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T-Helper 17 Cell Polarization in Pulmonary Arterial Hypertension

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BACKGROUND: Inflammation may contribute to the pathobiology of pulmonary arterial hypertension (PAH). Deciphering the PAH fingerprint on the inflammation orchestrated by dendritic cells (DCs) and T cells, key driver and effector cells, respectively, of the immune system, may allow the identification of immunopathologic approaches to PAH management.

METHODS: Using flow cytometry, we performed immunophenotyping of monocyte-derived DCs (MoDCs) and circulating lymphocytes from patients with idiopathic PAH and control subjects. With the same technique, we performed cytokine profiling of both populations following stimulation, coculture, or both. We tested the immunomodulatory effects of a glucocorticoid (dexamethasone [Dex]) on this immunophenotype and cytokine profile. Using an epigenetic approach, we confirmed the immune polarization in blood DNA of patients with PAH.

RESULTS: The profile of membrane costimulatory molecules of PAH MoDCs was similar to that of control subjects. However, PAH MoDCs retained higher levels of the T-cell activating molecules CD86 and CD40 after Dex pretreatment than did control MoDCs. This was associated with an increased expression of IL-12p40 and a reduced migration toward chemokine (C-C motif) ligand 21. Moreover, both with and without Dex, PAH MoDCs induced a higher activation and proliferation of $CD4^+$ T cells, associated with a reduced expression of IL-4 (T helper 2 response) and a higher expression of IL-17 (T helper 17 response). Purified PAH $CD4^+$ T cells expressed a higher level of IL-17 after activation than did those of control subjects. Lastly, there was significant hypomethylation of the IL-17 promoter in the PAH blood DNA as compared with the control blood.

CONCLUSIONS: We have highlighted T helper 17 cell immune polarization in patients with PAH, as has been previously demonstrated in other chronic inflammatory and autoimmune conditions.

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ABBREVIATIONS: CCL = chemokine (C-C motif) ligand; DC = dendritic cell; Dex = dexamethasone; GC = glucocorticoid; hPAH = heritable pulmonary arterial hypertension; IFN = interferon; iPAH = idiopathic pulmonary arterial hypertension; MHC = major histocompatibility complex; MLR = mixed leukocyte reaction; MoDC = monocyte-derived dendritic cell; PAH = pulmonary arterial hypertension; PBMC = peripheral blood mononuclear cell; Th = T-helper; tLT = tertiary lymphoid follicle; TNF = tumor necrosis factor

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Pulmonary arterial hypertension (PAH) is characterized by a progressive increase in pulmonary vascular resistance, leading to right ventricular failure and ultimately death.[1] PAH has a complex and multifactorial pathogenesis in which excessive migration and proliferation of pulmonary vascular cells (ie, endothelial cells and smooth muscle cells) and dysregulated immune responses are critical contributors to inappropriate pulmonary vascular remodeling.[2] Inflammation is a common denominator of the hallmark lesions of PAH[3-5] and of its animal models.[6] The heritable form of PAH (hPAH) is usually (> 80%) caused by mutations in the *BMPR2*.[7] In animal models, it has been shown that greater endothelial injury and enhanced inflammatory response could be the underlying causes of the sensitivity and may work in concert with *BMPR2* heterozygosity to promote the development of persistent pulmonary hypertension.[8]

Different studies have shown increased levels of cytokines in PAH, including the proinflammatory cytokines IL-1β, IL-2, IL-4, IL-6, IL-8, IL-12p70, tumor necrosis factor (TNF)-α, monocyte chemoattractant protein-1,[9,10] and the cytokine-like hormone leptin.[11] Our group has demonstrated that IL-1α, IL-1β, IL-6, TNF-α, and IL-13 are linked to death in PAH.[12] However, the specific role of these cytokines in the PAH pathogenesis remains elusive. They probably play a role in the self-perpetuating vicious cycle of endothelial cell activation[13] and in the local adaptive immune response that occurs in the tertiary (ectopic) lymphoid follicles (tLTs) developing along the remodeled pulmonary vasculature in PAH.[14] Glucocorticoids (GCs) are effective and clinically useful medicines for repressing inflammation in lung disease; however, except for a single successful case report,[15] there is no evidence of GC efficacy in patients with idiopathic PAH (iPAH), even though cases of PAH associated with autoimmune diseases such as systemic lupus erythematosus or mixed connective tissue disease had an improvement in clinical and hemodynamic characteristics after GC treatment.[16,17]

To bridge innate and adaptive immunity, there is a specialized population of innate immune antigen-presenting cells, dendritic cells (DCs), that express all the receptors of the innate immune system and at the same time have the potential to take up antigen, process it into small peptides, and present it in the cleft of major histocompatibility complex (MHC)-I and MHC-II molecules to be recognized by T-cell receptors.[18] T-lymphocyte differentiation is a highly organized process controlled by DCs, which secrete cytokines and induce polarized T helper (Th)1, Th2, or Th17 cell subsets that secrete

TABLE 1] Clinicopathologic Features of Subjects Whose Blood Was Used for MoDC Generation, Characterization, and Cellular Assays

Feature	Patients With iPAH (n = 5)	Control Subjects (n = 5)
Age at diagnosis, y	30.8 ± 4.6	N/A
Age at analysis, y	43.6 ± 8.05	34 ± 8.03
Sex, female (male) [ratio]	3 (2) [1.5]	1 (4) [0.25]
NYHA class, No. (%)		
I	1 (20)	N/A
II	3 (60)	N/A
III	1 (20)	N/A
6MWD	512.8 ± 125	N/A
mPAP	44.2 ± 13	N/A
PVR	10.6 ± 6.7	N/A
PAH therapy, No. (%)		
Prostacyclin	3 (60)	N/A
PDE5	5 (100)	N/A
ERA	5 (100)	N/A

Data are mean ± unless otherwise indicated. 6MWD = 6-min walk distance; ERA = endothelin receptor antagonist; iPAH = idiopathic pulmonary arterial hypertension; MoDC = monocyte-derived dendritic cell; mPAP = mean pulmonary artery pressure; N/A = not applicable; NYHA = New York Heart Association; PAH = pulmonary arterial hypertension; PDE5 = phosphodiesterases type 5; PVR = pulmonary vascular resistance.

TABLE 2] Clinicopathologic Features of Subjects Whose Blood Was Used for Study of Circulating Lymphocyte Subpopulations

Feature	Patients With iPAH (n = 17)	Control Subjects (n = 20)
Age at diagnosis, y	44.6 ± 14.8	N/A
Age at analysis, y	52.2 ± 13.3	41.6 ± 13.4
Sex, female (male) [ratio]	10 (7) [1.43]	11 (9) [1.2]
NYHA class, No. (%)		
II	10 (58.8)	N/A
III	6 (35.3)	N/A
IV	1 (5.8)	N/A
6MWD	477.6 ± 105.1	N/A
mPAP	43.5 ± 10.3	N/A
PVR	7 ± 2.9	N/A
PAH therapy, No. (%)		
Prostacyclin	4 (23.5)	N/A
PDE5	13 (76.5)	N/A
ERA	15 (88.2)	N/A

Data are mean ± unless otherwise indicated. See Table 1 legend for expansion of abbreviations.