Inflammatory Cytokines in the Pathogenesis of Pulmonary Arterial Hypertension

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Abstract

Interleukin-6 (IL-6) is a multifunctional pro-inflammatory cytokine elevated in the serum of pulmonary arterial hypertension (PAH) patients and can predict the survival of idiopathic (I)PAH patients. Previous animal experiments and clinical human studies indicate that IL-6 is important in the pathogenesis of PAH; however, the molecular mechanisms of IL-6-mediated pathogenesis of PAH have been elusive. We recently identified IL-21 as a novel downstream cytokine of IL-6-signaling in PAH. First, we found that IL-6 blockade by the monoclonal anti-IL-6 receptor antibody, MR16–1, ameliorated hypoxia-induced pulmonary hypertension (HPH) and prevented the hypoxia-induced accumulation of Th17 cells and M2 macrophages in the lungs. Furthermore, the hypoxia-induced upregulation of IL-17 and IL-21, which are primarily produced by Th17 cells, was also ameliorated by IL-6 blockade in mice. Whereas IL-17 blockade with an anti-IL-17 neutralizing antibody had no effect on HPH, IL-21 receptor-deficient mice were resistant to HPH and exhibited no significant accumulation of M2 macrophages in the lungs. Consistently, IL-21 indeed promoted the polarization of primary alveolar macrophages toward the M2 phenotype. Moreover, significantly enhanced expressions of IL-21 and M2 macrophage markers were detected in the lungs of IPAH patients who underwent lung transplantation. Together, the above data suggest that IL-21 promotes PAH through M2 macrophage polarization, downstream of IL-6-signaling. IL-6/Th17/IL-21-signaling axis might be a novel potential target for treating PAH.

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20.1   Background

Pulmonary arterial hypertension (PAH) is a serious disease characterized by arteri-
opathy in the small- to medium-sized distal pulmonary arteries, which is associated
with arterial muscularization, concentric intimal thickening, and the formation of
plexiform lesions [1, 2]. Inflammation and autoimmunity are currently thought of as
critical factors to the pathogenesis of PAH [3, 4]. Inflammatory cells such as T cells,
B cells, and macrophages infiltrate the plexiform lesions in patients with advanced
PAH. Thus, proinflammatory cytokines produced by these cells may be responsible
for the hyperproliferation of pulmonary artery endothelial cells and pulmonary
artery smooth muscle cells (PASMCs) [5, 6].

Interleukin (IL)-6 is a multifunctional proinflammatory cytokine linked to
numerous autoimmune diseases [7, 8]. Patients with idiopathic (I)PAH exhibit
increased IL-6 serum levels, which correlate with their prognoses [3, 9, 10].
Consistent with these findings, lung-specific IL-6 transgenic mice display spontane-
ous PH in normoxia and develop greatly exaggerated hypoxia-induced PH (HPH)
[11], whereas IL-6-deficient mice show resistance to HPH [12]. These findings sug-
gest that IL-6 has a significant role in the pathogenesis of PH; however, the down-
stream target(s) of IL-6 in HPH have been elusive when we started the following
study on the pathogenesis of PH. We recently reported that the IL-6/interleukin-
21(IL-21)-signaling axis played critical roles for the development of HPH in asso-
ciation with M2 macrophage polarization [13]. In this manuscript, we elaborate on
the role of IL-6/IL-21-signaing axis in the pathogenesis of PAH.

20.2   IL-6 in the Pathogenesis of HPH

We examined the effect of hypoxia (10% oxygen) on the expression of *Il-6* mRNA
in the lungs of C57BL/6 mice using quantitative (q)RT-PCR. The *Il-6* mRNA levels
peaked on day 2 and returned to basal levels by day 7 after hypoxia exposure. Next,
we investigated the effect of IL-6 blockade on HPH development by measuring the
right ventricular systolic pressure (RVSP), Fulton's Index (right/[left + septum]
ventricular weight), and the medial wall thickness index, which is estimated by
elastic Van Gieson staining of the pulmonary arterioles. In control antibody-treated
mice, hypoxia exposure for 4 weeks induced significant increases in the RVSP,
Fulton's Index, and medial wall thickness index compared to the values observed
under normoxic conditions. On the other hand, the hypoxia-induced elevation of
these parameters was significantly inhibited by treatment with MR16–1. Similarly,
thickened medial vascular walls were detected in the lung sections of control

antibody-treated mice but not in those of MR16–1-treated mice. These data indicate that IL-6 blockade by MR16–1 effectively prevents HPH.

20.3   IL-21 in the Pathogenesis of HPH

IL-21 is an IL-2 family cytokine produced by activated T cells, including Th17 cells, that regulates immune responses [14]. We found that the *Il-21* mRNA level peaked on day 2, remained elevated until day 14, and returned to the basal levels on day 28 after hypoxia exposure. In addition, hypoxia induced significantly increased numbers of Th17 cells, which produce both IL-17 and IL-21, in the lungs of mice treated with control antibody but not in those treated with MR16–1. These data suggest that IL-6 promotes the accumulation of IL-21-producing Th17 cells in the lungs after hypoxia exposure.

Next, we evaluated the effect of hypoxia on IL-21 receptor (IL-21R) knockout (IL-21RKO) mice [15]. Wild-type (WT) mice exposed to hypoxia for 4 weeks. exhibited significantly increased RVSP, Fulton's Index, and medial wall thickness compared with mice exposed to normoxia. In contrast, the hypoxia-induced elevation of these parameters was significantly inhibited in IL-21RKO mice. Similarly, remodeling of the medial vascular walls following hypoxia exposure in WT mice was also significantly inhibited in the lungs of IL-21RKO mice. These data suggest that IL-21R-deficient mice are resistant to HPH.

Alveolar macrophages have been reported to undergo M2 macrophage polarization in response to hypoxia [16]. We hypothesized that IL-21, which has been reported to be majorly secreted from Th17 cells in mice, might play a role in the hypoxia-induced generation of M2 macrophages in the lung. IL-21 treatment of BALF-isolated alveolar macrophages significantly upregulated the mRNA levels of M2 signature genes, such as *Fizz1*, *Arg1*, and *Cxcl12*, but had no effect on the mRNA levels of M1 signature genes, including *Nos2*, *Il-12β*, and *Tnf-α*, indicating that IL-21 specifically induces the M2 polarization of alveolar macrophages.

We next examined the effect of IL-21R deletion on the hypoxia-induced upregulation of M2 signature genes, including *Fizz1*, *Arg1*, and *Cxcl12*, in the alveolar macrophages isolated from the BALF after exposure to hypoxia for 4 days. All of these genes were significantly upregulated in the alveolar macrophages isolated from WT mice but not in those from IL-21RKO mice. Similarly, Fizz1 protein expression was increased in the lungs of WT mice but not in those of IL-21RKO mice after exposure to hypoxia for 1 week. In contrast, M1 signature gene expression was comparable in the WT and IL-21RKO mice after exposure to hypoxia. Consistent with these findings, the hypoxia-induced generation of M2 macrophages in the lung was significantly inhibited by treatment with an anti-IL-21-neutralizing antibody but not a control antibody. Collectively, these findings suggest that IL-21 is essential for hypoxia-induced M2 macrophage polarization in the lung.

We next examined the effect of IL-21 blockade on the in vivo hypoxia-induced proliferation of PASMCs. Hypoxia exposure significantly increased the number of anti-Ki67 antibody-positive PASMCs in the lungs of WT mice but not in those of
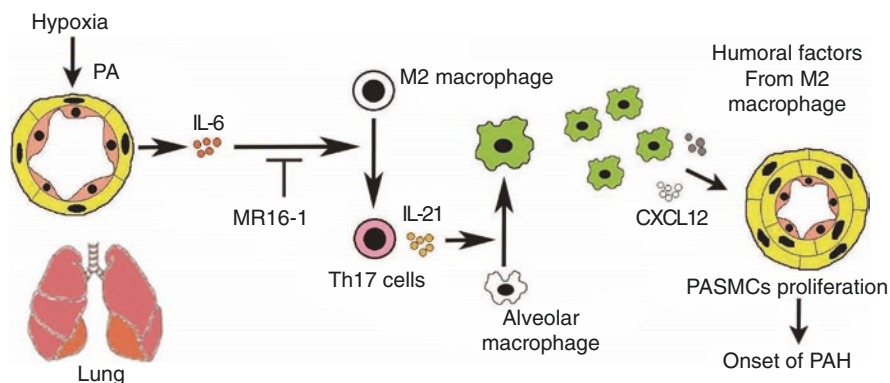
Fig. 20.1 Schematic illustration of the pathogenesis of PAH. IL-6/IL-21-signalling has a central role for the pathogenesis of PAH

IL-21RKO mice. Consistent with this finding, hypoxia exposure significantly increased the number of anti-phospho-Histone H3 antibody-positive PASMCs in the lungs of mice treated with control antibody but not in those treated with the anti-IL-21 neutralizing antibody. Together, these data suggest that IL-21 plays a critical role in the hypoxia-induced proliferation of PASMCs in association with M2 macrophage skewing (Fig. 20.1).

20.4   Increased Expression of IL-21 and M2 Macrophage Markers in the Lungs of IPAH Patients

Immunohistochemical analyses of the lung tissues from IPAH patients undergoing lung transplantation showed an excessive infiltration of IL-21-positive cells in the adventitia, obliterative intimal proliferative lesions, and plexiform lesions of the remodeled pulmonary arteries. In contrast, IL-21-positive cell infiltration was barely detected in the lungs of the control patient. We also observed both Arg1-positive and MRC-1-positive cells in all layers of the remodeled pulmonary arteries and alveolar areas examined in the lungs from IPAH patients but not in the tissue from control patients. Taken together, these findings strongly suggest that IL-6/IL-21-signaling axis is essential for the pathogenesis of human PAH in association with M2 macrophage polarization (Fig. 20.1).

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