A randomized clinical trial by the Acute Respiratory Distress Syndrome Network (1) demonstrated a 22% reduction in mortality in patients by reducing the tidal volume for mechanical ventilation from the conventional setting of 12 ml/kg to a lower setting of 6 ml/kg. This dramatic decrease in mortality has stimulated significant interest in the mechanisms of ventilator-induced lung injury and the development of lung protective strategies. One theory is that higher tidal volumes induce excessive stretch of lung tissue that results in stimulation of inflammatory responses and exacerbation of the underlying lung injury (reviewed in Refs. 12, 13, 23, 25, 26, 28). The central premise is that cells in the lungs sense changes in mechanical forces and transduce the mechanical signal into a biological response (mechanotransduction) (12, 21, 31). However, the mechanisms of mechanotransduction remain to be elucidated. A study by Ali et al. (Ref. 2a, page L486 in this issue), a current article in focus, examines one of the potential early mechanisms involved in mechanotransduction, the production of reactive oxygen species (ROS) in endothelial cells in response to mechanical stretch, and relates this signal to activation of inflammatory responses.

The foundation for the theory that changes in mechanical forces in the lungs stimulate inflammation can be found in studies of the development of atherosclerosis. Atherosclerotic plaques typically develop in regions of the circulation near branch points or areas of curvature that promote flow reversal or flow stagnation. Thus the hypothesis is that oscillatory shear stress or the loss of shear stress promotes proinflammatory pathways in endothelial cells in these regions (reviewed in Refs. 2, 4, 20, 27). Furthermore, it has been postulated that changes in shear stress regulate proinflammatory mechanisms in endothelial cells by stimulating the production of ROS (9–11, 15, 17, 24).

Stimulation of ROS production has been demonstrated in response to changes in shear stress (8, 9, 19, 24, 30, 33) and cyclic mechanical stretch (6, 7, 18, 22, 29, 32) in cultured endothelial cells. However, the source of ROS remains controversial. As described by Ali et al. (2a) ROS generation in response to mechanical forces may originate from an NAD(P)H oxidase system, from xanthine oxidase, from mitochondrial production, or from other oxidase systems such as NAD(P)H oxidase (22). Interestingly, mechanical stretch-induced stimulation of ROS production [measured by dichlorofluorescin (DCF) diacetate fluorescence] by vascular smooth muscle cells was not observed in cells isolated from mice deficient in the p47phox subunit of NAD(P)H oxidase (16). A recent study by McNally et al. (24) demonstrated that xanthin-dependent superoxide production was increased in endothelial cells exposed to oscillatory shear stress and that cells isolated from p47phox Yellow mice had low superoxide production and decreased xanthine oxidase expression and activity.

The study by Ali et al. (2a) shows that mechanical stretch stimulates ROS production by HUVEC through mitochondrial mechanisms. ROS production, assessed by DCF fluorescence, was significantly increased after 6 h of stretch, and the increase was abolished by the mitochondrial complex I inhibitor rotenone. Inhibitors of NAD(P)H oxidase (apocynin), xanthine oxidase (allopurinol), or nitric oxide synthase (N-nitro-L-arginine) did not block the stretch-induced increase in DCF fluorescence, suggesting that these pathways were not important in the response to stretch. To further examine the role of mitochondrial pathways in the stretch-induced stimulation of ROS, mutant HUVEC cells were generated that lacked mitochondrial DNA (ρ0 cells). When ROS production was measured in ρ0 cells, the baseline levels were substantially reduced, and there was a reduced increase in DCF fluorescence in response to stretch that was insensitive to rotenone. To determine whether the increased ROS production was functionally related to inflammatory pathways, the authors demonstrated that control HUVEC responded to stretch with increased activation of NF-kB and VCAM-1 expression. Cyclic stretch did not induce either of these proinflammatory events in ρ0 cells, but treatment with lipopolysaccharide did induce NF-kB activation and VCAM-1 expression, indicating that the respiration-deficient cells were capable of activating proinflammatory pathways.

The study by Ali et al. (2a) raises many interesting questions. Studies from different groups have now demonstrated that mechanosensitive stimulation of ROS is generated by NAD(P)H oxidase, xanthine oxidase, or mitochondrial pathways. Which of these pathways is predominant in pulmonary endothelial cells? The majority of work in the area of mechanosensitive ROS production has focused on HUVEC or other large vessel endothelial cells, but few studies have examined the sensitivity of pulmonary endothelial cells (3, 14, 30) or other lung cells such as epithelial cells, macrophages, eosinophils, or fibroblasts. It is also not clear what levels of mechanical forces are necessary to induce ROS production or proinflammatory pathways. The lungs typically undergo cyclic deformation during normal breathing, and this may be increased during mechanical ventilation, but the levels of stretch have not been well characterized and are likely to vary substantially throughout the lungs. Endothelial cells are exposed to a wide range of shear stresses in the pulmonary microcirculation since red blood cells must transit through capillaries that...
vary in size during both the respiration and cardiac cycles. Most of the studies with cultured cells have examined the response to the initiation of mechanical forces, but it may be just as important to examine the response of cells to changes in mechanical forces after conditioning with baseline levels, as in the studies by Fisher and colleagues (5, 14, 30). Does shear stress induce the same pathways as cyclic stretch? The unifying theme is that deformation of cellular structures at the cell membrane, at cell-cell or cell-substrate contacts, or through force transmitted through the cytoskeleton leads to activation of signaling pathways such as the generation of ROS. Although there appear to be many similarities in the signaling pathways induced by shear stress and mechanical stretch, there may also be differences in the initiating events. For example, does cyclic stretch preferentially activate mitochondrial production of ROS, as in the study by Ali and colleagues, because signaling pathways initiated by stretch target mitochondrial function or because mitochondrial structures are responsive to direct deformation under these conditions? The answers to some of these questions may provide important insights into the mechanisms by which changes in the mechanical environment of the lung, such as during mechanical ventilation, lead to proinflammatory conditions.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-064981.

REFERENCES