Chapter 12

Pulmonary Vascular Endothelial Cells

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Additional information is available at the end of the chapter

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Abstract

Pulmonary vascular endothelial cells (ECs) line the surface of the lung vasculature and accommodate the various levels of blood flow. Pulmonary endothelium is a critical regulator of vascular homeostasis by inhibiting coagulation of the blood. The ECs bind tissue factor pathway inhibitors (TFPI), modulate hemostasis with opposing effects such as antiplatelet, anticoagulant and fibrinolytic properties. Lung endothelium regulates synthesis and metabolism of vasoactive compounds such as nitric oxide and endothelin-1, both potent regulators of vascular tone. Cytokines, chemokines, interleukins, adhesion molecules, and growth factors can be secreted by pulmonary ECs with positive and adverse effects. Pulmonary endothelium exhibits heterogeneity with diverse expression of molecules and specific differences in signaling induced by various infections such as Gram-positive bacteria. The distinction of macro or microvascular endothelium occurs from the larger vessels to small capillaries in the lung alveoli system. Lectin-binding patterns discriminate between pulmonary artery and pulmonary microvascular capillary endothelium. The lung is one of the body's organs with the highest expression of vascular endothelial growth factor that stimulates small vessel formation of the microvascular endothelium. Acute respiratory distress syndrome and acute chest syndrome in sickle cell disease are two prototypes of devastating diseases caused by pulmonary EC dysfunction.

Keywords: pulmonary endothelial cells, pulmonary microvascular endothelial cells, pulmonary macrovascular endothelial cells, ARDS, Acute Chest Syndrome, Endothelial cell dysfunction

1. Introduction

Endothelial cells (ECs) line the interior surface of blood cells and lymphatic vessels forming an interface between circulating blood or lymph in the lumen and the vessel wall. ECs are a thin layer of squamous cells. The vascular EC line the entire circulatory system from the large vessels

to the smallest capillaries, thereby accommodating various levels of blood flow from the turbulent high pressures from large vessels entering and leaving the heart as well as small vessels such as that of the minute capillaries of the lungs, liver, kidneys, and the moderate vessels throughout the body. ECs from different blood vessels and microvascular ECs from different tissues have distinct and characteristic gene expression profiles. Pervasive differences in gene expression patterns distinguish the EC of large vessels from microvascular ECs [1].

2. Pulmonary endothelial cell dysfunction

Vascular endothelium is a critical regulator of vascular homeostasis. All EC inhibit coagulation of the blood. ECs bind tissue factor pathway inhibitors (TFPIs) that prevent the initiation of coagulation by blocking the actions of the factor (f) VIIa tissue factor (TF) complex [2]. Like other ECs, lung ECs modulate hemostasis with sometimes opposing effects such as antiplatelet, anticoagulant, and fibrinolytic properties; yet after injury or activation ECs are capable of exerting procoagulant functions. The balance between endothelial anti and prothrombotic activities determines whether thrombus formation, propagation, or dissolution occurs [3]. An intact endothelium in a healthy vessel inhibits the adhesion of platelets, platelet activation, and aggregation and adhesion of platelets and leucocytes to vessel wall through the release of nitric oxide (NO) [4]. On the other hand, injury or activation of ECs results in a procoagulant phenotype that contributes to localized clot formation.

Activation of the coagulation cascade is one of the early occurring events in lung injury, and it is initiated via the extrinsic pathway [5]. Endothelium activated by inflammation and/or injury releases the procoagulant molecule TF which binds with circulating coagulation fVII to form a TF/fVIIa complex that cleaves fIX and thrombin. (**Figure 1**). Thrombin further activates platelets

Figure 1. Schematic representation of pulmonary microvascular endothelial cells (MVEC) activated by inflammation and/or injury following release of the pro-coagulant molecule TF which binds with circulating coagulation factor VII (fVII) to form a TF/fVIIa complex that cleaves fIX, and generates thrombin.

and coagulation factors in the intrinsic coagulation pathway generating more thrombin and formation of a fibrin mesh. The adhesion of platelets is facilitated by von Willebrand factor (vWF). vWF is a product of normal EC and is not synthesized after endothelial injury. The clotting pathway is also induced by cytokines such as tumor necrosis factor alpha (TNF-α) or interleukin 1 (IL-1) or bacterial endotoxin such as lipopolysaccharide (LPS) to secrete TF which activates the extrinsic clotting pathway [3]. In a tightly regulated system, the proteases and molecules of the coagulation cascade can be inhibited by circulating protease inhibitors, such as antithrombin, heparin cofactor II, TF pathway inhibitor and C1 inhibitor. These bind with the active sites of proteases, thereby inactivating them. In addition, coagulation factors can be degraded through activation of the protein C and protein S complex, synthesized by ECs as a cofactor that is then catalyzed by the presence of thrombomodulin and endothelial protein C receptor (EPCR). Other pathways of coagulation factor degradation are disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13). ADAMTS13 cleaves the multimeric strands of vWF, thereby disrupting platelet adhesion. ECs synthesize tissue plasminogen activator (t-PA), promoting fibrinolytic activity to clear fibrin deposits from endothelial surfaces [3, 6]. Thrombin also binds to its protease activated receptor-1 (PAR-1) and induces a signaling cascade resulting in EC junctional gaps that lead to increased endothelial permeability [7].

Lung ECs also regulate the synthesis and metabolism of vasoactive compounds such as nitric oxide (NO) and endothelin-1 (ET-1), potent regulators of pulmonary vascular tone [8]. EC-derived NO, synthesized by the endothelial nitric oxide synthase (eNOS) from the precursor L-arginine, regulates the healthy endothelium. Antithrombotic effects of EC-derived NO are likely related to release of prostaglandin I_2 and inhibition of plasminogen activator inhibitor-1 (PAI-1), a prothrombotic protein [6, 9].

The enzyme eNOS depends on intracellular calcium (Ca^{2+}) level. In response to a rise in EC intracellular Ca^{2+} eNOS catalyzes the production of NO. The Ca^{2+}-dependent eNOS synthesizes small amounts of NO until the Ca^{2+} levels decrease. This Ca^{2+}-dependent eNOS provides the basal release of NO and is sufficient to inhibit the adhesion and activation of platelets providing homeostasis in unstimulated ECs [10].

Cytokines are small soluble proteins that are important in cell signaling and can change the behavior or properties of cells. Cytokines can be secreted by many cells including pulmonary ECs [11]. Cytokines can be grouped into families including the interferons, the chemoattractants (chemokines), the tumor necrosis factors (TNFs), the interleukins (IL-2, IL-3, IL-4 etc.), the epidermal growth factor family (EGF) and transforming growth factors-alpha and beta (TGF-α and β), the growth factors include vascular endothelial growth factor (VEGF and others) that are important in vasculogenesis and angiogenesis. The VEGF family of growth factors restores the oxygen supply to tissues in hypoxic conditions [12].

Pulmonary EC express adhesion molecules and pro and anti-inflammatory cytokines and are intricately involved in inflammatory processes [12, 13]. It was shown that there is a central role via the sphingosine-1-phosphate (S1P) receptor in pulmonary endothelium for regulating an excessive pro-inflammatory cytokine and chemokine production in an influenza virus-induced cytokine storm [14]. A deficiency of alpha 1-antitrypsin (A1AT), a protein that has been shown to trigger an inflammatory response leading to increased circulating concentrations of

pro-inflammatory cytokines such as TNF-α from ECs. In A1AT, cytokines activate their receptors and stimulate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) activity and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (IkBα), a regulatory protein that inhibits NF-kB degradation. The translocation of NF-kB increases the transcription of inflammatory genes, including an increased secretion of TNF-α from ECs [15]. The role of pulmonary EC cytokines has also been shown in the pathology of lung fibrosis where numerous cytokines have been implicated in pathogenesis including TGF-β, TNF-α, ET-1 and IL-1 and IL-8 [16].

ECs participate in the control of the adhesion and migration of inflammatory cells and the exchange of fluid from the vasculature into damaged tissue. Resting ECs do not interact with leukocytes; however, they store proteins such as P-selectin and chemokines in specialized secretory vesicles called Weibel-Palade bodies (WPBs) in microvascular ECs for interaction with leukocytes when needed [17]. ICAM-1 and E-selectin mediate the firm adhesion to ECs and are an obligatory step in neutrophil migration as neutrophils initially adhere to ECs, then migrate through the EC barrier [18]. Resting ECs also suppress the transcription of other adhesion molecules such as E-selectin, vascular cell-adhesion molecule 1 (VCAM) and intercellular adhesion molecule 1 (ICAM-1) [2]. Upon EC activation, the resulting inflammation is characterized by tissue infiltration of neutrophils, followed by macrophages [19, 20].

EC activation can be induced by endotoxin, cytokines and chemokines, and viruses and bacterial pathogens, all of which can activate NF-kB resulting in modulation of EC synthesis of pro-inflammatory cytokines and chemokines. When stimulated, the endothelium displays increased adhesiveness for monocytes, lymphocytes, and granulocytes mediated by endothelial leukocyte adhesion molecules such as ICAM-1, VCAM-1 and E-selectin. The secretion of inflammatory cytokines and of leukocyte specific chemo attractants such al IL-8 and MCP-1 also contributes to leukocyte recruitment during inflammatory responses [21]. In general, human endothelium can express a broad spectrum of pro-and anti-inflammatory cytokines, including IL-1, IL-5, IL-6 and IL-8, MCP-1 (monocyte chemotactic protein-1), CSFs (colony-stimulating factors), GM-CSF (granulocyte/macrophage CSF), G-CSF (granulocyte CSF), M-CSF (macrophage CSF), PDGF, and VEGF [12].

2.1. Pulmonary endothelial cell heterogeneity

ECs from various organs have distinctive vascular responses to chemokines, cytokines, and adhesions molecules and exhibit unique functional properties [22, 23]. Phenotypic heterogeneity among ECs may account for important organ-specific behaviors [24]. The unique features of pulmonary ECs allow them to function at multiple basic levels such as function as a dynamic barrier critical for lung gas exchange and the regulation of fluid and solute passage between the blood and interstitial compartments in the lung [25].

Pulmonary endothelium exhibits a high expression of adhesion molecules which contribute to the margination of the large intravascular pool of leucocytes in the lung [26]. Adhesion molecules are expressed by activated ECs in a sequential manner. Cytokines activate E-selectin promoter, which induces E-selectin and a prolonged contact among leukocytes, causing them to roll along the endothelium [27]. Other selectins such as platelet endothelial

cell adhesion molecule (PECAM, CD31), ICAM-1, and the inducible VCAM-1 are all essential to the subsequent firm attachment of leukocytes to and migration through the endothelium. Many studies of lung EC expression of molecules, including adhesion molecules, were initially accomplished on human pulmonary artery EC (HPAEC) or even human umbilical vein EC (HUVEC) due to the availability of these cells for culture. However, more recently there is a recognition that there are differences between the expression of pulmonary macrovascular and microvascular EC. For example, the study of adhesion molecules of macrovascular large vessel cells cannot be extrapolated to microvascular capillary cells. In vitro study of HPAEC is not comparable to study of the pulmonary microvasculature in vivo or in vitro [27].

Gram-positive bacterial pathogens cause lung inflammation and alterations in lung ECs. In macrovascular ECs, pharmacological inhibition of Rho kinase with the Rho kinase (ROCK) inhibitor Y27632 significantly suppressed p38 mitogen-activated protein kinase (MAPK) cascade activation, while inhibition of p38 MAPK with specific inhibitor p38α and β, SB203580 had no effect on Rho activation [28]. In contrast, inhibition of p38 MAPK in microvascular ECs suppressed lipoteichoic acid and peptidoglycan (LTA/PepG), found on the cell wall of Gram-positive bacteria induced activation of Rho, while Rho inhibitor suppressed activation of p38 MAPK [28]. These results demonstrate cell type-specific differences in signaling induced by *Staphylococcus aureus* derived pathogens in pulmonary endothelium. Thus, although Gram-positive bacterial compounds caused barrier dysfunction in both ECs types, it was induced by different patterns of crosstalk between Rho, p38 MAPK, and NFκB signaling [28].

The distinction of macro or microvascular endothelium is between those from the larger vessels to the small capillaries that feed the entire alveolar system in the lung [29]. Lectin-binding pattern discriminates between PAEC and Pulmonary microvascular EC (MVEC), and lectin protein agglutinins isolated from plant or animal sources are often used for distinguishing between cell phenotypes [23, 30]. It was identified that *Helix pomatia* (an agglutinin from the *Helix pomatia* snail) (HPA) and *Griffonia* lectins (a lectin from Bandeiraea simplicifolia (BS I), these lectins are isolated from a variety of natural sources including plants, mollusks, fish eggs) differentially bind to macro and microvascular EC. HPA preferentially binds macrovascular endothelium whereas BS I preferentially binds to microvascular endothelium (**Figure 2**) [31]. In cell culture experiments investigating diseases such as acute respiratory distress syndrome (ARDS), pulmonary edema, or acute chest syndrome in sickle cell disease (SCD), the distinction of macro versus microvascular cells could be important.

Majority of studies in the lung have been performed on macrovascular ECs from the HPAEC, bovine PAEC or human umbilical AEC. HPAEC are used to study various diseases of the lung involved in endothelial dysfunction such as hypoxia, inflammation, and environmental stresses. It was shown in one study in macrovascular HPAEC that thrombin induces protein kinase C (PKC)-dependent ezrin, moesin, radixin (ERM) phosphorylation on critical threonine residues ERM and translocation of phosphorylated ERM to the EC periphery and that the ERM proteins play differential roles in thrombin-induced modulation of EC permeability [32]. The results in this study are important to the knowledge of the EC barrier in the lung diseases; however, the critical EC in ARDS, pulmonary edema, and ACS are the pulmonary microvascular endothelial cells (PMVEC), and it is unclear if the results would be similar as our understanding of the molecular regulation of PMVEC permeability is incomplete [33].

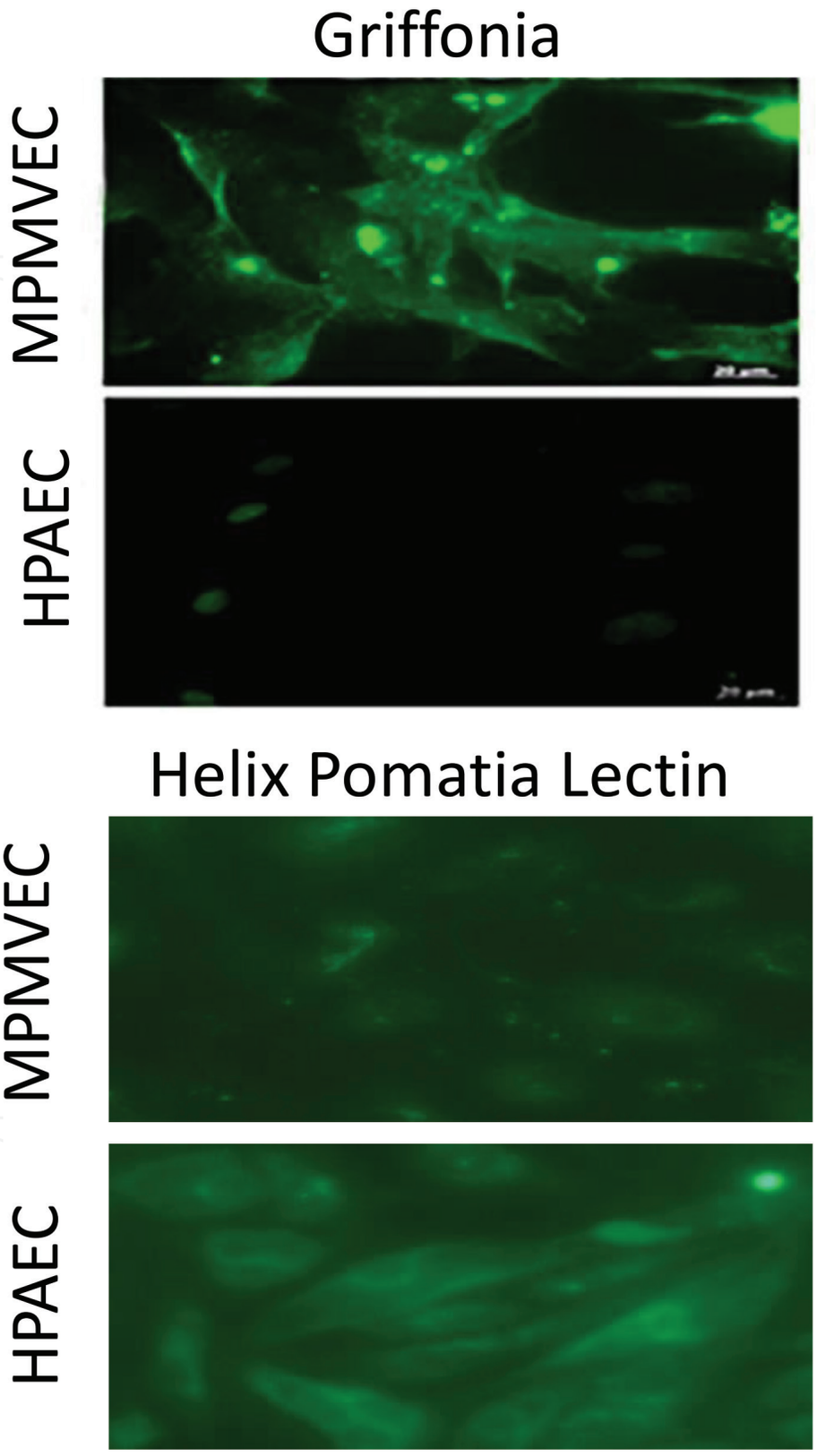

Figure 2. Lectin immunocytochemistry is used to identify pulmonary microvascular cells (top, Griffonia) compared to pulmonary macrovascular cells bottom, (*Helix pomatia* Lectin). ECs were cultured at 37°C in complete endothelial growth basal medium-2 until confluent. Endothelial cells were isolated from murine lungs or human pulmonary artery EC were used and stained with either *Top-Griffonia* to identify microvascular EC and compared to HPAEC or *Bottom-Helix pomatia* stain to identify human pulmonary macrovascular EC.

In another in vitro study comparing MVEC and PAECs, metabolic requirements for growth were studied in rat pulmonary cells. It was found that PMVEC populations had a higher metabolic function and grow faster than PAEC. PMVEC consumed threefold more glucose in cell culture over comparable time frame than PAECs. PMVECs but not PAECs generated a lactic acidosis, higher ATP concentrations, and lower oxygen consumption than PAECs [34]. The hydraulic conductance of rat PMVEC and PAEC were compared to study lung EC permeability, such as may occur in pulmonary edema to hydraulic stress. The results of these studies indicated dramatic differences in the baseline hydraulic responses to hydrostatic pressure between the two phenotypes with PAEC values averaged 22 times higher than PMVEC in new monolayers. It was speculated that the dramatic differences in PMVEC and RPAEC may be due to different embryologic origins, being derived respectively by vasculogenesis and angiogenesis [35]. The same group investigated the role of cytosolic calcium (Ca2+) in rat PMVECs and PAECs; they found that Thapsigargin (a non-competitive inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATPase) produced higher Ca2+ levels in PAECs than in PMVECs and increased permeability in PAEC but not in PMVEC monolayers. The significance is that whereas increased Ca2+ promotes permeability in PAECs it is not sufficient in PMVECs which show an apparent uncoupling of Ca2+ signaling pathways or dominant Ca2+ − independent mechanisms for controlling cellular gap formation and permeability [25].

Macrovascular ECs more abundantly express eNOS and generate more NO than do microvascular ECs [23, 30]. NOS and inducible NOS (iNOS) production in rat pulmonary macrovascular EC was shown to be greater than rat pulmonary microvascular ECs when cell cultures were stimulated with various combinations of TNF-α, interferon gamma (IFN-), and LPS, suggesting that differences between ECs populations may be substantial [24].

HPAEC are also useful to investigate the effects of various compounds and drugs. Other EC types such as human umbilical artery (HUVEC) or bovine pulmonary artery cells (BPAECs) have also been used previously due to their dependable, more robust nature and commercial availability.

2.2. Pulmonary microvascular endothelial cells

Pulmonary microvascular ECs (MVECs) are an active and dynamic layer of cells in the most delicate portion of the lung at the alveolar level (**Figure 3**) where they function to exert both specific and general endothelial function. In the microvascular circulation, the arteries are less than 70 μm in diameter, are nonmuscular arterioles, and extend into the alveolar capillaries. The walls of capillaries are composed of a single layer of MVECs. The general function of lung EC include regulation of systemic blood flow, tissue perfusion thorough changes in vessel diameter and vascular tone, performed in conjunction with underlying smooth muscle cells and pericytes [6] (**Figure 4**). The pulmonary microcirculation is less permeable to protein and water flux as compared to large pulmonary vessels [26]. Experiments have shown that the MVECs form a tighter barrier compared to the macrovascular barrier while showing less permeability to sucrose and albumin compared to macrovascular EC [36]. Lung injury or inflammation are associated with activation of mediators or secretion of cytokines that induce a prolonged increase in paracellular permeability and vessel wall leakiness. Endothelial barrier properties are known to be strictly dependent on the integrity of endothelial adherens and tight junctions [37]. The

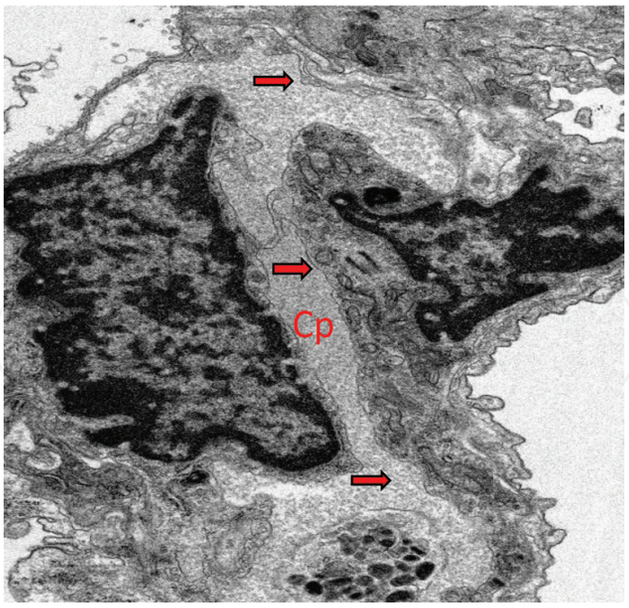

Figure 3. Transmission electron micrograph of a mouse alveolar capillary (Cp) with microvascular endothelial (EC) lining (arrows). Source is mouse alveoli from authors (JG) collection of images processed in the vascular biology laboratory, Augusta University health, electron microscope Core Laboratory (Libby Perry and Brendan Marshall PhD).

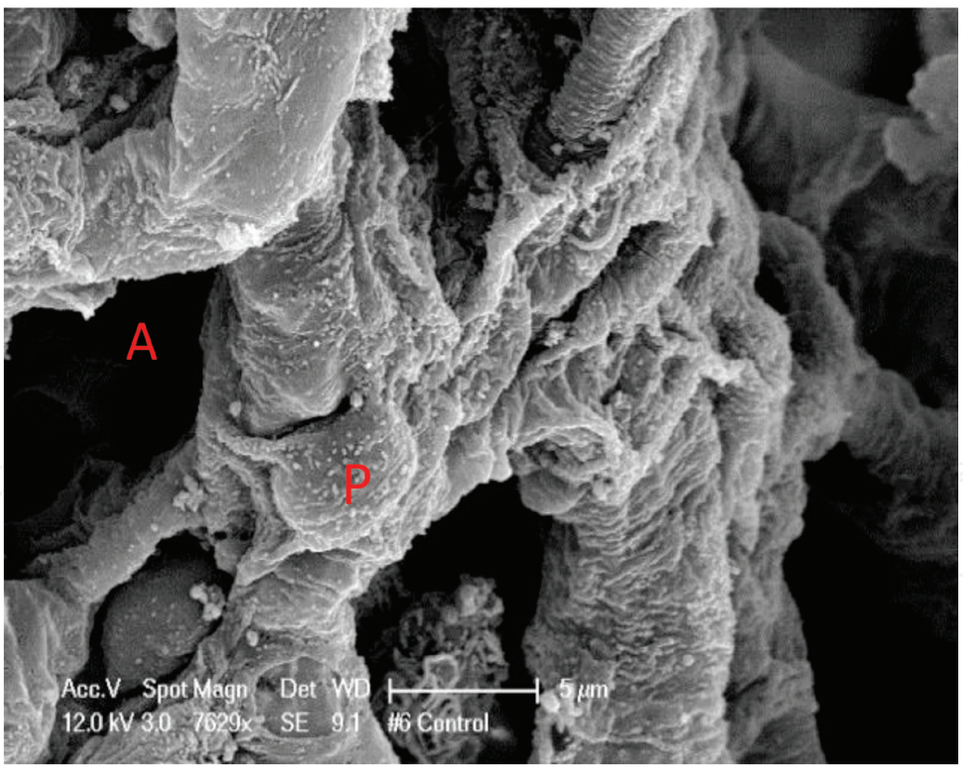

Figure 4. Scanning micrograph of mouse alveoli (a) and vessel with pericyte (P) surrounding the alveolar lining. Source is mouse alveoli from authors (JG) collection of images processed in the vascular biology laboratory, Augusta University health, electron microscope Core Laboratory (Libby Perry and Brendan Marshall PhD).

inter-endothelial junctions consist of adherens, tight and gap junctional complexes, and promote adhesion of opposing cells in the monolayer of microvascular ECs [38]. Microvascular EC gene clusters include genes related to lipid transport and metabolism [3].

The lung is one of the body's organs with the highest expression of VEGF [39]. VEGF stimulates small vessel formation of MVEC and is an essential component of the angiogenic process and MVECs survival. VEGF signaling orchestrates capillary development along the basement membrane of airway epithelium [23]. Excessive VEGF expression has a broad impact on ECs including vascular permeability increase [39]. VEGF can be induced by hypoxia-inducible factor (HIF) in cells under hypoxic conditions. Gene studies evaluating VEGF have shown a higher level of expression of actin binding proteins—Lin11, Isl-1 and Mec-3 (LIM) proteins 1, actinin-associated LIM protein, Arginase (Arg) binding protein 2, Slingshot, vav3, myosin IB, myosin 5C, myosin7A, and myosin light chain kinase in the microvascular ECs [3, 40]. These are proteins that play important roles in basic biological processes including cytoskeleton organization. This increase in cytoskeletal protein expression may be related to the ability of MVECs to undergo extensive cytoskeletal remodeling and migration during angiogenesis [3]. VEGF increases permeability by at least two different pathways: one, involving proto-oncogene (Raf-1), mitogen-activated protein kinase/ERK kinase (MEK), and extracellular signal-regulated kinases 1,2 (ERK); and the other involving endothelial nitric oxide synthase (eNOS). Protein kinase C (PKC) is a mediator of VEGF-induced ERK-1/2 phosphorylation and hyperpermeability which increases permeability via increased NO production [33]. Endothelin-1 (ET-1) is known to play a pathogenic role in pulmonary arterial hypertension (PAH). VEGF may have beneficial effects by decreasing ET-1 production in HLMVEC thereby modulating endothelin production in PAH [41].

Microvascular ECs are known to produce macrophage inflammatory protein-1beta (MIP-1β) and MIP-2 (the mouse equivalent to human interleukin-8) in the lung which act as major chemotactic factors responsible for the recruitment of neutrophils into the alveolar spaces during inflammation or infection [22, 41].

2.3. Pulmonary endothelial cells in ARDS and pneumonia

ARDS is a severe lung inflammatory disorder with a declining but still unacceptably high.

Mortality (25–46%) [42, 43]. The healthy alveolar-capillary barrier is formed by the microvascular endothelium, the alveolar epithelium, and the basement membrane. The homogeneous pulmonary microvasculature layer of ECs lining the pulmonary circulation forms a tight barrier [44]. The EC barrier dysfunction that occurs in acute lung injury is tightly linked to agonist-induced cytoskeletal remodeling resulting in the disruption of cell–cell contacts, paracellular gap formation, and EC barrier compromise [45, 46]. Tight junctions are formed by the fusion of the outer layers of the plasma membranes and are comprised of occludins, claudins, and junctional adhesion molecules that in turn bind to other protein partners in the actin cytoskeleton [8, 36]. Integrity of adherens junctions (AJs) is critical in regulating paracellular permeability and disruption of VE-cadherin homophilic adhesions leads to excessive accumulation of fluid in the interstitial space and is associated with inflammation, atherogenesis, and acute lung injury [38]. AJs are composed of VE-cadherin and its cytoplasmic binding partners: α-, β- γ-, p120 catenins, which link AJs to the actin cytoskeleton. The assembly of the VE-cadherin-catenin complex is regulated by phosphorylation, and their dissociation leads to cytoskeletal changes and loss of cohesive structure required for an intact EC barrier [36]. Therefore, the complex network of cytoskeletons is critical in the EC barrier regulation.

On the cellular level, in ARDS, there is increased pulmonary capillary EC permeability and fluid leakage into the pulmonary parenchyma that is followed by neutrophils, cytokines, and an acute inflammatory response [47]. Adhesion molecule upregulation on the vascular endothelium of the lung results from the systemic inflammatory cascade that occurs in EC activation. In fact, the expression of molecules that mediate adhesion and signaling of leukocytes is nearly synonymous with endothelial activation [48]. When ECs are activated by toxins such as LPS, other bacterial toxins, viral infections, thrombin or hypoxia, the ECs release cytokines such as TNF-α, IL-1ß or IL-8, and a shift toward a pro-inflammatory phenotype occurs [36]. There is a consecutive expression of adhesion molecules, including PECAM (CD-31), ICAM–1, VCAM, and E selectin, that plays a central role in the leukocyte endothelial adhesion. These adhesion molecules are responsible for recruiting and directing leukocytes to the sites of inflammation.

Muller et al. analyzed the autopsy lung specimen ECs for PECAM (CD-31), ICAM–1 in patients with Gram-negative sepsis-induced ARDS and found these adhesion molecules strongly expressed compared to normal lung autopsy specimen [49]. Another study demonstrated that blockade of the VCAM-1 receptor on the pulmonary vascular endothelium diminishes lung injury in established pancreatitis-induced ARDS [50]. In our own unpublished data, mice with lung injury induced by LPS and Gram-positive toxin, pneumolysin, an attenuation of ICAM-1 by a low-anticoagulant heparin, has been shown to attenuate neutrophils and acute lung injury (data not published). Neutrophil recruitment into the lung is the hallmark of acute lung injury (ALI) [51]. Neutrophils enter the interstitial spaces by rolling on the endothelium, and this is mediated by the selectins. The neutrophils adhere to the endothelium and affect the endothelial cytoskeleton inducing remodeling of the tight junctions and further facilitating the transmigration of neutrophils [51].

Once activated, ECs display recruited neutrophils in ARDS; there is considerable evidence that pro-and anti-inflammatory cytokines and chemokines play a major role in the pathogenesis of acute lung injury from sepsis and pneumonia [51]. There is a complex network of inflammatory cytokines and chemokines that play a major role in mediating, amplifying, and perpetuating the lung injury process. The pro-inflammatory cytokines IL–1 beta and TNF-α have been located in bronchoalveolar lavage fluid (BALF) from ARDS patients [52]. In influenza, early induction of the cytokines IFN-α, TNF-α, IL-1α, and IL-6 and the chemokines CCL2, CCL3, CXCL2 (IL-8), and CXCL10 are associated with clinical symptoms and morbidity in humans [14, 53, 54]. Simultaneous production of anti-inflammatory cytokines can counteract pro-inflammatory cytokine effects and modify the intensity of the inflammatory process in ARDS [52].

There is a search for biomarkers in ARDS to assess the activation and dysfunction of ECs. One marker may be endothelial progenitor cells (EPCs) as a marker of EC dysfunction and damage. It has been reported that there is an association between the EPC count and survival in ARDS [55]. The cells are present at very low levels in normal patients, but the number of EPCs increased significantly in conditions associated with vascular damage such as ARDS. In the study of Moussa et al. [56], EPC counts were increased in patients with moderate and severe ARDS compared with non-ARDS patients [56]. Higher EPC counts were also found in non-survivors of ARDS in this same study [56]. Other promising biomarkers are angiopoietin–2

(Ang-2), an endothelial growth factor. Ang-2, a mediator of pulmonary vascular permeability, binds to the tyrosine kinase receptor and plays a key role in endothelial junctional integrity [57]. Ang–2 levels have been shown to be higher in ARDS patients than in patients with hydrostatic pulmonary edema [58]. Increased levels have also been linked with the severity and mortality of ARDS [58]. Stimulation of PMVEC with IL–8 leads to cytoskeletal reorganization and cell retraction which in turn leads to gap formation between cells and IL-8 levels that are higher in non-survivors of ARDS [59]. Studies for biomarkers are ongoing with the potential that the EC biomarkers will aid in the diagnosis of acute lung injury.

2.4. Endothelial cells in acute chest syndrome in sickle cell disease

Sickle cell disease (SCD) is an inherited red blood cell disorder that affects millions of people throughout the world and in most common among those whose ancestors came from sub-Saharan Africa, Spanish-speaking regions in the Western hemisphere, Saudi Arabia, India, and Mediterranean countries [60]. SCD is caused by a mutant β-globin gene that substitutes valine for glutamic acid at position 6 in the β-globin chain of hemoglobin A. The resultant hemoglobin is called hemoglobin S and is characterized by red blood cells (RBCs) that are crescent or sickle shaped rather than the normal rounded disc shape [61]. One of the most common forms of acute pulmonary disease associated with morbidity and mortality in SCD is acute chest syndrome (ACS). Hypoxia induces abnormal hemoglobin S polymerization and RBC sickling, and the abnormal cells are rigid and unable to pass through narrow capillaries leading to vessel occlusion and ischemia [62]. ACS is the most common form of acute pulmonary disease associated with SCD. ACS is diagnosed by a new infiltrate on chest x-ray that is consistent with alveolar consolidation triggered by infection, fat embolization, or pulmonary sequestration of sickled erythrocytes [61, 62]. The patient experiences chest pain, fever, tachypnea, wheezing, or cough. Under hypoxic and infectious conditions, cell–cell junctions can be destabilized causing the passage of systemic inflammatory mediators into the lungs, producing pulmonary edema; in this sense, ACS is similar to ARDS [61, 62]. Other pathologies include alterations in activated EC metabolic functions that may contribute to the vaso-occlusive events in ACS [63]. The balance between vasoconstriction and vasodilation in ACS may be altered. The ET-1 gene is upregulated in the lung and is released by activated lung ECs in response to hypoxia and reduced NO bioavailability [64, 65]. In a transgenic mouse model consistent with chronic organ lesions, tissue lesions, and acute vaso-occlusive events analogous to human SCD, SAD mice [S(β6val) Antilles (β23lle) D-Punjab(β121Gln)] [66], it was found that ET-1 is produced at a higher level in the pulmonary MVEC of SAD mice than wild type (WT) mice. Further, in the SAD mice, bosentan, an ET receptor antagonist, was shown to prevent death of SAD mice exposed to a severe hypoxic challenge [64].

Painful vaso-occlusive crisis (VOC), one of the major and specific manifestations of SCD, is the most debilitating manifestation of SCD [67]. In VOC, the circulation of blood vessels is obstructed by sickled red blood cells causing ischemic injury and severe pain. Sickling and/or hypoxia associated with VOC in SCD may shift the balance of endothelial vasodilator and vasoconstrictor response in favor of vasoconstriction [68]. The study of Hammerman et al. [63] measured NO products from cultured pulmonary ECs exposed to red blood cells

and/or plasma from SCD patients during VOC [63]. Exposure to the plasma from SCD patients during VOC increased total NO production by both macro and microvascular lung ECs [63]. However, these increases were not accompanied by changes in eNOS or iNOS expression. Based on their findings, the authors suggested that altered NO production might contribute to the pathogenesis of ACS [63].

The vascular inflammation and increased thrombotic activity known to occur in patients with ACS in SCD may be associated with platelet activation of ECs through CD40, a platelet associated pro-inflammatory molecule that promotes ECs activation and is known to be elevated in the circulation of SCD patients [69]. Cluster of differentiation (CD)40, a protein found on antigen-presenting cells and its ligand (L), a protein receptor, are members of the TNF superfamily of molecules. The binding of CD40 to the endothelial cell induces a variety of downstream effects and initiates a variety of immune and inflammatory responses including the production of reactive oxygen species (ROS), chemokines, and cytokines and the expression of adhesion molecules such as E-selectin, ICAM-1 and VCAM-1. The inflammatory response then fosters recruitment of leukocytes around the EC [69]. Furthermore, the ROS generated by CD40L antagonizes NO synthesis and additionally promotes EC dysfunction [69]. A cohort of SCD patients was evaluated for the association of CD40L and inflammation with SCD clinical complications including ACS [69]. It was found that plasma CD40L was associated with ACS and that SCD patients with a lifetime history of ACS presented with significantly higher plasma CD40L than in SCD patients that had never experienced an episode of ACS [70]. Thrombospondin (TSP-1), also a platelet derived protein that activates ECs was found in the same study to correlate with increased ACS ECs activation of cytokines and chemokines [70].

One of the factors that have been identified in ACS is increased adherence between sickled red blood cells (RBC) and ECs [71]. Some investigators interpret abnormal endothelial adhesion as evidence of a pro-inflammatory state [72]. The pro-inflammatory state in SCD is associated with endothelial damage, increased production of ROS, hemolysis, and increased production of pro-inflammatory cytokines [73]. Transgenic SCD mice have been used to study the inflammatory responses that occur in SCD in many organs including the lung. The transgenic mice models have an active inflammatory response similar to human SCD patients [74]. Adhesion molecules VCAM, ICAM and PECAM have been shown to be upregulated in LPS-treated normal and transgenic-treated lungs [74]. IL-6 and NF-kB expressions were also increased in the lungs of transgenic SCD mice suggesting a vigorous inflammatory response with activated macro and microvascular ECs in the lungs [74]. LPS challenge is associated with increased mortality and increased levels of serum and BALF cytokines TNF-α, IL-1β and VCAM-1 in sickle mice compared with control subjects [72].

The role of the lung ECs and their interactions with sickle RBCs depend on multiple factors including the presence of inflammatory cells, cytokines, reactive oxygen species, hypoxic stress and infection that augment sickle cells' and white blood cells' (WBC) adherence to the endothelium. ACS is associated with infections, pneumonia, and fever, and in this setting, there is activation of pro-inflammatory factors such as cytokines that further activate the ECs and promote changes in vascular tone and permeability, anticoagulant-procoagulant balance, and leukocyte trafficking in the lungs of the SCD patient [74].

3. Conclusion

Endothelial cells are an active component of the lung and line the large and small vessels of the lung. They all engage in forming a barrier separation but also are an active constituent in the healthy and diseased lungs. The pulmonary ECs manifest disruption and breakdown under abnormal conditions such as hypoxia and infection and pathologic conditions such as infection, ARDS, and ACS. The ongoing research in pulmonary ECs has highlighted the significance of pulmonary microvascular and macrovascular EC in health and disease with continuing focus toward improving morbidity and mortality of disease involving the pulmonary microvasculature.

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Conflict of interest

We declare no author has any disclosure or conflict of interest for any product or result in this manuscript.

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