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ILC2 the Rescue?

In this issue of the *Journal*, Monticelli and colleagues (pp. 63–72) are the first to describe innate lymphoid cell (ILC) subsets in donor lungs before and after reperfusion in allograft transplantation, and they correlate the ILC subsets with primary graft dysfunction (PGD) (1). In a cohort of patients who underwent lung transplant for chronic obstructive pulmonary disease or interstitial lung disease at the University of Pennsylvania, there was a selective decrease in the percentage of group 2 ILC (ILC2s) in patients who developed PGD. Those patients who did not have PGD had an increased frequency of ILC2s after allograft perfusion, suggesting that these cells may protect against PGD.

ILC2s comprise one subset of the five major groups of ILC, which also include natural killer (NK) cells, lymphoid tissue inducer cells, ILC1s, and ILC3s (2). These subsets are defined by the transcription factors that regulate their differentiation and the cytokines that they secrete. Although NK cells were discovered more than 40 years ago (3, 4) and lymphoid tissue inducer cells were identified over 20 years ago (5), the other ILC subsets were first described within this decade. ILC1s produce IFN-γ as their signature cytokine and have Tbet as their master transcription factor. ILC3s produce IL-17A and IL-22 while using RORc as the key transcription factor (6).

The increased number of ILC2s in the lungs of patients who did not have PGD is particularly interesting and may be relevant to protection against PGD. ILC2s express the transcription factor GATA-3 while secreting IL-5, IL-9, IL-13, and amphiregulin, in addition to IL-4 under certain circumstances (6). It is tempting to consider the possibility that the increased number of ILC2s in patients who did not experience PGD may have provided protection against disease as a result of the cytokines they produce. Several cytokines produced by ILC2s may promote tissue repair in the lung. For instance, amphiregulin is a member of the EGF (epidermal growth factor) family and is related to TGF-α (transforming growth factor α) (7). Amphiregulin promotes the restoration of tissue integrity after damage from either acute or chronic inflammatory processes. Amphiregulin is produced not only by ILC2s but also by epithelial cells and immune cells that are predominantly, but not exclusively, associated with type 2 responses, such as mast cells, basophils, and eosinophils. IL-4 and IL-13 promote macrophage differentiation toward alternatively activated macrophages that produce TGF-β, and these cells are also important in tissue repair (8). A previous study demonstrated that IL-9 produced by ILC2 acts in an autocrine manner to amplify ILC2 survival and function, and in a mouse model of *Nippostrongylus brasiliensis* infection showed that IL-9 was crucial for restoring pulmonary tissue integrity and lung function (9). Although amphiregulin and IL-13 are involved in tissue repair, they also contribute to fibrosis by depositing connective tissue proteins such as collagen and fibronectin in sites of injury. The balance of the restoration/fibrosis response in the lung and the fine-tuning mechanisms that control repair versus an overexuberant fibrotic response are still being defined.

Although the data in the work by Monticelli and colleagues are very interesting, there are several caveats that must be recognized in interpreting their data (1). First, the number of subjects studied was very low. For example, the authors began with 18 subjects but were variably able to obtain meaningful pre- and postperfusion ILC data from as few as 3 subjects per endpoint. Data were obtainable from 3 to 5 subjects in the PGD group and from 3 to 11 subjects in the non-PGD group. This weakens some of the conclusions that were made regarding the significance of the associations with reperfusion injury and the development of PGD. Therefore, it is difficult to know the generalizability of the data. Another possible important confounding factor is that there was on average a sizable 94-minute difference in ischemia time between patients with and without PGD, which despite the low number of patients almost reached statistical significance. This could also be likely a critical reason for graft failure, perhaps even more so than the difference in ILC populations between patients with and without PGD.

Despite these limitations, this work makes some important contributions. For the first time, the authors demonstrate the feasibility of live-cell isolation and high-resolution flow-cytometric phenotyping of immune cell populations (CD4 T cells, NK cells, ILC1s, ILC2s, and ILC3s) from small biopsy specimens for up to 18 patients. Furthermore, the unique design of this cohort study gave the investigators an opportunity to track dynamic changes in the ILC family subset composition by examining donor grafts before and immediately after reperfusion, and is highly innovative. Lastly, although the sample size was small for patients who developed PGD, the investigators were still able to observe statistically significant changes among both ILC1s and ILC2s that provide preliminary support for an association between ILC population changes and PGD development. Thankfully, the authors do not overstate the strength of their findings and acknowledge that further analysis in a larger cohort will be necessary to examine whether these cells play a mechanistic role in lung injury or repair during graft rejection.

The data from this study complement previous publications in which the numbers of neutrophils, macrophages, and lymphocytes

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were enumerated in donor tissue in the setting of PGD or non-PGD. The authors are to be congratulated for their protocol of harvesting the tissue and processing the cells in a uniform fashion, freezing the cells, and then performing the flow cytometry concurrently to avoid the confounding factor of variation in flow compensation and other possible cytometer-related differences in the results. Another strength of the analysis is that the flow strategy for cell identification is appropriate based on the current state of the art. Thus, this work adds to a growing body of literature that describes the presence of ILC2s in human diseases. The presence of ILC2s has been described in allergic rhinitis, chronic rhinosinusitis, asthma, atopic dermatitis, pleural effusion, pulmonary fibrosis, psoriasis, and graft versus host disease (10). Although ILC2s are potent producers of cytokines that are critical for the pathophysiology of many diseases, the specific importance of these cells in pathogenesis is unknown given that no methods are currently available to eliminate or suppress the function of these cells without also directly targeting other effector cells. Until such methods are available, investigators are left with the association of ILC2s with a condition, and not a definitive cause-and-effect relationship. ■

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R. Stokes Peebles, Jr., M.D.
Division of Allergy, Pulmonary, and Critical Care Medicine
Vanderbilt University School of Medicine
Nashville, Tennessee

ORCID ID: 0000-0002-1429-7875 (R.S.P.).

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Early Detection of Pulmonary Vascular Dysfunction in Neonatal Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia (BPD), a paramount morbid lung disease typically complicating premature birth, is arguably one of the most vexing clinical problems in the neonatal ICU (NICU). Although the disease is characterized by truncated parenchymal growth, both vascular and alveolar, the pathogenic mechanisms involved remain poorly understood, and the early and late pulmonary complications, particularly development of pulmonary hypertension (PH), are ominous, as they limit survival. Although the definition of BPD is based simply on oxygen dependence at 36 weeks postmenstrual age (PMA), as agreed at a 2001 NIH workshop (1), several pre- and postnatal factors have been linked to its development, including identification of early disrupted pulmonary vascular growth in the form of pulmonary vascular disease (PVD) or PH (2, 3). In fact, these factors may have more relevance to later respiratory complications in early childhood than BPD itself (4) and have led investigators to question the usefulness of the NIH definition of BPD (5). In addition, what has become clearer over the past few years is that early identification of high-risk preterm infants is important for prognostication and possibly early therapy.

In this issue of the *Journal*, Critser and colleagues (pp. 73–82) tested the hypothesis that neonatal cardiac magnetic resonance imaging (MRI) correlates with BPD severity and can predict short-term clinical outcomes, including the need for PH therapies (6). Building on their previous work showing that an MRI scoring system, based on high- and low-signal intensity lung parenchyma, can detect quantifiable BPD structural abnormalities (7), the investigators retrospectively analyzed a mixed cohort of 52 infants with various degrees of BPD severity who underwent MRI between 39 and 47 weeks' PMA on a neonatal-sized, NICU-sited 1.5T MR scanner. MR left ventricular eccentricity index (MR-EI), main pulmonary artery to aorta diameter (PA/AO) ratio, and pulmonary

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