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Hypoxia Can Make Neutrophils Hyper, Potentially Wreaking Havoc during Exacerbations in Chronic Obstructive Pulmonary Disease

Patients with chronic obstructive pulmonary disease (COPD) often are hypoxic, as measured by O_2 percentage saturation, and neutrophilia can occur in both the blood and the lungs, particularly during exacerbations. Questions about the effect of systemic and local tissue hypoxia on the function of innate immune cells, particularly neutrophils and monocytes/macrophages, have been a concern for many years, but a clear understanding of the effect of hypoxia on immune cell function over the many years that our patients struggle with COPD has been very difficult to attain. Hypoxia may affect endothelial function directly or through effects on circulating myeloid cells to increase their propensity to induce endothelial injury (1–3). Hypoxia may also alter the response of myeloid cells to bacterial and viral pathogens, perhaps distorting the balance between beneficial pathogen destruction and deleterious host-cell injury (1, 2).

In this issue of the *Journal*, Lodge and colleagues (pp. 903–916) ask, what does hypoxia do to neutrophil function that injures endothelial cells (ECs) (4)? Their initial studies revealed a variable but significant increase in the cleavage products of NE (neutrophil elastase) and proteinase 3 activity in the plasma of subjects with exacerbating COPD compared with healthy donors. These proteases are both present in the azurophil granules of neutrophils. Thus, these observations suggest that exacerbating COPD neutrophils degranulate and that these proteinases are not immediately inhibited by the wide array of endogenous protease inhibitors present in the plasma.

These results led to a very interesting series of studies (4). Neutrophils from either healthy donors or subjects with nonexacerbating COPD and exposed to severe hypoxia (0.8% O_2) for 4 hours released more elastase than neutrophils kept in a normoxic environment (21% O_2), but only after brief stimulation with combined PAF (platelet-activating factor) and fMLP (*N*-formyl-methionyl-leucyl-phenylalanine) at the end of the hypoxic period. Inhibition of PI3Kγ (phosphatidylinositol-3-kinase-γ) but not PI3Kδ using selective inhibitors prevented elastase release from both normoxic and hypoxic neutrophils after brief exposure to fMLP and PAF. These studies were confirmed in hypoxic neutrophils from mice deficient in PI3Kγ.

Second, the authors mimicked *in vivo* circumstances by incubating ECs with supernatants from neutrophils exposed to hypoxia and then fMLP/PAF (4). Supernatants from hypoxic neutrophils induced more detachment and death of cultured human

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ECs from either the pulmonary artery or the lung microvasculature. This injury was only partially inhibited by α1AT (alpha-1 antitrypsin), particularly in microvascular ECs, suggesting that molecules other than proteases may contribute to injury. Furthermore, hypoxia alone, without stimulation with fMLP/PAF, induced endothelial apoptosis. These two observations prompted rigorous study of the neutrophils' secretome. Mass spectrometry revealed 63 differentially regulated proteins between supernatants from hypoxic compared with normoxic fMLP/PAF-stimulated neutrophils. Interestingly, the 35 that were more abundant in normoxic supernatants were predominantly cytoplasmic, whereas the 28 that were more abundant in hypoxic supernatants were mostly granular proteins (e.g., resistin, NGAL [neutrophil gelatinase–associated lipocalin], and MPO [myeloperoxidase]). Curiously, the cytoplasmic protein cyclophilin A and the nuclear protein histone H4 were greater in hypoxic supernatants. No difference in either neutrophil-derived microvesicle number or content was observed to explain the increase in cyclophilin A, and no difference in neutrophil extracellular trap formation (measured as extracellular DNA) in hypoxic neutrophils could be identified to explain the increase in histone H4.

The third series of studies examined neutrophils from participants with exacerbating COPD or healthy donors (4). The authors' data suggest that circulating neutrophils from subjects with COPD were not primed during exacerbations. However, after hypoxia and stimulation with fMLP and PAF, COPD neutrophils released more elastase, NGAL, and cyclophilin A compared with healthy neutrophils. Curiously, hypoxia caused MPO release similarly in healthy and COPD neutrophils.

Finally, to assess the potential to injure, these supernatants were incubated with cultured human pulmonary microvascular ECs (4). Supernatants from hypoxic stimulated COPD neutrophils induced more rolling and adhesion of healthy neutrophils than either normoxic stimulated COPD supernatants or hypoxic stimulated healthy neutrophil supernatants, suggesting that the effects of COPD on neutrophil function combined with neutrophil hypoxia and stimulation alter the adhesivity of ECs. These studies led to a search for a circulating signature of hypoxia-induced neutrophil protein secretion by comparing plasma from subjects with exacerbating COPD and healthy control subjects. Neutrophil granule proteins NE, MPO, and NGAL were increased, as well as biomarkers of vascular injury/activation (sICAM-1 [soluble intercellular adhesion molecule 1], sVCAM-1 [soluble vascular cell adhesion molecule 1]) and inflammation (SAA [serum amyloid], CRP [C-reactive protein]). These studies suggest exciting next steps to understand how this supernatant affects microvascular ECs and what components induce these changes.

The authors clearly show that *in vitro*, severe hypoxia alters neutrophil function and that the hypoxic neutrophil secretome alters EC responses to neutrophils and enhances EC activation and injury (4). Relating these observations to patients with COPD, exposure of a few circulating neutrophils to tissue hypoxia over time in the presence of a mediator (or mediators) that stimulates them may alter both neutrophil behavior and the function of nearby ECs. Components of the secretome in the plasma that are not quickly inhibited or cleared may interact with ECs at a distance. This may happen many times over the years required to develop COPD or the cardiovascular disease that can accompany COPD. Studies using cytometry by time of flight, flow cytometry, single-cell proteomics, and multiomics of neutrophils may help determine how many neutrophils show signs of hypoxia-induced changes at any point in time and to better define these changes (5). The *in vitro* system developed by the authors (4) may provide important information about the degree and duration of hypoxia required for changes in neutrophil secretomes and the presence of plasma, as well as mechanistic insight.

Curiously, stimulation of neutrophils with both PAF and fMLP was required for the increased release of elastase induced by hypoxia (4). PAF is a phospholipid that acts primarily through the PAF receptor. fMLP is an *N*-formylated tripeptide that binds to fMLP receptors. The PAF and fMLP receptors are G protein–coupled receptors (6–9). The signaling they induce is not identical, but both activate PI3K, and either can induce degranulation. Understanding why activation of both receptors is required for hypoxia to induce this effect may help elucidate the signaling pathways that hypoxia induces.

The authors suggest that the effect of hypoxia on the neutrophil secretome may contribute to the increased cardiovascular risk in COPD (4). Systemic hypoxia or hypoxia in areas of severe pulmonary obstruction may also lead to neutrophil activation and EC injury in alveolar capillaries or small arteries and arterioles, contributing to alveolar wall destruction and leading to emphysema and vascular injury leading to pulmonary hypertension and right heart failure. Their work brings us closer to understanding these mechanisms and enhances our interest in oxygen therapy and in drug therapies that suppress damaging effects of neutrophils while promoting their numerous beneficial effects. There is clearly much work to do! ■

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Dyspnea and Mechanical Ventilation
The Emperor Has No Clothes

Dyspnea, or breathing discomfort (1), is a common yet underappreciated problem for hospitalized patients. Although regulatory bodies in the United States require regular assessments of pain, similar requirements for evaluation of dyspnea do not exist. Dyspnea during a hospital admission has been shown to be associated with higher mortality during that admission and in the 2 years after discharge (2), and dyspnea after extubation is associated with greater risk of recurrent respiratory failure (3). For patients with acute respiratory failure, however, dyspnea has often been given less attention owing to difficulty assessing it in individuals who are frequently unable to communicate and the common use of sedation, and because the focus of care is directed at managing the underlying illness. Nevertheless, dyspnea among critically ill patients has been shown to be the most distressing of 10 symptoms studied (4) and has been found to afflict a significant percentage of patients during mechanical ventilation (5). In this issue of the *Journal*, Demoule and colleagues (pp. 917–926) extend earlier work of this group (5) to rectify this deficit and to demonstrate the relationship between breathing discomfort during mechanical ventilation and long-term mental health consequences (6).

Dyspnea has historically been associated with increased work of breathing and the now decades-old concept of length–tension inappropriateness (7). This has subsequently led many clinicians to equate dyspnea with mechanical loads on the inspiratory muscles and an increased effort to breathe. With this mindset, the institution of positive pressure ventilation, by relieving the work of breathing, should greatly relieve, if not eliminate entirely, respiratory distress. The reality is that the origins of dyspnea are more complicated; the breathing discomfort may arise from stimulation of pulmonary and vascular receptors, chemoreceptors, and other factors that may be the source of the discomfort and/or enhance the drive to breathe (1). Furthermore, dyspnea is worsened when the output of the ventilatory system (e.g., V_T and inspiratory flow) is not consistent with the expected or desired output. This concept has been termed neuromechanical uncoupling or efferent–reafferent dissociation (8, 9). For example, in individuals with normal lungs with high spinal cord lesions and quadriplegia requiring permanent mechanical ventilation, a decrease in V_T leads to breathing discomfort even in the absence of gas exchange abnormalities (10).

For the past 20 years, with the report of the ARDSNet (Acute Respiratory Distress Syndrome Network) study (11), critical care physicians have assiduously adhered to a low-V_T strategy, sometimes necessitating permissive hypercapnia and acute respiratory acidosis, to avoid ventilator-induced lung injury not only for patients with the acute respiratory distress syndrome but for most patients with acute respiratory failure necessitating mechanical ventilation. The restriction of V_T in patients with acute lung disease and a strong drive to breathe is likely to cause an intense sense of "air hunger" (12). Management of the ventilator in these patients may also be complicated by dyssynchrony. Although there are multiple types of dyssynchrony associated with different mechanisms (13), patient discomfort may play a role. Of those individuals who survive mechanical ventilation, a significant percentage will have emotional and behavioral problems, including symptoms consistent with post-traumatic stress disorder (PTSD), in the months and years after extubation (14), and a case has been made for dyspnea as a contributing factor for this outcome (15).

Sedation is commonly used to treat patients with acute respiratory failure, often triggered by apparent discomfort. When selecting medications, however, we need to distinguish drugs such as benzodiazepines, which may be anxiolytics but have little effect on dyspnea, from analgesics, such as opiates, which will reduce the intensity of dyspnea (15). Although an individual may be unconscious with sedation, experimental studies with propofol suggest that painful stimuli may still be "perceived," as evidenced by activation of the insular cortex, a part of the limbic system in which noxious experiences are processed, on brain imaging (16).

Can we assess dyspnea in a patient who is mechanically ventilated? Dyspnea, as with any symptom, is ideally reported by the individual. Yet, we suspect that unconscious patients can perceive pain based on their behavior (e.g., withdrawing from a noxious stimulus) and on the results of neural imaging (14). With respect to dyspnea, clinicians frequently make inferences from findings on physical exam that indicate "respiratory distress," although these findings are generally reflecting an increase in respiratory drive (e.g., use of accessory muscles of ventilation or nasal flaring). Validated instruments have been developed to incorporate facial

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