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REVIEW

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Pulmonary endothelium in acute lung injury: from basic science to the critically ill

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Abstract *Background:* Pulmonary endothelium is an active organ possessing numerous physiological, immunological, and metabolic functions. These functions may be altered early in acute lung injury (ALI) and further contribute to the development of acute respiratory distress syndrome (ARDS). Pulmonary endothelium is strategically located to filter the entire blood before it enters the systemic circulation; consequently its integrity is essential for the maintenance of adequate homeostasis in both the pulmonary and systemic circulations. Noxious agents that affect pulmonary endothelium induce alterations in hemodynamics and hemofluidity, promote interactions with circulating blood cells, and lead to increased vascular permeability and pulmonary edema formation. *Objective:* We highlight pathogenic mechanisms of pulmonary endothelial injury and their clinical implications in ALI/ARDS patients.

Keywords Endothelium · Lungs · Acute lung injury · Acute respiratory distress syndrome

Introduction

The intimal lining of all blood vessels is composed of a single continuous layer of simple squamous epithelial cells of mesenchymal origin which are called endothelial cells (ECs). In the human lung ECs occupy a surface area of approximately 130 m^2 [1]. Vascular endothelium was considered for many years to be nothing more than a nucleated layer, functioning as a semipermeable barrier that separates blood from the surrounding tissues and, in the lungs, blood from air. However, extensive research over the past 25 years has confirmed that vascular endothelium is a highly specialized metabolically active organ possessing numerous physiological, immunological, and synthetic functions (Table1). The strategic location of the lungs and the tremendous surface area of the pulmonary capillary endothelium allow the latter to filter the entire circulating blood volume before it enters the systemic circulation. Thus pulmonary endothelial functional and structural integrity are essential for adequate pulmonary and systemic cardiovascular homeostasis.

Pulmonary endothelium is a major component of the alveolar-capillary unit; it is therefore vulnerable to injury from noxious agents (mechanical, chemical, or cellular) that are either inhaled or delivered to the lung through the pulmonary circulation and may cause acute lung injury (ALI) in animals and humans (Table 2).

ALI represents a pathological continuum characterized by acute respiratory distress and severe oxygenation impairment, occurring as a consequence of the host response after exposure to noxious external or endogenous agents. The most severe extreme of ALI is the acute respiratory distress syndrome (ARDS), an overt noncardiogenic pulmonary edema that carries high morbidity and mortality

Table 1 Major pulmonary endothelial functions

Synthesis and release of several vasoactive compounds such as angiotensin II, prostacyclin, thromboxane A_2, nitric oxide (NO), and endothelins; regulation of vascular tone
Expression of enzymes such as angiotensin converting enzyme, endothelin converting enzyme, nucleotidases, NO synthase and lipoprotein lipase
Expression of receptors and signal transduction molecules
Cell surface redox activity (transplasma membrane electron transport systems)
Removal and biotransformation of drugs
Regulation of coagulation and thrombolysis; promotion of hemofluidity
Participation in immune reactions
Binding of immune complexes
Interaction with bacteria (phagocytosis) and blood components such as leukocytes and platelets
Expression of adhesion molecules
Production of growth factors
Production of cytokines and chemokines
Production of reactive oxygen species
Barrier function

Table 2 Major agents that cause pulmonary endothelial injury

Endotoxin
Cytokines, chemokines
Activated leukocytes
Proteolytic enzymes
Partially reduced O_2 species
Immune complexes
Microbes (e.g., rickettsial infection)
Hyperoxia
Radiation
Drugs
Ischemia/reperfusion
Hyperlipidemia
Fibrin split products
Actin and actin complexes
Toxins
Mechanical stretch

[2]. ALI/ARDS is caused by an autodestructive inflammatory process characterized by the activation of intrapulmonary and circulating cells and by a tremendous influx of neutrophils (although ARDS occurs in neutropenia) and cytokine production, resulting in a breakdown of the lung barrier and gas exchange functions. ALI pathogenesis is still only partly understood; however, pulmonary endothelium plays a major role by: (a) altering its metabolic activity, thus affecting pulmonary and systemic homeostasis; (b) mediating cell-cell adhesions, especially with neutrophils; and (c) changing its barrier permeability, thus promoting pulmonary edema formation [3].

Pulmonary endothelial functions

The various pulmonary endothelial metabolic properties were identified using isolated perfused lung preparations, in vivo animal studies, and EC culture techniques. It is well recognized now that the pulmonary endothelium possesses numerous enzymes, receptors, and transduction molecules, and that it interacts with other vessel wall constituents and circulating blood cells. Major physiological properties of the pulmonary endothelium include: (a) the promotion of antiaggregation and hemofluidity, (b) an enforced barrier function, and (c) the synthesis, metabolism, or uptake of vasoactive compounds that modulate the systemic (endocrinelike action) and/or pulmonary vascular tone (paracrinelike action) [4, 5]. The latter appears to contribute in the induction of hypoxic pulmonary vasoconstriction (HPV), a unique physiological feature of the pulmonary circulation that maintains proper ventilation/perfusion match and optimizes systemic oxygenation. Although the exact role of EC in HPV is still under investigation, EC-derived vasoactive compounds such as nitric oxide, endothelin (ET) 1, and a yet unidentified agent that may cause Ca^{2+} sensitization in the smooth muscle have been implicated [6]. Consequently, pulmonary endothelial injury is expected to compromise adequate HPV and contribute to the ventilation/perfusion abnormalities seen in ALI/ARDS.

The most important endothelial functions are presented in Table 1. Most of these functions are constitutive while others are induced upon endothelial activation after exposure to proinflammatory stimuli such as endotoxin and/or cytokines. In this respect the activated pulmonary endothelium (a) expresses leukocyte adhesion molecules, (b) produces cytokines, (c) induces changes in vascular integrity and tone, (d) becomes procoagulant, and (e) upregulates HLA molecules [7]. ALI is associated with an intense pulmonary inflammatory response with accumulation of both pro- and anti-inflammatory mediators [8]. If the proinflammatory process dominates, endothelial activation is followed by functional and, at a second stage, structural endothelial injury, leading to alterations in all the above critical metabolic functions that contribute to ARDS pathogenesis. ARDS-related structural endothelial injury has been identified in humans: Postmortem studies of patients who died of sepsis-related ARDS revealed patchy EC swelling and injury [9], while a recent study found circulating ECs to be increased (i.e., increased EC shedding) in sepsis and septic shock, suggesting a widespread endothelial damage that should also include the pulmonary endothelium [10].

Cytokines and pulmonary endothelium

Cytokines are soluble polypeptides serving as chemical messengers between cells; they are involved in processes such as cell growth and differentiation, tissue repair and remodeling, and regulation of the immune response [11]. ECs are both targets and cytokine producers. Among the more than 250 known cytokines, tumor necrosis factor

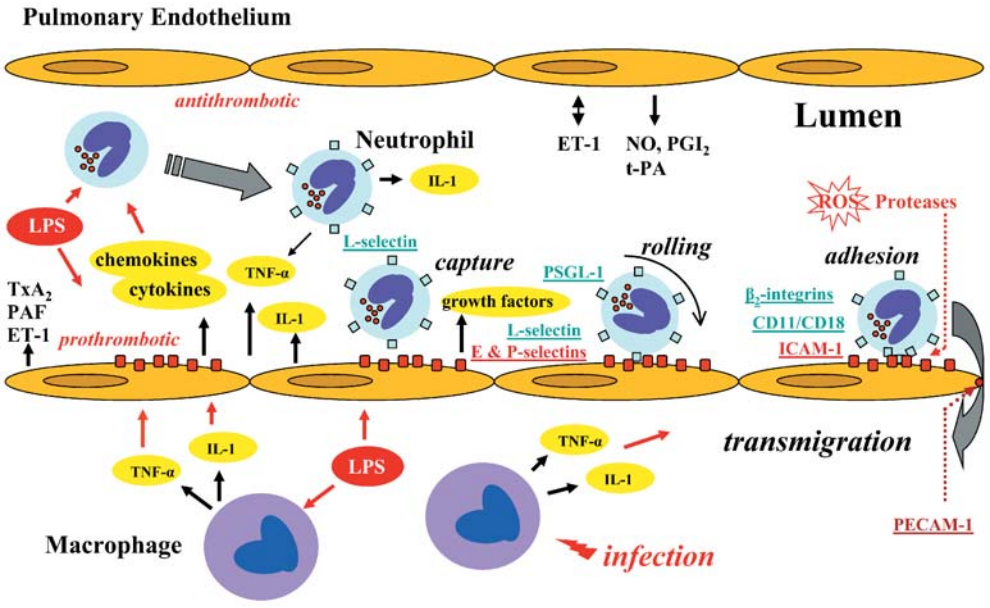

Fig. 1 Schematic illustration demonstrating major endothelial functional properties in the normal lung (upper endothelial cell layer), and mechanisms of pulmonary endothelial injury induced by infection, encompassing many of the major inflammatory interactions among cytokines, macrophages, neutrophils, and pulmonary endothelium. *LPS* Lipopolysaccharide; *TNF-α* tumor necrosis factor-α; *IL-1* interleukin-1; *NO* nitric oxide; *ET-1* endothelin-1; PGI_2 prostacyclin; TxA_2 thromboxane A_2; *PAF* platelet-activating factor; *t-PA* tissue plasminogen activator; *ROS* reactive oxygen species; *PSGL-1* P-selectin glycoprotein-1; *ICAM-1* intercellular adhesion molecule-1; *PECAM-1* platelet-endothelial cell adhesion molecule-1. *Underlined* Adhesion molecules; *red arrows* action; *black arrows* synthesis (and uptake for ET-1); *dotted arrows* location

(TNF) α and interleukin (IL) 1 are produced mostly by mononuclear phagocytes, and natural killer cells. In the lung they are mainly produced by activated interstitial and alveolar cells (primarily macrophages), as well as ECs, and have a major role in the early ALI stage. TNF-α and IL-1 share a number of biological properties and markedly amplify each other's biological actions. They act on EC mainly by inducing a functional program that promotes thrombosis and inflammation [12]. Among other things they induce (a) a prothrombotic EC phenotype, (b) the production of several cytokines including chemokines, colony-stimulating factors, IL-6 which has both pro- and anti-inflammatory properties, and IL-1 itself, (c) the production of several autacoids such as prostanoids including prostacyclin (PGI_2) and thromboxane A_2, platelet-activating factor (PAF) and nitric oxide (NO), and (d) the upregulation of adhesion molecules (Fig. 1). All these functions, with the latter being the most important, contribute to ALI/ARDS development [11, 12].

Several animal studies have revealed the pro- or anti-inflammatory contribution of cytokine–EC interaction in the pathogenesis of ALI occurring from different insults. In this respect acid aspiration induced lung injury in rabbits is mediated mainly by neutrophils recruited in the lung by IL-8 and the subsequent endothelial injury [13], while IL-8 also mediates injury from smoke inhalation to both pulmonary endothelium and epithelium in the same animal model [14]. In contrast to this, cardiotrophin-1, a member of the gp130 cytokine family that carries anti-inflammatory properties, appears to attenuate the endotoxin-induced impairment of endothelium-dependent pulmonary vasorelaxation in an ALI ex vivo rat model [15]. Partial liquid ventilation with perflubron decreases serum TNF-α concentrations in a rat acid aspiration model, thus reducing the systemic sequelae of ALI [16]. The above anti-inflammatory phenomenon might be related in part to attenuated leukocyte activation, which would consequently attenuate leukocyte–EC interaction. Interestingly, it has recently been shown that TNF-α installation into the alveolar space sends inflammatory signals to the adjoining capillary endothelium, which in minutes upregulates the expression of the adhesion molecule P-selectin enhancing leukocyte–EC interaction [17].

The cytokine-EC interaction in ALI pathogenesis has also been shown in humans or human tissues. Pulmonary microvascular EC (PMEC) from ARDS patients present an upregulation of TNF-R2 receptors and a higher constitutive production of IL-6 and IL-8 than control PMEC, suggesting either a stronger EC activation occurring during the ALI/ARDS process or that PMEC are constitutively more reactive in subjects who subsequently develop ARDS [18]. Additionally, TNF-α induces IL-8 production by pulmonary EC via the p38 mitogen-activated protein kinase pathway; the underlying mechanism is regulated by the EC redox status, suggesting that anti-oxidant therapy might be of value in the ALI treatment [19].

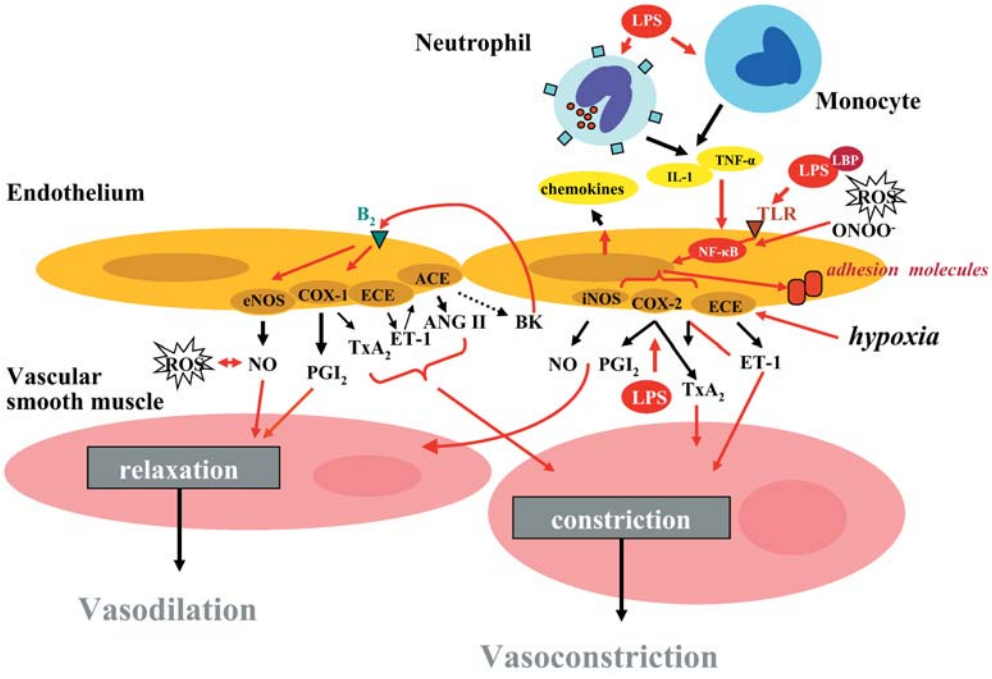

Fig. 2 Schematic illustration of major endothelial–smooth muscle interactions under normal conditions and after endothelial exposure to inflammatory stimuli. Inflammatory stimuli induce vasoactive mediator synthesis via the activation of nuclear factor-κB (*NF-κB*) or other transcription factors. Lipopolysaccharide (*LPS*) activates signaling pathways, leading to NF-κB through binding to Toll-like receptors (*TLR*) on the endothelial surface. TLR responsiveness depends on LPS-binding protein (*LBP*) and other factors. *TNF-α* Tumor necrosis factor-α; *IL-1* interleukin-1; *NO* nitric oxide; *ONOO⁻* peroxynitrite; *ET-1* endothelin-1; *PGI_2* prostacyclin; *TxA_2* thromboxane A_2; *ANG II* angiotensin II; *BK* bradykinin; *ROS* reactive oxygen species; *eNOS* endothelial NO synthase; *iNOS* inducible NO synthase; *COX-1* constitutive cyclooxygenase; *COX-2* inducible cyclooxygenase; *ACE* angiotensin-converting enzyme; *ECE* endothelin-converting enzyme; *B_2* B_2 kinin receptor. *Red arrows* Action; *black arrows* synthesis (and uptake for ET-1); *dotted arrow* breakdown

A particular pro-inflammatory process of high clinical importance is the ventilator-induced lung injury (VILI). This highly morbid clinical entity is believed to be caused by excessive mechanical stress that alters epithelial and endothelial barrier properties and stimulates pro-inflammatory responses of several cell types including macrophages and neutrophils [20]. Conventional mechanical ventilation in ARDS patients can induce ventilator-associated lung injury (VALI) that leads to pro-inflammatory cytokine production, attenuated by a protective ventilatory strategy [21]. In this respect "protective" low tidal volumes appear to attenuate epithelial and endothelial injury [estimated by plasma von Willebrand factor (vWF) and permeability to albumin] in a rat model of acid-induced ALI, demonstrating the role of endothelial injury in this pathology [22].

Transcriptional mechanisms in ALI

Transcription factors (i.e., DNA-binding proteins that regulate gene expression) are major components of the molecular mechanism underlying the cytokine-induced EC activation. Among these, nuclear factor-κB (NF-κB) is a crucial factor for the maximal expression of many cytokines involved in ALI pathogenesis. NF-κB enhances the transcription of several genes including cytokines, growth factors, vasoactive mediators, adhesion molecules, immunoreceptors, and acute-phase proteins (Fig. 2) [23]. NF-κB regulates the cytokine-mediated inducibility of adhesion molecules and cytokines in EC [24]. NF-κB activation is the final target of a signal transduction pathway that leads from the cell surface to the nucleous. Numerous inducers have been implicated in NF-κB stimulation including proinflammatory cytokines (mainly TNF-α and IL-1), bacterial and viral products [such as lipopolysaccharide (LPS)], and reactive oxygen species (ROS) [23].

ROS at low (subcytotoxic) concentrations function as important signaling molecules, while at higher concentrations they induce cell injury and death (see below) [25]. NF-κB activation is a major redox-sensitive transcription factor: Thiol antioxidants such as *N*-acetylcysteine abolish LPS-induced activation of NF-κB and improve lung function in ARDS patients [23]. Similarly, high intracellular glutathione concentrations inhibit NF-κB activation, an inhibition also induced by high levels of glutathione disulfide, the oxidized form of glutathione [25]. Redox

regulation of NF-κB appears to be complex and mediated by both oxidant and antioxidant mechanisms; it is cell-type specific and in several cases is more facilitatory than causal [23, 25]. Cathecholamines that are often administered in ALI/ARDS subjects also affect NF-κB activation via several mechanisms, including ROS generation [26].

Activation of NF-κB is a critical step in the initiation of neutrophilic inflammation in animals and has been linked to ALI/ARDS pathogenesis. NF-κB activation is inhibited in vivo by treatment with antioxidants, corticosteroids, and the induction of endotoxin tolerance [24]. In a similar respect NF-κB dependent expression of EC adhesion molecules were downregulated by antioxidant treatment [23]. Dexamethasone administration in isolated rat lungs inhibited the TNF-α and IL-1 induced upregulation of pulmonary vascular ET, possibly via NF-κB dependent mechanisms [27]. These findings suggest that a specific NF-κB inhibition would contribute to ALI/ARDS treatment.

Reactive oxygen and nitrogen species

Patients with ARDS are subjected to an oxidant burden that results in molecular/cellular damage and arises from an increased generation of ROS and reactive nitrogen species (RNS) and/or a deficiency of antioxidant defenses. ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH). Various ROS sources such as the mitochondrial respiratory chain, the protease-mediated enzyme xanthine oxidase, the metabolic cascade of arachidonic acid, and the oxidative burst of activated neutrophils are present in ARDS [28]. RNS consist of species such as NO, nitrogen dioxide (NO_2), and peroxynitrite ($ONOO^-$). NO is highly reactive with free radicals; the reaction between NO and O_2^- produces the very powerful and cell toxic $ONOO^-$ [28].

Following the exposure to various inflammatory stimuli, pulmonary endothelial, epithelial, and alveolar macrophages are among the lung cell types that contribute to the production of ROS and RNS, with deleterious effects on pulmonary endothelium [29]. Among other features, oxidant stress alters endothelial barrier function and increases endothelial permeability through activations of protein kinace C, myosin light chain kinase and other signaling pathways [25, 29]. ARDS nonsurvivors reveal higher levels of oxidative stress and damage than survivors as well as histochemical evidence of RNS-modified proteins in the lungs, while the antioxidant protective system in ARDS is severely compromised [28, 29].

Leukocytes and pulmonary endothelium

Pulmonary endothelial-leukocyte interaction is a key step in ALI/ARDS development since alterations in cell-cell adhesion is the initial step in leukocyte migration from the capillaries into the lung parenchyma, and the subsequent inflammatory response. Neutrophils appear to be the key cell type related to pulmonary injury in ALI/ARDS, while eosinophils [30, 31] and macrophages have also been implicated [30, 31, 32]. The latter might be responsible for ALI occurring in neutropenic patients [32].

Neutrophil adhesion to EC is a multistage process and a sine qua non for successful neutrophil migration and extravasation (Fig. 1). The initial phase, neutrophil capture and rolling, is mediated by cell adhesion molecules of the selectin family: L-selectin is constitutively expressed on neutrophils, P-selectin is found on platelets and EC, while E-selectin is expressed solely on EC [32, 33]. P-selectin is expressed within minutes on EC surface after EC activation by stimuli such as histamine, thrombin, bradykinin, leukotriene C_4 or free radicals; P-selectin interacts with neutrophil counterreceptors such as the P-selectin glycoprotein-1. E-selectin is rapidly synthesized by EC after cell activation by cytokines such as TNF-α and IL-1, or endotoxin [33].

The second phase is firm neutrophil adhesion (Fig. 1). It requires the interaction of the β_2 (CD18) integrin family (more specifically the CD11/CD18 integrins) expressed on neutrophils, mainly with the intercellular adhesion molecule (ICAM) 1, a member of the immunoglobulin superfamily expressed on EC [33]. ICAM-1 expression on EC is augmented by inflammatory mediators such as TNF-α, IL-1, γ-interferon, and endotoxin. Although ICAM-1 is constitutively expressed by EC in relatively high levels, it appears that the additional expression induced by cytokines is important for the neutrophil–EC interaction [33]. Oxidant stress promotes neutrophil adhesion [25]. Once neutrophils firmly adhere on the pulmonary endothelial layer, they create a microenviroment for injury, mainly via the production of proteases and ROS (i.e., oxidant burst) that induce cell injury and death. Neutrophil adherence to EC or matrix proteins appears to prime the former for a massive burst lasting 1–3 h in response to stimuli such as TNF-α. Activated EC also generate ROS, contributing in maintaining an oxidant-rich environment at injury site [25].

Neutrophil transmigration through the endothelium is the third phase of the adhesion cascade. It does not necessarily accompany firm adherence and depends on the presence of a chemeotactic gradient and the platelet–EC adhesion molecule 1 (PECAM-1) expressed on EC junctions [33]. ROS appear to increase endothelial permeability, facilitating leukocyte transmigration [25].

A large body of evidence has demonstrated the critical role of the neutrophil interaction with pulmonary endothelium in ALI in animals and humans and has examined potential therapeutic interventions. In this respect the dysfunction of endothelium-dependent and endothelium-independent pulmonary vasorelaxation in an endotoxin-induced ALI rat model is attenuated by

neutrophil depletion [34], while neutralization of CD18 attenuates ALI caused by acid installation in the rabbit [35]. Activated neutrophils, as revealed by elastase and superoxide production, are involved in an oleic acid induced ALI guinea pig model [36], while E-selectin and ICAM-1 play important roles in the bleomycin-induced ALI and the subsequent lung fibrosis, through the induction of neutrophil recruitment in the pulmonary circulation [37, 38]. Pulmonary endothelial P-selectin upregulation appears to play a crucial role in the leukocyte recruitment occurring in the pulmonary microcirculation in a pancreatitis-induced ALI rat model, a process that is possibly related to free radicals generated by xanthine-oxidase released by the injured pancreas. Constitutive pulmonary endothelial ICAM-1 contributes to the pathogenic process [39].

Numerous human studies have been performed focusing on EC–neutrophil interaction indices in ALI in an effort to both investigate the underlying pathogenic mechanisms and possibly provide endothelial markers that could predict ALI/ARDS development or outcome [3]. In this respect granulocyte aggregation occurring in the pulmonary microcirculation after activation by transfusion-derived antibodies or biologically active lipids appears to be involved in transfusion-related ALI in man [40]. Soluble plasma P-selectin was found elevated in ALI patients, especially in those who subsequently died [41], while plasma vWF antigen, soluble ICAM-1, and soluble E-selectin measured in patients at risk for ARDS were elevated in septic but not in trauma subjects [42].

In a different study, plasma soluble (s)L-selectin measured in ARDS at-risk patients were significantly lower in those who subsequently progressed to ARDS than in those who did not or in normal controls. Significant correlations were found between the above low sL-selectin levels and the requirement for ventilation, the degree of respiratory failure, and patient mortality, elucidating the interactions between neutrophils and ECs at the early ARDS stage [43]. Additionally, PMEC purified from ARDS patients who died revealed a significantly higher constitutive expression of ICAM-1 than in control human PMEC. When treated with TNF-α, both cell lines showed a dose-dependent increase in ICAM-1 expression that was significantly higher in the ARDS-derived EC [18]. The question remains, however, of whether the observed stronger EC activation occurred during the ALI/ARDS process, or, more importantly, whether PMEC are constitutively more reactive in subjects who will subsequently develop the syndrome.

A more recent study used the endothelial specific E-selectin promoter to express a selective β_2 CD11/CD18 integrin antagonist in a cell- and inflammation-specific manner. This treatment prevented neutrophil adhesion to human pulmonary artery ECs that had been activated by LPS; it additionally prevented neutrophil sequestration in the lungs and ALI development in mice that had received *Escherichia coli* intraperitoneally. These data suggest that conditionally blocking of β_2 integrin function at sites where the endothelium is activated is feasible and might offer in the future a means of locally preventing neutrophil activation that leads to ALI/ARDS [44].

Pulmonary endothelium and pulmonary vascular permeability

Increased pulmonary vascular permeability is a hallmark of ALI/ARDS pathogenesis since it is a sine qua non for noncardiogenic pulmonary edema formation. ARDS patients exhibit persistent pulmonary endothelial permeability that was revealed in vivo by means of a dual-isotope technique; the severity of vascular permeability appeared related to lung injury score as proposed by Murray et al. [45] and the number of neutrophils in bronchoalveolar lavage [46].

Increased pulmonary endothelial permeability may be induced by ALI-related cytokines, other agents, and via EC cytoskeletal-related mechanisms in response to stimuli such as thrombin or mechanical stretch. For a detailed analysis the reader is referred to [47]. Vascular endothelial growth factor (VEGF) is a potent vascular permeability inducer. VEGF was higher in the plasma of ARDS patients, especially in subsequent nonsurvivors as compared to that from patients at risk of ARDS or controls; VEGF may be another important factor in the pathogenesis of noncardiogenic pulmonary edema in ARDS [48].

Pulmonary endothelium and hemofluidity

ECs possess a sophisticated metabolic machinery of interactive factors that modulates all three components of the hemostatic system: platelet aggregation, blood coagulation, and fibrinolysis [49]. In the healthy lung the combined effect of these factors promotes hemofluidity, while under pathological conditions the injured pulmonary endothelium becomes thrombogenic.

Platelets and pulmonary endothelium

Pulmonary endothelium affects platelet function mainly through the production of the platelet aggregation inhibitors PGI_2 and NO; the production of vWF; the conversion of adenosine diphosphate (which can induce platelet aggregation) to adenosine monophosphate, mediated by the endothelial ectoenzyme adenosine diphoshatase; and the removal of serotonin from the pulmonary circulation [49]. In ALI all the above features may be altered leading to enhanced platelet aggregation. In this respect several agents that could cause ALI, such as oxidative injury generated from reactive oxygen spe-

cies and hyperoxia, alter the synthesis and release of PGI_2 [49], while the pulmonary endothelium-mediated extraction of serotonin is decreased in ARDS patients [50].

Numerous studies have shown that vWF is altered in ALI/ARDS, and that vWF is a sensitive marker denoting the existence of EC injury or activation [3]. vWF is synthesized predominantly by vascular ECs. Markedly elevated levels of plasma vWF were reported in patients with acute respiratory failure 22 years ago [51]; this phenomenon appears to occur in early ALI, prior to significant endothelial damage [52]. Since then investigators have focused on the validity of vWF as a predictor of ARDS development. Elevated plasma vWF in patients with nonpulmonary sepsis had a predictive value for ALI development, especially in patients who had concomitant dysfunction of at least one organ [53]. However, more recent studies confirmed that vWF is increased in ARDS at risk patients, but it does not predict ALI development in a heterogeneous patient population [54, 55].

Pulmonary endothelium and coagulation

Pulmonary endothelium possesses both anticoagulant and procoagulant properties. Antithrombin III (AT III) is a major inhibitor of blood coagulation that inhibits thrombin. EC possess heparinlike glycosaminoglycans and sulfated proteoglycans on their surface that sequester AT III and thrombin from the circulation, facilitating their reaction [49]. Additionally, AT III binding to glycosaminoglycans promotes PGI_2 release [56], a feature that among other things prevents LPS-induced pulmonary vascular injury in rats, possibly by inhibiting lung leukocyte accumulation [57].

Thrombomodulin (TM) is an anticoagulant proteoglycan located on the EC surface. TM reacts with thrombin producing a marked increase in the thrombin-catalyzed activation of protein C, which in turn inactivates coagulation factors VA and VIIIA [49]. Plasma TM is increased in ARDS patients, possibly through proteolytic release from the injured pulmonary endothelium, an event mediated by activated neutrophils [58]. Similarly, plasma TM is increased in preterm infants with respiratory distress syndrome, especially in those treated with mechanical ventilation [59]. The critical role of TM dysfunction on ALI/ARDS development was recently demonstrated by blocking pulmonary endothelial TM in mice by means of glucose oxidase (the H_2O_2 generating enzyme) immunotargeting. This treatment caused lung injury that combined oxidative, prothrombotic, and inflammatory components, characteristic of the ALI/ARDS pathology in humans [60].

Endothelial procoagulant properties under normal conditions are covered by its predominant anticoagulant activity. In this respect the activity of thromboplastin, an EC-associated procoagulant factor, is normally low but can be induced by various ALI-related stimuli such as endotoxin, IL-1, and thrombin [49].

Pulmonary endothelium and fibrinolysis

Pulmonary endothelium is actively involved in the fibrinolytic process, expressing tissue-type (t-PA) and urokinase-type (u-PA) plasminogen activators as well as plasminogen activator inhibitors [49]. The EC fibrinolytic activity appears to be affected by several ALI-related mediators including endotoxin, IL-1, TNF-α, and thrombin [49, 61]. In a more recent study human PMECs isolated from ARDS patients expressed higher procoagulant activity and plasminogen activator inhibitor (PAI) 1 as well as lower fibrinolytic potential (i.e., t-PA/PAI-1) than the controls, confirming the procoagulant pulmonary endothelial profile in ARDS [61].

Pulmonary endothelium-derived vasoactive mediators

Nitric oxide

NO is a free radical (RNS) with a very short half-life and is very unstable in biological systems. NO is formed from L-arginine by NO synthase (NOS). There are three known NOS isoenzymes: (a) neuronal (n) NOS, also expressed in pulmonary arterial smooth muscle cells (SMC), (b) inducible (i) NOS, induced by several pro-inflammatory mediators, which upon expression produces NO at very high rates with profound effects on cardiovascular homeostasis, and (c) endothelial (e) NOS, a constitutive isoenzyme expressed principally in EC (Fig. 2) [62]. The latter is the main isoenzyme involved in vascular tone regulation. Deficiency of L-arginine or the NOS cofactor tetrahydrobiopterin may result in eNOS-generated $O_2^{\bullet -}$ instead of or along with NO, promoting the formation of highly reactive RNS such as $ONOO^-$. NO activates soluble guanylate cyclase, thus producing 3,5-cyclic monophosphate (cGMP) and eliciting cGMP-mediated SMC relaxation and other cell-specific functions [62].

In addition to vascular SMC relaxation, NO inhibits (a) platelet aggregation, (b) leukocyte adhesion, and (c) cellular proliferation [7]. In the pulmonary circulation NO synthesis is reduced under hypoxia, and as such it may modulate HPV [63], a feature that is lost in ARDS. Several studies have reported that NO can in addition exert either pro- or anti-oxidative effects, depending on the type and the quantity of oxygen radicals present; NO can additionally attenuate ARDS-associated lung leak [64]. Therapeutic NO inhalation improves oxygenation in several ALI animal models and in responder ARDS patients, while in addition it inhibits neutrophil activation, platelet adhesion, and the production of inflammatory mediators in the injured lungs [64].

Endothelins

Endothelins (ETs) are the most potent naturally occurring vasoconstrictors. Three isoforms have been identified, ET-1, ET-2, and ET-3 all formed from "big endothelin" by ET-converting enzyme [7]. ET-1 is produced mainly by EC, and its production is induced by several factors including hypoxia, endotoxin, TNF-α, interferon, and epinephrine (Fig. 2) [7]. ET-1 release occurs mainly in the abluminal direction towards SMC, and its signaling is mediated by two distinct receptors, ET_A and ET_B. ET_A is expressed on SMC, signaling vasoconstriction; ET_B is expressed primarily on EC and elicits transient vasodilation by signaling NO and prostaglandin release, revealing a cross-talk between the ET-1 and NO pathways [62]. Similarly, EC activation is characterized by a reciprocal ET-1 and eNOS regulation, with most pro-inflammatory stimuli increasing ET-1 and decreasing eNOS expression [62].

The human lung is an important site for both ET-1 clearance and production: approximately 50% of circulating ET-1 is cleared in a single transpulmonary passage via the ET_B receptor, with a simultaneous equal production [65]. This balance between pulmonary ET-1 clearance and release was found decreased early in ALI, reversing in patients who subsequently recovered [66]. Additionally, plasma ET-1 values are increased in septic patients with and without ARDS [67], possibly contributing to the ALI-associated pulmonary hypertension.

Prostaglandins

Among the several cyclo-oxygenase (COX) products PGI_2 and thromboxane A_2 are probably the most important in ALI. PGI_2 is a potent vasodilator and an important inhibitor of platelet aggregation. Thromboxane A_2 is a potent pulmonary vasoconstrictor secondary to endotoxin infusion; Thromboxane A_2 also increases capillary permeability and platelet aggregation [7]. Prostaglandin E_1 (PGE_1) is another COX product with EC protective properties. As with PGI_2, PGE_1 is a vasodilator and platelet aggregation inhibitor, also impairing neutrophil chemotaxis and macrophage activation [68]. COX products contribute to HPV; however, their vasoactive action varies with the size of the artery and the species involved. A particular role of eicosanoids in several ALI models is their contribution to the regulation of perfusion redistribution that diverts blood flow to healthier lung regions. Pretreatment of rabbits with indomethacin, under partial lung microvascular recruitment, protects against PMA-induced pulmonary endothelial enzyme dysfunction, probably by diverting flow to previously unperfused (i.e., unexposed to PMA) capillaries. Under nearly full microvascular recruitment, the above protective effect of indomethacin is abolished [69]. In a similar respect selective inhibition of the inducible COX isoform protects against the endotoxin-related loss of perfusion redistribution in an oleic acid induced dog ALI model, an effect mediated by PGI_2 [70].

Platelet activating factor

Pulmonary ECs release the phospholipid PAF, a highly reactive mediator that has been reported to cause both vasodilation and vasoconstriction in vivo, depending on its concentration [49]. PAF has been additionally reported to increase lung permeability, to activate platelets, neutrophils, and macrophages and to cause EC release of t-PA and PGI_2. PAF synthesis by EC may be induced by H_2O_2 and other reactive oxygen species [49]. *E. coli* injection in rats induced pulmonary hypertension stimulated by PAF and partly mediated by ET-1; it additionally induced PAF-mediated microvascular injury and leak as well as neutrophil activation-sequestration in the lungs. Pretreatment with a PAF receptor antagonist completely blocked all the above events, suggesting a potential future therapeutic application for this compound [71].

Pulmonary endothelial angiotensin-converting enzyme

Angiotensin-converting enzyme (ACE) hydrolyzes angiotensin I to angiotensin II and breaks down bradykinin [72, 73]. Pulmonary endothelium-bound (PE) ACE has a central role in maintaining adequate local and systemic homeostasis, revealing the dynamic interaction between EC and other cell types schematically shown in Fig. 2. In this respect angiotensin II induces SMC constriction, proliferation, and growth. In contrast to this, bradykinin that escapes ACE inactivation exerts vasodilatory, anti-inflammatory and antithrombotic actions through stimulation of endothelial B_2 kinin receptors, causing the synthesis and release of substances such as NO and PGI_2, generated by eNOS and constitutive COX (COX-1), respectively [73]. PE-ACE pro-inflammatory action is further revealed by the fact that angiotensin II can generate $O_2^{\cdot -}$ via the activation of NADH/NADPH oxidases in EC and SMC [73]. Superoxide anions interact with NO to generate $ONOO^-$, while free radicals from several sources cause molecular and cellular damage and decrease ACE activity [74]. It has recently been proposed that the PE-ACE activity reduction seen in ALI is related to enzyme downregulation, mediated by overproduction of $ONOO^-$ and other ROS/RNS, aimed at reducing oxidant stress in the microenvironment [74]. The role of PE-ACE in lung injury and repair may be more complex since recent investigation provided evidence that ACE possesses characteristics of a signal transduction molecule, involved in EC outside-in signaling [75].

Pulmonary endothelial ACE is an ectoenzyme uniformly distributed throughout the luminal EC surface, with its catalytic site exposed to the blood stream; it is directly accessible to blood-borne substrates, and its activity may be measured in vivo by means of indicator-dilution type techniques [5, 72, 76]. Due to the very high enzyme concentrations in the capillaries, monitoring pulmonary endothelial ACE activity in this type of studies, is in practical terms equal to monitoring pulmonary capillary endothelium-bound (PCEB) ACE activity [72]. This method offers quantifiable indices that may distinguish between abnormalities secondary to endothelial dysfunction per se and decreased pulmonary vascular surface area. PCEB-ACE activity estimations have been recently validated in humans [77].

Plasma soluble ACE (sACE) activity is decreased in ARDS patients [78]. However, in contrast to PCEB-ACE, sACE activity is a surrogate index of pulmonary endothelial function. PCEB-ACE activity reduction is among the earliest signs in various ALI animal models, preceding changes in parameters such as acid-base balance, gas exchange, hemodynamic parameters, increased permeability, and morphological changes at the light and electron-microscopic level. This is the case following administration of bleomycin to rabbits [79], exposure of rabbits to hyperoxia [80], PMA administration to rabbits and dogs [69, 81], and chest irradiation to rabbits [82, 83]. Similarly, pulmonary endothelial ACE activity depression, determined by the decreased pulmonary uptake of an anti-ACE monoclonal antibody, occurs in rats secondary to normoxic lung ischemia/reperfusion [84].

PCEB-ACE activity was estimated in mechanically ventilated patients belonging to high-risk groups for ARDS development and suffering from various degrees of ALI/ARDS [85]. Enzyme activity was expressed as transpulmonary substrate hydrolysis (reflecting enzyme activity per capillary) and as the functional capillary surface area (FCSA) index A_{max}/K_m (reflecting enzyme activity per vascular bed) related to both enzyme quantity and functional integrity [72, 77]. Both indices decreased early during the ALI/ARDS continuum and were inversely related to the lung injury score [45], suggesting that the clinical severity of the syndrome is related to the degree of PCEB-ACE activity depression (i.e., the underlying pulmonary endothelial dysfunction). Further analysis of the FCSA data revealed two different profiles in the A_{max}/K_m vs. cardiac output relationships, probably distinguishing patients with reserves of healthy or mildly injured capillaries from those without; the former had better survival, raising the possibility that FCSA could be of value as an outcome predictor in ARDS [85].

Marshall et al. [86] have recently shown that ACE insertion/deletion polymorphism is associated with the susceptibility and outcome in ARDS, with the DD genotype frequency being increased and associated with mortality in these patients. This first description of a specific allele association with ARDS development suggests a major role for the renin-angiotensin system in the pathophysiology of the syndrome. Although the D allele has been associated with higher ACE activity, this does not necessarily contradict the reported PCEB-ACE activity reduction in ALI/ARDS [85]. The former could affect mostly other lung compartments or, alternatively, the latter might be related to either damaged ECs or to PCEB-ACE downregulation as a host response aimed at reducing local inflammation.

Endothelium-related therapies in ALI/ARDS

Pulmonary endothelial functional and structural alterations are key components of ALI pathogenesis. Consequently EC-related therapies may have beneficial effects in ALI/ARDS. Such therapies should restore adequate endocrine and paracrine EC functions, and protect ECs against harmful insults as well as against pro-inflammatory cell-cell interactions [68, 87]. Several therapeutic interventions, most of them related to endothelium-derived vasoactive (and anti-inflammatory) mediators are already in place, in an effort to improve arterial oxygenation and treat ARDS-related pulmonary hypertension [68]. In this respect inhaled or intravenous PGI_2, inhaled or intravenous (in either native or liposomal form) PGE_1, and, mainly, inhaled NO have been used, with mixed results [68, 87]. Additional agents include antioxidants such as *N*-acetylcysteine and hyperoncotic albumin, and the thromboxane synthetase inhibitor ketoconazole [87]. Several agents already in clinical use for treating pathologies other than ALI, such as ET-1 receptor antagonists, phosphodiesterase inhibitors, and ACE inhibitors might have beneficial effects. For a comprehensive analysis of this topic the reader is referred to [68] and [87]. Throughout this review several experimental studies with future therapeutic potential have been reported. More recent experimental work focus on unmasking EC diversity in an effort to develop means that will target the injured pulmonary endothelium and allow specific drug and/or gene delivery.

Conclusion and future directions

In addition to gas exchange, pulmonary vasculature filters the entire circulating blood before the latter enters the systemic circulation, affecting both local and systemic vascular tones, inflammatory processes, and whole-body homeostasis. Pulmonary circulation possesses two major distinct features: in health it responds to hypoxia with HPV to maintain adequate ventilation/perfusion match; in critical illness pulmonary hypertension may develop, as opposed to the often occurring systemic hypotension. As these responses strongly depend on pulmonary endothelial functional and structural integrity, further under-

standing of the pulmonary endothelial properties will provide insights into ALI pathophysiology and how the latter affects systemic cardiovascular homeostasis. In this respect recent investigations revealed the existence of pulmonary EC heterogeneity that might contribute to ALI pathogenesis: neutrophil adhesion through ICAM-1 induces cytosceletal changes in pulmonary microvascular ECs (where the site of neutrophil emigration) and not in pulmonary arterial ECs [88]. Additionally, Ca^{2+} communication from pulmonary septal capillaries appears to establish a pro-inflammatory state in downstream venular capillaries, probably amplifying lung inflammation [89]. Specific pulmonary ECs that locally enhance or amplify lung injury might be the targets of future genetic or other therapies.

A National Heart, Lung and Blood Institute workshop has recently proposed future research directions in ALI/ARDS [90]. Pulmonary ECs appear to be the first lung cells altered in ALI/ARDS generated by sepsis, trauma, and other systemic conditions. Among other things, EC heterogeneity should be further investigated along with the EC functional changes involving new gene expression or constitutive pathways, the molecular mechanisms that govern pulmonary EC responses, their interaction with alveolar epithelium, and the responses of systemic endothelium in ALI/ARDS. New EC-related therapies targeting pulmonary microvascular inflammation and thrombosis, and the value of newly implanted pulmonary ECs derived by intravenous infusion of bone marrow stem cells should be investigated [90]. In relation to the former, studies of activated protein C administration in ARDS patients are already under way.

In conclusion, pulmonary EC dysfunction is a key component of ALI pathogenesis. Future investigations of pulmonary endothelial dysfunction may provide additional information on ALI pathophysiology, markers that could predict ARDS development or outcome, and new therapeutic approaches.

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