Time and pressure dependence of transvascular Clara cell protein, albumin, and IgG transport during ventilator-induced lung injury in mice

Sawako Yoshikawa,1,3 Judy A. King,2,3 Susan D. Reynolds,4 Barry R. Stripp,4 and James C. Parker1,3

Departments of 1Physiology and 2Pathology and 3Center for Lung Biology, College of Medicine, University of South Alabama, Mobile, Alabama 36688; and 4Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Submitted 21 August 2003; accepted in final form 8 November 2003

Yoshikawa, Sawako, Judy A. King, Susan D. Reynolds, Barry R. Stripp, and James C. Parker. Time and pressure dependence of transvascular Clara cell protein, albumin, and IgG transport during ventilator-induced lung injury in mice. Am J Physiol Lung Cell Mol Physiol 286: L604–L612, 2004; 10.1152/ajplung.00283.2003.—We compared the transport of three proteins with different hydrodynamic radii with ultrastructural changes in lungs of intact mice ventilated at peak inflation pressures (PIP) of 15, 35, 45, and 55 cmH2O for 2 h and PIP of 55 cmH2O for 0.5 and 1 h. After 2 h of ventilation, significant increases were observed in plasma Clara cell secretory protein (1.9 nm radius) at 35 cmH2O PIP and in bronchoalveolar lavage fluid albumin (3.6 nm radius) at 45 cmH2O PIP and IgG (5.6 nm radius) at 55 cmH2O PIP. Increased concentrations of all three proteins and lung wet-to-dry weight ratios were significantly correlated with PIP and ventilation time. Clara cell secretory protein and albumin increased significantly after 0.5 h of 55 cmH2O PIP, but IgG increased only after 2 h. Separation of endothelium or epithelium to form blebs was apparent only in small vessels (15–30 μm diameter) at 45 cmH2O PIP and after 0.5 h at 55 cmH2O PIP but became extensive after 2 h of ventilation at 55 cmH2O PIP. Junctional gaps between cells were rarely observed. Ultrastructural lung injury and protein clearances across the air-blood barrier were related to ventilation time and PIP levels. Protein clearances increased in relation to molecular size, consistent with increasing dimensions and frequency of transmembrane aqueous pathways.

ventilator-induced lung injury (VILI) has been recognized as a significant contributing factor to the morbidity and mortality of patients with acute respiratory distress syndrome (6). Previous studies have noted that high airway pressures and lung volumes can increase pulmonary endothelial and epithelial permeability (8). Although preventative strategies and risk factors for VILI are under investigation (8, 10, 25), there is no indicator of acute lung injury after exposure to lipopolysaccharide (2), ozone (1, 5), high-volume ventilation (21), and arachis oil and α-naphthylthiocarbamide (17). However, the pattern of CCSP fluxes from lung to blood in relation to ventilation times and peak inflation pressures (PIP), as well as larger protein markers of permeability and lung edema, has not been systematically studied.

In the present study, we measured accumulation of three proteins of known hydrodynamic radii in response to ventilation for 2 h with a progressive increase in PIP and as a function of time during ventilation with a PIP observed to produce lung injury. We assessed vascular permeability by measuring changes in CCSP concentration in plasma as well as albumin and IgG in BALF. Because of the differences in molecular radii of CCSP (1.9 nm), albumin (3.6 nm), and IgG (5.6 nm), larger-diameter aqueous pathways would be required for exchange of IgG than albumin and for exchange of albumin than CCSP between blood and alveolar fluid. The clearance of each protein was calculated as an indicator of transvascular flux in relation to molecular size.
each group. We also used least-squares regression analysis after graded injuries with different PIP values and time periods to determine relations between plasma CCSP concentrations, BALF levels of albumin, IgG, and total protein, and lung wet-to-dry weight ratios. Transmission electron microscopy was used to assess structural changes in each group. We observed increases in protein transport related to molecular radii and ultrastructural changes in proportion to PIP levels and longer ventilation times. Increases in plasma CCSP concentration occurred at the lowest PIP and shortest ventilation time, whereas increases in IgG fluxes occurred at the highest PIP and longest ventilation time.

METHODS

Animal preparation. All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of South Alabama. Specific pathogen-free C57BL/6j mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice weighing 20.0–26.7 g (23.3 ± 0.3 g) were anesthetized with pentobarbitonal sodium (65 mg/kg), and a tracheostomy was performed. Then the cannula was inserted into the trachea, and mice were ventilated using a rodent ventilator (model 683, Harvard, South Natick, MA). Airway pressure was measured using a Cobe pressure transducer (Lakewood, CO), and an electrocardiogram was monitored using a polygraph (model 70, Grass, Quincy, MA).

Experimental protocol. Mice were ventilated with PIP of 15 cmH2O (n = 3), 35 cmH2O (n = 3), 45 cmH2O (n = 3), and 55 cmH2O (n = 7) with 2.5 cmH2O positive end-expiratory pressure (PEEP) for 2 h. Constant pH and PacO2 values were maintained at the tidal volume required to produce these PIP values by adjustment of the respiratory rates between groups (Table 1). A control group (n = 6) was ventilated only immediately after tracheal intubation for a very brief time during blood sampling. After the ventilation period, blood was sampled by cardiac puncture for gas analysis and measurement of CCSP, IgG, and albumin. Separate groups were ventilated with 55 cmH2O PIP for 30 min (n = 3) and 1 h (n = 3) to establish a time course for injury.

BAL. After collection of blood samples, the right lung and the lower left lung lobe were lavaged three times with 0.5 ml of phosphate-buffered saline.

Albumin and IgG concentrations in BALF and serum. Albumin and IgG concentrations were measured in BALF and serum using an ELISA kit (Bethyl, Montgomery, TX).

Total protein concentration. Total protein in BALF was assayed using the modified method of Bradford with bovine serum albumin as a standard.

CCSP. CCSP was assayed in BALF, plasma, and lung homogenate. After lavage, the right and left lower lobes were homogenized in 1 ml of RIPA buffer and centrifuged at 4°C to remove debris. CCSP was measured in BALF, plasma, and lung supernatant by an ELISA based on an anti-CCSP antibody previously described (28, 29).

Protein clearances. Changes in protein clearance from plasma to BALF produced by ventilation were calculated for albumin and IgG as follows

\[
V_{\text{BAL}} = \frac{(C_{\text{BAL,Vent}} - C_{\text{BAL,Unvent}})}{(C_p - t)}
\]

where \(V_{\text{BAL}}\) is BAL volume, \(C_{\text{BAL,Vent}}\) and \(C_{\text{BAL,Unvent}}\) are BAL concentrations in ventilated and unventilated control mice, \(C_p\) is plasma concentration, and \(t\) is ventilation period in hours.

Changes in protein CCSP clearance from airway fluid to plasma produced by ventilation were calculated as follows

\[
V_{p} = \frac{(C_{\text{P,Vent}} - C_{\text{P,Unvent}})}{(C_{\text{P,Vent}} - 1.800) \times t}
\]

where \(V_{p}\) is plasma volume estimated as 0.043 × body weight, \(C_{\text{P,Vent}}\) and \(C_{\text{P,Unvent}}\) are plasma concentrations in ventilated and unventilated control mice, \(C_{\text{P,Vent}}\) * 1,800 is the airway fluid concentration estimated to be diluted 1,800 times in the BAL (16), and \(t\) is the ventilation period in hours.

Table 1  Conditions of ventilation and blood gas analysis

<table>
<thead>
<tr>
<th>PIP, cmH2O</th>
<th>Rate, min⁻¹</th>
<th>pH</th>
<th>PacO₂, Torr</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>16.5</td>
<td>7.31 ± 0.04</td>
<td>33.1 ± 2.9</td>
<td>7</td>
</tr>
<tr>
<td>45</td>
<td>20</td>
<td>7.33 ± 0.03</td>
<td>32.0 ± 4.9</td>
<td>3</td>
</tr>
<tr>
<td>35</td>
<td>30</td>
<td>7.36 ± 0.5</td>
<td>35.6 ± 4.8</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>120</td>
<td>7.35 ± 0.01</td>
<td>35.0 ± 2.9</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n\), number of mice. PIP, peak inflation pressure.

RESULTS

High-PIP ventilation increases edema formation and vascular permeability. Figure 1 shows the effects of 2 h of ventilation with 15, 35, 45, or 55 cmH2O PIP on BALF albumin, total protein, and IgG concentrations as well as lung wet-to-dry weight ratios. Ventilation for 2 h with 15 cmH2O PIP did not increase any of these variables compared with the control group. However, BALF albumin concentration and wet-to-dry lung weight ratio increased only at 55 cmH2O PIP in a PIP-dependent manner, whereas BALF IgG and total protein concentrations were increased significantly at 45 and 55 cmH2O PIP in a PIP-dependent manner, whereas BALF IgG and total protein concentrations were increased significantly only at 55 cmH2O PIP compared with the control group. Regression analysis of individual experimental data points with PIP produced significant correlations for BALF albumin (\(P = 0.0001, r^2 = 0.89\)), total protein (\(P = 0.0001, r^2 = 0.71\)), and IgG (\(P = 0.003, r^2 = 0.48\)) concentrations as well as lung wet-to-dry weight ratios (\(P = 0.0001, r^2 = 0.71\)), suggesting a greater leak of fluid and protein into the air spaces as PIP was increased.
The relation of BALF protein concentration to edema formation is shown in Fig. 2. BALF albumin, total protein, and IgG concentrations significantly correlated with lung wet-to-dry weight ratios. These correlations suggest that convective coupling of protein transport to an increased fluid filtration across the alveolar-capillary barrier was a mechanism for protein movement into the airway fluid after mechanical injury to the lung.

**Effect of PIP on CCSP in BALF, lung homogenate, and plasma.** BALF CCSP concentrations were significantly decreased after ventilation for 2 h with 15 cmH₂O PIP, even though concentrations of BALF CCSP at 45 and 55 cmH₂O PIP were not significantly different from control values (Fig. 3A). Lung homogenate CCSP content (Fig. 3C) normalized to 1 mg of protein was not significantly different from control after ventilation with 15 and 55 cmH₂O PIP. Unfortunately,
samples for the measurement of BALF CCSP at 35 cmH₂O PIP and lung homogenate samples for the 35 and 55 cmH₂O PIP groups were lost. Plasma CCSP concentrations (Fig. 3B) increased in a PIP-dependent manner, with significant increases in plasma CCSP levels after ventilation with 35 cmH₂O PIP, the lowest PIP for any of the proteins. There was also a significant correlation of plasma CCSP with PIP (Fig. 3D) for individual data points ($r^2 = 0.69$). In addition, no significant differences were observed for CCSP mRNA in lung homogenate between PIP groups using a nuclease protection assay (data not shown).

**Time course of BALF protein concentrations and edema formation.** Figure 4 shows the effects of ventilation with 55 cmH₂O PIP for 0.5, 1, and 2 h on BALF albumin, IgG, and total protein concentrations and lung wet-to-dry weight ratios. All variables increased significantly as a function of time during high-PIP ventilation. BALF albumin and total protein concentrations increased significantly compared with control after only 0.5 h of ventilation, whereas BALF IgG concentrations and wet-to-dry lung weight ratios increased significantly only after 2 h of 55 cmH₂O PIP.

**Fig. 3.** Effect of 2 h of ventilation with graded increases in PIP on Clara cell secretory protein (CCSP) in BALF (A), plasma (B), and lung homogenate (C). D: correlation of individual data points between plasma CCSP and PIP. Lost samples account for missing data in A and C. *$P < 0.01$ vs. control mice.

**Fig. 4.** Time course of BALF albumin (A), total protein (B), and IgG (C) concentrations and lung wet-to-dry weight ratios (D) with 0.5–2 h of ventilation at 55 cmH₂O PIP. *$P < 0.01$ vs. control mice (ventilation period = 0 h).
Time course of plasma and BALF CCSP during ventilation.
Figure 5 shows the time course of CCSP in plasma and BALF in mice after 0, 0.5, 1, and 2 h of ventilation with 55 cmH2O PIP. A significant increase in plasma CCSP levels was observed at 0.5 h, and these levels remained constant for up to 2 h and did not increase progressively with time. BALF CCSP concentrations did not change significantly relative to control over the 2-h ventilation period.

Correlations of plasma CCSP with BALF proteins and lung edema.
When the plasma CCSP measurements from individual experiments were tested as a dependent variable against other indexes of lung injury (Fig. 6), significant correlations were observed with BALF albumin, total protein, and lung wet-to-dry weight ratios, but not with BALF IgG concentrations. The highest correlation was obtained between plasma CCSP and lung wet-to-dry weight ratio ($r^2 = 0.57$), which suggests a graded increase in CCSP with greater degrees of edema and injury.

Time and PIP dependence of protein clearances.
Changes in protein clearance from baseline unventilated control levels as a function of time and PIP are shown in Fig. 7. Clearances are shown as percentage of maximum mean values to compare all proteins on the same scale. Maximal clearances calculated were $2.08 \pm 0.36$, $0.053 \pm 0.012$, and $0.105 \pm 0.008 \mu l/h$ for albumin, IgG, and CCSP, respectively. Figure 7A shows protein clearances as a function of PIP after 2 h of ventilation. CCSP increased progressively even at 15 cmH2O PIP to a maximum at 45 cmH2O PIP, whereas clearance was maximal at 55 cmH2O PIP for albumin and IgG. BALF CCSP concentration in mice ventilated at 35 cmH2O PIP was assumed to equal that in mice ventilated at 45 cmH2O PIP for these calculations, because BALF CCSP concentrations did not change markedly between PIP groups. Figure 7B shows the time course of the changes in clearance for the three proteins across the alveolar-capillary barrier in the lung during ventilation with 55 cmH2O PIP. CCSP was cleared at a maximal rate at 0.5 h and then subsequently declined, albumin clearance was near maximal at all time periods, and IgG clearance increased progressively to a maximum at 2 h.

Ultrastructure of lung injury.
Figure 8 shows transmission electron micrographs of lungs from mice ventilated at the
different PIP levels and different time periods. In general, the Clara cells did not show obvious damage at any of the pressures or time periods investigated. Vessels 15–30 μm diameter were the structures affected first and the structures exhibiting the most severe injury. Capillaries were affected later and less severely, but in all cases the pattern of injury was heterogeneous, with apparently normal areas, even in the most severely injured group. Injuries consisted of endothelium and epithelium that were torn to form blebs and basement membrane that was disorganized or pulled apart. Some artifactual spaces were present because of loss of mitochondria and surfactant granules from type II cells during ventilation with 55 cmH₂O PIP. The Clara cells to alveolar capillaries. Blebs were not apparent filled inclusions in the Clara cells and the proximity of surfactant granules from type II cells during ventilation with 55 cmH₂O PIP. The next largest protein (3.6-nm radius), increased significantly in BALF at 45 cmH₂O PIP and also after only 0.5 h of ventilation with 55 cmH₂O PIP, whereas IgG, the largest molecule (5.6-nm radius), increased significantly only after 2 h of ventilation at the highest PIP of 55 cmH₂O. Clearance rate, which represents the actual rate of flux across the alveolar capillary barrier, suggested an increased flux in CCSP during ventilation with only 15 cmH₂O PIP compared with unventilated controls. Albumin clearances increased progressively with time and PIP, whereas IgG clearance increased only at 55 cmH₂O PIP after 2 h. This progressive loss of size-related selectivity suggests a progressive increase in diameter of fluid-filled pathways to reduce protein sieving. The significant correlation of BALF albumin, total protein, and IgG with lung wet-to-dry weight ratios suggests a filtration coupling of protein transport with edema formation, rather than protein transport at a fixed rate by transcytosis (26). The transfer of CCSP against a filtration gradient could be attributed to active transport but, more likely, represents passive diffusion down the large CCSP concentration gradient as pore dimensions increase with injury and pore surface area increases. The excellent correlation of BALF CCSP with wet-to-dry weight ratios and BALF albumin concentration would support this idea.

Early studies by Taylor and Gaar (31) reported equivalent pore radii of 0.6–1.0 nm for alveolar epithelium and 4.0–5.8 nm for the capillary endothelial barrier. Later studies of lung epithelium indicated the presence of infrequent larger pores of 3.4-nm radius for passage of macromolecules (3). Although active vesicular transport of protein across epithelium may contribute to transport (20), the size and concentration dependence of endogenous proteins in BALF and the size-selective clearance of solutes from the alveolar spaces reported in most

DISCUSSION

Transfer of proteins across the continuous capillaries and epithelium can occur by passive diffusion down a concentration gradient and convective coupling to filtration through fluid-filled pathways at the cell junctions or through cells via continuous pathways created by fused vesicles (22, 32). Active protein transport across epithelium and endothelium has also been proposed on the basis of observations of albumin-binding proteins in caveoli and uptake of albumin by these cell types (20, 23). However, size selectivity changes and filtration dependence of protein transport in most lung injury models suggest that active vesicular transport does not contribute significantly to the increased protein transport during injury (26). In the present study, the smallest protein molecule (CCSP, 1.9-nm radius) increased significantly in plasma at the lowest PIP (35 cmH₂O) and at the earliest time period (0.5 h) during ventilation with 55 cmH₂O PIP. Albumin, the next largest protein (3.6-nm radius), increased significantly in BALF at 45 cmH₂O PIP and after only 0.5 h of ventilation with 55 cmH₂O PIP, whereas IgG, the largest molecule (5.6-nm radius), increased significantly only after 2 h of ventilation at the highest PIP of 55 cmH₂O. The excellent correlation of BALF albumin, total protein, and IgG with lung wet-to-dry weight ratios suggests a filtration coupling of protein transport with edema formation, rather than protein transport at a fixed rate by transcytosis (26). The transfer of CCSP against a filtration gradient could be attributed to active transport but, more likely, represents passive diffusion down the large CCSP concentration gradient as pore dimensions increase with injury and pore surface area increases. The excellent correlation of BALF CCSP with wet-to-dry weight ratios and BALF albumin concentration would support this idea.
Fig. 8: A: small (≈15-μm-diameter) vessel with an edema cuff from group subjected to 2 h of ventilation at 15 cmH₂O PIP. V, vessel; AS, alveolar space; PE, perivascular edema cuff. Magnification ×1,900. B: small bronchiole lined with Clara cells (CC) adjacent to 2 alveoli (AS) from group subjected to 2 h of ventilation at 35 cmH₂O PIP. Note CCSP-filled inclusions in Clara cells and close proximity of Clara cells to alveolar capillaries (arrows). Magnification ×900. C: 15-μm-diameter lung vessel from group subjected to 2 h of ventilation at 45 cmH₂O PIP. Note minimal widening of basement membrane and no apparent bleb formation. WBC, polymorphonuclear leukocyte; RBC, red blood cell; BM, basement membrane; AS, alveolar space. Magnification ×2,750. D: small lung vessel (V) from group subjected to 2 h of ventilation at 55 cmH₂O PIP. Note extensive bleb formation (+); basement membrane surrounds nearly the total circumference of the vessel. Magnification ×1,900. E: higher magnification of a small vessel (V) and alveolar space (AS) from group subjected to 2 h of ventilation at 55 cmH₂O PIP. Note blebs (+) created by separation of endothelium and epithelium from a widened basement membrane (BM), with apparent separation of basement membrane layers. Magnification ×7,000. F: capillary (CAP) from group subjected to 2 h of ventilation at 55 cmH₂O PIP. Note extensive blebbing (+) and widening of basement membrane. Magnification ×1,900. G: effect of 30 min of ventilation with 55 cmH₂O PIP on a small (≈15-μm-diameter) vessel (V). Note limited regions of blebbing (+) and alveolar space (AS). Magnification ×1,900. H: endothelial blebbing (+) in a small (≈15-μm-diameter) vessel (V) after 1 h of ventilation with 55 cmH₂O PIP. Note relatively little injury to an adjacent capillary filled by the red cell. Magnification ×1,900.
studies suggest that paracellular pathways are the major transport route for proteins entering and leaving the alveolar compartment (16). Because the molecular radius of CCSP is ~1.9 nm (19), restriction of this small molecule by the epithelium would be easily lost at an early phase of lung injury. Égan (11) demonstrated a progressive increase in epithelial equivalent pore radius with lung overinflation. An equivalent pore radius of 2.0 nm occurred at 82% of vital capacity, and unrestrictive leaks occurred at 100% vital capacity. Thus CCSP would leak very rapidly as the lung was distended to total lung capacity. This was confirmed by the significant increases in plasma CCSP at the lowest PIP. The sensitivity of CCSP transport to injury was demonstrated by Broeckaert et al. (5), who observed that plasma CCSP increased in mice and humans breathing ozone levels below the permissible air quality standards, which did not cause respiratory distress. In patients with lung failure, plasma CCSP also was strongly inversely correlated to the arterial PO2-to-inspired O2 fraction ratio but less correlated to the histological indexes of severe lung injury, suggesting leak of CCSP before inflammatory processes sufficient to produce significant tissue disruption (9). In the present study, CCSP transport also occurred before microscopic evidence of injury. Because most macromolecules <40 kDa are believed to cross the air-blood barrier mainly by passive diffusion through paracellular pathways (7), the concentration gradient of CCSP between the epithelium lining fluid and blood of ~10,000 times provides a large driving force for diffusion (16). Paracellular epithelial pores sufficiently large to allow passage of CCSP (1.6-nm radius) would not allow passage of albumin or IgG, whereas pathways that permitted passage of albumin (3.6-nm radius) would not allow passage of significant amounts of IgG (5.6-nm radius). Thus significant transport of IgG occurred only at the highest PIP (55 cmH2O).

The bidirectional flux of proteins across the air-blood barrier, even in normal lungs, is indicated by the presence of trace amounts of serum albumin and IgG in BALF and of CCSP in plasma (12, 14). Because the concentration of CCSP in plasma reflects a balance between the rate of synthesis and/or CCSP leak from the air spaces and the rate of clearance from the circulation, it can be affected by glomerular filtration rate (9, 15). It is possible that high PIP ventilation might contribute to the increase in plasma CCSP levels by a reduced renal clearance if systemic blood pressure and cardiac output were significantly decreased by an increased intrathoracic pressure. However, a progressive increase in plasma CCSP concentrations with time would be expected with renal failure, and this was not observed over the time course of these experiments, even in mice ventilated with 55 cmH2O PIP.

We can exclude an increased secretion of CCSP into the air space at high PIP ventilation as a probable cause of the increase in plasma CCSP, because BALF CCSP concentrations were significantly lower in mice ventilated with 15 cmH2O PIP than in control mice, and BALF CCSP concentrations returned to control values in groups ventilated using higher PIP values. Because neither CCSP protein nor mRNA levels in lung homogenate changed as PIP increased, it is unlikely that the increase in plasma CCSP concentration is the consequence of increased synthesis of CCSP by the Clara cell. Also, there was no apparent ultrastructural injury to Clara cells in the present study. Lesur et al. (21) reported a decrease in BALF CCSP in rats during high-tidal-volume ventilation proportional to tidal volume that was suggestive of Clara cell injury. The use of 2.5 cmH2O PEEP in our experiments may have minimized the small airway injury and prevented a decrease in BALF CCSP (24).

Increases in plasma CCSP preceded any ultrastructural indication of vessel injury, because no ultrastructural evidence of endothelial and epithelial injury was observed at 15 and 35 cmH2O PIP. Injury became apparent as occasional bleb formation after 2 h of ventilation at 45 cmH2O PIP, and this was accompanied by significant increases in BALF albumin. The ultrastructural evidence of injury was greater after 0.5 h of ventilation at 55 cmH2O PIP than after 2 h at 45 cmH2O PIP, and BALF albumin was significantly increased by about the same amount in both groups. Increases in BALF IgG occurred only after 2 h of ventilation at 55 cmH2O PIP, which was associated with extensive endothelial blebbing of small (15- to 30-μm-diameter) vessels and separation of endothelium and epithelium with widening of basement membranes in the alveolar capillaries. Muscedere et al. (24) observed significant histological evidence of bronchiolar injury in rats ventilated without PEEP, but we did not observe significant small airway injury to the epithelium in our mice ventilated with 2.5 cmH2O PEEP. Although the extent of ultrastructural changes was related to PIP and time of ventilation, the exact pathway for movement of the proteins across the air-blood barrier could not be deduced. No obvious endothelial gaps were observed in the samples examined. The small plasma membrane breaks reported by Fu et al. (13) at high airway pressures were also not observed in our study. However, separation of endothelium from the basement membrane suggests that the cell junctions may be stronger than the focal adhesions binding endothelial cells to the basement membranes.

In summary, VILI in mice produced by high-PIP ventilation increased plasma CCSP levels, BALF albumin, IgG, and total protein, and lung edema formation in a time- and pressure-dependent manner. The smallest protein, CCSP, increased in plasma at the lowest PIP and earliest sampling period; the largest protein, IgG, increased significantly only at the highest PIP and the longest ventilation time of 2 h. The surface area of separated endothelium and epithelium in small vessels and capillaries was related to ventilation time and PIP levels and protein clearances, but plasma CCSP levels increased before any ultrastructural evidence of vascular injury. Therefore, plasma CCSP levels would be useful for early detection of injury and quantification of mild-to-moderate injury, BALF albumin concentration increases would be useful for detection of progressive development of injury, and BALF IgG would increase only during severe injury when obvious histological evidence of tissue destruction is present.

ACKNOWLEDGMENTS

The authors thank Anna Penton and Freida McDonald for technical assistance.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grant P01 HL-66299 and the Lung Biology Center.

REFERENCES


