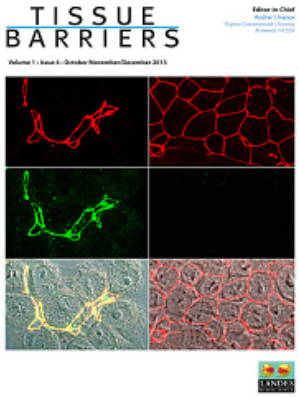

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Cytoskeletal mechanisms regulating vascular endothelial barrier function in response to acute lung injury

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Abbreviations: AJ, adherens junction; ALI, Acute Lung Injury; ARDS, Acute Respiratory Distress Syndrome; CaD, caldesmon; CPI-17, PKC potentiated inhibitory protein of 17 kDa; EC, endothelial cells; GJ, gap junction; HSP-27, small heat shock actin-capping protein of 27 kDa; IL, interleukin; LPS, lipopolysaccharide; MLC, myosin light chain; MLCK, Ca^{2+}/calmodulin (CaM) dependent MLC kinase; MLCP, myosin light chain phosphatase; MT, microtubules; MYPT1, myosin phosphatase targeting subunit 1; PKA, protein kinase A; PKC, protein kinase C; SM, smooth muscle; TLR4, toll-like receptor 4; TNFα, tumor necrosis factor α; TJ, tight junction.

Endothelial cells (EC) form a semi-permeable barrier between the interior space of blood vessels and the underlying tissues. In acute lung injury (ALI) the EC barrier is weakened leading to increased vascular permeability. It is widely accepted that EC barrier integrity is critically dependent upon intact cytoskeletal structure and cell junctions. Edemagenic agonists, like thrombin or endotoxin lipopolysaccharide (LPS), induced cytoskeletal rearrangement, and EC contractile responses leading to disruption of intercellular contacts and

EC permeability increase. The highly clinically-relevant cytoskeletal mechanisms of EC barrier dysfunction are currently under intense investigation and will be described and discussed in the current review.

Introduction. Lung endothelium forms a semi-permeable barrier between the blood and the interstitial space.[1] Disruption of endothelial barrier results in the movement of fluid and macromolecules into the interstitium and pulmonary air spaces causing pulmonary edema which is a common feature of Acute Lung Injury (ALI) and its more severe form Acute Respiratory Distress Syndrome (ARDS). The integrity of pulmonary EC monolayer is a critical requirement for tissue and organ homeostasis. EC barrier is heavily dependent upon the EC cytoskeleton network primarily microfilaments and microtubules which tightly linked to cell junction proteins.[1-4] This review will describe the cytoskeletal mechanisms of EC permeability increase, induced by various inflammatory conditions focusing on edemagenic agonists, like LPS and thrombin.

Clinical and physiological importance of the lung vascular barrier. The alveolar-capillary barrier is formed by the microvascular endothelium, the alveolar epithelium and the basement membrane. Direct or indirect injuries of the lung caused by inflammatory or toxic mediators can lead to pathophysiological syndromes such as severe pneumonia and ALI/ARDS. Despite recent therapeutic advances, these conditions still have high (30-40%) rates of patient mortality.[5] The acute phase of lung injury is characterized by a massive and rapid flood of protein rich edema fluid into the alveolar spaces as a consequence of increased endothelial permeability[5] (Fig. 1). Neutrophils are adhering to the injured endothelium and migrating through the interstitium into the alveoli,[6, 7] whereas the macrophages are secreting cytokines (IL-1, 6, 8 and 10) and TNFα.[8] ALI/ARDS leads to impaired gas exchange and may cause respiratory failure.[9] It is widely accepted that EC barrier dysfunction, a prominent feature of these clinical syndromes is tightly linked to agonist-induced cytoskeletal

remodeling resulting in the disruption of cell-cell contacts, paracellular gap formation and EC barrier compromise.[3, 4] Apart from ventilation strategies there is no standard treatment for pulmonary edema, making the investigation of regulatory mechanisms of endothelial barrier dysfunction highly clinically important.[5]

Endothelial barrier properties. The vascular endothelium serves as a semi-selective barrier lining in the vessel walls (Fig. 1). It dynamically regulates the liquid and macromolecule transport between the blood and the interstitial space.[10] The vasculature is lined by heterogeneous population of endothelial cells. This heterogeneity is derived from the origin of endothelial cells in the vascular tree. The barrier function, surface biochemistry, and morphology of confluent monolayers of microvascular and macrovascular endothelial cells are different for these two cell types.[11] In general, microvascular EC form a tighter barrier, compared to macrovascular one. It was found that permeability is ~16-fold less for sucrose and to 2-fold less for albumin in microvascular EC compared to macrovascular EC monolayers. [12] Conversely, primary cultures of microvascular EC produced 10 times higher transmonolayer electrical resistance (TER) compared to macrovascular one.[13] Although the precise mechanisms that regulate this variability are still under investigation, microarray analysis showed a significant variation in microvascular and macrovascular gene expression patterns.[14] Extracellular matrix proteins, collagen 4α1, collagen 4α2, and laminin were associated with microvessel endothelia, while fibronectin, collagen 5α1, and collagen 5α2 were seen with the large vessel endothelia.[14] Furthermore, electron microscopy revealed that microvascular EC have more developed intercellular junctions with more focal membrane adhesion sites per junction than the macrovascular cells.[12] Pulmonary artery endothelial cells (macrovascular EC) participate in blood homeostasis, blood-tissue exchange regulation under various conditions.[15] They share similarities in cell characteristics and in physiological properties with pulmonary microvascular EC. However, in vivo models of pulmonary edema

suggest that most fluid filtration occurs in the microcirculation.[16]

Endothelial permeability pathways. A variety of physical, inflammatory and bioactive stimuli alter the EC barrier leading to gap formation, increasing vessel permeability and compromising organ function. Permeability across endothelial and epithelial cell monolayers can involve transcellular, paracellular or the combination of both pathways (Fig. 2). The transcellular transport involves membrane-attached cytosolic caveolae that migrate through the endothelial cells and transfer macromolecules from the blood to the interstitium.[10] The main player in this process is the Src kinase, which can phosphorylate caveolin-1 on tyrosine residues inducing the migration of the vesicles across the endothelium.[17] Recent studies demonstrated that transcellular permeability increase precedes and may trigger paracellular permeability increase via signaling involved Src-mediated phosphorylation of caveolin-1.[18] However, majority of trafficking occurs through the paracellular route,[19] which will be described in this review in more details.

External stimuli leading to EC barrier compromise. The capillary endothelium is impermeable to macromolecules under basal conditions. This is due to the network of cytoskeletal and cell-junction elements which protect the endothelial barrier integrity. In state of acute or chronic inflammation, sepsis, diabetes, angiogenesis, or excessive level of mechanical alterations (stretch or shear stress), the EC barrier integrity is compromised. Inflammatory mediators such as LPS, thrombin, pro-inflammatory cytokines, or reactive oxygen species induce the loss of endothelial barrier function leading to permeability increase to solute and plasma proteins.[20-23]

LPS, a pro-inflammatory mediator and constituent of Gram-negative bacterial cell wall, directly disrupts macro- and microvascular EC barrier function in vitro and in vivo.[20, 24, 25] LPS primarily acts through the activation of toll-like receptor 4 (TLR4).[26] LPS-induced EC barrier dysfunction is correlated with actin reorganization and caspase-mediated cleavage of

cytoskeletal proteins that participate in cell-cell and cell-matrix adhesion.[25] Signal transduction mechanisms for LPS-induced EC permeability are not completely clear yet, but likely involve Tyr kinase(s), protein kinase C (PKC) as well as Rho signaling.[27-30] Murine lung injury induced by LPS is a model that has been shown to be largely consistent with sepsis-induced ALI.[31] Specifically, the injury elicited is characterized by neutrophil infiltration into the lung in association with increased inflammatory mediators including TNFα and NF-kB.[31]

Thrombin is a serine protease generated by injured endothelial cells by the cleavage of circulating prothrombin, participating in the prothrombinase complex which also contains factors X and V, Ca^{2+} and membrane phospholipids.[32] Thrombin not only induces coagulation, but also affects endothelial barrier function by releasing of inflammatory mediators and growth factors as well as inducing leukocyte adhesion on EC surface.[33] The cellular responses of EC to thrombin are mainly mediated through a thrombin-specific protease-activated receptor, PAR1. [34, 35, 36] In vitro, thrombin produces rapid, reversible, concentration-dependent increases in EC permeability as measured by the clearance rate of Evans blue dye-labeled albumin across EC monolayers[37, 38] or by changes in transendothelial electrical resistance.[21, 39, 40] Thrombin infusion in animals resembles that seen after LPS administration in several respects, including pulmonary hypertension and increased pulmonary vascular permeability.[41, 42] Interestingly, thrombin inhibitor, anti-thrombin III (AT III) prevents LPS-induced pulmonary vascular injury suggesting the involvement of thrombin in LPS-induced permeability response.[43]

Contractile mechanisms of EC permeability. Endothelial barrier integrity is maintained by the precisely regulated balance between actomyosin contractile forces and adhesive cell-cell, cell-matrix tethering forces.[4] Both competing forces are generated by the cytoskeleton comprising actin microfilaments, microtubules and intermediate filaments.[3, 4] Therefore, the

complex network of cytoskeleton is critical in the EC barrier regulation. Disruption of either intact actin or microtubule network leads to formation of paracellular gaps and permeability increase.[44, 45] Under quiescent conditions, when the balance is tilted towards tethering forces, a thick cortical actin ring can be observed, where endothelial cells can maintain tight connections with each other and the underlying matrix.[3, 4] Due to the effect of barrier-compromising agents like thrombin or LPS, the balance is shifted towards contractile forces (Fig. 3).

Thrombin cleaves and activates its G-protein-coupled receptor (PAR-1). Engagement of Gq protein leads to activation of phospholipase C resulting in intracellular [Ca^{2+}] increase.[46] Ca^{2+} elevation activates the Ca^{2+}/calmodulin (CaM) dependent myosin light chain (MLC) kinase (MLCK) that phosphorylates MLC and, consequently, actomyosin interaction and cell contraction will be evoked.[47, 48] Beside the Ca^{2+}/CaM-induced activation, endothelial (non-muscle) MLCK can be activated by Src-mediated Tyr phosphorylation on its unique N-terminal fragment, which is absent in smooth muscle (SM) MLCK.[49] Thrombin was shown to increase EC permeability in a Src/MLCK-dependent manner via MLC-mediated contractile mechanism.[37, 50]

Additionally, thrombin and LPS induced MLC-mediated EC contractile response and permeability via activation of Rho signaling pathway.[21, 29] The Ras homologous small GTPase Rho acts as molecular switch, cycling between an active GTP-bound and inactive GDP-bound state.[51] Rho activity is positively regulated by guanosine nucleotide exchange factors (GEFs) and inhibited by GTPase-activating proteins (GAPs), and GDP-dissociation inhibitors (GDIs).[52] Thrombin induced Rho activation involved $G_{12/13}$-mediated activation of p115RhoGEF, GEF-H1 activation, as well as PKC-mediated inhibition of GDI-1.[21, 53, 54] LPS-induced Rho activation dependent upon the activity of Src family kinases and direct nitration of RhoA at a Tyr side chain.[34 29, 55] GTP-bound Rho activates its downstream effector, Rho-

kinase, which increases MLC phosphorylation by two mechanisms: directly, via phosphorylation of MLC at Ser^{19} and indirectly, via phosphorylation of the targeting subunit (MYPT1) of the myosin phosphatase (MLCP). Phosphorylation of MYPT1 at the inhibitory Thr^{686} and Thr^{850} sites leads to the inhibition of MLCP, accumulation of phospho-MLC resulting in cell contraction.[56, 57 21]

Inhibition of MLCP also can be achieved through activation of CPI-17 (PKC potentiated inhibitory protein of 17 kDa). This soluble globular protein was first identified in SM cells, and later was found in several non-muscle cells including microvascular EC.[58, 59] Phosphorylation of CPI-17 at Thr^{38} by PKC increases its inhibitory potency toward MLCP ~1000-fold.[60,61] Histamine and thrombin (to a lesser extent) activate CPI-17 in PKC-dependent manner in ECs.[58] CPI-17 depletion significantly attenuates histamine-induced microvascular permeability increase implicating CPI-17-mediated mechanism of MLCP inhibition in EC barrier regulation[58] (Fig. 3).

EC barrier dysfunction and cytoskeletal rearrangement are not always associated with triggering contraction by an increase in MLC phosphorylation. Some agonists, like direct PKC activators induced EC permeability without increasing MLC phosphorylation at Ser^{19}/Thr^{18}.[62, 63] Phorbol ester-induced EC barrier dysfunction is accompanied by increased phosphorylation of a cytoskeletal protein, caldesmon (CaD).[63-65] CaD contains distinct binding sites for actin and myosin, thereby potentially regulating actomyosin interactions and promoting actin filament formation in the absence of MLC phosphorylation.[66-68] Phorbol ester-induced phosphorylation of CaD correlates with contraction and has been postulated as an on/off switch regulating actomyosin interactions in smooth muscle.[67] It is clear that CaD is directly involved in EC cytoskeletal arrangement and migration,[69] however, the functional significance of CaD phosphorylation in the regulation of EC barrier function have not been fully investigated.

Interestingly, PKC does not directly phosphorylate CaD. Phorbol ester-induced EC barrier dysfunction includes complex signaling involving sequential activation of Ras, Raf-1 and MEK resulting in activation of ERK1/2 MAP kinases,[70] which phosphorylate CaD and are responsible for CaD-mediated contractile response in smooth muscle.[67] Aside of ERK 1/2, another MAPK family member, p38 kinase is also directly involved in EC cytoskeletal remodeling and permeability.[71-73] p38, but not ERKs, is involved in thrombin-induced EC barrier compromise[73] and p38 signaling is involved in several in vivo models of lung injury including the LPS model of ALI.[74-76] p38 MAPK downstream targets contain several cytoskeletal proteins such as CaD and HSP-27.[77, 78]

Small heat shock actin-capping protein, HSP-27, is phosphorylated by MAP kinase-activated protein kinase 2 (MAPKAP kinase 2), that is in turn phosphorylated by p38 MAPK.[77, 79] Phosphorylation of HSP-27 promotes F-actin formation, membrane blebbing and mediates actin reorganization and cell migration in human endothelium.[89, 80-82] However, the role of HSP-27 in the regulation of EC permeability remains controversial. For example, pertussis toxin-induced EC permeability is temporally linked to p38 MAPK activation and phosphorylation of HSP-27 in EC[72]; and LPS-induced endothelial barrier dysfunction correlates with HSP-27 phosphorylation in vivo.[76] In contrary, depletion of HSP-27 did not prevent p38-mediated TGFβ-induced EC barrier dysfunction.[83] Therefore, the exact cytoskeletal targets of p38 MAPK in endothelium remain undetermined. The putative targets include ezrin/radixin/moesin (ERM) proteins, which may be phosphorylated through p38-dependent mechanisms,[84] but apparently, the role of ERM phosphorylation in EC barrier regulation is agonist-specific.[84-87] A few studies implicated the involvement of p38 activity in the activation of Rho/Rho kinase pathway and EC barrier dysfunction induced by TGFβ and Staphylococcus aureus-derived toxins.[83, 88] In contrast, inhibition of p38 has no effect on thrombin-induced MLC phosphorylation, which involves Rho activation.[21, 73] Finally, recent

study supports the cross-talk between p38 and Rho pathways in the regulation of microvascular permeability.[88]

Crosstalk between microtubules and microfilaments in EC permeability regulation. Paracellular gap formation evoked by barrier-disruptive agents resulting in increased endothelial permeability is governed by the coordinated communication among cytoskeletal elements. Disruption of microtubule (MT) structure leads to an increase in transendothelial permeability associated with a characteristic loss of the peripheral actin band as well as an increase in the density of actin stress fibers, increased levels of MLC phosphorylation, consistent with actomyosin contraction, and paracellular gap formation.[45, 89] Further, microtubule dissolution increased vascular permeability in murine model.[75] Vice versa, stabilization of microtubules protects EC monolayer in vitro and in vivo.[75, 90, 91] Edemagenic agonists like thrombin, LPS, TNFα and TGFβ induce partial microtubule dissolution accompanied by activation of EC contraction and permeability increase.[91-94] The effect of microtubule dissolution on actin reorganization is attributed to stimulation of Rho and p38 MAPK pathways, but not to an increase in $[Ca^{2+}]$, neither to MLCK or ERK1/2 activation.[45, 93, 95]

In the thrombin model of EC permeability microtubule disassembly precedes actin stress fiber formation.[96] Thrombin may induce microtubule dissolution via stimulation of $G_{12/13}$/p115RhoGEF cascade, followed by Rho/Rho kinase activation, resulting in phosphorylation of the microtubule-associated protein, tau.[93] In its unphosphorylated form, tau promotes assembly of microtubules and inhibits the rate of depolymerization.[97-99] Phosphorylation of tau decreases its capacity to bind microtubules and promotes MT assembly. [99, 100] Interestingly, p38 MAPK is also able to phosphorylate tau in vitro.[101] Inhibition of p38 attenuates microtubule dissolution and permeability increase induced by various agonists[94, 102] suggesting that thrombin-induced p38 activation may also be involved

in MT destabilization via tau phosphorylation.

Thrombin may also destabilize microtubules via Rho kinase-mediated phosphorylation and activation of LIM kinase (LIMK).[103] In quiescent conditions, LIM kinase is associated with microtubules. Thrombin treatment or ectopic expression of Rho kinase leads to dissociation of LIM kinase from microtubules accompanied by MT destabilization, phosphorylation/inhibition of cofilin, an actin depolymerization factor, resulting in F-actin assembly.[103]

It was also recently reported that thrombin may destabilize microtubules via dephosphorylation of stathmin, a MT-associated protein, which in its phosphorylated form stabilizes the microtubules.[104] However, the thrombin-induced phosphatase, which is able to dephosphorylate stathmin and is involved in thrombin-induced permeability increase, is not known yet.

Thrombin-induced microtubule dissolution may further activate Rho pathway via GEF-H1, which has been recently characterized as a Rho-specific GEF localizing on microtubules. [105] In its MT-bound state, GEF-H1 is inactive, whereas GEF-H1 release caused by MT disassembly stimulates its activity towards Rho.[106] Importantly, GEF-H1 is directly involved in thrombin-induced permeability increase.[53]

Microtubule dissolution may also affect cellular localization and activity of cytoskeletal regulatory proteins like CaD, which can be involved in EC barrier regulation. CaD co-purifies with microtubules from brain and potentiates tubulin polymerization.[107, 108] Phosphorylation of CaD by cell cycle-dependent cdc2 kinase (Pro-directed kinase, similar to MAPK) eliminates MT-binding activity of CaD, and also decreases CaD-mediated inhibition of actomyosin ATPase, consistent with contraction.[107, 108] Ectopic expression of CaD in fibroblasts eliminates the increase in focal adhesions and microfilament bundles induced by MT dissolution and Rho activation.[109]

Current findings describing the role of microtubule/microfilament crosstalk in thrombin permeability model are summarized on Fig. 4. Thrombin may induce cytoskeletal reorganization leading to permeability increase in two phases. In the initial phase thrombin-induced engagement of heterotrimeric G-proteins activates Rho (via p115RhoGEF) and p38 MAPK pools associated with microtubules, resulting in phosphorylation/activation of MT-associated proteins, like LIMK, tau and CaD. In addition, thrombin destabilizes microtubules by dephosphorylation of stathmin. In the final stage MT dissolution releases MT-associated protein complexes, further activating Rho (via GEF-H1) and p38 MAPK pathways, leading to increased phosphorylation of cytoskeletal targets, stress fiber formation, and barrier compromise.

Endothelial cell junctions and barrier regulation. The vascular endothelium is constantly exposed to hemodynamic stimuli, such as shear stress, contraction or dilation of the vessels. The continuous reorganization of cell junctions and the cytoskeleton have key importance in the maintenance of the endothelial barrier integrity. Reshaping of the cells allows the endothelial monolayer to adapt to the dynamic conditions to which it is exposed.[110] Inter-endothelial communicating structures mainly comprise of adherens junctions (AJ), tight junctions (TJ) and gap junctions (GJ) (Fig. 5).

AJs are critical in the maintenance of endothelial integrity providing connection between neighboring ECs, thus regulating endothelial barrier function. AJs represent the majority of cell junctions comprising the endothelial barrier, in contrast with epithelial cells where tight junctions dominate.[10] AJs are composed of VE-cadherin and its cytoplasmic binding partners: α-, β- γ-, p120 catenins, which link AJs to the actin cytoskeleton. The assembly of the VE-cadherin-catenin complex is regulated by phosphorylation, and their dissociation leads to EC barrier dysfunction.[111]

VE-cadherin is a transmembrane protein that mediates hemophilic binding of adjacent

cells in a Ca^{2+}-dependent manner.[111] The extracellular region contains five repeating domains which coordinate with calcium ions and form a rod-like structure. The intracellular tail of VE-cadherin has two domains, the juxtamembrane domain (JMD) and the C-terminal domain (CTD). JMD binds p120 catenin, while CTD binds β-catenin or plakoglobin (γ-catenin) which attach α-catenin to link the cadherin-catenin complex to the actin cytoskeleton. α-catenin also interacts with other actin-binding proteins, specifically, α-actinin, vinculin, TJ zonula occludin proteins: ZO-1, ZO-2, ZO-3 and possibly spectrin. VE-cadherin is critical for the proper assembly of AJs, and for normal endothelial barrier function.[112] VE-cadherin impairing results in interstitial edema and inflammation in lung and heart microvasculature.[113]

Catenins also play an important role in the regulation of AJ assembly. β-catenin has a dual role in cells. First it was identified as a component of AJs in the late '80s. Kemler and colleagues were able to isolate β-catenin together with α-catenin and plakoglobin.[114] Later genetic and embryogenic studies revealed β-catenin as a component of the Wnt signaling pathway playing an important role in embryonic development and tumorogenesis.[115] Recent study implicates the involvement of Wnt signaling in EC barrier regulation.[116]

Plakoglobin plays an important role in cadherin/catenin complex assembly, as a linker between this complex and F-actin cytoskeleton.[117] Plakoglobin is an intracellular binding partner for VE-cadherin in ECs and its main function is to stabilize the AJ complex.[117, 118] Through α-catenin, plakoglobin is in connection with actin-binding proteins, like α-actinin and ZO-1.[119] Plakoglobin is closely related to β-catenin, sharing 80% sequence identity[120] and can bind the cytoplasmic domains of the classical cadherins. Both β-catenin and plakoglobin were shown to stabilize the linkage between VE-cadherin and the actin cytoskeleton, thus regulating endothelial barrier function.[10] Thrombin-induced release of β-catenin and p120 catenin from the cell membrane has been described recently in human endothelium.[121] Interestingly, recent studies implicated the involvement of p120 catenin in inhibition of Rho

signaling in ECs.[122]

Regulation of AJs assembly and junctional permeability by reversible phosphorylation. The dynamic assembly and disassembly of AJs depends on protein-protein interactions regulated by reversible phosphorylation. Histamine, tumor necrosis factor (TNF) and vascular endothelial growth factor induced tyrosine phosphorylation of VE-cadherin, β-catenin and p120 thus increasing endothelial barrier permeability.[123] For instance, tyrosine phosphorylation on Tyr^{860} of VE-cadherin and Tyr^{654} on β-catenin leads to disassembly of the catenin-cadherin complex.[124] G_{12} binding to VE cadherin stimulates Src-mediated VE-cadherin phosphorylation at Tyr^{658} leading to AJ disassembly.[125] Recent studies revealed the possibility of AJ regulation by Ser/Thr phosphorylation as well. For example, activation of PKCα leads to phosphorylation of p120 catenin at Ser^{879} resulting in AJs disassembly.[126] The cytoplasmic domain of VE-cadherin is phosphorylated at $Ser^{684,-686,-692}$ creating more interaction sites for β-catenin binding.[111] Huber and Weis identified two residues in cadherin (Ser^{684} and Ser^{699}) which are phosphorylated by casein kinase 2 (CK-2) and glycogen synthase kinase 3 (GSK-3). This phosphorylation of cadherin could stabilize and strengthen the catenin-cadherin complex by several hundred folds.[111, 127, 128] However, there are some reports indicating that cadherin phosphorylation can be a negative factor for binding to β-catenin.[129] E-cadherin phosphorylation mediated by CK-2 leads to the disruption of AJs in keratinocytes.[130, 131]

Multiple kinases are involved in β-catenin phosphorylation such as casein kinase I (CK-I) and GSK-3β.[132, 133] These kinases induce the phosphorylation of β-catenin on $Ser^{33/37}$ and Thr^{41}, respectively, leading to its ubiquitination and proteosomal degradation.[132, 133] Wnt and other stimuli lead to the inactivation of GSK-3β, thus decreasing β-catenin phosphorylation, translocation into the nucleus and binding to transcription factors.[115] In contrary, Ser^{552} phosphorylation of β-catenin is not implicated in the Wnt signaling. In quiescent cells the

phosphorylation level of this serine residue is very low and phospho-β-catenin Ser[552] could be detected at the cell periphery of adjacent ECs. Phosphorylation of β-catenin at Ser[552] by AKT leads to its dissociation from cell contacts.[134, 135] Finally, the inhibition of Ser/Thr phosphatases caused hyperphosphorylation of β-catenin on Ser/Thr residues and resulted in the loss of cell-cell contacts[131] implicating the involvement Ser/Thr phosphatases in AJ assembly.

TJs regulate the transport of ions and solutes through the paracellular pathway.[136] They comprise of two families of transmembrane proteins, occludins and claudins as well as their cytoplasmic partners, zonula occludens (ZO) proteins, which connect TJs to actin cytoskeleton.[137] Compared to AJs, mechanisms regulating TJs are far less understood. AJs assembly precedes tight junction formation and in some in vivo cases cadherin is required for the formation of TJs, as it controls the recruitment of ZO-1 to TJ complexes.[138] Up-regulation of EC-specific claudin-5 isoform is involved in EC barrier enhancement in some, but not all models.[139, 140] Conversely, edemagenic agonists decreased claudin-5 and ZO-1 expression accompanied by translocation of ZO-1 from the cytoskeleton to the membrane/nuclear fractions.[141, 142] Recent study implicated the involvement of PKCε/Erk1/2 MAPK axis in phosphorylation of ZO-1 at Thr[770/772].[143] This phosphorylation is accompanied by dissociation of ZO-1 from occludin resulting in EC barrier dysfunction.[143] In contrary, cyclic-strain-induced enhancement of EC barrier function involved increased PKC-dependent ZO-1-occludin association.[144] In addition, Tyr phosphorylation of ZO-2 is involved in its dissociation from TJs and barrier dysfunction.[145]

Gap junctions (GJ) form intercellular channels involving in the passages of ions and macromolecules between neighboring cells. They also present in ECs and play an important role in endothelial functions; however, information regarding the involvement of GJ in EC permeability regulation is limited and somewhat controversial. Recent studies on pulmonary

EC demonstrated that the expression of TJ protein, connexin 43, is involved in LPS-induced permeability increase.[146] Consistent with these observations, connexin 43 inhibition blocked thrombin-induced permeability increase in lung capillaries.[147] In contrary, other report demonstrated that thrombin-induced permeability is accompanied by internalization (inhibition) of TJ communications in vascular endothelium.[148] Further studies are needed to define the involvement of TJ in the EC permeability regulation.

Conclusion. Molecular basis of ALI and ARDS is still poorly understood. Based on the existing literature we proposed complex mechanisms involving crosstalk between microtubule and microfilaments accompanied by activation/phosphorylation cytoskeletal proteins following by re-arrangement of cell junctions. Further studies are needed to define cytoskeletal-specific structure/function relationships and enhance our understanding of the lung vascular barrier regulation.

Disclosure of Potential Conflicts of Interest

The authors have declared that no competing interests exist.

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Figure Legends.

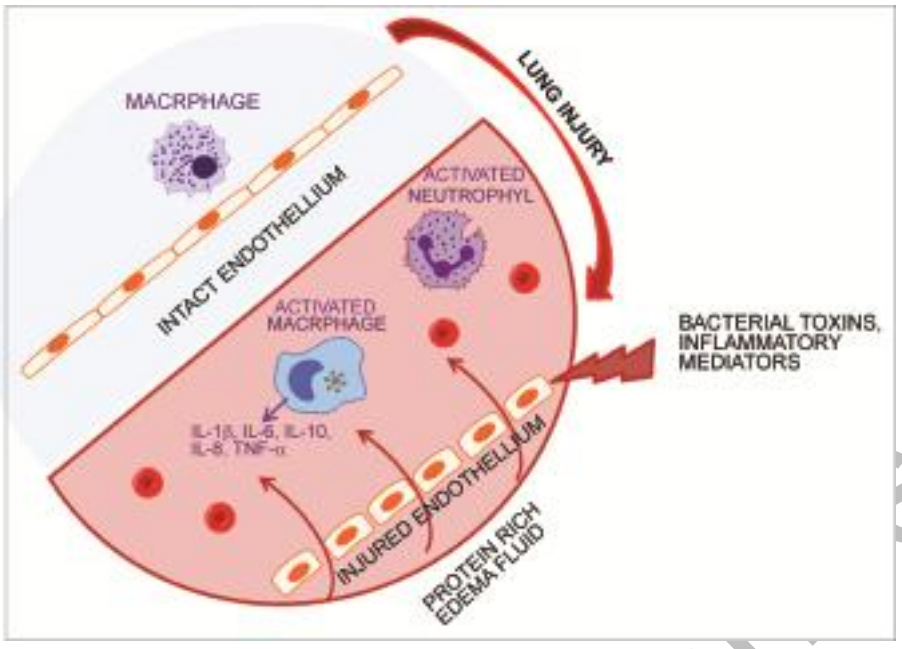

Figure 1. Endothelial activation in ALI. Edemagenic agents like bacterial toxins (LPS) or inflammatory mediators (thrombin) disrupt endothelial barrier leading to EC permeability increase accompanying by inflammatory response

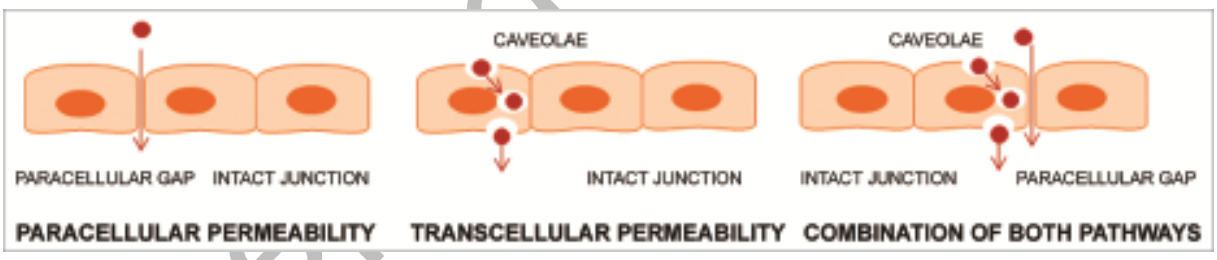

Figure 2. EC permeability pathways. Edemagenic agonists can increase endothelial permeability via caveolae-mediated transcellular route or (and) via increased intercellular gaps (paracellular route).

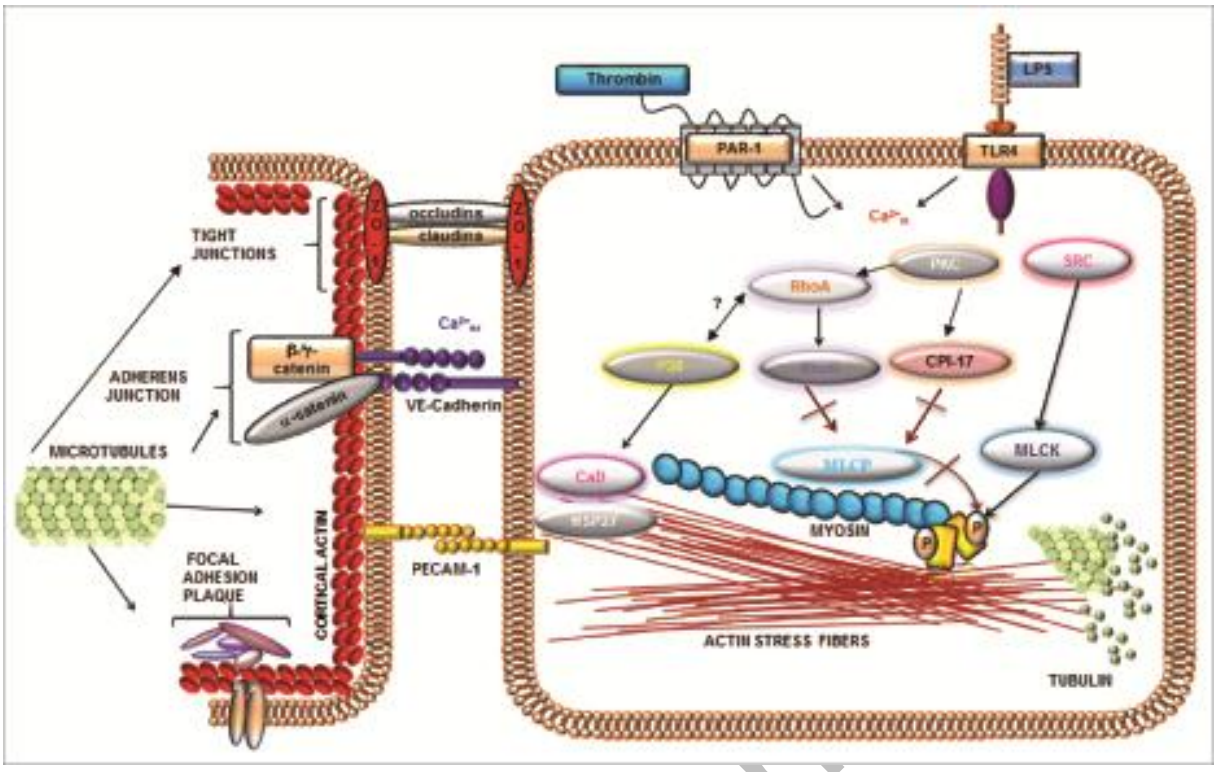

Figure 3. EC cytoskeletal rearrangement in response to edemagenic agonists. Thrombin or LPS activates their receptors (PAR-1 and TLR4, respectively) leading to activation of pro-inflammatory intracellular cascades (intracellular Ca^{2+} increase, activation of Rho, PKC and Src signaling) following by microtubule dissolution, increased MLC phosphorylation (MLCK activation, MLCP inhibition) and phosphorylation of regulatory cytoskeletal proteins, CaD and HSP-27 (via p38 MAPK activation). These events result in actomyosin contraction, actin rearrangement and disruption of intercellular contacts, following by EC permeability increase.

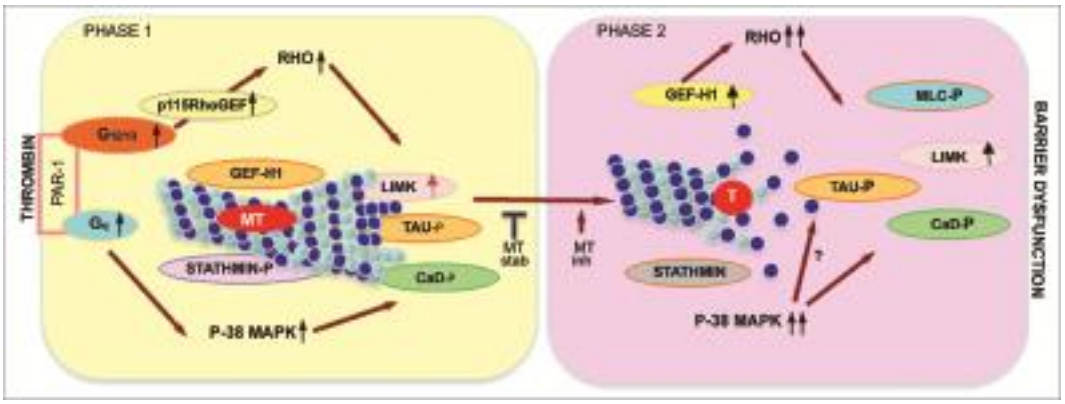

Figure 4. Hypothetic mechanism of thrombin-induced microtubule-mediated EC barrier compromise. Thrombin activates its receptor (PAR-1) leading to activation of trimeric G-proteins ($G_{12/13}$ and Gq), following by initial activation (phase 1) of Rho and p38 MAPK signaling and resulting in disruption of microtubule structure via activation of MT-binding proteins (phosphorylation of tau, CaD and LIMK and dephosphorylation of stathmin). At phase 2 MT dissolution leads to further activation of Rho (via release and activation of GEF-H1) and p38 MAPK followed by additional phosphorylation of cytoskeletal targets and their relocation to actin cytoskeleton resulting in actin rearrangement and permeability increase. MT stab: MT stabilization, MT inh: microtubule inhibition.

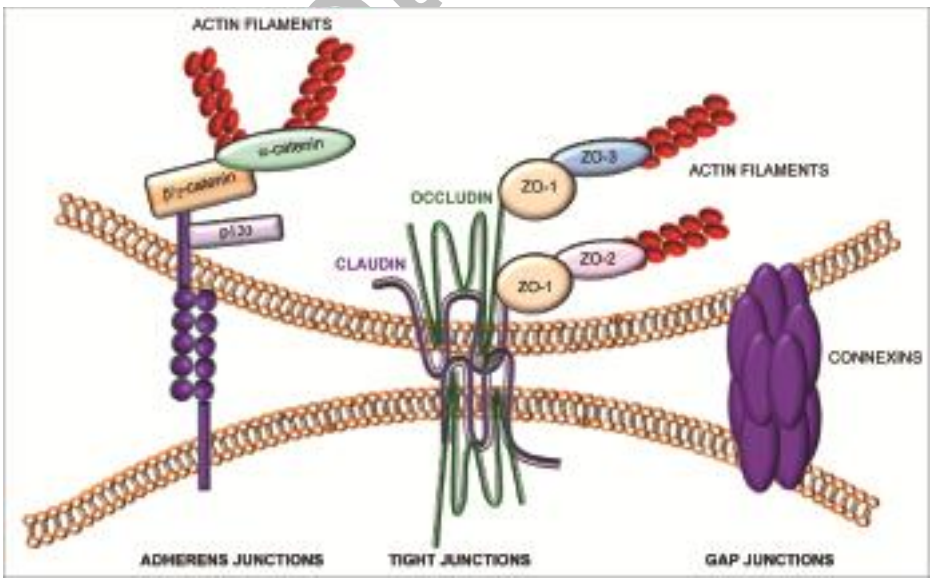

Figure 5. Schematic representation of major intercellular contacts.