples were obtained from the venous reservoir every hour in the high-PIP group and every 2 h in the low-PIP group. Lungs were weighed at the end of the experiments.

**Intact animal preparations.** All intact animal ventilation protocols were also approved by the Institutional Animal Care and Use Committee of University of South Alabama. Specific pathogen-free Balb/c, B6/129J, and TNF-α receptor knockout (TNFRKO) mice (p55−/−, p75−/−) were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were anesthetized by intraperitoneal injection of pentobarbital sodium (65 mg/kg). A tracheostomy was performed, and the cannula was inserted into the trachea. Mice were ventilated by use of a rodent ventilator (model 683, Harvard) with various PIP. Anesthesia was maintained by intermittent intraperitoneal injection of pentobarbital sodium. Airway pressure was measured using a Cobe pressure transducer (Lakewood, CO), and electrocardiograph was monitored using a polygraph (model 70, Grass).

**Cytokine measurement.** Cytokines were measured using commercially available ELISA kits and plates read on an automated microtiter plate reader from Dynatech Laboratories (Chantilly, VA 20021). The following kits were obtained: mouse ELISA kits for IL-1α, IL-6, and TNF-α were obtained from Endogen (Woburn, MA); and those for IL-1β and MIP-2 were obtained from R&D Systems. Minimal detection limits for these kits were <6 pg/ml for IL-1α, <3 pg/ml for IL-1β, <15 pg/ml for IL-6, <10 pg/ml for TNF-α, and 1.5 pg/ml for MIP-2. Cytokine concentrations were expressed as picograms per milliliter.

**Statistics.** All values are expressed as means ± SE unless otherwise stated. The pressures and resistances were compared using an ANOVA with repeated measures and either a Newman-Keuls or Bonferroni posttest. A least-squares regression analysis was used where indicated. A significant difference was determined where \( P < 0.05 \).
RESULTS

Microvascular permeability and edema. Figure 1 shows the effects of high and low PIP ventilation on $K_{fc}$ in isolated perfused lungs from BALBc mice. A baseline period of ~30 min at a 10 cmH$_2$O PIP was followed by a 20-min ventilation period with either 10 cmH$_2$O PIP (low-PIP group) or 27 cmH$_2$O PIP (high-PIP group). $K_{fc}$ was unchanged by continued ventilation with 10 cmH$_2$O PIP but increased significantly by threefold after high PIP ventilation for only 20 min. Terminal lung weights increased by 83 ± 9 and 104 ± 7% relative to predicted lung weights for the low-PIP group and high-PIP group, respectively, but the weight increases were not significantly different between the high- and low-PIP groups.

A rapid increase in permeability also occurred in intact BALBc mice ventilated with approximately the same PIP as used in the isolated BALBc mouse lungs. Figure 2 shows that the BALF albumin concentration in these mice was increased by 7.3-fold after 30 min and 9-fold at 2 h compared with baseline after ventilation with respective PIP values of 25.7 ± 0.6 and 25.8 ± 0.5 cmH$_2$O. There was a trend toward increased edema with time ($P < 0.056$) because the respective lung W/D ratios were 4.44 ± 0.04, 4.53 ± 0.04, and 4.71 ± 0.10 for the respective time groups. The time groups also exhibited a trend toward an increase in red cell numbers in BALF ($P < 0.10$) of 0.95 ± 0.52, 6.3 ± 2.1, and 5.9 ± 2.3 × 10$^4$ in the respective time groups, but no significant change in BALF neutrophils was observed. Increases in edema formation and red cell extravasation suggest rapid microvascular damage with these PIP levels after only 30 min.

In preliminary experiments on B6/129 wild-type mice, ventilation with PIP of 7 cmH$_2$O at rate of 120 breaths/min for 2 h did not cause an increase in BALF albumin concentrations. In contrast, a PIP of 45 cmH$_2$O consistently produced lung injury. The use of BALF albumin as a permeability marker was validated by the highly significant relationship between BALF albumin concentration and PIP after 2 h of ventilation of intact B6/129 wild-type mice with a range of PIP values (Fig. 3). At higher tidal volumes, respiratory rate was decreased to maintain a constant minute volume.

As shown in Table 1, BALF albumin concentrations increased significantly by ~45-fold in both wild-type and TNFRKO mice ventilated for only 30 min at 45 cmH$_2$O PIP and remained elevated over 2 h. However, there were no statistical differences between high PIP-ventilated wild-type and TNFRKO groups. There were also no significant differences between BALF albumin concentrations in the low-PIP group at 2 h compared with unventilated mice.

Calb calculated from BALF and plasma concentrations also indicate a rapid permeability response to high PIP ventilation (Table 1). After an initial peak, Calb decreased in a time-dependent manner, indicating that albumin flux actually decreased with continued high PIP ventilation. However, there was no statistical difference between the wild-type B6/129 and TNFRKO groups at any time point during high PIP ventilation. Calb in the low-PIP group at 2 h was 0.06 ± 0.02 ml/h, whereas Calb in high-PIP wild-type and TNFRKO groups were 3.84 ± 0.71 and 3.89 ± 0.84 ml/h, respectively.

Lung W/D ratios also increased as a function of time in both the wild-type and TNFRKO high-PIP ventilation groups (Table 1).
1) Lung W/D ratios increased by 1.66-fold in wild-type and by 1.50-fold in TNFRKO mice after 2 h of high PIP ventilation. No significant differences were observed in lung W/D ratios between nonventilated mice and the low-PIP group after 2 h of ventilation.

**Arterial blood gases.** Table 2 summarizes the arterial blood gases obtained at 0 and 2 h. Arterial blood gases did not change significantly from baseline over the 4-h perfusion period in either the low-PIP or high-PIP groups. In the wild-type high-PIP ventilation group, BALF MIP-2 and IL-6 but not IL-1β levels were increased significantly at 2 h compared with the nonventilated controls and the low-PIP ventilated group. In the TNFRKO high-PIP group, MIP-2 and IL-6 also increased at any time period. BALF MIP-2 increased to detectable levels at 2 h only in 2 of 5 animals in this group (data not shown).

The time course of BALF cytokines measured in intact B6/129 wild-type and TNFRKO mice ventilated at 46 cmH2O PIP are shown in Fig. 5. TNF-α was not detected in BALF of low- or high-PIP groups at any time period. In the wild-type high-PIP ventilation group, BALF MIP-2 and IL-6 but not IL-1β levels were increased significantly at 2 h compared with the nonventilated controls and the low-PIP ventilated group. In the TNFRKO high-PIP group, MIP-2 and IL-6 also increased

<table>
<thead>
<tr>
<th>Time, h</th>
<th>PIP, cmH2O</th>
<th>BALF Albumin Concentration, mg/ml</th>
<th>Albumin Clearance, ml/h</th>
<th>Lung Wet-to-Dry Weight Ratio</th>
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<tr>
<td>Low PIP WT</td>
<td>0</td>
<td>12.0±1.2</td>
<td>3.73±0.71</td>
<td>4.47±0.19</td>
</tr>
<tr>
<td>2</td>
<td>13.1±1.4</td>
<td>4.21±0.81</td>
<td>4.48±0.16</td>
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<tr>
<td>High PIP WT</td>
<td>0</td>
<td>46.3±0.6</td>
<td>2.73±0.71</td>
<td>4.47±0.19</td>
</tr>
<tr>
<td>0.5</td>
<td>47.0±0.7</td>
<td>169.2±37.4*</td>
<td>10.62±2.12</td>
<td>5.63±0.57*</td>
</tr>
<tr>
<td>1</td>
<td>47.2±1.1</td>
<td>187.2±51.2*</td>
<td>5.89±1.16*</td>
<td>5.77±0.58*</td>
</tr>
<tr>
<td>2</td>
<td>48.2±1.4</td>
<td>201.0±130.7*</td>
<td>3.84±0.71*</td>
<td>7.45±0.81*</td>
</tr>
<tr>
<td>High PIP KO</td>
<td>0</td>
<td>46.3±0.9</td>
<td>4.31±0.35</td>
<td>4.21±0.13</td>
</tr>
<tr>
<td>0.5</td>
<td>47.1±0.7</td>
<td>121.4±12.2*</td>
<td>8.52±2.32</td>
<td>4.99±0.41*</td>
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<tr>
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<td>47.4±0.8</td>
<td>142.6±26.6*</td>
<td>7.47±1.38</td>
<td>5.51±0.72*</td>
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<tr>
<td>2</td>
<td>48.6±0.7</td>
<td>210.8±43.9*</td>
<td>3.88±0.84*</td>
<td>6.31±0.84*</td>
</tr>
</tbody>
</table>

Values are means ± SE. BAL, bronchoalveolar lavage; TNF, tumor necrosis factor; PIP, peak inflation pressure; WT, wild type; KO, knockout. *P < 0.05 vs. 0-h group. †P < 0.05 vs. 0.5-h group.
Both capillaries and larger vessels had a normal endothelial ultrastructure. Both type I and type II epithelial cells are present. No abnormalities were noted in the epithelial cells. Figure 7B indicates that separation of the endothelium from the basement membranes by bleb formation in small vessels (30–50 μm in diameter) occurs within 30 min after onset of high PIP ventilation. Rare subendothelial blebs are seen in the lung capillaries with few changes identified in the epithelial cells. After 1 h of high PIP ventilation (Fig. 7C), the lungs exhibit separation of the endothelium from the basement membrane (blebs) in both capillaries and larger vessels with some epithelial separation from the basement membrane. Figure 7D shows lungs from wild-type mice after 2 h of high PIP ventilation. Widespread separation of the epithelial cells from the basement membrane was evident with a rare appearance of apoptotic endothelial cells. Significant bleb formation is not observed in the lung capillaries or larger vessels.

Similar microscopic changes were observed in lungs from TNF receptor knockout mice after high PIP ventilation. Subendothelial and intracellular blebs were evident in the larger vessels, whereas the capillary endothelial cells did not show significant changes. Epithelial separation from the capillary basement membranes was also observed.

**Discussion**

There is overwhelming experimental evidence that ventilation with high pressures and volumes can cause increased microvascular permeability in both normal and impaired lungs (4, 5, 8, 10, 28). Recent clinical trials of a reducing tidal volume from 12 to 6 ml/kg during mechanical ventilation of patients with ARDS resulted in a reduction of the predicted mortality rate by 22% (4). Proinflammatory cytokines are generally elevated in patients with ARDS (18, 25), and a reduced tidal volume also reduced the cytokine levels in these patients. However, clinical trials of anti-cytokine antibodies have proved unsuccessful in reducing the morbidity and mortality of sepsis or ARDS (9), and the relationship of proinflammatory cytokines to vascular injury in the lung remains con-

**Electron microscopy.** Figure 7, A–D, shows electron micrographs of lungs from wild-type mice. The lungs from an unventilated control mouse were well preserved (Fig. 7A). Both capillaries and larger vessels had a normal endothelial ultrastructure. Both type I and type II epithelial cells are present. No abnormalities were noted in the epithelial cells. The lungs from wild-type mice ventilated at low PIP, there was a small but significant increase in BALF IL-6, but MIP-2 and IL-1 levels did not change during ventilation. The cytokine levels in serum did not change significantly with time in any group, and there were no differences observed between groups.

**Relationship of Calb to cytokines.** To determine whether there was a possible relationship between Calb and the BAL concentrations of MIP-2 and IL-6 a least squares regression analysis was performed on simultaneous measurements from each experiment. Because there were no apparent differences in MIP-2, IL-6, or IL-1β levels between the wild-type and TNFR KO mice, the data was pooled for each cytokine. Measurements were expressed as percent of maximum to allow comparison on the same scale, and a natural log transform permits linear fit of logarithmic functions. A line of best fit for regressions of Calb (CLR) on MIP-2 and IL-6 were CLR = 5.4−0.713·MIP-2, \( r = 0.52, P = 0.002, \) and CLR = 4.1−0.128·IL-6, \( r = 0.41, P = 0.019. \) Because Calb is related to actual albumin transport and reflects vascular permeability, the presence of significant negative correlations implies that increased cytokine concentrations did not enhance albumin fluxes.

**Measurement of albumin clearances.** Figure 7, E–G, shows albumin clearances as a function of BAL cytokine concentrations in individual experiments. Measurements are expressed as percent of maximum to allow comparison on the same scale, and a natural log transform permits linear fit of logarithmic functions. The lines of best fit for regressions of albumin clearances (CLR) on MIP-2 and IL-6 were CLR = 5.4−0.713·MIP-2, \( r = 0.52, P = 0.002; \) and CLR = 4.1−0.128·IL-6, \( r = 0.41, P = 0.019. \)
Some authors have proposed that the early response cytokines TNF-α and IL-1β may have a causal role in the vascular permeability increase observed in VILI (7, 46). However, the findings of the present study of a rapid onset of the vascular permeability increase in both isolated ex vivo and in vivo lung preparations, the delayed and variable cytokine responses in the different preparations and mouse strains, the lack of difference in BAL albumin responses between wild-type and TNFRKO mice, the lack of a detectable change in BAL or serum TNF-α in the intact animal models, and the negative correlation between lung Calb and BAL MIP-2 and IL-6 concentration data all argue against such a role. The proinflammatory cytokines and chemokines undoubtedly will influence the ultimate degree of injury but do not appear to initiate the permeability increase.

**Rapid onset of vascular permeability increases.** In the isolated perfused Balb/c mouse lungs, microvascular permeability (∆K̇c) increased by threefold in the high-PIP group after only 20 min of ventilation with a PIP of 27 cmH₂O compared with both the low-PIP and baseline groups. Lung vascular permeability,
as measured by BAL albumin concentration in intact Balb/c mice, also increased after only 30 min of ventilation with a similar PIP. Likewise, ventilation of intact B6/129 wild-type and TNFRKO mice for only 30 min with a somewhat higher PIP resulted in equivalent increases in BAL albumin concentration, which remained elevated for 2 h. Calculated Calb indicates that the albumin flux rate was highest at 30 min and waned significantly at 1 and 2 h of high PIP ventilation. Ventilation of B6/129 mice over a range of PIP for 2 h indicates that significant BAL albumin increases were also observed at lower PIP values (Fig. 3). Thus the presence of TNF-α receptors had no measurable effect on the permeability response to high PIP. Quantitative differences in the threshold PIP required for lung injury differ between mouse strains because lung compliance of B6/129 mice is approximately half that of Balb/c mice (44). However, the very high compliance of the chest wall in mice suggests that the susceptibility to injury of isolated mouse lungs and intact mice should be comparable (24, 44), unlike other species where the chest wall significantly limits lung volume and the potential for high PIP injury (20).

Variable cytokine response to lung overdistention. In contrast to the consistently rapid increase in lung vascular permeability measured by Kf and BAL albumin, the cytokine response to lung overdistention was delayed and highly variable. In isolated perfused Balb/c lungs ventilated at 25 cmH₂O PIP, a small but significant increase in TNF-α was observed in perfusate at 30 min. IL-1β, TNF-α, and MIP-2 were significantly increased by 1 h, and IL-1β, TNF-α, IL-6, and MIP-2 were present in high concentrations at 2 h. In the low-PIP group, TNF-α, IL-6, and MIP-2 were only increased significantly after 2 h of ventilation and were present at concentrations several times lower than present in perfusate of the high-PIP group of lungs. Our observations confirm those of von Bethmann (49), who also reported significant increases in TNF-α and IL-6 in perfusate from isolated perfused Balb/c lungs after 2 h of ventilation with a PIP of 25 cmH₂O and who also observed a small increase in cytokines at low PIP, possibly due to tissue manipulation during the isolation procedure. Tremblay et al. (45) previously observed high levels of IL-1β, TNF-α, IL-6, and MIP-2 in the BALF of isolated unperfused rat lungs ventilated at a PIP of 42–45 cmH₂O for 2 h. However, Ricard et al. (39) could not repeat these observations in isolated ischemic rat lungs ventilated under similar conditions and reported elevations of MIP-2 but not IL-1β and TNF-α. The reported increase in TNF-α after ischemia/reperfusion of isolated perfused rat lungs suggest that an added insult of ischemia may contribute to the cytokine response of isolated lungs (22).

Unlike the isolated lung preparations, the intact mice ventilated with high PIP had no detectable levels of TNF-α in BALF and no significant increase in TNF-α in serum in either Balb/c or B6/129 mouse strains. Two previous studies in intact rats ventilated at a PIP of 45 cmH₂O also failed to detect elevated TNF-α in BALF (39, 47). The lack of increases in TNF-α and IL-1β levels in BALF and serum in this study is unlikely due to lack of sensitivity in the ELISA because TNF-α was detectable in serum and BALF when intraperitoneal lipopolysaccharide was administered (data not shown). In addition, minimal detection limits were <6 pg/ml for IL-1α, <3 pg/ml for IL-1β, <15 pg/ml for IL-6, <10 pg/ml for TNF-α, and <1.5 pg/ml for MIP-2. Ricard et al. (39) found negligible TNF-α and IL-1β in BALF of intact rats ventilated at high tidal volumes.
despite an increase in microvascular permeability that resulted in a 47-fold increase in BALF total protein concentration. We show here that high PIP ventilation of intact mice not only is not associated with lung production of TNF-α and IL-1β but that binding of TNF-α to both p55 and p75 receptor types is not necessary for the acute vascular permeability increase. The distinction between ventilation-associated cytokine production and receptor-mediated effects is significant because Xavier et al. (52) demonstrated that only minute amounts (<0.5 ng/ml) of TNF-α were required to stimulate the release of MIP-2 from alveolar epithelial cells. In the present study, BALF albumin concentration increased 46-fold after 2 h of ventilation with 42 cmH₂O PIP with or without the presence of TNF-α receptors. Kuebler et al. (23) observed that a Ca²⁺ signal induced by application of TNF-α to the alveolar space was communicated via gap junction between lung epithelial and endothelial cells in intact lungs. Thus the effect of TNF-α produced anywhere in the lung would be rapidly spread between lung cells. A lack of receptors would preclude the possibility of low but effective local concentrations of TNF-α that could not be detected after dilution in the BALF. The observed increase in MIP-2 in BALF after 2 h of high PIP ventilation also was not affected by the presence or absence of TNF-α receptors. In patients, 1 h of high tidal volume ventilation did not cause significant changes of TNF-α levels in the systemic circulation, and blockade of TNF-α with antibody receptor ligand domains did not increase survival in sepsis (13, 51).

Many of the previous studies implicating TNF-α in VILI involve ventilation after preexisting injuries. In rats with preexisting lung injury due to intratracheal hydrochloric acid instillation or cecal ligation with perforation that were ventilated with high volumes, progressive increases in BALF and serum TNF-α and MIP-2 levels were reported (6, 26). Application of TNF-α to monolayers of endothelial cells from human umbilical vein (12) or bovine pulmonary artery (35) consistently produced increases in monolayer permeability, primarily attributed to binding of the p55 TNF receptor (12). In contrast, infusion of TNF-α into intact animals or isolated, perfused lungs without preexisting injury either does (16) or does not produce increases in lung vascular permeability (2, 22), but intratracheal instillation of anti-TNF-α antibodies attenuated high PIP injury in surfactant-depleted rabbits (21). Therefore, the mechanical trauma and ischemia during the isolation procedure for isolated lung preparations may also constitute a preexisting injury that primes lung cells to produce the acute response cytokines TNF-α and IL-1β and account for the similarities of the cytokine profiles between isolated lung studies and preexisting injury studies subsequently ventilated with high lung volumes. Thus mechanical ventilation and these insults may activate common pathways for priming inflammatory cells and endothelium as suggested in the “two-hit” hypothesis of lung injury (8).

MIP-2, the murine analog of IL-8 in humans, was significantly increased after 2 h of ventilation with 42 cmH₂O PIP in our intact wild-type and TNFRKO mouse models but only marginally detected in intact Balb/c mice ventilated at 25 cmH₂O PIP. This potent chemoattractant for neutrophils is the most consistently reported cytokine after mechanical stress in isolated and intact lung studies and cultured monolayers of alveolar epithelial cells, fetal lung cells, and lung endothelial cells (17, 36–38). The importance of this cytokine in VILI is emphasized by the attenuated injury in CXC receptor knockout mice and after neutrophil depletion (1). However, neutrophils probably augment the lung injury with inflammation after the initial vascular fluid leak. Yoshikawa et al. (54) reported that increases in BAL albumin and Clara cell secretory protein preceded microscopic evidence of the tissue injury and inflammation in a similar mouse model of VILI. The time course for lung injury and inflammation that we observed was similar to that previously reported by Quinn et al. (37) for rats ventilated for 2 h at 20 ml/kg. Although lung W/D ratios significantly increased, BAL MIP-2 and neutrophil counts did not change significantly at 2 h. Only after waiting an additional 6 h after ventilation were the BAL MIP-2 increased by fourfold and the neutrophil counts increased significantly over the low-volume group. Lung MPO was not elevated after 2 h of ventilation in their study but increased significantly after the additional 6-h wait. Likewise, Belperio et al. (1) only observed attenuation of VILI using CXC receptor knockout mice and CXC receptor antibodies after 6 h of high-volume ventilation after there was significant histological evidence of tissue destruction and edema. We found that there was actually a significant negative correlation of normalized Calb with normalized BAL MIP-2 and IL-6 concentration. We do not infer that these inflammatory cytokines inhibit albumin flux rates but rather that elevated concentrations of these cytokines are unlikely to cause the initial increases in Calb that were measured. Because we did not detect BAL TNF-α in any of the intact mouse models and the absence of TNF-α receptors did not affect susceptibility to lung injury, we can conclude that these cytokines are unlikely to induce the acute permeability response reported here. The full-blown inflammatory response takes time to develop, and we would expect further increases in permeability and tissue injury with neutrophil infiltration during longer ventilation times.

These studies suggest that the acute proinflammatory cytokines do not play a major role in the initial vascular leak of fluid and protein in the absence of preexisting injury or inflammation. Rather, the initial vascular leak probably results from acute signaling events such as calcium entry and phosphorylation of focal adhesion, and junctional and cytoskeletal proteins that alter endothelial contraction and tethering (15). Protein leak and edema may then cause release of growth factors, proteases, arachidonic acid derivatives, and other mediators that facilitate neutrophil influx and activation. A consequence of such a sequence of events would infer that pharmacological blockade of initial signaling events may be more effective in preventing mechanical injury than immunologic interventions targeted at specific proinflammatory cytokines after inflammation is well established.

GRANTS

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REFERENCES


