Published in final edited form as:

Trends Immunol. 2009 November ; 30(11): 547–556. doi:10.1016/j.it.2009.07.012.

Opening the flood-gates: how neutrophil-endothelial interactions regulate permeability

Matthew R. DiStasi and Klaus Ley

La Jolla Institute of Allergy & Immunology, 9420 Athena Circle, La Jolla, CA 92037

Abstract

Many diseases have an inflammatory component, where neutrophil interactions with the vascular endothelium lead to barrier dysfunction and increased permeability. Neutrophils increase permeability through secreted products like the chemokines CXCL1, 2, 3 and 8, through adhesion-dependent processes like β_2 integrins interacting with endothelial ICAM-1, and combinations, where β_2 integrin engagement leads to degranulation and secretion of heparin-binding protein (HBP), which in turn increases permeability. Some neutrophil products like arachidonic acid or leukotriene (LT) A4 are further processed by endothelial enzymes through transcellular metabolism before the resulting products thromboxane A2, LTB4 or LTC4 can activate their cognate receptors. Neutrophils also generate reactive oxygen species that induce vascular leakage. This review focuses on the mechanisms of neutrophil-mediated leakage.

Inflammation, neutrophils, and vascular permeability

Many diseases involve neutrophil recruitment into the interstitial space, including chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), glomerulonephritis, ulcerative colitis, psoriasis, and autoimmune vasculitis [1]. A key event in these inflammatory processes is the interaction of the circulating neutrophils with the endothelium, which has profound effects on both the neutrophils and the endothelial cells (ECs). One common consequence of this interaction is increased vascular permeability resulting in the accumulation of protein-rich edema fluid in the interstitial tissues. A controlled increase in vascular permeability upon inflammation is a natural physiologic response that aids in clearing the inducing stimulus. As permeability increases in the inflamed area, fluid and proteins leak out of the blood into the interstitial space, thus producing an inflammatory exudate that contains complement proteins and antibodies [2]. Complement proteins can kill or opsonize microorganisms and produce additional chemoattractants such as C5a and C3a. Antibodies can neutralize viruses and toxins, opsonize microorganisms and promote phagocytosis. If unchecked, edema can lead to hospitalization or death in cases such as influenza-induced pneumonia, increased intracranial pressure, myocarditis, or severe burns. This review will focus on the interaction of neutrophils with the endothelium and its consequences for endothelial phenotype and vascular permeability, a subject not reviewed since 2003 [3].

Under conditions of inflammation, increased vascular permeability occurs primarily via changes in the integrity of interendothelial cell junctions. Endothelial cell contraction creates more space between adjacent ECs for macromolecules and fluid to pass [4]. Indeed, a seminal study by Majno and Palade in 1961 demonstrated the formation of these paracellular gaps via electron microscopy at sites of inflammation [5]. A number of soluble factors have long been

known to increase vascular permeability through effects on the endothelium, among them thrombin, bradykinin, histamine, oxygen free radicals, vascular endothelial growth factor (VEGF), tumor necrosis factor- α (TNF α), and lipopolysaccharides (LPS) from gram-negative bacteria [4]. Most permeability-inducing factors bind to plasma membrane receptors, activate heterotrimeric G-proteins and cause an increase in intracellular Ca^{2+} , which brings about myosin light chain (MLC) kinase activation, myosin phosphorylation, myosin-driven EC contraction, and junctional disruption [4,6]. Ca^{2+} -dependent activation of endothelial protein kinase C α (PKC α) activates RhoA and its downstream effector Rho kinase (ROCK), thus modulating actin polymerization, increasing stress fiber formation, which causes EC contraction and junctional disruption [4,6] (Box 1). The molecular components and interactions within the interendothelial junctional complexes are also critical for vascular permeability [4, 6,7]. The adherens and tight junctions (Box 2) are responsible for interendothelial barrier maintenance, and the junctional constituents most directly involved in altered permeability are the transmembrane protein vascular endothelial (VE-) cadherin with its associated cytosolic proteins α -, β -, and p120-catenins [6].

Box 1

Signaling cascade mediating increased permeability in inflammation

Permeability-inducing factors (such as thrombin) bind to plasma membrane receptors (like the protease-activated receptor (PAR) -1 for thrombin) and activate heterotrimeric G-proteins which then signal through PLC to produce diacylglycerol (DAG) and inositol triphosphate (IP_3), which results in an increase in intracellular Ca^{2+} via receptor- or store-operated transient receptor potential canonical (TRPC) channels (TRPC6 for extracellular Ca^{2+} or TRPC1 for endoplasmic reticulum Ca^{2+} pools, respectively) [4,6]. The increased intracellular [Ca^{2+}] activates serine/threonine protein kinases like isoforms of PKC and tyrosine kinases like those of the Src family, all of which activate myosin light chain kinase (MLCK) causing increased myosin phosphorylation and myosin-driven EC contraction as well as junctional disruption [4]. Furthermore, activation of the Ca^{2+} and DAG-dependent PKC α results in activation of the monomeric GTPase RhoA and its downstream effector Rho kinase (ROCK), which then modulates actin polymerization, inhibits MLC phosphatase activity, and increases stress fiber formation allowing for further EC contraction and junctional disruption [4,6].

Box 2

Components of endothelial junctions

The adherens and tight junctions are responsible for interendothelial barrier maintenance. Endothelial junctions contain the transmembrane protein vascular endothelial (VE-) cadherin as well as the cytosolic proteins α -, β -, and p120-catenins [6]. The intracellular attachment of VE-cadherin to the actin cytoskeleton is mediated through α - and β -catenins; the extracellular domain of VE-cadherin interacts in a homotypic and Ca^{2+} -dependent fashion with the extracellular domains of adjacent EC VE-cadherins; and p120-catenin regulates the interaction of kinases, phosphatases, and GTPases with the junctional proteins through its scaffolding function [6]. Additionally, vascular endothelial protein tyrosine phosphatase (VE-PTP) associates with VE-cadherin, helping to keep it dephosphorylated, and enhancing adhesive function [87] (see Figure 2). Tight junctions are composed of the transmembrane proteins occludin, claudins, and junctional adhesion molecules (JAMs) – all of which adhere to adjacent ECs through homotypic interactions – as well as the linker protein zona occludin-1 (ZO-1) allowing attachment to the actin cytoskeleton [6].

Wedmore & Williams were the first to suggest that neutrophils can control blood vessel permeability [8]. Numerous subsequent studies have demonstrated that activated neutrophils induce increased interendothelial gap formation. This increased permeability is associated with MLC phosphorylation, phosphorylation and dissociation of EC junctional proteins, actin stress fiber formation, as well as RhoA, ROCK, and focal adhesion kinase (FAK) activation [9–14]. Neutrophils regulate vascular permeability via secreted neutrophil products, through adhesion dependent mechanisms, based on the route of neutrophil transmigration (paracellular versus transcellular), and by alterations in the balance between reactive oxygen species (ROS) and nitric oxide (NO) [15].

Secreted neutrophil products

When interacting with the endothelium leukocytes receive signals through G-protein coupled receptors [16], integrins [17], P-selectin glycoprotein ligand 1 (PSGL-1) and L-selectin [18]. All these inputs are involved in leukocyte activation, but none of these pathways alone can fully activate all neutrophil functions. Full activation of a neutrophil induces respiratory burst and degranulation of at least four types of neutrophil granules [19]. Primary (azurophilic) granules are enriched in bactericidal and cytotoxic mediators and contain myeloperoxidase (MPO), elastase, cathepsins, defensins, acid phosphatase, heparin-binding protein, neutral serine proteases as well as lysosomal hydrolases [19–21]. Secondary (specific) granules contain lactoferrin, collagenase, lysozyme, adhesion molecules (like Mac-1), and chemotactic receptor molecules (like FPR1) [19–21]. Tertiary granules contain gelatinase B (matrix metalloproteinase, MMP-9). Finally, secretory granules contain plasma proteins (like albumin), integrins (Mac-1), complement receptor 1, heparin-binding protein, and alkaline phosphatase [1,19–21]. The granules most easily mobilized upon neutrophil activation are the secretory vesicles, with the tertiary, then the secondary, and finally the azurophilic granules being the least mobilizable compartment [1,3]. Thus, based on both the order in which these granules are released and their contents, it is generally thought that secretory vesicles enhance firm adhesion of neutrophils, tertiary granules aid in the degradation of basement membranes, secondary granules enhance neutrophil phagocytosis, and primary as well as secondary granules contribute to the reactive oxygen-dependent and -independent bactericidal activity [22]. Recent studies suggest there is much more heterogeneity to the neutrophilic granules in regards to their content and function than previously appreciated [23]. Indeed, both a granule's contents and its propensity to be released can be viewed as a continuum, with the least mobilizable azurophilic granules being generated earliest in the neutrophil development cycle and tertiary and secretory granules being generated at the latest stages [23]. Several secreted neutrophil products have been implicated in increased endothelial permeability (Figure 1), but it is not clear that adherent or transmigrating neutrophils release granules beyond secretory vesicles.

Neutrophil elastase (NE) and cathepsin G can increase vascular permeability *in vitro* [24]. Both can lead to VE-cadherin digestion, evidenced by VE-cadherin proteolytic fragments found in culture medium after EC exposure to activated neutrophils or elastase, a process inhibited by elastase activity inhibition [24]. However, neutrophil elastase-deficient (*Ela2^{-/-}*) mice show increased, not decreased vascular permeability [25]. This phenomenon may be due to an inability of *Ela2^{-/-}* neutrophils to degrade intercellular adhesion molecule 1 (ICAM-1), extending the interaction time between the neutrophils and the ECs, and resulting in increased endothelial damage from neutrophil granule contents [25]. Relatively long incubation times are needed for neutrophil elastase to mediate EC dysfunction *in vitro*, which argues against a role for elastase in physiologic neutrophil-induced increases in vascular permeability *in vivo* [3]. Indeed, depletion of either elastase or cathepsin G had virtually no effect on the decrease in transendothelial electrical resistance (TEER) observed in cultured endothelial monolayers exposed to activated neutrophil supernatant [26,27]. However, the role of NE *in vivo* remains

controversial as various models have shown either alterations in or no effect of NE on neutrophil trafficking [25]. A critical role for NE was demonstrated in a vasculitis model of inflammatory injury, but neutrophil recruitment was not altered by deletion of NE and vascular permeability was not directly assessed [28].

Arachidonic acid (AA) derivatives such as prostaglandins and leukotrienes have long been implicated in altering EC permeability. Prostaglandins can be formed by most cells while leukotrienes (LT) are predominantly made by inflammatory cells like neutrophils, macrophages, and mast cells (Box 3) [29]. The vasodilation and increased permeability seen in inflammation correlate with locally derived prostaglandin and LT production [29]. Neutrophil-derived LTA₄ leads to the biosynthesis of biologically active leukotrienes such as LTB₄ or LTC₄ [30]. Although LTB₄, *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), or complement protein C5a do not elicit overt edema on their own, they do when combined with PGE₂ [8]. The effects of bradykinin and histamine on endothelial permeability are also significantly enhanced in combination with PGE₂ [8]. The inability of LTB₄ to directly induce increased endothelial permeability has been verified in additional studies, but a permeability increasing effect was observed when LTB₄ was in the presence of neutrophils adhering to the vascular endothelium [31,32]. Neutrophils activated by LTB₄ release heparin-binding protein, which subsequently causes endothelial contraction and thus increased permeability [33]. Since accumulation of extravascular fluid depends on both the properties of the vessel wall (i.e., its permeability) as well as the pressure head driving the fluid through the vessel (which is controlled by pre-capillary vasoconstriction), relaxation of pre-capillary arterioles would increase blood supply to the inflamed tissue and potentiate plasma exudation [8]. Taken together, these findings suggest that low capillary pressure can curb transendothelial fluid loss, and the vasodilatory activity of PGE₂ can overcome this limitation. Furthermore, since LTB₄, C5a, and fMLP do not mediate increased permeability by directly interacting with the endothelium, it is the potent chemoattracting capacity of these mediators to neutrophils, and the neutrophils' subsequent ability to induce increased endothelial permeability, that results in overt edema in the presence of a vasodilator such as PGE₂.

Box 3

Prostaglandins, thromboxane, and leukotrienes: generation from arachidonic acid

Cytosolic phospholipase A₂ (cPLA₂) releases arachidonic acid (AA) from membrane phospholipids and into the cytoplasm where they can be enzymatically modified into either the reactive intermediate prostaglandin H₂ (PGH₂) or leukotriene A₄ (LTA₄) via the cyclooxygenases (COX-1 or -2) or 5-lipoxygenase (5-LO), respectively [30]. PGH₂ is converted into biologically active prostaglandins (PGD₂ – E₂, via prostaglandin isomerase), prostacyclin (PGI₂, via prostacyclin synthase), or thromboxane (TXA₂, via thromboxane synthase) [30]. LTA₄ can be metabolized into leukotriene B₄ (LTB₄) by neutrophil leukotriene A₄ hydrolase (LTA₄H) [120] or into cysteinyl leukotriene C₄ (LTC₄) by endothelial leukotriene C₄ synthase (LTC₄S) [30]. Additional cysteinyl leukotrienes D₄ (LTD₄) and leukotriene E₄ (LTE₄) can be generated by γ -glutamyl transpeptidase and dipeptidase, respectively [30].

An important intracellular enzyme involved in controlling vascular permeability is cytosolic phospholipase A₂ (cPLA₂). Genetic disruption of the gene encoding cPLA₂ significantly decreased lung permeability in both LPS or zymosan- and HCl-induced models of acute lung injury (ALI) [34]. Also, genetic disruption of *Alox5*, the gene encoding 5-lipoxygenase (5-LO), led to significantly decreased local and systemic protein leakage [35]. Blockade of either cyclooxygenase (COX) or of EC thromboxane receptors almost completely normalized the

pathologically increased permeability observed in HCl-induced ALI. Conversely, stimulation of cultured ECs with a thromboxane receptor agonist induced increased F-actin polymerization as well as cell retraction [36]. In contrast to the indirect effects of LTB₄, the cysteinyl leukotrienes LTC₄ and LTD₄ have been shown to directly induce increased endothelial permeability both *in vitro* and *in vivo* [37,38], a response mediated through the cysteinyl leukotriene receptor 2 (CysLT₂R) [38]. Thus, neutrophil-derived AA produces downstream thromboxane and leukotrienes, which increase endothelial cell permeability (Figure 1).

A series of seminal studies have identified heparin-binding protein (HBP, also called CAP37/neutrophil azurocidin, *AZU1*) as a critical mediator of neutrophil-dependent endothelial leakage (Figure 2). HBP is from the serprocidin family of cationic glycoproteins (a family of serine proteases with similar overall structure that includes elastase, cathepsin G, and proteinase 3) and is primarily localized in neutrophil azurophilic granules [21]. However, up to 18% of neutrophil HBP is located in the readily mobilizable secretory granules [21]; therefore, HBP can be more rapidly released from neutrophils upon activation than can elastase or other azurophilic granule components. Indeed, HBP is the only matrix protein stored in secretory vesicles (apart from plasma proteins), is enzymatically inactive (due to mutations in two of the three essential amino acids forming the highly conserved catalytic triad observed in all serine proteinases), and can be almost completely released (~90%) upon neutrophil activation [39]. HBP has been shown to bind to endothelial cell surface proteoglycans, probably via a concentration of numerous positively charged amino acids which create a strong dipole moment [39]. HBP binding results in internalization [40]. It was previously shown that neutrophil-mediated vascular permeability was CD18 (β₂ integrin component of LFA-1) dependent [26,41] LFA-1 engagement results in release of HBP, recombinant HBP produces an identical response to activated neutrophilic media (i.e. supernatants from activated neutrophil cultures), and HBP depletion of activated neutrophil media completely eliminates the permeability effect [27] (Figure 2). Recently, LTB₄-mediated permeability has been shown to require BLT1 receptor activation on neutrophils, resulting in release of neutrophil HBP, which triggers EC intracellular Ca²⁺ mobilization and increased EC permeability both *in vitro* and *in vivo* [33].

Activated neutrophils also release a number of cytokines and chemokines [42,43] which can directly modulate EC permeability (Figure 1). However, these cytokines and chemokines are not stored in large amounts in the neutrophil granules, but instead are generated from a burst of synthesis and secretion which is activated when the neutrophil emigrates from the circulation into the tissues [23]. Human neutrophils secrete TNF-α, as well as the chemokines CCL3, CCL4, CCL20, CXCL2, and CXCL8 upon stimulation with LPS [42]. TNF-α can directly modulate EC permeability through PKC- and p38 mitogen-activated protein kinase (MAPK)-induced phosphorylation of ezrin, radixin, and moesin (ERM) proteins [44] as well as p38 MAPK-mediated microtubule destabilization [45]. TNF-α leads to actin stress fiber and intercellular gap formation and increased EC permeability [45]. Members of the CXC chemokine family containing the conserved ELR amino acid motif (Glu-Leu-Arg), such as CXCL1, 2, 3, 5, 6, 7 and 8, can activate pro-angiogenic processes in endothelial cells including directional chemotaxis [46], which are associated with increased permeability. TNF-α, fMLP, C5a, platelet activating factor, LTB₄, and some proteolytic enzymes also induce neutrophil chemokine expression [43]. Several studies have shown that the chemokine receptor CXCR2 on both leukocytes and endothelium is critically involved in the increased vascular permeability associated with different models of sepsis and acute lung disease [47–49]. Neutrophil recruitment into the lung interstitium and alveolar spaces is decreased along with permeability upon CXCR2 inhibition [50]. Indeed, endothelial CXCR2 is critical for LPS-induced protein leakage in lung injury [47]. A key regulatory molecule for these responses is p21-activated kinase (PAK) in both ECs and neutrophils working through a Rac, PAK, β PAK-interacting exchange factor (βPIX), G-protein coupled receptor interacting protein 1 (GIT1) protein

complex, mitogen-activated protein kinase kinase (MEK) 1/2, extracellular signal-regulated kinase (Erk), and MLCK pathway [51–53]. The Rac/PAK pathway in ECs can be activated by CXCL8 signaling through CXCR2 and phosphoinositol 3-kinase γ (PI3K γ) to increase permeability both *in vitro* and *in vivo* via VE-cadherin phosphorylation and internalization, an effect that can be blocked by pharmacological inhibitors of CXCR2 and PI3K γ [54].

Complement activation results in the production of complement proteins C3a, C4a, and C5a. Increased plasma concentrations of these proteins in sepsis is associated with poor clinical outcomes, and C3a and C5a have a wide range of proinflammatory activities [55,56]. Neutrophil- and COX-1-dependent increased lung permeability to radiolabeled iodine was observed in a model of complement activation [57]. Immune complex-induced increased lung permeability is reduced by pharmacologic inhibition of the C5a receptor (C5aR) [58], or the complement system [59]. However, neither C3a nor C5a are responsible for the increased permeability observed in an intratracheally administered LPS model of ALI [60]. Neutrophils can accelerate complement activation and C5a production [61]. In addition, C5aR has been found on ECs, and its expression is upregulated in the lungs in sepsis, and receptor engagement increases intracellular Ca²⁺ [56], EC retraction, and increased permeability *in vitro* [62] (Figure 1). While C3a receptors (C3aR) have also been found on ECs *in vitro* [62,63] and in certain models of chronic inflammation [63,64], one study which investigated EC permeability could find no involvement of the C3aR [62].

Extracellularly released nucleotides, such as adenosine, comprise another class of mediators for neutrophil-EC regulation of vascular permeability (Figure 1). Extracellular adenosine accumulates in inflamed or damaged tissue [65] and endogenous protective mechanisms in response to acute injury become activated via adenosine [66]. Adenosine triphosphate (ATP) released by neutrophils through connexin 43 hemichannels (Cxn 43) is converted to adenosine monophosphate (AMP) by CD39 on the neutrophil surface and then to adenosine by CD73 on the EC surface [67]. Adenosine then can bind adenosine receptor A2b to induce enhanced barrier function which counteracts vascular permeability [67,68]. Other studies have implicated adenosine receptor A2a in the regulation of EC permeability in inflammation or hypoxia [65,69–72]. Pharmacologic activation of adenosine receptor A2a attenuates the increased permeability seen in mouse lungs after exposure to LPS [73]. A recent study of EC permeability in response to hypoxia revealed that A2b is expressed on macrophages, neutrophils, lymphocytes, and is more important *in vivo* than A1 or A3 as shown by lung water accumulation or Evan's Blue extravasation [66]. Conversely, the precursor to adenosine, ATP, and adenosine diphosphate (ADP) can bind P2 purinoceptors and induce increases in EC intracellular Ca²⁺, cell shape changes, and increased endothelial permeability *in vitro* (via MLCK and ROCK) [74,75] and *in vivo* [76]. These effects have been shown to be mediated through P2Y receptors [74–76] and not P2X₇ receptors [77]. However, the specific function of the many P2 receptor subtypes and their role in cell physiology remains poorly understood and little is known about how neutrophil-endothelial interactions are affected by nucleotides [78].

Endothelial cell injury or death also results in increased permeability via breakdown of the vascular wall. This EC death may be due to apoptosis or necrosis and molecules of the adaptive immune response as well as various immune effector cells are known to induce EC death [79]. EC death or injury from innate or adaptive immunity can occur through either secretion of injurious molecules or engagement of death receptors by immune cells [79]. Indeed, neutrophil secretion products causing the death or injury of ECs and resultant edema have been implicated in numerous disease states. While a detailed discussion is beyond the scope of this review, recent work demonstrating the involvement of angiopoietins in ALI is relevant to this discussion. Angiopoietin 2 (Ang2) has been shown to destabilize blood vessels [80] and has been implicated in hyperoxic ALI (HALI) [81]. Ang2 mRNA and protein expression was

increased *in vivo* in mice exposed to HALI, Ang2 null mice showed reduced BAL cell recruitment and lower BAL total protein concentrations, and the plasma and alveolar fluid of humans with ALI or pulmonary edema showed increased Ang2 protein concentrations [81]. Furthermore, mice exposed to HALI and injected with recombinant Ang2 had increased apoptosis while those given Ang2 siRNA in addition to HALI and rAng2 demonstrated reduced apoptosis [81]. Angiopoietins can activate both ECs and neutrophils through the Tie2 (epidermal growth factor homology domain 2) receptor leading to increased P-selectin translocation in ECs as well as platelet-activating factor (PAF) synthesis, functional up-regulation of β_2 -integrin complex, and increased adhesion to ECs by the neutrophils [82].

Endothelial cell signaling following neutrophil adhesion

In addition to soluble molecules secreted by activated neutrophils mediating increased EC permeability, firm adhesion of neutrophils to the endothelium ligates EC adhesion molecules including ICAM-1, vascular cell adhesion molecule 1 (VCAM-1), or E-selectin and P-selectin [15]. A rapid and transient increase in intracellular free Ca^{2+} can occur in ECs upon docking of neutrophils to the luminal surface [41,83] and blockade of this Ca^{2+} influx with cell permeant calcium chelators prevents neutrophil-mediated permeability [3,26,84]. Neutrophil adherence to microvascular ECs results in actin polymerization and cytoskeletal reorganization probably via ICAM-1 engagement [15]. Indeed, ICAM-1 blocking antibodies completely inhibit neutrophil adherence-induced cytoskeletal changes *in vitro* [15]. Neutrophils bind ICAM-1 via the β_2 integrin leukocyte function-association antigen 1 (LFA-1) and macrophage receptor 1 (Mac-1) (Figure 2) [3]. Antibody-mediated cross-linking of endothelial ICAM-1 can activate PKC, Rho, Src family kinases and Ca^{2+} , which may then act upon actin-associated proteins like paxillin, cortactin, p130Cas, and FAK to induce changes in the cytoskeleton of brain or venular ECs [15]. Recent work has demonstrated that engagement of ICAM-1 on ECs via leukocytes (through β_2 integrin) or ICAM-1 antibody-coated beads resulted in Src- and Pyk2-dependent phosphorylation of VE-cadherin on tyrosines 658 and 731 (the binding sites for p120- and β -catenin, respectively) [85]. Phosphorylation of these residues led to an inability of p120- and β -catenin to bind VE-cadherin and an increase in permeability *in vitro* [86]. Neutrophil adhesion to ECs results in the rapid dissociation of VE-PTP from VE-cadherin, increased VE-cadherin phosphorylation, and increased EC permeability [87]. A recent review examines in further detail the outside-in signaling events mediating ICAM-1 clustering, subsequent kinase recruitment, and phosphorylation of junctional proteins that result in junctional destabilization primarily in the context of neutrophil transmigration [88]. In addition to the role of paracellular permeability in pathogenesis, a large component of the permeability increase due to ICAM-1 engagement on ECs was mediated by Src phosphorylation of caveolin-1, resulting in increased caveolae-dependent transcytosis of labeled albumin both *in vitro* as well as *in vivo* [89].

Another immunoglobulin superfamily transmembrane glycoprotein shown to play a role in the maintenance of endothelial barrier function in a model of ALI is CD47 (integrin-associated protein, IAP) [90]. CD47 is expressed by virtually all hematopoietic cells and its absence reduced neutrophil recruitment and EC permeability after LPS exposure, but the mechanism through which CD47 exerts its effect is still unclear [90].

Similar to the ICAM-1 response, ligation of VCAM-1 can induce increased intracellular Ca^{2+} and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation in ECs, which leads to actin cytoskeletal reorganization [15]. Elevated H_2O_2 production by endothelial NADPH oxidase [91,92] stimulated EC PKC α activation [93], resulting in protein tyrosine phosphatase (PTP)-1B phosphorylation and activation [94], which has been associated with remodeling of the actin cytoskeleton [95]. However, neutrophil-induced ligation of VCAM-1

as a modulator of EC permeability is less likely to be clinically relevant, since human neutrophils do not constitutively express the VCAM-1 binding integrin $\alpha_4\beta_1$ [96].

In vitro studies with human umbilical vein endothelial cells (HUVECs) have shown that ligation of E-selectin with monoclonal antibodies increased intracellular Ca^{2+} , stress fiber formation, and cell shape changes [15]. Ligation also induced the association of E-selectin's cytoplasmic domain with cytoskeletal proteins (such as α -actinin, vinculin, filamin, FAK and paxillin [97]), as well as Erk activation, up-regulation of *c-fos* mRNA, and phosphorylation of the E-selectin cytoplasmic tail (reviewed in [15]) (Figure 2). Ligated E-selectin colocalized to cholesterol-rich lipid rafts in the plasma membrane with caveolin-1 and $\text{PLC}\gamma$, which became phosphorylated and activated [98].

Both endothelial cells and leukocytes express gap junctional subunits (called connexins, Cxs) and can couple through various Cxs [99]. Stimulated human neutrophils express Cxs37, 40, and 43 which can mediate bi-directional dye transfer between neutrophils adherent to HUVEC monolayers [100]. However, the broad gap junction inhibitor SRPTEKTVFTV did not alter neutrophil adhesion or HUVEC permeability [100], so the role of connexins remains controversial [101] (Figure 2).

Transmigration mechanisms

It was previously thought that the increased permeability observed in association with neutrophil recruitment was due to leakage of the plasma through the same channels used by the emigrating leukocyte [3]. However, adhesion of activated neutrophils is sufficient to induce alterations in activated EC barrier function without transmigration [3,41]. Conversely, adhesion *per se* does not always result in increased permeability [32,102]. Indeed, it has been shown in human lungs that increased neutrophil recruitment into the alveolar space in response to LTB₄ instillation did not significantly alter the epithelial barrier protein permeability [103]. Neutrophils predominantly migrate through the endothelial barrier via a paracellular route (i.e. between cells), but a transcellular route (i.e. through cells) is favored when ICAM-1 expression is high [104,105]. *In vivo*, the route of neutrophil emigration had no effect on the increase in permeability [102,106]. “Endothelial domes” encapsulate the migrating neutrophil and minimize increases in permeability during both types of leukocyte emigration [102]. These domes are thought to arise from the previously described “transmigratory cups” [107], consisting of membrane projections on ECs enriched with ICAM-1 and VCAM-1 found surrounding all types of emigrating leukocytes including neutrophils [107]. During transmigration, neutrophil LFA-1 and EC ICAM-1 redistribute to the contact site at the EC junctions such that they form a distinct ring-like structure, which is maintained around the neutrophil as it migrates through the endothelium [88,108]. These rings, cups, and domes are in such close apposition to the migrating neutrophil that it is no longer a surprise there is minimal loss of barrier integrity during the processes of leukocyte trans- or para-cellular diapedesis.

Reactive oxygen species (ROS) and nitric oxide (NO) mechanisms

As a thorough review of this subject was published earlier this year [109], our treatment of this subject will be brief. Activated neutrophils can generate large amounts of reactive oxygen species, which can induce alterations in barrier function through direct EC damage [3]. However, ROS also activate signaling pathways, which can subsequently cause increases in EC Ca^{2+} , activation of MLCK, and reorganization of junctional proteins [3]. ROS can induce proinflammatory mediator production from ECs, which in turn enhances neutrophil-dependent permeability increases [3,109]. ROS produced from activated neutrophils might therefore play an important role in edema formation and pathology in a number of conditions including ALI, ARDS, sepsis, and lung ischemia-reperfusion injury [109]. The important role of ROS in edema

formation prompted clinical trials with antioxidant therapy, which have been disappointing so far [109]. In addition, reactive nitrogen species, mostly in the form of NO, can offset thrombin-induced increased permeability and can attenuate oxidant-induced barrier dysfunction in ARDS patients [109]. Large amounts of NO can lead to nitrosative stress and endothelial dysfunction [109].

Enhancers of barrier function

There is an ever-growing list of molecules being identified that are capable of enhancing endothelial barrier function [4]. The extent of organ dysfunction and edema which occurs depends on the balance between inflammatory injury and the capacity of endogenous barrier enhancing mechanisms to restore vascular barrier stability [110].

Probably the best characterized of these barrier enhancing agents is sphingosine 1-phosphate (S1P). S1P effectively enhances endothelial barrier function both *in vitro* and in animal models of ALI and sepsis [111]. S1P is primarily released from erythrocytes and platelets and binds to G-protein coupled receptors S1P1-S1P5 (with S1P1, S1P2, and S1P3 being expressed on vascular endothelium). Ligation of S1P1 signals through G_{α_i} to recruit PI3K, T-lymphoma invasion and metastasis gene 1 (Tiam1), and Rac1 (a Rho family GTPase) to lipid rafts, activate Rac1 and increase intracellular Ca^{2+} through PLC activation [111]. Rac mediates cortactin translocation and interaction with MLCK at the cell periphery to strengthen cortical F-actin rings, binds with PAK to activate LIM-domain kinase (LIMK), which in turn inactivates the F-actin destabilizer cofilin, induces increased VE-cadherin and β -catenin expression, zona occludens-1 redistribution to the cell periphery, assembly of tight junctions and association of GIT1 with paxillin [111]. Focal adhesion redistribution to the cell periphery is associated with enhanced endothelial barrier integrity [111]. Physiologic concentrations of S1P (0.5–1 μ M) will signal through the S1P1 receptor to induce endothelial barrier enhancement via Rac activation and the mechanisms listed above; however, supra-physiologic concentrations of S1P (>5 μ M) will signal through the S1P3 receptor, which can couple to G_q and $G_{12/13}$, induce Rho activation, stress fiber formation and barrier disruption [111]. Signaling through the S1P2 receptor also leads to increased permeability *in vitro* and *in vivo* through a Rho-ROCK-PTEN dependent pathway [112]. The long-lasting effects of single dose S1P therapy on permeability barrier enhancement and the fact that S1P receptors can be transactivated by other molecules makes S1P an interesting choice for intervention [111]. Indeed, FTY720 (an S1P analog and agonist of S1P1, S1P3, S1P4, and S1P5 [113]) has reached Phase III clinical trials for kidney graft rejection [114] and multiple sclerosis [115].

Another known enhancer of barrier function and also a molecule capable of transactivating S1P1 is activated protein C (aPC). aPC has been shown to improve the outcome of severe sepsis [116] and is known to downregulate thrombin generation via binding to protein S as well as cleavage of coagulation factors VIIIa and Va [117]. However, recent *in vitro* work has shown that aPC will bind the endothelial protein C receptor (EPCR) and induce increased MLC phosphorylation, peripheral actin reorganization, and central stress fiber reduction resulting in endothelial barrier enhancement [118]. This study also showed that aPC engagement of EPCR induced Rac activation and phosphorylation of the S1P1 receptor, which is required for the barrier-protective effect of aPC [118]. aPC binding to EPCR induced PAR-1 activation leading to sphingosine kinase 1 (SK1) generation of S1P and subsequent barrier enhancement via S1P1 [119]. Interestingly, this study also showed that while high concentrations of thrombin induced increased permeability through the classical activation of the PAR-1 receptor, low concentrations of thrombin (~40 pM) activated the PAR-1 receptor in a fashion similar to aPC and mediated barrier enhancement through S1P1 transactivation [119]. A slight increase in basal permeability was observed with supraphysiologic concentrations of aPC (200 nM) but concentrations which inhibited thrombin did not induce this increased permeability [119]. It

is likely that at least some of the aPC effects occur in the presence of adherent and transmigrating neutrophils.

Adenosine is also a potent barrier enhancing molecule and binding of adenosine to A2a or A2b adenosine receptors protects the endothelial barrier in models of inflammation [66,73].

Conclusion

Inflammatory alterations of vascular permeability are generally associated with the formation of paracellular gaps between endothelial cells due to the disruption of intercellular junctions and endothelial cell contraction. Upon stimulation and interaction with the endothelium, neutrophils are capable of inducing endothelial barrier disruption through several mechanisms. These include molecules which can be secreted (such as leukotrienes or HBP), direct signaling into the EC via adhesion-dependent mechanisms (like outside-in signaling via ICAM-1), and production of ROS. The formation of endothelial rings, cups, and domes help maintain barrier integrity during neutrophil extravasation. Several molecules (such as S1P and aPC) enhance endothelial barrier integrity and serve as an attractive foundation on which potential therapies could be built.

References

1. Dallegri F, Ottonello L. Tissue injury in neutrophilic inflammation. *Inflamm Res* 1997;46 (10):382–391. [PubMed: 9372309]
2. Markiewski MM, Lambris JD. The role of complement in inflammatory diseases from behind the scenes into the spotlight. *Am J Pathol* 2007;171 (3):715–727. [PubMed: 17640961]
3. Lindbom L. Regulation of vascular permeability by neutrophils in acute inflammation. *Chem Immunol Allergy* 2003;83:146–166. [PubMed: 12947983]
4. Mehta D, Malik AB. Signaling mechanisms regulating endothelial permeability. *Physiol Rev* 2006;86 (1):279–367. [PubMed: 16371600]
5. Majno G, Palade GE. Studies on inflammation. 1. The effect of histamine and serotonin on vascular permeability: an electron microscopic study. *J Biophys Biochem Cytol* 1961;11:571–605. [PubMed: 14468626]
6. Vandenbroucke E, et al. Regulation of endothelial junctional permeability. *Ann N Y Acad Sci* 2008;1123:134–145. [PubMed: 18375586]
7. Komarova YA, et al. Dual regulation of endothelial junctional permeability. *Sci STKE* 2007;2007 (412):re8. [PubMed: 18000237]
8. Wedmore CV, Williams TJ. Control of vascular permeability by polymorphonuclear leukocytes in inflammation. *Nature* 1981;289 (5799):646–650. [PubMed: 7464931]
9. Tinsley JH, et al. Activated neutrophils induce hyperpermeability and phosphorylation of adherens junction proteins in coronary venular endothelial cells. *J Biol Chem* 1999;274 (35):24930–24934. [PubMed: 10455168]
10. Yuan SY, et al. Myosin light chain phosphorylation in neutrophil-stimulated coronary microvascular leakage. *Circ Res* 2002;90 (11):1214–1221. [PubMed: 12065325]
11. Tinsley JH, et al. Src-dependent, neutrophil-mediated vascular hyperpermeability and beta-catenin modification. *Am J Physiol Cell Physiol* 2002;283 (6):C1745–1751. [PubMed: 12388068]
12. Breslin JW, Yuan SY. Involvement of RhoA and Rho kinase in neutrophil-stimulated endothelial hyperpermeability. *Am J Physiol Heart Circ Physiol* 2004;286 (3):H1057–1062. [PubMed: 14630629]
13. Guo M, et al. Focal adhesion kinase in neutrophil-induced microvascular hyperpermeability. *Microcirculation* 2005;12 (2):223–232. [PubMed: 15824042]
14. Breslin JW, et al. Involvement of ROCK-mediated endothelial tension development in neutrophil-stimulated microvascular leakage. *Am J Physiol Heart Circ Physiol* 2006;290 (2):H741–750. [PubMed: 16172166]

15. Wang Q, Doerschuk CM. The signaling pathways induced by neutrophil-endothelial cell adhesion. *Antioxid Redox Signal* 2002;4 (1):39–47. [PubMed: 11970842]
16. Alon R, Ley K. Cells on the run: shear-regulated integrin activation in leukocyte rolling and arrest on endothelial cells. *Curr Opin Cell Biol* 2008;20 (5):525–532. [PubMed: 18499427]
17. Abram CL, Lowell CA. The ins and outs of leukocyte integrin signaling. *Annu Rev Immunol* 2009;27:339–362. [PubMed: 19302044]
18. Zarbock A, Ley K. Neutrophil adhesion and activation under flow. *Microcirculation* 2009;16 (1):31–42. [PubMed: 19037827]
19. Lacy P, Eitzen G. Control of granule exocytosis in neutrophils. *Front Biosci* 2008;13:5559–5570. [PubMed: 18508605]
20. Culpitt, SV. Neutrophils: Collection, Separation, and Activation. In: Rogers, DFaDLE., editor. *Human Airway Inflammation: Sampling Techniques and Analytical Protocols*. Humana Press Inc; 2001. p. 177–189.
21. Tapper H, et al. Secretion of heparin-binding protein from human neutrophils is determined by its localization in azurophilic granules and secretory vesicles. *Blood* 2002;99 (5):1785–1793. [PubMed: 11861296]
22. Borregaard N, Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* 1997;89 (10):3503–3521. [PubMed: 9160655]
23. Borregaard N, et al. Neutrophil granules: a library of innate immunity proteins. *Trends Immunol* 2007;28 (8):340–345. [PubMed: 17627888]
24. Hermant B, et al. Identification of proteases involved in the proteolysis of vascular endothelium cadherin during neutrophil transmigration. *J Biol Chem* 2003;278 (16):14002–14012. [PubMed: 12584200]
25. Kaynar AM, et al. Neutrophil elastase is needed for neutrophil emigration into lungs in ventilator-induced lung injury. *Am J Respir Cell Mol Biol* 2008;39 (1):53–60. [PubMed: 18276796]
26. Gautam N, et al. Signaling via beta(2) integrins triggers neutrophil-dependent alteration in endothelial barrier function. *J Exp Med* 2000;191 (11):1829–1839. [PubMed: 10839800]
27. Gautam N, et al. Heparin-binding protein (HBP/CAP37): a missing link in neutrophil-evoked alteration of vascular permeability. *Nat Med* 2001;7 (10):1123–1127. [PubMed: 11590435]
28. Hirahashi J, et al. Mac-1 signaling via Src-family and Syk kinases results in elastase-dependent thrombohemorrhagic vasculopathy. *Immunity* 2006;25 (2):271–283. [PubMed: 16872848]
29. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 2001;294 (5548):1871–1875. [PubMed: 11729303]
30. Folco G, Murphy RC. Eicosanoid transcellular biosynthesis: from cell-cell interactions to in vivo tissue responses. *Pharmacol Rev* 2006;58 (3):375–388. [PubMed: 16968946]
31. Bjork J, et al. Increase in vascular permeability induced by leukotriene B4 and the role of polymorphonuclear leukocytes. *Inflammation* 1982;6 (2):189–200. [PubMed: 6179872]
32. Rosengren S, et al. Leukotriene B4-induced neutrophil-mediated endothelial leakage in vitro and in vivo. *J Appl Physiol* 1991;71 (4):1322–1330. [PubMed: 1661722]
33. Di Gennaro A, et al. Leukotriene B4-induced changes in vascular permeability are mediated by neutrophil release of heparin-binding protein (HBP/CAP37/azurocidin). *FASEB J* 2009;23 (6):1750–1757. [PubMed: 19151333]
34. Nagase T, et al. Acute lung injury by sepsis and acid aspiration: a key role for cytosolic phospholipase A2. *Nat Immunol* 2000;1 (1):42–46. [PubMed: 10881173]
35. Benjamim CF, et al. Opposing and hierarchical roles of leukotrienes in local innate immune versus vascular responses in a model of sepsis. *J Immunol* 2005;174 (3):1616–1620. [PubMed: 15661924]
36. Zarbock A, et al. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *J Clin Invest* 2006;116 (12):3211–3219. [PubMed: 17143330]
37. Hui Y, et al. Directed vascular expression of human cysteinyl leukotriene 2 receptor modulates endothelial permeability and systemic blood pressure. *Circulation* 2004;110 (21):3360–3366. [PubMed: 15545522]
38. Moos MP, et al. Cysteinyl leukotriene 2 receptor-mediated vascular permeability via transendothelial vesicle transport. *FASEB J* 2008;22 (12):4352–4362. [PubMed: 18779380]

39. Soehnlein O, Lindbom L. Neutrophil-derived azurocidin alarms the immune system. *J Leukoc Biol* 2009;85 (3):344–351. [PubMed: 18955543]
40. Olofsson AM, et al. Heparin-binding protein targeted to mitochondrial compartments protects endothelial cells from apoptosis. *J Clin Invest* 1999;104 (7):885–894. [PubMed: 10510329]
41. Gautam N, et al. Kinetics of leukocyte-induced changes in endothelial barrier function. *Br J Pharmacol* 1998;125 (5):1109–1114. [PubMed: 9846652]
42. McColl SR, et al. Immunomodulatory impact of the A2A adenosine receptor on the profile of chemokines produced by neutrophils. *FASEB J* 2006;20 (1):187–189. [PubMed: 16280366]
43. Scapini P, et al. The neutrophil as a cellular source of chemokines. *Immunol Rev* 2000;177:195–203. [PubMed: 11138776]
44. Koss M, et al. Ezrin/radixin/moesin proteins are phosphorylated by TNF-alpha and modulate permeability increases in human pulmonary microvascular endothelial cells. *J Immunol* 2006;176 (2):1218–1227. [PubMed: 16394012]
45. Petrache I, et al. The role of the microtubules in tumor necrosis factor-alpha-induced endothelial cell permeability. *Am J Respir Cell Mol Biol* 2003;28 (5):574–581. [PubMed: 12707013]
46. Strieter RM, et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. *J Biol Chem* 1995;270 (45):27348–27357. [PubMed: 7592998]
47. Reutershan J, et al. Critical role of endothelial CXCR2 in LPS-induced neutrophil migration into the lung. *J Clin Invest* 2006;116 (3):695–702. [PubMed: 16485040]
48. Zarbock A, et al. Therapeutic inhibition of CXCR2 by Reparixin attenuates acute lung injury in mice. *Br J Pharmacol* 2008;155 (3):357–364. [PubMed: 18587419]
49. Strieter RM, et al. The role of CXCR2/CXCR2 ligands in acute lung injury. *Curr Drug Targets Inflamm Allergy* 2005;4 (3):299–303. [PubMed: 16101537]
50. Belperio JA, et al. Critical role for CXCR2 and CXCR2 ligands during the pathogenesis of ventilator-induced lung injury. *J Clin Invest* 2002;110 (11):1703–1716. [PubMed: 12464676]
51. Stockton RA, et al. p21-activated kinase regulates endothelial permeability through modulation of contractility. *J Biol Chem* 2004;279 (45):46621–46630. [PubMed: 15333633]
52. Reutershan J, et al. Blocking p21-activated kinase reduces lipopolysaccharide-induced acute lung injury by preventing polymorphonuclear leukocyte infiltration. *Am J Respir Crit Care Med* 2007;175 (10):1027–1035. [PubMed: 17322107]
53. Stockton R, et al. Induction of vascular permeability: beta PIX and GIT1 scaffold the activation of extracellular signal-regulated kinase by PAK. *Mol Biol Cell* 2007;18 (6):2346–2355. [PubMed: 17429073]
54. Gavard J, et al. A role for a CXCR2/phosphatidylinositol 3-kinase gamma signaling axis in acute and chronic vascular permeability. *Mol Cell Biol* 2009;29 (9):2469–2480. [PubMed: 19255141]
55. Rittirsch D, et al. Harmful molecular mechanisms in sepsis. *Nat Rev Immunol* 2008;8 (10):776–787. [PubMed: 18802444]
56. Ward PA. Role of the complement in experimental sepsis. *J Leukoc Biol* 2008;83 (3):467–470. [PubMed: 17875813]
57. Younger JG, et al. Systemic and lung physiological changes in rats after intravascular activation of complement. *J Appl Physiol* 2001;90 (6):2289–2295. [PubMed: 11356794]
58. Huber-Lang MS, et al. Protection of innate immunity by C5aR antagonist in septic mice. *FASEB J* 2002;16 (12):1567–1574. [PubMed: 12374779]
59. Lister KJ, et al. Immune complexes mediate rapid alterations in microvascular permeability: roles for neutrophils, complement, and platelets. *Microcirculation* 2007;14 (7):709–722. [PubMed: 17885996]
60. Rittirsch D, et al. Acute lung injury induced by lipopolysaccharide is independent of complement activation. *J Immunol* 2008;180 (11):7664–7672. [PubMed: 18490769]
61. Huber-Lang M, et al. Generation of C5a by phagocytic cells. *Am J Pathol* 2002;161 (5):1849–1859. [PubMed: 12414531]
62. Schraufstatter IU, et al. Complement C3a and C5a induce different signal transduction cascades in endothelial cells. *J Immunol* 2002;169 (4):2102–2110. [PubMed: 12165538]

63. Monsinjon T, et al. Regulation by complement C3a and C5a anaphylatoxins of cytokine production in human umbilical vein endothelial cells. *FASEB J* 2003;17 (9):1003–1014. [PubMed: 12773483]
64. Oksjoki R, et al. Receptors for the anaphylatoxins C3a and C5a are expressed in human atherosclerotic coronary plaques. *Atherosclerosis* 2007;195 (1):90–99. [PubMed: 17234193]
65. Ohta A, Sitkovsky M. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature* 2001;414 (6866):916–920. [PubMed: 11780065]
66. Eckle T, et al. A2B adenosine receptor dampens hypoxia-induced vascular leak. *Blood* 2008;111 (4):2024–2035. [PubMed: 18056839]
67. Eltzschig HK, et al. ATP release from activated neutrophils occurs via connexin 43 and modulates adenosine-dependent endothelial cell function. *Circ Res* 2006;99 (10):1100–1108. [PubMed: 17038639]
68. Eltzschig HK, et al. Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: role of ectonucleotidases and adenosine A2B receptors. *J Exp Med* 2003;198 (5):783–796. [PubMed: 12939345]
69. Fredholm BB. Adenosine, an endogenous distress signal, modulates tissue damage and repair. *Cell Death Differ* 2007;14 (7):1315–1323. [PubMed: 17396131]
70. Sitkovsky MV, et al. Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. *Annu Rev Immunol* 2004;22:657–682. [PubMed: 15032592]
71. Fredholm BB, et al. Aspects of the general biology of adenosine A2A signaling. *Prog Neurobiol* 2007;83 (5):263–276. [PubMed: 17804147]
72. Thiel M, et al. Oxygenation inhibits the physiological tissue-protecting mechanism and thereby exacerbates acute inflammatory lung injury. *PLoS Biol* 2005;3 (6):e174. [PubMed: 15857155]
73. Reutershan J, et al. Therapeutic anti-inflammatory effects of myeloid cell adenosine receptor A2a stimulation in lipopolysaccharide-induced lung injury. *J Immunol* 2007;179 (2):1254–1263. [PubMed: 17617618]
74. Tanaka N, et al. P2Y receptor-mediated Ca(2+) signaling increases human vascular endothelial cell permeability. *J Pharmacol Sci* 2004;95 (2):174–180. [PubMed: 15215641]
75. Tanaka N, et al. Myosin light chain kinase and Rho-kinase participate in P2Y receptor-mediated acceleration of permeability through the endothelial cell layer. *J Pharm Pharmacol* 2005;57 (3):335–340. [PubMed: 15807989]
76. Tanaka N, et al. ATP participates in the regulation of microvessel permeability. *J Pharm Pharmacol* 2006;58 (4):481–487. [PubMed: 16597365]
77. McClenahan D, et al. Effects of extracellular ATP on bovine lung endothelial and epithelial cell monolayer morphologies, apoptoses, and permeabilities. *Clin Vaccine Immunol* 2009;16 (1):43–48. [PubMed: 18987163]
78. Weissmuller T, et al. Dynamic purine signaling and metabolism during neutrophil-endothelial interactions. *Purinergic Signal* 2005;1 (3):229–239. [PubMed: 18404508]
79. Pober JS, et al. Mechanisms of endothelial dysfunction, injury, and death. *Annu Rev Pathol* 2009;4:71–95. [PubMed: 18754744]
80. Jain RK. Molecular regulation of vessel maturation. *Nat Med* 2003;9 (6):685–693. [PubMed: 12778167]
81. Bhandari V, et al. Hyperoxia causes angiotensin 2-mediated acute lung injury and necrotic cell death. *Nat Med* 2006;12 (11):1286–1293. [PubMed: 17086189]
82. Lemieux C, et al. Angiotensins can directly activate endothelial cells and neutrophils to promote proinflammatory responses. *Blood* 2005;105 (4):1523–1530. [PubMed: 15498854]
83. Kunkel EJ, et al. Leukocyte arrest during cytokine-dependent inflammation in vivo. *J Immunol* 2000;164 (6):3301–3308. [PubMed: 10706723]
84. Huang AJ, et al. Endothelial cell cytosolic free calcium regulates neutrophil migration across monolayers of endothelial cells. *J Cell Biol* 1993;120 (6):1371–1380. [PubMed: 8449983]

85. Allingham MJ, et al. ICAM-1-mediated, Src- and Pyk2-dependent vascular endothelial cadherin tyrosine phosphorylation is required for leukocyte transendothelial migration. *J Immunol* 2007;179 (6):4053–4064. [PubMed: 17785844]
86. Potter MD, et al. Tyrosine phosphorylation of VE-cadherin prevents binding of p120- and beta-catenin and maintains the cellular mesenchymal state. *J Biol Chem* 2005;280 (36):31906–31912. [PubMed: 16027153]
87. Nottebaum AF, et al. VE-PTP maintains the endothelial barrier via plakoglobin and becomes dissociated from VE-cadherin by leukocytes and by VEGF. *J Exp Med* 2008;205 (12):2929–2945. [PubMed: 19015309]
88. Alcaide P, et al. Neutrophil recruitment under shear flow: it's all about endothelial cell rings and gaps. *Microcirculation* 2009;16 (1):43–57. [PubMed: 18720226]
89. Hu G, et al. Intercellular adhesion molecule-1-dependent neutrophil adhesion to endothelial cells induces caveolae-mediated pulmonary vascular hyperpermeability. *Circ Res* 2008;102 (12):e120–131. [PubMed: 18511851]
90. Su X, et al. CD47 deficiency protects mice from lipopolysaccharide-induced acute lung injury and *Escherichia coli* pneumonia. *J Immunol* 2008;180 (10):6947–6953. [PubMed: 18453616]
91. Matheny HE, et al. Lymphocyte migration through monolayers of endothelial cell lines involves VCAM-1 signaling via endothelial cell NADPH oxidase. *J Immunol* 2000;164 (12):6550–6559. [PubMed: 10843714]
92. Tudor KS, et al. Cytokines modulate endothelial cell intracellular signal transduction required for VCAM-1-dependent lymphocyte transendothelial migration. *Cytokine* 2001;15 (4):196–211. [PubMed: 11563880]
93. Abdala-Valencia H, Cook-Mills JM. VCAM-1 signals activate endothelial cell protein kinase C α via oxidation. *J Immunol* 2006;177 (9):6379–6387. [PubMed: 17056569]
94. Deem TL, et al. VCAM-1 activation of endothelial cell protein tyrosine phosphatase 1B. *J Immunol* 2007;178 (6):3865–3873. [PubMed: 17339486]
95. Dadke S, Chernoff J. Protein-tyrosine phosphatase 1B mediates the effects of insulin on the actin cytoskeleton in immortalized fibroblasts. *J Biol Chem* 2003;278 (42):40607–40611. [PubMed: 12902327]
96. Davenpeck KL, et al. Rat neutrophils express α 4 and β 1 integrins and bind to vascular cell adhesion molecule-1 (VCAM-1) and mucosal addressin cell adhesion molecule-1 (MAdCAM-1). *Blood* 1998;91 (7):2341–2346. [PubMed: 9516132]
97. Yoshida M, et al. Leukocyte adhesion to vascular endothelium induces E-selectin linkage to the actin cytoskeleton. *J Cell Biol* 1996;133 (2):445–455. [PubMed: 8609175]
98. Kiely JM, et al. Lipid raft localization of cell surface E-selectin is required for ligation-induced activation of phospholipase C γ . *J Immunol* 2003;171 (6):3216–3224. [PubMed: 12960351]
99. Oviedo-Orta E, Howard Evans W. Gap junctions and connexin-mediated communication in the immune system. *Biochim Biophys Acta* 2004;1662 (1–2):102–112. [PubMed: 15033582]
100. Zahler S, et al. Gap-junctional coupling between neutrophils and endothelial cells: a novel modulator of transendothelial migration. *J Leukoc Biol* 2003;73 (1):118–126. [PubMed: 12525569]
101. Scerri I, et al. Gap junctional communication does not contribute to the interaction between neutrophils and airway epithelial cells. *Cell Commun Adhes* 2006;13 (1–2):1–12. [PubMed: 16613775]
102. Phillipson M, et al. Endothelial domes encapsulate adherent neutrophils and minimize increases in vascular permeability in paracellular and transcellular emigration. *PLoS ONE* 2008;3 (2):e1649. [PubMed: 18297135]
103. Martin TR, et al. Effects of leukotriene B₄ in the human lung. Recruitment of neutrophils into the alveolar spaces without a change in protein permeability. *J Clin Invest* 1989;84 (5):1609–1619. [PubMed: 2553777]
104. Petri B, et al. The physiology of leukocyte recruitment: an in vivo perspective. *J Immunol* 2008;180 (10):6439–6446. [PubMed: 18453558]
105. Yang L, et al. ICAM-1 regulates neutrophil adhesion and transcellular migration of TNF- α -activated vascular endothelium under flow. *Blood* 2005;106 (2):584–592. [PubMed: 15811956]

106. Phillipson M, et al. Intraluminal crawling of neutrophils to emigration sites: a molecularly distinct process from adhesion in the recruitment cascade. *J Exp Med* 2006;203 (12):2569–2575. [PubMed: 17116736]
107. Carman CV, Springer TA. A transmigratory cup in leukocyte diapedesis both through individual vascular endothelial cells and between them. *J Cell Biol* 2004;167 (2):377–388. [PubMed: 15504916]
108. Shaw SK, et al. Coordinated redistribution of leukocyte LFA-1 and endothelial cell ICAM-1 accompany neutrophil transmigration. *J Exp Med* 2004;200 (12):1571–1580. [PubMed: 15611287]
109. Boueiz A, Hassoun PM. Regulation of endothelial barrier function by reactive oxygen and nitrogen species. *Microvasc Res* 2009;77 (1):26–34. [PubMed: 19041330]
110. Garcia JG. Concepts in microvascular endothelial barrier regulation in health and disease. *Microvasc Res* 2009;77 (1):1–3. [PubMed: 19232241]
111. Wang L, Dudek SM. Regulation of vascular permeability by sphingosine 1-phosphate. *Microvasc Res* 2009;77 (1):39–45. [PubMed: 18973762]
112. Sanchez T, et al. Induction of vascular permeability by the sphingosine-1-phosphate receptor-2 (S1P2R) and its downstream effectors ROCK and PTEN. *Arterioscler Thromb Vasc Biol* 2007;27 (6):1312–1318. [PubMed: 17431187]
113. Lee JF, et al. Balance of S1P1 and S1P2 signaling regulates peripheral microvascular permeability in rat cremaster muscle vasculature. *Am J Physiol Heart Circ Physiol* 2009;296 (1):H33–42. [PubMed: 19011048]
114. Brinkmann V, et al. FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. *Am J Transplant* 2004;4 (7):1019–1025. [PubMed: 15196057]
115. Trial watch: Phase III promise for oral multiple sclerosis therapy. *Nat Rev Drug Discov* 2009;8 (2): 98.
116. Bernard GR, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001;344 (10):699–709. [PubMed: 11236773]
117. Jacobson JR, Garcia JG. Novel therapies for microvascular permeability in sepsis. *Curr Drug Targets* 2007;8 (4):509–514. [PubMed: 17430121]
118. Finigan JH, et al. Activated protein C mediates novel lung endothelial barrier enhancement: role of sphingosine 1-phosphate receptor transactivation. *J Biol Chem* 2005;280 (17):17286–17293. [PubMed: 15710622]
119. Feistritzer C, Riewald M. Endothelial barrier protection by activated protein C through PAR1-dependent sphingosine 1-phosphate receptor-1 crossactivation. *Blood* 2005;105 (8):3178–3184. [PubMed: 15626732]
120. Houard X, et al. Differential inflammatory activity across human abdominal aortic aneurysms reveals neutrophil-derived leukotriene B4 as a major chemotactic factor released from the intraluminal thrombus. *FASEB J* 2009;23 (5):1376–1383. [PubMed: 19136615]

Figure 1.

Regulation of vascular permeability by neutrophils: role of soluble mediators. Following interaction with endothelium, activated neutrophils release a variety of soluble mediators. Neutrophils help convert complement protein C5 to C5a, which binds C5a receptor (C5aR) on the endothelial cell surface. Neutrophil-secreted tumor necrosis factor α (TNF α) binds its receptors TNFR1, 2. Arachidonic acid (AA) released by neutrophils can be processed by endothelial COX-1/2 into thromboxane A2 (TXA2) and by 5-lipoxygenase (5-LO) into leukotriene A4 (LTA4). LTA4 is then metabolized by LTA4 hydrolase (LTA4H) into LTB4 or by LTC4 synthase (LTC4S) into leukotriene LTC4. TXA2 binds to the thromboxane receptor (TP) and LTC4 binds the cysteinyl leukotriene receptor subtype 2 (CysLT2R). Neutrophil-derived chemokines such as CXCL1, 2, 3, and 8 bind their endothelial receptor (CXCR2). Engagement of all these receptors leads to endothelial retraction (red arrows). Activated neutrophils also release adenosine triphosphate (ATP) through connexin 43 hemichannels (Cxn 43), which is processed by CD39 on the neutrophil surface and by CD73 on the endothelial surface to adenosine monophosphate (AMP) and adenosine (ADO), respectively. ADO binds adenosine receptors A2a or A2b blocking endothelial retraction. Unprocessed ATP or adenosine diphosphate (ADP) can couple to P2 purinoceptors (P2Y) and induce endothelial retraction. C5a and CXCL1, 2, 3, and 8 can also activate the neutrophil in an autocrine loop. Neutrophil-derived LTA4, after conversion to LTB4 by neutrophil LTA4H, activates BLT1 which can lead to release of heparin-binding protein (HBP) from adherent neutrophils and mediate increased endothelial permeability.

Figure 2.

Regulation of vascular permeability by neutrophils: adhesion-dependent signals. β_2 integrins leukocyte function-associated antigen 1, LFA-1, and macrophage receptor 1, Mac-1 engagement with their endothelial ligands intercellular adhesion molecule 1 or 2 (ICAM-1, 2) is required for release of heparin-binding protein (HBP) from neutrophils, which binds to an as yet unidentified receptor (possibly cell surface heparin sulfate proteoglycans, PG) to increase endothelial intracellular Ca^{2+} . ICAM-1 engagement induces an increase in endothelial intracellular Ca^{2+} and phosphorylation of the junctional protein vascular endothelial (VE) cadherin, causing junctional disruption and cell retraction (red arrow). ICAM engagement also induces dissociation of vascular endothelial protein tyrosine phosphatase (VE-PTP, dashed arrow) from VE-cadherin, allowing for VE-cadherin phosphorylation. In addition, E-selectin engagement via P-selectin glycoprotein ligand 1 (PSGL-1) or other glycoprotein ligands induces increased endothelial intracellular Ca^{2+} , extracellular signal-regulated kinase (Erk) phosphorylation, and cytoskeletal changes. As both neutrophils and endothelial cells are known to express connexins, some studies have suggested a role for gap junction formation between the two cells, however, this is still controversial.