D uring the past 2 decades, it has become evident not only that the vascular endothelium is a physical barrier separating blood flow from the underlying vessel wall but also that it plays a crucial role in maintaining intravascular and extravascular homeostasis. Under physiological conditions, the endothelium works in concert with other vessel wall cells to maintain a balance in the regulation of vascular tone, cell growth, coagulation, leukocyte adhesion and migration and the production of cytokines or other paracrine signaling molecules. Endothelial cells, as eukaryotic cells in general, are dependent on aerobic metabolism, inasmuch as mitochondrial respiration offers greater efficiency for the extraction of energy charge from glucose than does anaerobic glycolysis. Importantly, reduction of tissue oxygen tension, as seen in hypoxia, is a common factor in many diseases, including pulmonary disorders, occlusive vascular disease, and septic shock (conditions in which the arterial blood supply becomes compromised).

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Although endothelial cells have developed protective mechanisms to withstand ischemic challenge, severe hypoxia has been found to trigger profound changes in endothelial phenotype, leading to a state of endothelial activation that may turn into an uncontrolled state of endothelial dysfunction. Dysfunctional endothelium is characterized by altered vascular homeostasis, disrupted homeostatic balance in coagulation, increased vascular permeability, and increased vascular tone. Subsequent microcirculatory failure and local inflammation may ultimately lead to end-organ damage in the setting of hypoxia.

Important mechanisms of hypoxia-induced vascular dysfunction include increased vasoconstriction due to enhanced endothelin-1 production or ACE expression, decreased NO synthase III expression, decreased NO bioavailability, and disruption of cAMP and cGMP second-messenger signaling, as well as induction of leukocyte adhesion. In addition, secretion of proinflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF)-α from endothelial cells in response to hypoxia has been reported.

Many of these hypoxia-induced changes are prevented by cotreatment with antioxidants, suggesting that reactive oxygen species (ROS) may play a crucial role in mediating these phenomena. ROS initiate intracellular redox signaling, leading to a broad range of adaptive responses, such as ischemic preconditioning and activation of transcription factors, such as hypoxia-inducible factor-1, nuclear factor (NF)-κB, and p53 (see review). In particular, the activation of hypoxia-inducible factor-1, a transcription factor, has been shown to influence multiple properties of vascular homeostasis during hypoxia through the activation of multiple genes, including erythropoietin, vascular endothelial growth factor, glucose transporters, heme oxygenase-1, and NO synthase II.

Although several studies have demonstrated an induction of ROS generation by hypoxia (see review), the precise source of ROS still remains to be determined. In general, ROS may be generated by the NAD(P)H oxidase system, xanthine oxidase, NO synthase, or the mitochondrial electron transport chain (ET).

In the present issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Pearlstein et al have provided us with a very important study addressing the mechanisms underlying hypoxia-induced IL-6 production. Human umbilical vein cells exposed to hypoxia markedly increased the production of ROS, as detected with dichlorofluorescein fluorescence and with the fluorescent dye dihydroethidine. In these studies, the authors found that specific inhibitors of ET, rotenone and diphenylene iodonium (inhibitor of flavoprotein-dependent oxidoreductases), but not allopurinol, apocynin, or N^{2}-nitro-L-arginine were able to block hypoxia-induced superoxide production. Thus, the study by Pearlstein et al clearly points to a crucial role of mitochondrial ET as the predominant superoxide source and also makes a significant contribution of xanthine oxidase, the NO synthase, or an NADPH-oxidase-like enzyme unlikely.

How does hypoxia stimulate mitochondria to produce superoxide? Under physiological conditions, mitochondrial respiration leads to chemical reduction of oxygen to water by the transfer of 4 electrons at cytochrome oxidase, resulting in the synthesis of ATP. Approximately 2% to 3% of the oxygen that is consumed by mitochondria is converted to superoxide. Although superoxide can potentially be generated at different sites within the mitochondria, there is a growing body of evidence indicating that under hypoxic conditions superoxide is formed at the ubisemiquinone site of complex III (see Figure) and, therefore, by a complex that already produces superoxide under normoxic conditions. Ubisemiquinone is a free radical that has been shown to transfer electrons to molecular oxygen, yielding superoxide. Inhibitors that block ET from that site (diphenylene iodonium [DPI] and rotenone) prevent the formation of ubisemiquinone and, thereby, diminish ROS generation. Other inhibitors, such as
cyanide or antimycin A, which act further downstream, tend to augment ROS generation by increasing the generation of ubisemiquinone. In addition to superoxide, mitochondria also produce NO and CO₂. Thus, superoxide production stimulated by conditions of hypoxia may lead to the generation of reactive nitrogen species, such as peroxynitrite and ONOOCO₂, which may lead to further mitochondrial and, therefore, endothelial dysfunction via secondary oxidation and nitration reactions.

The article by Pearlstein et al also represents a significant advance in our understanding of the link between hypoxia-induced superoxide production and the subsequent increases in endothelial cell permeability. In general, hypoxia leads to the release of cytokines, such as TNF-α, IL-1, IL-6, and IL-8, and also stimulates the formation of edema fluid. Recent studies have indicated that monoclonal antibodies against IL-6 (but not antibodies against TNF-α, IL-1, or IL-8) and antioxidants such as N-acetylcysteine are able to inhibit hypoxia-induced increases in endothelial cell permeability.

The presented findings may have important pathophysiological implications. They clearly indicate that conditions such as ischemia/reperfusion and also the preceding hypoxia alone can induce marked damage to endothelial cells by stimulating mitochondrial superoxide production. The demonstration of a predominant role of IL-6 in hypoxia-induced increases in cell permeability may explain why strategies with so-called cytokine modifiers, such as monoclonal antibodies against TNF-α or TNF receptor fusion proteins, were quite ineffective in antagonizing endothelial cell leakage or improving the prognosis in patients with septic shock in adult respiratory distress syndrome. The discovery of a mitochondrial superoxide source in the setting of hypoxia as well as the demonstration of it being linked to the production of proinflammatory cytokines may help to develop new strategies for future, more successful therapeutic interventions.

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