Is It Still "Idiopathic"? Features of Autoimmunity in Idiopathic Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a rare but devastating cardiopulmonary disease marked by progressive remodeling of the pulmonary vascular bed, which typically leads to right ventricular failure and death and/or lung transplantation (1). Over the preceding 25 years, multiple therapies, all aimed at pulmonary vasodilation, have been approved for use and implemented in care guidelines. Although vasodilation extends transplantation-free survival and improves functional performance for most patients, curative approaches remain elusive (2). Elucidating the underlying pathophysiologic events that initiate and/or perpetuate the PAH state may open new avenues of impactful therapies. For many years, substantial effort has focused on the potential contribution of immune dysregulation and/or overt autoimmune activity to PAH pathogenesis, with associated therapeutic approaches ranging from systemic steroid exposure to advanced biologic manipulations (e.g., specific cytokine inhibitors). To date, no therapeutic approach to modify the immune response has proved definitively effective, although comprehensive randomized clinical trials are lacking in most circumstances.

Although idiopathic PAH (IPAH), by definition, has no definitive disease association, it is a member of the broader classification group of entities hemodynamically linked together as PAH. Among these is connective tissue disease (CTD)–associated PAH, which of course by definition is PAH in the setting of a detectable CTD, such as systemic sclerosis (3). PAH entities not only share hemodynamic properties, but they are characterized by a female predominance as well as features of exuberant immune cell activity, if not overt autoimmunity. Histologic and molecular studies of lung tissue, lung-derived cells, and circulating cells suggest perturbed regulation of immune cell distribution and perhaps activity in PAH. Systemically, numerous studies of biologic markers indicate an exuberant inflammatory milieu and/or a lack of inflammatory control (recently reviewed in Reference 4). Intriguingly, and in support of the notion of immune cell–associated pathogenesis, a growing number of studies have published results potentially supportive of the prospect of bone marrow transplantation as a therapeutic approach to PAH or that at least implicate bone marrow–derived cells in pathogenesis.

On this scientific background, in their article in this issue of the *Journal*, "Autoimmunity Is a Significant Feature of Idiopathic Pulmonary Arterial Hypertension," Jones and colleagues (pp. 81–93) demonstrate, using multiple methodological approaches in the largest such study of IPAH conducted to date, that patients with IPAH possess an autoimmune signature (5). Specifically, the investigators report IPAH is associated with an altered humoral immune response, which may ultimately propel a change in how patients with IPAH are approached and treated if confirmed and expanded.

The study showed that patients with IPAH have an immune phenotype defined by a shift in the adaptive immune response axis. The investigators characterized IPAH by a distinct immune profile of altered B-cell frequencies, increased circulating T follicular helper (cT_{FH}) cells, and an altered regulatory T-cell profile, an immune phenotype indicative of an activated immune response and previously shown to be perturbed in PAH (recently reviewed in Reference 6). Consistent with this, the authors report that IL-21 concentrations were significantly increased ($P < 0.0001$) in subjects with IPAH. IL-21 is an autocrine cytokine produced predominantly by cT_{FH} and T-helper cell type 17 cells, not previously measured in subjects with IPAH. It plays an important role in the immune system by promoting proliferation and development of cT_{FH} and T-helper cell type 17 cells, balancing T-helper cell type subsets, inducing B-cell generation and differentiation into plasma cells, and enhancing the production of immunoglobulin. Consistent with this, IL-21 is implicated in the promotion of autoimmune disease, with increased concentrations positively associated with cT_{FH} cells, plasma cells, autoantibodies, and disease activity in CTD (7).

Although Jones and colleagues found that the major subclasses remained unchanged compared with healthy control subjects, IgG3 concentrations ($q = 0.0392$) were elevated in subjects with IPAH. This is an interesting finding, as IgG3 concentrations and proportions of IgG3 in serum are identified as potential prognostic markers of B-cell dysfunction in people with a clinically isolated syndrome with immunopathogenesis that rapidly converts to multiple sclerosis (8). IgG3 is often one of the earliest subclasses to be elicited against protein antigens upon infection, so IgG3 concentrations might indicate an active antibody response, for example, IgG3 concentration elevation in response to a viral pathogen that triggers an immune response (9). Although infectious disease features and/or lymphopenia are not reported in this IPAH cohort, autoantibodies of ribonucleic protein (RNP) complex are found to be elevated. Anti-RNP antibodies bind and penetrate lymphocytes, which can cause impairment in lymphocyte function and may interfere with cellular immunity (10, 11).

Autoantibodies are recognized as a component of classification criteria for CTD (12–14). By definition, IPAH does not meet criteria for CTD (15). The clinical differentiation of CTD-related PAH in patients with systemic sclerosis, mixed CTD

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and systemic lupus erythematosus and IPAH is important because of possible endpoint differences in treatment response, exercise capacity, oxygen requirements, dyspnea, quality of life, and, most important, survival (16–18). Jones and colleagues found that multiplexed autoantibodies were significantly raised in subjects with IPAH, and clustering demonstrated three distinct clusters: "high autoantibody", "low autoantibody", and a small "intermediate" cluster exhibiting high concentrations of RNP complex. The high-autoantibody cluster had worse hemodynamics for pulmonary vascular resistance and cardiac output but improved survival despite no significant differences in treatment. This is particularly interesting in the context of mixed CTD regardless of PAH, in which the presence of high titers of antibodies to U1RNP is believed to modify the expression of the CTD relevant to prognosis and treatment, but U1RNP can disappear during the course of the disease (13, 19).

The authors extended these findings with a focus on the predominant molecular pathway associated with PAH, BMPR2 (bone morphogenetic protein receptor 2) signaling (5). Putative serum autoantibodies against BMPR2 were detected in patients with IPAH ($n = 5$) but not in patients with other autoimmune etiologies ($n = 11$). The authors then screened 350 subjects with IPAH and heritable PAH as well as 55 healthy donor control subjects to detect IgG reactivity against a peptide of the extracellular domain of human BMPR2, and they found that bound immunoglobulins were significantly increased in patients with IPAH ($P = 0.038$). These results support the role of autoantibody screening in the evaluation of patients with PAH and the possible value of longitudinal assessment, although additional studies of the impact on long-term outcomes in larger cohorts are warranted.

The work by Jones and colleagues advances the field while raising many new questions. In addition to the inherent need for validation and expansion in IPAH and other PAH subtypes, the findings may have implications for both therapy and comorbid disease screening approaches. We resist the urge to speculate on the use of broad-based or focused immune modifiers as both beyond the scope of this editorial and in need of additional study, while recognizing the value of immune system control for subjects with true CTD-associated PAH. However, this work does raise the question as to whether some of our patients with IPAH are truly idiopathic or with occult autoimmune features. And is our CTD screening detailed enough at diagnosis? Furthermore, should CTD screening be revisited more frequently over time? Although the role of IL-21 and IgG3 concentrations in the generation of autoantibodies demands additional study, Jones and colleagues provide new potential mechanisms to improve the accuracy of IPAH diagnosis and ultimately individualize treatment strategies, adding more reason for optimism from patients and providers. ■

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Mycobacterium tuberculosis: A Pathogen That Can Hold Its Breath a Long Time

In this issue of the *Journal*, Bucşan and colleagues (pp. 94–104) present an extensive comparative analysis of two widely used, virulent strains of *Mycobacterium tuberculosis* (Mtb) in the nonhuman primate (NHP) model (1). They found that the Erdman strain was considerably more virulent than the CDC1551 strain by multiple parameters: larger areas of necrotic granulomas, poorer survival, increased bacterial lung burden, increased lung pathology associated with greater systemic inflammation, and a high and early inflammatory myeloid cell influx to the lung with evidence of greater macrophage and T-cell activity in the lung. Comparison of gene expression signatures in the lungs of infected animals by RNA sequencing revealed that pathways associated with the hypoxia response and lung tissue remodeling were induced to higher degrees in Erdman-infected animals.

Combining the observation of hypoxia response genes being elevated in the Erdman-infected animals with the fact that the Erdman-induced necrotic granulomas demonstrated impressively larger volumes and higher bacterial burdens than those from CDC1551, the authors hypothesized that the Erdman strain might be better able to survive and proliferate under hypoxic conditions. To evaluate this, bacterial RNA sequencing was used to study the responses of the two strains to hypoxia *in vitro*. The authors found that transcription of the DosR (dormancy survival regulator) regulon was significantly elevated in the Erdman strain compared with CDC1551. Even when *in vitro* hypoxia was terminated and the bacteria were grown in reaerated conditions, the DosR regulon gene members remained highly expressed in the Erdman strain, whereas expression of these genes returned to basal levels in CDC1551.

DosR is part of a bacterial two-component gene regulatory system that controls the expression of a 48-gene regulon first identified by Sherman and colleagues as a key regulator of hypoxia in Mtb (2). Indeed, it is already known that Mtb DosR regulon mutants fail to persist or cause disease in the NHP model (3). However, before this study, the impact of DosR expression magnitude and its response to reaeration had not been studied in detail in the NHP model. Hence, the importance of this study is that it underscores a key

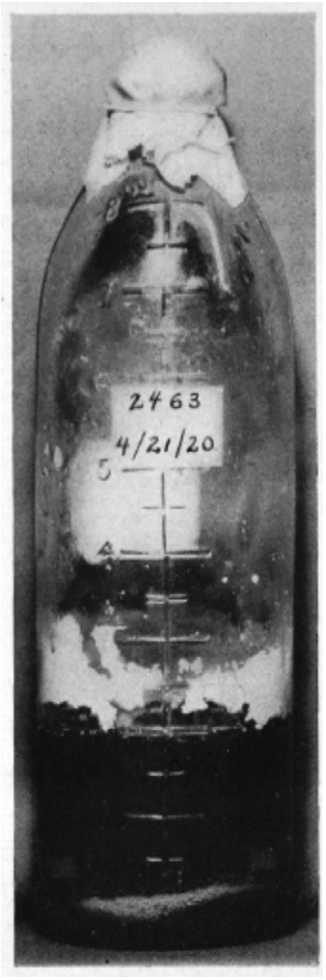

Figure 1. A sealed culture of *Mycobacterium tuberculosis* inoculated on April 4, 1920, and held at 37°C until the spring of 1932. Cultures from the sediments of this bottle grew viable *M. tuberculosis*, which was shown to be virulent in guinea pigs. Reprinted from Reference 7.

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