Stretching the lung and programmed cell death

ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) is a clinical condition characterized by impaired gas exchange as a result of the accumulation of edema in the alveolar space. The clinical management of ARDS patients includes mechanical ventilation with high oxygen concentrations. Mechanical ventilation alleviates the work of breathing and hypoxemia while allowing time for the lungs to recover from the initial injury. Although positive-pressure mechanical ventilation is commonly used as part of the care for respiratory failure, under some circumstances, it may cause or worsen lung injury. A recent trial conducted by the National Institutes of Health-sponsored ARDS Network reported that patients ventilated with high tidal volumes had a significantly higher mortality rate than patients ventilated with low tidal volumes (1). This large multicenter study and another clinical study (8) provide evidence of the clinical significance of ventilator-associated lung injury. Early insight into the ventilator-associated lung injury came from studies in the 1970s by Webb and Tierny (13) and in the 1980s by Dreyfuss et al. (3), who demonstrated that lung injury and pulmonary edema occur during ventilation with high inflation volumes. In the 1990s, a study (10) showed that high tidal volume ventilation causes lung injury and stimulates the release of proinflammatory mediators. Collectively, these studies stress the importance of maintaining alveolar function during ventilation by not overstretching the lungs.

Recently, investigators have focused on the effects of stretching alveolar epithelial cells (AECs). Several of these studies have employed the commercially available Flexercell strain unit device. This equipment uses a vacuum pump to pull a cell-covered flexible membrane downward and allows the frequency of cyclic stretch to be adjusted. In this issue, Edwards et al. (4) report using the Flexercell to expose alveolar type II cells (ATII) to a 30% strain at 60 cycles/min and demonstrate that these cells undergo apoptosis. Furthermore, ATII cells cocultured with macrophages were protected against cyclic stretch-induced apoptosis. The ability of macrophages to protect ATII cells against stretch-induced apoptosis was completely ablated by nitric oxide inhibitors, suggesting that the antiapoptotic effects of macrophages were mediated by nitric oxide. These results were corroborated when exogenous nitric oxide donors protected ATII cells against stretch-induced apoptosis. The observations by Edwards et al. raise two fundamental questions. First, what are the strain levels that AECs encounter during a normal breathing pattern? Second, what are the intracellular signaling mechanisms causing or preventing stretch-induced apoptosis?

The normal range of strain an AEC encounters varies with the changes in tidal volume associated with normal breathing. Edwards et al. (4) argue that 30% strain at 60 cycles/min may resemble strain levels on ATII cells during the normal breathing pattern of rats in vivo and that antiapoptotic factors such as nitric oxide being released from macrophages surrounding the alveolar environment contribute to the protection against cyclic stretch. Although this is an intriguing concept, currently there are no definitive studies quantifying the levels of strain that the ATII cells are exposed to during tidal breathing or high tidal volume ventilation. It is generally estimated that AECs are exposed to 1–5% strain during normal breathing, and levels of 30% strain might represent a very high tidal volume mechanical ventilation associated with ventilation-induced lung injury. We reason that there may be a both a threshold and duration of strain required to trigger apoptosis. Thus because AECs are subject to increasing levels of stretch, they may trigger adaptive responses until the strain levels reach a threshold where AECs undergo apoptosis. One such response might be the stimulation of Na\(^{+}\)-K\(^{-}\)-ATPase activity. To this effect, Waters et al. (12) demonstrated that cyclic stretch at 30 cycles/min with a mean strain of 10% stimulated Na\(^{+}\)-K\(^{-}\)-ATPase activity, an important mechanism keeping the alveoli free of edema. We reason that low tidal volume ventilation with normal or slightly higher strain levels than those associated with a normal breathing pattern might be beneficial by stimulating the surfactant system and Na\(^{+}\)-K\(^{-}\)-ATPase without triggering apoptosis of AECs. However, high strain levels trigger detrimental effects by increasing the release of proinflammatory mediators and initiating AEC apoptosis. Cytokines in combination with cyclic stretch might provide potent proapoptotic signals for AECs, resulting in lung injury.

What are the intracellular mechanisms that participate in regulating adaptive and apoptotic responses in AECs? Adaptive responses are mediated by changes in the phosphorylation status of the cells and intracellular calcium levels (7). Recently, Ashino et al. (2) demonstrated that lung expansion induced synchronous intracellular calcium oscillations in alveolar cells and exocytosis of lamellar bodies from ATII cells. The intracellular signaling pathways leading to apoptosis involve the loss of mitochondrial integrity associated
with cytochrome c and the activation of caspases (11). Nitric oxide can inhibit caspase activation and cytochrome c release from mitochondria in response to various death stimuli such as tumor necrosis factor-α (6). Although Edwards et al. (4) did not measure changes in mitochondrial integrity or caspase activation, their observations that nitric oxide prevents stretch-induced apoptosis suggests that caspase activation may be an important mediator of stretch-induced cell death.

What determines whether cyclic stretch in AECs invokes adaptive responses or apoptosis? We reason that the cytoskeletal network is directly affected by cyclic stretch and plays a role in the intracellular pathways regulating either apoptotic or adaptive responses. The actin cytoskeleton, the microtubule transport system, and the intermediate filaments appear to be important modulators of changes in cell shape. In epithelial cells, intermediate filaments are the structural backbone of the cytoplasm and are considered to be the major contributor to the mechanical integrity of the cell. Intermediate filaments form a continuous, dynamic structural network extending from the nuclear surface to the cell periphery and are connected to other parts of the cytoskeletal network such as microfilaments, hemidesmosomes, and focal adhesions (5). Mitochondria are associated with microtubules and motor proteins such as kinesin, which participates in the shuttle movement of mitochondria between the cell body and the periphery. Disruption of kinesin results in abnormal perinuclear clustering of mitochondria (9).

Low tidal volume mechanical ventilation might trigger responses by modifying the cytoskeletal network and activating signaling pathways involving calcium and kinases. In contrast, high tidal volume ventilation might disrupt focal adhesions and depolymerize microfilaments, initiating a loss in mitochondrial integrity, thereby triggering apoptosis. Thus the magnitude of changes in the cytoskeletal network could determine whether ATII cells produce surfactant and stimulate Na⁺-K⁺-ATPase activity or die by apoptosis.

The fundamental question regarding stretch-induced responses is how stretch sensing at the plasma membrane is transmitted throughout the cell. For example, is there a universal sensor that triggers an adaptive response and initiates apoptosis? Although mechanical stretch activates multiple second messenger systems, it is not known which molecules are directly modulated by stretch and which molecules are indirectly activated by upstream targets. A mechanosensitive molecule should have some interaction with the plasma membrane to sense the tension of the membrane. Models to explain mechanotransduction include stretch-activated ion channels that modulate intracellular calcium levels or stretch-activated receptor-type tyrosine kinases that initiate phosphorylation cascades. However, not all of the responses to stretch are likely to involve calcium or kinases; thus a more universal mechanism could exist to account for the variety of cellular responses ranging from apoptosis to surfactant production. Therefore, as new information is gathered on the effects and effectors of normal and high stretch in the lungs, lessons from other stretch-exposed cells point to the cytoskeletal network as an important component of the stretch-sensing mechanisms.

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