Factors controlling vascular permeability: transmitting mechanical signals.
Focus on “mechanical induction of group V phospholipase A2 causes lung inflammation and acute lung injury”

Judy Creighton
Departments of Anesthesiology and Cell and Molecular Biology, and the Pulmonary Injury and Repair Center, University of Alabama at Birmingham, Birmingham, Alabama

MECHANICAL VENTILATION HAS been used in the management of critically ill patients for more than 60 years. During this time knowledge of the physiology of ventilation and gas exchange evolved from a relatively primitive state to an appreciation that molecular pathways contribute to the pathology of respiratory failure. One hypothesis of the basis of lung injury associated with mechanical ventilation that has received recent attention is the two-hit model (4, 5). This hypothesis holds that injurious mechanical ventilation alone may not be sufficient to promote the inflammatory response but will do so in combination with another assault. However, it remains unclear which molecular pathways might be involved or even whether or to what extent mechanical ventilation tilts the inflammatory balance toward a proinflammatory state (9, 14). In this focus article, we highlight the study by Meliton et al. “Mechanical induction of group V phospholipase A2 causes lung inflammation and acute lung injury” (10), wherein the authors demonstrate a connection between activation of secretory group V phospholipase A2 (gVPLA2) in pulmonary vascular endothelium exposed to excessive mechanical stretch and onset of ventilator-induced lung injury (VILI). This evidence provides a new link in the chain of events associated with this process and strengthens the idea that pathological stretch contributes to the activation of the inflammatory response through a molecular pathway.

Although well described in ancient texts, acute respiratory distress syndrome (ARDS) was first described in modern times by military physicians as a complication of battlefield casualties during World Wars I and II (11). These same surgeons developed mechanical positive-pressure breathing methods to facilitate oxygenation in their patients. This concept led to the development of mechanical ventilation, which is still considered the cornerstone of ARDS treatment today. The primary goal of ventilatory management is to achieve oxygenation adequate to support organ function. However, the use of mechanical ventilation, especially high-tidal-volume ventilation, is not without risk. Nondependent regions of the lung retain greater compliance than atelectic and liquid-filled regions and so receive a greater proportion of the tidal volume. Thus normal unaffected regions of the ARDS lung are susceptible to alveolar rupture from overdistension. VILI occurs when the epithelial and endothelial barriers are damaged, allowing enhanced vascular permeability and increased accumulation of protein-rich fluid into the alveolar space.

It can be argued the most important advance in ARDS research has been the recognition that high-tidal-volume mechanical ventilation can itself cause lung damage. This led to a number of studies and the ARDS Network Trial, which demonstrated a 22% higher survival rate in patients who received lower-tidal-volume ventilation (6 ml/kg) than those who received larger tidal volumes. The ventilation strategy that has gained the most support has been low tidal volumes with a relatively fast rate, with or without more generous positive end-expiratory pressure (1). These strategies have had a positive impact on patient health. Prognosis of ARDS patients has improved dramatically over the past several decades, with mortality rates dropping from a high of 90% in the 1970s to lows of around 40% today. However, patient mortality rates have remained relatively stable since 1994 and despite considerable effort there is still no standard treatment for patients with ARDS (13).

Other factors contribute to the lack of continued improvement in patient survival. In addition to direct structural damage, mechanical force is thought to trigger a complex array of inflammatory cascades that propagate injury. Abnormal cyclic opening and closing of alveolar units causes release of cytokines and reinforces and amplifies local and systemic inflammatory responses.

However, studying these processes is not without difficulty. The point at which stretch becomes pathological can only be estimated because the extent to which lung tissue stretches during breathing cannot be determined directly. In experimental models of high-tidal-volume mechanical ventilation that activate the inflammatory response and increase vascular permeability, endothelial cells are estimated to undergo as much as 17–22% increase in elongation (3). Thus, experimentally, this level of stretch is considered an appropriate surrogate for the pathological stretch that occurs during high-tidal-volume ventilation.

How the inflammatory response is triggered under these conditions still remains an incompletely understood process. Endothelial cells exposed to excessive cyclic stretch (18% elongation) undergo cytoskeletal rearrangement and alterations in gene expression. One of the genes upregulated is Rho, which plays a central role in the control of actomyosin-based stress fibers. These structures contract when the myosin regulatory light chain is phosphorylated and generate sufficient centripetal tension to increase vascular permeability. In this way, Rho signaling plays a part in the mechanosensitive response of endothelial cells. This mechanism may serve a dual function and contribute to the release of propagators of inflammation...
from endothelial cells. Meliton et al. (10) suggest that the Rho pathway may be involved in the activation and release of \( \text{gVPLA2} \) in pulmonary vascular endothelium exposed to excessive mechanical stretch.

The \( \text{gVPLA2} \) enzyme is part of a superfamily of \( \text{PLA}_2 \) proteins, which hydrolyze fatty acid from the sn-2 position of membrane phospholipids. The sn-2 position frequently contains polyunsaturated fatty acids and, when released, can form various eicosanoids and related bioactive lipid mediators. Some of these bioactive mediators are proinflammatory. The \( \text{gVPLA2} \) phospholipase preferentially hydrolyzes phosphatidylcholine substrates and releases arachidonic acid, which is further metabolized to form several inflammatory and thrombogenic molecules (8). In this manner, secretion of \( \text{gVPLA2} \) is thought to initiate the inflammatory response at the site of injury and propagate inflammation through paracrine action on neighboring cells (15).

The authors show that \( \text{gVPLA2} \) levels and surface expression are increased in endothelial cells exposed to pathologically relevant levels of cyclic stretch. They further demonstrate that this increase in \( \text{gVPLA2} \) stimulates IL-8 production by lung epithelial and endothelial cells and generates a chemotactic signal that promotes neutrophil recruitment to the site of damage. Stretch-induced \( \text{gVPLA2} \) also acts on the endothelium itself by promoting endothelial activation and surface expression of the adhesion molecules ICAM-1, VCAM, and E-selectin, which are required for leukocyte adhesion to lung endothelium. These in vitro results were confirmed in vivo by use of \( \text{gVPLA2} \) knockout mice. The knockout animals were resistant to high-tidal-volume mechanical ventilation-induced injury; significant lung vascular leak present in the lungs of \( \text{gVPLA2} \) knockout animals, which was a corresponding decrease in the production of inflammatory cytokines as well as a reduction in the accumulation of leukocytes in the lungs of the knockout animals. These data are consistent with major roles for \( \text{gVPLA2} \) at multiple points in the inflammatory process and indicate that \( \text{gVPLA2} \) is a mechanosensitive activator of VILI.

The molecular events through which cyclic stretch induces activation and release of \( \text{gVPLA2} \) in pulmonary endothelium have not been fully resolved. The action of heparin-binding \( \text{sPLA}_2 \) enzymes, such as \( \text{gVPLA2} \), has been attributed to their ability to bind to glypicans located within specialized lipid raft membranes called caveolae (2). Lipid rafts compartmentalize cellular processes by serving as organizing centers for the assembly of signaling and effector molecules. In this way, lipid rafts and caveolae facilitate discrete physiological outcomes through colocalization of component molecules into signaling microdomains (6, 7). Many of these signaling mechanisms are involved in sensing and responding to a variety of vascular disruptive stimuli including stretch. In particular, lipid rafts and caveolae are known to serve roles as flow-regulated mechanosensors in endothelium and stretch-sensing triggers for cell cycle progression in smooth muscle (12). There is evidence linking the mechanosensitive actions of \( \text{gVPLA2} \) to caveolae. When activated, \( \text{gVPLA2} \) translocates from a submembrane compartment to the outer cell membrane. Meliton et al. (10) postulate that this process may be mediated by cellular mechanisms that control exocytosis or microparticle release. Actin and the microtubule structural proteins, along with several motor proteins, are implicated in these events. Once the vesicles reach their targets, they come into contact with the anchoring protein caveolin that constrains them to caveolar membranes.

How might the mechanical stretch signal be transmitted? (Fig. 1). The cholesterol-enriched composition of lipid rafts generates a “stiff patch” on the cell membrane that responds to mechanical stretch differently from the surrounding, more fluid, bulk membrane. It is possible these stiff patches provide the signaling impetus for stretch-specific \( \text{gVPLA2} \) release through other components of \( \text{gVPLA2} \) signaling, like Rho kinase and the calpain system, which are known to interact through other components of \( \text{gVPLA2} \) signaling, like Rho kinase and the calpain system, which are known to interact.
REFERENCES


