Pulmonary arterial hypertension (PAH) is a progressive cardiopulmonary disease in which extensiveobliterative changes are prevalent in the small to midsized pulmonary arteries. Alterations in structure and function of the endothelium occur in conjunction with growth of neointimal, medial, and adventitial layers, culminating in an occlusive arteriopathy associated with high resistance to blood flow and right heart failure and death. Currently approved PAH therapies focus on dilating the partially occluded vessels and are weak antiproliferative agents. However, they have not resulted in a strategy that is effective in reversing vascular remodeling and preventing deterioration and the need for a lung transplant. In recent years, greater attention has been focused on the frequently observed perivascular inflammation in patients with all forms of PAH, from idiopathic (I) PAH to PAH associated with systemic autoimmune diseases. An expanding body of knowledge has related genetic susceptibility, inflammation, and metabolic (glycolytic) shifts in vascular cells to PAH pathogenesis. In fact, the inflammatory processes are inextricably linked to altered vascular and inflammatory cell metabolism. Thus, there is a strong rationale to identify genetic factors that predispose to impaired resolution of inflammation and to determine how immune-mediated vascular injury initiates and propagates alterations in metabolic function and in the phenotype of PAH vascular cells. Based on clinical and animal studies, described below, there is now reason to suggest that advanced vascular remodeling may be reversed by approaches that address specific inflammatory and immune processes.

Abstract: This review summarizes an expanding body of knowledge indicating that failure to resolve inflammation and altered immune processes underlie the development of pulmonary arterial hypertension. The chemokines and cytokines implicated in pulmonary arterial hypertension that could form a biomarker platform are discussed. Preclinical studies that provide the basis for dysregulated immunity in animal models of the disease are reviewed. In addition, we present therapies that target inflammatory/immune mechanisms that are currently enrolling patients, and discuss others in development. We show how genetic and metabolic abnormalities are inextricably linked to dysregulated immunity and adverse remodeling in the pulmonary arteries. (Circ Res. 2014;115:165-1745.)

Key Words: hypertension, pulmonary □ leukotriene B4

Inflammation and Immunity in the Pathogenesis of Pulmonary Arterial Hypertension

Marlene Rabinovitch, Christophe Guignabert, Marc Humbert, Mark R. Nicolls

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Histopathology and Biomarker Evidence of Inflammation in PAH

Pulmonary vascular lesions occurring in patients with PAH as well as in animal models of pulmonary hypertension (PH) are characterized by varying degrees of perivascular inflammatory infiltrates, comprising T- and B-lymphocytes, macrophages, dendritic cells, and mast cells. (By convention, animal models are still referred to as having PH rather than PAH). Figure 1 illustrates representative histopathology and the observed inflammatory cells implicated in PAH. Recently, correlations of the average perivascular inflammation score with intima plus media and adventitia thickness, respectively, and with mean pulmonary arterial pressure have been reported; these associations support a role for perivascular inflammation in the processes of pulmonary vascular remodeling.6 Moreover, these studies also indicate that in the presence of a mutation in bone morphogenetic protein type 2 receptor (BMPR2), the inflammatory pathology was more advanced. The fact that inflammation precedes vascular remodeling in experimental PH suggests that altered immunity is a cause rather than a consequence of vascular disease.7 Beyond increased perivascular immune cells accumulation and intravascular infiltration, circulating levels of certain cytokines and chemokines are abnormally elevated. These include interleukin (IL)-1β, IL-6, IL-8, monocyte chemokine receptor-1, fractalkine, CCL5/RANTES, and tumor necrosis factor (TNF)-α. Some of these cytokines and chemokines correlate with a worse clinical outcome in PAH patients and may serve as biomarkers of disease progression. Some, such as IL-1β and TNF-α, have been related to an accumulation of extracellular matrix proteins such as fibronectin,8 observed in PAH lesions9 and others such as IL-6 have been related to the proliferation of smooth muscle cells.10 The cytokines implicated in PAH11,12 and in related studies are summarized in Table 1.

Chemokines, Cytokines, and PAH

Recent investigations provide evidence that both pulmonary vascular cells and inflammatory cells are important local sources of chemokines and cytokines that can lead to pulmonary vascular remodeling in PAH.20a,b Indeed the increased expression of cytokines and chemokines contribute to exaggerated contractility and proliferation of vascular cells. IL-1 can induce fibroblast growth factor-221 and both fibroblast growth factor-2 and IL-6 and play an integral role in mediating the proliferative response in the smooth muscle-like cells and fibroblasts of the pulmonary vasculature22–27 and contribute to the increased pericyte coverage in PAH.2b Mutations

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMPR2</td>
<td>bone morphogenetic protein type 2 receptor</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>PAH</td>
<td>pulmonary arterial hypertension</td>
</tr>
<tr>
<td>PH</td>
<td>pulmonary hypertension</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
</tbody>
</table>

Figure 1. Pulmonary vascular changes in pulmonary arterial hypertension includes infiltrating adaptive and innate immune cells. In the top panel is a representative histopathology of a vessel with severe neointimal formation represented below by a diagrammatic illustration. The histopathology shows a single endothelial layer and an eccentric neointima (pale pink) that contains cells that have markers of inflammatory cells and others that stain with markers of smooth muscle but appear poorly differentiated. The medial muscular layer is expanded and there is an abundant adventitial layer. This vessel is decorated with complement and autoantibodies, infiltrated by neutrophils in the lumen attacking the vessel wall and other inflammatory cells binding to the endothelium and infiltrating. The neointima is comprised of pale cells and matrix and infiltrating T and B cells and in the adventitia there are dendritic cells, macrophages, and mast cells and in the perivascular space tertiary lymphoid follicles characterized by T cells, B cells, and plasmacytoid dendritic cells (APC). NK indicates natural killer; PH, pulmonary hypertension; and SMC, smooth muscle cell.
BMPR2 signaling pathway seen in all forms of PAH can contribute to the development of T cells from thymocytes. Bone morphogenetic proteins (BMPs) can modulate the immune response in vascular cells, suggesting a role in immune-mediated vascular remodeling. The BMPR2 signaling pathway plays an important role in the development of pulmonary hypertension (PH).

Loss of BMPR2 signaling results in increased p-p38 signaling, leading to heightened production of IL-6. IL-6 is a key cytokine in PH, contributing to vascular remodeling and angiogenesis.

GM-CSF can prevent hypoxia-induced PH. It is also known that osteoprotegerin, a protein regulated by BMP and serotonin, is involved in the pathogenesis of PAH.

Complement depletion has been shown to have a protective effect in experimental models of PH, suggesting the relevance of exploring complement depletion in clinical settings.

Recent evidence suggests that increased production of macrophage migration inhibitory factor (MIF) plays a pivotal role in the pathogenesis of PAH. MIF inhibits factor-CD74 interaction, leading to the activation of Src-family kinase, MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase), PI3K/Akt (phosphatidylinositide 3-kinase/protein kinase B), and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathways.

The protective effect of complement depletion in experimental models of PH is related to the prevention of disease pathology. MIF inhibits the proliferation of pulmonary artery smooth muscle cells and contributes to hypoxic PH.

Table 1. Serum Cytokine/Chemokine Levels in Patients With Pulmonary Arterial Hypertension

<table>
<thead>
<tr>
<th>Cytokine/Chemokine</th>
<th>PAH</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α*</td>
<td>†</td>
<td>13</td>
</tr>
<tr>
<td>IL-1β*</td>
<td>†</td>
<td>14</td>
</tr>
<tr>
<td>IL-2†</td>
<td>†</td>
<td>15</td>
</tr>
<tr>
<td>IL-4</td>
<td>†</td>
<td>16</td>
</tr>
<tr>
<td>IL-5</td>
<td>†</td>
<td>17</td>
</tr>
<tr>
<td>IL-6†‡</td>
<td>†</td>
<td>18</td>
</tr>
<tr>
<td>IL-8†</td>
<td>†</td>
<td>19</td>
</tr>
<tr>
<td>IL-10†</td>
<td>†</td>
<td>20</td>
</tr>
<tr>
<td>IL-12†</td>
<td>†</td>
<td>21</td>
</tr>
<tr>
<td>IL-13*</td>
<td>†</td>
<td>22</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>†</td>
<td>23</td>
</tr>
<tr>
<td>TNF-α*</td>
<td>†</td>
<td>24</td>
</tr>
</tbody>
</table>

Table 1 indicates chemokine ligands; IL, interleukin; MCP, monocyte chemotactant protein; PAH, pulmonary arterial hypertension; RANTES, regulated on activation, normal T cell expressed and secreted; and TNF, tumor necrosis factor.

Cytokines implicated in survival: *16; †12,18, ‡18.

In BMPR2 seen in heritable PAH and dysfunction of the BMPR2 signaling pathway seen in all forms of PAH can lead to inappropriate expression of growth factors and proinflammatory responses in vascular cells as described both in experimental and human PH. For example, in pulmonary arterial endothelial cells, loss of BMPR2 causes repression of apelin and this reduces a microRNA that normally represses fibroblast growth factor-2. However, in addition, increased p-p38 signaling resulting from loss of BMPR2 in vascular cells causes heightened production of IL-6. Loss of BMPR2 also results in the enhanced secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF) in response to TNF-α. The mechanism is related to the increased translation of GM-CSF mRNA associated with subverted stress granule formation. Infusion of GM-CSF in rats can exaggerate, and administration of neutralizing antibodies to GM-CSF can prevent, hypoxia-induced PH. It is also known that BMPR2 signaling plays an important role in the development of T cells from thymocytes.

Bone morphogenetic protein (BMP)-2/4 (ligands for BMPR2) and transforming growth factor-β have a synergistic effect on the induction of Foxp3 regulatory T cells. BMP-2/4 affects Smad signaling molecules, including phosphorylated extracellular signal-regulated kinase (ERK) and JNK (c-Jun N-terminal kinase), which may promote the differentiation of Foxp3 Treg cells induced by transforming growth factor-β. These cells protect against autoimmune responses that lead to severe PH in the experimental and clinical setting.

Several cytokines can directly control cell proliferation, migration, and differentiation of pulmonary vascular cells. IL-6 is prominent among these multifunctional proinflammatory cytokines and has been linked to the pathogenesis of PAH. Delivery of recombinant IL-6 protein in rodents is sufficient to cause pulmonary vascular remodeling and to exaggerate the pulmonary hypertensive response to chronic hypoxia. Furthermore, IL-6-overexpressing mice spontaneously develop PH and pulmonary vascular remodeling and an obliterative form of remodeling in hypoxia resembling human disease, whereas IL-6 knockout mice are more resistant to the development of PH induced by chronic hypoxia. IL-6 also induces pulmonary artery smooth muscle cell proliferation via induction of fibroblast growth factor-2 by the transcription factor KLF-5 (Kruppel-like factor). TNF-related apoptosis inducing ligand has recently been identified as playing a key role in apoptosis of endothelial cells and proliferation of smooth muscle cells in many PH experimental models and its inhibition is related to prevention of disease pathology. In addition, osteoprotegerin, a protein regulated by BMP and serotonin and IL-1 signaling, is highly expressed in smooth muscle cells and serum of patients with PH and can stimulate their migration and proliferation.

Recent data from our group demonstrated that increased production of macrophage migration inhibitory factor plays a pivotal role in the pathogenesis of PAH. Migration inhibitory factor is a critical upstream inflammatory mediator with pleiotropic actions partly explained by its binding to the extracellular domain of CD74. In endothelial cells, a migration inhibitory factor-CD74 interaction can lead to the activation of Src-family kinase, MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase), PI3K/Akt (phosphatidylinositide 3-kinase/protein kinase B), and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathways, and to apoptotic resistance via elevated BCL2 and BCL-xL and repressed p53. In addition, migration inhibitory factor can bind to CXC receptor (CXCR)2 and CXCR4 and lead to the proliferation of pulmonary artery smooth muscle cells and contribute to hypoxic PH. In elevated production of cytokines and chemokines, phenotypic alterations and functional defects in cytotoxic T and natural killer cells are linked to human PAH and experimental PH as well as pulmonary veno-occlusive disease. Recent data show deposition of complement C3 in idiopathic PAH patients and the protective effect of complement depletion in experimental models of PH, emphasizing the relevance of exploring complement-mediated vascular injury in the pathobiology of PAH.

### Immune Dysregulation, T Cells, B Cells, and Dendritic Cells

Additional analyses of immunity in PAH support the notion that maladaptation of the immune response exists and may explain both the accumulation of perivascular inflammatory cells and the overabundance of cytokines and chemokines. Indeed, a delicate balance between immunity and tolerance exists and any disturbance may result in chronic inflammation or autoimmunity. Several types of autoantibodies directed against antinuclear antigens, endothelial cells, and fibroblasts have been found in idiopathic and systemic sclerosis-associated PAH. These autoantibodies may play an important role in endothelial cell apoptosis and in the expression of cell adhesion molecules but more studies are necessary to characterize their pathogenic importance.
The role of T cells and more specifically of Treg cells in the control of self-tolerance is well-established, and altered Treg function has been demonstrated in patients with PAH. Tregs not only control other T-cells but also regulate monocytes, macrophages, dendritic cells, natural killer cells, and B cells; decreased Treg function may predispose individuals to PAH, as it does in animals. For example, conditions associated with PAH, such as HIV, systemic sclerosis, systemic lupus erythematosus, Hashimoto thyroiditis, Sjögren syndrome, and the antiphospholipid syndrome, are characterized by abnormal CD4+ T-cell number and function. In animals with a congenital absence of T cells (athymic nude rats), vascular injury causes the lungs to become infiltrated with macrophages, mast cells, and B cells, similar to human PAH lesions, and disease is prevented with Treg reconstitution. Similarly, natural killer cells have recently been implicated as having a beneficial effect on the pathogenesis of PH but a phenotypic switch results in impaired production of interferon-γ and elevated levels of MMP9 (matrix metalloproteinase 9).

In experimental PH and clinical PAH, accumulation of immature dendritic cells in remodeled pulmonary arteries has been demonstrated, suggesting that they may contribute to PAH immunopathology. In addition, a recent study described lymphoid neogenesis in lungs from patients with idiopathic (1) PH. In this study, pulmonary tertiary lymphoid tissues were identified, ranging from small lymphoid follicles to highly organized lymphoid follicles. Thus, the presence of pulmonary tertiary lymphoid tissues in idiopathic pulmonary arterial hypertension (IPAH) lungs could provide a structural basis for a local autoimmune response occurring in this apparently idiopathic disease. In addition, circulating autoantibodies are commonly detected in IPAH patients without evidence of an associated autoimmune condition. Moreover, several autoimmune and infectious diseases such as systemic sclerosis, systemic lupus erythematosus and HIV, herpes, and schistosomiasis infection are recognized causes of PAH. In many of these conditions, the PAH is not reversible with the treatment of the causal disease. The sequence of events from initiation or recurrence of inflammation to pulmonary vascular disease development remains unknown in idiopathic and even in autoimmune and infectious forms of PAH. A recent study, indicated that passive transfer of IgG from rats after monocrotaline injection and documentation of antifibroblast antibodies resulted in PH and vascular changes in naïve rats.

**Macrophages and Pulmonary Vascular Pathobiology**

Recent attention has focused on the role of perivascular macrophages macrophage infiltrating pulmonary arterioles. The role of the macrophage in processes as diverse as limb or cardiac regeneration and pathogen defense indicates that it is important to comprehend the behavior of this cell in the context of the vascular milieu. CD68+ macrophages are prominent in advanced oblitative plexiform lesions observed in experimental and clinical PAH, and macrophage depletion or inactivation prevents PAH in several model systems including experimentally induced hypoxic PH and portopulmonary hypertension.

Macrophages have been implicated in infectious causes such as HIV-associated experimental PAH, and human herpes virus infection is associated with vascular remodeling, perivascular macrophages, and lung fibrosis. However, even in patients with IPAH, recruitment of lung macrophages is evident and has been associated with the unfolded protein response. Rare conditions, such as the vHL-Chuvash mutation, are characterized by the association of PAH with macrophage infiltration. Activation of macrophages is also closely linked to epigenetic changes that stimulate and induce proliferation of vascular fibroblasts in patients and in experimental models of PAH. These features involve histone deacetylase 1 (HDAC1)-mediated activation of a host of proinflammatory cytokines. Altered metabolism involving a switch to glycolysis, fatty acid oxidation, and production of reactive oxygen species underlies the abnormal interaction of fibroblasts and macrophages. Reversing the metabolic phenotype can also reverse the pathological features of PH in terms of macrophage recruitment and activation. There is recent evidence for macrophage GM-CSF and LTβ signaling pathways in PH development, consistent with a paradigm of cooperative interaction between these 2 pathways in regulating gene expression in inflammatory macrophages.

The absence of normal Treg activity in athymic rats leads to activated macrophage recruitment after vascular injury with the vascular endothelial growth factor receptor inhibitor, SU5416; this macrophage recruitment occurs in close association with the development of PH. As described, these macrophage secrete LTβ, that induces pulmonary artery endothelial cell injury and apoptosis as well as pulmonary artery smooth muscle cell proliferation/hypertrophy. Blocking macrophage-derived LTβ, biosynthesis or signal transduction reverses experimental PH, and depleting CD68+ macrophages prevents PH from developing in this model. A useful oversimplification of the athymic/immune dysregulation model is that after vascular injury in animals with a healthy immune system, normal Treg activity skews macrophage responses to promote injury resolution, but that without this Treg activity (as observed in T-cell deficient athymic rats) pulmonary macrophages become activated and participate in injury evolution (Figure 2).

**TH1/TH17 and TH2 Immunity in PAH**

The effector responses of CD4+ T cells are roughly divided into TH1, TH2, and TH17 responses; all are important in the pathogenesis of autoimmune disorders. TH1 effector cells are induced in parallel to TH1 (producing interferon-γ, TNFs and IL-2), and, such as TH1, polarized TH17 cells have the capacity to cause inflammation and autoimmune disease. Both TH1 and TH17 colocalize regionally and may require each other for recruitment into the region. In addition to IL-17, TH17 cells produce IL-6, TNF-α, GM-CSF, IL-21, and IL-22. By distinction, Th2 cells, which produce the cytokines IL-4, IL-5, and IL-13, are involved in allergic responses and the clearance of extracellular pathogens, such as worms. When immune dysregulation favors TH1/TH17 immunity reactions, TNF-α and IL-6 seem harmful mediators promoting vascular remodeling whereas IL-6 may exert a protective effect in pulmonary vascular injury induced by Schistosomiasis.

**References**

Plasma IL-13 is associated with PAH in systemic sclerosis and overexpressing IL-13 induces experimental PH. Figure 2 postulates that when immune dysregulation occurs, perhaps because of a deficiency in normal Treg or regulatory natural killer function or both, there can be a skewing toward TH1/TH17 immunity including a predominance of LTB₄-secreting GM-CSFR⁺ macrophages that can directly induce vascular injury by inducing endothelial cell apoptosis, and smooth muscle hypertrophy and proliferation. Alternatively, such as in the case of Schistosomiasis, TH2 immunity also induces vascular remodeling, but apparently by unique pathways.

**Neutrophils and Neutrophil Elastase**

Little attention has been given to the neutrophil in the pathogenesis of PAH, but it is evident both in experimental and clinical studies that neutrophil elastase can influence pathogenesis. We have recently shown enhanced neutrophil elastase in smooth muscle cells from patients with IPAH and experimental models of PH, including genetic models such as the S100A4/Mts1 overexpressing mouse, where the elastase inhibitor elafin repressed the development and progression of PH. Elevated neutrophil elastase is also present in pulmonary artery smooth muscle cells from mice exposed to chronic hypoxia. Repressing neutrophil elastase both arrests progression and induces regression of experimentally induced PH secondary to injection of the toxin monocrotaline. In addition to the release of biologically active growth factors from the extracellular matrix by elastase, elastin and fibronection fragments that result from elastase activity are highly chemotactrant, and elastase can also activate components of the complement system, further contributing to the immune inflammatory response.

**Therapeutic Considerations**

Addressing the immune/inflammatory component of PAH, through novel therapeutic approaches, may prove sufficient to prevent progression of the disease. Elastase inhibition is one strategy that has proved effective in reversing advanced PH in the inflammatory monocrotaline model, and a highly selective elastase inhibitor, human recombinant elafin, has received Food and Drug Administration approval as an orphan drug to treat PAH. Elafin also suppresses NFkB activation.
and the subsequent inflammatory response associated with experimental PH.107 We have recently shown that low-dose FK506 not only is an immunosuppressant, but also can reverse severe PH in the SUGEN/hypoxia model, in addition to its other function of activating the BMPR2 receptor by removing FKBP12 from the BMPR type 1 coreceptor.108 In human endothelial cells from patients with PAH, low-dose FK506108 improved function by restoring BMPR2 signaling as evidenced by elevated pSMAD, Id1, and apelin. FK506 activated the BMPR2 pathway in PAH cells whereas the normal ligand BMP4, failed to do so. Inhibition of the hydrolase that produces leukotriene B4 by bestatin can in fact reverse PH in the athymic rat/SUGEN and monocrotaline models,86 and this compound has a long record of clinical use with minimal toxicity.109,110 B cell depletion with rituximab is another strategy that is being investigated in patients with PAH associated with systemic sclerosis, in a currently enrolling National Institutes of Health trial. Apelin can promote endothelial cell homeostasis and is in the clinic to treat heart failure.111 Apelin, like elafin and like naturally occurring peroxisome proliferator-activated receptor (PPAR)γ adducts (nitro fatty acids),27 can also inhibit NFkB,112 and induce Nrf2, the transcription factor for antioxidant genes such as hemoxygenase 1. Antioxidants that revert vascular cells to a normal metabolic phenotype, such as dichloroacetate, show promise. HDAC1 inhibitors, tested to date in the experimental setting can revert activated to normal fibroblasts and can prevent adverse interactions with macrophages.91 Although inherently anti-inflammatory, transforming growth factor-β is a pleiotropic cytokine, that is also associated with TH2 immunity, endothelial injury, and now implicated in the development of PH associated with Schistosoma mansoni infection.94 Other experimental studies previously described in this review have used GM-CSF inhibition to show that suppression of macrophage recruitment can prevent hypoxia-induced PH.36 Table 2 highlights preclinical studies that have found a pathogenic role for immunity in the development of PH and the responsiveness to therapeutics.

Table 2.  Selected Preclinical Studies Demonstrating Role for Immunity in PH

<table>
<thead>
<tr>
<th>Preclinical Immune Target</th>
<th>Drug</th>
<th>PH Model</th>
<th>Prevented PH</th>
<th>Reversed PH</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>NA</td>
<td>Spontaneous PH in mice overexpressing IL-6</td>
<td>NA</td>
<td>NA</td>
<td>24</td>
</tr>
<tr>
<td>TNF-α</td>
<td>NA</td>
<td>Spontaneous PH in mice overexpressing TNF-α</td>
<td>NA</td>
<td>NA</td>
<td>114</td>
</tr>
<tr>
<td>OX-40 L</td>
<td>NA</td>
<td>Spontaneous PH in mice overexpressing OX-40 L</td>
<td>NA</td>
<td>NA</td>
<td>115</td>
</tr>
<tr>
<td>CD20</td>
<td>Anti-Rat CD20</td>
<td>SU5416/ovalbumin rat</td>
<td>X</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td>IL-1 receptor antagonist</td>
<td>Monocrotaline rat</td>
<td>X</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>TGF-β</td>
<td>TGF-β</td>
<td>1D11</td>
<td>Schistosoma mouse</td>
<td>X</td>
<td>94</td>
</tr>
</tbody>
</table>

| Purine synthesis | Mycophenolate mofetil | Monocrotaline rat | X | 118 |
| Target(s) of steroids | Dexamethasone | Monocrotaline rat | X | 119,120 |
| Target of rapamycin | Rapamycin | Monocrotaline pneumonectomy rat | X | X | 121 |
| NFAT                   | Cyclosporine | Monocrotaline rat, hypoxia rat | X | X | 122,123 |
| TRAIL                  | TRAIL-antibodies | Monocrotaline rat, hypoxia mouse, ApoE−/− mouse | X | 124 |
| 5-LO/FLAP              | MK886 | Hypoxic mouse, monocrotaline adeno-5-LO rat | X | 125,126 |
|                         | Zileuton | Monocrotaline/adeno-5-L0 rat | X | 125 |
| LTB4                   | BLT1 | LY293111/ON04057 | SU5416/athymic rat, monocrotaline rat | X | X | 86,127 |
| LTA4H                  | Bestatin | SU5416/athymic rat, monocrotaline rat | X | X | 86 |

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FLAP indicates 5-lipoxygenase activated protein; IL, interleukin; LO, lipooxygenase; NA, not applicable; NFAT, nuclear factor of activated T-cells; PH, pulmonary hypertension; TGF, transforming growth factor; TNF, tumor necrosis factor; and TRAIL, tumor necrosis factor–related apoptosis inducing ligand.

and the subsequent inflammatory response associated with experimental PH.107 We have recently shown that low-dose FK506 not only is an immunosuppressant, but also can reverse severe PH in the SUGEN/hypoxia model, in addition to its other function of activating the BMPR2 receptor by removing FKBP12 from the BMPR type 1 coreceptor.108 In human endothelial cells from patients with PAH, low-dose FK506108 improved function by restoring BMPR2 signaling as evidenced by elevated pSMAD, Id1, and apelin. FK506 activated the BMPR2 pathway in PAH cells whereas the normal ligand BMP4, failed to do so. Inhibition of the hydrolase that produces leukotriene B4 by bestatin can in fact reverse PH in the athymic rat/SUGEN and monocrotaline models,86 and this compound has a long record of clinical use with minimal toxicity.109,110 B cell depletion with rituximab is another strategy that is being investigated in patients with PAH associated with systemic sclerosis, in a currently enrolling National Institutes of Health trial. Apelin can promote endothelial cell homeostasis and is in the clinic to treat heart failure.111 Apelin, like elafin and like naturally occurring peroxisome proliferator-activated receptor (PPAR)γ adducts (nitro fatty acids),27 can also inhibit NFkB,112 and induce Nrf2, the transcription factor for antioxidant genes such as hemoxygenase 1. Antioxidants that revert vascular cells to a normal metabolic phenotype, such as dichloroacetate, show promise. HDAC1 inhibitors, tested to date in the experimental setting can revert activated to normal fibroblasts and can prevent adverse interactions with macrophages.91 Although inherently anti-inflammatory, transforming growth factor-β is a pleiotropic cytokine, that is also associated with TH2 immunity, endothelial injury, and now implicated in the development of PH associated with Schistosoma mansoni infection.94 Other experimental studies previously described in this review have used GM-CSF inhibition to show that suppression of macrophage recruitment can prevent hypoxia-induced PH.36 Table 2 highlights preclinical studies that have found a pathogenic role for immunity in the development of PH and the responsiveness to therapeutics.

Table 3.  Currently Enrolling Clinical Trials in Pulmonary Hypertension That Target Immunity

<table>
<thead>
<tr>
<th>Drug Intervention</th>
<th>Drug Target</th>
<th>Patient Population</th>
<th>Clinical Trial Sponsor and Collaborator</th>
</tr>
</thead>
<tbody>
<tr>
<td>FK506</td>
<td>NFAT inhibitor</td>
<td>PAH</td>
<td>Stanford University</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20+ B cells</td>
<td>Systemic sclerosis PAH</td>
<td>National Institute of Allergy and Infectious Disease/Division of Allergy, Immunology, and Transplantation</td>
</tr>
<tr>
<td>Anakinra</td>
<td>Interleukin-1 receptor antagonist</td>
<td>PAH</td>
<td>Virginia Commonwealth University</td>
</tr>
</tbody>
</table>

NFAT indicates nuclear factor of activated T-cells; and PAH, pulmonary arterial hypertension.

and the subsequent inflammatory response associated with experimental PH.107 We have recently shown that low-dose FK506 not only is an immunosuppressant, but also can reverse severe PH in the SUGEN/hypoxia model, in addition to its other function of activating the BMPR2 receptor by removing FKBP12 from the BMPR type 1 coreceptor.108 In human endothelial cells from patients with PAH, low-dose FK506108 improved function by restoring BMPR2 signaling as evidenced by elevated pSMAD, Id1, and apelin. FK506 activated the BMPR2 pathway in PAH cells whereas the normal ligand BMP4, failed to do so. Inhibition of the hydrolase that produces leukotriene B4 by bestatin can in fact reverse PH in the athymic rat/SUGEN and monocrotaline models,86 and this compound has a long record of clinical use with minimal toxicity.109,110 B cell depletion with rituximab is another strategy that is being investigated in patients with PAH associated with systemic sclerosis, in a currently enrolling National Institutes of Health trial. Apelin can promote endothelial cell homeostasis and is in the clinic to treat heart failure.111 Apelin, like elafin and like naturally occurring peroxisome proliferator-activated receptor (PPAR)γ adducts (nitro fatty acids),27 can also inhibit NFkB,112 and induce Nrf2, the transcription factor for antioxidant genes such as hemoxygenase 1. Antioxidants that revert vascular cells to a normal metabolic phenotype, such as dichloroacetate, show promise. HDAC1 inhibitors, tested to date in the experimental setting can revert activated to normal fibroblasts and can prevent adverse interactions with macrophages.91 Although inherently anti-inflammatory, transforming growth factor-β is a pleiotropic cytokine, that is also associated with TH2 immunity, endothelial injury, and now implicated in the development of PH associated with Schistosoma mansoni infection.94 Other experimental studies previously described in this review have used GM-CSF inhibition to show that suppression of macrophage recruitment can prevent hypoxia-induced PH.36 Table 2 highlights preclinical studies that have found a pathogenic role for inflammation in the development of PH and the responsiveness to therapeutics.

Table 3 highlights ongoing clinical trials using immunotherapies to reverse PH.

While the targets of harmful immunity are numerous in PAH, some may prove ineffective, and, for others, the risk/benefit ratio is likely unacceptable. For example, targeting transforming growth factor-β may limit certain TH2-mediated processes,94 but could, if given chronically, be
proinflammatory and harmful. On the other hand, directly addressing TH cytokines IL-4, IL-5, and IL-13 could, in the correct PAH subtype, prove highly useful as suggested by protection from disease in genetic knockout mouse studies. While TNF-α seems to be clearly implicated in PAH pathogenesis, the results of antagonizing this cytokine has been mixed in preclinical studies and it predisposes patients to severe infectious complications such as tuberculosis. Some approaches that are putatively useful for limited duration, such as i.v. cyclophosphamide or high-dose steroids, must be balanced against the risks of profound systemic immunosuppression if considered as a more chronic therapy. Finally, different subtypes of PAH are characterized by distinct inflammatory profiles, a phenomenon that suggests that immunotherapies should be tailored to each patient’s disease. In this respect, the addition of adjunctive immunotherapies will likely require higher selectivity than what is currently required for vasodilators. Targeting IL-13 may be appropriate in a helminthic disease causing TH2 immunity, whereas therapies addressing B cell immunity will likely be more effective for a disease process dominated by pathogenic antibodies (and so on).

**Factors Linking Genetics, Inflammation, and Metabolism**

Recently, a call for a unifying theory of PAH pathogenesis was made to the research community to consolidate the ever-growing diversity of scientific findings. To this end, we here consider immunity as a unifying hypothesis explaining this disease. Why does inflammation cause PAH, and how is it linked to all the other noninflammatory and genetic factors implicated in disease pathogenesis? There are many ways of looking at this. If we start with the BMPR2 mutation, we now know that downstream effectors of BMPR2 signaling in both endothelial and smooth muscle cells can activate PPARγ-mediated gene regulation. The targets of PPARγ such as apelin and PGC (peroxisome proliferator-activated receptor gamma coactivator)-1α profoundly affect inflammation (macrophage recruitment) and metabolism. Dysregulated immunity (as illustrated in Figure 2) can arise from other genetic causes (such as, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy), viruses (such as HIV), or may be an idiopathic process (such as with the connective-tissue diseases). Dysregulated immunity may occur when the normal means of controlling inflammation via Tregs is functionally impaired. When there is a predilection to harmful immunity, an injury to the pulmonary circulation, whether induced by a vasculopathic virus or through altered shear stress or by local hypoxia, transforms a self-limited inflammatory response into a propagated injurious process. For some conditions, inflammation is limited to the lungs, such as in idiopathic PAH, while for other conditions, such as in systemic sclerosis, the vasculopathy is systemic. It is possible that the differences observed between local and systemic presentations reflect processes that are, variably, either antigen-specific or nonantigen specific. A clear case of localized injury is the TH2 adaptive immune response mounted against *Schistosoma* eggs physically lodged in the pulmonary circulation, whereas a nonantigen-specific cause may possibly apply to anorexigen-induced PAH. Inflammation confined to lungs suggests that the injury occurs there specifically because of unique aspects of the pulmonary circulation or autoantigen localization or both. In scleroderma associated with PAH, there is likely a global physiological derangement perhaps reflecting more widespread autoantigen presence. In this manner, the concept of inherited or acquired immune dysregulation could explain the protean manifestations of PAH.

Two themes form the focus of this PAH compendium: inflammation and metabolism. A cogent case has been made for how abnormal metabolism is a separate and credible unifying theory of PAH pathogenesis, and because the 2 unifying theories of inflammation and metabolism in PAH are not mutually exclusive, an important future goal of research will be to show the relationship between dysregulated immunity and altered metabolism in the development of this disease. Inflamed tissues are characterized by significant changes in metabolic activity that are attributable to the recruitment of monocytes and neutrophils, in addition to locally proliferating lymphocyte populations. Immune cells, implicated in PAH pathogenesis, obtain energy by different means. Lymphocytes predominantly use oxidative phosphorylation, whereas myeloid lineage cells derive their energy almost exclusively from glycolysis. Lymphocytes divide and quiesce in accordance with strictly controlled levels of essential metabolites that support anabolic growth, whereas innate immune cell survival is dependent on different metabolic cues. For example, neutrophil mitochondria maintain a transmembrane potential via the glycerol-3-phosphate shuttle leading to increased aerobic glycolysis (a peculiar mitochondrial phenotype also observed in the lung vasculature and right ventricle of PAH patients); this pattern of increased aerobic glycolysis is acquired during neutrophil differentiation from myeloid precursors. The differences in the metabolic requirements of adaptive and innate immune cells can help shape the characteristics of the immune response in a specific microenvironment. Dysregulated immunity observed in PAH may directly lead to alterations in mitochondrial metabolism, and, conversely, altered mitochondrial energetics in cardiopulmonary tissue may directly influence participating immune cells. Beyond cell energy utilization, inflammation is linked to other facets of metabolic derangement including insulin resistance. Macrophages are highly implicated in PAH pathogenesis, and their infiltration into adipose tissue is associated with insulin resistance, which is also a clinical feature of PAH. In summary, these studies cumulatively illustrate how bridging research in the fields of genetics, metabolism, and immunity will yield a more holistic theory of PAH pathogenesis with perhaps stronger insights than those afforded by separate approaches to the problem.

**What Does This Mean for the Patient or Clinician?**

The focus on inflammation and altered immunity in PAH lends itself to a whole new strategy for preventing and potentially reversing disease progression.
**A Patient Asks Questions…**

I read that there is a lot of inflammation in my blood and in my lungs, as if I had an infection, but I do not have fever. What does this mean and how important is it for my condition?

Inflammation is now increasingly recognized to be important for the development of pulmonary arterial hypertension (PAH). Although some viruses have been implicated as potential triggers of PAH, this inflammation may result from other causes. We do now know that inflammation around the lung vessels in PAH patients (i.e., accumulation of activated white blood cells around and within the lung blood vessels) may explain many of the features of the disease, including the overgrowth of the cells in the wall of the lung blood vessels. Blood cells that go through the lungs are activated and secrete substances (called cytokines) designed to organize our defense and response to infections. In this case, however, they have adverse effects in the lung blood vessels. It is not known why in PAH they may affect the lung blood vessels but not blood vessels outside the lungs. This activation of white blood cells is important but low grade, perhaps explaining why PAH patients do not have a fever. It may, however, cause weakness and malaise, similar to the malaise one feels during a viral infection, contributing to the PAH clinical symptoms.

Animal work has shown that normalizing the function of some of these white blood cells can also normalize PAH, opening a new window in the treatment of the disease. It is also promising in terms of developing accurate tools to monitor the activity of the disease. Clinical trials are ongoing with several approaches designed to inhibit the function of white blood cells selectively and hold promise that they may reverse PAH without increasing susceptibility to infections in the treated patients.

For the case description, see introductory article by E.D. Michelakis, page 109.

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**Disclosures**

None.

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