Severe Angioproliferative Pulmonary Hypertension: The Lung Circulation Between Vasoconstriction and Cancer

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Analysis of Lung Tissue in Severe Pulmonary Hypertension Has Changed Concepts of Pathogenesis

The rather recent development of specific antibodies and molecular probes allows the identification, localization, and colocalization of growth factor genes and proteins, transcription factors, enzymes, oncogenes, and markers of cell growth and cell death.[1-5] Standard hematoxylin/eosine staining continues to be used for routine screening of abnormalities of the lung tissue, but special stains permit answers to specific questions. Lung tissue extracts from patients with pulmonary hypertension (PH) have also been used for Western blot protein analysis[3,6] or for mass spectroscopy analysis of lipid mediators[7] and for RNA and DNA work;[6,8,9] quantitative PCR allows the detection of low levels of expressed genes,[6,8] and microarray analysis can establish a signature gene expression profile that can distinguish different forms of severe PH.[10] Some investigators observed the emergence of tissue-based information with skepticism because they believed the data related only to end stage disease and scar tissue. This is clearly not the case, since the tissue displays the full spectrum of early and late lesions and different degrees of inflammation, and contains relatively uninvolved areas.

Which Vascular Lesions? Which Cells?

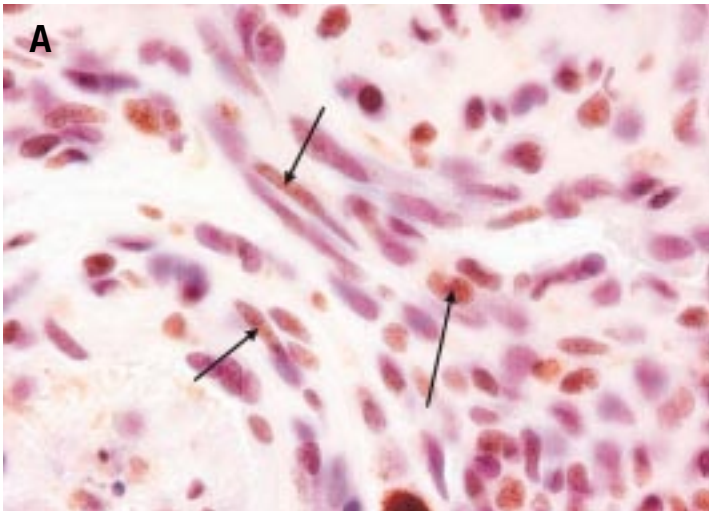
Controversies still exist regarding the importance of the complex vascular lesions, which include the so-called plexiform (or glomeruloid) lesions, concentric intima fibrosis (or onion skin lesions in scleroderma), and angiomatoid lesions.[11] Are these lesions hemodynamically important or just markers of severe PH? Some patients seem to have more of these lesions than others, yet the "pruning" of the pulmonary arteriolar tree, which can be documented radiographically and is seen in all patients with severe PH, can be explained as a drop out or loss of precapillary resistance vessels by an unknown mechanism or by angiogenic lumen obliteration. In the peripheral subpleural regions of the lungs the vascular pruning is most evident and complex, and lumen-obliterating lesions are likely detected here.

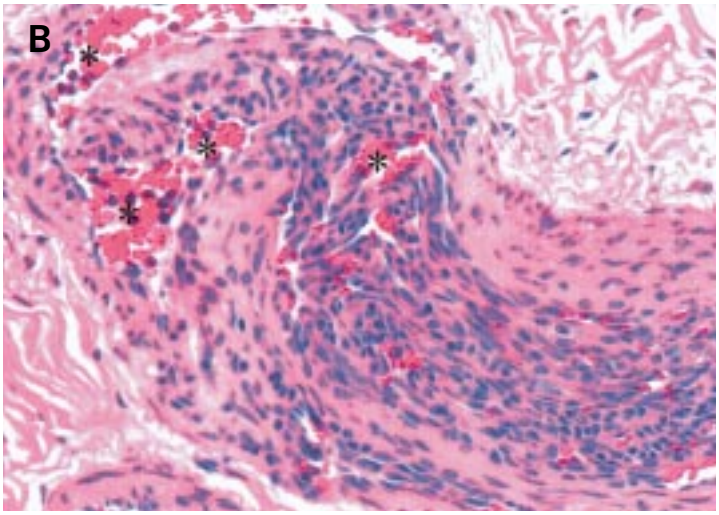
Muscularization of the arterioles alone is (in the opinion of these authors) not sufficient to explain severe and progressive PH. In the aggregate, a number of recent studies point to an angioproliferative process with a preference for particular sites of the vascular tree, namely bifurcations of precapillary arterioles, which makes severe PH a group of diseases characterized by structural alterations of microscopically small vessels. These alterations do not span a great length of an individual vessel; instead, three-dimensional reconstruction analysis shows there is a patent lumen proximal and distal to a "plug."[12] Careful examination of many serial sections of many subpleural lung tissue samples would likely result in the detection of many regionally obliterated vessels and lead to the conclusion that the occlusion or partial blockage of literally millions of these peripheral microvessels can explain why the pulmonary artery pressure is so high (usually 50 mm Hg), and why at the time of the first hemodynamic study only 20% to 25% of patients with severe PH display a significant acute vasodilator response.

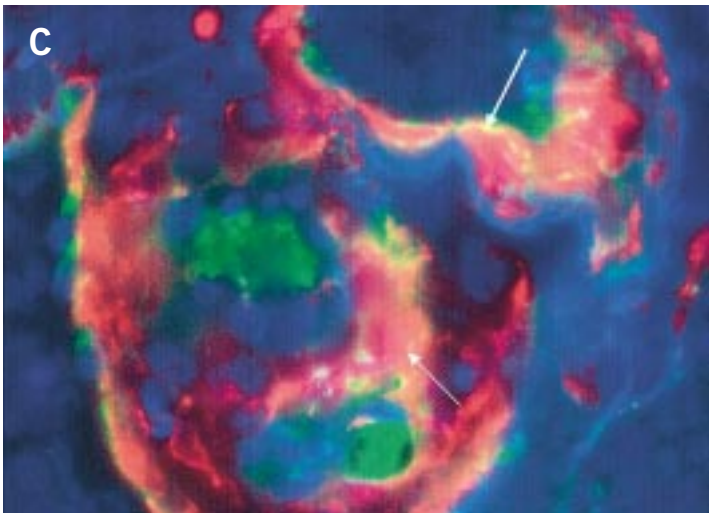
Many of our earlier pathogenetic concepts were based on physiologic parameters, pressure, flow, and vasoconstriction causing an increase in precapillary vascular resistance. Understandably our thought models were those of 19th and 20th century physiology, and the still fascinating acute hypoxia-induced pulmonary vasoconstriction[13] or chronic hypoxic pulmonary hypertension[14,15] animal experiments provided tools to study pulmonary vascular tone regulation and pulmonary vascular remodeling.[16] Yet these models infringe on the human condition of severe angioproliferative PH (SAPH) only peripherally, and it is interesting that the development of the first endothelin receptor blocker for clinical use in "primary" PH was based to a large degree on the positive results of preclinical studies in hypoxia and monocrotaline rat models.[17,18] The assumption clearly was that these rodent models bore enough resemblance to human SAPH, and further, that endothelin was not only "bad" but also a central and very critical actor in a drama played out by a large score of characters. Because human SAPH occurs only in individuals with a still ill-defined genetic predisposition (or susceptibility) and because the pulmonary vascular biology and pathobiology are most certainly very complex, these assumptions are likely wrong or at least too simple.

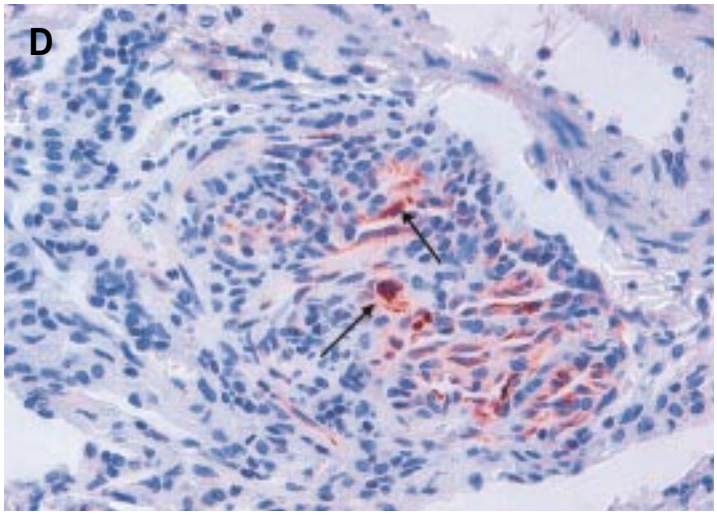
New Concept of Severe Angioproliferative Pulmonary Hypertension

Histologically all the layers of the pulmonary arterioles—intima, media, and adventitia—and a great number of cell types (including lymphocytes, myofibroblasts, macrophages, mast cells, and bombesin-positive cells) are involved and present in









A. **Plexiform lesion from a patient with severe angioproliferative pulmonary hypertension. The nuclei of the phenotypically altered cells in the plexiform lesion demonstrate punctuate, brown staining (arrows) with antibodies directed against latency associated nuclear antigen-1 (LANA-1), which is constituitively expressed in lytic and latent human herpes virus 8 (HHV-8) infection. LANA-1 immunohistochemical stain, 1000X.** ***B.*** **H&E stain of plexiform lesion demonstrating the characteristic exuberant endothelial cell proliferation. A disordered arrangement of plump, spindle-shaped endothelial cells line the multiple slit-like lumens (*). 400X.** ***C.*** **Plexiform lesion co-stained with Factor VIII-related antigen (FVIII-r.ag - an endothelial cell marker) and a-smooth muscle actin (a-SMA), followed by Alexa Fluor labeled secondary antibodies. The slides are coverslipped with fluorescent mounting media containing DAPI. Factor VIII-r.ag positive cells fluoresce green (FITC), the cell nuclei fluoresce blue (DAPI), and the smooth muscle fluoresces red (rhodamine). The transitional cells, defined by their coexpression of Factor VIII-r.ag and a-SMA, fluoresce yellow to orange (arrows). 1000X.** ***D.*** **Vascular endothelial growth factor (VEGF) immunostaining of a plexiform lesion. Some of the cells of the plexiform lesion show a high level of expression for this angiogenic growth factor (arrows), while the surrounding lung tissue is negative. 400X.**

SAPH. The vascular lesions in SAPH are truly complex and it is now clear that they contain not only normal but also very abnormal, phenotypically altered cells. Why is this important? We suggest that it is the nature of these phenotypically altered, apoptosis-resistant cells[2,6,19] that sets human SAPH apart from most of the rodent models and makes human angioproliferative PH so difficult to treat.

A growing number of reports have provided evidence for clonal cell growth and somatic endothelial cell mutations as well as for loss of tumor suppressor genes and expression of proteins usually not found in endothelial cells, like 5-lipoxygenase and 5-lipoxygenase-activating protein (FLAP).[19-21] Most likely a number of different trigger factors can induce programmed cell death/apoptosis of vascular endothelial cells. We hypothesize that "exuberant endothelial cell proliferation"[2] may involve the participation of usually quiescent stem or precursor cells and/or perhaps bone marrow-derived hemeangioblasts. Circulating endothelial cells occur in greater numbers in severe PH;[22] whether these cells have been sheared off in the lung circulation or originate from other sites is unclear. Regardless, in theory, precursor cells or phenotypically altered endothelial cells could—perhaps through shunts—gain access to the systemic circulation and also recirculate into the lung.

Peripheral Blood Cell Analysis in Diagnosis of Severe Pulmonary Hypertension

Patients with severe PH have elevated plasma levels of norepinephrine, vascular endothelial growth factor (VEGF), IL-1, IL-6, and endothelin;[23-25] elevated levels of serotonin have also been described.[26] This altered cytokine and growth factor milieu of

Clinical Algorithm

Trigger Factors Leading to Death of Vascular Endothelial Cells

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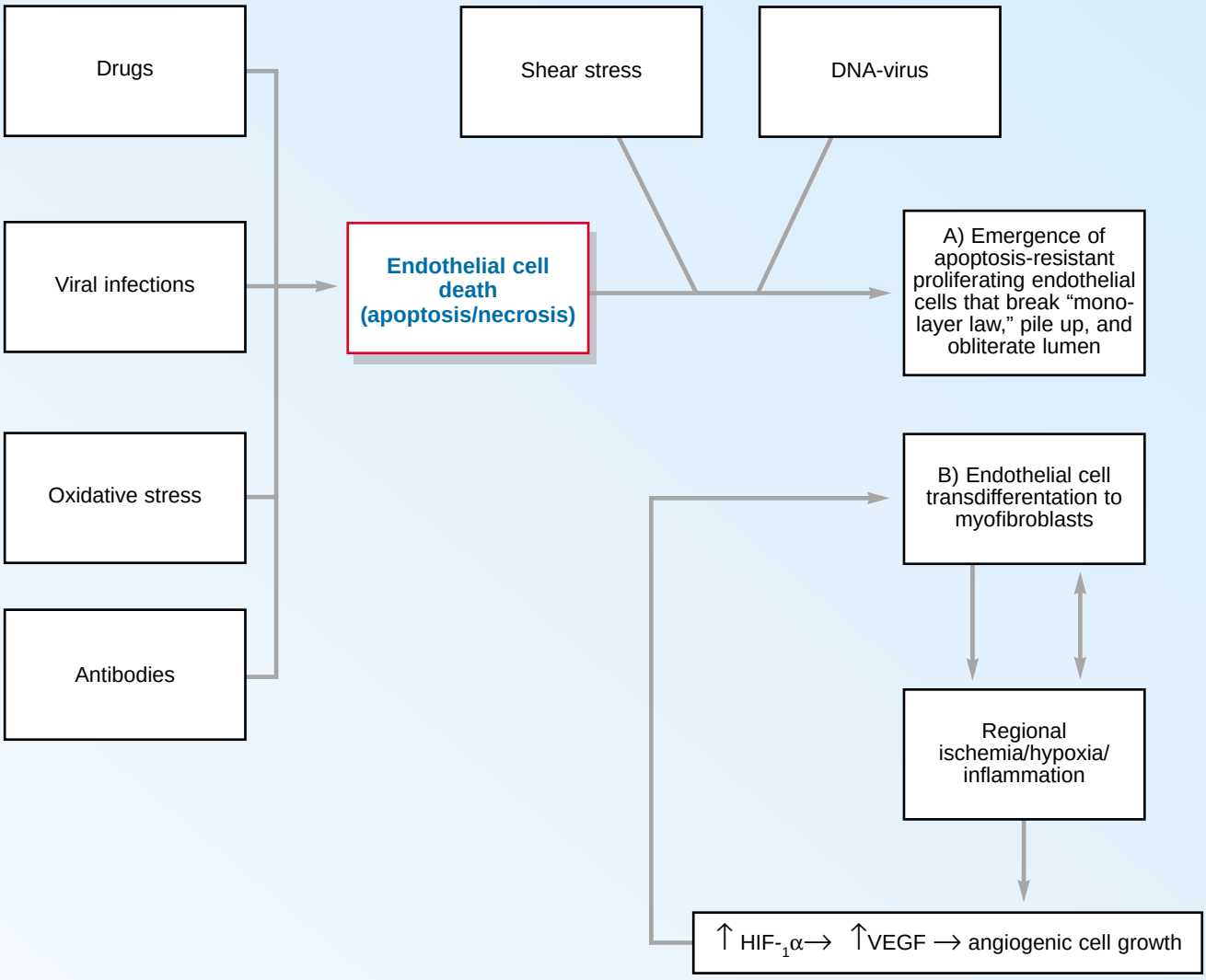

the peripheral blood cells is bound to affect the gene expression pattern and behavior of these cells.

Bull et al[27] recently acted on this concept and performed microarray gene expression analysis of peripheral blood monocytes (PBMC) in patients with severe PH and found that the patients' PBMC gene expression was clearly different from that of normal PBMCs. In addition, they identified a small number of genes that were differently expressed when patients with so-called primary PH and patients with secondary forms of severe PH were compared. It is likely that analysis of PBMCs will allow subgroup identification and possibly prediction of treatment response. We have entered only the first phase of this exploration.

Severe Angioproliferative Pulmonary Hypertension and Viral Infection

The first recognized association of viral infection and severe PH, a disease histologically indistinguishable from so-called primary PH, was the association with human immunodeficiency virus (HIV) infection.[28] This association and the recently recognized second association with human herpes virus 8 (HHV-8, or Kaposi's sarcoma virus) infection,[29] raise many new questions. These questions when answered will lead to a more complete understanding of the pathobiology of SAPH.

The first question is whether there is only an association with certain viral infections—based on an altered immune system—or whether the virus *causes* severe PH because of death of some endothelial cells and "transformation" of surviving cells as in Kaposi's sarcoma. Next, are there other viruses that need to be discovered? What is the mode of infection in HHV-8-associated severe PH? Does severe PH also occur in virally infected primates? Is the occurrence of severe PH in splenectomized patients[30] also associated with viral infections, etc?

Infection, Inflammation, Phenotypically Altered Pulmonary Vascular Cells, and Vasodilator Therapy

As we[31] and hopefully others, perhaps with a measure of reluctance, accept this new pulmonary vascular pathobiology, our patients expect and deserve new treatment strategies. These will likely not result in "complete molecular repair" of all of the lung lesions or a molecular remission, but in a partial desobliteration of precapillary arterioles because of removal of lumen obliterating cells (perhaps by induction of apoptosis in apoptosis-resistant cells). There is enough collective experience to say that the drugs currently in use—all vasodilators—do not reopen occluded vessels.[32] It is not clear whether antiangioproliferative agents will be able to do the job. They may prevent further progression because of inhibition of further angioproliferation, but they may not remove already established lesions.

A rodent model of severe PH (mean pulmonary artery pressure >50 mm Hg) due to lumen obliteration of precapillary arterioles has been established.[33] Inhibition of apoptosis with a broad-spectrum caspase inhibitor prevents the development of severe PH in these animals, but reversal of established SVP in this model is as difficult as in the human SAPH group.[34] Patients with SAPH have millions of obliterating lesions and may not benefit from antiviral drugs. Instead, proteins expressed only in abnormal apoptosis-resistant vascular cells, not in normal endothelial and smooth muscle cells, are drug targets and cell-specific homing strategies can be developed.

Outlook

The continuous infusion of prostacyclin was the first real breakthrough in the treatment of severe PH,[32;35;36] and this treatment has improved the survival of many patients afflicted with this rare and deadly disease. To accept that angioproliferative obliteration in severe PH is both pathobiologically and hemodynamically important is the first step. We hope that many investigators will be able to take this step. The next step is to take the fact seriously that the lumen-obliterating cells in SAPH are abnormal cells and actually cancerlike.[19,29] Primary PH was once called "the cardiologists' cancer" (Greg Eliot). The removal of these quasimalignant cells from established vascular lesions is our new treatment goal.[34] If successful, such treatment would shorten the transplantation list.

Acknowledgment

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Profiles

(continued from page 3)

The discovery of the efficacy of nitric oxide was based on Dr Higenbottam's work in physiology. "We were using inhaled nitric oxide to measure the lung diffusion capacity alongside carbon monoxide. We had PH patients inhale the same concentrations that we had used for the gas diffusion studies and we were able to show that nitric oxide is a selective pulmonary vasodilator. We then developed a device for use in ambulatory patients because prior to that they could not use it except through a ventilator. The new technique involved a pulsing device delivering nitric oxide every time they breathed."

In his new role at Astra Zeneca, Dr Higenbottam remains on the frontier of new approaches to lung disease. "My job is to apply some of these thoughts on disease and make the final link between the molecule and affecting the disease process itself." In PH, "the major problem is how to restore function to the chronically damaged lung, enhancing the reparative process. This will lead us to the next generation of treatments—looking at growth factors and various signaling systems that work not only in the exogenous stem cells but on endogenous stem cells in the organ. Growth and repair are the main issues in chronic lung disease. The current generation of treatments is beneficial in terms of the horrendous pathophysiology. The treatments are improving the circulation of the blood through the lung, but we need to move beyond that. We need to ask, how we can restore some compromised vessels back to normal."

The introduction of bosentan has been a major advance. "It has profound effects, and the approach of endothelin-1 antagonism is a very strong one, clearly addressing a persistent abnormality present in all forms of PH. Bosentan in this regard is probably the best, because it is oral, but it's in the same category as other agents—it's just better.

"We need to think in terms of real function, not just vessels where there's a narrowing as a result of a particular deficiency, but actually restoring the structure and architecture of the vessels to normal. I believe that in the next 5 to 10 years we will have that therapy. The key will be restoring the blood vessel and airway back to normal."