



Pulmonary vascular endothelium: the orchestra conductor in respiratory diseases

Highlights from basic research to therapy

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ABSTRACT The European Respiratory Society (ERS) Research Seminar entitled “Pulmonary vascular endothelium: orchestra conductor in respiratory diseases - highlights from basic research to therapy” brought together international experts in dysfunctional pulmonary endothelium, from basic science to translational medicine, to discuss several important aspects in acute and chronic lung diseases. This review will briefly sum up the different topics of discussion from this meeting which was held in Paris, France on October 27–28, 2016. It is important to consider that this paper does not address all aspects of endothelial dysfunction but focuses on specific themes such as: 1) the complex role of the pulmonary endothelium in orchestrating the host response in both health and disease (acute lung injury, chronic obstructive pulmonary disease, high-altitude pulmonary oedema and pulmonary hypertension); and 2) the potential value of dysfunctional pulmonary endothelium as a target for innovative therapies.

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Introduction

The lung is supplied by two distinct circulatory systems, the systemic bronchial circulation system and the pulmonary circulation system. From a functional point of view, the pulmonary circulation system is unique among vascular beds in the human body, characterised as it is by high-flow, low-resistance and low-pressure. Although all cardiac output is pumped through the pulmonary circulation system, the measured values of mean pulmonary arterial pressure (mPAP) in pulmonary arteries for a resting, healthy adult human are about 14 ± 3 mmHg, whereas systemic arterial blood pressure is about 100 ± 20 mmHg. This unique feature of the pulmonary circulation system is mainly explained by three critical characteristics of the pulmonary vasculature: 1) a low impedance to blood flow; 2) a high compliance of pulmonary precapillary arterioles characterised by a thin vessel wall; and 3) a high capacity to recruit available vessels that help to accommodate an increase in flow. Indeed, this circulatory system can accommodate flow rates ranging from approximately $6 \text{ L}\cdot\text{min}^{-1}$ under resting conditions to $25 \text{ L}\cdot\text{min}^{-1}$ under strenuous exercise, with minimal increases in pulmonary pressure [1].

Due to their strategic localisation at the interface between the bloodstream and lung tissue, pulmonary endothelial cells (ECs) play key roles not only in optimising gas exchange and in controlling barrier integrity and function, but also in regulating pulmonary vascular tone (*i.e.* through the nitric oxide (NO), prostacyclin (PGI_2), endothelin (ET) and serotonin (5-HT) pathways) [2]. Moreover, the pulmonary endothelium functions as an active and dynamic receptor–effector tissue and responds to different chemical, physical, or mechanical stimuli by secreting the correct substance(s) by which it may maintain vasomotor balance and vascular-tissue homeostasis. Indeed, pulmonary ECs are highly metabolically active, sensing and responding to signals from extracellular environments. All these interactions with adjacent cells (and circulating cells and/or mediators) aim to maintain a thrombosis-free surface, so as to control inflammatory cell adhesion and trafficking to assure normal angiogenesis and the integrity of the vascular wall [3]. As a vital part of the respiratory system, alteration of the pulmonary endothelium plays a central role in the pathogenesis of several chronic and acute lung diseases, both common and rare. The main characteristics of pulmonary endothelial alteration (or dysfunction) are increased permeability that can lead to vascular leakage and oedema formation; altered balance between vasoconstriction and vasodilation; acquisition of a pro-inflammatory phenotype with increased expression of adhesion molecules for inflammatory cell recruitment, increased activation of pro-inflammatory transcription factors, release of inflammatory mediators and oxidative stress; pro-thrombotic phenotype; pro-proliferative and anti-apoptotic phenotype; and miscommunication with adjacent vascular cell walls.

Although considerable progress has been made in the understanding of pulmonary EC dysfunction, the triggers, mechanisms and consequences of a dysfunctional endothelium in both acute and chronic lung disease are still not completely understood. A better knowledge of these key aspects would help in finding new disease biomarkers and/or novel therapeutic targets to preserve homeostasis in response to injury and disease.

The importance of genetic and environmental factors

Most, if not all, acute and chronic lung diseases result from interaction between multiple environmental exposures and many genetic risk factors, an aspect that has been critically evaluated in a recent review from the European Lung White Book [4]. The role of vasculature in the development, lifelong repair and maintenance of lung tissue homeostasis is of paramount importance. Indeed, many diseases involving the lung, such as emphysema, chronic obstructive pulmonary disease (COPD) [5, 6] and pulmonary hypertension (PH) [7], display major EC abnormalities. A better understanding of how pulmonary ECs contribute to normal lung development and how these mechanisms are deregulated during chronic disease is of crucial importance for the development of new therapeutic strategies for debilitating lung conditions. However, individual differences in subject response to the same stimulus, such as environmental hypoxia at high altitude, are well documented. In addition, it is well established that the pulmonary vasopressor response varies greatly between humans and animals (and even between different species), supporting the importance of unknown genetic influences.

Beyond the genetic background and specific gene mutations, other factors are known to determine structural and functional modifications to the pulmonary vasculature (such as exogenous exposure to drugs and toxins, hormones and aging), resulting in decreasing repair capacity [8–10] and impaired production of different vasoreactive mediators [11].

Another potential agent that is capable of causing endothelial impairment is cigarette smoke (tobacco products), which play a major role in COPD, since “healthy smokers” (without COPD) already show significant abnormalities in their pulmonary arteries including: vessel remodelling, inflammatory cell infiltrate, endothelial dysfunction, reduced expression of endothelial NO synthase (eNOS), increased expression of growth factors and a similar gene expression profile to that observed in COPD patients [12, 13].

It has also been demonstrated in experimental models of COPD that chronic exposure to cigarette smoke induces endothelial dysfunction in pulmonary arteries [14], along with changes in the expression of eNOS [15] and soluble guanylate cyclase (sGC) [16]. Interestingly, vascular changes precede the development of pulmonary emphysema [17]. Altogether, these findings strongly support the relationship between smoking and pulmonary vascular damage in COPD.

Properties of dysfunction of the endothelial barrier

The vascular endothelium forming the innermost lining of all pulmonary blood vessels is the major barrier that protects air spaces against vascular fluid entry. The integrity of the permeability of this endothelial barrier is critical for the maintenance of homeostasis for all the tissues and organs of the body and changes in its permeability are a hallmark of acute and chronic lung disease.

The vascular endothelium forms a continuous uninterrupted layer of ECs that is held together by complex junctional structures, namely adherens junctions, tight junctions and gap junctions, which are known as gate keepers for trans-endothelial movements of fluid, protein and cells. It is well established that endothelial barrier permeability under basal conditions and in response to different stimuli or certain agents, varies considerably among the different vascular beds [18]. Loss of the endothelial barrier causes pulmonary oedema, the characteristic feature of acute lung injury (ALI), as well as high-altitude pulmonary oedema (HAPE). The pathogenesis of both conditions is still unclear and no drugs have been approved so far for these life-threatening conditions.

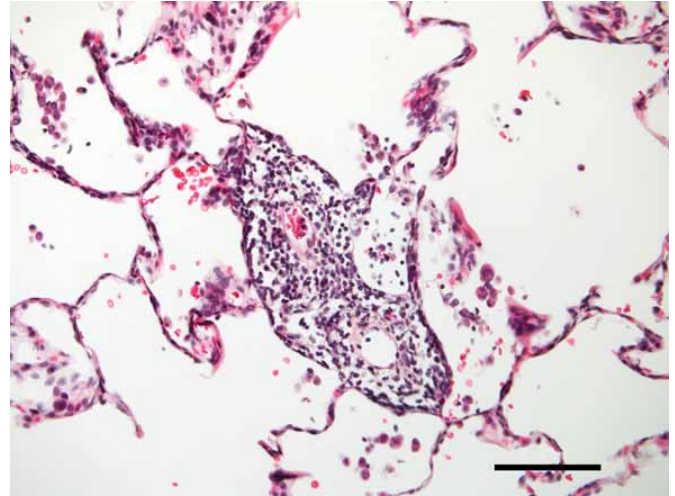
ALI can progress to acute respiratory distress syndrome (ARDS) in which multi-organ failure accounts for a high degree of mortality and morbidity. ARDS can be precipitated by either direct insults to the lung (*i.e.* pneumonia or aspiration of gastric contents) or indirect insults (*i.e.* sepsis or multiple trauma). Sepsis is the most common cause of ARDS in humans and it is also well-established that a pulmonary cause carries with it the highest mortality compared with other ARDS aetiologies. The incidence of ARDS varies widely, from 15 to 70 cases per 100 000 persons per year, representing approximately 5% of hospitalised, mechanically ventilated patients [19].

Being at the interface between the vascular and lung airspaces, the pulmonary endothelium is positioned to play a critical role in establishing immune responses that lead to ALI. The mechanistic understanding underlying these processes remains inadequate and is considered here in light of new findings. The lung's innate immune response involves recruitment of immune cells to pulmonary vessels across which they migrate to the air space. Injury to the endothelial barrier occurs through the paracrine effect of mediators (such as arachidonate [20], ATP [21] and peroxide [22]) released from adjoining epithelial cells, mechanical stresses such as vascular stretch resulting from increased vascular pressure [23], peroxide release from hypoxic red cells [24] and pro-coagulant proteins deposited by platelets [25].

A surprising yet robust role for endothelial mitochondria in the lung's immune response is becoming increasingly evident, even though mitochondria are generally considered to be major sites of cellular ATP generation and little is known about the functional significance of lung endothelial mitochondria in the context of the normal or the diseased lung. The old impression that ECs lack mitochondria, as derived from cultured cells, has been dispelled by the more recent real-time fluorescence imaging (RFI) of live lungs in studies that provide definitive evidence for the presence of lung endothelial mitochondria [26]. As a result of enhanced cytosolic Ca^{2+} oscillation, either spontaneous [27] or induced by conditions associated with ALI [26, 28], oscillation of mitochondrial Ca^{2+} increases causing mitochondrial hydrogen peroxide production and therefore nuclear factor- κB (NF- κB) pathway activation [26, 28]. The resulting gene transcription leads to endothelial expression of P-selectin and leukocyte adhesion receptor E-selectin, the defining marker of pro-inflammatory activation in the lung vasculature. In the pulmonary endothelium, mitochondrial density tends to be somewhat higher at capillary branch points than in the septal capillaries [23]. As such, inflammation initiates at capillary branch points, which are also major sites of microvascular filtration, a process which generates the interstitial fluid required to drive lymph flow. Thus, lung endothelial mitochondria activate innate immune mechanisms that in turn induce physiological responses to facilitate lymphatic delivery of antigens to lymph nodes, promoting adaptive immunity (figure 1).

A protective role for endothelial mitochondria has also emerged through RFI studies addressing the expression of an endothelial pro-inflammatory receptor, tumour necrosis factor (TNF) receptor 1 (TNFR1), which is expressed as a trans-membrane protein on the luminal membrane [28]. The mitochondrial release of hydrogen peroxide resulting from tumour necrosis factor- α (TNF- α) induced Ca^{2+} oscillations activates a metalloproteinase, TNF- α converting enzyme (TACE), that is also expressed on the luminal endothelial membrane, causing shedding of TNFR1 ectodomains and limiting the pro-inflammatory effects of TNF- α .

FIGURE 1 Inflammation and pulmonary microcirculation. Inflammation-mediated events occurring in pulmonary arterioles, capillaries and venules contribute to impaired capillary perfusion. The sample presented was taken from the lungs of a patient with chronic thromboembolic pulmonary hypertension. Note the moderate to severe microvascular inflammation (in the absence of any major vascular wall remodelling). The scale bar represents 100 μ m.



The new understanding that mitochondria are immune sensors may have implications beyond the pathology of ALI to inflammatory lung diseases in general [29]. Further research is required to understand disease processes that damage lung mitochondria, particularly in the pulmonary endothelium and to develop therapeutic strategies for improving mitochondrial function to cure lung diseases.

Loss of endothelial barrier properties in the pulmonary circulation system can also cause a high-permeability form of pulmonary oedema, defined as HAPE, that can occur in previously healthy, fit, but not acclimatised individuals who ascend rapidly to altitudes above 3000–4000 m [30, 31]. It is a multifactorial disease involving both environmental (altitude and rate of ascent) and genetic risk factors [32–34]. In addition, oxygen deprivation causes vasoconstriction of the small pulmonary arteries when systemic arteries dilate under hypoxic conditions. This phenomenon, known as hypoxic pulmonary vasoconstriction (HPV), is more pronounced as the vessel diameter decreases and represents an important physiological mechanism by which pulmonary arteries constrict in hypoxic lung areas to redirect blood flow to areas with greater oxygen supply. It is now well recognised that chronic exposure to hypoxia (*i.e.* COPD or living at high altitude) causes continuous alveolar hypoxia, resulting in sustained HPV that is thought to play a crucial role by exposing the pulmonary capillaries to high pressure, damaging their walls and leading to pulmonary vascular remodelling, and possibly to PH [35, 36].

Abnormally high pulmonary arterial pressure (PAP) in hypoxia has been shown to be a hallmark of HAPE. HAPE susceptibility can be revealed at low altitude during a brief hypoxic challenge in people with a history of HAPE [37–39]. Right-heart catheterisation performed at high altitude showed that all individuals developing pulmonary oedema had capillary pressures above a leakage threshold of 19 mmHg, indicating that abnormally high PAP in HAPE translates to the capillary bed [40–42]. Areas with less HPV have higher perfusion causing flow-mediated increased capillary pressure and leakage, while areas with more vigorous vasoconstriction have lower perfusion and are thus protected from oedema formation. This hypothesis is supported by perfusion scan results in HAPE which show that pulmonary oedema occurs in areas of high blood flow [31], the observation that oedema has a patchy distribution in HAPE [43] and by analysis of lung perfusion in hypoxia by magnetic resonance imaging (MRI) [44, 45]. In addition, bronchoalveolar lavage (BAL) performed at high altitude in individuals at the outset of HAPE reported an absence of inflammatory markers in the BAL fluid [46]. However, inflammatory cells and cytokines were found in BAL fluid in more advanced cases [47] and in patients hospitalised with HAPE in Japan [48]. HAPE is thus a pressure-induced noninflammatory oedema that may induce a secondary inflammatory response [30, 49].

A recent meta-analysis of high-altitude studies, in which PAP was measured by echocardiography in people living at altitudes between 3600 and 4350 m, concluded that altitude-induced PH appears to be rare [50]. Mean systolic PAP at high altitude was 25.3 mmHg, which was modestly elevated compared to that seen in healthy individuals from the general population living below 1200 m. Nonetheless, there is a wide distribution and some people are protected from PH at altitude compared to others [51]. Others have reported that the magnitude of HPV in humans can vary almost five-fold between individuals [52, 53]. This has a genetic basis and understanding the genetic mechanisms involved offers important insights into the molecular regulators of pulmonary vascular tone and structure which can be exploited as drug targets. Studies examining extreme phenotypes in high-altitude populations and rat strains have begun to support this approach and open up new areas of research.

HAPE-susceptible individuals display endothelial dysfunction in forearm blood flow studies in hypoxia but not in normoxia, reduced exhaled NO at high altitude [54, 55], decreased pulmonary NO bioavailability [31, 46] and an increase in production of reactive oxygen species (ROS) across the lung (particularly at the pulmonary artery smooth muscle cell (PA-SMC) level in hypoxia) [56]. In addition, plasma levels of endothelin-1 (ET-1) increase in parallel with increased PAP in HAPE-susceptible individuals at high altitude [57]. Besides these endothelium-dependent mechanisms accounting for increased HPV in HAPE-susceptible individuals, additional factors must be considered such as a low hypoxic ventilatory response [58] and a smaller capillary bed as suggested by lower lung volumes [59, 60], which can both contribute to increased PAP in hypoxia.

There is a need to better understand the mechanisms underlying the differences in endothelial permeability of various vascular beds, the different signalling pathways that modulate inter-endothelial junctions and how tissue–fluid homeostasis is controlled under normal conditions and in pathological processes. Indeed, the restoration of the endothelial barrier is critical for tissue homeostasis and also for recovery following exposure to acute inflammatory events or injury. However, our understanding of how this process occurs at the molecular level remains obscure. More effort should be geared toward developing drugs that facilitate the formation of inter-endothelial junctions or that enhance the function of key mediators of barrier integrity.

Altered balance between vasoconstriction and vasodilation

As already underlined, the pulmonary circulation system has unique haemodynamic features, characterised by a more relaxed vasomotor tone in the normal physiological state, in contrast to the systemic circulation system. In the fetus, where gas exchange occurs in the placenta, HPV acts to maintain high pulmonary vascular resistance and to divert blood flow away from the lungs through the *ductus arteriosus*. Different ion channels, including potassium (K^+) channels, are expressed and distributed in several types of pulmonary vascular cells (including PA-SMCs) and contribute to the large functional diversity in the hypoxic response.

Pulmonary ECs synthesise a variety of paracrine and endocrine factors that are capable of controlling vascular tone, either by favouring relaxation (*i.e.* PGI₂ and NO) or by causing contraction (*i.e.* ET-1 and 5-HT) under basal conditions and in response to different stimuli or certain agents [61]. There is evidence to suggest that background release of NO contributes to the normally low pulmonary vascular tone in normoxia [61]. Although there are theoretical grounds to hypothesise that hypoxia reduces the synthesis of NO, lack of the latter does not seem to account for the acute HPV. Instead, there is evidence to suggest that NO activity is increased in order to modulate the pulmonary vasopressor response to acute alveolar hypoxia. The impaired NO production, whilst reducing the ability of the pulmonary vasculature to relax, also favours the occurrence of excessive pulmonary vasoconstriction [5]. Lack of NO synthesis might also permit mitogenesis and proliferation of various cell types within the vascular wall [62]. It is also well established that the RhoA/Rho-associated kinase (ROCK) pathway also regulates pulmonary vascular tone through both NO-dependent and NO-independent mechanisms [63]. Hypoxia-induced activation of ROCK pathways increases the Ca^{2+} sensitivity of the contractile myofilaments in PA-SMCs, thereby increasing vascular tone and causing sustained HPV and increased pulmonary vascular resistance [63–65].

In the adult, HPV has at least two phases: the initial constrictor response starts within seconds and reaches a maximum within minutes, followed after 30–120 min by a sustained phase [52, 64, 66]. These phases are regulated, at least in part, by different signalling pathways. The second phase of HPV is influenced by EC function through changes in the release of vasoactive mediators such as ET-1, PGI₂ and NO. *In vivo*, neurohumoral mediators, red blood cells and lung innervation may also influence the response. The mechanisms underlying the sensing of low oxygen levels by cells have been the subject of discussion. Both mitochondria and NAHD/NADPH oxidases have been suggested as oxygen sensors. A change in the levels of ROS is thought to be important but there is a lack of agreement about whether the signal is an increase or decrease in ROS. Hypoxia-inducible factor 1 (HIF-1) and HIF-2 that transactivate a number of common as well as distinct downstream target genes are clearly candidates for explaining variability in the pulmonary vascular response to chronic hypoxia [67]. In addition to differing in terms of downstream targets, their α -subunit expression levels are also known to vary in cells and tissues. Recently, a comparison of two rat strains, F344 and WKY, which differ in their response to chronic hypoxia, has highlighted the importance of zinc in several cell types, particular PA-SMCs, in regulating pulmonary vascular homeostasis [68]. A mutation in the solute carrier family 39 member 12 gene (*Slc39a12*), which encodes the zinc transporter ZIP12, predicts a truncated and inactive protein in the F344 rat strain. Introduction of a similar mutation in the WKY rat strain reduces its responsiveness to hypoxia. Upregulation of ZIP12 expression in the lungs of humans living at high altitude (and patients with idiopathic pulmonary arterial hypertension (PAH)) has now stimulated a search for small molecule inhibitors of ZIP12, as well as interest in the role of intracellular labile zinc in pulmonary vascular disease.

From dynamic to unadapted pulmonary vascular remodelling

The pulmonary circulation system responds to alveolar hypoxia by increasing pulmonary vascular resistance and HPV is responsible for the initial rise in pulmonary pressures [4, 52, 53]. However, with continued exposure to hypoxia, other mechanisms drive structural changes in resistance vessels which contribute to the elevated pressures, thus explaining why after 2 or 3 weeks of hypoxia there is little response to rebreathing 100% oxygen. Notably, pulmonary pressures decrease progressively with time on re-exposure to a normal oxygen environment, suggesting that the pulmonary vascular remodelling induced by chronic hypoxia is reversible. Histological examination of lungs from mammals, including rats, cows and humans exposed chronically to hypoxia, demonstrate structural remodelling of pulmonary arterioles [69]. All layers of the vascular wall are involved in the remodelling, including fibroblasts, but the hallmark of the vascular response to chronic hypoxia is increased muscularisation of distal vessels with extension of muscle into previously non-muscularised arterioles (figure 2). The relative contributions of HPV and vascular remodelling to chronic hypoxia-induced PH are the subject of debate [70]. Histological studies in rats contest the extent to which remodelling narrows vascular lumen and provides an obstruction. A recent consideration in this debate is vascular stiffness, where the changes in vascular structure lead to an increase in stiffness. This stiffness alters the transmission of pulse waves along affected vessels and their reflection from branch points and the right heart then senses an increased pressure load from the combination of advancing and reflected waves. The role of haemodynamic forces and shear stress in initiating and sustaining pulmonary vascular remodelling in hypoxia has been highlighted recently by studies in rats. Pulmonary artery banding reduces haemodynamic shear stress and not only prevents the development of vascular remodelling but also reverses occlusive lesions and perivascular inflammation in the Sugen hypoxia model [71]. It is easy to speculate that haemodynamic stress and occlusive vascular lesions could form a vicious cycle. If so, these observations suggest that mechanisms to reduce shear stress, for example with vasodilator therapies, should not be ignored in the search for treatments for PH.

Defined as $mPAP \geq 25$ mmHg at rest as assessed by right-heart catheterisation, PH can present in a number of clinical scenarios including several with a high mortality rate [72, 73], as follows: PAH (group 1); PH due to lung diseases such as COPD and emphysema and/or hypoxia (group 3); chronic thromboembolic pulmonary hypertension (CTEPH) (group 4); PH with unclear multifactorial mechanisms, such as haematological disorders (group 5) [74]. In all of these PH subgroups, pulmonary endothelial dysfunction contributes to pulmonary vascular remodelling [75, 76]. In particular, major functional alterations in the pulmonary vascular endothelium have been demonstrated in PAH, including among others: 1) a transition from a quiescent state without adhesive capacity to an activated state with adhesive capacity [77]; 2) an aberrant pro-proliferative and apoptosis-resistant phenotype [78]; 3) a pro-inflammatory phenotype characterised by an excessive release of various key cytokines and chemokines such as interleukin (IL)-1 α , IL-6, IL-8, IL-12 and chemokine (C-C motif) ligand 2 (CCL2)/monocyte chemoattractant protein-1 (MCP-1) [77]; and 4) an excessive production and secretion of various key growth factors including fibroblast growth factor-2 (FGF-2) [78, 79], angiotensin (Ang II) [80] and leptin [81–83]. Additional insights into the altered pulmonary EC phenotype and endothelial communication with both resident vascular cells (*i.e.* PA-SMCs and myofibroblasts) and immune cells are a prerequisite for a better understanding of PAH pathogenesis that could lead to novel therapeutic strategies.

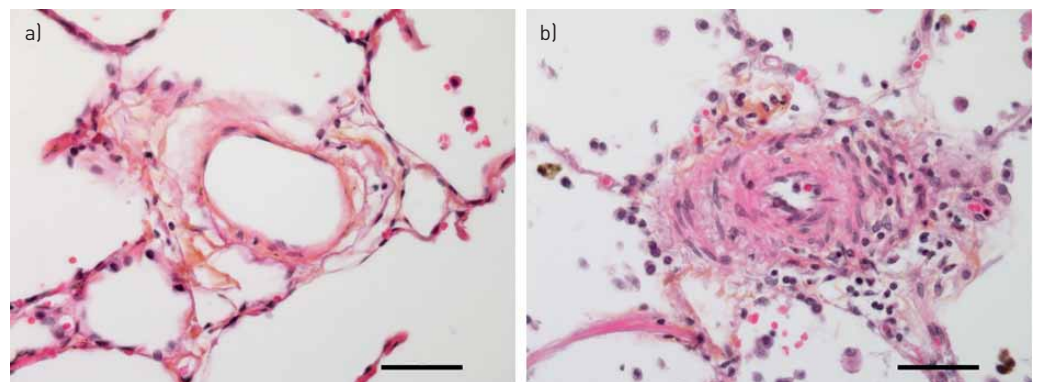


FIGURE 2 Vascular response to chronic hypoxia. Previously nonmuscularised arterioles (a) show “muscularisation” with recruitment of smooth muscle cells and myofibroblasts (b). Note the mild inflammatory infiltrate [predominating on the right side of the vessel] which accompanies the remodelling process. The scale bars represent 50 μ m.

More recently, endothelial communication was reported with pericytes, a key type of progenitor cell, which are found around pre-capillary arteries, capillaries and post-capillary venules. They also occupy a strategic position at the interface between circulating blood and interstitial space, and in close proximity to ECs and PA-SMCs. RICARD *et al.* [84] have demonstrated excessive pericyte coverage (a two to three-fold increase) in distal pulmonary arteries in human PAH, a phenomenon that contributes to pulmonary vascular remodelling as a source of smooth-muscle-like cells. Pericytes are central regulators of vascular development, stabilisation and maturation, as well as modulation of remodelling by affecting: 1) EC growth, proliferation, differentiation and migration; 2) PA-SMC contraction; 3) immune cell function; 4) acting as progenitor cells and possible differentiation in SMC-like cells and dendritic cells.

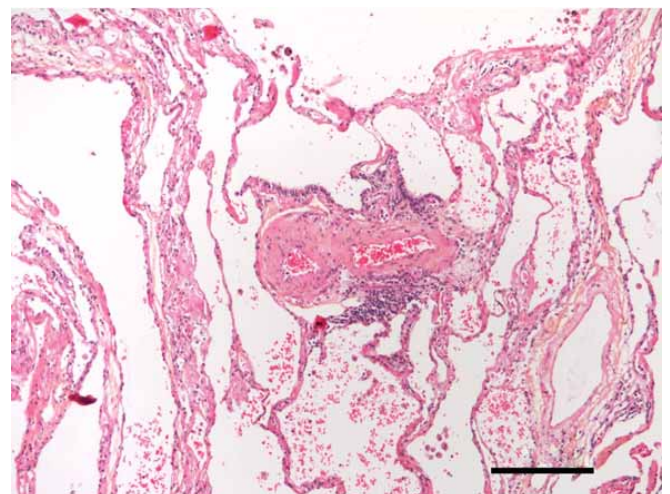
Deciphering the intracellular pathways that modulate pulmonary vascular remodelling has paved the way for precise molecular targeting strategies in new treatments for PH [85]. For example, one can now choose between specific and nonspecific ET-1 receptors, or stimulation of synthesis or inhibition of degradation of the key intracellular second messenger, cyclic guanosine monophosphate (cGMP), to alter pulmonary vascular tone in diseased states. Even though the prognosis remains poor, these additional pathways may represent innovative tools for the treatment of PH in the future.

In PH due to a lung disease, such as COPD, pulmonary vascular impairment is highly prevalent with about 40% of patients with Global Initiative for Chronic Obstructive Lung Disease (GOLD) Stage 4 COPD and 27% of patients with GOLD Stage 3 COPD displaying an increased mPAP [86]. Among patients without PH when at rest, a significant proportion develop it during exercise [87, 88]. These findings concur with the frequent observation of histological changes, mainly consisting of intimal hyperplasia, in the small pulmonary arteries in patients with end-stage disease who underwent lung transplantation [5], even in patients with mild-to-moderate disease [6, 89]. In addition to vascular remodelling, dysregulated vascular tone is a major contributor to PH in COPD: the expression of eNOS [90], sGC [16] and PGI₂ synthase [36] are reduced in the pulmonary arteries of COPD patients, thus decreasing their vasodilator and antiproliferative capacity (figure 3).

With this background, it is interesting to note that some patients with extensive emphysema develop severe PH [91], suggesting a link between the emphysematous destruction of lung parenchyma and the severity of pulmonary vascular impairment. In fact, in explanted lungs of COPD patients who underwent lung transplant, the severity of emphysema was significantly higher in those patients who had PH [92]. Accordingly, the combination of vascular remodelling, endothelial dysfunction and parenchymal derangement might explain the development of severe PH in a subset of patients with COPD [93].

The pulmonary endothelium represents an interesting therapeutic target in COPD. Studies conducted in experimental models show that targeting the NO–cGMP signalling pathway with sGC stimulators [16] or phosphodiesterase-5 (PDE5) inhibitors [94, 95] prevents the development of PH, pulmonary vascular remodelling and right-ventricle hypertrophy. Interestingly, sGC stimulators and PDE5 inhibitors also attenuated the development of pulmonary emphysema [16, 95], an effect that was associated with a reduction in the number of neutrophils and alveolar macrophages in lung tissue, as well as a reduction in leukocyte adhesion [16]. Taken together, these findings suggest that therapeutic interventions in the NO–cGMP signalling pathway exert a dual effect: 1) relaxation and eventually attenuation of cell proliferation

FIGURE 3 A pulmonary artery in a patient with chronic obstructive pulmonary disease. The vessel (shown here at a dichotomous branching point) presents with important intimal fibrosis. Note the mild perivascular infiltrate (predominating beneath the artery). The surrounding pulmonary parenchyma displays emphysematous remodelling. The scale bar represents 200 μ m.



through protein kinase G dependent mechanisms in smooth muscle cells; 2) reduction of leukocyte adhesion in ECs, presumably by downregulating P-selectin [96].

These results have been obtained using a “preventive experimental design” but it remains to be shown as to what extent the therapeutic intervention exerts the same effects when lung damage is already established (*i.e.* using a “therapeutic design”). In fact, treatment of COPD patients with PH using PDE5 inhibitors or sGC stimulators has been ineffective in improving exercise tolerance [86]. However, exercise tolerance might not be the most adequate surrogate for the effects of treatment on the vascular endothelium. Further experimental and clinical studies addressing the effects of therapeutic interventions on the disturbed endothelium are warranted to elucidate the significance of this approach in COPD.

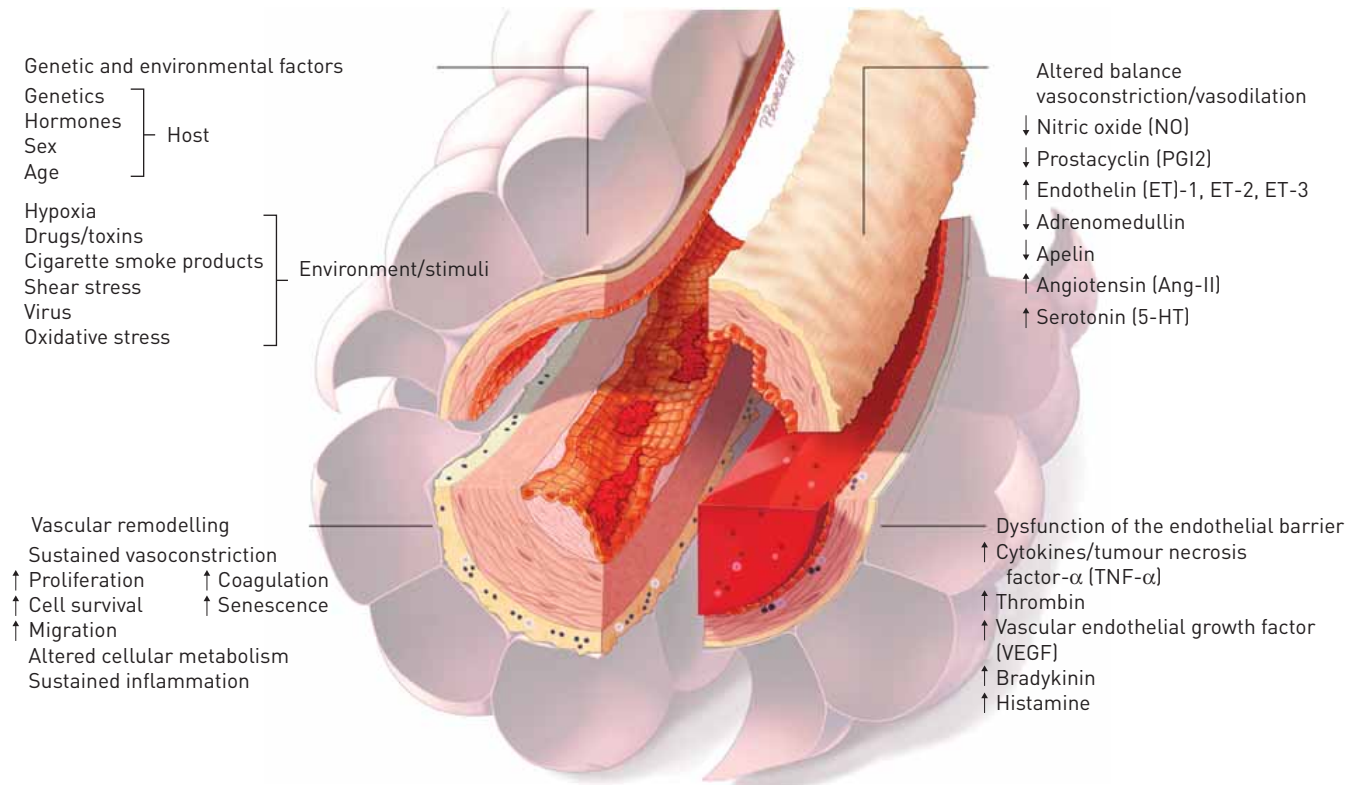
In PH due to hematological disorders, such sickle cell disease (SCD) [97–101], the mechanistic linkage between haemolytic anaemia and vasculopathy has been the subject of extensive study in pre-clinical animal models and in vascular studies in patients, as well as in large human cohort studies [99, 102, 103]. Intravascular haemolysis releases cell-free haemoglobin (Hb) in the plasma that can scavenge NO and generate ROS, impairing redox balance and leading to proliferative systemic and pulmonary vasculopathy [103, 104]. It has recently been noted that products released from red cells during haemolysis can be considered as danger-associated molecular patterns (DAMPs) or erythrocyte DAMPs (eDAMPs) [105]. These studies in SCD support a more general pathological role for intravascular haemolysis and cell-free Hb in various human diseases and in transfusion medicine [106].

Biological factors to assess pulmonary endothelial dysfunction

In light of the critical role played by the pulmonary endothelium in acute and chronic lung diseases, identification of biological factors to assess its (dys)function in the field is needed; however, this represents a real challenge for clinicians and researchers. Indeed, a broad appreciation of the numerous functions of the endothelium requires the assessment of a large panel of molecules of endothelial origin in circulating blood (table 1) and comprehensive prior assessment and validation in large and well-characterised cohorts of patients before routine clinical use. These EC products could include, among others: measures of NO biology, circulating miRNAs, cytokines, chemokines, circulating adhesion molecules, growth factors and

TABLE 1 A partial list of endothelium-derived pulmonary factors

Pulmonary factor	Type
Vasoconstrictors	Serotonin (5-HT)
	Endothelin-1 (ET-1), ET-2, ET-3
	Angiotensin (Ang-II)
	Thromboxane A2
	Oxidative stress
Vasodilators	Nitric oxide (NO)
	Prostacyclin (PGI ₂)
	Apelin
	Adrenomedullin
Cell accumulation	Fibroblast growth factor-2 (FGF-2)
	Vascular endothelial growth factor (VEGF)
	Platelet derived growth factor (PDGF)
	Tumour growth factor (TGF)
	Epidermal growth factor (EGF)
	Nerve growth factor (NGF)
Inflammation	Interleukin-1 (IL-1), IL-6, IL-8
	Tumour necrosis factor (TNF)
	Interferon
	Leptin (Ob)
Other proteins	Leukotrienes
	Plasminogen activator inhibitor-1 (PAI-1)
	Soluble E-selectin
	Soluble intercellular adhesion molecule-1 (ICAM-1)
	Soluble vascular cell adhesion molecule-1 (VCAM-1)
	Matrix metalloproteinase-2 (MMP-2), MMP-9, MMP-10
	Tissue plasminogen activator (tPA)
	Platelet-activating factor
Thrombomodulin	



Assessment of pulmonary endothelial dysfunction

- Pulmonary endothelial-derived factors (table 1)
- Circulating endothelial miRNA or endothelial microparticles (EMPs)
- Circulating adhesion molecules
- Circulating markers of endothelial damage (*i.e.* detachment of mature endothelial cells) and repair (*i.e.* number and function of endothelial progenitor cells)

FIGURE 4 A schematic overview of the main characteristics of pulmonary endothelial dysfunction.

regulators of thrombosis, as well as markers of endothelial damage (*i.e.* detachment of mature ECs or derived endothelial microparticles (EMPs)) and endothelial repair (*i.e.* the number and functional characteristics of circulating endothelial progenitor cells).

Besides changes in vasoactive mediators, COPD patients present with structural damage in the pulmonary endothelium. Areas of cell denudation and detachment between ECs have been shown in the pulmonary arteries of COPD subjects [107]. The presence of endothelial damage in COPD has also been demonstrated by increased numbers of EMPs [108, 109]. Circulating EMPs may originate from EC apoptosis or activation, which can be identified by the expression of the CD31 and CD62E markers, respectively. The number of circulating EMPs is inversely related to the forced expiratory volume in 1 s [109]. Interestingly, whereas CD31 EMPs (apoptotic) are already significantly elevated in patients with mild COPD, CD62E EMPs (activated) are increased only in patients with severe disease. The number of circulating EMPs further increases during exacerbation episodes [110] and also correlates with the severity of emphysema [109], pointing to a connection between parenchymal destruction and endothelial damage. Furthermore, in COPD, circulating EMPs are also correlated with the vascular function of systemic arteries. An increased number of circulating EMPs is associated with impaired endothelial function and increased stiffness in systemic arteries [111]. COPD patients also show reduced numbers of bone marrow derived circulating endothelial progenitor cells (EPCs) [112–115], which play a key role in the repair of a damaged endothelium and are closely related to endothelial function [116]. Studies carried out during the last years have made great effort to try and identify, define and characterise cell populations with phenotypic, biochemical, molecular and functional properties of EPCs [117, 118]. EPCs have been suggested as a precious source for generating functionally competent ECs, candidates for various clinical applications. However, the paucity of these progenitor cells and the technical difficulties involved in their *in vitro* growth represent a significant limitation to their use. Interestingly, recent studies have led to the identification in haematopoietic tissues (blood, bone marrow and cord blood) of very rare EPCs not

originating from bone marrow. These endothelial colony-forming cells (ECFCs) or “late outgrowth ECs” are capable of generating a large progeny of phenotypically and functionally competent mature ECs *in vitro* and also of sustaining *in vivo* angiogenic processes [119, 120]. Such EPCs are also present at the level of the vessel wall, particularly in the vascular endothelial intima [117, 118]. This methodology can be easily extended to the study of circulating EPCs and can facilitate and improve the functional characterisation of EPCs in various pathologic conditions, particularly in various lung diseases.

Conclusion

In conclusion, it is now well established that the pulmonary vascular endothelium is involved in most if not all acute and chronic lung disease, either as a primary determinant of these disease processes or as a victim of collateral damage (figure 4). Although considerable progress has been made in the understanding of pulmonary EC (dys)function, the triggers, mechanisms and consequences of a dysfunctional endothelium in lung disease, both acute and chronic, are still not completely understood. In addition, further efforts are also needed to elucidate the remarkable heterogeneity of the pulmonary endothelium in space and time, in structure and function, and in health and disease. An understanding of the molecular basis for these key aspects would help in finding new disease biomarkers and/or novel therapeutic targets to preserve homeostasis in response to injury and disease and to reduce the “bench-to bedside” gap in endothelial biomedicine.

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