Animal models of pulmonary arterial hypertension: the hope for etiological discovery and pharmacological cure

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Pulmonary hypertension (PH) is not a disease per se but rather a pathophysiological parameter defined by a mean pulmonary arterial pressure exceeding the upper limits of normal, i.e., ≥25 mmHg at rest (9). PH occurs in a variety of clinical situations and is associated with a broad spectrum of histological patterns and abnormalities. Because of this diversity, a classification system for PH has been developed and recently modified to organize the diseases into categories based on common clinical parameters, potential etiologic mechanisms, and responses to treatment. At present, six groups of chronic PH are described (Refs. 146, 147; Table 1). Among these, group I (and I') comprises a group of diverse diseases termed pulmonary arterial hypertension (PAH) that have several pathophysiological, histological, and prognostic features in common. PAH is a particularly severe and progressive form of PH that frequently leads to right heart failure and premature death. The diagnosis of PAH must include a series of defined clinical parameters, which extend beyond mere elevations in pulmonary arterial pressures and include precapillary PH, pulmonary hypertensive arteriopathy (usually with plexiform lesions), slow clinical onset (months or years), and a chronic time course (years) characterized by progressive deterioration. What appears to distinguish PAH from other forms of PH is the severity of the arteriopathy observed, the defining characteristic of which is “plexogenic arteriopathy.” The pathogenesis of this arteriopathy remains unclear despite intense investigation in a variety of animal model systems. The most commonly used animal models (“classic” models) are rodents exposed to either hypoxia or monocrotaline. Newer models, which involve modification of classic approaches, have been developed that exhibit more severe PH and vascular lesions, which include neointimal proliferation and occlusion of small vessels. In addition, genetically manipulated mice have been generated that have provided insight into the role of specific molecules in the pulmonary hypertensive process. Unfortunately, at present, there is no perfect preclinical model that completely recapitulates human PAH. All models, however, have provided and will continue to provide invaluable insight into the numerous pathways that contribute to the development and maintenance of PH. Use of both classic and newly developed animal models will allow continued rigorous testing of new hypotheses regarding pathogenesis and treatment. This review highlights progress that has been made in animal modeling of this important human condition.

bone morphogenetic protein; hypoxia; inflammation; progenitor cells

What Do We Need to Model? Clinical and Pathological Findings in Human Pulmonary Arterial Hypertension

Pulmonary hypertension (PH) is not a disease per se but rather a pathophysiological parameter defined by a mean pulmonary arterial pressure exceeding the upper limits of normal, i.e., ≥25 mmHg at rest (9). PH occurs in a variety of clinical situations and is associated with a broad spectrum of histological patterns and abnormalities. Because of this diversity, a classification system for PH has been developed and recently modified to organize the diseases into categories based on common clinical parameters, potential etiologic mechanisms, and responses to treatment. At present, six groups of chronic PH are described (Refs. 146, 147; Table 1). Among these, group I comprises a group of diverse diseases termed pulmonary arterial hypertension (PAH) that have several pathophysiological, histological, and prognostic features in common (Table 1). Distinction between the various groups of patients with PH is of significance because they differ in etiology, prognosis, histological appearance, and response to various therapies. Potentially, and importantly, differences in micro-anatomic site and histological features of the different vascular lesions in these various conditions, summarized in Table 2, point to differences in pathogenesis, the elucidation of which is key to the design of effective therapeutic strategies. As noted, the classification system developed and recently updated is centered largely on clinical parameters rather than histological features. However, from the view of the pathologist, it should be noted that some diseases, which belong or should be placed in the same group, are split across different groups, whereas others such as plexogenic arteriopathy and pulmonary veno-occlusive disease, which are essentially different diseases histopathologically, have, in the past, been grouped together in group I (PAH). Such lumping together of different diseases might indeed constitute an impediment to the elucidation of pathogenesis. Recognizing this, the new World
Health Organization (WHO) classification has addressed this issue, at least in part, by putting pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis into a new group termed group 1’ (147). In addition, other classification systems of PH based on pathological appearance have been developed and are helpful in evaluating PH from a pathophysiological point of view (Table 3; Ref. 83).

Although any form of PH can contribute to increases in patient morbidity and mortality, PAH (group 1) is a particularly severe and progressive form that frequently leads to right heart failure and premature death (26, 61, 68, 101, 176). Thus, when it is stated that a patient has PAH, this diagnosis must include a series of defined clinical parameters, which extend beyond mere elevations in pulmonary arterial pressures. For instance, diseases categorized as PAH share some common characteristics such as precapillary PH, pulmonary hypertensive arteriopathy (usually with plexiform lesions), slow clinical onset (months or years), and a chronic time course (years) characterized by progressive deterioration. Furthermore, patients with PAH often demonstrate similar responses to the currently available treatment [endothelin receptor antagonists (ERAs)], phosphodiesterase type 5 (PDE5) inhibitors, and prostanoids. However, patients within this group also exhibit

### Table 1. Updated clinical classification of pulmonary hypertension (Dana Point, 2008; Ref. 147)

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pulmonary arterial hypertension (PAH)</td>
</tr>
<tr>
<td>1.1</td>
<td>Idiopathic PAH</td>
</tr>
<tr>
<td>1.2</td>
<td>Heritable</td>
</tr>
<tr>
<td>1.2.1</td>
<td>Type 2 bone morphogenetic protein receptor (BMPR2)</td>
</tr>
<tr>
<td>1.2.2</td>
<td>Activin receptor-like kinase-1 (ALK1), endoglin (with or without hereditary hemorrhagic telangectasia)</td>
</tr>
<tr>
<td>1.2.3</td>
<td>Unknown</td>
</tr>
<tr>
<td>1.3</td>
<td>Drug and toxin-induced</td>
</tr>
<tr>
<td>1.4</td>
<td>Associated with</td>
</tr>
<tr>
<td>1.4.1</td>
<td>Connective tissue diseases</td>
</tr>
<tr>
<td>1.4.2</td>
<td>Human immunodeficiency virus (HIV) infection</td>
</tr>
<tr>
<td>1.4.3</td>
<td>Portal hypertension</td>
</tr>
<tr>
<td>1.4.4</td>
<td>Congenital heart diseases</td>
</tr>
<tr>
<td>1.4.5</td>
<td>Schistosomiasis</td>
</tr>
<tr>
<td>1.4.6</td>
<td>Chronic hemolytic anemia</td>
</tr>
<tr>
<td>1.5</td>
<td>Persistent pulmonary hypertension of the newborn</td>
</tr>
<tr>
<td>1.6</td>
<td>Pulmonary veno-occlusive disease (PVOD) and/or pulmonary capillary hemangiomatosis (PCH)</td>
</tr>
<tr>
<td>2.</td>
<td>Pulmonary hypertension owing to left heart disease</td>
</tr>
<tr>
<td>2.1</td>
<td>Systolic dysfunction</td>
</tr>
<tr>
<td>2.2</td>
<td>Diastolic dysfunction</td>
</tr>
<tr>
<td>2.3</td>
<td>Valvular disease</td>
</tr>
<tr>
<td>3.</td>
<td>Pulmonary hypertension owing to lung diseases and/or hypoxia</td>
</tr>
<tr>
<td>3.1</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>3.2</td>
<td>Intersitial lung disease</td>
</tr>
<tr>
<td>3.3</td>
<td>Other pulmonary diseases with mixed restrictive and obstructive pattern</td>
</tr>
<tr>
<td>3.4</td>
<td>Sleep-disordered breathing</td>
</tr>
<tr>
<td>3.5</td>
<td>Alveolar hypoventilation disorders</td>
</tr>
<tr>
<td>3.6</td>
<td>Chronic exposure to high altitude</td>
</tr>
<tr>
<td>3.7</td>
<td>Developmental abnormalities</td>
</tr>
<tr>
<td>4.</td>
<td>Chronic thromboembolic pulmonary hypertension (CTEPH)</td>
</tr>
<tr>
<td>5.</td>
<td>Pulmonary hypertension with unclear multifactorial mechanisms</td>
</tr>
<tr>
<td>5.1</td>
<td>Hematologic disorders: myeloproliferative disorders, splenectomy</td>
</tr>
<tr>
<td>5.2</td>
<td>Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis: lymphangioleiomomatosis, neurofibromatosis, vasculitis</td>
</tr>
<tr>
<td>5.3</td>
<td>Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders</td>
</tr>
<tr>
<td>5.4</td>
<td>Other: tumoral obstruction, fibrosing mediastinitis, chronic renal failure on dialysis</td>
</tr>
</tbody>
</table>

### Table 2. Main histological features of pulmonary hypertensive vascular disease

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:</td>
<td>Pulmonary arterial hypertension (PAH)</td>
</tr>
<tr>
<td>1.1–1.4</td>
<td>Pulmonary plexogenic arteriopathy</td>
</tr>
<tr>
<td>Early phase:</td>
<td>Medical hypertrophy</td>
</tr>
<tr>
<td></td>
<td>Cellular intimal proliferation of muscular pulmonary arteries</td>
</tr>
<tr>
<td></td>
<td>Appearance of muscle in normally nonmuscular arteries</td>
</tr>
<tr>
<td>Late phase:</td>
<td>Centric laminar intimal fibrosis</td>
</tr>
<tr>
<td></td>
<td>Loss of luminal vascular volume</td>
</tr>
<tr>
<td></td>
<td>Dilatation lesions (vein-like branches, angiomatoid lesions)</td>
</tr>
<tr>
<td></td>
<td>Plexiform lesions</td>
</tr>
<tr>
<td></td>
<td>Recanalization of arteries</td>
</tr>
<tr>
<td></td>
<td>Fibrinoid necrosis</td>
</tr>
<tr>
<td></td>
<td>Arteritis</td>
</tr>
<tr>
<td>1.4.2</td>
<td>PVOD</td>
</tr>
<tr>
<td></td>
<td>Foci of intense congestion of pulmonary parenchyma</td>
</tr>
<tr>
<td></td>
<td>Patchy hemosiderosis associated with areas of congestion</td>
</tr>
<tr>
<td></td>
<td>Encrustation of elastin with iron and calcium salts in congested areas</td>
</tr>
<tr>
<td></td>
<td>Duplication of elastic laminae</td>
</tr>
<tr>
<td></td>
<td>Obliterative fibrosis of small veins and of venules, associated with congested areas</td>
</tr>
<tr>
<td></td>
<td>Abnormalities set against a background of normal or near normal lung tissue</td>
</tr>
<tr>
<td></td>
<td>Prominence of capillaries, associated with increased numbers of capillaries, in some cases, blurring the distinction from pulmonary capillary hemangiomatosis (group 1.4.2)</td>
</tr>
<tr>
<td>1.4.1</td>
<td>PCH</td>
</tr>
<tr>
<td></td>
<td>Marked increase and prominence of capillary vessels in alveolar walls, interlobular septa, bronchovascular bundles, and pleura. Masses of capillaries may bulge into lumina of airways and vessels.</td>
</tr>
<tr>
<td></td>
<td>Associated features of PVOD in some cases</td>
</tr>
<tr>
<td>2:</td>
<td>Pulmonary hypertension with left heart disease</td>
</tr>
<tr>
<td></td>
<td>Arterialization of large or middle-sized pulmonary veins</td>
</tr>
<tr>
<td></td>
<td>Intrestitial edema and fibrosis</td>
</tr>
<tr>
<td></td>
<td>Hemosiderosis</td>
</tr>
<tr>
<td></td>
<td>Medial hypertrophy and adventitial thickening of pulmonary arteries</td>
</tr>
<tr>
<td>2.1</td>
<td>Pulmonary hypertension due to chronic thrombotic and/or embolic disease</td>
</tr>
<tr>
<td></td>
<td>Thromboembolic obstruction of distal pulmonary arteries</td>
</tr>
<tr>
<td></td>
<td>Eccentric intimal fibrosis</td>
</tr>
<tr>
<td></td>
<td>Recanalized organized thrombi forming bands and webs</td>
</tr>
<tr>
<td></td>
<td>Fresh thrombi very rare</td>
</tr>
<tr>
<td></td>
<td>Nota bene: lesion may be focal, requiring extensive search in multiple sections</td>
</tr>
<tr>
<td></td>
<td>Nonthrombotic pulmonary embolism</td>
</tr>
<tr>
<td></td>
<td>Nonthrombotic material or tissue (foreign bodies, bone marrow)</td>
</tr>
<tr>
<td></td>
<td>Fat embolism: many dilated optically empty blood vessels (down to capillary size)</td>
</tr>
<tr>
<td>3:</td>
<td>Pulmonary vascular disease associated with lung disease and/or hypoxemia</td>
</tr>
<tr>
<td>3.1 and 3.3–3.5</td>
<td>Hypoxic pulmonary vasculopathy</td>
</tr>
<tr>
<td></td>
<td>Muscularization of arterioles</td>
</tr>
<tr>
<td></td>
<td>Medial hypertrophy of muscular pulmonary arteries, especially of smaller branches</td>
</tr>
<tr>
<td></td>
<td>Longitudinally oriented intimal smooth muscle cells</td>
</tr>
<tr>
<td></td>
<td>Slight medial hypertrophy of veins</td>
</tr>
<tr>
<td>3.2</td>
<td>Pulmonary vasculopathy associated with intersitial lung disease</td>
</tr>
<tr>
<td></td>
<td>Features of hypoxic pulmonary vasculopathy</td>
</tr>
<tr>
<td></td>
<td>Eccentric intimal fibrosis of arteries and, to a lesser extent, veins</td>
</tr>
<tr>
<td>4:</td>
<td>Pulmonary hypertension due to chronic thrombotic and/or embolic disease</td>
</tr>
<tr>
<td></td>
<td>Thromboembolic obstruction of distal pulmonary arteries</td>
</tr>
<tr>
<td></td>
<td>Eccentric intimal fibrosis</td>
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<tr>
<td></td>
<td>Fat embolism: many dilated optically empty blood vessels (down to capillary size)</td>
</tr>
<tr>
<td>5:</td>
<td>Miscellaneous [sarcoidosis, compression of pulmonary vessels (adenopathy), tumor, fibrosing mediastinitis]</td>
</tr>
<tr>
<td></td>
<td>Heterogeneous group of disorders, some showing the features of congestive vasculopathy, some with features of postthrombotic vasculopathy, some with combinations</td>
</tr>
</tbody>
</table>

[Adapted from Mooi and Grunerg (114).]
some relevant differences, for example with regard to etiology in the case of congenital heart disease associated PAH and pulmonary veno-occlusive disease.

The pathogenesis of increased precapillary pulmonary vascular resistance in PAH is generally ascribed to the combined effects of sustained vasoconstriction, arterial wall remodeling, and in situ thrombosis (23, 69, 133, 188). What appears to distinguish PAH from other forms of PH is the severity of the arteriopathy. Although all forms of PH exhibit arterial changes that include increased intimal, medial, and adventitial thickness, appearance of muscle-like cells in the walls of smaller and more peripheral arteries than normal, and, arguably, loss of peripheral vascular volume, the defining characteristic of this specific subcategory of disease is the so-called pulmonary plexogenic arteriopathy (i.e., the arterial disease that ultimately gives rise to plexiform lesions).

The early phases of PAH are thought by some to be histologically nonspecific with pulmonary arterial medial hypertrophy, adventitial thickening, and appearance of muscle in the walls of normally nonmuscular arteries representing the sole abnormalities (Table 3; Fig. 1). The apparently later, more progressed stage involves formation of complex cellular and fibrotic neointimal and plexiform lesions that obstruct and obliterate medium and small pulmonary arteries and arterioles such that blood flow through the pulmonary arteries is severely limited (Refs. 24, 106, 114, 130, 173, 178–180, 196; Table 3; Fig. 1). In fact, PAH is said by some to have an early reversible and a late irreversible and/or progressive phase based on observations of progressive intimal fibrosis and plexiform lesion formation in children vs. adults with PAH (60, 114, 180, 193). Thus the diagnosis of this group of PH cannot be made solely on histological grounds in cases that have not advanced to this histologically pathognomonic stage. Furthermore, although many say that plexiform lesions are always present in patients with late PAH, others find such lesions are not always present (131). Furthermore, it is evident that the lungs of some idiopathic PAH (IPAH) patients examined after the advent of new therapeutic approaches exhibit more severe remodeling, perhaps contributing to the more frequent reports regarding the occurrence of plexiform lesions in more recent publications. Whether this is because these treated patients are living longer or because current therapies contribute to the emergence of patients with a more severe arteriopathy (or both) is currently unclear.

The plexiform lesion is generally observed in arteries <300 μm in diameter and is often situated just distal to an arterial branching site, most commonly just distal to the origin of the supernumerary artery (Fig. 1). It consists of a plexus of slit-like channels lined by small, flat endothelial cells and subjacent myofibroblasts (149, 150, 192, 196), enclosed within, or in continuity with a greatly dilated segment of the affected small pulmonary artery (Fig. 1). Some thrombus fragments are common within the lesion, and VEGF-1 expression is often markedly increased (62, 172). Distal to the plexus, there is often marked dilation of the affected arterial branch. Similar dilation may occur without the formation of the plexus that characterizes the plexiform lesion. The latter situation results in so-called vein-like branches; clusters of these have been termed angiomatoid lesions (Refs. 59, 114, 178; Fig. 1). Arteries in patients with PAH may also display cellular intimal proliferation and concentric laminar intimal fibrosis, which significantly narrows the lumen of small arteries (Fig. 1). These changes are commonly observed in axial arteries (rather than supernumerary arteries; Ref. 192). However, intimal fibrosis is conspicuous in many forms of PAH and may occur in the absence of plexiform lesions (197). In fact, Overbeek et al. (126) have recently reported that although the lungs of 8 patients with systemic sclerosis-associated PAH show intimal fibrosis of pulmonary arteries/arterioles and veins/venules, no convincing example of a plexiform lesion was found. In contrast, 10 of 11 IPAH lungs had clear evidence of plexogenic arteriopathy. Furthermore, in many of the lesions observed in PAH patients, inflammatory cell infiltrates are apparent, a finding that is becoming increasingly common (173). Cell infiltrates consisting of monocytes/macrophages, T and B lymphocytes, and dendritic cells are reported (16, 129). Recently, cells exhibiting endothelial or mesenchymal precursor characteristics have been observed in these lesions, raising the possibility that these cells contribute to the arteriopathy (5, 99). It is believed by many that the cellular and fibrotic luminal obliteration described accounts for the poor responsiveness to the acute administration of conventional pulmonary vasodilators that most adult PAH patients exhibit as well as the irreversibility of PH following corrective surgery in some PAH patients with congenital heart diseases (92, 128, 148, 179, 196). However, Tudor (171) has recently emphasized the heterogeneity of vascular lesions in PAH and pointed out that it remains unknown which lesion in which vessel segment contributes most to the estimated 80% reduction in overall pulmonary vascular luminal area, which is apparently necessary to cause hypertension.

### Table 3. Classification of pulmonary hypertension from a pathological point of view

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>Idiopathic (primary)</td>
</tr>
<tr>
<td></td>
<td>Familial</td>
</tr>
<tr>
<td></td>
<td>Secondary conditions (congenital left-to-right shunt, collagen vascular disease, portal hypertension, HIV infection, drugs, and toxins)</td>
</tr>
<tr>
<td>Venous</td>
<td>Obstruction of intrapulmonary veins (Pulmonary veno-occlusive disease, capillary hemangiomatisis)</td>
</tr>
<tr>
<td></td>
<td>Cardiac abnormality (left-sided atrial or ventricular heart disease or left-sided valvular disease)</td>
</tr>
<tr>
<td></td>
<td>Extrinsic compression of central pulmonary veins (fibrosing mediastinitis)</td>
</tr>
<tr>
<td>Lung disease and/or hypoxemia</td>
<td>COPD, interstitial lung disease, sleep apnea, alveolar hyperventilation, high altitude, developmental anomalies</td>
</tr>
<tr>
<td>Chronic thrombotic or embolic</td>
<td>Chronic thromboembolic occlusion of proximal pulmonary arteries</td>
</tr>
<tr>
<td>disease</td>
<td>Obstruction of distal pulmonary arteries (thrombi, thromboemboli, foreign materials, and parasites)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Sarcoïdosis, lymphangiomatomyositis, eosinophilic granuloma, and tumors</td>
</tr>
</tbody>
</table>

COPD, chronic obstructive pulmonary disease. [Adapted from Katzenstein (83).]
Death in patients with chronic PH (including those with PAH) is the result of high right ventricular (RV) afterload and ultimately right heart failure. It is now commonly accepted that RV afterload is determined by both changes in resistance (a steady-state parameter that measures opposition to continuous flow that is determined largely by changes in tone and structure of distal vessels) as well as changes in the dynamic compliance of large proximal vessels. The importance of decreased vascular wall compliance (stiffening) has been highlighted by studies demonstrating that impaired pulsatile arterial function (a result of large vessel stiffening) is an independent predictor of risk for cardiovascular events in many diseases including PH (12, 22, 40, 46, 50, 98, 144, 181, 183, 204). Thus assessment of the dynamic (capacitance and compliance) components of the arterial system has been suggested (if not shown) to provide important prognostic and therapeutic information beyond that provided by traditional blood pressure or vascular resistance measurements.

The reasons for this are becoming clearer. Under normal conditions, the elastic conduit vessels constitute a hydraulic buffer, converting the intermittent cardiac output into steady flow, which not only reduces cardiac workload during systole and conserves energy expenditure for the heart, but also alleviates pulsatile stress for the perfused organs. Stiffening of the conduit arteries increases the pulsatile component, exerting detrimental hemodynamic load on the heart, thus limiting coronary perfusion, impairing ventricular performance, and eventually exacerbating the development of heart failure (13, 181, 204). Increases in the pulsatile waves, generated in stiff vessels, can perpetuate remodeling of distal vessels, and, reciprocally, abnormal resistance created by remodeling of distal vessel can cause further stiffening of large vessels. Abnormalities in the pulsatile characteristics of arteries occur early in a variety of disease processes that are associated with increased cardiovascular risk and can be favorably modified by therapeutic interventions (4, 181, 194, 204). Several groups have now demonstrated that measuring input impedance in both the pulmonary and systemic circulations is an important “new” assessment of vascular properties because it integrates both static (resistance) and dynamic (compliance and wave reflection components) changes in vascular properties and thus provides comprehensive information about the state of the vascular bed (40, 50, 98, 117, 145, 183). Published data suggest that impedance is a better predictor of severity of disease and response to therapeutic intervention than measurement of pulmonary vascular resistance alone in PH patients (40, 70, 183). It thus becomes extremely important to understand the mechanisms contributing to stiffening of proximal
vessels as well as to remodeling of distal vessels if we are to
device better therapies for the treatment of PAH and ultimately
heart failure.

A diverse group of risk factors has been identified for
patients with PAH. Perhaps the strongest association is with
congenital heart disease with a posttricuspid shunt where the
obstructive lesions of the distant vessels manifest early in life
and may progress to the late and irreversible phase within 2–3
yr, although the “clinical” disease may run a far more pro-
trated course. PAH has also been associated with anorexigens
and other toxic compounds, portal hypertension, human im-
munodeficiency virus (HIV) infection, and collagen vascular
diseases (67–69). Some cases of PAH occur in familial clusters
where they seem to be associated with germ-line mutations in
the type 2 bone morphogenetic protein receptor (BMPR2) and
activin receptor-like kinase-1 (ALK1) receptor genes (7, 33).
Perhaps the most intriguing group of patients in this category
has no apparent associated cause or risk factor, i.e., those
patients with IPAH. However, even in these cases, ~20% of
patients have been shown to have mutations in BMPR2.

It has been only relatively recently that attempts have been
made to arrest or reverse this very debilitating and life-threaten-
ing disease. The treatment goals of PAH are to reduce pulmo-
nary vascular resistance and pulmonary arterial pressure
and thereby reverse the pressure overload on the RV to prevent
its failure and ultimately death of the patient. In addition to
adjunctive therapy with anticoagulants, diuretics, ionotropic
drugs, and supplemental oxygen, PAH patients are currently
treated with one or a combination of three specific classes of
agents, which include prostacyclin analogs, endothelin-1 re-
ceptor antagonists, and/or PDE5 inhibitors. The effect of these
treatments on function and survival has been assessed in recent
meta-analysis of randomized trials in PAH patients (48, 96).
Previously, we (48) showed active treatments were associated
with a reduction in mortality of 43%. Sensitivity analysis
confirmed a reduction in mortality of 38%. The conclusion of
this study, as opposed to another recent meta-analysis that
found no statistically significant effects on mortality (96),
suggests an improvement in survival of the patients treated
with the targeted therapies approved for PAH. However, both
studies concluded that the current treatment strategy for PAH
patients remains inadequate because mortality rate continues to
be high and the functional and hemodynamic impairments are
still extensive in many patients. This disappointing situation
indicates that important pathogenic cellular and molecular
signaling mechanisms have neither been identified nor thera-
peutically targeted. It is hoped that new insights regarding
pathogenesis and thus new, more effective pharmacological
approaches will be forthcoming based on the mechanistic
insights provided by better animal models of PAH. It is toward
the discussion of these animal models that the remainder of this
review is focused.

Classic Animal Models of PH

Numerous animal models of PH are currently available to
the interested investigator. The most commonly used animal
models of PH are the chronic hypoxic model and the mono-
crotaline injury model. These animal models have been used
for quite some time and have undoubtedly contributed to a
better understanding of the pulmonary hypertensive process.
However, as discussed more thoroughly below, whether these
are models of human PAH is becoming less controversial, as
most would agree that they probably are not.

Chronic hypoxia. Normo- and hypobaric hypoxia are fre-
quently utilized to induce PH in a wide variety of animal
species. This model is useful because it is very predictable and
reproducible within a selected animal strain. However, there is
variability in the responses to chronic hypoxia between species
(Fig. 2). Responses are also significantly affected by age, as
younger individuals with rapidly maturing lungs are more
susceptible to this trigger (154). The most commonly used
hypoxic animal systems are those in rats and mice. In contrast
to the monocrotaline model discussed below, hypoxia-induced
PH in almost all mammals investigated so far is associated with
very similar (albeit of differing magnitude) structural changes.
Muscularization of small, normally nonmuscular arteries in the
alveolar wall begins quickly, and increases in the appearance
of cells expressing α-smooth muscle actin (α-SM actin) in the
walls of previously nonmuscularized arterioles rapidly occurs.
Many possibilities could account for these changes in addition
to the differentiation of pericytes and/or “migration” of smooth
muscle cells (SMC), including recruitment and differentiation
of local fibroblasts, mononuclear cell/progenitor cell recruit-
ment, and transdifferentiation of endothelial cells into mesen-
chymal-like cells (77, 154). Rapidly (but subsequently), there
is increased thickening of the previously muscularized precap-
illary pulmonary arteries. This may be due to medial SMC
proliferation, although the magnitude in rats is small, with an
~2-fold increase in labeling index transiently noted (108).
Hypertrophy of SMC, however, is marked in these vessels
(108). In addition, inflammation appears to play a significant
role in the hypoxia-induced remodeling process in at least
some strains of rats. Recently, it was reported that hypoxia
induced an early and persistent pulmonary artery-specific vas-
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mokine/chemokine receptors preceded the appearance of in-
flammatory cells, which, in the case of hypoxia, are primarily
mononuclear. A persistence of adhesion molecules and cyto-
kines within the vessel wall was noted along with persistent
inflammatory infiltrates. Contained within the mononuclear
cell infiltrates are mesenchymal precursor cells, which deple-
tion studies demonstrate play a critical role in the remodeling
(47). In addition, there is significant thickening and fibrosis of
the large proximal pulmonary arteries, and these vessels have
been documented to have significant stiffening (38). After 2 wk
of hypoxia, rats develop moderate PH with a doubling of mean
pulmonary artery pressure that seems to correlate with the
progression of structural changes. RV hypertrophy occurs, but
there is little evidence of RV failure.

It should be noted that fawn-hooded (FH) rats develop more
severe PH and remodeling than other strains with exposure to
hypoxia and represent the most severe spectrum of hypoxia-
induced PH in rodents (15, 116, 140). The FH rat strain has an
inducible PH in almost all mammals investigated so far is associated with
very similar (albeit of differing magnitude) structural changes.
Muscularization of small, normally nonmuscular arteries in the
alveolar wall begins quickly, and increases in the appearance
of cells expressing α-smooth muscle actin (α-SM actin) in the
walls of previously nonmuscularized arterioles rapidly occurs.
Many possibilities could account for these changes in addition
to the differentiation of pericytes and/or “migration” of smooth
muscle cells (SMC), including recruitment and differentiation
of local fibroblasts, mononuclear cell/progenitor cell recruit-
ment, and transdifferentiation of endothelial cells into mesen-
chymal-like cells (77, 154). Rapidly (but subsequently), there
is increased thickening of the previously muscularized precap-
illary pulmonary arteries. This may be due to medial SMC
proliferation, although the magnitude in rats is small, with an
~2-fold increase in labeling index transiently noted (108).
Hypertrophy of SMC, however, is marked in these vessels
(108). In addition, inflammation appears to play a significant
role in the hypoxia-induced remodeling process in at least
some strains of rats. Recently, it was reported that hypoxia
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It should be noted that fawn-hooded (FH) rats develop more
severe PH and remodeling than other strains with exposure to
hypoxia and represent the most severe spectrum of hypoxia-
induced PH in rodents (15, 116, 140). The FH rat strain has an
inherited platelet storage disorder characterized by the deficient
uptake of serotonin into platelets. FH rats develop PH from the
age of 4 wk, and this can be accelerated by exposure to
hypoxia. For instance, only ~70% of FH rats show increases in
RV pressure and remodeling at 130-m altitude, whereas at
1,600 m all FH rats had elevated pulmonary artery pressure
compared with Sprague-Dawley rats (85). FH rats also have
more immature lungs with decreased number of alveoli at

http://ajplung.physiology.org/ by 10.220.035.5 on November 6, 2017
birth, and this decrease persists into adulthood (91). In addition, pulmonary artery SMC of these rats show increased endothelin production, which may account for their heightened pressure and remodeling responses to hypoxia (200). Thus FH rats allow study of genetic factors that contribute to PH. However, the FH rat does develop systemic hypertension as well, whereas this is usually not a feature of the human patient with PAH.

Exposure of mice to chronic hypoxia, although causing an elevation in pulmonary artery pressure, is associated with only minimal vascular remodeling, certainly less than the rat (37, 45, 121, 152). The most common findings are muscularization of previously nonmuscularized vessels and a minimal medial thickening of muscular resistance vessels. Only brief increases in SMC proliferation are noted, with some reports demonstrating an overall decrease in SMC numbers late in the exposure (127). In addition, thickening of proximal pulmonary arteries is observed with adventitial thickening and fibrosis and functional increases in the stiffening of these arteries (42, 169). From a molecular point of view, definite differences between the response of the rat and mouse to hypoxia have also been shown. Microarray analysis of the lung tissue demonstrates distinct differences in gene expression induced by hypoxia between the species (19). Chronic hypoxic exposure in the rat increased expression of genes involved in endothelial cell proliferation and decreased expression of those associated with apoptosis. The lung tissue from rats showed a >7-fold upregulated gene expression in both major histocompatibility antigen class II and osteopontin, whereas tissue expression of these genes in mice did not change. Furthermore, the phosphoinositide-3 kinase gene (often associated with proliferation and inflammation) was greatly upregulated (6-fold) in its expression in rat lungs but not in mouse lungs. The gene expression pattern of mouse lungs was notable for downregulation of genes for vascular SMC proliferation such as IGF binding protein (36, 65). However, it should be acknowledged that recent studies demonstrate that the responses in hypoxia in mice are strain-specific and that these intraspecies comparisons could vary significantly depending on strains compared (157).

In contrast, neonatal calves exposed to chronic hypobaric hypoxia, even at less severe conditions of hypoxia (12.5 vs. 10%), develop severe PH with pulmonary artery pressures equal to or exceeding systemic pressures and vascular remodeling that is far more striking in both distal and proximal pulmonary arteries than that observed in the rat or mouse (125, 154, 155). In some animals there is significant intimal thickening, especially in proximal vessels, and in distal vessels there is remarkable thickening of the media and adventitia. Excessive proliferation of the vasa vasorum occurs in the adventitia of these animals, to the extent that they may even be confused with the plexiform lesions that have been found in some of the rat models described below. In addition, there is marked accumulation of mononuclear cell infiltrates and mesenchymal progenitors (32, 47). Again, in this model, significant stiffening of the conduit vessels has been noted (89). However, despite these severe inflammatory and fibrotic lesions, and in distinct contrast to the PAH described in humans, the disease is reversible with return to normoxic conditions (K. R. Stenmark, Fig. 2. Hypoxia-induced vascular and perivascular remodeling [medial and adventitial (adv.) thickening and accumulation of myofibroblasts and inflammatory cells] varies significantly among species, ranging from minimal perivascular changes in mice to marked changes in neonatal calves. Note the strong correlation between remodeling and inflammation. αSMA and α-SM-actin, α-smooth muscle actin; S-D strain, Sprague-Dawley rat strain; WKY strain, Wistar-Kyoto rat strain; expr., expression; PB-MNC, peripheral blood mononuclear cell.
unpublished observations), a finding that is also true for the hypoxic rat and mouse models. It should also be noted that people who develop significant PH at altitude, so-called Monge’s disease, improve markedly when returned to sea level conditions.

In summary, there is no indication that irreversible intimal fibrosis or plexogenic lesions, similar to those in human PAH, occur in any of these chronically hypoxic models. These limitations have previously been discussed (10, 58, 177, 186, 199). It is also apparent that the severity of the structural modification in hypoxic lungs is under the influence of factors other than just hypoxia. Pulmonary artery banding experiments have been demonstrated to modulate the changes induced by hypoxia, indicating a role for hemodynamics (135). Hypoxia-induced remodeling in PH is also dependent, at least in part, on inflammatory and progenitor cell recruitment (47). Whether chronic hypoxia leads to loss or rarefaction of pulmonary microvessels as is thought to be the case in PH is currently controversial (134). Thus the chronic hypoxic models of PH in rodents could be regarded as models for less severe PH (not PAH) and should be regarded as having relevance to human PH associated with hypoxia as it occurs in pulmonary parenchymal disease, sleep disordered breathing, severe chronic obstructive pulmonary disease (COPD), and residence at high altitude.

**Monocrotaline injury.** Monocrotaline is a toxic pyrrolizidine alkaloid present in the plant Crotalaria spectabilis. Ingestion of monocrotaline results in the progressive development of PH in various animal species and was first described after repeated oral ingestion in laboratory rats more than 40 yr ago (84). It is known that the monocrotaline pyrrole (MCTP) has to be activated in vivo by mixed function oxidases in the liver to form the reactive bifunctional cross-linking compound MCTP, which leads to vascular injury. MCTP models, particularly in rats, can now be achieved by injection with a single subcutaneous or intraperitoneal injection of MCTP, making this a very simple and thus technically appealing animal model available to a wide spectrum of investigators. Unfortunately, the response to monocrotaline is variable among species, strains, and even animals because of differences in the hepatic metabolism by cytochrome P-450. The preferred species for the study of monocrotaline-induced PH is currently the rat. Varied results have been obtained in mice even when the active MCTP has been administered. In larger mammals such as dogs, investigators have generated PH by directly injecting the toxic metabolite dihydromonocrotaline (124). Young (12-wk-old) beagles injected with monocrotaline developed PH, and significant vascular remodeling with neointimal proliferation was reported in 42% of small pulmonary arteries (56).

Although the exact mechanism through which monocrotaline causes PH is not known, it is speculated by many that it causes direct endothelial damage that then triggers the inexorable development and progression of severe and eventually lethal PH (75). This is based on observations showing that the onset of increased pulmonary arterial pressures and vascular remodeling is delayed until 1–2 wk after the initiating dose (107). Other investigators have suggested that the increases in pulmonary artery pressure and vascular remodeling are caused by early and often dramatic accumulation of mononuclear inflammatory cells in the adventitial sheath of the small intracinar vessels (190). This change occurs in both the pulmonary arteries and veins and precedes the evidence of smooth muscle hypertrophy in the media. Thus adventitial inflammation, particularly macrophage accumulation, is suggested by some to have more important effects on the pathogenesis of PH than the endothelial cell (153, 189, 190). Furthermore, some investigators have also noted changes in the veins, raising questions about this as a model of PH (109, 189, 190). Other reports also describe significant liver and kidney damage, with a recent report documenting that monocrotaline reliably produces hepatic veno-occlusive disease in rats (25, 136). There is significant RV hypertrophy and RV dysfunction, which is important for study in PH models. Following high doses of monocrotaline injection, RV systolic pressures reaching 80 mmHg after 5 wk have been reported, which was associated with a low survival rate of 35% (75, 107, 136). Unfortunately, there is evidence that the monocrotaline causes myocarditis affecting both the right and left ventricle, which complicates the study of the RV hypertrophy/failure commonly associated with severe PH (112).

In summary, with regard to the relevance of MCTP models to PAH, we stress that at least in the rat there is no formation of obstructive intimal lesions in the peripheral pulmonary arteries. The model is therefore considered by some to be an acute toxic model characterized by acute/subacute damage of the peripheral vasculature of the lung and other organs (kidney, liver, and heart). It is also important to note that the MCTP model in the rat seems to be curable by almost every intervention tried. So far, more than 30 agents have been reported to prevent and some even reverse established monocrotaline-induced PH (Refs. 1, 8, 11, 27–30, 39, 54, 55, 63, 66, 71, 72, 74, 78–82, 86, 87, 90, 94, 102–104, 111, 113, 118, 119, 122, 132, 141, 143, 156, 165, 167, 168, 191, 198, 201–203; Table 4).

Intriguingly, the list includes agents that have been implicated in the development of PH including dexfenfluramine and elastase (81, 111). It thus appears quite easy to therapeutically improve this model, likely due to its acute/subacute nature, which is very unlike the human with PAH.

**Alternative Animal Models of PAH**

As stated above, patients with PAH are characterized clinically by a progressive deterioration with severe PH and pathologically by a neointimal and plexogenic arteriopathy. Since the monocrotaline and hypoxic models do not recapitulate this clinical or pathological picture, efforts have been made to modify these classic models to induce progressive pulmonary vascular disease with neointimal changes.

**Monocrotaline and pneumonectomy.** In 1996, Tanaka et al. (159) tested the hypothesis that by changing the hemodynamic conditions in the pulmonary arteries (i.e., raising pressures toward systemic levels), the effects of monocrotaline on the pulmonary vasculature would be increased. Indeed, they found that in animals in which a subclavian to pulmonary artery anastomosis had been created, monocrotaline administration produced neointimal changes in the large elastic pulmonary arteries. They further showed that in the absence of endothelial cell injury, systemic levels of pressure alone did not induce significant pulmonary vascular remodeling, at least not in large vessels. They concluded that elevated pressure per se is not a sufficient stimulus for remodeling the uninjured elastic pulmonary artery, as measured by matrix protein synthesis. However, they did show that if injury is severe, such as after balloon...
Table 4. Agents shown to prevent and/or reverse monocrotaline-induced PH in rats

<table>
<thead>
<tr>
<th>Agent</th>
<th>Reference</th>
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<tbody>
<tr>
<td>ACE inhibitors</td>
<td>11, 80, 113</td>
</tr>
<tr>
<td>Angiotensin receptor antagonists</td>
<td>82</td>
</tr>
<tr>
<td>Dexamfluramine</td>
<td>111</td>
</tr>
<tr>
<td>Dichloroacetate</td>
<td>103</td>
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<tr>
<td>Difluoromethylornithine (DFMO)</td>
<td>203</td>
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<tr>
<td>Elastase</td>
<td>81</td>
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<tr>
<td>Elastase inhibitors</td>
<td>28</td>
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<tr>
<td>Epidermal growth factor inhibitor</td>
<td>104</td>
</tr>
<tr>
<td>Endothelin receptor antagonists</td>
<td>27, 30, 132, 198</td>
</tr>
<tr>
<td>Gene therapy (prostacyclin synthase, antisurvivin)</td>
<td>71, 102, 118, 141</td>
</tr>
<tr>
<td>Guanylate cyclase activators</td>
<td>39</td>
</tr>
<tr>
<td>HMG-CoA reductase inhibitors</td>
<td>66</td>
</tr>
<tr>
<td>Isosorbide dinitrate</td>
<td>81</td>
</tr>
<tr>
<td>K⁺ channel openers</td>
<td>168</td>
</tr>
<tr>
<td>PDGF inhibitors</td>
<td>143</td>
</tr>
<tr>
<td>Phosphodiesterase (4 and 5) inhibitors</td>
<td>74, 78, 94</td>
</tr>
<tr>
<td>Propylthiouracil</td>
<td>156</td>
</tr>
<tr>
<td>Prostanoids</td>
<td>72, 122</td>
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<tr>
<td>Prostacyclin receptor agonants</td>
<td>87</td>
</tr>
<tr>
<td>Ramipril</td>
<td>119, 202</td>
</tr>
<tr>
<td>Rho kinase inhibitors</td>
<td>1, 165</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>29</td>
</tr>
<tr>
<td>Serine/threonine kinase inhibitor</td>
<td>86</td>
</tr>
<tr>
<td>Serotonin transport inhibitors</td>
<td>55</td>
</tr>
<tr>
<td>Stem cells (“EPCs” and mesenchymal)</td>
<td>8, 79, 191, 201</td>
</tr>
<tr>
<td>Steroids</td>
<td></td>
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<tr>
<td>• Methylprednisolone</td>
<td>90</td>
</tr>
<tr>
<td>• Prednisolone</td>
<td>81</td>
</tr>
<tr>
<td>• Estradiol</td>
<td>167</td>
</tr>
<tr>
<td>• Dihydroepiandrostone</td>
<td>63</td>
</tr>
<tr>
<td>Trimetazidine</td>
<td>54</td>
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</table>

PH, pulmonary hypertension.

Because of formation of neointimal pulmonary vascular occlusive lesions, consisting of proliferating endothelial and SM-like cells, and the development of more severe PH, this pneumonectomy plus monocrotaline model has subsequently been utilized by a number of groups to investigate etiologic mechanisms and responses to treatment (Fig. 3; Refs. 63, 115, 118–120). In addition, this model of neointimal pulmonary vascular disease also shows histological evidence of inflammation. Array analysis also demonstrated marked upregulation of inflammatory transcriptional molecules in this model. Although extensive studies evaluating etiologic mechanisms involved in neointimal formation in this model have not been pursued, it is clear that in addition to inflammation, BMPR signaling is apparently inactivated, and this may be important in the pathogenesis (120).

This model has also been used by many investigators to test new drug treatments. Importantly, the 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitor simvastatin has been found to attenuate the development of PH and to reverse established disease and promote survival in this model (118, 120).
Similar results, albeit not with complete reversal of hypertension and neointimal lesions, have been observed by treatment with triptolide (an agent that has antitumor, antiangiogenic, and antiproliferative effects), rapamycin (an immunosuppressant and antiproliferative agent), and the naturally occurring steroid hormone, dehydroepiandrosterone (DHEA) (44, 63, 119, 175). However, it should be stressed that differences exist between this rat model of pulmonary vascular disease and human PAH. Most notable are the rate of disease progression (i.e., weeks vs. years), the absence of plexiform lesions in these rats, and perhaps the absence of genetic mutations. Furthermore, it does not appear that thorough studies have been performed to evaluate the problems raised regarding the monocrotaline rat model, i.e., involvement of veins and effects on other organs, especially the heart and liver.

Recently, White et al. (187) reasoned that in younger rats pneumonectomy plus monocrotaline might result in even more exaggerated findings. Indeed, in addition to the previously observed medial thickening and formation of occluding neointimal lesions, these younger rats also developed perivascular proliferative lesions. These lesions, which comprise a diffuse population of cells that express VEGF receptor 2 and/or α-SM actin, appear to form outside the arterial lumen and were occasionally permeated by disorganized vascular channels lined with von Willebrand factor-positive endothelial cells (Fig. 4). Microangiography indicated that these channels were connected to the pulmonary circulation. Interestingly, these lesions were reported to occasionally be observed within 1 wk of monocrotaline injection, i.e., before severe hypertension had developed, and to become more numerous over time. Tissue factor was implicated in the pathogenesis of the lesion formation in this study. However, its specific role in the neointimal lesion formation remains unclear, as increases in tissue factor have also been reported in hypoxic models of PH, which lack intimal changes and which reverse spontaneously (121, 195). At present, no studies evaluating treatments to attenuate or reverse the pulmonary vascular disease in this model have been reported.

The fact that the young animals develop more significant lesions, particularly in the perivascular spaces where there is likely inflammation, is not surprising. It has been known for some time that infants and children display greater adventitial changes than adults with many forms of PH (154). The adventitia is prominent in the fetal pulmonary circulation and normally regresses following birth. Interruption of this process has been speculated to play a role in the excess medial and adventitial thickening observed in neonatal and infant models. Furthermore, fetal and neonatal fibroblasts have been shown to exhibit exaggerated responses to hypoxia and other stimuli compared with adult fibroblasts (31, 35, 154). In addition, similar perivascular channels in the presence of severe inflammation have been noted in the hypoxic calf model of PH (32).

**Sugen 5416 and Hypoxia**

Taraseviciene-Stewart and colleagues (161) developed a model of severe PAH to better address the etiologic mechanisms involved in the endothelial cell hyperproliferation that they believe characterizes the plexogenic arteriopathy of human PAH. Based on the concept that VEGF is an important maintenance and differentiating factor for vascular endothelial cells, these investigators designed experiments to inhibit
VEGF signaling in rats exposed to normoxia or chronic hypoxia. They found that a VEGF receptor inhibitor, Sugen 5416 (SU-5416), caused mild PH and pulmonary vascular remodeling in normoxic rats but a severe, irreversible PH associated with precapillary arterial endothelial proliferation in chronically hypoxic rats (161). They presented evidence that the SU-5416-injected animals also exhibited increased vascular SMC proliferation, indicating that endothelial cell VEGF receptors can regulate pulmonary vascular SMC growth. Because they found that the VEGF receptor blockade caused endothelial cell apoptosis, which under chronic hypoxic conditions triggered endothelial cell proliferation in the lumen of small vessels, they speculated that chronic hypoxia per se and/or hypoxic vasoconstriction stimulated the proliferation of a subset of apoptosis-resistant endothelial cells. Importantly, in this model, it was shown that chronic hypoxia plus VEGF receptor blockade caused severe PH, which persisted and in fact progressed after the animals had been removed from the hypoxic stimulus. The persistence and progression of pulmonary vascular disease and right heart failure with death of some but not all animals is somewhat similar, although obviously different in its time course, to the inexorable progression of human severe pulmonary hypertensive disorders.

SU-5416 injection plus chronic hypoxia for 2 wk led to a decrease in the mRNA expression of the prosurvival molecules Bcl-2, Bcl-X, and IGF-I compared with an untreated chronically hypoxic lung. Furthermore, the investigators studied other organs and came to the conclusion that it was remarkable that VEGF receptor blockade by SU-5416 affected only the lung and not other organs. Also remarkable, compared with all the above-mentioned models, was the fact that the investigators did not find perivascular infiltration of monocytes/macrophages in this model. This is somewhat surprising as chronic hypoxia alone has clearly been demonstrated to cause both an early and persistent influx of mononuclear cells into the pulmonary arterial wall (20).

Because of the severity of PH, the formation of intimal lesions (Fig. 5), and the progressive nature of this model, other investigators have used it to evaluate new treatments for PAH. So far, several drugs, including the bradykinin antagonist B-9430, the caspase inhibitor Z-Asp-2,6-dichlorobenzoyl-oxymethylketone, and the anticancer drug sorafenib, have been demonstrated to prevent the development of PAH (115, 160, 161). In addition, attempts to reverse established PH in this model have also been made. Treatment with the bradykinin or receptor antagonist B9972 and simvastatin arrested the progression of established PH but did not reverse the hypertension or neointimal lesions (163, 164). Several other drugs with a variety of actions, including the anticancer drugs cyclophosphamide and paclitaxel, the angiotensin converting enzyme inhibitor lisinopril, the angiotensin-2 type I receptor blocker irbesartan, the bradykinin antagonist B-9430, the antiangiogenic agent thalidomide, the peroxisome proliferator-activated receptor-γ agonist PGJ2, and the calcium channel blocker nifedipine, failed to arrest progression of PAH. No reversal experiment with sorafenib has been reported yet.

With regard to its relation to PAH, Moreno-Vinasco et al. (115) compared gene expression in the hypertensive lungs of this model to that reported by Geraci et al. (51) in human PAH lungs and found only four genes common to both cases.

Fig. 5. Occlusive neointimal lesions in rats treated with Sugen 5416 (SU-5416) and chronic hypoxia. Representative images for each group (H&E staining) with inset (anti-vWF staining) demonstrate that, compared with normoxic rats (A), rats exposed to hypoxia alone for 3.5 wk displayed only mild lung vascular remodeling (B). In contrast, hypoxia/SU-5416-exposed rats showed marked vascular remodeling with medial wall thickening, endothelial cell hyperproliferation, and formation of lesions with exuberant vWF-positive endothelial cell proliferation (arrow in 3 representative insets, C). Sorafenib treatment completely prevented the chronic hypoxia/SU-5416-induced vascular remodeling (H-SU-Sor; D). [Adapted from Taraseviciene-Stewart et al. (161).]
Additional Rat Models

Rats with an endothelin B (ET\(_B\)) receptor deficiency have been reported to develop more severe PH and to exhibit obstructive neointimal lesions consisting of endothelial-like and SM-like cells when treated with monocrotaline (73). In many ways, these animals resemble the traditional monocrotaline model, with perivascular inflammatory infiltrates being prominent. In addition, athymic rats treated with the VEGF receptor inhibitor SU-5416 also develop severe PH (in the absence of hypoxia) and exhibit distal obstructive neointimal formation largely comprising SM-like cells with prominent perivascular inflammatory cell infiltrates (162). No studies investigating etiologic mechanisms or responses to treatment have been reported in these models.

Mouse Models Used in the Investigation of PAH

Because of the ability to manipulate specific gene expression using overexpression or knockout strategies, the mouse lends itself well to the study of etiologic mechanisms in PH. BMPR2 model. Genetic studies have shown that BMPR2 signaling plays a critical role in the pathogenesis of IPAH and familial PAH (FPAH). FPAH accounts for $\geq 6\%$ of all cases of PAH and shows an autosomal dominant manner of inheritance (7, 33). Approximately 80\% of FPAH patients carry germline BMPR2 mutations (7). Furthermore, 11–40\% of apparently sporadic patients also carry such mutations. In addition, PAH develops in a subset of hereditary hemorrhagic telangiectasia patients harboring heterozygous mutations in the ALK1 or endoglin gene (ENG) (57). Surprisingly, pedigree studies of FPAH families have shown that only $\sim 20\%$ of people harboring a heterozygous BMPR2 mutation exhibit PH. These genetic data suggest that heterozygous BMPR2 mutations are by themselves insufficient to account for the clinical manifestation of PAH and that multiple environmental or genetic hits are required to trigger the disease. This information has led to several attempts to investigate the impact of BMPR2 deficiency on the development of PAH in mice. Because the clinical and functional characteristics of BMPR2 mutations in human PAH patients indicate haploinsufficiency as the molecular mechanism of the disease, study of mice heterozygous for the BMPR2 allele (BMPR2 \(+/-\)) provide insight to the disease (14, 151, 166). One study suggested BMPR2 \(+/-\) mice showed moderately elevated mean pulmonary artery pressure and pulmonary vascular resistance under basal conditions (14). However, other studies using the same mouse strain showed no significant difference in RV systolic pressure between BMPR2 \(+/-\) and control mice (95, 151). Liu et al. (93) also found that mice in which BMPR2 was constitutively knocked down using short hairpin RNA did not exhibit increases in pulmonary vascular resistance. Mice with SM-specific downregulation of BMPR2 signaling using a dominant-negative form of BMPR2 showed elevated RV systolic pressure, minimal remodeling, and inflammation (184). None of these mice developed intimal fibrosis occlusion of arteries or plexiform lesions. However, West et al. (185) recently reported that mice expressing a BMPR2 mutation in the tail domain in SMC [SM22-rtTAxTet0 (7)-BMPR2 (R899x)] develop PH, significant vascular remodeling, pruning, and adventitial perivascular inflammation.

Based on a report showing that BMPR2 expression is almost completely absent in the endothelial cells of plexiform or concentric vascular lesions of FPAH patients harboring heterozygous BMPR2 mutations (6, 97), studies were carried out by Hong et al. (64) in mice in which the BMPR2 gene was deleted in pulmonary endothelial cells, using a conditional knockout approach. These investigators found that some, but not all, mice lacking BMPR2 in pulmonary endothelial cells had elevated RV systolic pressures, developed RV hypertrophy, and had certain histopathological features observed in human PAH. Most predominant of these was the accumulation of $\alpha$-SM actin-positive cells in combination with a marked inflammatory infiltrate, consisting mostly of monocytes/macrophage-like cells, in muscular pulmonary arteries. In addition, some vessels were occluded by lesions resembling concentric fibrosis. Marked thrombosis in many of the vessels was also reported.

Thus these genetic models of BMPR2 mutations, although in some ways disappointing in that they do not recapitulate human disease, serve as useful genetic resources to further the knowledge regarding gene mutations in PAH. The incomplete penetrance in the Hong et al. (64) model could be considered a limitation for studying PAH pathogenesis, but as the authors suggested, it also presents opportunities to further identify environmental and genetic factors that influence PAH pathogenesis in terms of frequency, time of onset, and severity.

Overexpression of S100A4/Mts1 in mice. The S100A4/Mts1 gene was identified because of its differential expression in highly metastatic mouse mammary adenocarcinoma cells (41). Its expression appears to confer a metastatic phenotype and correlates with advanced stages of human tumors (53, 158). In addition to interacting with intracellular proteins, S100A4 may also exert effects extracellularly as a secreted protein. It is a stimulator of angiogenesis and inflammatory responses (2). When studying the tumor biology of this gene, it was noted that a subset ($\sim 5\%$) of transgenic mice overexpressing S100A4 in all tissues developed pulmonary arterial changes resembling plexogenic lesions (3). In this model, there was seemingly no progressive development of vascular lesions, as the mice either lacked pulmonary lesions altogether or developed plexiform-like arteriopathy (53). The investigators also reported minimal S100A4 expression in human lungs with no PH or with evidence of early-stage disease but marked expression of S100A4 in late-stage plexogenic arteriopathy, suggesting that S100A4 is not involved in the initial responses but may be functionally significant in the development of the more severe arterial lesions seen in end-stage disease. The plexogenic lesions found in these S100A4-overexpressing mice are composed of SM-like and endothelial-like cells (Fig. 6). However, in mice it was noted that S100A4 is expressed in the endothelial cell, whereas in human vessels S100A4 is expressed in the intimal SMC. Furthermore, in those animals that develop plexogenic arteriopathy, there is a marked periarterial inflammatory response, suggesting again that an inflammatory insult triggers the development of plexogenic arteriopathy in these mice. The specific roles of S100A4 in lesion development remains unclear, as S100A4 overexpression has also been noted in hypoxic models of PH.
that do not demonstrate plexogenic arteriopathy and that also spontaneously resolve on return to normoxia (88).

When exposed to hypoxia, S100A4-overexpressing mice had greater pulmonary arterial pressure increases and more RV hypertrophy, which, unlike in control mice exposed to hypoxia, was sustained, even 3 mo after return to room air (105). The S100A4 mice did not develop more severe peripheral vascular disease than control mice in response to hypoxia, but those changes that did develop did not regress on return to room air. Interestingly, one protein, fibulin-5, might be linked to these findings. Fibulin-5 is a matrix component necessary for elastin fibrin assembly (105). The decreased vascular distensibility observed in these mice is similar to that observed in the chronic hypoxia models (89, 170).

IL-6 overexpression in mice. Several reports document that IL-6 is elevated in the serum and lungs of patients with PAH. Therefore, Steiner et al. (152) recently investigated the role of IL-6 in the pathogenesis of pulmonary vascular disease using lung-specific, IL-6-overexpressing transgenic mice under both normoxic and chronic hypoxic conditions. IL-6 was overexpressed under the direction of the CC10 (Clara cell secretory protein) promoter. The transgenic mice exhibited elevated RV systolic pressures and RV hypertrophy with corresponding pulmonary vasculopathic changes, all of which were exacerbated by chronic hypoxia. Interestingly, in the IL-6-overexpressing mice exposed to chronic hypoxia, a proliferative arteriopathy with inflammation was observed in the distal arterial vessels (Fig. 7). No specific evidence for a plexogenic arteriopathy was observed. These occlusive vascular lesions appear to be composed of endothelial cells and excessive accumulation of T lymphocytes. The IL-6-induced arteriopathic changes were accompanied by activation of the proangiogenic factor VEGF and the proproliferative transcription factors c-Myc and Max, up-regulation of the antiapoptotic proteins survivin and Bcl-2, and downregulation of the growth inhibitor TGF-β (152). In addition, in response to chronic hypoxia, there was marked remodeling in the proximal pulmonary arteries with dramatic increases in the number of elastic lamellae (Fig. 7B). These proximal vessel changes appear distinct and, to our knowledge, have never been reported in a semiacute model of PH. In further support of an important role for IL-6 in the PH process is a recent report demonstrating that RV systolic pressure, RV hypertrophy, and both the number and medial thickness of muscular pulmonary vessels were decreased in IL-6−/− mice compared with wild-type controls after 2 wk of hypoxia (142). Hypoxic IL-6−/− mice showed less inflammatory cell recruitment in the lungs compared with hypoxic wild-type mice as determined by protein levels and immunostaining for the specific macrophage marker F4/80, adding further support to the idea that IL-6 is actively involved in hypoxia-induced lung inflammation and pulmonary vascular remodeling (142). Additional studies have recently linked IL-6 expression to reductions in TGF-β signaling (18).

Neprilysin and vasoactive intestinal peptide knockout mice. Neprilysin is a transmembrane metalloendopeptidase that degrades neural peptides important for both growth and contraction of SMC. Dempsey et al. (37) recently demonstrated that neprilysin null mice exhibit exaggerated PH and striking increases in muscularization of distal vessels in response to chronic hypoxia. No specific description of occlusive arteriopathy was made, although marked perivascular inflammation was observed.

Vasoactive intestinal peptide (VIP) is emerging as a critical regulator of tone and structure in the pulmonary circulation (138). It was recently shown that deletion of the VIP gene leads to significant increases in RV systolic pressures, vascular remodeling, and inflammation in male mice breathing room air. Both the vascular and RV remodeling were attenuated by 4-wk treatment with VIP. Furthermore, activation of calcineurin-nuclear factor of activated T cells (NFAT) was observed in the VIP null mice and thus speculated to be under control of VIP (139). The calcineurin-NFAT signaling pathway has also been suggested by others to be important in the development of PH with recent studies showing NFATc3 knockout mice being protected against hypoxia-induced PH (34, 110).

Thus genetic manipulation of mice is providing important insights regarding the role of specific genes in the pulmonary hypertensive process. Continued work in this area will un-
doubtedly lead to further insights into mechanisms involved in PH and perhaps even PAH.

Models of PAH: Group 1.4: Associated with PAH

Patients with high-pressure systemic-to-pulmonary shunts will develop PAH if left untreated (49). It is commonly accepted that persistent exposure of the pulmonary vasculature to increased blood flow and increased pressure can lead to an obstructive pulmonary arteriopathy, resulting in increases in pulmonary vascular resistance that approach or even exceed systemic resistance, ultimately causing reversal of flow from the pulmonary to the systemic circulation, the so-called Eisenmenger’s syndrome. Many of the pathobiological changes seen in patients with PAH associated with congenital systemic-to-pulmonary shunts, such as endothelial dysfunction and plexogenic arteriopathy, are considered similar to those observed in idiopathic forms of PAH (49). These can be well-modeled in both large animals and...
rodents. High flow and pressure shunts in both pigs and calves have been reported (17, 43, 49, 135, 182). Significant vascular remodeling is consistently observed, although the development of severe occlusive arterioles is not universally observed. Work in these important models can and should be pursued and will likely lead to further insights into the pathogenic mechanisms involved in this very important subset of patients with PAH.

Another important subset of patients with PAH are HIV-infected patients with PH. These patients have histological manifestations that are indistinguishable from those found in patients with IPAH. A model for this important problem was recently described Marecki et al. (100), who showed the development of complexplexiform-like lesions characterized by medial hypertrophy, luminal obliteration, and thrombosis in macaques infected with SHIV-nef (a chimeric viral construct containing the HIV-nef gene in a simian immunodeficiency virus (SIV) backbone) but not in animals infected with SIV alone. Some cells in the intimal lesions were factor VIII-positive, and others expressed muscle-specific and SM actin. Large numbers of mononuclear cells were present and positive for HIV-nef. Endothelial cells in both the SHIV-nef macaques and patients with HIV-related PH, but not in patients with IPAH, were also nef-positive. This appears to be an important model to study the pathogenesis of PAH in this subgroup of human patients with PAH (100).

**Conclusion**

It is clear that there is no currently available perfect preclinical model of human PAH. The similarities and differences between the animal models and human PAH are summarized in Table 4. Arguably, there is probably no animal model that accurately reproduces all the clinical pathological features of any of the groups of human PH (Table 1). However, it is also clear that animal models have provided, and will continue to provide, valuable insight into the numerous pathways that contribute to the development and maintenance of PH. These models will allow us to investigate important interactions between the various triggers, which have been implicated in PH, their impact on signaling pathways, and their temporal evolution into the structural and functional abnormalities, which characterize the pulmonary hypertensive disease process. Use of both classic and newly developed animal models will allow us to continue to rigorously test new hypotheses regarding pathogenesis and also evaluate the ability of newly developed agents to not only prevent, but also, more clinically important, reverse established disease. Limitations of the model used should always be acknowledged. For instance, studies have clearly demonstrated interspecies differences in the responses to stimuli capable of promoting PH. Furthermore, for each given biological pathway studied to date, the relative importance in a specific animal strain appears influenced by not only the inciting stimulus and/or the disease, but also by age, sex, environment, and species-specific counterregulatory modifications in cells and tissues. Precise comparisons between animal species and the human condition are therefore difficult (Table 5).

Several additional points are worth considering as work continues in animal models: 1) when considering new treatment approaches, prevention studies provide useful information, but the more clinically relevant experiment is to determine whether treatment reverses neointimal arteriopathy and hypertension once they are established; 2) complete hemodynamic assessment of the animals used is essential, including assessment of both static and dynamic parameters (resistance and impedance) of the vasculature and the relative roles of vasoconstriction and structural remodeling in determining the magnitude of these parameters as well as assessment of right and left ventricular cardiac performance; 3) standards for defining PH in the models should be considered, and care must be taken when defining the conditions under which hemodynamics are assessed; 4) assessment of the functional capacity of both diseased and treated animals to determine whether improvement in hemodynamics and cardiac function afforded by pharmacological intervention is clinically significant; 5) long-term studies on the effects of treatment must be performed with effects on survival and toxicity being included. Finally, we

### Table 5. **Comparison of animal models with human PAH with respect to physiological and pathological parameters**

<table>
<thead>
<tr>
<th>Condition/Characteristic</th>
<th>Precapillary Arteriopathy</th>
<th>Alveolar Hypoxia</th>
<th>Pulmonary Hypertensive Arteriopathy with Plexiform Lesions</th>
<th>Vascular/Perivascular Inflammation</th>
<th>Slow Clinical Onset (Month/Year) and Chronic Time Courses (Years) with Progressive Deterioration</th>
<th>Similar Response to Drugs (ERAs, Prostanoids)</th>
<th>Response to O2 Therapy</th>
<th>Conduit Artery Stiffening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical human PAH</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y Y N Y</td>
<td>Y Y N Y Y Y Y</td>
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<tr>
<td>Hypoxic PH animal models</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y Y N Y</td>
<td>Y Y Y Y Y Y Y Y</td>
<td>Y Y Y Y Y Y Y Y</td>
<td></td>
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<tr>
<td>Monocrotaline rat model</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y Y N Y</td>
<td>Y Y Y Y Y Y Y Y</td>
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<tr>
<td>Monocrotaline rat +</td>
<td>Y</td>
<td>N</td>
<td>N</td>
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<tr>
<td>pneumectomy</td>
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<tr>
<td>Monocrotaline rat +</td>
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<tr>
<td>pneumectomy + young age</td>
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<tr>
<td>Sugen 5416 + hypoxia</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y Y N Y</td>
<td>Y Y Y Y Y Y Y Y</td>
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<tr>
<td>S100A4-overexpressing mice</td>
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<td></td>
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<tr>
<td>IL-6-overexpressing mice</td>
<td>Y</td>
<td>Y/N*</td>
<td>Y</td>
<td>Y</td>
<td>Y Y N Y</td>
<td>Y Y Y Y Y Y Y Y</td>
<td>Y Y Y Y Y Y Y Y</td>
<td></td>
</tr>
<tr>
<td>Left-to-right shunt in piglets and calves</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y Y N Y</td>
<td>Y Y Y Y Y Y Y Y</td>
<td>Y Y Y Y Y Y Y Y</td>
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<tr>
<td>SHIV-nef-infected macaques</td>
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</tbody>
</table>

ERAs, endothelin receptor antagonists; PDE5, phosphodiesterase type 5; SHIV-nef, a chimeric viral construct containing the HIV-nef gene in a simian immunodeficiency virus (SIV) backbone; Y, yes/present or similar; N, no or not present; ?, no data available; Y ±, yes in the opinion of the authors of this manuscript, not, however, reliably reproduced by multiple investigators. *Both normoxic and hypoxic conditions have been studied.
must always keep in mind that cellular and molecular pathogenesis of even the obstructive vascular lesions described in new animal models may not duplicate that which occurs over months and, more likely, years in human PAH. Careful and rigorous clinical trials will be required to establish safety and efficacy of any new PAH therapy in human patients (52, 110).

DISCLOSURES
No conflicts of interest are declared by the author(s).

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ANIMAL MODELS OF PULMONARY ARTERIAL HYPERTENSION

Review

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