Tissue Barriers 3:1-2, e978720; January-June 2015; © 2015 Taylor & Francis Group, LLC

Blood cells and endothelial barrier function

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Keywords: Endothelial barrier, erythrocytes, leukocytes, platelets, vascular permeability

Abbreviations: AJ, Adherens junctions; ANG-1, Angiopoietin 1; AQP, Aquaporins; BBB, blood brain barrier; cAMP, 3'-5'-cyclic adenosine monophosphate; CNS, Central nervous system; COPD, Chronic obstructive pulmonary disease; EAE, Experimental autoimmune encephalomyelitis; EPAC1, Exchange protein activated by cyclic AMP; ERK1/2, Extracellular signal-regulated kinases 1 and 2; FA, Focal adhesions; FAK, focal adhesion tyrosine kinase; FoxO1, Forkhead box O1; GAG, Glycosaminoglycans; GDNF, Glial cell-derived neurotrophic factor; GJ, Gap junctions; GPCR, G-protein coupled receptors; GTPase, Guanosine 5'-triphosphatase; HMGB-1, High mobility group box 1; HRAS, Harvey rat sarcoma viral oncogene homolog; ICAM-1, Intercellular adhesion molecule 1; IL-1β, Interleukin 1 beta; IP3, Inositol 1,4,5-triphosphate; JAM, Junctional adhesion molecules; MEK, Mitogen-activated protein kinase kinase; MLC, Myosin light chain; MLCK, Myosin light-chain kinase; MMP, Matrix metalloproteinases; NO, Nitric oxide; OSM, Oncostatin M; PAF, Platelet activating factor; PDE, Phosphodiesterase; PKA, Protein kinase A; PNA, Platelet-neutrophil aggregates; pSrc, Phosphorylated Src; Rac1, Ras-related C3 botulinum toxin substrate 1; Rap1, Ras-related protein 1; RhoA, Ras homolog gene family, member A; ROS, Reactive oxygen species; S1P, Sphingosine-1-phosphate; SCID, Severe combined immunodeficient; Shp-2, Src homology 2 domain-containing phosphatase 2; SOCS-3, Suppressors of cytokine signaling 3; Src, Sarcoma family of protein kinases; TEER, Transendothelial electrical resistance; TJ, Tight junctions; TGF-beta1, Transforming growth factor-beta1; TNF-, Tumor necrosis factor alpha; VCAM-1, Vascular cell adhesion molecule 1; VE, Vascular endothelial; VEGF, Vascular endothelial growth factor; VE-PTP, Vascular endothelial receptor protein tyrosine phosphatase; VVO, Vesiculo-vacuolar organelle; ZO, Zonula occludens

The barrier properties of endothelial cells are critical for the maintenance of water and protein balance between the intravascular and extravascular compartments. An impairment of endothelial barrier function has been implicated in the genesis and/or progression of a variety of pathological conditions, including pulmonary edema, ischemic stroke, neurodegenerative disorders, angioedema, sepsis and cancer. The altered barrier function in these conditions is often linked to the release of soluble mediators from resident cells (e.g., mast cells, macrophages) and/or recruited blood cells. The interaction of the mediators with receptors expressed on the surface of endothelial cells diminishes barrier function either by altering the expression of adhesive proteins in the inter-endothelial junctions, by altering the organization of the cytoskeleton, or both. Reactive oxygen species (ROS), proteolytic enzymes (e.g., matrix metalloproteinase, elastase), oncostatin M, and VEGF are part of a long list of mediators that have been implicated in endothelial barrier failure. In this review, we address the role of blood borne cells, including, neutrophils, lymphocytes, monocytes, and platelets, in the regulation of endothelial barrier function in health and disease. Attention is also devoted to new targets for therapeutic intervention in disease states with morbidity and mortality related to endothelial barrier dysfunction.

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Submitted: 08/20/2014; Revised: 10/13/2014; Accepted: 10/15/2014
http://dx.doi.org/10.4161/21688370.2014.978720

Endothelial Barrier Function

An intact layer of healthy endothelial cells is essential for normal blood vessel function. The close apposition and alignment of endothelial cells in the vessel wall accounts for their ability to form a barrier that restricts the movement of water, proteins and blood cells between the intravascular and interstitial compartments. This barrier is formed by a layer of endothelial cells that are joined laterally by cell-cell junctions, while the basolateral aspect of this layer is attached to a basement membrane composed of collagen, fibronectin, laminin, and glycosaminoglycans (GAG). Cell-surface expressed integrins, which form regions called focal adhesions (FA), bind the endothelial cells to the extracellular matrix. The resulting barrier is semi-permeable to water and non-lipophilic molecules, and is both size- and charge-selective for solutes passage.[1] Precise regulation of the restrictive properties of the endothelial barrier is essential for normal organ function. Indeed, diminished barrier function (and increased vascular permeability) is associated with organ dysfunction and can lead to serious pathological consequences, as evidenced in diseases such as sepsis, as well as inflammatory and neurodegenerative diseases. Restoration of endothelial barrier integrity in these conditions can significantly impede disease progression.[1,2]

Several different pools of proteins are assembled on endothelial cells to form membrane domains that create the cohesive structure that accounts for the barrier properties of the vessel wall. Among the barrier-forming adhesive structures, the most important are the adherens junctions (AJ), gap junctions (GJ), and tight junctions (TJ). These domains collectively form the paracellular junctional structure that regulates the partitioning of water and solutes between

blood and interstitium, and an alteration in these membrane components often underlie the increased vascular permeability that accompanies inflammation.[3] In addition to their barrier function, signaling mediated through these adhesive membrane proteins contribute to a variety of endothelial cell processes, such as cell growth, cell polarity and their interactions with other cell types such as smooth muscle cells and pericytes.[4] Consequently, an alteration in endothelial adhesive proteins affects not only vascular permeability but also the vascular responses to changes in the perivascular environment.

Adherens junctions mediate cell-cell contact among endothelial cells in all types of blood vessels, and is composed mainly of VE-cadherin, a member of a transmembrane Ca^{2+}-dependent adhesion molecule family that regulates vascular permeability.[5] VE-cadherin on one cell strongly binds (homotypically) VE-cadherin on an adjacent cell, which leads to a reorganization of the cytoskeleton in both cells via an interaction with actin filaments after cadherin-catenin binding (β-catenin, p120 catenin and α-catenin).[6,7] VE-cadherin recruits α-catenin, via β-catenin, to sites of adherens junction assembly. Other actin-binding, such as vinculin, α-actinin, and eplin are also recruited to the adherens junction by following conformational changes in α-catenin, which serve to reinforce the adherens junction.[8] The main function of VE-cadherin is to seal the paracellular space, but it also modulates angiogenesis, inhibits growth (cell contact inhibition), and protects cells from apoptosis.[9] VE-cadherin has also been implicated in the transcriptional regulation of claudin-5, another component of the endothelial tight junction, via a mechanism that involves the phosphorylation of Fox01.[10] Phosphorylation and dephosphorylation of AJ residues, including the intracellular tail of VE-cadherin, regulates the function of VE-cadherin.[11] The enzymes responsible for the VE-cadherin phosphorylation include tyrosine kinase Src, protein kinase C, and focal adhesion tyrosine kinase (FAK),[12-14] while the dephosphorylating enzymes include Shp-2 and vascular endothelial (VE) receptor protein tyrosine phosphatase (VE-PTP).[15,16] Wessel[17] have recently demonstrated that selective phosphorylation/dephosphorylation of specific tyrosine residues (Tyr685 or Tyr731) on VE-cadherin allows for the differential regulation of vascular permeability and leukocyte extravasation.

Gap junctions allow for cell-cell communication via the formation of clusters of intercellular hemi-channels that link to each other to connect the cytoplasm of the adjacent cells.[18] Gap junctions are formed by proteins from the connexin family. Molecules less than 1000 daltons, such as ions, simple sugars, amino acids, nucleotides, and second messengers (cAMP, calcium, IP3) can move between cells via these channels.[19] GJ are also involved in several cellular events, including metabolic transport, electrical coupling, apoptosis, differentiation, and tissue homeostasis,[20] and phosphorylation of the inner tail of this junctional structure can affect these functions of GJ.[21] In addition to allowing for communication between endothelial cells,[22] GJ also allow for cross-talk between the endothelium and smooth muscle cells in the vessel wall.[23]

The cerebral vasculature contains an additional component of the endothelial barrier called tight junctions (TJ), which closely fuses adjacent endothelial cells and further restricts the exchange of fluid and solutes through the paracellular spaces.[24,25] TJ contribute to the highly selective properties of the blood brain barrier (BBB), which significantly limits the passage of substances from blood to brain interstitium. TJ are comprised of different proteins such as occludin, claudin family members, zonula occludens (ZO) family members and junctional adhesion molecules (JAM),[24] which form a charge selective pore that only allows for the passage of small ions and uncharged molecules.[26] Zonula occludens are scaffolding proteins that interact with intracellular components such as F-actin to influence cytoskeleton mobility and other functions.[27] The claudin family is comprised of more than 20 proteins and endothelial cells in the BBB are particularly rich in claudins 4, 5 and 16.[28] TJ permeability is significantly influenced by the type(s) of claudin present or absent in the endothelial cells.[29] For example, in the absence of claudin-5 BBB permeability is profoundly comprised.[30] Occludin is a phosphoprotein of 65-kDa located in the cytoplasmic membrane of endothelial cells in brain.[31] Phosphorylation of occludin amino acid residues can strongly influence vascular barrier function.[32] However, selective deletion of occludin has been shown not to affect vessel permeability, suggesting overlapping functions of the different TJ proteins.[33] JAM family members, including JAM-A, JAM-B and JAM-C, are also present in endothelial cells found in different vascular beds including liver, brain, intestine and lungs,[34] and are expressed by circulating blood cells, including platelets, lymphocytes and neutrophils.[35] Known functions of JAMs include signaling to cytoskeletal proteins, assembly of TJ, and gathering cell-polarity proteins to the TJ.[36] Alterations in either of these TJ constituents members may result in endothelial barrier failure.

As a connective structure that links vascular endothelial cells to extracellular matrix proteins, focal adhesions (FA) are comprised of integrins, which participate in different cell functions such as adhesion, movement, and matrix remodeling. FA are connected to actomyosin bundles and serve as extracellular sensors.[37] While FA do not directly form cell-cell junctions, these structures act as mechano- and chemo-sensors that modulate cytoskeleton tension. Intracellular signaling events associated with FA include the recruitment and activation of kinases that can modulate the binding affinity of integrins via phosphorylation.[38] Immunoblockade of these integrins or interference with their binding to extracellular matrix constituents results in an increased vascular permeability,[39,40] confirming the critical role of integrins in the regulation of endothelial barrier function. The relative importance of integrins in modulating endothelial barrier function appears to increase in conditions associated with angiogenesis or inflammation.[41,42]

Another structural feature of endothelial cells that has been implicated in the modulation of vascular permeability is the glycocalyx, a 200–500 nm thick layer on the luminal surface of the cell that is comprised of proteoglycans with GAG side chains (e.g., heparan sulfates).[43,44] A reduction in glycocalyx thickness caused by enzymatic degradation is associated with an increased transendothelial albumin flux,[44] while stabilization of the glycocalyx with angiopoietin-1 reduces albumin permeability.[45] The negative charge of GAGs in the glycocalyx is believed to impose a significant barrier to protein movement, while offering little resistance to the movement of water across the endothelial barrier.[44,46]

While most attention devoted to vascular permeability has been given to modulation of the intercellular junctions (paracellular pathway), solutes and water can also cross the endothelial barrier via a transcellular pathway. Vesicules (or calveolae) have long been considered a pathway for the exchange of plasma proteins between the blood and interstitial compartment.[47] The transcytosis process is regulated by different factors that target components of the vesicle, such as caveolin-1, which serves as a scaffold for albumin-binding proteins as well as different signaling molecules that regulate transcytosis.[48] In the cerebral microvasculature, with its tight junctions, the transcellular route is also important for the exchange of water. Aquaporins (AQP), cell membrane channels in vascular endothelium, have been shown to contribute to water exchange across the BBB under both basal conditions and in certain pathological states.[49-51] However, for most vascular beds, the quantitative importance of the transcellular pathway for the exchange of water and plasma protein exchange across endothelial cells appears small.[47]

cAMP, a second messenger that is constantly formed in most cells, including endothelial cells, plays an important role in the modulation of endothelial barrier function. It is generated by the membrane-associated enzyme adenylyl cyclase following activation of G-protein coupled receptors (GPCR) by either endogenous (e.g., inflammatory mediators, hormones, neurotransmitters)[52,53] or exogenous (e.g., drugs, xenobiotics, germs)[54-56] stimuli. cAMP degradation is mediated by phosphodiesterase (PDE).[57] The accumulation of cAMP in endothelial cells can result in either barrier-destabilization or -preservation, depending on the intracellular locus of cAMP generation, with cytosolic accumulation leading to increased vascular permeability, while increased cAMP in vacuoles appears to protect against barrier dysfunction.[58] At least part of the endothelial barrier preserving effect of cAMP reflects its influence on junctional proteins.[59] cAMP-induced barrier preserving signaling includes: 1) activation of cAMP-dependent protein kinase A (PKA) and phosphorylation of downstream proteins, such as ERK1/2 and myosin light-chain kinase (MLCK), important modulators of vascular permeability; and 2) binding to intracellular proteins involved in inflammation, such as the exchange protein activated by cyclic AMP (EPAC1).[60] EPAC1 is known to induce immunomodulatory genes such as suppressors of cytokine signaling 3 (SOCS-3) and to reduce integrin-mediated permeability responses.[60] Furthermore, both PKA and EPAC1 are known to activate Rac1, a small GTPase involved in endothelial barrier protection via inhibition of RhoA, which regulates the MLCK, a protein whose activation leads to endothelial cell contraction.[61] EPAC1 activation by cAMP also results in the activation of Rap1, via a PKA-independent pathway, and ultimately leads to enhanced endothelial barrier function by inducing the reorganization of cortical actin, redistribution of VE-cadherin and other junctional proteins to cell-cell contacts.[62] Consequently, cellular events that alter the bioavailability of cAMP can exert a major influence on the barrier function of vascular endothelial cells.

A variety of chemical and physical factors (e.g., shear stress) act constantly on endothelial cells to influence its barrier properties.[7,63] To some extent, the factors that act on endothelial cells are derived from other cell populations that comprise the vessel wall (e.g., podocytes, smooth muscle) or from neighboring cells that lie in the immediate perivascular space (e.g., mast cells, macrophages). Endothelial cells are also able to synthesize and release factors, such as adrenomedullin, that act to stabilize the endothelial barrier thereby opposing the actions of inflammatory mediators on vascular permeability.[64] However, when mediator release from these other cell populations is excessive, endothelial barrier dysfunction or failure may result. There is also mounting evidence that blood cells are capable of exerting a similar influence on endothelial barrier function, and may account for the barrier failure evidenced in different pathological conditions. In the following sections, we briefly summarize evidence implicating different blood cell populations in the modulation of endothelial barrier function, address their potential role in the vascular permeability responses in different disease states, and discuss potential therapeutic targets for prevention of endothelial barrier dysfunction.

Pericytes, which heavily populate the vessel wall in some vascular beds, such as brain, lie in close contact with endothelial cells.[65] The proximity between pericytes and endothelial cells allow for cross-talk between the 2 cell types, and accounts for the ability of pericytes to regulate the expression of junctional proteins.[66] In the brain, pericytes also influences astrocyte cell organization/polarization, thereby maintaining the restrictive properties of the BBB.[67] Some pericytes-derived mediators also exert a modulating influence on BBB function by regulating the expression of endothelial junction proteins. There include transforming growth factor-beta1 (TGF-beta1),[68] glial cell-derived neurotrophic factor (GDNF),[69] and angiopoietin 1 (ANG-1).[70]

Leukocytes and Endothelial Barrier Function

Neutrophils

Neutrophils have been implicated as mediators of the increased vascular permeability that accompanies a variety of pathological conditions, including ischemia-reperfusion,[71] sepsis,[72] cancer[73,74] and neurological diseases.[75] A role for neutrophils in these conditions is largely based on 2 observations: 1) neutrophils are recruited into the diseased/injured tissue, and 2) interfering with the neutrophil accumulation minimizes or prevents the endothelial barrier dysfunction.[76,77] Activated neutrophils release an impressive mixture of chemicals that can impair endothelial barrier function, including reactive oxygen species (ROS), proteolytic enzymes, and cytokines (**Fig. 1**). These mediators and other products of neutrophil activation can alter barrier function by acting on the endothelial cell cytoskeleton, junctional proteins, and the endothelial glycocalyx. For example, endothelial cells exposed to ROS exhibit an increased permeability response that has been linked to disruption of the inter-endothelial junction, actomyosin contraction, gap formation, and an altered expression and phosphorylation state of junctional adhesion molecules.[78-80] Since superoxide is known to rapidly interact with (and inactivate) nitric oxide, some have attributed the effects of ROS on endothelial barrier function to an alteration in NO

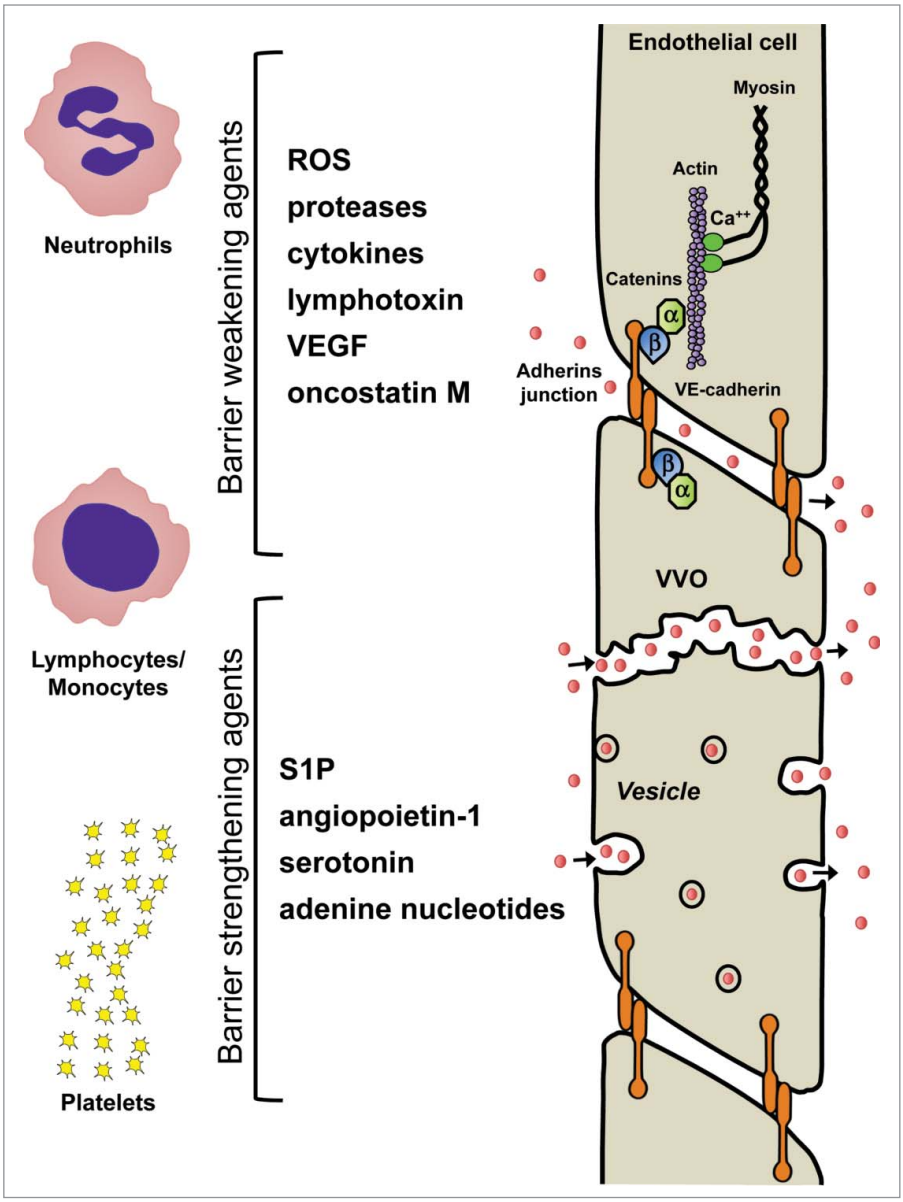

Figure 1. The endothelial barrier. Mediators released from neutrophils, lymphocytes, monocytes, and platelets act on endothelial cells to either weaken or strengthen the barrier. The mediators exert their effects on barrier function by altering the width of the intercellular junctions, either through changes in junctional proteins, the endothelial cell cytoskeleton, or both. Adherens junctions regulate paracellular leakage. Transcellular (vesiculo-vacuolar organelle [VVO] or vesicular) transport of water and solutes also occurs across the endothelial barrier. However, the quantitative significance of this pathway and its responsiveness to barrier altering agents remain unclear. Arrows designate the direction of transport across the barrier.

bioavailability.[81] However, NO has been implicated as both a negative and a positive modulator of endothelial barrier function,[82,83] with the protective role of NO attributed to its ability to inhibit leukocyte-endothelial cell adhesion.[84] Nitric oxide synthase inhibition increases the permeability of endothelial cell monolayers, a response that is associated with the formation of stress fibers and disruption of adherens junctions.[85]

Neutrophils are also able to enhance transendothelial protein exchange by releasing proteases, like elastase and matrix metalloproteinases (MMP), which appear to alter barrier function by disrupting junctional complexes and inducing endothelial cell retraction.[86-88] Elastase has also been shown to promote the adhesion and transendothelial migration of leukocytes in the microcirculation,[89] suggesting that the permeability enhancing effect of the protease may also be related to an enhancement of neutrophil-endothelial cell adhesion. This possibility is supported by reports describing diminished endothelial barrier function, resulting from junctional disassembly and cytoskeletal reorganization, following the ligation of neutrophil adhesion molecules with their counter-receptors on endothelial cells, such as the binding of β-2 integrins with either ICAM-1 or VCAM-1.[83,90,91] It has also been proposed that neutrophils can diminish barrier function due to physical disruption of the paracellular pathway caused by the passage of these cells through the junctions.[92,93] This appears to occur despite the fact that endothelial cells can extend projections to envelop the migrating neutrophils, forming endothelial domes, with the leakage response resulting from the transfer of entrapped plasma proteins within the "dome."[83,94] It has also been reported that the endothelial barrier dysruption caused by transmigrating leukocytes are detected by the endothelial cells as a release of isometric tension, which results in protective actin remodeling that is dependent on the production of reactive oxygen species.[95] Furthermore, the results of a recent study reveal that extravasating leukocytes deposit microparticles on the subendothelium during their passage through the junctions and that the microparticle deposition serves to maintain barrier function; inhibition of neutrophil-derived microparticle formation resulted in dramatically increased vascular leakage.[96]

Another consequence of neutrophil activation within the microcirculation is capillary no-reflow, which is manifested as a reduced number of perfused capillaries and tissue hypoxia.[97] The capillary malperfusion is worsened when neutrophil-dependent increases in vascular permeability lead to enhanced capillary fluid filtration and excessive accumulation of fluid in the interstitial compartment. The accompanying increase in interstitial fluid pressure leads to compression of the microvasculature, which further exacerbates the no-reflow response. This mechanism is supported by studies describing reductions in vascular permeability and intersititial edema, and an improvement of capillary

perfusion following neutrophil depletion or prevention of leukocyte-endothelial cell adhesion.[98]

Lymphocytes

Less is known about the role of lymphocytes in the modulation of endothelial barrier function. Because T-cells are known to influence neutrophil function and to enhance the endothelial cell dysfunction mediated by neutrophils,[99] it is often assumed that the contribution of T-cells to inflammation-induced vascular protein leakage largely reflects the ability of T-cells to enhance the recruitment and reactivity of neutrophils.[77] However, studies in severe combined immunodeficient (SCID) mice,[100] CD3+ T-cell deficient[101] mice and wild type mice treated with CD4+ T-cell depleting antibody[102] have revealed an important role for T-lymphocytes in mediating the increased vascular permeability induced by ischemia-reperfusion in the intestine, kidney and lung. T-cells have also been implicated in mediating the blood-brain barrier (BBB) disruption that is associated with experimental autoimmune encephalomyelitis (EAE).[103] In this model of neurological disease, CD4+ T cells appear to elicit changes in tight junction architecture and BBB permeability by inducing astrocytes to release vascular endothelial growth factor (VEGF). Studies of a CD8+ T-cell dependent model of BBB disruption that mimics multiple sclerosis have revealed that stimulation of CNS infiltrating CD8 T cells leads to astrocyte activation, alteration of BBB tight junction proteins and increased BBB permeability in a non-apoptotic manner, but these responses were not observed in perforin deficient mice.[104] While other lymphocyte-derived products, such as lymphotoxin, have been shown to increase the permeability of endothelial cell monolayers in vitro,[105] the role of these products in T-cell dependent modulation of vascular permeability in vivo remains unclear.

Monocytes

Monocytes are known to produce and release a variety of mediators of endothelial barrier dysfunction, notably factors such as oncostatin M (OSM) and VEGF. Oncostatin M (OSM), a member of the IL-6 superfamily, has been shown to reduce trans-endothelial electrical resistance (TEER) of monolayers comprised of cultured rat cerebral microvascular endothelial cells.[106] OSM may also promote BBB dysfunction by stimulating brain cells to produce cytokines and prostaglandins,[107,108] and to increase the expression of cell adhesion molecules on endothelial cells.[109] While monocytes are the dominant source of OCM produced by blood cells, activated microglia and astrocytes are additional sources of OCM in the brain. Monocytes are also a rich source of VEGF.[110] Monocyte-derived VEGF has been implicated in the enhanced vascular leakage that accompanies breast tumor metastasis to the lung.[111] This mechanism may also contribute to the endothelial barrier dysfunction detected in other disease models that includes the recruitment of monocytes, such as atherosclerosis. Other monocyte-derived mediators that have been shown to increase vascular permeability include high mobility group box 1 (HMGB-1), TNF-α and IL-1β.[112,113]

The engagement of some inflammatory cells with integrins expressed on the endothelial cell surface can initiate a series of responses that will facilitate the transendothelial migration of the attached blood cell. For example, the binding of integrins present on monocytic cells with adhesion molecules on endothelial cells induces HRas\Raf\MEK\ERK signaling, which leads to myosin light chain (MLC) activation. This results in the recruitment of Src to VE-cadherin and phosphorylation, the dissociation of VE-cadherin/β-catenin complex, and ultimately gap junction formation.[114]

There is also evidence that supports a protective role for monocytes in the maintenance of endothelial barrier function. As described above for neutrophils,[96] it has been reported that microparticles released from activated monocytes enhance the tightness of endothelial cell monolayers after exposure to bacterial endotoxin.[115] While this microparticle mediated response was associated with inhibition of pSrc (tyr416) signaling, a cause-effect relationship with endothelial barrier function was not demonstrated. In another study,[116] a different mechanism of monocyte-mediated protection was demonstrated. CD14+ peripheral monocytes, cultured under angiogenic conditions, were shown to acquire phenotypic and functional properties similar to cerebral microvascular endothelial cells. The features acquired by the monocytes included the expression of tight junction proteins, high transcellular electrical resistance and low permeability to solutes. It was proposed that CD14+ blood monocytes may play an important role in repairing (sealing) the BBB after brain injury.[116]

Platelets and endothelial barrier function

Recently, much attention has been devoted to addressing the role of platelets in inflammation, and the evolving consensus is that platelets tend to amplify diffrent components of the inflammatory response, most notably the expression of endothelial cell adhesion molecules and the recruitment of leukocytes.[117,118] While there are some reports that describe the ability of platelets to diminish endothelial barrier function,[119] there is a larger body of evidence that supports an anti-permeability effect of platelets.[120] For example, thrombocytopenia appears to elicit an increased vascular permeability in resting microvessels and this response is reversed following the restoration of blood platelet count.[121] Some of the beneficial effects of platelets in support of barrier function have been attributed to a purely physical effect resulting from adherent platelets covering gaps in the endothelial lining of injured blood vessels,[120,122] however, soluble factors released by platelets are a more likely to explain the ability of these cells to maintain vascular wall integrity in the setting of inflammation or other pathological conditions.[77,120] Platelet-conditioned media[123,124] and different molecules released from platelets, including sphingosin-1-phosphate (S1P),[123] serotonin,[125] angiopoietin-1,[126] and adenine nucleotides,[127] have been shown to enhance the barrier properties of endothelial cells either in vivo or in vitro. S1P is believed to be continuously secreted into the blood stream by platelets as well as erythrocytes under physiological conditions.[128] The S1P subsequently binds to its receptor on the surface of endothelial cells thereby activating Rac1, which acts to preserve endothelial barrier function. The importance of platelet and erythrocyte-derived S1P in modulating vascular permeability is evidenced by reports that describe a high basal leak of proteins in pulmonary microvessels of mutant

mice that selectively lack S1P in plasma,[129] and the observation that the increased permeability observed in intact microvessels perfused with an erythrocyte-free solution is reversed following the administration of exogenous S1P.[130]

Platelets also hold the potential to influence endothelial barrier function by forming heterotypic aggregates with leukocytes. For example, platelet-neutrophil aggregates (PNA) have been implicated in the increased pulmonary vascular permeability in mice with sickle cell disease.[131] In this model of human disease, interfering with PNA formation with a P-selectin blocking antibody decreased the lung vascular permeability response. While it is not clear how the aggregate formation leads to altered barrier function, the response may be related to the observation that neutrophils and monocytes with attached activated platelets produce more than twice the amount of superoxide than their platelet-free counterparts, and P-selectin mediated signaling underlies this response.[132] Similarly, it has been demonstrated that the generation of platelet activating factor (PAF) by the combination of platelets and neutrophils is 2-times higher than that detected in either cell activated separately, but this amplification effect on PAF production results from transcellular phospholipid metabolism between the 2 cells, and does not require cell–cell adhesion.[133] PAF, which is known to increase vascular permeability when engaged with its receptor on endothelial cells, disrupts the inter-endothelial junctions via Rac1-dependent relocation of junctional proteins (e.g., VE-cadherin, ZO-1) and actin polymerization.[134]

Diseases associated with endothelial barrier dysfunction

An injured or dysfunctional endothelial barrier has the potential to significantly impact tissue function and viability. Discontinuities or breaks in the endothelial lining can impair blood flow regulation by interfering with vasodilatory responses that are dependent on endothelial cell-cell communication (e.g., ascending vasodilation).[135] Clot formation can also result if the breach in the barrier is sufficient to expose platelets to the collagen layer that normally lies beneath the endothelial cell lining.[120] However, endothelial barrier dysfunction is more commonly associated with subtle changes in the inter-endothelial junctions (discussed above) that can result in the excessive loss of water and proteins into the extravascular compartment.[136] The magnitude of the leakage of fluid and protein that accompanies an increased vascular permeability can lead to edemagenic responses that range from small, reversible and without a long-lasting effect on tissue function to a severe and irreversible response that leads to tissue necrosis and organ failure. The entire range of permeability-dependent edemagenic responses is evidenced in human disease states. As noted in Table 1, increased vascular permeability has been implicated in a variety of pathological conditions, including both acute and chronic diseases. In some conditions, the permeability response is largely manifested in one organ system (e.g., COPD, nephrotic syndrome, Alzheimer disease) while a more widespread (systemic) permeability response is noted in other diseases (e.g., sepsis, diabetes mellitus).

The contribution of the endothelial barrier dysfunction to disease morbidity and mortality appears to be condition- and organ-dependent. For example, while the vascular permeability increases that accompanies sickle cell disease and hypertension are not likely to contribute significantly to disease induction, progression and/or mortality, a significant contribution to disease outcome may be expected of the endothelial barrier failure that is associated with conditions such as sepsis, acute kidney injury, dengue hemorrhagic fever, and stroke. Two organs that appear to be most vulnerable to the deleterious consequences of endothelial barrier dysfunction are the brain and lungs. In both tissues, excessive fluid loss across a leaky endothelial cell layer has the potential to profoundly impact organ function and/or viability. This is commonly manifested in the lungs as an accumulation of interstitial fluid in the alveolar spaces (pulmonary edema), which results when the alveolar membrane is ruptured due to excessive interstitial fluid accumulation (and an elevated interstitial pressure) secondary to capillary fluid leakage.[163] A similar phenomenon has been described in the intestine, with excessive capillary fluid and protein leakage resulting in mucosal barrier disruption and the movement of interstitial fluid in the gut lumen.[164] However, the response in gut is not as immediately life-threatening as pulmonary edema, which impairs gas exchange and may cause respiratory failure. The rapidly evolving and often fatal (despite mechanical ventilation) pulmonary edema that is associated with Hantavirus infection likely results from endothelial barrier failure.[165]

The structurally unique and highly restrictive endothelial barrier in the brain offers a level of tissue protection that is beyond that manifested in other organs. The BBB is largely impermeable to water, ions, plasma proteins, inflammatory mediators (e.g., cytokines), immune cells, and a variety of drugs. Consequently, BBB disruption in the brain can be associated with more profound local and systemic detrimental effects than observed in other tissues following endothelial barrier failure. The fact that

Table 1. Pathological conditions associated with endothelial barrier dysfunction

Local response	Systemic response
Acute conditions/diseases	
Stroke[71,137,138]	Sepsis[139]
Acute respiratory distress syndrome[140]	Dengue fever[141]
Nephrotic syndrome[142]	Malaria[143]
Myocardial infarction[144]	Ebola[145]
Hantavirus pulmonary syndrome[146]	Preeclampsia[147]
Anaphylaxis[148]	
Chemical/thermal injury[149]	
Chronic conditions/diseases	
Atherosclerosis[150]	Hypertension[151]
Inflammatory bowel disease[152]	Diabetes mellitus[153]
COPD[1] [154]	Sickle cell disease[155]
Tumors[156,157,137]	Hereditary angioedema[158]
Arthritis[159]	
Asthma[154]	
Neurological diseases	
Alzheimer[160]	
Multiple sclerosis[137]	
Amylotrophic lateral sclerosis[161]	
Epilepsy[138]	
Major depressive disorder[162]	

[1]Chronic obstructive pulmonary disease (COPD).

the brain is encased in a vault (the skull) results in significantly larger increases in interstitial pressure when high fluid filtration rates result from BBB failure, which can result in blood vessel compression and blood flow restriction.[166] Macrophages that normally reside in the brain, like microglia and astrocytes, no longer enjoy an "immunoprivileged" environment following BBB disruption. Consequently, inflammatory response elicited by a pathological insult is greatly amplified when the BBB loses its ability to impede the egress of immune cells and mediators. Many of these manifestations of BBB dysfunction are evidenced following an ischemic stroke and this response is believed to promote expansion of the infarcted area.[167] While restoration of BBB function has gained attention as a potentially useful therapeutic goal in stroke patients,[168] BBB disruption has also been exploited for enhanced delivery of imaging agents to optimize the detection and quantification of brain edema and infarct size following stroke.[169]

Drugs targeting endothelial barrier dysfunction

Our understanding of the cellular and molecular events that regulate vascular permeability has advanced significantly over the past few decades. However, this expanded knowledge base has not translated into the identification or development of therapeutic approaches that can be widely used to enhance endothelial barrier function in patients with life-threatening conditions that are linked to barrier dysfunction or failure. While efforts to target individual barrier-enhancing agents (e.g., cytokines, reactive oxygen species), derived from either circulating blood cells, cellular components of the vessel wall or perivascular cells (mast cells, macrophages) have shown promise in some animal models of human disease, this strategy has limited effectiveness in more complicated pathological conditions that involve a role for multiple mediators of endothelial barrier dysfunction. Hence, more recent efforts have focused on identifying therapeutically relevant agents that directly target the endothelial barrier to render it more restrictive to fluid and solute exchange. Table 2 summarizes endothelial barrier enhancing agents that have been proposed as potential drugs for the clinical management of patients suffering from a condition characterized by vascular hyper permeability.[1,170] Some of these agents (e.g, sphingosine-1-phosphate, activated protein C) appear to act on the barrier to stabilize both the junctions and the cytoskeleton, while other agents target either specific receptors for known potent barrier-altering agents (e.g., VEGF, vasopressin type 1a receptor) or act to interfere with key signaling molecules that promote changes in the junction and/or cytoskeleton to produce a hyperpermeability state (e.g, protein kinase C and RhoA inhibitors).[1,170,171] While these strategies hold promise, additional work is needed to translate the existing knowledge on endothelial barrier regulation to the development of a therapeutic agent that can be routinely used to protect or enhance endothelial barrier function.

Table 2. Agents that protect or enhance endothelial barrier function*

Sphingosine-1-phospate
Activated protein C
Angiopoietins
Protein kinase C inhibitors
RhoA inhibitors
Corticosteroids
Antihistamines
Vasopressin type 1a agonists

*1,170,171.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

The authors are supported by grants from the National Heart Lung and Blood Institute (HL26441) (DNG) and from the Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP, 2011/02438–8) and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq, 470895/2011–0) in Brazil (SFR).

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