Dendritic Cells in Pulmonary Hypertension: Foot Soldiers or Hidden Enemies?

Dendritic cells (DCs) constitute a heterogeneous and versatile group of hematopoietic antigen-presenting cells that are indispensable for a healthy immune system. At the crossroads of innate and adaptive immunity, DCs have indeed a prominent role in immune surveillance for self- and non–self-antigens, and in the initiation and orchestration of different types of antigen-specific adaptive immune responses. This first line of defense is therefore critical at barrier sites, particularly in the lungs. However, they are also involved in the pathogenesis and progression of highly prevalent respiratory conditions (1). In this issue of the *Journal*, Koudstaal and colleagues (pp. 665–680) provide evidence that enhanced NF-κB activation in DCs in mice is sufficient to cause IL-6–dependent pulmonary arterial muscularization and mild pulmonary hypertension (PH) (2).

Even if DCs express high expression of major histocompatibility complex class II molecules and CD11c, additional markers are essential to identify DCs and to categorize them into specific subtypes. A multitude of phenotypically and functionally distinct DC subtypes have been described; they are classically divided into five distinct major subsets, namely the conventional DCs (cDCs), comprising cDC1s and cDC2s, the plasmacytoid DCs, Langerhans cells, and the monocyte-derived DCs (moDCs) (3). The cDC1 subset, identified in humans by the expression of IRF8, CD141, and XCR1, is most efficient at priming cytotoxic $CD8^+$ T cells to exogenously derived antigens (cross-presentation) and polarizing $CD4^+$ T cells toward a T-helper cell type 1 (Th1) phenotype (4). IRF4, CD1c, and CD172a identify human cDC2s that are the most efficient in differentiating $CD4^+$ T cells into Th1, Th2, and Th17 in specific contexts. Human plasmacytoid DCs, positive for CD303, CD304, and CD123, secrete high amounts of IFN and support B-cell differentiation. However, such identification can be challenging because DC subset composition, maturation, and migration is dependent on multiple tissue-specific factors and can rapidly be adapted depending on the microenvironmental milieu (5, 6).

Alterations in DC subset composition and activation state are present in lungs of patients with pulmonary arterial hypertension (PAH) (7–14). Although DCs accumulate around remodeled pulmonary vessels (7, 10, 12, 13), their numbers decrease in the blood of patients with PAH (8, 9, 11), suggesting that they are likely recruited in pulmonary lymph nodes and tertiary lymphoid organs. Consistent with this notion, Perros and colleagues (15) have reported increased concentrations of CCL20, a potent chemoattractant for immature DCs, in explanted lungs of patients with PAH. They also reported the presence of CCR7 and its ligands CCL19 and CCL21, an axis that is essential for the development and maintenance of bronchus-associated tertiary lymphoid organs (15). In addition to increased DC numbers and complexity in PAH lungs, Hautefort and colleagues have demonstrated that PAH moDCs are functionally different from control moDCs, as reflected by a higher capacity to induce activation and proliferation of $CD4^+$ T cells, a lower IL-4 expression (Th2 response), and a higher IL-17 expression level (Th17 response) (8). Finally, as previously reviewed (14), additional DC alterations are also found in other PAH clinical subgroups, especially those associated with connective-tissue disorders. Collectively, these findings support the idea that DC homeostasis is altered in PAH; however, it is unclear whether such alterations are the cause of disease development and/or progression or a consequence of it, and precisely how DCs contribute to PAH pathogenesis remains unknown.

In their study, Koudstaal and colleagues (2) examined the lung and cardiac effects of enhanced cDC activation in adult mice, through DC-mediated deletion of the NF-κB negative regulatory protein A20 (encoded by *Tnfaip3*). To accomplish this aim, they crossed mice with loxP-flanked *Tnfaip3* alleles with mice expressing Cre under the control of a promoter region of *Clec9a* (encoding DNGR1) that has a restricted expression profile in cDCs. Using histology and immunohistology, they found that $Tnfaip3^{DNGR1}$-knockout (KO) mice display pronounced lung accumulation of activated cDC1s, cDC2s, and moDCs, accompanied by peribronchial and perivascular infiltration of activated T and B cells, macrophages, and IgA-producing plasma cells. Furthermore, they show that these mice developed spontaneous PH with mild right ventricular (RV) hypertrophy. They also noted high levels of IL-1β, IL-6, IL-10, and TGFβ and high proportions of IL-10– and IFNγ-producing $CD4^+$ and $CD8^+$ T cells and of IL-17A–producing $CD4^+$ T cells in $Tnfaip3^{DNGR1}$-KO mouse hearts and lungs. Using an indirect immunofluorescence assay on human epithelial type 2 cells and *Rag1*-deficient mice, the authors show that autoreactive IgA recognizing lung vasculature is present in the serum of $Tnfaip3^{DNGR1}$-KO mice. Remarkedly, the PH phenotype was markedly attenuated in the groups receiving IL-6–neutralizing antibodies versus control antibodies.

A key finding of this study is the demonstration that changes in DC composition, maturation, and migration in mice are sufficient to cause structural and functional changes of the pulmonary vasculature. Spontaneous DC activation and subsequent T- and B-cell activation induced by the loss of A20 in DCs were indeed found to be sufficient to produce the PH phenotype in ∼50% of $Tnfaip3^{DNGR1}$-KO mice in the absence of other stimuli. These findings, taken with the observation that arterial DC accumulation precedes vascular and hemodynamic alterations in monocrotaline-injected rats (13), are strong arguments supporting the idea that DCs are critical in the maintenance of immune surveillance and lung homeostasis, as well as in PAH pathogenesis. Interestingly,

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mice lacking the DC homing receptor CCR7 also display a marked lung inflammatory phenotype and develop spontaneous PH (16). Moreover, although anti–IL-6 receptor therapy does not produce beneficial effects on pulmonary vascular resistance in patients with PAH, in this study, the anti–IL-6 treatment was associated with a normalization of pulmonary pressures and RV hypertrophy in *Tnfaip3*^{DNGR1}-KO mice. Because IL-6 influences antigen-specific immune responses and inflammatory reactions, as well as prosurvival signals in pulmonary vascular smooth muscle cells (17), these findings offer new insight into the complexity of the link between dysregulated immunity and adverse PAH pulmonary arterial remodeling.

Although the findings presented in this study are exiting, a number of questions remain. For obscure reasons, only ∼50% *Tnfaip3*^{DNGR1}-KO mice develop PH with variable phenotypic severity. Because these mice display chronic liver inflammation associated with collagen deposition (18), it would be important to explore the possible relationships between these two different manifestations. Similarly, because *Tnfaip3*^{DNGR1}-KO mice display RV hypertrophy before hemodynamic changes develop, further work is also needed to dissect the role of DCs in cardiac remodeling and function. To reinforce the translational relevance of these findings, it would also be important to determine how the local PAH environment and current therapies affect the NF-κB pathway and the specific characteristics of DC subsets.

Despite these remaining questions, the observations reported by Koudstaal and colleagues (2) should encourage a better understanding of the contribution of DCs in PAH. Clarifying these questions would be a milestone in the development of effective antiinflammatory and immunomodulatory therapies for PAH and other cardiovascular disorders. ■

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