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Inflammation, Growth Factors, and Pulmonary Vascular Remodeling

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Inflammatory processes are prominent in various types of human and experimental pulmonary hypertension (PH) and are increasingly recognized as major pathogenic components of pulmonary vascular remodeling. Macrophages, T and B lymphocytes, and dendritic cells are present in the vascular lesions of PH, whether in idiopathic pulmonary arterial hypertension (PAH) or PAH related to more classical forms of inflammatory syndromes such as connective tissue diseases, human immunodeficiency virus (HIV), or other viral etiologies. Similarly, the presence of circulating chemokines and cytokines, viral protein components (e.g., HIV-1 *Nef*), and increased expression of growth (such as vascular endothelial growth factor and platelet-derived growth factor) and transcriptional (e.g., nuclear factor of activated T cells or NFAT) factors in these patients are thought to contribute directly to further recruitment of inflammatory cells and proliferation of smooth muscle and endothelial cells. Other processes, such as mitochondrial and ion channel dysregulation, seem to convey a state of cellular resistance to apoptosis; this has recently emerged as a necessary event in the pathogenesis of pulmonary vascular remodeling. Thus, the recognition of complex inflammatory disturbances in the vascular remodeling process offers potential specific targets for therapy and has recently led to clinical trials investigating, for example, the use of tyrosine kinase inhibitors. This paper provides an overview of specific inflammatory pathways involving cells, chemokines and cytokines, cellular dysfunctions, growth factors, and viral proteins, highlighting their potential role in pulmonary vascular remodeling and the possibility of future targeted therapy. (J Am Coll Cardiol 2009;54:S10–9) © 2009 by the American College of Cardiology Foundation

Pulmonary arterial hypertension (PAH) constitutes a heterogeneous group of clinical entities sharing similar pathologies that have been subcategorized as idiopathic pulmonary arterial hypertension (IPAH), familial PAH, pulmonary hypertension (PH) associated with other diseases such as connective tissue diseases, (e.g., systemic sclerosis [SSc]), portopulmonary hypertension, and PH related to human immunodeficiency virus (HIV) infection, drugs, and toxins (1). Although modifications to this classification are reviewed elsewhere in this series, this review focuses on inflammatory processes in PAH and other forms of PH, highlighting specific components of inflammation in the development of PH, as well as potential targets for therapy.

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Inflammation in PAH

Inflammation plays a significant role in various types of human PH, such as IPAH and PAH associated with connective tissue diseases and HIV infection and in experimental animal models (e.g., monocrotaline [MCT]-induced PH). A subset of PAH patients have circulating autoantibodies, including antinuclear antibodies (2), and elevated circulating levels of the proinflammatory cytokines interleukin (IL)-1 and IL-6 (3). Although there are serologic and pathologic features suggestive of inflammation in both IPAH and PAH related to SSc (PAH-SSc) or other connective tissue diseases, it is likely that inflammatory pathways and autoimmunity are more pronounced in PAH-SSc. This might explain survival discrepancies and differential response to therapy between the 2 syndromes (4). As such, PAH-SSc might be considered the prototypic syndrome in which to study inflammatory processes potentially operative in the pathogenesis of PAH.

A role for inflammation in PAH is based on the finding of inflammatory cells, including macrophages and T and B lymphocytes, and dendritic cells around the plexiform lesions of PAH (5). Levels of macrophage inflammatory protein-1α, IL-1β and -6 (3,6), and P-selectin (7) are increased in severe IPAH. Involvement of leukocytes, macrophages, and lymphocytes in the complex vascular lesions of IPAH was initially described by Tuder et al. (8) and confirmed in more recent studies by Dorfmüller et al. (9). Cytokine- and chemokine-dependent mechanisms leading to inflammatory cell recruitment in human PAH are also prominent in PAH.

Cytokines and chemokines in PAH. Balabanian et al. (10) demonstrated that fractalkine (CX3CL1), a unique chemokine that promotes the chemokine (C-X3-C motif) receptor 1 (CX3CR1)-expressing leukocyte recruitment, is upregulated in circulating $CD4^+$ and $CD8^+$ T lymphocytes from PAH patients as compared with control subjects. These patients also have elevated soluble CX3CL1 plasma concentrations; their lung tissue samples demonstrate increased CX3CL1 messenger ribonucleic acid (mRNA) expression as compared with control subjects, and pulmonary artery (PA) endothelial cells (ECs) from these lungs express CX3CL1 protein.

Regulated upon Activation, Normal T cell expressed and secreted (RANTES, also known as CCL5) is an important chemoattractant for monocytes and T-cells. CCL5 plays a key role in several vascular inflammatory processes such as glomerulonephritis, Kawasaki disease, and Takayasu's arteritis. CCL5 might also play an indirect role in PAH through the induction of endothelin (ET)-converting enzyme-1 and ET-1, a potent endothelium-derived factor with strong vasoconstrictive and mitogenic action. Indeed, CCL5 mRNA expression is increased in lung samples from PAH patients as compared with control subjects and probably originates from ECs, as demonstrated by in situ hybridization and immunohistochemistry (11). The exact relevance of these findings to the pathophysiology of PAH requires further investigation.

Two recent studies further suggest that chemokines produced from small PAs of PAH patients might contribute to inflammatory cell recruitment and PA smooth muscle cell (SMC) proliferation. Perros et al. (12) demonstrated that CX3CL1 is expressed by inflammatory cells surrounding PA lesions and that SMCs from these vessels have increased CX3CR1 expression. In addition, cultured rat PA-SMCs express CX3CR1, and CX3CL1 induces proliferation but not migration of these cells. Therefore, fractalkine might act as a growth factor for PA-SMCs. The hypothesis that chemokines might play a role in PA remodeling was further studied by Sanchez et al. (13). Compared with control subjects, IPAH patients have elevated levels of CCL2, also known as monocyte chemotactic protein (MCP)-1, in plasma and lung tissue. In addition elevated CCL2 release from pulmonary ECs or PA-SMCs was demonstrated. Monocyte migration was markedly increased in the presence of pulmonary ECs (particularly from patients with IPAH) and significantly reduced by CCL2-blocking antibodies. Finally, compared with control subjects, PA-SMCs from patients exhibited stronger migratory and proliferative responses to CCL2, in keeping with the finding that CCR2 was markedly increased in PA-SMCs in these patients (13).

Growth factors and inflammation in PAH. Several growth factors, including platelet-derived growth factor (PDGF) (14,15), epidermal growth factor (EGF) (16), and vascular endothelial growth factor (VEGF) (17), have been implicated in the abnormal proliferation and migration of PA vascular cells. They act as potent mitogens

Abbreviations and Acronyms

AECA = anti-endothelial cell antibody
bcl = B-cell lymphoma
COPD = chronic obstructive pulmonary disease
EC = endothelial cell
EGF = epidermal growth factor
ET = endothelin
HCV = hepatitis C virus
HHV = human herpes virus
HIV = human immunodeficiency virus
5-HT = serotonin
5-HTT = serotonin transporter
IL = interleukin
IPAH = idiopathic pulmonary arterial hypertension
Kv = voltage-dependent potassium channel
MCT = monocrotaline
mRNA = messenger ribonucleic acid
NFAT = nuclear factor of activated T cells
PA = pulmonary artery
PAH = pulmonary arterial hypertension
PCR = polymerase chain reaction
PDGF = platelet-derived growth factor
PDGFR = platelet-derived growth factor receptor
PH = pulmonary hypertension
RV = right ventricular
SIV = simian immunodeficiency virus
SMC = smooth muscle cell
SSc = systemic sclerosis
TGF = transforming growth factor
TN = tenascin
TNF = tumor necrosis factor
VEGF = vascular endothelial growth factor

and chemoattractants for SMCs, fibroblasts, and ECs and cause resistance to apoptosis.

VEGF. Cool et al. (18) demonstrated intense expression of the VEGF receptor KDR, coupled with a reduced expression of p27/kip1, a cell cycle inhibitory protein, in the ECs of plexiform lesions. Other markers of angiogenesis, such as VEGF and hypoxia inducible factor-1 subunits α and β, are highly expressed in ECs of plexiform lesions in severe PAH (19). In addition, expression of C-Src kinase (19), a protein that mediates VEGF-induced production of prostacyclin and nitric oxide in ECs, is decreased in PAH. Taken together, these findings suggest a central role in PAH for VEGF, a mediator of angiogenesis but also a factor involved in permeability and inflammatory processes in the vascular endothelium.

PDGF. Platelet-derived growth factor is synthesized by many different cell types including SMCs, ECs, and macrophages. PDGF induces the proliferation and migration of SMCs and fibroblasts and has been proposed as a key mediator in the progression of several fibroproliferative disorders such as atherosclerosis, lung fibrosis, and PH (14). As a result, novel therapeutic agents, such as tyrosine kinase inhibitors, have been tested in experimental models of PH (15) and more recently in clinical trials. The rationale for use of these agents is discussed in more detail in later sections. The pathogenic role of PDGF was demonstrated by increased expression of PDGF and platelet-derived growth factor receptors (PDGFRs) by reverse transcription-polymerase chain reaction (PCR) performed on laser-captured microdissected PAs from native lungs of patients with severe IPAH who underwent lung transplantation (20). The PDGF-A, PDGF-B, PDGF-R α, and PDGF-R β mRNA expression is increased in small PAs from patients with severe IPAH as compared with control subjects. In small PAs, PDGF-B is mainly expressed in ECs, SMCs, and in some perivascular inflammatory cells, and PDGFR-β is mainly expressed in SMCs. The PDGF-BB–induced proliferation and migration of PA-SMCs is inhibited by imatinib (20). Taken together, these data support the concept that PDGF is overproduced and promotes PA remodeling in PAH.

EGF. The EGF-dependent proliferation and migration of SMCs is dependent on the extracellular matrix component tenascin C (TN-C). In addition, EGF colocalizes with TN-C in PAH lesions (21), suggesting a direct role in disease progression. It is noteworthy that the EGF receptor inhibitor PKI166 reverses established MCT-induced PH in rats (16).

SEROTONIN AND SEROTONIN TRANSPORTER. In addition to its vasoactive effects, serotonin (5-HT) exerts mitogenic and co-mitogenic effects on PA-SMCs. In contrast to the constricting action of 5-HT on SMCs, which is mainly mediated by 5-HT receptors (5-HT 1B/D, 2A, and 2B) (22), the mitogenic and co-mitogenic effects of 5-HT require internalization of indoleamine by serotonin transporter (5-HTT) (23). Accordingly, drugs that competitively inhibit 5-HTT also block the mitogenic effects of 5-HT on SMCs (24).

Serotonin transporter is abundantly expressed in the lung, where it is predominantly located in PA-SMCs (24). Direct evidence that 5-HTT plays a key role in PA remodeling is supported by studies showing that mice with targeted 5-HTT gene disruption develop less severe hypoxic PH than wild-type control subjects (25) and that selective 5-HTT inhibitors attenuate hypoxia- and MCT-induced PH (26). Conversely, increased 5-HTT expression is associated with increased severity of hypoxic PH (27). Transgenic mice with selective overexpression of 5-HTT in SMCs spontaneously develop PH (28). Pulmonary hypertension seems to develop in these mice without any alterations in 5-HT bioavailability and as a sole consequence of the increased expression of 5-HTT in SMCs. Taken together, these observations suggest a close correlation between 5-HTT expression and/or activity and the extent of PA remodeling during experimental PH.

Serotonin transporter expression is increased in platelets and in the media of thickened PAs in IPAH (24). The PA-SMCs from patients with IPAH grow faster than PA-SMCs from control subjects when stimulated by 5-HT or serum, as a consequence of increased 5-HTT expression (24). In the presence of 5-HTT inhibitors, the growth-stimulating effects of serum and 5-HT are markedly reduced, and the difference between growth of PA-SMCs from patients and control subjects was abolished. Taken together, 5-HTT overexpression and/or activity in PA-SMCs from IPAH patients seem responsible for the increased mitogenic response to 5-HT. The 5-HT is synthesized by ECs in the normal lung as a result of tryptophan hydroxylase-1 enzyme activity and seems to be the main growth factor produced by ECs, acting on PA-SMCs in a paracrine fashion. In conclusion, PA-SMC hyperplasia in IPAH seems to result from both dysregulation of 5-HT production by ECs due to overexpression of tryptophan hydroxylase-1 and from an increased PA-SMC response to 5-HT due to overexpression of the 5-HTT (29).

SURVIVIN. Survivin (16.5 kDa) is the smallest member of the mammalian inhibitor of the apoptosis family. Several malignant processes have been linked to dysregulation of survivin expression. The normal absence of survivin from healthy tissues suggests it is a potential target for therapy. Survivin is overexpressed in PAs from PAH patients and in rats with MCT-induced PAH, compared with control subjects (30). Wild-type survivin delivered via an inhaled adenovirus to normal rats causes PH. Conversely, gene therapy with an adenovirus carrying a phosphorylation-deficient survivin mutant with dominant-negative properties (T34A survivin) reverses established MCT-PAH and prolongs survival (30). Administration of the survivin mutant reduces pulmonary vascular resistance, right ventricular

(RV) hypertrophy, and PA medial hypertrophy. Both in vitro and in vivo, inhibition of endogenous survivin induces PA-SMC apoptosis, depolarizes mitochondria, causes efflux of cytochrome c in the cytoplasm, translocates apoptosis-inducing factor into the nucleus, and increases voltage-dependent potassium channel (Kv) current, whereas the opposite effects are observed with gene transfer of wild-type survivin. Survivin also induces the production of the PDGF receptor in human vascular SMCs (31). Therefore, the proposed causative role of survivin in PAH and the lack of its expression in normal PA wall and systemic vasculature make this gene attractive for future targeted therapy in PAH.

Transcriptional factors: the nuclear factor of activated T cells in inflammation and vascular remodeling. The nuclear factor of activated T cells (NFAT), originally described in T cells, is a master activator of T cells, increasing the transcription of multiple inflammatory mediators, including many interleukins and tumor necrosis factor (TNF)α, and activating T and B cells (32). Increased $[Ca^{2+}]_i$ activates calcineurin, which dephosphorylates cytoplasmic NFAT, allowing its entry to the nucleus, where it forms complexes with other important transcription factors (e.g., GATA or activator protein-1) and regulates gene transcription (32).

Several recent observations suggest that NFAT might be involved in PAH. The NFAT activation causes downregulation of Kv1.5 (33), which plays a preponderant role in pulmonary vasoconstriction. Second, ET (upregulated in PAH) activates NFAT, which in turn increases B-cell lymphoma (bcl)-2 expression, contributing to the prosurvival and antiapoptotic effects of ET in the heart (34). Third, NFAT directly or indirectly regulates the transcription of several genes that regulate mitochondrial function (e.g., pyruvate decarboxylase and the electron transport chain enzyme cytochrome C oxidase) (35).

The NFAT is upregulated and activated (i.e., translocated in the nucleus) in circulating inflammatory cells in patients with PAH, including IPAH and PAH-SSc. The CD3-positive cells with activated NFAT are also seen in remodeled PAs. Intriguingly, NFAT is also activated in the PA-SMCs of remodeled arteries. The PA-SMCs isolated from PAH patients maintain in culture a unique phenotype (downregulated Kv1.5, upregulated bcl-2, hyperpolarized mitochondria), which is associated with activated NFAT and resistance to apoptosis. The NFAT is not activated in normal lungs and PA-SMCs. The unique phenotype of PAH PA-SMCs is normalized by selective inhibition of NFAT.

Inhibition of NFATc2 (predominant NFAT isotype in PAH) by VIVIT (a competitive peptide that inhibits the docking of NFAT to calcineurin) or cyclosporine (inhibitor of calcineurin), restores Kv1.5 expression and current and decreases $[Ca^{2+}]_i$, $[K^+]_i$, bcl-2, and mitochondrial membrane potential ($\Delta\psi m$), leading to increased apoptosis in vitro (36). In vivo, cyclosporine treatment decreases established MCT-induced PAH in the rat (36). Intriguingly, PA-SMCs exposed to chronic hypoxia display NFAT activation, hyperpolarized mitochondria, and downregulated Kv1.5, similar to the SMC phenotype of PAH. Inhibition with VIVIT or cyclosporine reverses this phenotype, normalizing the mitochondrial membrane potential and level/function of Kv1.5 in these cells. There has been recent interest in developing specific NFAT inhibitors for the treatment of cardiac hypertrophy and failure (37). Therefore, in PAH, NFAT inhibitors might contribute to reversing RV hypertrophy and pulmonary vascular remodeling through their effects on cardiomyocytes, PA-SMCs, and inflammatory cells.

Viral and Other Infectious Etiologies in PAH

Hypothetically, PH is caused by latent viral infections, because associations between Epstein Barr virus infection and Hodgkin's disease and parvovirus and cytomegalovirus infection and SSc have been described (38); both diseases have also been associated with PH. Infectious organisms can affect the lung circulation directly, by obliterating lung vessels, or indirectly, by causing and maintaining inflammation.

However, there is little evidence for a "direct" role for infectious agents in the pathogenesis of severe PH. Even in schistosomiasis-associated PH, it is unclear to what extent liver disease and therefore portopulmonary hypertension dominate the pathobiology of PH. Schistosoma eggs modulate regulatory T-cell activity and express a novel member of the transforming-growth factor (TGF)-β superfamily, *Schistosoma mansoni* inhibin/activin (SmInAct) (39). Recently a mouse model of pneumocystis-induced PH associated with muscularized PAs has been reported (40), and Daley et al. (41) reported a mouse model of highly muscularized PAs after a regimen of aspergillus antigen (ag) immunization.

Role of human herpes virus-8, HIV, and SHIV-Nef in pulmonary vascular remodeling. Pulmonary arterial hypertension has a prevalence of 0.0002% in the general population, but in HIV-infected individuals the prevalence is 0.46% in France (42). The HIV-related PAH (HRPAH) is independent of $CD4^+$ T cell counts (43) and antiviral drug treatment. The clinical features of HRPAH are similar to PAH of other etiologies. Although highly active antiretroviral therapy might have decreased the incidence of HRPAH and might partially reverse PH in a small number of HIV-1–infected individuals only when combined with PH-specific treatment such as bosentan (44), this disease remains a significant clinical complication in the HIV-1–infected population. Other studies showed no correlation between viral load and right heart changes (45).

Most of the pathways involved in virus pathogenesis converge on either prosurvival or proangiogenic signals, the same signals associated with PH. In the lung, HIV-1 infects primarily macrophages, providing a potential reservoir for the transmission of the virus to circulating T-cells, and is a source

for localized viral proteins such as *Nef*, *Tat*, and *gp120*, which might have direct or indirect effects. Chronic exposure to these viral products as well as deficiency in regulatory T cells and altered production of chemokines/cytokines might contribute to pulmonary vascular dysfunction.

Macaques infected with chimeric SHIV-*nef* virions (simian immunodeficiency virus $[SIV]_{mac239}$ Δ*nef* virus containing a cloned HIV-1 *nef* gene) demonstrate lung vascular changes characteristic of PAH, whereas macaques infected with parental SIV strains containing the native SIV *nef* allele show no vascular remodeling (46). The *Nef* was also demonstrated by immunohistochemistry in lungs of HIV-infected patients with PH (47). Thus, HIV-1 Nef protein, perhaps in conjunction with host genetic factors and/or persistent immune dysregulation, contributes to the development of pulmonary vascular remodeling. Foci of mononuclear cells and ectopic lymphoid tissues adjacent to the lesions might be sources of this viral protein.

The HIV-1 Nef is 1 of the accessory proteins made early in HIV infection and whose major effects are downregulating CD4 (48) and blocking major histocompatibility antigen-I trafficking to the membrane (49), allowing the infected cells to evade immune surveillance (50). In human monocyte-derived macrophages, Nef activates the STAT1 pathway and the secretion of MIP-1, IL-1-α, IL-6, and TNFα (51).

Human gamma herpes virus 8. Human gamma herpes virus 8 (HHV8), also known as Kaposi's sarcoma-associated herpes virus, has been associated with angioproliferation (52). The HHV8 is unquestionably associated with proliferative disorders, including multicentric Castleman's disease and Kaposi's sarcoma. Evidence of HHV8 was found in a large percentage of plexiform lesions of one cohort of PH patients, suggesting for the first time that this virus was a contributing factor (53). However, a number of other investigators have attempted without success to find evidence of latent HHV8 infection in lung tissue sections from patients with idiopathic PAH, with immunohistochemistry and PCR methodology (54–57).

Hepatitis C virus. Finally, PH represents one of the extrahepatic complications of hepatitis C virus (HCV) infection, with a prevalence of 1% to 5% (58). In the majority of patients, portal hypertension precedes PH (58,59). The pathogenesis is poorly understood, but the histologic hallmarks are similar to IPAH. Whether these lesions are secondary to increased inflammatory cytokine production, direct viral replication, or presence of viral products in the lung remains to be determined. In contrast, an observational study of 823 HIV-infected patients with and without HCV concluded that although age, baseline $CD4^+$ cell count, and duration of highly active antiretroviral therapy were significantly associated with survival, HCV infection was not (60). An associated immune dysregulation might trigger uncontrolled intrapulmonary angiogenesis, as in HIV-mediated PH.

In summary, very little is known about the natural history of any form of virus-related PH or the molecular mechanisms that account for the pathogenesis. Cell biological studies with recombinant viral proteins or with cloned virions might shed some light as to potential molecular mechanisms whereby viral proteins induce angioproliferation.

PAH-SSc as a Prototypic Inflammatory Disease

Vascular changes in SSc and evidence for autoimmunity as a central component of remodeling. Vascular changes occur at an early state in SSc and include apoptosis (61), EC activation with expression of cell adhesion molecules, inflammatory cell recruitment, procoagulant state (62), and intimal proliferation and adventitial fibrosis leading to vessel obliteration. Endothelial cell injury is reflected by increased levels of soluble vascular cell adhesion molecule-1 (63), disturbances in angiogenesis as reflected by increased levels of circulating VEGF (64), and presence of angiostatic factors (64). Dysregulated angiogenesis in PAH-SSc, whether driven by the inflammatory process or other mechanisms, seems to be a predominant feature of the disease and should be a focus of future studies.

Autoantibodies in scleroderma-related PAH. A role for an autoimmune process has been proposed in the pathogenesis of PAH-SSc. Antifibrillarin antibodies (anti–U3-RNP) are frequently found in PAH-SSc patients (65), and the poorly characterized anti-endothelial cell antibodies (AECAs) correlate with digital infarcts (66). Antibodies to fibrin-bound tissue plasminogen activator in patients with limited cutaneous SSc (67) and in IPAH patients with HLA-DQ7 antigen (68) and antitopoisomerase II-α antibodies, particularly in association with HLA-B35 antigen (69), are found in PAH-SSc. Nicolls et al. (5) suggested that AECAs—which can activate ECs, induce the expression of adhesion molecules, and trigger apoptosis—play a role in PAH pathogenesis. In vitro experiments using autoantibodies from patients with connective tissue diseases (anti–U1-RNP and –dsDNA) can upregulate adhesion molecules (e.g., endothelial leukocyte adhesion molecule-1) and histocompatibility complex class II molecules on human PA ECs (70), suggesting that an inflammatory process could lead to proliferative and inflammatory pulmonary vasculopathy.

Fibroblasts are essential components of remodeling of the pulmonary vascular wall in PAH and can be found in the remodeled neointimal layer in both PAH-SSc and IPAH. The detection of antifibroblast antibodies in the serum of PAH-SSc and IPAH patients (71,72) has significant pathogenic importance, because these antibodies can activate fibroblasts and induce collagen synthesis, thus potentially contributing directly to the remodeling process. Antibodies from sera of patients with SSc induce a proadhesive and proinflammatory response in normal fibroblasts (72). Immunoglobulin G antifibroblast antibodies are present in sera of patients with IPAH and PAH-SSc and have distinct reactivity profiles in these 2 conditions (71). With

2-dimensional immunoblotting technique, several antigens recognized by serum immunoglobulin G from IPAH and PAH-SSc patients were identified, including proteins involved in regulation of cytoskeletal function, cell contraction, cell and oxidative stress, cell energy metabolism, and different key cellular pathways (73). Although the specific membrane antigens targeted by these autoantibodies remain to be determined, it is likely that they react to membrane components, because they typically bind to unpermeabilized fibroblasts, and might mediate the release of cytokines and growth factors which in turn might contribute to the pathogenesis of vascular remodeling in PAH (71).

Taken together, particularly in light of the positive response to immunosuppressive therapy for one-third of patients with PAH associated with systemic lupus erythematosus and mixed connective tissue disease (74), these studies suggest that inflammation and autoimmunity could play a major role in the pathogenesis of PAH. Thus, a search for specific biomarkers of inflammation could be a focus of future studies in IPAH, PAH-SSc, and other autoimmune conditions associated with PAH.

Inflammatory genes in SSc and scleroderma-related PAH. An increasing number of candidate genes have been reported to be associated with SSc in different populations: a variant in the promoter of MCP-1 (75); 2 variants in CD19 (−499G>T, and a GT repeat polymorphism in the 3′-UTR region) (76); a promoter and coding polymorphism in TNF-α (TNF-α 238A>G, TNF-α 489A>G) (77); a variant in the promoter of the IL-1α gene (IL1-α −889T) (78); and a 3-single nucleotide polymorphism haplotype in IL-10 (79). Thus, compelling data support a genetic basis for SSc. Despite these recent advances in genetics, little is known about genetic involvement in PAH-SSc. *BMPR2* mutations have not been identified in 2 small cohorts of PAH-SSc patients (80,81).

Recently, an association between an endoglin gene (*ENG*) polymorphism and PAH-SSc was identified (82). Endoglin, a homodimeric membrane glycoprotein primarily present on human vascular endothelium, is part of the TGF-β receptor complex. The functional significance of the *ENG* polymorphism in SSc patients remains to be determined.

Aside from the few examples cited in the preceding text, the genes relevant to the pathogenesis and generally poor outcome associated with PAH-SSc have not been identified. Their definition will require robust, well-characterized patient populations to provide adequate power for analysis.

Inflammation in PH Associated With Chronic Obstructive Pulmonary Disease

Pulmonary vascular remodeling is a common finding in chronic obstructive pulmonary disease (COPD) and in heavy smokers with normal lung function (83). Inflammatory cells might contribute to the alterations of pulmonary vessels. Indeed, the extent of pulmonary vascular remodeling correlates with the severity of the inflammatory cell infiltrate in small airways (84). Furthermore, patients with COPD have an increased number of inflammatory cells infiltrating the adventitia of muscular PAs, as compared with nonsmokers (85). This inflammatory infiltrate is largely constituted by activated T lymphocytes with a predominance of the $CD8^+$ T cell subset (85) without change in neutrophils, macrophages, and B-lymphocytes.

VEGF. Patients with mild-to-moderate COPD show increased expression of VEGF in PAs compared with control nonsmokers (86). The VEGF expression correlates with arterial wall thickness, suggesting a potential role of VEGF in the pathogenesis of pulmonary vascular remodeling in COPD. In patients with advanced COPD and severe emphysema, the expression of VEGF in PAs is lower than in patients with mild-to-moderate disease and does not differ from control nonsmokers (86), suggesting downregulation of VEGF in patients with emphysema that might lead to EC apoptosis.

TGF-β. In COPD, TGF-β has been implicated in connective tissue deposition (87) and airway macrophage recruitment (88). In patients with very severe COPD, the expression of type II receptor (TGF-β RII) but not TGF-β is increased in the tunica media and intima of PAs (89), along with a normal cell proliferation rate in both layers of the vessel wall, suggesting that TGF-β might exert a protective role (restraining cell proliferation) and that growth factors other than TGF-β might be involved in pulmonary vascular remodeling (89).

Targeting Signaling Pathways: The Role of Antineoplastic Drugs in the Control of Vascular Remodeling in PAH

The concept of "targeted" therapy holds popular appeal for advancing cancer treatment. Imatinib, an inhibitor of Bcr-Abl kinase, has dramatically changed prognosis for patients with chronic myeloid leukemia (90). Although imatinib is the archetype for targeted cancer therapeutics, it does not exclusively inhibit Bcr-Abl but also inhibits PDGFR (91). Schermuly et al. (15) tested the effects of imatinib in rodent models, on the basis of evidence that PDGF signaling is an important process in the pathophysiology of PAH (92). The effects of MCT on RV systolic pressure, cardiac index, RV hypertrophy, and overall survival were reversed in dose-dependent fashion with administration of imatinib, along with downregulation of phosphorylated PDGFRβ and extracellular signal-related kinase in lung tissue homogenates. Clinical validation of imatinib as PAH therapy was first suggested in case reports (93–95). These led to a Phase II trial to evaluate the safety, tolerability, and efficacy of imatinib in patients with PAH that, at the time of the PH World Congress, was open to accrual at multiple centers in the U.S. and Europe.

Disrupting PDGF and VEGF signaling. Although the role for specific disruption of PDGFR signaling in cancer

therapeutic regimens is still under investigation, the efficacy of 2 U.S. Food and Drug Administration-approved agents, sunitinib and sorafenib, is attributed in part to their dual inhibition of VEGF and PDGF signaling pathways. Whereas PDGF is a validated specific target in PH, the rationale for testing antiproliferative drugs in advanced human PAH is also based on the presence of dysregulated proliferation of microvascular ECs and SMCs, monoclonal EC expansion (96), increased expression of secreted growth factors such as VEGF and basic fibroblast growth factor (97), and the fact that this condition—with its poor prognosis—is reminiscent of advanced solid tumors (98). Also at the time of the PH World Congress, a Phase I clinical trial to determine the safety and tolerability of

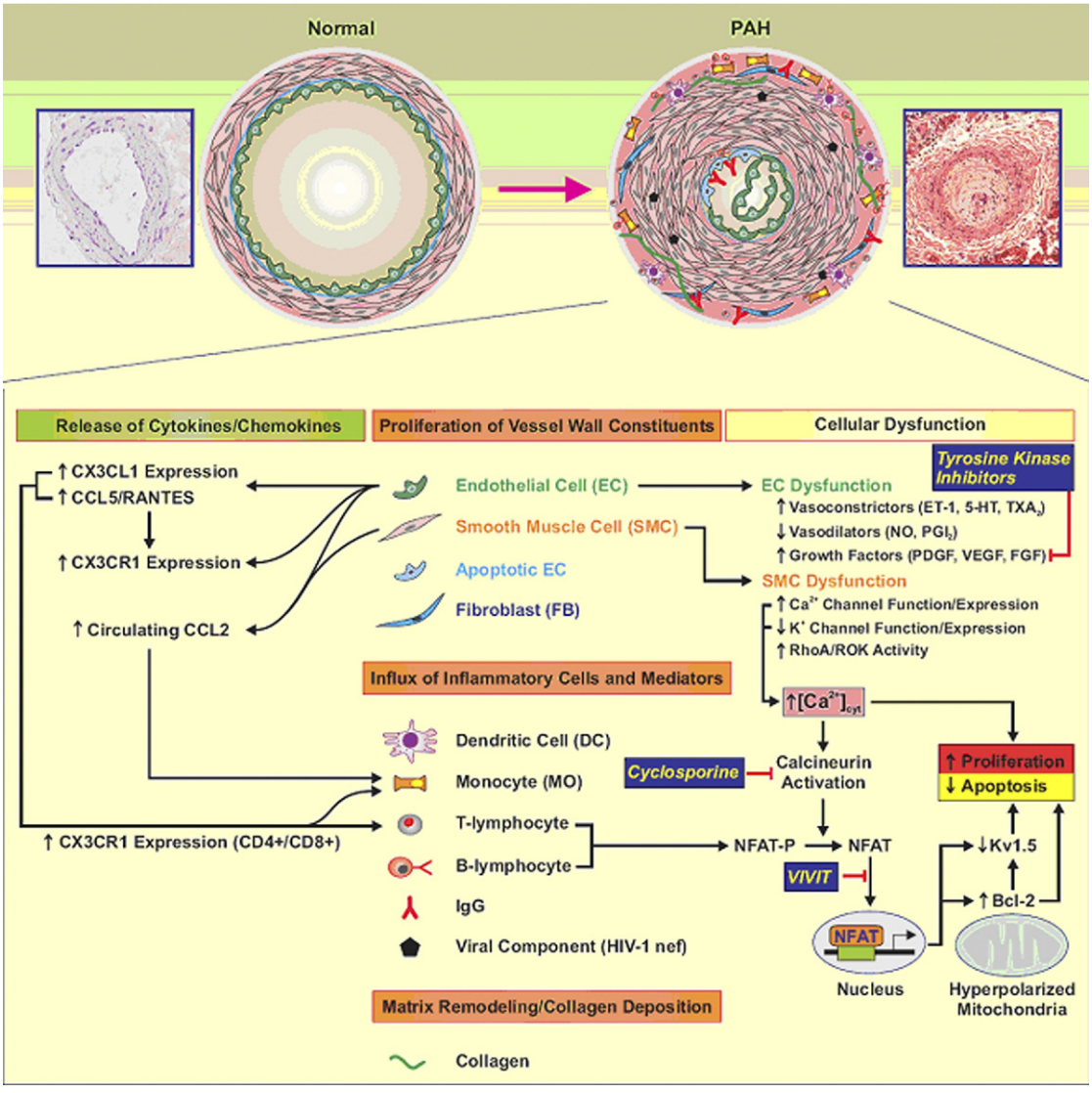

Figure 1 **Mechanisms of Inflammation-Mediated Remodeling**

This schematic features inflammatory mediators, cells, and mechanisms involved in pulmonary vascular remodeling as well as potential therapeutic targets. Release of cytokines and chemokines in remodeled vessels (e.g., plexiform lesions) or in the circulation, from activated endothelial cells (ECs) and smooth muscle cells (SMCs), mediate the influx of inflammatory cells (e.g., monocytes, T and B lymphocytes). Cellular dysfunction (particularly involving EC and SMC) contributes to release of vasomotor and growth mediators, activation of transcriptional factors (e.g., nuclear factor of activated T lymphocytes [NFAT]), influx of calcium, and mitochondrial dysfunction. The net effect is a shift of balance in favor of cell proliferation and decreased apoptosis, leading to remodeling and narrowing of the pulmonary vascular lumen. Potential therapeutic target sites include inhibition of growth factors with tyrosine kinase inhibitors, calcineurin with cyclosporine, and prevention of NFAT activation with VIVIT polypeptide (a competitive peptide that inhibits the docking of NFAT to calcineurin). Specific mechanisms are further detailed in the text. bcl2 = B-cell lymphoma 2; CCL2 = chemokine (C-C motif) ligand 2; CCL5 = chemokine (C-C motif) ligand 5 or RANTES (Regulated upon Activation, Normal T cell expressed and secreted); CX3CL1 = chemokine (C-X3-C motif) ligand 1 (fractalkine); CX3CR1 = chemokine (C-X3-C motif) receptor 1; DC = dendritic cells; ET_1 = endothelin 1; FB = fibroblasts; FGF = fibroblast growth factor; 5-HT = serotonin; HIV-1 = human immunodeficiency virus 1; IgG = immunoglobulin G; MO = monocyte; NO = nitric oxide; PAH = pulmonary arterial hypertension; PDGF = platelet-derived growth factor; PGI2 = prostacyclin; ROK = Rho kinase; VEGF = vascular endothelial growth factor.

sorafenib in PAH patients with stable clinical and hemodynamic status on prostacyclin-based therapy was open and now has since been completed at the University of Chicago. The results of these trials should help advance development of this therapeutic strategy in PAH.

Conclusions

It has become clear that inflammatory processes involving cellular effectors, chemokines, cytokines, and growth factors play a preponderant role in the vascular remodeling characteristic of PAH (Fig. 1). Recognition of these specific pathways should allow development of additional targeted therapy in this disease, with the hope of altering a prognosis that has been all too dismal in spite of significant recent progress.

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