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Pulmonary Hypertension Caused by Interstitial Lung Disease
A New iNK(T)ling into Disease Pathobiology

Interstitial lung disease (ILD) encompasses a heterogeneous group of conditions characterized by restrictive lung physiology and impaired gas transfer caused by lung parenchymal destruction with varying degrees of inflammation and fibrosis. The development of pulmonary hypertension (PH) in the context of ILD (PH-ILD) has a substantial impact on morbidity and mortality (1–3). In fact, among PVDOMICS (Redefining Pulmonary Hypertension through Pulmonary Vascular Disease Phenomics) study subjects with Groups 1–5 PH according to the World Symposium on Pulmonary Hypertension classification, those with Group 3 PH (>50% with PH-ILD) had the lowest transplant-free survival (4). Therefore, a sense of cautious optimism has emerged since the results of the INCREASE study (5, 6) and U.S. Food and Drug Administration approval of inhaled treprostinil, the first and only U.S. Food and Drug Administration–approved treatment for PH-ILD. Nonetheless, the substantial impact of PH-ILD on quality of life, functional capacity, and survival underscores the urgent need for translational studies that elucidate additional treatment paradigms.

In this issue of the *Journal*, Jandl and colleagues (pp. 981–998) describe a novel link between natural killer T (NKT) cell deficiency and pulmonary vascular fibrosis (7). Perivascular type I collagen deposition was increased in lung tissue from patients with PH-ILD, a cohort composed mainly of patients with idiopathic pulmonary fibrosis, chronic hypersensitivity pneumonitis, and unclassified ILD, compared to samples from ILD patients without PH and donor lung controls. Multicolor flow cytometric analysis of immune cell subsets from isolated pulmonary arteries revealed an overall increase in perivascular $CD3^+$ lymphocytes in patients with PH-ILD compared to samples from donor lungs but a significantly lower proportion of NKT cells ($CD3^+/CD56^+$). Lower concentrations of IL-15, responsible for NKT cell maturation and survival, in lung tissue and plasma from patients with PH-ILD compared to controls further substantiated the observed perivascular NKT cell deficiency. Notably, NKT cell activation with a synthetic analog of α-galactosidase (KRN7000) not only preserved NKT cell ($CD3^+/NK1.1^+/TCR\beta$) number but also reduced pulmonary vascular muscularization, right ventricular systolic pressure, and right ventricular hypertrophy in mice with bleomycin-induced pulmonary fibrosis. Previous studies have demonstrated that NKT cell deficiency worsens lung fibrosis and increases mortality in the bleomycin-induced pulmonary fibrosis murine model (8) and that NKT cell activation with KRN7000 in mice attenuates lung fibrosis induced by intratracheal bleomycin (9). However, the impact of NKT cell activation on the pulmonary vasculature in this model was previously unrecognized.

The authors then determined that STAT1 expression was significantly reduced in isolated pulmonary arteries from patients with PH-ILD compared to both donor controls and vessels from ILD patients without PH. Expression appeared specifically decreased in

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pulmonary artery smooth cells (PASMCs). In contrast, although KRN7000 treatment increased STAT1 expression, baseline STAT1 concentrations in lung homogenates from vehicle-treated mice after intratracheal bleomycin instillation was similar to those in healthy control mice. Coculture of KRN7000-treated human peripheral blood mononuclear cells (PBMCs) was sufficient to increase STAT1 expression and activation as well as block transforming growth factor (TGF)-β–induced type I collagen production in human PASMCs, thus linking NKT cell stimulation with activation of STAT1 signaling and reduction of TGF-β–induced fibrosis in PASMCs. Culture of precision-cut lung slices (PCLSs) from end-stage fibrotic lung explants with KRN7000-treated PBMCs similarly decreased parenchymal collagen and vascular remodeling, albeit with variable efficacy across patient samples.

The authors then explored the secretome of KRN7000-treated healthy donor PBMCs to determine candidate antifibrotic mediators. Interestingly, while both plasma protein concentration and lung mRNA expression of CXCL9 was reduced in patients with PH-ILD, treatment with KRN-7000 increased CXCL9 secretion from human PBMCs. Expression of CXCL9 was also increased in lung tissue of mice treated with KRN7000 before bleomycin-induced fibrosis and in PH-ILD PCLSs after coculture with KRN7000-treated PBMCs. Additional *in vitro* mechanistic studies revealed that, similar to coculture with KRN7000-treated PBMCs, CXCL9 stimulation also blocked TGF-β–induced type I collagen production in human PASMCs, and this antifibrotic effect was reversed by a CXCR3 inhibitor.

Jandl and colleagues are to be commended for their use of several complementary experimental approaches, including clinical specimens (blood and lung tissue), an animal model, an *in vitro* coculture system, and an *ex vivo* assay of lung parenchymal fibrosis using PCLSs. With these tools in hand, the authors identified a lower fraction of perivascular NKT cells, reduced STAT1 expression in isolated pulmonary arteries, and decreased CXCL9 concentrations in the blood and lung tissue of patients with PH-ILD. Activation of NKT cells (and potentially other immune subsets such as monocytes) with KRN7000 was associated with reduced PASMC collagen production, increased PASMC STAT1 expression and activation, and increased PBMC CXCL9 production, observations that were largely mirrored in an animal model and *ex vivo* PCLSs.

Although PASMC STAT1 concentrations appear to be reduced in patients with PH-ILD, STAT1 expression was highest within endothelial cells of remodeled vessels. Constitutive STAT1 activation (Ser701 phosphorylation) and an IFN-biased gene signature were recently shown to be a direct consequence of caveolin-1 deficiency in pulmonary artery endothelium, and endothelial STAT1 activation was also increased in explanted lung tissue from patients with idiopathic pulmonary arterial hypertension (PAH) (10). Likewise, the development of PAH in a subset of patients treated with IFN (11) suggests that the implications of pulmonary vascular STAT1 activation are likely cell type and context dependent. Although alterations in circulating NK cell abundance and function have been observed in PAH (12) and NK cell–deficient mice spontaneously develop mild PH (13), here the proportion of lung perivascular NK cells ($CD3^{-}/CD56^{+}$) was unaltered in PH-ILD.

A major limitation of the study by Jandl and colleagues is the relatively small sample size, and therefore their findings need to be assessed in a larger PH-ILD cohort. In addition, the study cohort was composed largely of patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis as well as several samples from patients in whom the underlying etiology of ILD could not be further classified. As a result, their findings may not be generalizable to other fibrotic lung diseases. For example, patients with connective tissue disease–associated PH-ILD were particularly underrepresented. Reconciling whether NKT cell/STAT1/CXCL9 deficiency exists in this population would be particularly interesting, given evidence of IFN gene activation in lung tissue from these patients (14).
In addition, future studies should include the assessment of KRN7000 treatment initiated after the establishment of disease as well as testing NKT activation in alternative PH-ILD models to further substantiate the efficacy of this approach. As we enter a new therapeutic era for patients with PH-ILD, the present study not only advances our understanding of pulmonary vascular fibrosis but also offers hope for future treatment paradigms that target mechanisms beneath the surface (15). ■

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Do Coarse Mass Particles Increase Daily Mortality? New Findings from a Multi-Country, Multi-City Study

The thousands of liters of air inhaled daily contain myriad particles that are diverse in size, origin, composition, and potential toxicity. For regulatory and air pollution control purposes, airborne particulate matter (PM) is classified by aerodynamic diameter (1, 2). At present, air pollution control is focused on reducing PM $\leqslant$ 2.5 μm in aerodynamic diameter ($PM_{2.5}$), a size range encompassing combustion-generated particles that reach the smaller airways and alveoli. For $PM_{2.5}$, the U.S. Environmental Protection Agency (EPA) first promulgated an annual National Ambient Air Quality Standard (NAAQS) in 1997 at 15.0 $\mu g/m^3$, which was tightened to 12.0 in 2012. The recently revised World Health Organization (WHO) Air Quality Guidelines propose an $PM_{2.5}$ level at 5.0 $\mu g/m^3$, lower than levels in most urban areas. There is also a NAAQS for PM $\leqslant$10 μm in aerodynamic diameter (PM_{10}), which includes both $PM_{2.5}$ and larger particles in the size range from $PM_{2.5}$ up to PM_{10}.

In the United States, the increasingly stringent NAAQS for PM have driven improvements in air quality (3). However, PM in the 2.5 to 10 μm band is not specifically regulated and the potential toxicity of particles in this size range has not received the attention directed at smaller particles. The larger particles are primarily crustal in origin, and include road and desert dust and some bioaerosols, e.g., pollens (1). These larger particles are deposited in the upper airway and the larger airways of the lung, where they can cause injury through inflammation and other mechanisms. The most common indicator for particles in this size range, generally referred to as coarse mass PM, is the mass difference between PM_{10} and $PM_{2.5}$, i.e., $PM_{10-2.5}$. Given their differing sources, strategies to address $PM_{2.5}$ may have little impact on $PM_{10-2.5}$.

To date, the evidence on $PM_{10-2.5}$ has not been considered as sufficient to warrant regulation. The most recent EPA Integrated Science Assessment (ISA) for PM found the evidence for adverse health effects of $PM_{10-2.5}$ to be unconvincing and the WHO Guidelines, while covering PM_{10}, did not consider $PM_{10-2.5}$ (1).

In this issue of the *Journal* (pp. 999–1007), Liu and colleagues, a large international collaborative team, report the findings of a pooled daily time-series analysis that assesses associations of $PM_{10-2.5}$ with daily counts for all deaths, respiratory deaths, and cardiovascular deaths in 205 cities in 20 countries (4). The investigators find significant positive associations with each of the three outcomes and the associations are robust to consideration of other pollutants. Additionally, as found with $PM_{2.5}$ in other studies, the modeled exposure-response relationships showed an association down to the lowest concentrations, weighing against thresholds that might anchor regulations and guidelines (5). The 20 countries span a range of environments, although most are high- or middle-income. There was significant but explained variation across the three WHO regions considered. For the 20 countries included, desert dust was not a major source of $PM_{10-2.5}$ so that new insights were not gained on this problematic contributor to coarse mass PM.

The Multi-City Multi-Country (MCC) Collaborative Research Network performed this study (6). The group has used a large pooled data set to carry out multiple analyses directed at air pollution and temperature and health. The Network's analyses

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