Vascular endothelial cells actively participate in high inflation pressure-induced permeability and edema

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TRADITIONAL THERAPY FOR PATIENTS with acute lung injury and the acute respiratory distress syndrome (ARDS) utilized mechanical ventilation with tidal volumes in the range of 10–15 ml/kg body wt coupled with high inflation pressure to adequately maintain blood gases in lungs with low compliance and many atelectatic and edema-filled alveoli. For years, clinicians and experimental investigators have been concerned that the remaining open alveoli in ARDS patients may be overinflated and damaged by ventilation with high pressure. A number of investigators have reported increases in transvascular protein flux and fluid filtration, damaged endothelium and alveolar epithelium, interstitial and alveolar edema, severe hypoxemia, decreased dynamic compliance, hyaline membranes, and increased mortality after subjecting animal lungs to inflation pressures ranging from 20 to 45 cmH₂O (4, 13, 17). In the year 2000, a multicenter, randomized clinical trial spearheaded by the recently formed ARDS network discovered early on in the trial that a reduction in tidal volume from 12 ml/kg body wt (with an end-inspiration pressure of 50 cmH₂O or less) to 6 ml/kg body wt (with <30-cmH₂O end-inspiratory pressure) was beneficial to patients with acute lung injury and ARDS (2). This reduction in tidal volume and end-inspiratory pressure led to a decrease in mortality and an increase in the number of days without ventilator use. As the result of this study, it is now highly recommended that ARDS patients be ventilated at lower tidal volumes.

Although ventilator-induced lung injury may become a nonissue in the near future, understanding the mechanism of this injury will further our knowledge of the barrier function of the vascular endothelium derived from microvessels and large, conduit vessels. Initially, ventilator-induced lung injury was ascribed to increases in transvascular protein and water fluxes, indicating a permeability defect in the vascular and alveolar barriers (13). Morphological evidence of damage to the vascular endothelium as well as alveolar epithelium was demonstrated microscopically by separation of the endothelium from the basement membrane within 5 min of high pressure and by destruction of the alveolar epithelium after 20 min of high pressure (4). The ensuing interstitial then alveolar edema was attributed to these mechanical disruptions in the alveolar capillary barrier mediated by overstretching of the lung and pulmonary blood vessels. The vascular endothelium was, therefore, considered a passive partner in this high pressure-induced injury.

In two studies involving high inflation pressure-induced pulmonary injury, Parker and co-workers (11, 12) brought forth the intriguing notion that the vascular endothelium actively and not passively participates in the loss of barrier function. Furthermore, this active process appears to require signaling pathways involving calcium and tyrosine phosphorylation. Gadolinium chloride, a trivalent lanthanide used as an inhibitor of stretch-activated cation channels, prevented the increase in the capillary filtration coefficient (Kf) induced by high peak inflation pressure. This finding suggests a role for calcium entry via mechanogated channels, as has been proposed for ligand stimulation, in the regulation of endothelial barrier function. However, the role of calcium signaling in the pulmonary microvasculature, which comprises most of the vascular surface area of the lung, is under question. Endothelial cell monolayers derived from the pulmonary microvasculature are tighter with regard to the passage of protein, respond differently to inflammatory mediators, and, most importantly, have a diminished response to changes in intracellular calcium compared with cell monolayers derived from conduit vessels such as the pulmonary artery. For example, an elevation in intracellular calcium induced by three different processes, store-operated calcium entry, intracellular release of calcium, and extracellular influx of calcium, increases the permeability of dextran molecules across endothelial cell monolayers derived from conduit vessels but not from lung microvessels (8). The potential involvement of calcium implied by the inhibitory effect of gadolinium and the apparent diminished response to calcium by microvessel cells suggest that ventilator-
induced lung injury may occur in specific lung segments due to phenotypic differences in the endothelium.

In light of these recent findings, Parker and Yoshikawa, in one of the current articles in focus (Ref. 15, see p. L1203 in this issue), set out to determine which vascular segments of the lung, alveolar vs. extra-alveolar, contribute to the overall increase in fluid conductance in isolated lungs subjected to high peak inflation pressure-induced lung injury. These authors demonstrated that fluid conductance (Lp) was segmentally distributed by 18% in the arteries, 41% in the veins, and 41% in the microvessels under baseline conditions. High peak inflation pressure of 45 cmH2O increased total Kf by 680% with segmental increases in the arteries, veins, and microvessels of 398, 589, and 975%, respectively. Gadolinium attenuated the increase in Kf in all three vascular segments. After factoring in the large surface area of the pulmonary microvasculature, the authors concluded that Lp across the alveolar endothelium, although increased, was lower than for the other two extra-alveolar segments. This latter finding coincides with an earlier report by Parker and Trenkle (14) comparing basal Lp and the filtration rate of endothelial cell monolayers derived from pulmonary arteries and pulmonary microvessels subjected to a constant hydrostatic pressure of 40–45 cmH2O for 2 h. The large vessel monolayers had a 12-fold higher basal fluid conductance or Lp, assuming a constant surface area, and a 97-fold higher filtration rate at the high pressure.

Inhibition with gadolinium once again points to the role of cations, in particular, calcium in signaling responses of the endothelium, albeit from the alveolar or extra-alveolar segments of the lung. In their discussion, Parker and Yoshikawa (15) focus on calcium and suggest that calcium entry via stretch-activated cation channels may initiate actin-myosin contraction, rearrangement of cytoskeletal elements, and phosphorylation of intracellular proteins involved in cell-cell and cell-matrix adhesions. The role of contraction or relaxation of actin-myosin filaments in the regulation of endothelial barrier function, however, is controversial. An increase in centripetal tension generated by actin-myosin motors after an elevation of intracellular calcium has been hypothesized to unbalance competing adhesive forces between cells (adherens junction) and the underlying matrix (focal contacts), causing contraction or retraction of cells, gaps within the intercellular junctions, and an increase in paracellular permeability. The opposite scenario may explain the barrier-enhancing activity of cAMP-enhancing agents.

Recent findings, however, have challenged the contraction/relaxation hypothesis. Moy et al. (9, 10) have directly measured cellular isometric tension as well as myosin light chain (MLC) phosphorylation in endothelial cell monolayers treated with histamine, thrombin, or cAMP-enhancing agents. Histamine and thrombin initially decreased endothelial electrical resistance, indicative of a loss of barrier function, in association with an increase in phosphorylation of MLC (10). Histamine had no effect on isometric cellular tension, and thrombin increased cellular tension. But the cellular tension induced by thrombin developed after the initial decrease in electrical resistance and was steadily rising after restoration of electrical resistance back toward basal levels. Furthermore, inhibition of MLC phosphorylation with ML-7 blocked the thrombin-induced increases in MLC phosphorylation and tension but not the initial decrease in electrical resistance. Interestingly, ML-7 caused a more rapid reversal of the decreased electrical resistance. It was concluded that the initial disruption of barrier function induced by thrombin occurred independently of actin-myosin contraction and that the reversal of electrical resistance was opposed by centripetal force generated by actin-myosin contraction. In a separate article, Moy et al. (9) demonstrated that an increase in intracellular cAMP enhanced barrier function independently of MLC-dependent tension development. They speculated that cell-signaling events initiated by cAMP could directly affect cell-cell adhesion and/or directly influence microtubules or intermediate filaments and increase compressive-resistive forces and maintain cell spreading.

Tyrosine phosphorylation is also involved in ventilator-induced lung injury. In a previous paper, Parker et al. (11) attenuated the increased Kf induced by high inflation pressure with genistein, an inhibitor of tyrosine kinase activity, and augmented the increased Kf with phenylarsine oxide, an inhibitor of phosphotyrosine phosphatase activity. Tyrosine phosphorylation contributes to the disassembly of the adherens junction between endothelial cells and the focal contacts connecting cells to the underlying matrix. The endothelial adherens junction comprises a linear complex of proteins, vascular endothelial (VE)-cadherin, and catenins (p120-catenin, β-catenin, plakoglobin, and α-catenin) that are linked to the actin cytoskeleton. These proteins, except for α-catenin, are major substrates for protein tyrosine kinases. Tyrosine phosphorylation of the adherens junction has been demonstrated to influence cell-cell adhesion. For example, tyrosine phosphorylation and dephosphorylation of β-catenin have been associated with the loss and gain, respectively, of adherens junction integrity (1). It has been proposed that tyrosine phosphorylation of the catenins recruits phosphotyrosine binding proteins to the adherens complex, which in turn alters the affinity or conformation of the complex resulting in weakening of the adherens junction (3). IQGAP1, an effector of Rac1 and Cdc2, small GTPases proposed to be involved in the assembly and stabilization of the adherens junction, may be one of those phosphotyrosine binding proteins recruited by tyrosine phosphorylated catenins. Fukata et al. (6) have hypothesized that IQGAP1 competes with α-catenin for binding to β-catenin and uncouples the linkage of the complex via α-catenin to actin, thus weakening cell-cell adhesion. Furthermore, activated Rac1 or Cdc42 interacts with IQGAP1 at the junction and sequesters IQGAP1 away from β-catenin, thus strengthening cell-cell adhesion. The barrier-disrupting activity of phorbol esters and heptacyte growth factor
(scatter factor) has been associated with the accumulation and loss of IQGAP1 and α-catenin, respectively, at epithelial cell junctions as well as the loss of Rac1 activity (5).

It is possible that increases in intracellular calcium could trigger phosphorylation events that would compromise the endothelial barrier after exposure to high inflation pressure. A clear linkage between intracellular calcium signaling and tyrosine phosphorylation in the regulation of the adherens junction has not been described, although calcium can activate protein tyrosine kinases such as Src and Fyn, which have the potential to induce tyrosine phosphorylation of adherens junction proteins. A recent publication links intracellular calcium to disassembly of focal contacts that connect cells to the underlying matrix (7). An increase in intracellular calcium induced the phosphorylation and activation of focal adhesion kinase, an integral protein in focal contacts. Association of calcium-related disassembly of focal contacts and the rapid activation of focal adhesion kinase led the authors to conclude that calcium signaling via activated focal adhesion kinase alters the dynamics of focal contacts, resulting in cell motility. Disassembly of focal contacts could contribute to the microscopic observation of separated endothelium from the underlying interstitium in lungs exposed to high inflation pressure (4).

The mechanism by which hyperinflated lungs initiate active cellular responses in adjacent endothelial cells remains to be determined. It is well known that hemodynamic forces modulate the structure and function of the vascular endothelium. Shear stress causes realignment of the actin cytoskeleton in endothelial cells to the axis of blood flow. Thus endothelial cells act as mechanical transducers. The components of the cell that act as mechanical transducers are under intensive study. Caveolae, G proteins, ion channels, integrin components such as focal adhesion kinase and Src, tyrosine kinase receptors, and calcium and phosphorylation mechanisms are often mentioned as putative mechanotransducers (16). Recently, it has been suggested that the receptor 2 for vascular endothelial growth factor in complex with VE-cadherin and β-catenin acts as a mechanotransducer that couples shear stress to endothelial cell responses (16). Interestingly, Parker and Yoshikawa (15) demonstrate in this issue that both extra-alveolar and alveolar vessels respond to high inflation pressure. Longitudinal tension generated in overinflated alveoli could transmit to the alveolar vessels, thereby increasing circumferential tension. The tension generated on the extra-alveolar vessels is somewhat different as they are pulled and opened by the stretch of elastin and collagen fibers. Mechanical sensors of the endothelial cell, which may differ in alveolar vs. extra-alveolar vessels, could then transduce this signal to an active, cellular process that alters fluid conductance across the endothelial barrier.

Utilization of the isolated lung model presented in this issue with the addition of targeted probes may enhance our fundamental understanding of the function of the vascular endothelium in microvessels vs. large, conduit vessels.

REFERENCES


