The pathogenesis of pulmonary hypertension (PH) involves a complex and multifactorial process. Endothelial dysfunction seems to play an integral role in mediating the structural changes in the pulmonary vasculature. Disordered endothelial cell proliferation along with concurrent neangiogenesis, when exuberant, results in the formation of glomeruloid structures known as the plexiform lesions, which are common pathological features of the pulmonary vessels of patients with pulmonary arterial hypertension (PAH). In addition, an altered production of various endothelial vascular mediators, such as NO, prostacyclin, endothelin-1 (ET-1), serotonin, and thromboxane, has been increasingly recognized in patients with PH. Because most of these mediators affect the growth of the smooth muscle cells, an alteration in their production may facilitate the development of pulmonary vascular hypertrophy and structural remodeling characteristic of PH. It is conceivable that the beneficial effects of many of the treatments currently available for PAH, such as the use of prostacyclin, NO, and ET antagonists, result at least in part from restoring the balance between these mediators. However, the ultimate cellular and physiological targets of these treatments remain unknown.

In addition to the potential consequences of an imbalance in the endothelial production of various mediators, injury to the endothelium may expose the underlying vascular tissue to diverse blood-borne factors that may further promote pathological changes. Endothelial dysfunction may also have adverse consequences on pulmonary vascular hemostasis by altering the production of anticoagulant factors. Recent reports of genetic mutations in the endothelial cells of patients with PH further underscore the role of these cells in the disease pathogenesis.

**The Endothelium in Normal Lung**

The endothelium lining the normal lung is characterized by significant heterogeneity. Not only is it vastly different from systemic endothelium in ultrastructure and function, but it varies in various vessel types in the pulmonary vasculature itself. The main functions of the pulmonary endothelium include maintenance of vascular tone, homeostasis, leukocyte trafficking, transduction of luminal signals to abluminal vascular tissues, production of growth factors and cell signals with autocrine and paracrine effects, and barrier function. The normal endothelium is considered a genetically stable, “quiescent” cell line. Once “activated” by disease states, the endothelial cells may express specific markers and proteins, such as E-selectin, intercellular adhesion molecule (ICAM)-1, tissue factor, and vascular endothelial growth factor (VEGF) receptors. Such changes may herald increased coagulability, proliferation, and vasoconstriction (Figure 1).

**The Endothelium in PH**

**Phenotype**

Cool et al used immunohistochemistry and three-dimensional reconstruction to study the different endothelial cell phenotypes in pulmonary arteries affected by severe PH. An intense expression of VEGF receptor KDR coupled with a reduced expression of p27kip1, a cell cycle inhibitory protein (ie, marker of low growth potential), was noted in the endothelial cells of plexiform lesions. However, phenotypically distinct endothelial cells lined the plexiform lesions and the sites of neangiogenesis, suggesting differential angiogenic activity within the plexiform lesion. Other markers of angiogenesis, such as VEGF and hypoxia inducible factor (HIF-1) subunits α and β, are highly expressed in endothelial cells of plexiform lesions in patients with severe PH. Both HIF-1α and HIF-1β are responsible for the hypoxia-dependent induction of VEGF. However, although HIF-1β is usually constitutive, the finding of an increased expression of HIF-1α, which is usually seen specifically in response to hypoxia, suggests a decreased oxygen availability around the plexiform lesion, perhaps as a result of vessel obliteration by proliferating endothelial cells. In addition, the lungs of patients with PAH are characterized by a decreased expression of e-Src kinase, a protein that mediates VEGF-induced production of prostacyclin and NO in the endothelial cells.

**Circulating Endothelial Cells**

Although circulating endothelial cells (CEC) may participate in processes such as reparative response to vascular injury and tumorigenesis, the role of these cells in the pathobiology of PH is yet to be elucidated. An increase in the number of CEC has been reported in patients with sickle cell disease. These cells represent an “activated phenotype” and are characterized by CD36 positivity and by an expression of the markers ICAM-1, vascular cell adhesion molecule-1, E-selectin, and P-selectin. A similar increase in CEC may

From the Division of Pulmonary, Critical Care, and Sleep Medicine, Department of Medicine, Tufts—New England Medical Center, Tufts University School of Medicine, Boston, Mass (R.B.), and Division of Cardiopulmonary Pathology, Department of Pathology (R.M.T.), and Division of Pulmonary and Critical Care Medicine (P.M.H.), Department of Medicine, Johns Hopkins Hospital and Johns Hopkins University School of Medicine, Baltimore, Md.

Correspondence to Dr Paul M. Hassoun, 5501 Hopkins Bayview Circle, Baltimore, MD 21224. E-mail phassoun@jhmi.edu

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occur in patients with idiopathic PPH. Whether these cells are derived from the endothelial progenitor cells obtained from the mobilization of bone marrow by growth factors such as VEGF, or they are sloughed off (or actively shed) from injured pulmonary vessels, is not known. Study of endothelial cell ultrastructure, and use of vascular bed-specific markers such as lung endothelial cell adhesion molecules and DANCE (developing arteries and neural crest derivatives with multiple epidermal growth factor-like domains) may help clarify the origin of these cells and further improve our understanding of the angiogenesis/vasculogenesis process in the hypertensive lungs.

The Sentinel Event
The mechanisms responsible for endothelial activation are yet to be fully elucidated. However, a number of stimuli, including shear stress from increased pulmonary blood flow, viral infection (HIV), and alveolar hypoxia, may potentially activate such a response in genetically predisposed individuals. The nature of the initial stimulus that precedes the vasoconstrictive/vasoproliferative events is unknown. However, an attractive hypothesis is that injury to the endothelium leads to apoptosis of the usually quiescent cells, destabilization of the pulmonary vascular intima, preferably at branching points, and uncontrolled proliferation of endothelial cells. Most of the physiological consequences of PH would then emanate from the resultant narrowing of the pulmonary vessels. A decrease in the vascular lumen increases the pulmonary vascular resistance, consequentially raising the pulmonary vascular pressure. The endothelium senses the increased mechanical stretch and responds by enhancing collagen tissue of the vessel wall. This response is lost in arteries from which the endothelium has been stripped. Endothelial injury may also cause a leakage of proliferative mediators such as fibroblast growth factor. Endogenous vascular elastase may permeate the vascular wall and initiate, via degradation of matrix elements, growth signals to medial smooth muscle cells. Furthermore, adaptive hypertrophy of vascular smooth muscle and adventitia may occur in response to increasing luminal pressure. These phenomena constitute the so-called “remodeling” of the pulmonary arteries.

Critical to the understanding of the increase in pulmonary vascular impedance will be the elucidation of the relative contribution of vasoconstriction and cell growth. Furthermore, it is still unclear whether vascular impedance is affected, and to what extent, by either endothelial cell (intimal) or smooth muscle (medial) proliferation.

Endothelium-Derived Vasoactive Mediators
The endothelium releases diverse growth factors and vasoactive mediators, which regulate the physical and biochemical properties of the pulmonary vessels and affect vascular contractility and cell growth. In healthy individuals, a balance between these mediators is thought to mediate the low basal pulmonary vascular tone, homeostasis, and vascular injury repair and growth. Hypothetically, an alteration in this crucial balance might alter the architectural and tensile properties of the pulmonary vessels. Genetic mutations have been recently recognized in patients with primary PH (PPH or idiopathic PAH) and may render the patient more susceptible to intrinsic biological processes and/or external factors. Indeed, there is recent evidence that bone morphogenetic proteins (BMPs) render cultured endothelial cells more resistant to apoptotic stimuli.

Nitric Oxide
The role of endothelium-derived NO in the pathogenesis of PH remains poorly delineated. Available evidence suggests that NO is at least partially responsible for resting pulmonary vasorelaxation. Endothelial NO synthase (eNOS) catalyzes the conversion of L-arginine to citrulline, producing NO. NO activates guanylate cyclase and increases cyclic GMP
levels in smooth muscle cells, causing vasodilatation. Apart from a baseline constitutive expression, eNOS can be modulated by diverse stimuli such as shear stress and increased pulmonary blood flow.\textsuperscript{13}

The specific role of eNOS in pulmonary vascular tone regulation is best demonstrated in animal models. Overproduction of eNOS in transgenic mice prevents hypoxia-induced PH.\textsuperscript{14} Conversely, exposure to mild hypoxia results in severe PH in eNOS-deficient mice.\textsuperscript{15,16} Human studies, however, have reported variable production of eNOS in patients with idiopathic PPH with reduced expression in pulmonary vessels\textsuperscript{17} or increased expression in the endothelium.\textsuperscript{18} Patients with severe idiopathic PAH, portopulmonary hypertension, and HIV-associated PAH,\textsuperscript{25} and urinary levels of prostacyclin metabolites are decreased in patients with PH.\textsuperscript{26} Decreased prostacyclin levels may thus explain pulmonary vasoconstriction, smooth muscle cell proliferation, and enhanced coagulation.

The role of prostacyclin in PAH is perhaps best demonstrated by the remarkable success of this drug in the treatment of this disease. Several trials have consistently shown an improvement in the exercise capacity, cardiopulmonary hemodynamics, New York Heart Association functional class, symptoms, as well as survival in patients with PAH treated with continuous infusion of intravenous prostacyclin (epoprostenol).\textsuperscript{27-29} Epoprostenol infusion also improves the balance between ET-1 release and clearance,\textsuperscript{30} increases VEGF levels,\textsuperscript{31} and restores normal homeostasis by increasing plasma levels of soluble P-selectin and thrombomodulin, which are reportedly low in patients with PAH.\textsuperscript{32}

**Endothelin-1**

ET-1, a 21-amino-acid peptide with potent vasoconstrictor activity and platelet-aggregating properties, is widely distributed in the human endothelium.\textsuperscript{33} ET-1 expression is elevated in animal models of PH\textsuperscript{34,35} and in patients with PH.\textsuperscript{36} Furthermore, there is a strong correlation between ET-1 expression and pulmonary vascular resistance in patients with PAH\textsuperscript{37} and PH related to Eisenmenger syndrome.\textsuperscript{38} The pulmonary arterial-to-venous ratio of ET-1 is significantly increased in patients with PH, suggesting an overproduction or a decrease in clearance of this molecule.\textsuperscript{39}

In mammals, two ET receptors have been described, as follows: ET\textsubscript{A}, through which vasoconstriction is elicited, and ET\textsubscript{B}, which can mediate either vasoconstriction by its effects on smooth muscle or vasodilation through action on endothelial cells. Expression of mRNA for both receptor types is increased in animal models of PH.\textsuperscript{34,35} In humans, a significant increase in the gene expression of ET\textsubscript{B} receptor has been reported in severe thromboembolic PH.\textsuperscript{39} ET-1 receptor antagonists, such as bosentan, improve a patient’s functional status and other indices of PH-related morbidity, and are currently being used clinically for this condition.\textsuperscript{40} Collectively, these findings suggest a possible role for ET-1 in the pathogenesis of PH.

**Thromboxane**

Thromboxane is produced by endothelial cells and platelets. It is a potent vasoconstrictor, a smooth muscle mitogen, and an inducer of platelet aggregation. An increased production of thromboxane A\textsubscript{2} metabolites is seen in PPH.\textsuperscript{26} Furthermore, thromboxane-receptor density is increased in the right ventricle of patients with PPH.\textsuperscript{41} Thromboxane inhibition produced a modest improvement in pulmonary hemodynamics in a small study of patients with PPH.\textsuperscript{42} However, a larger trial involving a thromboxane inhibitor, terbogrel, had to be interrupted prematurely because of severe leg pain in the treatment group.\textsuperscript{43} Nevertheless, significant inhibition of thromboxane in this study suggests a potential therapeutic role for a thromboxane inhibitor with a better safety profile.

**Vascular Endothelial Growth Factor**

Normal endothelial cells do not characteristically secrete VEGF. However, the endothelial cells in PH have been shown to express VEGF. Platelet levels of VEGF are also elevated in PH.\textsuperscript{31} The putative role of VEGF may depend on the very nature of the normal or diseased endothelial cells. In the resting or homeostatic state, VEGF is a survival and differentiating factor for lung endothelial cells and, therefore, VEGF elevation in PH may represent a protective response. VEGF blockade results in severe PH\textsuperscript{44} and, overexpression is protective against the disease.\textsuperscript{45} However, in the diseased setting, VEGF may be primarily growth promoting, thus contributing to endothelial cell clusters or plexiform lesion formation.

The dual nature of VEGF actions is best illustrated by the fact that VEGF attenuates neointima formation in systemic vessels at the site of tissue injury,\textsuperscript{46} promotes the expression of plasminogen activator\textsuperscript{37} and collagenases,\textsuperscript{46} and induces tissue factor expression and monocyte infiltration. However,
VEGF also restores endothelial vasoreactivity in injured tissue.61 Furthermore, VEGF induces the production of NO and prostacyclin in aortic endothelial cells.5 By virtue of similar effects in the pulmonary vasculature, VEGF may be protective against some of the pathological changes of PH.

Polyamines
Polyamines are a group of biologically active amines that include diamines (putrescine and cadaverine) and oligoamines (spermidine and spermine).62 Increased pulmonary endothelial biosynthesis of polyamines is associated with the development of PH in monocrotaline-treated mice,50 and inhibition of this production attenuates vascular remodeling.50,51 Similarly, an increase in lung polyamine content occurs in hypoxia-induced PH in rats, and α-difluoromethylornithine, an inhibitor of polyanamine synthesis, attenuates hypoxia-induced medial thickening in this model.52 However, it is unclear at this time whether the increase in pulmonary polyamine production and uptake is the cause or the result of the changes in the pulmonary vascular microenvironment.53

Xanthine Oxidoreductase
Xanthine oxidoreductase (XOR) catalyzes the oxidation of hypoxanthine to uric acid, generating superoxide anion and hydrogen peroxide in the process.54 The role of this enzyme in modulating the tensile properties of the systemic vasculature has been increasingly recognized.55 XOR may also be an effector in pulmonary vascular pathology. Inflammatory cytokines and hypoxia (a potential modulator in the later stages of PH) stimulate the expression of this enzyme.56,57 Lower levels of NO may further potentiate the hypoxia-induced increase in lung XOR levels.58 XOR-derived free oxygen radicals have been shown to cause a dose-dependent contraction of rabbit pulmonary arterial rings.59 Hoshikawa et al60 demonstrated elevated lung XOR activity in a model of hypoxia-induced PH in which the associated pulmonary hypertensive changes were significantly attenuated by the XOR inhibitor allopurinol. Furthermore, uric acid, the product of XOR activity, is a marker of PPH severity and predictor of mortality.61

Endothelial and Inflammation
The role of inflammation in development of PH has been suggested.62 Inflammatory cells, including macrophages and lymphocytes, are increased in the plexiform lesions of hypertensive pulmonary vessels.63 Elevated levels of macrophage inflammatory protein-1α, interleukin-1β, and interleukin-6 are also found in patients with severe PPH.64,65 Levels of P-selectin, which are expressed on leukocytes and platelets and bind to their ligands on the endothelial cells, are increased in patients with PH.66

Wright et al67 recently demonstrated an increased expression of 5-lipoxygenase (5-LO) and 5-LO activating protein (FLAP), mediators of leukotriene synthesis in the pulmonary endothelium of patients with PPH. Furthermore, deletion of the 5-LO enzyme or FLAP inhibition in mice is protective against the development of hypoxia-induced PH.68

Endothelial Dysfunction and Hypercoagulability
The endothelium plays a key role in maintenance of the normal coagulation through elaboration of various substances such as humoral factors, heparan sulfates, thrombomodulin, tissue-type plasminogen activator, urokinase-type plasminogen activator, and von Willebrand factor (vWF).22,69 Endothelial dysfunction may, hence, contribute to the thrombotic process, a feature of most forms of PH. A relative deficiency of the antithrombotic molecules prostacyclin and NO (see above) and slowing of blood flow in the pulmonary circulation secondary to luminal narrowing further enhances thromogenicity.3 Thrombosis leads to narrowing of the pulmonary vessel lumen, thus worsening PH.70 Various hemostatic markers have been studied to correlate the endothelial dysfunction in patients with PH with the presence of a hypercoagulable state.

Selectins
P-selectin is a glycoprotein produced by both the endothelium and the platelets. An increased P-selectin level is a marker of endothelial dysfunction and/or platelet activation and may indicate the presence of a hypercoagulable state.71 Elevated levels of P-selectin and decreased levels of thrombomodulin are found in patients with primary as well as secondary PH.66

Thrombomodulin
The binding of thrombin to its endothelial membrane receptor thrombomodulin results in activation of the anticoagulant protein C. Several studies have now documented a reduction in the levels of thrombomodulin in patients with PH,66,72 again suggesting that altered hemostasis is an important attendant, if not a direct pathogenic factor, in PH.

Von Willebrand Factor
vWF, a large multimeric glycoprotein, is synthesized by the endothelium and megakaryocytes. In the endothelial cells, the Weibel-Palade bodies act as storehouses for both vWF and P-selectin. Increased plasma levels of vWF antigen as well as factor VIII in patients with PH further highlight a state of altered hemostasis and endothelial-platelet dysfunction in this disease.66 In addition, elevated vWF levels have been correlated with mortality in PPH.73

Loss of Endothelial Barrier Function
The normal endothelium shields the underlying vascular layers from the growth factors present in serum. A change in the endothelial cell permeability may result from direct injury; from the exuberant production of VEGF by alveolar epithelium in response to hypoxia74; or from actions of inflammatory mediators, cytokines, and oxidants.75 Mechanical stress as well as mediators such as thrombin can mediate morphological changes in endothelial cells and widening of the intercellular junctions.76 The resulting loss of endothelial barrier integrity may provide a surreptitious avenue for proliferative mediators to come in direct contact with the subendothelium, leading to cell proliferation in the medial and adventitial vascular layers. Platelet activation may also take place when platelets come in contact with the subendo-
thelial structures, resulting in release of vasomediators and growth factors (e.g., serotonin), which may help propagate the changes of PH.

**Genetics**

Present research suggests that a genetic predisposition may confer an increased risk for the occurrence of PH in up to 10% of individuals with the disease. Mutations in the BMPR gene, accompanied by a decrease in the alveolar density of BMPR expression in patients with PH, have been reported. In addition, various other candidate genes have been proposed to influence the pathogenesis of PH.

Polymorphisms of the activin-receptor-like kinase 1 (ALK1) gene, which encodes the transforming growth factor (TGF)–β receptor, have been reported in patients with hereditary hemorrhagic telangiectasia and PH. Immunohistochemical analysis revealed the presence of ALK-1 protein in diseased pulmonary vascular endothelium, again suggesting the role of endothelial dysfunction in both PH and hemorrhagic telangiectasia.

Errors in the DNA mismatch repair pathway, a group of genes responsible for detecting and repairing short segments of mismatched or unmatched base pairs, may lead to insertion/deletion mutations in stretches of repetitive DNA known as microsatellites. Such mutations (termed “microsatellite instability” or MSI) were first described in patients with hereditary nonpolyposis colorectal cancers, and a high frequency of such mutations has been suggested to predispose to an increased resistance of tumor cells to chemotherapy. In a study inspired by the demonstration of MSI in the TGF-β receptor gene in atherosclerotic smooth muscle cells, Yeager et al demonstrated such mutations in TGF-β receptor gene in plexiform lesions of patients with PPH. In addition to resulting in a truncated protein product, these mutations may somehow provide a survival advantage to the cells, producing an apoptosis resistant, TGF-β–unresponsive cell line. It is yet to be determined whether such mutations play a significant role in the pathogenesis of PAH, and whether they have any prognostic implications.

A recent study reported aberrant production of angiopoietin-1 (Ang-1) in the lungs of patients with nonfamilial PH. Ang-1 is an angiogenic molecule secreted by mesenchymal cells that binds and activates the Tie-2 receptors on endothelial cells. The resultant secretion of growth factors leads to muscle cell recruitment and endothelial cell stabilization. Ang-1 regulates embryonic vasculogenesis and is not present in the adult lung. Du et al found elevated levels of Ang-1 mRNA and protein in patients with primary or secondary PH, and an increase in phosphorylation of the endothelial receptor Tie-2. Although these patients did not have BMPR-2 mutations, they had diminished levels of BMPR1a, an endothelial coreceptor of BMPR-2, in the lung. Coincubation of Ang-1 with cultured human endothelial cells resulted in a significant diminution of BMPR1a expression and an increase in the Tie-2 phosphorylation. These results imply a unifying genetic basis for familial and sporadic PH, i.e., abnormal BMPR1 signaling (Figure 2). However, the mechanisms that would lead to Ang-1 upregulation are presently unclear. Ang-1 is an inducer of angiogenesis, endothelial cell survival, and vessel stabilization. A recent study demonstrated attenuation of monocroteline-induced PH in a murine model by administration of Ang-1. In light of these observations, Ang-1 might play a role similar to that of VEGF, i.e., protective of a normal vasculature and pathological in the abnormal endothelium in PAH.
These recent genetic studies have served to further our understanding of the mechanistic features of PAH and the unique role of the endothelium in this disease. Future studies focused on analyzing the prevalence of such mutations and their impact on severity of PH as well as responsiveness to therapy, will further elucidate the complex pathobiology of this disease. Along with a better mechanistic understanding, one can envision the development of newer therapies and perhaps even a gene-modifying approach to “cure” this disease.

**Conclusion**

Current evidence strongly suggests a central role for endothelial dysfunction in the initiation and progression of PH. Drugs that improve the endothelial function or restore the altered balance of endothelium-derived vasoactive mediators are currently used to treat this disease with some success. A greater understanding of the role of the endothelium in PH will facilitate the evolution of newer, targeted therapies. Drugs such as 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitors, angiotensin-converting enzyme inhibitors, antioxidants, and l-arginine supplementation reverse endothelial dysfunction in coronary artery diseases and may well find a use as adjunct therapies in PH. However, the future of PH therapy clearly lies in a better understanding of the genetics of this syndrome as it involves the endothelial cell and other important protagonists of the pulmonary vascular remodeling. Mutations in tumor suppressor genes, alterations in thresholds for apoptosis, and transdifferentiation of vascular cells are becoming prominent concepts as we move along in our quest to understand and ultimately treat this disease.

**References**


Endothelial Dysfunction in Pulmonary Hypertension
Rohit Budhiraja, Rubin M. Tuder and Paul M. Hassoun

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