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Chasing the "Holy Grail": Modulating Neutrophils in Inflammatory Lung Disease

Acute respiratory distress syndrome (ARDS) is a devastating condition characterized by severe hypoxemia, the accumulation of noncardiogenic pulmonary edema, and lung inflammation. ARDS affects >10% of all patients admitted to ICUs worldwide, and 35–45% of these patients die (1), predominantly of multiorgan failure. Survivors of ARDS are often left with significant long-term morbidities, and the healthcare costs associated with ARDS even 3–5 years after diagnosis match those of chronic conditions such as cardiac failure and chronic obstructive pulmonary disease (COPD) (2). Despite decades of research and many clinical trials, there remains no effective pharmacotherapy for ARDS.

Patients with neutropenia illustrate only too well the critical role of neutrophils in the defense of the host against invading microbes. However, although neutrophils are the "first responders" of the innate immune system, dysregulated neutrophilic inflammation occurs in a variety of acute and chronic lung conditions, including ARDS, COPD, bronchiectasis, and some subtypes of asthma (3). Neutrophils have long been recognized as key cells in the pathogenesis of ARDS, with clinical studies showing that neutrophil accumulation within the pulmonary vasculature occurs early in the evolution of the condition (4), and neutrophilic alveolitis is a histological hallmark (5). Neutrophilia is common in the BAL fluid of patients with ARDS (6), and the extent correlates with clinical outcome (7). Experiments using *ex vivo* neutrophils have shown marked alterations in the phenotype and function of cells obtained from the blood and alveolar compartments of patients with ARDS, including reduced rates of constitutive apoptosis (8, 9).

Neutrophils rely almost exclusively on anaerobic glycolysis for the generation of ATP and are therefore exquisitely adapted to undertake their functions in hypoxic environments (10). However, hypoxia has been shown to have marked effects on neutrophil function, including prolonging the lifespan (reducing apoptosis), enhancing degranulation, and impairing reactive oxygen species generation and oxidase-dependent killing of organisms such as *Staphylococcus aureus* (11). The effect of hypoxia on neutrophil survival has been shown to be mediated via stabilization of the transcription factor HIF-1α (hypoxia-inducible factor-1α), which can itself be regulated via a family of enzymes that includes the PHDs (prolyl hydroxylase domain–containing enzymes).

The therapeutic "holy grail" for neutrophil-associated inflammatory lung disease is a treatment that can reduce the unwanted effects of neutrophils without compromising host defense. Building on their previous work demonstrating the critical role of PHD2 in human neutrophils (12), in this issue of the *Journal*, Harris and colleagues (pp. 235–246) show for the first time in both murine models and human neutrophils that IL-4 is able to ameliorate the delayed apoptosis of neutrophils resulting from exposure to hypoxic environments (13). The observed IL-4–induced effect on neutrophil apoptosis was mediated via STAT 3/6 (signal transducer and activator of transcription 3/6) signaling, and was dependent on the expression of PHD2, which downstream led to the degradation of HIF-1α and increased rates of neutrophil apoptosis. Interestingly, IL-4 increased neutrophil apoptosis both under hypoxic conditions and after LPS challenge, without reducing the number of neutrophils initially recruited to sites of inflammation or compromising reactive oxygen species generation, raising the possibility that this may be an axis via which the delayed clearance of neutrophils observed in inflammatory disease may be targeted without impairing the host defense.

Of particular note, IL-4 was effective in modulating neutrophil lifespans even when administered after the onset of inflammation, supporting the concept that it may be suitable as a therapy for patients with established neutrophilic inflammation. To investigate whether the observations made in mice and purified human neutrophils were relevant to human disease, Harris and colleagues sampled BAL from patients with ARDS. They found that the lavage contained elevated concentrations of IL-4 compared with samples obtained from the lungs of healthy control subjects, and furthermore that circulating blood neutrophils from patients with ARDS exhibited increased expression of IL-4 receptor α.

Translating IL-4 into a therapy for inflammatory lung disease will require much more work, including exploring whether IL-4 supplementation can, in addition to modulating neutrophil lifespans, improve organ dysfunction and ultimately clinical outcomes. However, the incidence of neutrophil-associated inflammatory disease in which this therapeutic strategy may be of benefit is high and currently represents a pressing and unmet clinical need. The required initial steps along the translational path will likely include validating the current finding of elevated concentrations of IL-4 in the lungs of patients with inflammation, as well as demonstrating increased expression of the IL-4 receptor on neutrophils obtained from the same compartment—something that

Originally Published in Press as DOI: 10.1164/rccm.201902-0333ED on March 13, 2019

was not achieved in the current study due to technical limitations. Methods for purifying neutrophils from the lungs of patients with respiratory disease are now well developed (14) and could readily be applied. Given the syndromic nature of conditions such as ARDS, COPD, and asthma, it is possible that not all patients would benefit from therapeutically targeting neutrophil clearance via IL-4, and there may be subgroups of patients for whom this mechanism is more or less relevant. Work on defining mechanistic subtypes of inflammatory lung disease is underway by a number of investigators, and it would be interesting to determine whether there are groups of patients in whom delayed neutrophil clearance is particularly important.

Although targeted therapies for inflammatory lung disease are currently a long way from the bedside, the work from Harris and colleagues highlights the vital importance of basic science in providing deep mechanistic insights that will allow us to more fully understand the nuances of complex cellular behaviors and that are essential for the development of such therapies. To finally, after many decades, make meaningful progress toward developing pharmacotherapy for conditions such as ARDS will require collaboration among basic science, translational, and clinical researchers, and Harris and colleagues should be congratulated for setting out on this journey. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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