The endothelium in acute lung injury/acute respiratory distress syndrome

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Purpose of review
Since pulmonary edema from increased endothelial permeability is the hallmark of acute lung injury, a frequently encountered entity in critical care medicine, the study of endothelial responses in this setting is crucial to the development of effective endothelial-targeted treatments.

Recent findings
From the enormous amount of research in the field of endothelial pathophysiology, we have focused on work delineating endothelial alterations elicited by noxious stimuli implicated in acute lung injury. The bulk of the material covered deals with molecular and cellular aspects of the pathogenesis, reflecting current trends in the published literature. We initially discuss pathways of endothelial dysfunction in acute lung injury and then cover the mechanisms of endothelial protection. Several experimental treatments in animal models are presented, which aid in the understanding of the disease pathogenesis and provide evidence for potentially useful therapies.

Summary
Mechanistic studies have delivered several interventions, which are effective in preventing and treating experimental acute lung injury and have thus provided objectives for translational studies. Some of these modalities may evolve into clinically useful tools in the treatment of this devastating illness.

Keywords
ALI, ARDS, endothelium, lung

Introduction

The intimal lining of all blood vessels is a single layer of functionally and structurally heterogeneous endothelial cells depending on organ and vascular bed location [1•]. Pulmonary microvascular endothelium is a metabolically active organ essential for maintaining adequate pulmonary and systemic cardiovascular homeostasis [1•,2]. Noxious stimuli compromise pulmonary endothelial functional and structural integrity leading to noncardiogenic pulmonary edema and parenchymal inflammation. Thus, pulmonary endothelium has a key role in the development of acute lung injury (ALI) and its most severe form, the acute respiratory distress syndrome (ARDS). While no therapeutic breakthroughs in the lung endothelial field were reported over the past 12 months, several experimental contributions to the understanding of endothelial mechanisms of injury and repair were made. We will therefore focus on these recent advances and present some novel endothelial-based treatments.

Major endothelial properties

Endothelial cells in lung vessels are arranged in a continuous monolayer forming a tight barrier [1•]. Major determinants of endothelial fluid permeability are intercellular protein bridges called tight and adherens junctions (AJ) and contact sites to the substratum called focal adhesions [3]. These structures allow paracellular exchange of very small amounts of water and solutes. An exquisitely developed system of vesicular carriers allows uptake of macromolecules into and transport across endothelial cells in a highly selective manner. Besides permeability regulation, pulmonary endothelial cells modulate vascular tone, ventilation–perfusion matching, hemofluidity, and interactions with blood-borne cells. Inflammatory states such as sepsis induce a phenotypic shift in endothelial cells with secretion of inflammatory and chemotactic substances, expression of adhesion molecules, increased permeability and loss of anticoagulant functions [2]. A schematic representation of major lung endothelial functions in health and

Figure 1 Schematic illustration of major pulmonary vascular endothelial functions in health (a) and in acute lung injury (b)
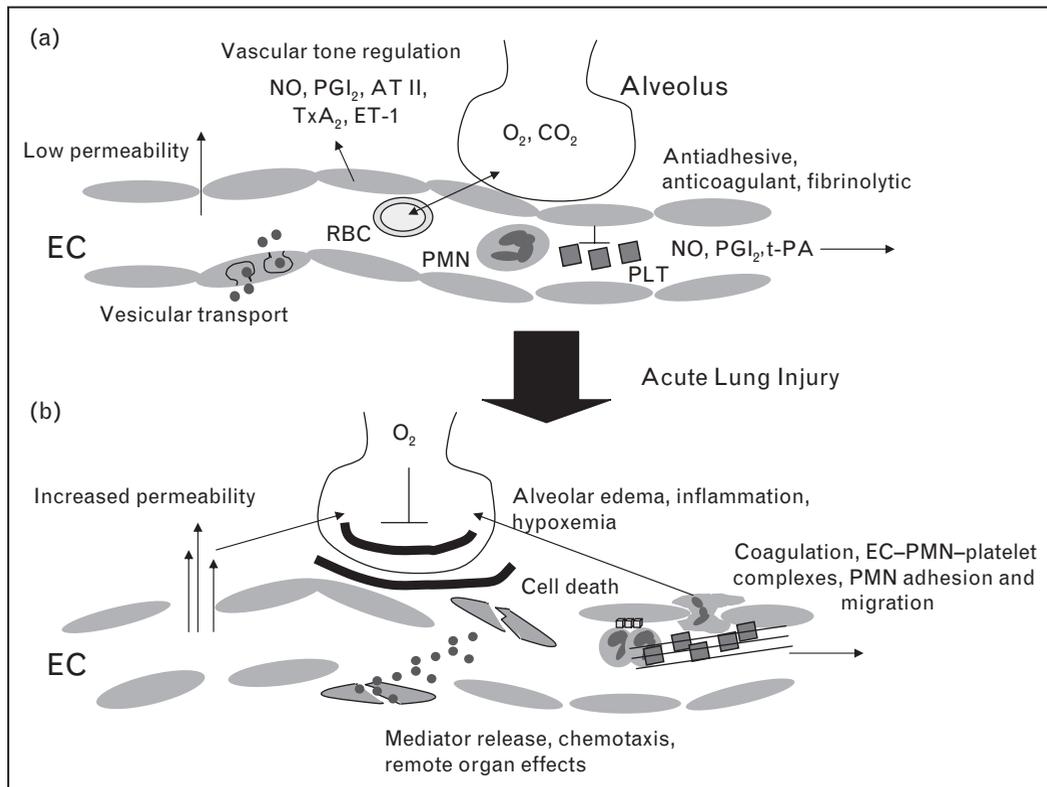
(a) Pulmonary vascular endothelium forms a single-layer interface separating blood from the extravascular space. The layer is composed of tightly connected endothelial cells (ECs) and allows minimal filtration of fluid, thus preventing alveolar flooding and facilitating gas diffusion. An extensive network of vesicles is responsible for the highly selective, receptor-mediated uptake and transport of macromolecules (e.g. albumin) across the barrier and the secretion of macromolecules (e.g. von-Willebrand factor) in the blood. Lung endothelial cells are largely responsible for regulating vascular smooth muscle (VSM) tone, maintaining low pulmonary vascular resistance and appropriate ventilation–perfusion matching. This is achieved by balancing the secretion of vasodilators also possessing antiproliferative and anti-inflammatory effects (e.g. nitric oxide, NO; prostacyclin, PGI_2) against that of vasoconstrictors also inducing cell proliferation/hypertrophy and inflammation (e.g. thromboxane A_2, TxA_2; angiotensin II, AT II; endothelin-1, ET-1). Intravascular clotting is prevented by agents counteracting platelet adhesion such as nitric oxide and prostacyclin and lysis of endovascular thrombi is facilitated by fibrinolytic agents such as tissue plasminogen activator (t-PA). (b) The exposure of lung endothelial cells to noxious stimuli, including bacterial toxins, inflammatory mediators or excessive mechanical forces triggers a shift towards a 'pro-inflammatory' and thrombophilic endothelial cell phenotype, characterized by platelet and leukocyte adhesion molecule expression, leading to tissue infiltration and intravascular clotting. These processes are augmented by endothelial cell apoptosis and necrosis, which produce further inflammatory mediators and allow clot formation on denuded surfaces. Endothelial cell cytoskeleton contraction, triggered by inflammatory mediators (e.g. thrombin), increases endothelial permeability. The ensuing plasma extravasation leads to pulmonary edema, the hallmark of acute lung injury and the acute respiratory distress syndrome. ; PLT, platelet; PMN, polymorphonuclear leukocyte; RBC, red blood cell.

disease is given in Fig. 1. For a more detailed analysis of pulmonary endothelial metabolic properties and endothelial cell contribution to ALI/ARDS pathophysiology, the reader is referred to [2].
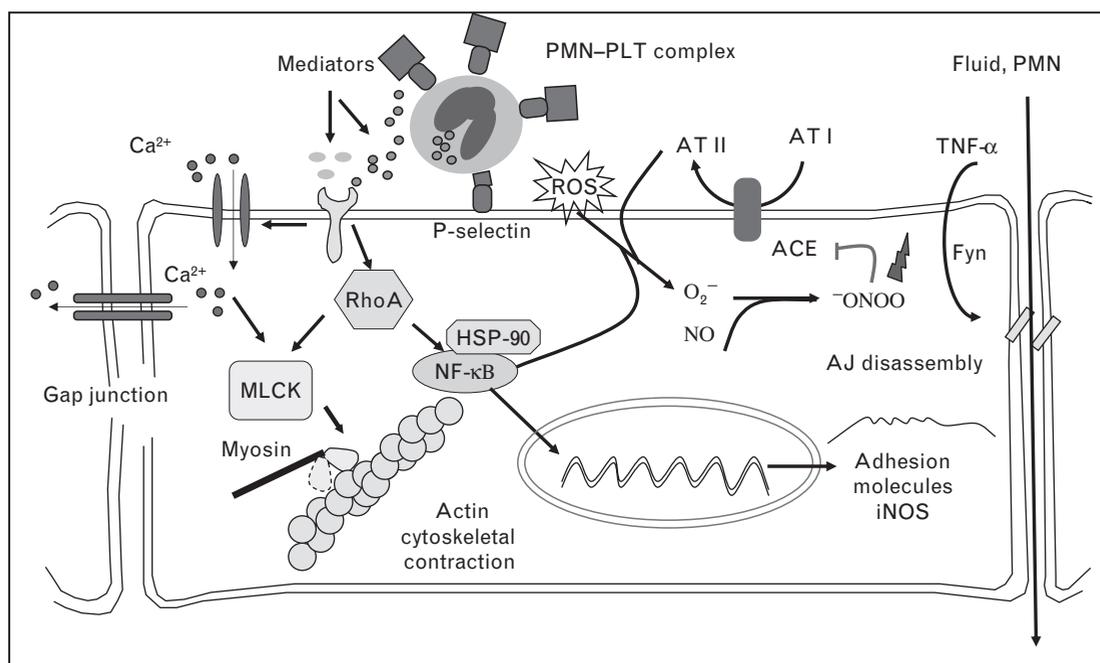
Mechanisms of endothelial barrier disruption in acute lung injury

Endothelial barrier disruption is a *sine qua non* of ALI/ARDS pathogenesis; related pathological mechanisms are presented in Fig. 2, along with additional proinflammatory endothelial cell pathways implicated in lung injury.

Cytoskeleton

Endothelial cell actin cytoskeleton is a complex network of actin molecules polymerized into filaments. Multiple connections of the cytoskeleton to junctional proteins, the glycocalyx, and focal adhesions exist. Stimulation of endothelial cells by agonists, such as thrombin, signals actin filament rearrangement into thicker actin cables or stress fibers, and enhances actin–myosin interactions. Cytoskeletal contraction, facilitated by the motion of myosin along actin fibers, generates tension that pulls cells away from each other creating inter-endothelial gaps and increasing permeability. Myosin light chain (MLC) phosporylation by MLC kinase (MLCK) augments cytoskeletal force generation [4]. Small GTPase-induced activation of MLCK and other cytoskeletal-remodeling compounds, such as p21-activated kinase, lead to pulmonary endothelial barrier compromise and assist leukocyte migration in sepsis [5]. Genetic deletion or pharmacologic inhibition of MLCK210, the endothelial

Figure 2 Schematic illustration focusing on recently described endothelial-based molecular aspects of acute lung injury (ALI) pathogenesis

Binding of inflammatory mediators, including thrombin, thromboxane A_2 and transforming growth factor-β on their receptors on endothelial cell (EC) surface activates a cascade of signaling events that lead to endothelial barrier disruption and modulation of gene expression. Sources of these mediators include platelets (PLTs), leukocytes and other tissue cells. Calcium ion (Ca^{2+}) influx following endothelial cell surface receptor ligation raises Ca^{2+} levels, activating downstream targets and propagating the signal to adjacent endothelial cells via gap junctions. The small GTPase RhoA and its downstream effectors mediate actin stress fiber formation and contraction of the actin cytoskeleton, allowing leukocyte transmigration and plasma leakage in the extravascular space. Nuclear factor κB (NF-κB) activated by RhoA stimulates expression of leukocyte and platelet adhesion molecules. Molecular chaperone heat shock protein-90 (HSP-90) promotes NF-κB activation. Adherent leukocytes, primarily polymorphonuclear leukocytes (PMNs), release superoxide radicals (O_2^-) with potent bactericidal and cytotoxic effects. Reactive oxygen species (ROS) bind with nitric oxide (NO) to form further aggressive molecules such as peroxynitrite (−ONOO). Angiotensin converting enzyme (ACE) is critical in ALI pathogenesis by producing angiotensin II (AT II), which has proinflammatory actions. In addition to promoting NF-κB activity and apoptosis, tumor necrosis factor-α (TNF-α) can directly disrupt endothelial cell adherens junctions (AJ) by activating tyrosine kinase Fyn, which subsequently phosphorylates various adherens junction protein components. MLCK, myosin light chain kinase; iNOS, inducible nitric oxide synthase; →, activation; ⊥, inhibition.

cell-specific MLCK isoform, protects mice from experimental pulmonary edema, inflammation and death [6]. Furthermore, activation of nuclear factor κB (NF-κB; i.e. a central transcription factor of inflammation-related genes) and expression of the inducible isoform of nitric oxide synthase (iNOS) were attenuated in endotoxemic MLCK210 knockout mice, translating into a reduction of nitric oxide, reactive oxygen species and vascular hyporeactivity, and pointing to a link between cytoskeletal and paracrine endothelial cell pathways [7•].

A second, less well studied component of the endothelial cell cytoskeleton, the microtubules, comprises a system of polymerized tubulin molecules in rod-shaped arrangements that extend from the center to the periphery of the cell. Microtubule destabilization by the experimental chemotherapeutic agent 2-methoxyestradiol increases endothelial cell permeability [8]. Conversely, inhibition of microtubule destabilization with paclitaxel leads to microvascular barrier enhancement in mice challenged with lipopolysaccharide (LPS) [9].

Intracellular signaling

Inflammatory mediators bind to their endothelial cell receptors to activate signaling events culminating in cytoskeletal contraction and increased microvascular permeability. These ligands include thrombin, vascular endothelial growth factor (VEGF), transforming growth factor-β and thromboxane A_2 (TxA_2). In many cases, a complex interplay between cell junctional elements and focal adhesions results in rapidly occurring actin cytoskeletal remodeling [10]. More delayed effects comprise the induction of enzymes favoring actin stress fiber formation such as small GTPase RhoA [11].

Injured tissues may serve as the origin of inflammatory mediators, promoting the expansion of inflammatory processes. The proinflammatory cytokine tumor necrosis factor-α (TNF-α) is secreted by lung endothelial cells following ischemia/reperfusion, and induces pulmonary edema in the nonischemic contralateral lung [12•]. TNF-α may promote disruption of endothelial cell junctions by phosphorylation of AJ

proteins such as vascular endothelial cadherin and β-catenin [13•].

Intracellular Ca^{2+} is an important second messenger in pathways that signal permeability increases. Cytosolic Ca^{2+} concentration is the composite of calcium ions from intracellular stores and the extracellular space. A route of Ca^{2+} entry into endothelial cells via the vanilloid transient receptor potential channel was recently uncovered [14•]. Activation of this mode of Ca^{2+} influx increased permeability in isolated mouse lungs and produced disruption of the alveolocapillary membrane at the electron microscopy level. In addition to activating MLCK, Ca^{2+} can trigger the expression of cell surface adhesion molecules, enabling docking of circulating polymorphonuclear leukocytes (PMNs) to endothelial cells and their transmigration in the interstitium and airspace. Ca^{2+} signals can be propagated along interconnected endothelial cells via gap junctions [15••], explaining how inflammation can rapidly spread across large areas of the lung and become manifest as extended patches of consolidation in imaging studies.

NF-κB is an important transcription factor of genes induced in inflammatory states, including sepsis and ventilator-induced lung injury (VILI). Heat-shock protein 90 (HSP-90), an abundantly expressed molecular chaperone, is required for NF-κB uncoupling from its associated inhibitory IκB and consequent activation. By administering the HSP-90 inhibitors radicicol or a geldanamycin analog to endotoxemic mice, Chatterjee et al. [16••] reduced NF-κB activation, resulting in decreased lung inflammation and endothelial permeability, as well as in improved lung function, systemic homeostasis and survival.

Endothelial interactions with blood-borne cells

An important endothelial cell proinflammatory function is the secretion of the potent chemotactic cytokine interleukin (IL)-8 [17]. This and other substances secreted by endothelial cells are implicated in causing cytoskeletal PMN alterations, thus reducing their deformability and promoting sequestration at sites of inflammation [18,19]. In addition, activated PMNs have been shown to directly induce vascular leak by free radical production [20•].

Platelet and PMN binding to endothelial cells is required for intravascular clotting and PMN transendothelial migration, respectively. Platelet P-selectin facilitates platelet–endothelial cell binding [21]. Bound platelets complexed with PMNs release mediators such as TxA_2 and soluble CD40 ligand, leading to endothelial cell and PMN activation, respectively [22••,23•]. In a similar respect, PMN diapedesis across endothelial cells was found to be dependent on signaling pathways involving endothelial cell G proteins, specifically Gα(i), which transduce signals downstream of G-protein-coupled receptors [24•]. The interaction of adherent PMNs with endothelial cell AJ was studied by Orrington-Myers et al. [25•]. AJ disassembly by expression of dysfunctional vascular endothelial cadherin, the extracellular AJ component, reduced PMN migration, NF-κB activity and intracellular adhesion molecule (ICAM)-1 expression in LPS-treated mice. It seems, therefore, that the presence of intact endothelial cell AJ is required for proper PMN binding and migration.

Reactive oxygen and nitrogen species

Oxygen and nitrogen-based free radicals including superoxide anion and nitric oxide (NO) are important biological endothelial cell signaling molecules but can cause tissue damage at high concentrations. Interventions depleting sources of superoxide anion such as xanthine oxidoreductase or aldehyde 4-hydroxy-2-nonenal reduce free radical production and the associated microvascular leak in various in-vivo and in-vitro injury models [26–28]. PMN-derived free radicals induce pulmonary endothelial cell dysfunction in the setting of transfusion-related acute lung injury: when blood containing antibodies to human neutrophil antigen 3a, expressed on the surface of recipient PMNs, is transfused to susceptible patients, PMN stimulation may elicit toxic effects on endothelial cells via reactive oxygen species (ROS) production [29•].

Catalytically active iron can induce oxidative stress in lung tissue. Such alterations in the redox state of lung endothelial cells [30] are accompanied by NF-κB activation leading to ICAM-1 expression, and downregulation of vascular endothelial cadherin expression.

Inhaled nitric oxide has been used in the treatment of ALI/ARDS to improve gas exchange and lower pulmonary vascular resistance, despite concerns for potential local or distant harmful effects [31•]. In a rat model of Pseudomonas pneumonia endothelial and epithelial permeability were separately quantified in vivo using a double flux technique of radio-labeled albumin. Inhaled nitric oxide was found to increase endothelial permeability [32] independent of tissue PMN infiltration, suggestive of a direct effect on pulmonary endothelium. This notion was corroborated in additional in-vitro and in-vivo studies, in which iNOS inhibition was associated with endothelial cell barrier protective effects [33,34].

Renin–angiotensin–aldosterone system

The angiotensin converting enzyme (ACE)-I, responsible for the conversion of angiotensin I to angiotensin II is expressed primarily on the surface of pulmonary microvascular endothelial cells [1•]. Even though pulmonary

endothelial ACE activity reduction has been documented in various animal models of lung injury as well as in patients with ALI/ARDS [2], evidence is accumulating that ACE is directly involved in the pathogenesis of the disorder. It has been proposed that the above-mentioned ACE activity reduction may be related to enzyme downregulation mediated by overproduction of peroxynitrite and other ROS/reactive nitrogen species [2], as schematically presented in Fig. 2. ACE inhibition in rats appears to ameliorate oleic acid-induced ALI and VILI [35,36•]. In the latter study, increased angiotensin II levels were detected in lung tissue following VILI, suggesting that ACE may contribute to lung injury via angiotensin II production. The same group followed Chinese ICU patients with ARDS and compared clinical outcomes among carriers of the three ACE genotypes: II, ID, DD. ARDS patients with the II genotype appeared to have a survival advantage over patients with the ID or DD genotype [37•]. These findings are in agreement with previous reports showing that the D allele is associated with higher ACE activity and that the DD genotype is more frequent and carries higher mortality in ARDS [2]. These observations underscore the involvement of ACE in ALI pathogenesis, although the nature of this involvement needs to be further clarified.

Angiopoietins

Angiopoietins are a novel class of angiogenic growth factors whose contribution in sepsis and ALI pathophysiology has recently drawn increasing attention [38]. Angiopoietin-1 is a Tie2 receptor agonist that reduces vascular permeability and inflammation, while angiopoietin-2 has context-dependent effects acting as a Tie2 agonist or antagonist. Angiopoietin-1 has been recently shown to exert an inhibitory effect on the synthesis and release of the proinflammatory and vasoconstrictor endothelin-1 by endothelial cells [39••]. Angiopoietin-2 acts mainly as a modulator of endothelial barrier disruption, promoting vascular leak in septic ALI [40] and hyperoxia-induced lung injury [41••]; in contrast, an autocrine protective effect on activated endothelial cells that blocks vascular leak has also been suggested [42••]. We have recently shown that serum angiopoietin-2 levels in critically ill patients are increased in severe sepsis and correlate with serum TNF-α and disease severity. Human lung microvascular endothelial cells treated with LPS, TNF-α, and IL-6 responded by angiopoietin-2 reduction, implying that human pulmonary endothelium may not be the source of increased angiopoietin-2 in human sepsis. In contrast, LPS and TNF-α stimulated angiopoietin-2 release by bovine lung microvascular endothelial cells, pointing to species-specific differences [38].

Endothelium-related protective mechanisms and interventions in acute lung injury

Several compounds which are either synthesized by or act on endothelial cells have been used for lung injury treatment in experimental studies or in human trials. An overview of recent reports is given below.

Activated protein C

Activated protein C (APC) is used in the treatment of patients with severe sepsis. The mechanisms of action of this drug are under intense investigation. Endothelial protein C receptors (EPCRs) are expressed on endothelial, vascular smooth muscle, and immune cells. Receptor-deficient mice appeared more sensitive to LPS administration than receptor-expressing animals, while EPCRs offered protection when expressed on endothelial cells and not on hematopoietic cells, indicating that the mechanism of APC action is primarily endothelial cell related [43•]. Animal studies by us and others, using various routes of APC administration and dosing regimens, documented lung-protective effects of the drug [44,45•,46•], despite apparent variations in the mode of APC action probably related to differences in experimental designs. Finally, of interest is the report of a homozygous 23 base pair insertion in the gene encoding EPCR in an 8-month-old boy with severe sepsis, pointing to the presence of a susceptibility-conferring mutation in this gene [47•].

Antioxidants

In order to counteract excessive oxidant production, cells possess defensive antioxidant mechanisms. The transcription factor Nrf2 induces the expression of antioxidant enzymes. Nrf2 transcription can be activated by actin cytoskeletal remodeling in response to cyclic stretch, and by stimulation of the epidermal growth factor receptor signaling pathways [48]. A point mutation (−617 C/A) in the promoter region of the Nrf2 gene, which decreased promoter activity, was identified in trauma patients, and was associated with increased risk for ARDS development [49•]. The protective role of the antioxidant enzyme catalase was elucidated in an ischemia–reperfusion rat lung model; catalase conjugation to anti-ACE antibodies allowed lung endothelial cell targeting and attenuated lung injury [50••].

Nitric oxide

A number of publications have dealt with the protective role of NO in lung injury. This is in contrast to data revealing permeability-increasing and cytotoxic effects. The discrepancy may be due to differences in experimental conditions, high versus low NO levels, as well as the site and source of NO release. Additionally, nitric oxide appears to exert either pro or antioxidative (i.e. harmful or protective) effects, depending on the type and concentration of ROS present in the microenvironment

[2] (see Figs 1 and 2). A most recent metaanalysis of studies on ARDS patients treated with inhaled NO revealed initial oxygenation improvement (a fact that supports the use of inhaled NO as a rescue therapy), no difference in clinical outcome, and an increased risk for renal dysfunction [31•]. The reason why the beneficial action of inhaled NO on the lungs appears neither sustained nor outcome improving is still puzzling and may be related, among other things, to dose–response changes with time and extrapulmonary organ toxicity. The observation that ALI patients with higher NO levels have reduced mortality, more ventilator-free days and more organ failure free days indicates that endogenous NO may, in fact, be cytoprotective due to immunomodulation or preservation of cell viability [51•]. Alternatively, since nitric oxide has bactericidal properties, patients with higher levels may be able to clear infections more efficiently.

The issue of the immune-modulating properties of endothelial nitric oxide synthase (eNOS)-derived NO was mechanistically addressed in a number of experimental studies using various lung injury models. eNOS-produced nitric oxide translated into: suppressed NF-κB activation in LPS models [52•]; lower activity levels of matrix metalloproteinase-9 (an enzyme which digests interstitial matrix) and thus a lower degree of fibrosis in mice receiving bleomycin [53•]; and abrogated PMN sequestration in mouse lungs primed with hypoxia and subsequently injured by ischemia–reperfusion [54].

Carbon monoxide
Low levels of carbon monoxide (CO) are released during enzymatic breakdown of heme by heme oxygenase-1 (HO-1) to perform important endothelial cell signaling functions. VEGF has been shown to induce HO-1 transcription and protect against hyperoxic ALI [55•]. Two additional studies revealed the protective effect of HO-1-produced CO and underscored the endothelial cell importance in hyperoxic injury [56•,57•]. In a model of murine thermal injury, CO donor drugs markedly reduced lung inflammation and edema, supporting the role of CO in lung endothelial cell cytoprotection and immune modulation against various insults [58•].

Adenosine
An important vasodilator, adenosine, has been shown to be released by mechanically stretched cells [59•]. Ecto-apyrase (CD39) and ecto-5′-nucleotidase (CD73) are enzymes producing extracellular adenosine and were both found upregulated in VILI. Underexpression of both enzymes in mice resulted in worse VILI outcome, underscoring the role of adenosine in counteracting the deleterious effects of mechanical ventilation [59•]. The mode of adenosine protective action in ALI may be related to preservation of intracellular energy pools [60] and prevention of apoptosis [61].

Cytoskeleton-stabilizing interventions
Newly developed pharmacologic agents have been reported to have endothelial barrier enhancing properties by strengthening endothelial cell–cell contacts via the actin cytoskeleton. This is presumably achieved by shifting the balance of actin polymerization towards cortical actin rings which reinforce cell–cell attachment, and away from stress fibers which pull cells away from each other [4]. Critical to these events is the function of small GTPases Rac and Cdc42. Compounds under study for this kind of effect are oxidized phospholipids, prostaglandins E_2 and I_2 and sphingosine-1 phosphate. These drugs have been tested in various lung injury models and were found to reduce lung edema and inflammation [62,63•,64,65].

Mechanisms of lung endothelial repair
Few studies addressed the vital issue of endothelial repair after the onset of injury. Since in clinical practice ALI is commonly diagnosed after its onset, understanding how to augment endothelial cell repair may be of critical importance to patient care. Zhao et al. [66•] showed that systemic LPS causes endothelial cell apoptosis in murine lungs. Transcription factor forkhead box M1 (FoxM1), known for its role in DNA replication and mitosis, is crucial to endothelial regeneration after LPS [66•]. In a clinically relevant animal experiment, recombinant VEGF administration enhanced alveolar and vascular regeneration in hyperoxic rat pups, albeit at the expense of transiently increasing permeability [67•]. Additionally, Quadri and Bhattacharya [68] used hydrogen peroxide to induce loss of cell–cell contacts in cultured endothelial cell monolayers, and showed that endothelial restoration was dependent on activation of focal adhesion kinase, an enzyme found in focal adhesions.

Endothelial–epithelial interactions
Kinniry et al. [69•] described a novel role for KL(4)-surfactant in ALI protection. KL(4)-surfactant, containing the novel peptide sinapultide, administered intranasaly diminished PMN infiltration in lungs of mice challenged with hyperoxia or LPS. This effect appeared related to blockade of PMN transmigration across the endothelium.

Novel lung-targeting therapies
Two already presented studies demonstrated the feasibility of lung targeting of genes or proteins with endothelial cell protective properties. In the work by McCarter et al. [39••], rat dermal fibroblasts transfected in vitro with the gene coding for angiopoietin-1 were injected intravenously to the intact animal and were rapidly incorporated in the lung microvessel walls. ALI was induced 24 h later by LPS intratracheal instillation. Animals overexpressing angiopoietin-1 sustained less lung injury than controls, a

fact attributed to the barrier-sealing effect of angiopoietin-1 and/or to endothelin-1 decreased expression. In the second study, the antioxidant enzyme catalase was conjugated to monoclonal antibodies against rat ACE, thus concentrating the enzyme on lung endothelial cells. Rats treated with this conjugate exhibited a significant attenuation of ischemia–reperfusion lung injury [50••].

Conclusion

The endothelial cell is central to the pathogenesis of ALI. Endothelial-targeted interventions are able to prevent lung injury in several experimental models. Keeping in mind that there is usually a long way to go from bench to bedside, some of these modalities may in the long term prove to be useful in the clinical setting and improve the management and prognosis of this devastating complication.

Acknowledgements

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