Linking VEGF Deficiency and IL-33 Upregulation in Chronic Obstructive Pulmonary Disease

Vascular endothelial growth factor (VEGF) is a critical regulator of epithelial cell integrity, angiogenesis, and repair in lung tissues (1). VEGF expression is higher in the lungs than in other organs at baseline, and an increase in the levels of VEGF expression in the airspace has been associated with the repair mechanisms that follow acute lung injury (2). Thus, lower levels of VEGF are found in BAL from patients with acute respiratory distress syndrome compared with healthy control subjects or ventilated patients without acute respiratory distress syndrome. In the case of chronic obstructive pulmonary disease (COPD), sputum levels of VEGF were found to be inversely related to the severity of COPD and biomarkers of oxidative stress (3), indicating that a deficiency in VEGF may render the lung tissues more susceptible to oxidant injury, although the cause-and-effect relationship of VEGF expression and oxidant formation is still unclear. By extension, experimental blockade of VEGF receptors with the receptor tyrosine kinase inhibitor SU5416 in rats resulted in alveolar enlargement and emphysema (4). In this model, SU5416-induced emphysema was characterized by an increase in alveolar epithelial cell apoptosis and pruning of the pulmonary arterial tree, which developed independently of macrophage and immune cell infiltration.

Embryonic deletion of *Vegf*, *Vegfr1*, and *Vegfr2* is lethal in mice. To more specifically examine the role of VEGF ligand in alveolar structure, Tang and colleagues previously used a conditional knockout approach (5). Mature VEGF*loxP* mice were treated intratracheally with AAV-Cre, an adenovirus that expresses Cre recombinase, to delete VEGF expression. Within 5 weeks of AAV-Cre delivery, lung expression of VEGF was reduced by 86%. Similar to the effects of VEGF receptor 2 (VEGFR2) blockade with SU5416 in rats, lungs from $Vegf^{f/f}$ mice had increased bronchiolar and alveolar septal wall apoptosis, air space enlargement, and loss of elastic recoil consistent with an emphysema phenotype. However, as with the SU5416 model, these effects were observed independently of inflammatory cell infiltration, and were temporal as VEGF levels returned to baseline. The observations from the SU5416-treated rat and $Vegf^{f/f}$ mouse models highlighted the importance of VEGF-VEGFR2 signaling in maintaining the airway epithelium, but these models still fell short in replicating the obligate inflammatory components that are essential for the development of emphysema, and more broadly COPD, in humans.

In this issue of the *Journal*, Lee and colleagues (pp. 567–574) take a step further (6). In their study, after intratracheal AAV-Cre–mediated deletion of VEGF, mice were exposed to tobacco smoke for 4 months. Lungs from smoke-exposed $Vegf^{f/f}$ mice demonstrated greater static compliance and less elastic recoil than those from non-smoke–exposed and VEGF-intact mice, consistent with the hyperinflation and air trapping noted in humans with emphysema. Moreover, an increase in neutrophil infiltration was noted in the lungs from smoke-exposed $Vegf^{f/f}$ mice. These results are in accord with the "two-hit paradigm" that a compromised airway epithelium (in this case $Vegf^{f/f}$) and an inflammatory stimulus (in this case tobacco smoke) result in the more severe forms of disease.

IL-33 is an alarmin cytokine of the IL-1 superfamily that has been linked to the pathogenesis of COPD (7–9). IL-33 expression is increased in mouse models of COPD after cigarette smoke (9, 10) and virus infection (7, 9), and blockade of the IL-33/suppression of tumorigenicity 2 receptor (ST2) axis can prevent the chronic disease from developing (7, 10). IL-33 exerts multiple effects depending on its structure (11). At baseline, full-length IL-33 is constitutively expressed in the nuclei of barrier epithelial cells, and histone-binding motifs underscore a function for full-length IL-33 in nuclear regulation. After cellular injury, IL-33 is released and rapidly cleaved to a "mature" and potent alarmin that binds to the IL-33 receptor, ST2, on macrophages and other innate immune cells to drive type 2, as well as type 1, inflammatory responses. Inactivation of IL-33 can occur in the extracellular space via the oxidation of free cysteines to form disulphide bonds (DSBs) in IL-33 that prevent binding to ST2 (12). A recent report indicated that a relative increase of mature IL-33 compared with DSB–IL-33 in bronchial washings may serve as a biomarker of IL-33 activity in asthma and related airway diseases (12). In the $Vegf^{f/f}$ model, Lee and colleagues found an increase in IL-33 staining on lung macrophages from smoke-exposed $Vegf^{f/f}$ mice compared with controls (6). The increase in IL-33 staining was associated with a decrease in the oxidizing capacity of bronchial washings from smoke-exposed $Vegf^{f/f}$ mice to convert active IL-33 to the inactive DSB–IL-33 form. Consistent with several other reports, IL-33 expression was also found in the airway epithelial cells of control and smoke-exposed $Vegf^{f/f}$ mice. Further characterization of IL-33, matrix metalloproteinse-12, triggering receptor expressed on myeloid cells-2, and related markers of macrophage activation is still needed to clarify the contributions of immune cells versus epithelial cells in this chronic disease model (13, 14).

In summary, VEGF deficiency and IL-33 upregulation have been independently linked to the development of COPD. This report by Lee and colleagues is the first to examine these factors together. Both VEGF and IL-33 are expressed by lung epithelial cells and are upregulated within hours of lung injury (6, 7). It is still unclear how the initial upregulation of VEGF after tobacco exposure ultimately leads to VEGF deficiency in the long term. A compelling hypothesis is that VEGF deficiency corresponds to alveolar destruction in emphysema, perpetuated by IL-33–mediated macrophage activation, neutrophil infiltration, and oxidative stress, and the resulting apoptosis of epithelial cells (3). VEGF is essential for the development and maintenance of alveolar

integrity, and nuclear IL-33 is prominently expressed in lung progenitor epithelial cell populations (7, 14). The relationship between VEGF and IL-33 in progenitor cell activation in alveolar and bronchial cell populations after lung injury should also be examined in other models. VEGF deficiency in COPD was a focus of research by several groups in the 2000s, and IL-33 upregulation in COPD has been a focus for several groups over the past decade. Now, the tobacco-smoke *Vegf*$^{f/f}$ model developed by Lee and colleagues may provide a new opportunity to examine the relationship of these two pathways in the pathogenesis of COPD. ■

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