Our Compliments to the Authors: Decay Accelerating Factor and the Complement System in Pulmonary Fibrosis

The complement system is a complex branch of the innate immune system that comprises more than 60 circulating proteins that become activated by means of proteolytic cascades and are responsible for the opsonization and lysis of pathogens; for regulating inflammatory responses, vasodilation, and phagocyte recruitment; and for modulating cellular and humoral immunity (1). Activation of the complement system is mediated by three pathways: the classical pathway (by immune complexes and apoptotic cells), the lectin pathway (by microbial carbohydrates), and the alternative pathway (by spontaneous tickover activation). The end result of these cascades is the activation of C3 (complement component 3) and C5 (complement component 5) convertases. C3 convertase cleaves C3 into C3a (a chemotactic peptide) and C3b (an opsonin). C5 convertase cleaves C5 into C5a (a chemotactic peptide) and C5b (which seeds the membrane attack complex, promoting cell lysis) (1).

In addition to its role in immune defense, the complement system plays an important role in wound healing and tissue regeneration after injury. C3a and C5a are required for liver regeneration after partial hepatectomy (2). Moreover, topical application of components of the complement system promotes angiogenesis, inflammatory cell recruitment, collagen deposition, and healing in cutaneous wound models (3, 4). Although these findings suggest a positive role for the complement system in the wound-healing process, local membrane attack complex deposition and increased local and systemic C3 have been shown to be associated with chronic ulcers (5). Moreover, *C3* knockout mice exhibit improved cutaneous wound healing, compared with wild-type mice (6). Thus, the role of the complement system in wound healing is complex and likely depends on a tight balance of activation and regulatory components.

Fibrosis is a state of abnormal wound healing. Aberrant complement system activation has been shown to be associated with fibrosis in the lung for more than 40 years (7). Complement system activation has been shown to be elevated in idiopathic pulmonary fibrosis (IPF), as well as in patients with familial fibrosis due to MUC5B mutation (8, 9). Moreover, cleavage of factor B, which plays a role in alternative pathway activation, was shown to correlate with disease progression in IPF (8). Although complement system activation is associated with pulmonary fibrosis, the mechanisms and regulators of this activation are poorly understood.

In this issue of the *Journal*, Vittal and colleagues (pp. 459–470) present evidence that decay accelerating factor (DAF), a negative regulator of C3 complexes, is an important regulator of complement system activation and lung fibrosis (10). They show that DAF expression was markedly reduced in alveolar epithelial cells from patients with IPF compared with cells from those without IPF. DAF levels were also significantly reduced in bronchoalveolar lavage fluid from patients with IPF. Consistent with these data, bleomycin-induced lung injury resulted in loss of DAF expression in mouse lungs. The authors then used intranasal lentiviral delivery to promote DAF expression in the lungs of bleomycin-treated mice. Lentiviral DAF expression led to decreased complement system activation and significantly reduced lung fibrosis. Conversely, *in vivo* siRNA-mediated knockdown of DAF resulted in increased bleomycin-induced complement system activation and markers of fibrosis.

What is the mechanism by which DAF deficiency—and, thus, complement system activation—worsens fibrosis? The authors provide evidence that DAF protects the alveolar epithelium from apoptosis as lentiviral DAF expression reduced TUNEL staining and caspase activity after bleomycin. It is important to note that the authors instilled lentiviral DAF into the lungs of mice at 14 days after bleomycin treatment. Thus, the rescue of epithelial apoptosis and fibrosis in these mice is independent of the effects of DAF and complement on the acute lung injury and inflammatory phases of the bleomycin model. DAF expression was associated with reduced markers of endoplasmic reticulum (ER) stress in lung epithelial cells both *in vivo* and *in vitro*. Although not explicitly proven, the authors' data suggest that DAF deficiency leads to ER stress–induced epithelial dysfunction and apoptosis resulting from elevated C3 activity.

The study by Vittal and colleagues sheds light on an underappreciated mechanism underlying the pathogenesis of lung fibrosis. Although complement system activation has been associated with lung fibrosis for decades, the regulators of this activation are poorly understood. As the etiology of IPF remains unknown and disease incidence and prevalence increase, a thorough understanding of the regulation of complement system in IPF becomes even more important. This study suggests that DAF is a major regulator of the complement system in the context of lung fibrosis. Further study into the regulators of DAF in fibrotic lungs is thus warranted. The authors' current and past studies suggest a role for complement system–mediated release of DAF from epithelial cell surfaces as well as regulation by transforming growth factor β signaling and the ER stress response (11, 12); however, it is far from clear whether these or other pathways are the major regulators of DAF in lung epithelial cells. Hypoxia, for instance, is a condition that exists in fibrotic lungs and has been shown to inhibit DAF expression in epithelial cells (13).

This study also raises questions about the role of DAF in regulating the complement system in earlier phases of lung injury. Although lentiviral expression of DAF allowed the authors to target the fibrotic phase of bleomycin-induced lung injury, how DAF

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contributes to the acute phase of lung injury is unknown. Previous studies have shown that C5-deficient mice exhibit greatly increased inflammation and reduced survival after bleomycin-induced lung injury. However, when these mice received a low dose of bleomycin, they displayed reduced fibrosis compared with wild-type mice (14). Thus, the specific condition of the lung may play an important role in determining whether targeting the complement system is beneficial or detrimental to outcomes.

Fibrosis is a condition that can affect all tissues. DAF downregulation and complement system activation have also been associated with glomerulosclerosis, suggesting a common link (15). Moreover, patients with severe coronavirus disease (COVID-19) show prominent activation of the complement system in their lungs, skin, and sera, and single-nucleotide variants of DAF are risk factors for adverse clinical outcomes after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (16, 17). How these genetic variants may regulate SARS-CoV-2–induced lung fibrosis or play a role in the pathogenesis of IPF would be interesting future questions to address. The study by Vittal and colleagues is, thus, very timely and a good starting point on which to build. ■

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