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Review

Endothelial pathomechanisms in acute lung injury

Nikolaos A. Maniatis [a], Anastasia Kotanidou [a,b], John D. Catravas [d], Stylianos E. Orfanos [a,c,*]

[a] "M. Simou" Laboratory, University of Athens Medical School, Athens, Greece
[b] 1st Department of Critical Care, Evangelismos Hospital, University of Athens Medical School, Athens, Greece
[c] 2nd Department of Critical Care, Attikon Hospital, University of Athens Medical School, Athens, Greece
[d] Vascular Biology Center, Medical College of Georgia, Augusta, GA, United States

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ABSTRACT

Acute lung injury (ALI) and its most severe extreme the acute respiratory distress syndrome (ARDS) refer to increased-permeability pulmonary edema caused by a variety of pulmonary or systemic insults. ALI and in particular ARDS, are usually accompanied by refractory hypoxemia and the need for mechanical ventilation. In most cases, an exaggerated inflammatory and pro-thrombotic reaction to an initial stimulus, such as systemic infection, elicits disruption of the alveolo-capillary membrane and vascular fluid leak. The pulmonary endothelium is a major metabolic organ promoting adequate pulmonary and systemic vascular homeostasis, and a main target of circulating cells and humoral mediators under injury; pulmonary endothelium is therefore critically involved in the pathogenesis of ALI. In this review we will discuss mechanisms of pulmonary endothelial dysfunction and edema generation in the lung with special emphasis on the interplay between the endothelium, the immune and hemostatic systems, and highlight how these principles apply in the context of defined disorders and specific insults implicated in ALI pathogenesis.

Contents

* Corresponding author. 2nd Department of Critical Care, Attikon Hospital, 1, Rimini St., 124 62, Haidari, Athens, Greece. Tel.: +30 210 7235521; fax: +30 210 7239127.
E-mail address: sorfanos@med.uoa.gr (S.E. Orfanos).

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1. Introduction

Acute lung injury (ALI) is characterized by hypoxemic respiratory insufficiency from non-cardiogenic pulmonary edema caused by increased pulmonary vascular permeability. ALI and its more severe form, the acute respiratory distress syndrome (ARDS), comprise a uniform response of the lung to infectious, inflammatory or chemical insults and are therefore commonly associated with systemic illness such as sepsis or major trauma; ALI/ARDS may also evolve following pulmonary insults such as pneumonia, gastric acid aspiration or toxic gas inhalation (Matthay and Martin, 2005; Ware and Matthay, 2000). Acute-onset dyspnea, tachypnea and hypoxemia are the cardinal clinical features of ALI and mechanical ventilation is often required to maintain oxygenation and ventilation in these patients. Despite positive survival trends noted in the past two decades in ALI patients, mortality remains between 30 and 50%, particularly among elderly patients with sepsis and prior co-morbidity (Avecillas et al., 2006; Rubenfeld et al., 2005). Even though relatively few patients with ALI actually die of respiratory failure and the majority appear to succumb to multiple organ dysfunction, many ALI survivors sustain long-term neuro-psychiatric and respiratory impairment (Davidson et al., 1999a, b; Hopkins and Herridge, 2006; Vincent and Zambon, 2006). It is thus evident that the syndrome is a major public health burden that causes substantial morbidity, mortality and healthcare cost.

Endothelial alterations in the form of shifts in activity or serum levels of endothelial-specific proteins, including angiotensin converting enzyme (ACE), tissue factor pathway inhibitor and von Willebrand factor, as well as permeability increases have been noted in patients with ALI *in vivo*, and most experts consider the endothelial cell as a major actor in host responses to insults related to ALI (Calandrino et al., 1988; Orfanos et al., 2000; Sabharwal et al., 1995b; Ware et al., 2004). We will present some of the prevailing general concepts of ALI pathogenesis that are pertinent to the endothelium of the lung and then examine how these concepts apply to individual disease entities. Even though a significant portion of the findings discussed in this review has been generated in animals or *in vitro* systems, and despite the shortcomings of lab experiments in duplicating the real world, we feel that many of these findings help elucidate important concepts relevant to human disease and identify potential therapeutic strategies to be tested in patients.

2. Basic principles of endothelial biology

The pulmonary endothelium is a continuous monolayer of squamous cells that internally lines blood vessels and, together with the collagen-rich basement membrane, separates but also selectively connects tissue micro-environment and blood, thus helping to maintain tissue homeostasis. The main site of pulmonary gas exchange, which is the chief responsibility of the lung, is the <0.2 μm thin part of the alveolo-capillary membrane, which consists of an endothelial and an alveolar epithelial cell with their individual basement membranes fused together (Burns et al., 2003; Weibel, 1984). This arrangement facilitates diffusion of gases across the alveolo-capillary wall, allowing rapid equilibration of blood and alveolar oxygen and carbon dioxide tensions at rest and on exertion (Wagner and West, 2005).

The pulmonary endothelium participates in the exchange of water and solutes between the blood and the interstitium. Under normal conditions, a small amount of fluid is filtered across the endothelial monolayer and drained by the lymph. Fluid filtration is limited by the continuous nature of the endothelium, with multiple connections between the cells called tight and adherens junctions (Bazzoni and Dejana, 2004; Mehta and Malik, 2006). Thus, water and solute flux is tightly regulated and occurs passively between endothelial cells (EC) (paracellular pathway), driven by the hydrostatic pressure gradient between the intra- and perivascular space (Garcia and Malik, 2005; Matthay and Martin, 2005; Mehta and Malik, 2006). Albumin and other macromolecules are actively transported through cells by an elaborate system of vesicles (transcellular pathway) (Mehta and Malik, 2006; Minshall and Malik, 2006; Minshall et al., 2003; Predescu et al., 2007). Water can also permeate EC via endothelial water channels called aquaporins (Agre, 2006; Verkman, 2002). Permeability properties of lung EC from various anatomic locations vary significantly, with microvascular EC being less permeable than macrovascular EC, which has been attributed to structural differences in interendothelial junctions and may be a mechanism to restrict alveolar flooding under increased vascular pressures (Parker et al., 2006; Parker and Yoshikawa, 2002; Stevens, 2005). A schematic representation of major lung endothelial properties in health is given in Fig. 1.

Lung EC fulfil important metabolic functions. Some substances (bradykinin, endothelin, angiotensin I) are cleared from the blood by lung EC while others (Angiotensin II, Nitric Oxide—NO, prostacyclin) are produced in response to a variety of stimuli and regulate among others vascular tone, angiogenesis and cell proliferation (Barnes and Liu, 1995; Michiels, 2003) (Fig. 1). In addition, pulmonary EC form a barrier that separates the blood-borne humoral (coagulation factors) and cellular (platelets) elements of coagulation from the thrombogenic sub-endothelial tissues. The endothelial glycocalyx, a complex layer composed primarily of proteoglycans and glycoproteins forms an anti-adhesive surface; in addition, mediators secreted by EC and other cells, including NO, prostacyclin, heparin and activated protein C prevent platelet aggregation and clotting (Michiels, 2003; Weinbaum et al., 2007). EC damage exposes the basement membrane components to the blood, thus initiating coagulation. EC actively regulate hemostasis by producing pro-thrombotic substances (von Willebrand factor, P-selectin) and restrict coagulation by producing anti-thrombotic (heparan sulfate, thrombomodulin, NO, prostacyclin, tissue factor pathway inhibitor) and fibrinolytic (plasminogen activators) factors (Edelberg et al., 2001). Finally, EC can be actively involved in regulating innate immune system responses by secreting inflammatory mediators and expressing the molecular machinery that allows them to be activated and participate in host defence (Orfanos et al., 2004).

The above functions are not performed by each lung EC to the same degree and considerable heterogeneity exists between lung EC from different vascular segments of bronchial and pulmonary circulation in respect to structure and function; differences include cell shape, nuclear orientation, barrier properties, adhesion molecule expression, proliferation rates, leukocyte trafficking, and responses to intracellular Ca^{2+} increases and physical stretch (Aird, 2003; Chetham et al., 1999; Doerschuk, 2000; Gebb and Stevens, 2004; Parker et al., 2006; Parker and Yoshikawa, 2002; Stevens, 2005). These differences seem to reflect the presence of anatomically and functionally distinct EC subpopulations, as can be demonstrated by the differential gene expression and surface marker profile maintained by EC from different vascular segments when the cells are cultured under the same *in vitro* conditions. However, the influence of local micro-environment factors,

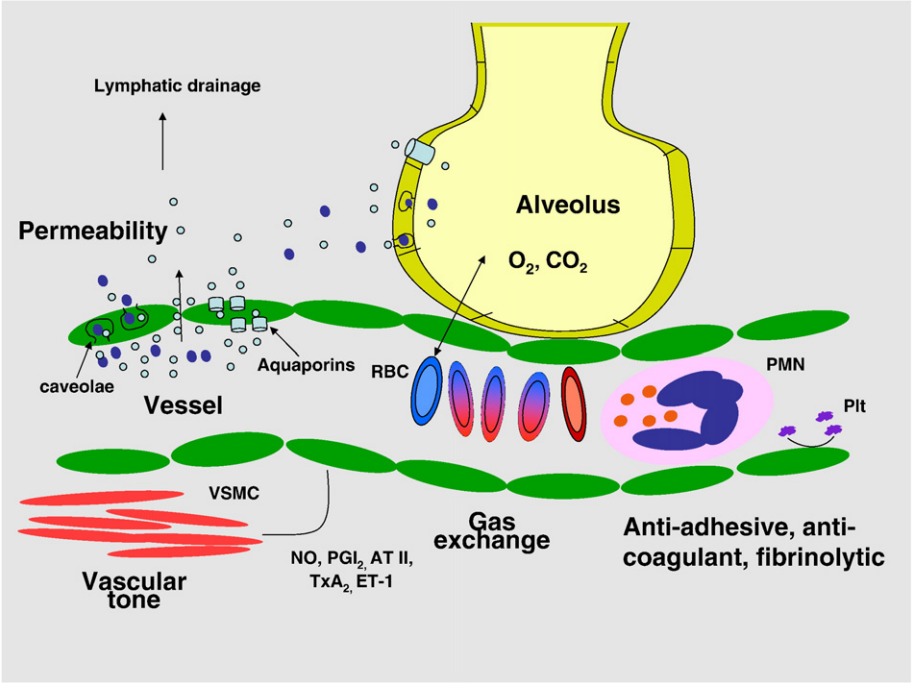

Fig. 1. Major pulmonary endothelial functions in health: The structure of pulmonary microvessels and endothelial cells is adapted in such a way as to enable efficient gas exchange of the entire cardiac output at baseline and under exertion. To this end, permeability and vascular tone are kept low and perfusion is matched to ventilation by synthesis and release of several vasoactive compounds such as angiotensin II (AT II), prostacyclin (PGI_2), thromboxane (Tx) A_2, nitric oxide (NO), and endothelin-1 (ET-1). The high vascular compliance of the lung enables it to act as a blood reservoir. Regulation of coagulation and thrombolysis and promotion of hemofluidity by a number of systems aids in maintaining rapid and unobstructed blood flow. In addition, lung endothelial cells (EC) are metabolically highly active, expressing enzymes (such as angiotensin converting enzyme, endothelin converting enzyme, nucleotidases, NO synthase and lipoprotein lipase), receptors, and signal transduction molecules, and synthesizing anti-coagulant and hemostatic factors. Other important functions of lung EC include removal and biotransformation of drugs, participation in immune reactions, binding of immune complexes, internalization of microorganisms and blood components such as leukocytes and platelets. RBC: red blood cells, PMN: polymorphonuclear granulocytes; VSMC: vascular smooth muscle cells, Plt: platelets, dark blue circles: albumin; light blue circles: water. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

such as paracrine and endocrine signals from matrix or other EC, oxygen tension, blood pressure and flow is also considered crucial to EC phenotypic differentiation (Aird, 2003, 2007a,b; Garlanda and Dejana, 1997; Stevens, 2005).

3. Pathogenesis of ALI

3.1. Coagulation and inflammation cross-talk

Inflammation and coagulation are critical host responses to infection and injury and are involved in ALI pathogenesis. The lung endothelium provides the surface that integrates inflammatory pathways of the innate immune system with the coagulation cascade (Levi et al., 2002). Clinical observations documented the presence of fibrin deposition as a marker of hemostasis in addition to intravascular clots and inflammatory markers in the lungs of ALI patients (Fuchs-Buder et al., 1996; Quinn et al., 1987; Tomashefski, 1983). It has been recognized that EC orchestrate the immune and hemostatic response by shifting from their normal anti-thrombotic and anti-inflammatory phenotype to an "activated" state of endothelial "dysfunction" (Félétou and Vanhoutte, 2006), characterized by pro-thrombotic and pro-adhesive properties. Key events to this transformation are the expression of adhesion molecules to leukocytes and platelets on the EC surface in addition to the expression of activators of the humoral clotting system, including tissue factor (Scarpati and Sadler, 1989) and von Willebrand factor (Sabharwal et al., 1995a). The above processes are launched by a variety of stimuli including hypoxia, cytokines and chemokines, inflammatory mediators and activated platelets and neutrophils. Key aspects of pulmonary endothelial pathobiology under lung injury are illustrated in Fig. 2.

3.2. Endothelial interactions with blood-borne cells

Recent evidence points to a pivotal role of platelet–neutrophil interactions in ALI. Platelets can rapidly stimulate neutrophils by excreting their vast arsenal of stored mediators (Brandt et al., 2000). It was recently shown in mouse models of acid aspiration and endotoxemia that the process of neutrophil activation is enhanced by binding to activated platelets with subsequent formation of platelet–neutrophil complexes and release of the inflammatory, vasoconstricting and permeability-enhancing prostaglandin thromboxane A_2 (Zarbock et al., 2006).

Stimulated neutrophils transmigrate along chemotactic gradients into lung tissue across the endothelium *en route* to sites of pathogen invasion or tissue damage due to a toxic or physical insult. In contrast to the systemic circulation, where neutrophils seem to migrate primarily through post-capillary venules, a major site of neutrophil sequestration in the lung appears to be the alveolar capillary bed (Burns et al., 2003; Doerschuk, 2000). This is likely a consequence of the unique pulmonary capillary microanatomy, since many pulmonary capillaries are narrower than neutrophils, forcing them to deform in order to pass through (Burns et al., 2003; Doerschuk, 2000; Worthen et al., 1989). Activated neutrophils undergo cytoskeletal rearrangements that render them stiff and unable to deform; they are thus trapped in capillaries, where they can adhere on activated EC. Neutrophils and other leucocytes in the lung may also marginate at larger vessel sites, including arterioles or post-capillary venules (Ichimura et al., 2005). Ligands of the selectin family, comprising L-selectin (expressed on leukocytes), E-selectin (expressed on EC) and P-selectin (EC and platelets) tether circulating leucocytes reversibly on the EC surface and facilitate rolling of leucocytes along the endothelium to the point of transmigration,

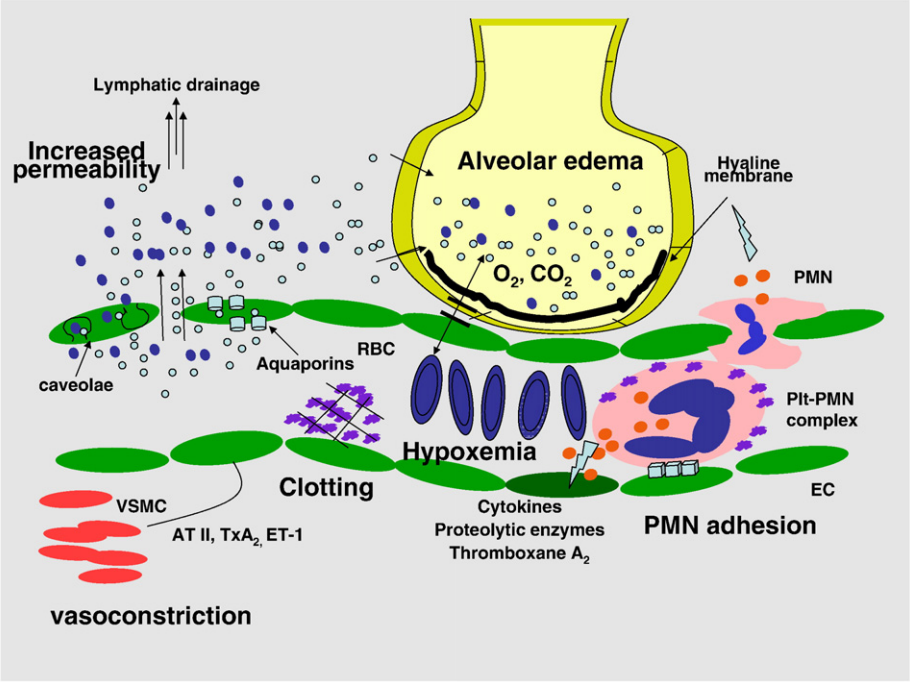

Fig. 2. Endothelial responses in acute lung injury: EC respond to a variety of insults by converting from an anti-adhesive, low permeability barrier to an adhesive, high permeability cell layer, as described in detail in the text. Common endothelial insults include microbes (e.g., bacteria, viruses, protozoa), hyperoxia, radiation, immune complexes, drugs, ischemia/reperfusion, toxins, mechanical stretch and microemboli. Adhesion molecules on the surface of EC orchestrate transmigration of circulating immune cells, which secrete microbicidal and cytotoxic substances such as oxygen free radicals and proteolytic enzymes. Increased endothelial permeability and vascular tone is mediated by vasoactive substances, including thrombin, angiotensin II (AT II), endothelin-1 (ET-1), thromboxane (Tx) A_2, tumor necrosis factor-α and interleukin-8, which are secreted by a number of cell types (EC, platelets, neutrophils, airway epithelia, macrophages). The rationale for these responses seems to be the clearance of infectious agents and damaged host cells; however, life-threatening pulmonary dysfunction and respiratory failure can occur as a consequence of lung flooding and hemodynamic compromise. These alterations trigger a set of anti-inflammatory and repair processes, which, in many cases, successfully restore vascular integrity. RBC: red blood cells, PMN: polymorphonuclear granulocytes; VSMC: vascular smooth muscle cells, Plt: platelets, dark blue circles: albumin; light blue circles: water. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

which is usually the junction of three adjacent EC (Burns et al., 2003). P-selectin is stored on Weibel–Palade bodies in EC and appears on the surface following exposure to stimuli such as bacterial components, ventilator-induced distension, acid aspiration, hypoxia, angiotensin II, thrombin etc. in a tyrosine kinase- and Ca^{2+}-dependent manner (Bhattacharya et al., 2003; Cleator et al., 2006; Ge et al., 2007; Into et al., 2007; Pinsky et al., 1996; Yiming et al., 2005; Zarbock et al., 2006). Firm adherence of marginated neutrophils on EC seems to be essential for subsequent transmigration. An important event in neutrophil–EC adherence is the binding of neutrophil surface integrins on their EC ligands (Intracellular Adhesion Molecule-1-[ICAM-1] and Vascular Cell Adhesion Molecule-1-[VCAM-1]) (Burns et al., 2003; Carlos and Harlan, 1994). Data from animal studies indicate that the type of integrin expressed by the neutrophils may vary depending on the noxious stimulus. For example, *E. coli* endotoxin administration in the lung seems to induce CD11b/CD18-dependent neutrophil migration; in contrast, CD11b/CD18 was not required for neutrophil diapedesis in a model of *Streptococcus pneumoniae* infection (Burns et al., 2003; Doerschuk et al., 1990). Ligation of EC adhesion molecules by neutrophil binding triggers downstream signaling pathways in EC that induce cytoskeletal contraction and junctional opening to allow neutrophil migration (Hordijk, 2006). A significant fraction of neutrophils and lymphocytes can migrate across the endothelium by traversing the EC cytoplasm engulfed in large vesicles. These vesicles have been shown to have a caveolin-positive coat in lymphocyte transmigration experiments, although it is unknown if neutrophils can also be transported through these giant caveolar structures (Millán et al., 2006). Ligand binding to adhesion molecules such as ICAM-1 also induces nuclear translocation of Nuclear Factor κB (NF-κB) and induction of adhesion molecules and other inflammation-related gene products (Hordijk, 2006). Infiltrating activated neutrophils release proteolytic enzymes (neutrophil elastase), which digest tissue, and large amounts of oxygen-based free radicals, which have bactericidal and cytotoxic effects.

3.3. Coagulation and fibrinolysis

Intravascular coagulation in ALI signifies the preponderance of hemostatic factors over anti-coagulant factors, thus disturbing the equilibrium that maintains hemofluidity. Intravascular thrombi may form on denuded vessel walls following EC desquamation via activation of the intrinsic pathway. They may also form by activation of the extrinsic pathway, which is initiated by tissue factor expression on EC and other cells, including macrophages. Cytokines such as interleukin-6 induce tissue factor expression, while tumor necrosis factor-α (TNF-α) blocks coagulation-inhibiting and fibrinolytic pathways (Schultz et al., 2006). Initiation of the extrinsic coagulation pathway by tissue factor leads to proteolytic cleavage of prothrombin and thrombin release, which has important downstream effects, including cleavage of fibrinogen to fibrin and platelet activation by binding to proteinase-activated receptors (PAR). Thrombin acts on EC via the same receptors and evokes several effects, including Ca^{2+} release, EC contraction and permeability increase (Coughlin, 2005; Garcia and Malik, 2005). EC actively regulate hemostasis by producing pro-thrombotic substances (von Willebrand factor, P-selectin) and restricting coagulation by liberating anti-thrombotic (heparan sulfate, thrombomodulin, NO, prostacyclin, tissue factor pathway inhibitor) and fibrinolytic (plasminogen activators) factors (Edelberg et al., 2001). It follows therefore that EC play a primary role in ALI pathogenesis by implementing the cross-talk between inflammatory and clotting pathways (Levi et al., 2002). Finally, ALI resolution also depends on preservation of EC function

(Komarova et al., 2007). Intravascular clotting is counteracted by EC on multiple levels. Tissue factor is inactivated by tissue factor pathway inhibitor up-regulation. Thrombin binding to thrombomodulin and the endothelial protein C receptor (EPCR) on the surface of EC releases activated protein C (APC), which degrades thrombin and elicits cytoprotective effects, intriguingly through PAR ligation (Esmon, 2004, 2006; Looney and Matthay, 2006). Tissue plasminogen activator promotes fibrinolysis. Activated EC also respond by synthesis of a whole host of substances aimed at inactivating cytotoxins, including antioxidants (Maniatis and Orfanos, 2008).

3.4. Vasoactive compounds

The normal balance between pulmonary vasodilators and vasoconstrictors is disrupted in ALI in favor of the latter, thus resulting in increased pulmonary vascular resistance and pulmonary hypertension (Matthay and Martin, 2005; Moloney and Evans, 2003). Most vascular alterations in ALI are driven by mediators secreted by immune cells, EC or platelets, which bind to their receptors on EC and vascular smooth muscle cells and influence permeability, vascular tone and gene expression. These mediators are thoroughly reviewed in several related articles (Garcia and Malik, 2005; Kosmidou et al., 2008; Maniatis and Orfanos, 2008; Moloney and Evans, 2003; Orfanos et al., 2004) and include among others thrombin, oxygen and nitrogen free radicals (NO, superoxide radical), prostaglandins, leukotriens, cytokines and growth factors (TNF-α, interleukin-8, transforming growth factor-β, vascular endothelial growth factor-1), endothelins and angiotensin II. The precise role for most of these substances in human ALI is largely unknown, particularly since each molecule can trigger many different effects on different cell types and most of the current understanding is derived from animal experiments performed under more or less strictly controlled conditions. However, emerging evidence is beginning to shed light into the uncertainty. Endothelial NO synthase (eNOS), an enzyme abundantly expressed in the lung, constitutively produces NO from L-arginine and oxygen in an NADPH-dependent fashion (Dudzinski et al., 2006). NO production is stimulated by Ca^{2+}-dependent and independent pathways (Maniatis et al., 2006; Sessa, 2004). NO is a potent vasodilator and inhibitor of hypoxic pulmonary vasoconstriction and platelet aggregation. It also regulates gene expression and protein function. NO may regulate NF-κB activity (Peng et al., 1995) and increased levels of eNOS-derived NO have been correlated with improved lung outcomes in endotoxemia, ventilator injury and ischemia–reperfusion models (Garrean et al., 2006; Kaminski et al., 2007, 2004; Takenaka et al., 2006; Yamashita et al., 2000; Zhang et al., 2006). Conversely, NO may have deleterious effects, such as hypotension and cytotoxicity (Förstermann and Münzel, 2006). Animal studies seem to support the notion that high-output NO from inducible NO synthase (iNOS), in addition to its bactericidal effects, can worsen lung injury, presumably by direct effects of highly reactive and toxic NO metabolites on various proteins and lipids (Förstermann and Münzel, 2006; Gow et al., 1998).

Prostacyclin is produced downstream of cyclooxygenase by prostacyclin synthase in EC and, like NO, is also a potent vasodilator and inhibitor of platelet aggregation acting primarily through cyclic AMP as opposed to NO, which predominantly activates cyclic GMP production (Egan and FitzGerald, 2006). In small studies in children and adults with ALI, aerosolized prostacyclin improved oxygenation (Dahlem et al., 2004; van Heerden et al., 2000). In a study addressing the mechanism of action of prostacyclin and PGE_2 on EC, it was shown that these compounds enhanced the barrier properties of cultured EC at baseline, attenuated thrombin effects, and conferred lung protection in an experimental model of ventilator injury (Birukova et al., 2007). Another product of cyclooxygenase secreted by platelets or EC, thromboxane A_2, has opposite effects, including platelet aggregation, vasoconstriction and barrier disruption (Moloney and Evans, 2003).

3.4.1. Renin–angiotensin–aldosterone system (RAAS)

RAAS is a potent biological system that plays a key role in pulmonary and systemic vascular homeostasis. Angiotensin converting enzyme (ACE), the key RAAS enzyme, is highly expressed on the surface of pulmonary microvascular EC (Aird, 2007b); ACE is mainly responsible for the hydrolysis of angiotensin I to angiotensin II and the breakdown of bradykinin. In addition, ACE bears a distinct outside–in pathway involving c-Jun N-terminal kinase (JNK) (Ryan and Sigmund, 2004). Angiotensin II induces smooth muscle constriction (SMC), proliferation and growth, while in contrast non-inactivated by ACE bradykinin exerts vasodilatory, anti-inflammatory and anti-thrombotic actions (Orfanos et al., 2004). Angiotensin II bears a variety of additional pro-inflammatory actions: acting via the angiotensin receptor 1 (AT_1), it stimulates the expression of interleukins-1, -6 and -8, of adhesion molecules such as ICAM-1 and VCAM-1, and it induces NF-κB transcription. In contrast, stimulation of angiotensin receptor 2 (AT_2) is mainly associated with anti-inflammatory actions, such as decreases in IL-6 and NF-κB, and increased expression of IL-10 (Unger and Stoppelhaar, 2007).

Accumulating information over the last years has provided evidence for the implication of RAAS, and more in particular of ACE, in the pathogenesis of acute lung injury. ACE D allele has been associated with higher enzyme activity and ACE insertion/deletion polymorphism appears related to patients' susceptibility and outcome in several ALI/ARDS studies, with the DD genotype being associated with worse prognosis (Adamzik et al., 2007; Lee et al., 2005; Marshall et al., 2002b). Although the aforementioned association is not always present (Villar et al., 2008), it does point to a link between higher ACE activity and the severity of lung injury.

Increasing experimental evidence suggests that RAAS activation in the lungs and in particular angiotensin II enhance ALI. In this respect Yamamoto and co-workers have shown in the late 90s that angiotensin II induces pulmonary edema in the rabbit (Yamamoto et al., 1997). More recently several research groups have provided evidence that ACE inhibition or AT_1 blocking ameliorates lung injury induced by oleic acid administration or animal exposure to VILI (He et al., 2007; Jerng et al., 2007; Yao et al., 2008). Angiotensin II has been additionally implicated in the pathogenesis of the fibroproliferative response that follows lung injury, an effect also attenuated by ACE inhibition and AT_1 blocking (Marshall et al., 2002a). Thus, blocking the deleterious effects of angiotensin II in the lung might offer protection against ALI development and its sequelae.

3.4.2. Pulmonary endothelial ACE

Pulmonary endothelial ACE is an ectoenzyme uniformly distributed along 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 (Orfanos et al., 1997, 2004). Due to the very high enzyme concentrations in the capillaries, monitoring pulmonary endothelial ACE activity in this type of studies equals in practical terms to monitoring pulmonary capillary endothelium-bound (PCEB) ACE activity (Catravas and Orfanos, 1997; Orfanos et al., 1999). 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 reduction has been among the earliest signs in different ALI animal models, preceding changes in parameters such as acid–base balance, gas exchange, hemodynamic parameters, increased permeability, and morphologic changes at the light and electron-microscopy level. This is the case following administration of bleomycin to rabbits, exposure of rabbits to hyperoxia, phorbol myristate acetate (PMA) administration to rabbits and dogs, and chest-irradiation to rabbits (Orfanos et al., 2004).

Ten years ago, similar techniques were introduced to humans and, like in the animal studies, they offer quantification of pulmonary

endothelial function and estimates of pulmonary functional capillary surface area in health (Orfanos et al., 1999) and disease. In relation to the latter, PCEB-ACE activity has been validated in mechanically ventilated patients belonging in high risk groups for ARDS development and suffering from various degrees of ALI/ARDS (Orfanos et al., 2000). Indices reflecting enzyme activity per capillary as well as the functional capillary surface area (FCSA) index A_{max}/K_m decreased early during the ALI/ARDS continuum and were inversely related to Lung Injury Scores, implying that the clinical severity of the syndrome is related to the degree of PCEB-ACE activity depression (i.e. the underlying pulmonary endothelial dysfunction). In a similar respect, PCEB-ACE activity was assessed in patients undergoing total knee arthroplasty; enzyme activity depression occurred in association to lung injury induction secondary to embolic lung syndrome (Jules-Elysee et al., 2004). In addition to the aforementioned PCEB-ACE activity reductions seen in acute lung vascular pathologies, enzyme activity depressions appear to occur in chronic lung vasculopathies related to systemic sclerosis and pulmonary arterial hypertension (Langleben et al., 2008; Orfanos et al., 2001).

3.4.3. The ACE paradox

The fact that ACE and angiotensin II promote lung injury which is attenuated by ACE inhibitors and/or AT_1 blocking appears in apparent contrast with the constant and well established finding that PCEB-ACE activity reductions occur under ALI in both animals and humans. One possible explanation of the observed paradox might be the following: Angiotensin II can generate O_2^- via the activation of NADH-/NADPH-oxidases in ECs and SMCs (Linz et al., 1999). Superoxide anions will then interact with NO to generate $ONOO^-$, which along with free radicals from several sources will cause molecular and cellular damage and decrease ACE activity. It is possible that the observed PCEB-ACE activity reduction seen in ALI may be related to an enzyme compensatory down-regulation, mediated by the aforementioned mechanism, aiming to limit ACE-mediated pro-inflammatory processes in the micro-environment, which would be more potent if ACE activity were higher. Alternatively and/or in addition, PCEB-ACE reductions may be related to crude endothelial losses, while viable ECs maintain or even over-express ACE, thus contributing to the local inflammatory process (see below, under radiation-induced lung injury).

3.4.4. Angiotensin converting enzyme-2

In 2000 an ACE homologue, named ACE2, was identified. ACE2 functions as a carboxypeptidase but possesses different substrate specificity than ACE; it cleaves a single residue from angiotensin I to generate angiotensin-(1–9), and a single residue from angiotensin II to generate angiotensin-(1–7) (Kuba et al., 2006). Information accumulated thus far suggests that this novel RAAS axis counterbalances the ACE angiotensin II pro-inflammatory action. In this respect, loss of ACE2 expression in mutant mice increases lung vascular permeability and edema formation, as well as neutrophil accumulation, while treatment with recombinant ACE2 appears to improve ALI in both wild-type and ACE2 knockout mice (Kuba et al., 2006). Of interest is the discovery that ACE2 serves as a receptor for the SARS corona-virus, a fact that may trigger ACE2 dysfunction contributing to the severe ARDS under SARS (Imai et al., 2007).

3.5. Angiogenic growth factors

Vascular development strongly depends on the collaboration of growth factors, which are either produced by EC and/or act on the latter; three different compound families are mainly involved in the process: vascular endothelial growth factors (VEGFs), angiopoietins and ephrins (Gale and Yancopoulos, 1999). Increasing attention has been drawn over the last few years on the contribution of the former two families in the pathogenesis and pathobiology of ALI/ARDS.

3.5.1. Vascular endothelial growth factor

VEGF is a potent mediator of vascular regulation in angiogenesis and of vascular permeability to water and proteins, bearing a central role in the growth and survival of ECs and their functions. The VEGF gene family includes several members; the central and best studied member is VEGF-A also referred to as VEGF. In the lung, VEGF acts as a pluripotent growth factor essential for lung development and efficient regulation of vascular permeability and angiogenesis (Papaioannou et al., 2006). VEGF appears to increase microvascular permeability approximately 20,000 times more potently than histamine; its effects on the endothelium comprise among others the release of NO and prostacyclin, the inhibition of apoptosis, and the promotion of cell-survival and differentiation, angiogenesis and vasculogenesis (Lahm et al., 2007). Hyperoxia has been shown to decrease pulmonary VEGF levels (Maniscalco et al., 1997), while VEGF mRNA expression is induced by several stimuli like hypoxia, endotoxin, growth factors and cytokines such as IL-1 and IL-6. For a detailed related analysis the reader is referred to the reviews by Lahm et al. (2007) and Papaioannou et al. (2006). VEGF appears to have a contributing but complex role in ALI/ARDS pathophysiology, depending on the disease stage and organ compartment. In this respect, decreased levels of VEGF were associated with EC apoptosis in early ARDS (Abadie et al., 2005), while in a different study plasma levels were found elevated and appeared associated with patients' mortality (Thickett et al., 2001). The aforementioned complex role of VEGF in ALI/ARDS has been thoroughly reviewed by Medford and Millar (2006) and has been demonstrated by a very recent investigation where recombinant VEGF administration enhanced alveolar and vascular regeneration in hyperoxic rat pups, albeit at the expense of transiently increasing permeability (Kunig et al., 2006). VEGF gene therapy has been additionally studied revealing promising results: hyperoxia-induced lung injury in newborn rats is among others accompanied by capillary loss that is associated with decreased lung VEGF expression. Intratracheal VEGF gene transfer appears to improve survival and promote lung capillary formation (Thebaud et al., 2005).

3.5.2. Angiopoietins

Angiopoietins (Angs) are a novel class of angiogenic growth factors, which are essentially implicated in the pathophysiology of sepsis and ALI. Three out of the 4 identified family members, namely Ang-1, 2 and 4 are expressed in humans; Ang-1 and Ang-2 are so far the best characterized compounds. Ang-1 is a Tie2 receptor agonist that induces EC migration and formation of capillary-like structures, inhibits EC apoptosis, reduces vascular permeability and inflammation, and promotes vascular integrity. In contrast, Ang-2 has context-dependent effects acting as either a Tie2 agonist or antagonist (Tsigkos et al., 2003). Ang-1 has been shown to exert an inhibitory effect on endothelin-1 transcript and protein levels *in vitro*, while cell-based Ang-1 gene transfer into the pulmonary circulation of experimental ARDS models, markedly reduced lung inflammation conferring protection against lung injury (McCarter et al., 2006, 2007). In a more recent report, the same investigators provided evidence that syngeneic mesenchymal stem cells transfected with human Ang-1 gene can reverse lung injury in mice pretreated with endotoxin, probably via an EC-related mechanism (Mei et al., 2007). In a similar respect, Huang et al. (2008) have recently shown that administration of an adenoviral vector expressing Ang-1 in a murine ALI model protects against the development of lung capillary protein leak and improves survival.

Angiopoietin-2 mainly acts as a modulator of endothelial barrier disruption, promoting vascular leak in ALI developed in septic and surgical critically-ill patients (Gallagher et al., 2007; Parikh et al., 2006), as well as in hyperoxia-induced lung injury (Bhandari et al., 2006). However, an Ang-2 autocrine protective effect on activated ECs that blocks vascular leak, has been additionally suggested (Daly et al., 2006). We have recently investigated the pattern of circulating Ang-2

levels in critically-ill subjects; serum Ang-2 levels were increased in severe sepsis and correlated with serum TNF-α and disease severity. Human lung microvascular ECs treated with endotoxin, TNF-α, and IL-6 responded by Ang-2 reduction, implying that human pulmonary endothelium should not be the source of increased Ang-2 in septic humans. In contrast, endotoxin and TNF-α stimulated Ang-2 release by bovine lung microvascular ECs, pointing to species-specific differences (Orfanos et al., 2007b). In a similar context, Gallagher et al. (2007) have more recently reported that circulating Ang-2 levels correlate with mortality in surgical patients suffering from ALI/ARDS.

3.6. Mechanisms of increased permeability

Increased-permeability edema due to disruption of the alveolo-capillary membrane and alveolar flooding with protein-rich fluid (Fein et al., 1979) is the hallmark of ALI (Matthay and Martin, 2005). As early as 1961 it was noted that vasoactive substances could induce extravascular fluid leak by inducing the formation of gaps between EC (Majno and Palade, 1961). In lungs from septic patients, the alveolar spaces contain abundant amorphous material, presumably extravasated plasma proteins and debris, leukocytes and erythrocytes (Bachofen et al., 1988). Ultrastructurally, epithelial cells showed a high degree of damage, compared to EC, which seemed much less affected. This was attributed by Bachofen et al. not to the lesser degree of injury sustained by EC, but more to the potential of this cell type to regenerate. It was later shown by Zhao et al. that EC of endotoxemic mice actually possess regenerative capacity, which depends on the up-regulation of transcription factor FoxM1 (Zhao et al., 2006).

Experiments addressing the mechanisms of endothelial permeability increases in ALI have focused on endothelial responses to agonists such as thrombin or histamine. Binding of such mediators to their cell-surface receptors elicits a series of signaling events that culminate in cell rounding and interendothelial gap formation, which represents disruption of interendothelial junctions (Garcia and Malik, 2005; Mehta and Malik, 2006). These cell-shape changes, which are reversible in most cases due to the initiation EC barrier-restorative processes (Broman et al., 2007), are thought to occur when EC come in contact with inflammatory mediators capable of inducing leakiness of the endothelial membrane and edema formation in ALI. The concept of actin cytoskeleton modulation by small GTPase RhoA and Myosin Light Chain Kinase (MLCK) is central to the paradigm of permeability increase by EC contraction (Dudek and Garcia, 2001, 2003; Garcia and Malik, 2005). Activation of RhoA by G-Protein-Coupled Receptors (Lutz et al., 2007) by a variety of substances including cytokines (interleukin-8), vasoactive molecules such as thrombin, histamine, bradykinin, thromboxane A_2, growth factors (VEGF) etc. results in actin polymerization and bundling of actin filaments to form thicker actin cables or stress fibers. Phosphorylation of Myosin Light Chain by MLCK accelerates cytoskeletal contraction by activating myosin, a molecular motor protein that is bound to actin stress fibers and generates the force required for cytoskeletal contraction (Dudek and Garcia, 2001). Support for the role of MLCK in sepsis pathophysiology was provided by studies in *ex vivo* perfused mouse lungs using pharmacological inhibitors (Parker, 2000), as well as *in vivo* in mice lacking the endothelial isoform of MLCK, which were found to be resistant to ALI elicited by endotoxin and mechanical ventilation (Ralay Ranaivo et al., 2007; Rossi et al., 2007; Wainwright et al., 2003). Endothelial MLCK-disrupted endotoxemic or septic mice showed reduced activation of NF-κB, inducible (i)NOS expression and oxidant production, and increased survival (Ralay Ranaivo et al., 2007). These findings suggest a link between cytoskeletal processes and inflammatory mediator production or, alternatively, a direct role of MLCK in regulating the endothelial response in sepsis in addition to permeability increases, as described in cultured cell studies (Petrache et al., 2001). The involvement of MLCK in sepsis was further solidified by the detection of genetic polymorphisms in humans. In this respect, polymorphisms of the MYLK gene, which encodes smooth muscle and non-smooth muscle MLCK, were found associated with sepsis and ALI (Gao et al., 2006).

Mechanisms of barrier breakdown not involving the action of MLCK have also been described; this is the case with TNF-α, which can induce junctional instability by Src-family tyrosine kinase-mediated phosphorylation of junctional protein vascular endothelial cadherin independent of MLCK activation and actin stress fiber formation (Angelini et al., 2006; Petrache et al., 2001).

4. Pulmonary endothelial responses to specific insults

4.1. Endotoxin

Endotoxin, or lipopolysaccharide, is a component of the outer membrane of Gram-negative bacteria. By inducing an exaggerated host immune reaction, endotoxin is the key mediator of the Gram-negative sepsis syndrome. Levels of endotoxin have been correlated with sepsis outcomes (Brandtzaeg et al., 1989) and extracorporeal adsorption treatments aimed at removal of endotoxin from the circulation of septic patients presently undergo clinical testing (Cruz et al., 2007). Endothelial cells are directly stimulated by endotoxin binding to Toll-like receptor-4 (TLR-4), activating transcription of a number of mostly NF-κB-dependent genes that regulate inflammation and apoptosis (Bannerman and Goldblum, 2003; Zhao et al., 2001). Systemic endotoxin administration causes ALI in animals and is associated with neutrophil margination in the lungs. Endothelial TLR-4, as opposed to TLR-4 expressed on hematopoietic cells, appears to be critical in this process (Andonegui et al., 2003).

Whether lung injury is a direct effect of endotoxin action on EC or a consequence of margination and stimulation of neutrophils is not entirely clear. However, the available evidence points to a synergism between these two cell types and it seems that pulmonary edema in Gram-negative sepsis may be the composite of actions of endotoxin, immune cells and humoral mediators on lung EC. Endotoxin stimulation of cultured pulmonary endothelia from various species can among others induce gene transcription, cytokine release, adhesion molecule expression, apoptosis, actin cytoskeletal rearrangements and activation of endocytosis (Bannerman and Goldblum, 2003; Heckel et al., 2004; Tiruppathi et al., 2007; Zhao et al., 2001). Infusion of endotoxin to perfused lungs induces no changes (Held and Uhlig, 2000; Uhlig et al., 1995) or relatively mild vasoconstriction and increases in microvascular permeability, depending on species and dose (Salzer and McCall, 1990; Urbain et al., 1992; Walmrath et al., 1994). All studies though document that endotoxin pretreatment potentiates the effects of vasoactive substances or exotoxin (Salzer and McCall, 1990; Urbain et al., 1992; Walmrath et al., 1994). Since endotoxin triggers the endothelial release of molecules capable of altering permeability and vascular pressure in intact lung systems, including TNF-α, thromboxane A_2 and endothelin-1, it has been argued that most of the vasoactive effects are most likely due to these mediators and not due to direct TLR-4 signaling (Held and Uhlig, 2000; Horgan et al., 1993; Salzer and McCall, 1990; Schmeck et al., 2000; Urbain et al., 1992).

What is the significance of these data to the understanding of ALI pathogenesis by endotoxin *in vivo*? Is endotoxin alone enough to cause ALI or is the inflammatory response also required? A large number of studies addressing this issue have focused on the pathogenetic role of neutrophils in this process although the role of other immune cells is also beginning to emerge (O'Dea et al., 2005). Despite clear evidence that ALI can occur in neutropenic patients with sepsis (Laufe et al., 1986; Ognibene et al., 1986), the majority of the published literature seems to favor a central role of the neutrophil as an important effector cell that induces alveolo-capillary barrier disruption in ALI (Abraham, 2003). In this context, animal

experiments have shown that interference with neutrophil function on various levels, ranging from total neutrophil depletion to disruption of surface integrins or oxidant production, is associated with blunted lung vascular permeability increases (Gao et al., 2005; Gao et al., 2002; Xu et al., 2002). Tampering with neutrophil function comes at a price, however, and this is a severe impairment of host defence resulting in reduced bacterial clearance (Gao et al., 2002; Ong et al., 2005; Sadikot et al., 2004).

If interference with neutrophil function might be a risky enterprise, then why not intervene in some or other hemostatic mechanism? In order to answer this question, one would need to review evidence on the role of clotting in septic ALI. Studies in mice have documented the importance of clotting factors in ALI development secondary to endotoxin (Cruz-Topete et al., 2006; Xu et al., 2006). The relevance of platelet-derived mediators in activating neutrophils and coagulation has also been demonstrated (Clark et al., 2007; Voelkel et al., 1992). Large clinical trials utilizing these approaches were conducted in the past ten years. Trials studying antithrombin III administration, in an effort to target thrombin, a central mediator of the septic process (Warren et al., 2001), and tissue factor inhibitor administration in an effort to block the related pathway (Abraham et al., 2003) did not reveal mortality benefits. In contrast APC administration improved patients' outcome (Bernard et al., 2001).

4.2. Activated protein C administration in severe sepsis

Treatment with APC had been promising in a number of animal studies and its use relies on solid scientific background (Mosnier et al., 2007): APC appears to play a major role in sepsis, being an important regulator of the coagulation system (anticoagulant protein C pathway), while in addition numerous investigations have provided evidence that APC exerts direct cytoprotective effects on various cell types, and more specifically on EC. These cytoprotective effects appear mostly related to modulation of gene expression, anti-inflammatory and antiapoptotic activity and EC barrier stabilization (Mosnier et al., 2007; Orfanos et al., 2008). Most cytoprotective effects require the activation of protease activated receptor-1 (PAR-1), while the endothelial protein C receptor (EPCR) serves as a co-receptor. Animal studies by us and others, using APC inhalation documented lung-protective effects of the drug (Kotanidou et al., 2006; Slofstra et al., 2006), despite apparent variations in the mode of APC action probably related to differences in experimental designs.

APC administration in patients with severe sepsis has been one of the most promising and discussed topics in the Critical Care setting. In human trials APC has been efficacious in reducing mortality in severe sepsis, but this beneficial action appears restricted to the most severely affected patients (Martí-Carvajal et al., 2007). In the most recent guidelines of the Surviving Sepsis Campaign, the use of APC in adults was given a grade 2B recommendation (Dellinger et al., 2008), indicating lack of clarity regarding the risk–benefit ratio, and the European Union has mandated that this issue be addressed in a placebo-controlled clinical trial. Interestingly, pediatric patients do not appear to benefit from APC administration, and its use was clearly discouraged for this group (Dellinger et al., 2008). The fact that interventions that seem to work in the laboratory appear less efficacious in the clinical setting should probably not be interpreted as a failure of experimental studies to deliver valid disease concepts; they rather represent the discrepancy between the simple animal models of sepsis used in basic research and their shortcomings in reflecting the complexity of the clinical situation.

In an attempt to come up with a summary statement on the role of the pulmonary endothelium in endotoxemia, it seems that endotoxin through induction of mediators 'primes' EC to attract, capture and activate immune cells, which then act in concert with platelets and coagulation factors in mounting the inflammatory response that accompanies sepsis-derived ALI.

4.3. Biotrauma

Mechanical ventilation for respiratory failure is a critical mode of life support for many ALI/ARDS patients. Gravity forces distribute pulmonary edema in ALI in the dependent areas, resulting in low-compliance, poorly ventilated regions, and higher compliance regions, which receive the bulk of the gas volume delivered by the ventilator (Gattinoni et al., 2003; Gattinoni and Pesenti, 2005). To add insult to injury, increased ventilation is required for many patients in order to maintain adequate oxygenation in the face of a large physiologic dead space and to effectively eliminate the excess CO_2 produced under an activated state of metabolism. Thus, physicians end up forced to excessively ventilate lungs with limited airspace available for gas exchange, causing tissue overdistention and injury, and leading to a form of ALI termed "ventilator-induced lung injury" (VILI) (Gattinoni et al., 2003; Gattinoni and Pesenti, 2005). As demonstrated in animal and human studies, VILI can exacerbate pre-existing ALI, cause systemic inflammation, remote organ dysfunction and increase mortality (dos Santos and Slutsky, 2006; Petrucci and Iacovelli, 2007; The Acute Respiratory Distress Syndrome Network, 2000).

EC is a contractile cell possessing structures that sense mechanical forces and translate them to biochemical signaling events (mechanotransduction). Due to their coupling to alveolar cells, EC are exposed to stretch by mechanical ventilation that elicits a large number of EC biochemical and structural alterations. Researchers have therefore been engaged in the question of which are the key signaling pathways that govern cellular responses to VILI, and what would happen if EC were prevented from responding to stretch. These questions are particularly important in the setting of VILI; in contrast to ALI/ARDS, VILI represents an iatrogenic entity of known temporal onset and as such, it could be preventable by interventions targeted at the initial signaling pathways. Data from cultured pulmonary EC and isolated intact lungs consistently demonstrate increases in tyrosine phosphorylation which lead to P-selectin-dependent neutrophil margination (Bhattacharya et al., 2003; Ichimura et al., 2005; Shikata et al., 2005; Yiming et al., 2005). Phospho-Inositide-3-Kinase and Src non-receptor tyrosine kinase have been shown to be implicated in some of these events, as well as in enhancing microvascular permeability by direct action on EC (Miyahara et al., 2007; Okutani et al., 2005). Studies addressing the effects of endothelial Ca^{2+} influx clarified the significance of transient receptor potential V4 (TRPV4) Ca^{2+} channels in acute permeability alterations due to overdistention (Alvarez et al., 2006; Hamanaka et al., 2007; Townsley et al., 2006). In addition, excessive cyclic stretch (18% elongation) induces actin stress fiber formation due to RhoA-dependent MLCK activation in cultured human pulmonary artery EC (Birukov et al., 2003). Cells stretched in this protocol for 48 h and subsequently challenged with thrombin responded with greater permeability increases than cells not or physiologically stretched (5% elongation) (Birukov et al., 2003; Birukova et al., 2006). Further work using isolated rat lungs exposed to ventilator overdistention showed that MLCK inhibition prevented permeability increases (Parker, 2000). Finally, in a mixed model of endotoxin and VILI it was demonstrated that mice lacking EC MLCK had improved outcomes than wild-type controls (Ralay Ranaivo et al., 2007; Rossi et al., 2007). The above studies have elucidated mechanistically the direct effects of cyclic stretch on EC acute permeability alterations, and most likely on subsequent events including NF-κB activation and inflammatory gene expression (Held et al., 2001). They have thus provided important targets for pharmacologic interventions that could be clinically useful.

4.4. Transfusion-related acute lung injury

Transfusion of red blood cell concentrates (RBC) and blood products is a common practice in intensive care unit patients due to anemia of critical illness or blood loss. Transfusions can elicit ALI in a

small subset of patients and transfusion-related acute lung injury (TRALI) is the most common cause of death related to transfusions (Holness et al., 2004; Silliman, 2006). TRALI, which is otherwise clinically or pathologically indistinguishable from ALI occurs almost immediately (within 6 h but mostly in the first 1 to 2 h) (Silliman et al., 2005) following transfusion of various blood products including RBC and fresh frozen plasma (Holness et al., 2004). The identification of donor antibodies to human leukocyte antigens (Popovsky et al., 1983) in the recipient has spawned the hypothesis that TRALI is mediated by an immune reaction (Curtis and McFarland, 2006; Sachs, 2007; Silliman, 2006; Silliman et al., 2005). Further studies in the plasma of donated blood revealed the presence of mainly three types of antibodies possibly implicated in TRALI, including antibodies to HLA-I, HLA-II, and to Human Neutrophil Antigens (HNA) a class of neutrophil surface markers with poorly understood function (Curtis and McFarland, 2006).

The neutrophil appears to be the important effector cell in this process (Dry et al., 1999; Looney et al., 2006; Silliman et al., 2007). Cell culture and animal studies using models of TRALI have detected the release of inflammatory mediators and cytokines by neutrophils, which are incriminated in the pathogenesis of the syndrome (Grimminger et al., 1991; Wyman et al., 2002). Activation of neutrophils by anti-HLA antibodies induces endothelial adhesion molecule expression and cell death in cultured EC (Nishimura et al., 2007; Silliman et al., 2007), and permeability increases in perfused intact microvessels (Seeger et al., 1990); the mechanism of neutrophil activation however is not well understood. Since many patients receiving transfusions with antibodies to their white blood cells do not develop TRALI, it has been postulated that prior neutrophil activation and adhesion to lung EC in the setting of sepsis or other inflammatory condition is necessary for TRALI development (Silliman, 2006). In one of the largest clinical case series published, Gajic et al. showed that transfused patients with sepsis or history of alcohol use were more likely to develop TRALI (Gajic et al., 2007). Additionally in this same study TRALI was more often observed in transfused blood products from multiparous women, who are more likely to harbour anti-neutrophil antibodies due to immunization from fatal blood, providing indirect support for the role of antibodies as TRALI mediators.

However, not all cases of TRALI can be attributed to antibodies, since the syndrome has been seen with transfusion of products not containing antibodies or with autologous blood (Covin et al., 2004). These observations have led to the identification of another cause of TRALI, namely bioactive lipids (Silliman et al., 1994, 1998). Lyso-phosphatidylcholines are phospholipids that are released in stored blood components, including RBC and platelets. They can bind to their cell-surface receptors on neutrophils and activate oxidant release by NADPH-oxidase in a Ca^{2+}-dependent mechanism (Silliman et al., 2003b). In a prospective observational study, bioactive lipids accounted for the majority of TRALI cases (Silliman et al., 2003a). It seems therefore that TRALI is an uncommon but serious complication of transfusions that can lead to neutrophil-mediated EC toxicity and increased pulmonary microvascular permeability.

4.5. Pulmonary microembolism

Embolism into arteries with a diameter less than 200 μm can cause reversible increases in pulmonary microvascular permeability (Malik, 1983). This is explained by a combination of factors acting on EC, including the release of vasoactive EC mediators, the clotting system (thrombin), and inflammatory cells (Malik, 1983). The degree of lung dysfunction depends on the amount and nature of embolized material. For example, embolized tumor cells are not known to cause edema, as opposed to amniotic fluid, which contains antigenic fetal substances and often leads to respiratory failure. This pathophysiologic concept may be relevant to a variety of disease processes such as sickle cell crisis, fat embolization from fractured bones or fat-containing internal organs, and embolism by amniotic fluid, gas, silicone or cement used in surgical or endovascular procedures, although in most cases the pathomechanism remains incompletely understood (Barie and Malik, 1982; Fabian, 1993; Fukaya and Hopf, 2007; Mirski et al., 2007; Moore and Baldisseri, 2005; Schmid et al., 2005). In the following section we will briefly discuss some of the salient features of sickle cell acute chest syndrome.

4.6. Acute chest syndrome

The acute chest syndrome is a devastating and relatively common complication of sickle cell disease and related hemoglobinopathies (hemoglobin [Hb] SS, Hb SC, Hb S-thalassemia) (Melton and Haynes, 2006). It usually occurs during a vaso-occlussive episode during which the lung microvessels are occluded by adherent sickle cells and white blood cells. Emboli from infarcted bone marrow further obstruct pulmonary microvessels (Maitre et al., 2000; Melton and Haynes, 2006; Vichinsky et al., 2000). These events complicate a state of pre-existing endothelial dysfunction in sickle cell patients characterized by increased oxidant production by Hemoglobin S, membrane lipid peroxidation, decreased NO bioavailability, increased endothelin-1 secretion and adhesiveness (Brown et al., 2001; Nath et al., 2000; Phelan et al., 1995; Pritchard et al., 2004; Shiu et al., 2000; Telen, 2007).

4.7. Ischemia/reperfusion lung injury

Numerous publications over the last years along the clinical experience accumulated from humans undergoing lung transplantation or resuscitation from hemorrhagic shock (HS) have revealed the impact of ischemia/reperfusion (I/R) on the lung. ALI post-I/R is mainly associated with the formation of reactive oxygen and nitrogen species (Hamvas et al., 1992; Ischiropoulos et al., 1995), as well as with leukocyte interactions with the activated pulmonary endothelium (Martinez-Mier et al., 2001). The lung may in addition be the target of pro-inflammatory mediators released by distal I/R. In this respect mesenteric I/R in the rat impaired pulmonary endothelial-dependent vasorelaxation, and induced increased neutrophil lung accumulation (Fullerton et al., 1996), while in a more recent publication Boutros et al. (2005) have shown that I/R of the lower extremities in the rat was associated with lung injury characterized by neutrophilic infiltration and increased vascular permeability. ALI in that model was accompanied by an increase in pulmonary expression of inducible heme-oxygenase, while inhibition of the latter enhanced ALI (Boutros et al., 2005). The harmful I/R effect on the endothelium was further revealed by the observed shedding of the endothelial glycocalyx in patients undergoing surgical procedures involving global ischemia, and regional ischemia of the heart and the lungs; both procedures induced significant increases of the endothelial glycocalyx components syndecan-1 and heparin sulfate in blood (Rehm et al., 2007).

Several investigations have focused on the effect of I/R and related interventions on pulmonary endothelium-bound ACE. Atochina and coworkers have provided evidence that normoxic lung I/R induces ACE shedding from the pulmonary endothelium of isolated perfused rat lungs, an event associated with increases in lung vascular permeability (Atochina et al., 1997). Shedding of pulmonary endothelial ACE following I/R in a similar animal model appears to be attenuated by the anesthetic agent propofol which also carries anti-oxidant properties (Balyasnikova et al., 2005). In a preliminary recent report, we have provided evidence that normoxemic resuscitation of HS in rabbits is associated with decreases in PCEB-ACE activity a phenomenon associated with higher ICAM-1 and VCAM-1 lung expression; resuscitation with hypoxic mixtures preserved endothelial enzyme activity and attenuated lung inflammation (Orfanos et al., 2007a). An additional important role of pulmonary endothelium-bound ACE in I/R lung injury was recently shown by Nowak and coworkers; conjugation of the anti-oxidant enzyme catalase to anti-ACE antibodies allowed lung endothelium targeting and attenuated lung injury in a rat I/R model (Nowak et al., 2007).

Resuscitation from HS is a major global I/R phenomenon frequently met in the clinical emergency setting. The lung is among the principle organs affected, and the central role of cell adhesion molecules in I/R pathophysiology, leading to enhanced leukocyte–endothelial interaction and inflammation promotion, is well established (Martinez-Mier et al., 2001). Interestingly, numerous reports have provided evidence on the beneficial effects of several fluid resuscitation regimens used, and on their specific effects on leukocyte and endothelial adhesion molecules in the lung and other tissues. In this respect, resuscitation of HS by hypertonic saline in mice has been shown to diminish neutrophil rolling and adherence to endothelium *in vivo*, altering EC–PMN interactions and diminishing vascular permeability (Pascual et al., 2002). As a thorough analysis of this field is beyond the scope of this review, the reader is referred to Martinez-Mier et al. (2001).

4.8. Ionizing radiation and acute lung injury

Pulmonary tissue exhibits high sensitivity to ionizing radiation, an effect that limits the use of actinotherapy for lung and other thorax-related tissues. Exposure of the chest to ionizing radiation will produce a dose-dependent acute injury affecting endothelial, epithelial and interstitial cell types. Endothelial cells appear to be among the first cell types to undergo functional and structural injury. An initial acute radiation pneumonitis occurs, characterized by bronchoalveolar and endothelial swelling associated with increased capillary permeability, interstitial and intraalveolar edemas, and accumulation of inflammatory cells, and fibrosis. Several patients who have either undergone radiation pneumonitis development or did not develop the acute reaction will develop lung fibrosis in a later stage (Epperly et al., 2002).

Accumulating research has provided evidence that ionizing radiation affects the expression of various pro-inflammatory and growth-inducing genes such as those controlling the production of IL-1, TNF-α, transforming growth factor-beta (TGF-β), fibroblast growth factor, and the transcription of NF-κB (Giaid et al., 2003). Pulmonary radiation in mice appears to induce the expression of the adhesion molecules VCAM-1 and ICAM-1, initially detected in endothelin-positive ECs. These phenomena were associated with the late development of lung fibrosis and were delayed by the administration of manganese superoxide dismutase-plasmid/liposomes, suggesting that pulmonary endothelium may be a potential target for intervention against fibrosis (Epperly et al., 2002). In a similar respect, radiation induces persistent pulmonary endothelial PECAM-1 up-regulation, an event that probably leads to sustained platelet–EC adhesion and thrombosis (Gaugler et al., 2004), while increased formation of iNOS-synthesized NO and nitrotyrosine occur in an irradiated lung mouse model (Giaid et al., 2003).

Pulmonary endothelial ectoenzyme activity measurements have been shown to provide early and sensitive indices of radiation-induced endothelial lung injury. In this respect, rabbits exposed to 30 Gy of ionizing radiation produced early decreases of PCEB-ACE and -5′-Nucleotidase affinities for their substrates *in vivo* in the absence of structural endothelial damage (Catravas et al., 1988). The aforementioned early dysfunction of pulmonary endothelial ectoenzymes was further confirmed under a broad range of pulmonary blood flows in the rabbit heart-bypass model (i.e. hemodynamically independent), while animal treatment with indomethacin appeared to prevent most of these alterations implying a probable role for arachidonic acid metabolites (Orfanos et al., 1994). Several other investigators have reported pulmonary unilateral or bilateral ACE decreases post-irradiation, while plasma ACE levels measured either pre- or during-radiotherapy to the lungs were found lower in patients who developed radiation pneumonitis (Ward et al., 1987; Zhao et al., 2007).

In apparent contrast with the above-mentioned information and in agreement with the previously analyzed ACE paradox, treatment of radiated rats with a novel angiotensin II blocker and the ACE inhibitors captopril or enalapril protected the lungs from radiation-induced pneumonitis and fibrosis by interfering on pathways evolving TGF-β and alpha-actomyosin (Molteni et al., 2007). The ACE paradox in the setting of radiation-induced lung endothelial injury might be, at least partly, explained by the findings of Papapetropoulos and coworkers: exposure of bovine pulmonary arterial endothelial cells to radiation resulted in decreases of viable ECs leading to subsequent decreases of ACE activity per culture well, while in contrast ACE activity per surviving EC increased in a time- and radiation-dose manner (Papapetropoulos et al., 1993). It is therefore probable that the observed ACE activity reductions are related to EC losses, while the increased enzyme activity in the surviving cells may promote inflammation and cell proliferation in a paracrine pattern, phenomena that are attenuated by the administration of ACE inhibitors and/or angiotensin II receptor antagonists.

5. Conclusion

Acute lung injury and acute respiratory distress syndrome are “umbrella terms” to describe increased endothelial permeability-induced pulmonary edema and respiratory insufficiency in a variety of clinical circumstances. Endothelial cells are central in the pathogenesis of ALI. Research so far has been successful in providing a basic understanding of the disease mechanisms and has led to significant improvements in the treatment of ALI, most notably in regard to the proper way to ventilate and resuscitate these patients. Still, several important aspects remain unclear, especially in the field of lung repair processes. An important hurdle in the research is the difficulty in designing experiments that reflect the complexity of critically illness. In most laboratory studies, a defined stimulus is applied in a relatively short-term experiment and an outcome is measured. However, in clinical practice, several factors could predispose a patient to ALI at the same time, meaning that many redundant pathways studied individually in the lab would be concomitantly activated in real-life patients. For example, a patient with polytrauma may develop lung injury due to fat embolism, blood transfusion and health-care-associated infection; a patient with abdominal sepsis may develop lung injury due to endotoxin, ventilator stretch, ventilator-associated pneumonia and toxicity due to oxygen-rich gas mixtures. Nevertheless, as the understanding of the pathomechanisms advances, it will be more likely to develop therapeutic modalities that protect the lung in critical illness, as has been demonstrated by experimental endothelial targeted treatments (Christofidou-Solomidou et al., 2003; Nowak et al., 2007). This progress is imperative in order to deal with the still very high mortality and morbidity associated with the condition.

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