Emerging therapeutic strategies to prevent infection-related microvascular endothelial activation and dysfunction

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Recent evidence suggests that loss of endothelial barrier function and resulting microvascular leak play important mechanistic roles in the pathogenesis of infection-related end-organ dysfunction and failure. Several distinct therapeutic strategies, designed to prevent or limit infection-related microvascular endothelial activation and permeability, thereby mitigating end-organ injury/dysfunction, have recently been investigated in pre-clinical models. In this review, these potential therapeutic strategies, namely, VEGFR2/Src antagonists, sphingosine-1-phosphate agonists, fibrinopeptide Bβ$_{15-42}$, slit2N, secinH3, angiopoietin-1/tie-2 agonists, angiopoietin-2 antagonists, statins, atrial natriuretic peptide, and mesenchymal stromal (stem) cells, are discussed in terms of their translational potential for the management of clinical infectious diseases.

Introduction

Microvascular leak caused by compromised vascular barrier function plays an important role in the pathogenesis and disease progression for a range of infectious syndromes, including sepsis,[1] acute lung injury,[2] dengue hemorrhagic fever and shock syndrome,[3] viral hemorrhagic fevers,[4] and hantavirus pulmonary syndrome.[5] The main component of the vascular barrier is the endothelial cell monolayer, which is comprised of endothelial cells themselves and associated endothelial cell–cell junctions, including both adherens junctions and tight junctions, as well as a variety of extracellular components (e.g., the glycocalyx and the basement membrane).

Tight junctions, also referred to as zonula occludens, are predominantly composed of occludins and claudins, and are commonly located at the apical surface of the inter-endothelial cell cleft. Vascular surfaces that require tight regulation of endothelial cell permeability, such as the blood–brain barrier, are typically comprised of well-developed tight junctions. Adherens junctions are predominantly composed of vascular endothelial cadherin (VE-cadherin). VE-cadherin contains an extracellular domain that connects to adjacent endothelial cells and an intracellular domain that connects to the actin cytoskeleton via a family of catenins (α-, β-, γ-, and p120 catenins).[6] VE-cadherin is regulated by the Rho family of GTPases, including Rho1, Rac1, and Cdc42. Specifically, Rho mediates endothelial cell permeability and junction disassembly, while Rac enhances vascular endothelial cell barrier integrity.[7]

The semipermeable endothelial barrier allows for transport of fluids and solutes from blood vessels into tissues. However, in pathological states, increased endothelial cell permeability results in excess transit of proteins and solutes between endothelial cells (paracellular leak), thereby causing edema. Such gaps in the vascular barrier are predominantly regulated by VE-cadherin,[8,9] but may also be regulated by additional components of the adherens junctions, as well as modification of tight junctions and distortion of the endothelial cell structure due to cytoskeletal remodeling.

Recent evidence suggests that preventing microvascular leak may represent a viable therapeutic strategy to decrease infection-related end-organ injury/dysfunction in infectious diseases, thereby improving clinical outcome. A number of therapeutic strategies have emerged that are intended to strengthen vascular barrier integrity (**Table 1 and Fig. 1**). The focus of this review is to summarize these emerging therapeutic strategies and highlight their reported effects in pre-clinical models of infectious diseases.

VEGFR2/Src Antagonists

Vascular endothelial growth factor (VEGF) is a glycoprotein that is generated and released by endothelial cells, lung epithelial cells, platelets, and leukocytes. VEGF is a well-established regulator of vascular permeability and exerts its effects through binding endothelial cell-specific membrane tyrosine kinase receptors, VEGFR1–3.[10] Upon activation by its cognate ligand, VEGFR2

Table 1. Emerging microvascular barrier-enhancing agents

Agent	Mechanism of action
VEGFR2/Src antagonists	• Decrease activation of Src family kinases
	• Inhibit VEGF-induced VE-cadherin internalization
	• Regulate $\alpha_v\beta_3$ integrins
Sphingosine-1-phosphate agonists	• Bind endothelial receptor $S1P_1$ to activate Rho and enhance cadherin expression
	• Activate $\alpha_v\beta_3$ integrins through Rac to stabilize the endothelial cytoskeleton
	• Block thrombin-activated PAR-1 signaling
	• Block VEGF induced VE-cadherin internalization
	• Downregulate IFN-α thereby dampening innate immune responses
Fibrinopeptide $B\beta_{15–42}$	• Binds VE-cadherin to stabilize interendothelial junctions
	• Increases binding of the Src kinase Fyn with p190RhoGAP in parallel with decreasing Fyn association with VE-cadherin
Slit2N	• Binds to Robo4 to reduce p120-catenin phosphorylation and increases p120 catenin association with VE-cadherin at the cell surface
	• Inhibits ARF6 and VEGF signaling
	• Attenuates endothelial cytoskeletal elements via Rac1
SecinH3	• Inhibits guanine nucleotide exchange factors such as ARNO to increase cell surface VE-cadherin
Angiopoietin-1/Tie2 agonists	• Bind Tie2 to downregulate VCAM-1 and E-selectin
	• Decrease NFκB-dependent gene expression
	• Block VEGFR2 signaling thereby decreasing VE-cadherin internalization
Angiopoietin-2 antagonists	• Decrease Ang-2 antagonism of Ang-1-induced endothelial stabilization
	• Inhibit Ang-2 induced activation of endothelial cell adhesion molecules and proinflammatory cytokines
Statins	• Downregulate P-selection and ICAM-1
	• Decrease NFκB-dependent gene expression
Atrial natriuretic peptide	• Attenuates p38 MAPK, NFκB and Rho-dependent signaling
	• Increases Rac-dependent p21-activated kinase (PAK1) phosphorylation, resulting in endothelial cell barrier enhancement
	• Stabilizes VE-cadherin at the adherens junction
Mesenchymal stromal (stem) cells	• Increase expression of genes involved in tightening gap junctions, calcium signaling, and focal adhesions
	• Secrete endothelial stabilizing factors including Ang-1 and KGF
	• Restore β-catenin, VE-cadherin, occludin-1 and claudin-1 by producing soluble paracrine factors
	• Decrease activation of innate immunity

Abbreviations: VEGFR2, vascular endothelial growth factor receptor 2; S1P, sphingosine-1-phosphate; PAR-1, protease activated receptor 1; ARF6, ADP ribosylation factor 6; ARNO, ARF nucleotide binding site opener; VCAM-1, vascular cell adhesion protein 1; Ang-1/2, angiopoietin-1/2; ICAM-1, intercellular adhesion molecule 1; KGF, keratinocyte growth factor.

increases vascular permeability by promoting dissociation of VE-cadherin from the adherens junction through a VEGFR2-Src-VE-cadherin signaling pathway.[11,12]

In sepsis, elevated plasma soluble VEGFR1 (sVEGFR1) levels have been reported to predict 28-d mortality and multi-organ dysfunction.[13] It has been hypothesized that increased production of sVEGFR2 promotes binding and neutralization of VEGF, thereby strengthening the endothelial barrier. However, the precise function of VEGF in sepsis is controversial, with studies implicating contrasting roles for VEGF in the pathophysiology of sepsis.[13-17] An ongoing clinical trial evaluating the effects of a neutralizing anti-VEGF antibody (bevacizumab) in patients with septic shock should provide further insight regarding the potential use of VEGF-targeted therapeutics in sepsis.[18]

Targeting the VEGF pathway has also been of interest in the treatment of viral hemorrhagic fever syndromes, including hantavirus-induced hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. Administration of inhibitory VEGFR2 antibody decreased hantavirus-directed endothelial cell permeability in vitro[19] via inhibition of VEGF-induced VE-cadherin internalization.[5] Similarly, pazopanib and dasatinib, FDA-approved inhibitors of VEGFR2 and Src family kinases, respectively, decreased endothelial permeability induced by pathogenic hantavirus in vitro, via inhibition of VE-cadherin internalization.[20] These results suggest the possibility that targeting the VEGF pathway may be a therapeutic strategy for multiple infectious diseases characterized by endothelial barrier disruption.

Sphingosine-1-Phosphate (S1P) Agonists

Sphingosine-1-phosphate (S1P) is a signaling sphingolipid that is released from platelets and binds to G-protein-coupled S1P receptors, $S1P_{1–5}$ (formerly known as $Edg_{1–5}$).[21-25] Plasma S1P contributes to the maintenance of microvascular integrity by signaling through $S1P_1$ on endothelial cells.[26] $S1P_1$ signals through
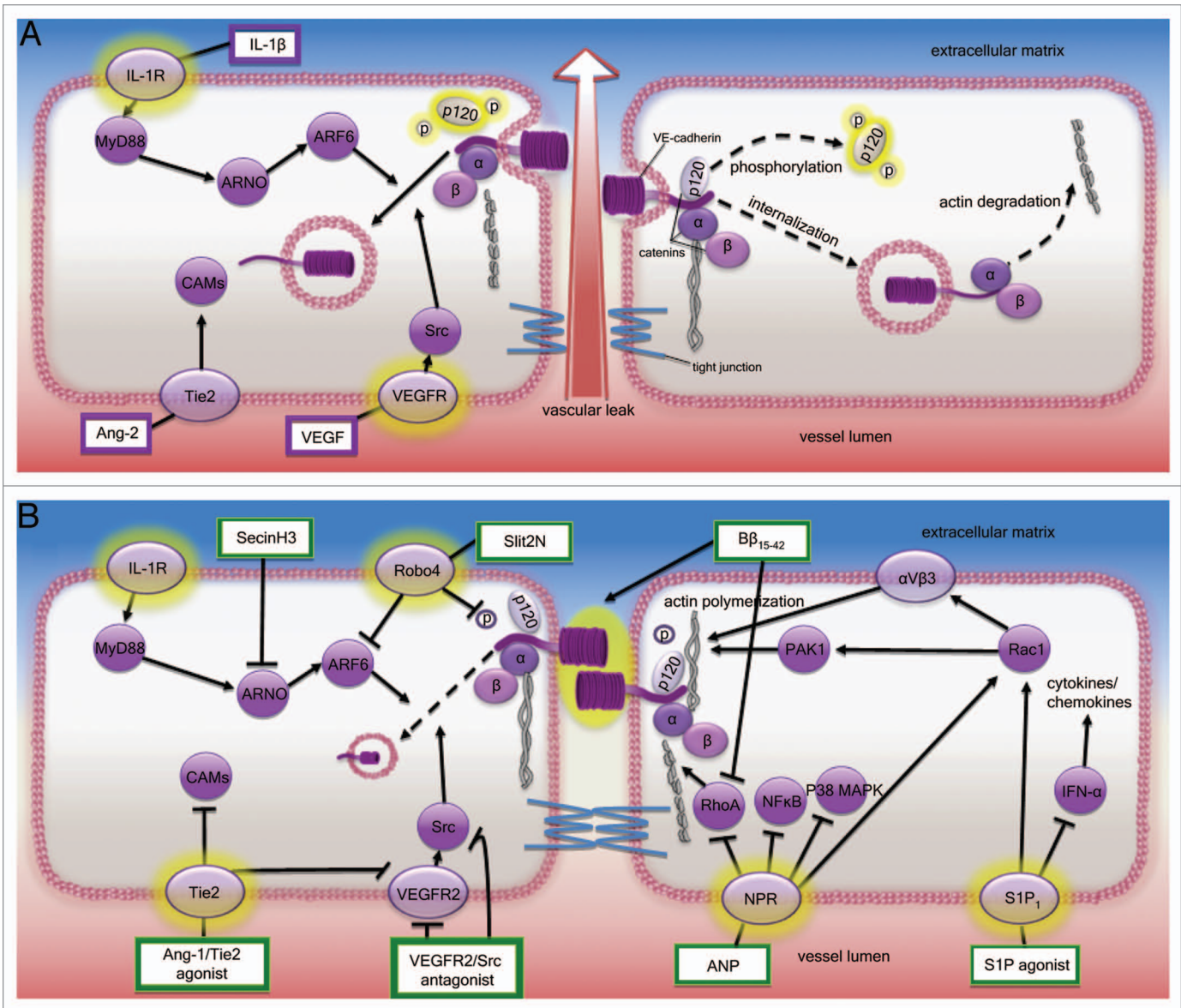
Figure 1. (**A**) Endothelial cell barrier dysfunction. During severe infections, endothelial activation and dysfunction may lead to the loss of microvascular endothelial barrier integrity, resulting in edema, multiple organ failure and death. Phosphorylation of adherens junction protein p120 catenin precipitates VE-cadherin internalization/junction disassembly. Endothelial integrity may also be compromised by rearrangements/degradation of the actin cytoskeleton. Molecular pathways implicated in this response include MYD88-ARNO binding that results in enhanced ARF6 signaling and decreased VE-cadherin localization at the cell surface. Endothelial activation may also cause an increase in the Ang-1 antagonist, Ang-2. Ang-2 binds to its cognate receptor, Tie2, and impedes the vascular stabilizing effects of Ang-1 by promoting proinflammatory endothelial responses and upregulating cell surface adhesion molecules. Vascular permeabilizing VEGF binds to VEGFR2 resulting in increased dissociation of VE-cadherin from the adherens junction through a VEGFR2-Src-VE-cadherin signaling pathway. (**B**) Endothelial cell barrier enhancement. SecinH3, a GEF (e.g., ARNO) inhibitor, inhibits ARF6-induced VE-cadherin internalization. Ang-1/Tie2 agonists activate Tie2 resulting in increased vascular quiescence via strengthening of endothelial cell junctions, downregulation of surface adhesion molecules and transdominate blockade of VEGR2 signaling. Fibrinopeptide Bβ_{15-42} provides barrier protection via maintenance of membrane VE-cadherin and inhibition of actin degradation via RhoA signaling inhibition. Upon binding its receptor Robo4, Slit2N reduces p120 catenin phosphorylation and inhibits ARF6, thereby increasing VE-cadherin retention at the cell surface. ANP administration decreases microvascular permeability via inhibition of RhoA-induced actin degradation and NFκB/P38 MAPK inhibition. S1P agonist administration reinforces the endothelial barrier via Rac1 and $\alpha_v\beta_3$ integrin signaling, resulting in formation and stabilization of cortical actin. Administration of S1P agonist enhances cortical actin formation (via Rac1 and $\alpha_v\beta_3$ integrin signaling) and downregulates IFN-α, thereby decreasing cytokine/chemokine production and enhancing endothelial cell barrier stability.

the Rho family GTPase Rac, which activates $\alpha_v\beta_3$ integrins, to increase cortical actin formation, thereby enhancing stability of the endothelial cytoskeleton.[27,28] Decreased plasma S1P has been demonstrated in several pathological conditions associated with vascular barrier dysfunction and microvascular leak, including cerebral malaria in children.[29]

Protease-activated receptor-1 (PAR-1) is an important mediator of $S1P_1$ signaling that contributes to both endothelial barrier stability and dysfunction.[30] PAR-1 activation by the serine protease thrombin can lead to increased endothelial cell permeability, while conversely, PAR-1 activation by activated protein C (APC) can lead to endothelial cell barrier protection.[30] This finding suggests that APC may serve as an effective endothelial stabilizing therapeutic agent. However, in a murine model of hyperoxic lung injury, prophylactic or therapeutic administration of recombinant murine APC was unable to ameliorate lung injury.[31] More importantly, several clinical trials in patients with severe sepsis or septic shock failed to demonstrate a therapeutic benefit of recombinant human APC on 28-d mortality, including one study in which recombinant human APC administration was associated with a higher risk of bleeding.[32-36] These studies precipitated the withdrawal of recombinant human APC (drotecogin alfa) from the worldwide market.[36] A modified APC variant with minimal anticoagulant activity but preserved cell signaling functionality (5A-APC) was capable of reducing mortality by approximately 40% after bacterial infection or LPS challenge in murine models of sepsis,[37] suggesting further testing of anticoagulant APC variants may be of interest.

Administration of S1P or pharmacological analogs has been demonstrated to preserve or enhance vascular integrity in a number of infectious diseases in which microvascular leak plays an important pathologic role. In vitro, addition of S1P to Andes virus (hantavirus)-infected cells blocked VE-cadherin internalization in response to VEGF, thereby increasing endothelial integrity.[20] S1P administration has also been shown to stabilize the microvascular endothelium in several pre-clinical animal models. In a murine model of ventilator-induced lung injury and a canine model of LPS-induced ventilator associated acute lung injury, S1P administration attenuated lung vascular leak as documented by decreased Evans blue dye extravasation in the lung, decreased protein level in bronchoalveolar lavage fluid and decreased lung tissue volume (i.e., decreased "wet-to-dry" lung weight).[38] Furthermore, S1P antagonism has been reported to increase vascular leak in vivo under physiological conditions.[39]

Recently, the pharmacologic agent FTY720, a potent S1P receptor agonist[40,41] licensed as an experimental drug (Gilenya™) by the FDA, was evaluated in phase III clinical trials of multiple sclerosis.[42,43] Phosphorylated FTY720 (FTY720-P) and its analog (R)-AAL ([R]-AFD) exert similar effects to S1P. In vitro, both agents induced β-catenin and localization of VE-cadherin to adherens junctions, as well as antagonized VEGF-induced endothelial cell permeability.[44] Similar effects were confirmed in vivo in a murine model of VEGF-induced vascular leak.[44]

In a murine model of influenza, (R)-AAL administration one hour after influenza virus inoculation decreased pulmonary edema and inflammation.[45] Notably, (R)-AAL therapy exerted a greater therapeutic benefit than oseltamivir, the most widely used antiviral drug for the specific treatment of influenza. Furthermore, administration of (R)-AAL in combination with oseltamivir provided additional benefit over (R)-AAL administration alone. A follow-up study by the same group demonstrated that the benefits of S1P agonist administration ([R]-AAL, CYM-5442, or RP-002) were due to its ability to downregulate excessive cytokine/chemokine production by the host, an immunopathological feature of severe influenza disease, in response to decreased IFN-α production, an upstream regulator of early cytokine production.[46] In a murine model of cerebral malaria, FTY720 was also shown to improve clinical outcome when administered therapeutically, either alone or as an adjunctive therapy in combination with the anti-malarial drug artesunate.[29] This effect was attributed to increased integrity of the blood-brain barrier and enhanced endothelial stability, demonstrated by decreased Evans blue extravasation in the brain, reduced plasma sICAM-1 (a marker of endothelial activation), and increased angiopoietin-1 (a marker of endothelial stability).[29]

In contrast, Puneet et al.[47] reported that blockade of sphingosine kinase 1 (sphk1) protected mice from experimental sepsis by enhancing bacterial clearance without altering systemic S1P levels required to maintain vascular barrier integrity. Intriguingly, because S1P receptor stimulation can lead to subsequent receptor downregulation, S1P agonists can potentially serve as functional $S1P_1$ antagonists in certain circumstances.[48] Thus, it is likely that S1P-targeted agents can exert both deleterious and beneficial effects depending on the contextual pathophysiological state. Taken together, current pre-clinical evidence warrants further evaluation of Sphk1/S1P modifying therapeutic agents in the treatment of infectious disorders associated with excessive cytokine production and consequent microvascular leak, such as influenza and sepsis.

Fibrinopeptide Bβ$_{15-42}$

The fibrin N-terminal peptide Bβ$_{15-42}$ is a 28 amino acid cleavage product of fibrin that binds VE-cadherin and stabilizes interendothelial junctions.[49,50] Because of its endothelial barrier stabilizing properties, the therapeutic use of fibrinopeptide Bβ$_{15-42}$ (also known as FX06) has recently been investigated. In murine models of vascular leak, including pneumonitis and shock (intranasal LPS and intravenous LPS administration, respectively), FX06 administration attenuated capillary leak in the lungs.[51] In addition, FX06 administration improved survival by approximately 40% in a murine model of dengue shock.[51] These effects were mediated by FX06-induced dissociation of the Src kinase Fyn from VE-cadherin, in parallel with Fyn association with p190RhoGAP, a RhoA antagonist. In a murine model of polymicrobial sepsis (cecal ligation and puncture), treatment with FX06 attenuated leukocyte infiltration and reduced proinflammatory cytokines in the lung, liver, and blood.[52] Decreased tissue inflammation was attributed to FX06-sustained vascular integrity, thereby suppressing vascular leakage and subsequent inflammatory cell trafficking into the lungs. In support of this hypothesis, FX06 pretreatment of macrophages and endothelial cells was unable to reduce TLR2- and TLR4-induced inflammation; however, microvascular permeability was not specifically investigated in this study.[52] These data suggest that FX06 therapy may represent a novel and effective adjunctive therapy to increase vascular stability, thereby preventing end-organ inflammation, edema, and dysfunction in disorders associated with vascular activation/dysfunction and microvascular leak.

Slit2N

Binding of the ligand Slit to its cognate endothelial receptor Robo4 inhibits inflammation-induced endothelial permeability by strengthening adherens junctions and modulating cytoskeletal dynamics.[53-56] Endothelial cell monolayer permeability induced by several mediators in vitro, including VEGF, LPS, TNF, and IL-1β, was counteracted by treatment with Slit2N, the active fragment of Slit.[54,55] This effect correlated with increased cell surface expression of VE-cadherin. Specifically, Slit2N increased p120-catenin-VE-cadherin association by reducing p120-catenin phosphorylation.[55] Slit2N-Robo4 signaling may also increase cell surface VE-cadherin via inhibition of ARF6 signaling.[54,57] These findings were substantiated in several experimental models of infectious diseases characterized by microvascular activation/dysfunction, including sepsis, LPS-induced lung injury, and H5N1 avian influenza. In each of these pre-clinical models, administration of Slit2N enhanced microvasculature integrity and improved survival without dampening inflammation (or altering viral load in H5N1 experimental avian influenza).[55] These findings suggest that therapeutic targeting of Robo receptors represents a promising strategy to improve clinical outcome in infectious diseases associated with endothelial dysregulation and microvascular leak.

SecinH3

Recently, Zhu et al.[57] described a novel cytokine-mediated pathway involved in endothelial barrier stability that functions independently of MYD88-induced NFκB signaling. In vitro, various NFκB pathway inhibitors failed to rescue IL-1β-induced endothelial permeability or endothelial cell surface VE-cadherin internalization. The results from a series of elegant experiments demonstrated a requirement for the adaptor protein MYD88 in IL-1β induced endothelial permeability, suggesting that MYD88 mediates a distinct NFκB-independent pathway involved in endothelial activation/dysfunction.[57] The investigators implicated a pathway involving MYD88 activation of the ARF guanine nucleotide-exchange factor inhibitor (GEF) ARNO, based on the observations that ARNO-ARF6 signaling decreased cell surface VE-cadherin and increased vascular permeability. Furthermore, administration of SecinH3, a GEF inhibitor, restored endothelial barrier function in murine models of inflammatory arthritis and acute inflammation, without affecting global cytokine expression.[57] SecinH3 administration has also been shown to decrease endothelial leak in a murine model of vascular eye disease.[54] Therapeutic agents that target the ARNO-ARF6 pathway, such as Slit2N and SecinH3, are emerging as important potential therapies for conditions associated with vascular destabilization.

Angiopoietin-1/Tie-2 Agonists

Angiopoietin-1 (Ang-1) is a ligand for the endothelial-specific receptor tyrosine kinase Tie2,[58] a potent mediator of angiogenesis that functions post-development to prevent vascular leakage and promote vascular quiescence via strengthening of endothelial cell junctions and downregulation of surface adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and E-selectin.[59-62] The Ang-1/Tie2 axis also transdominantly blocks VEGFR2-mediated microvascular permeability.[63] A variety of Ang-1/Tie2 targeted strategies have been investigated in pre-clinical models to reduce complications of infectious diseases. Both cell- and viral vector-based Ang-1 gene therapeutic strategies, including Ang-1 specifically engineered to potently induce Tie2 phosphorylation,[64] have been reported to reduce microvascular leak in murine models of acute lung injury and acute kidney injury.[2,64-67]

A synthetic Tie2 agonist peptide known as vasculotide (VT) has been shown to protect against vascular leak and mortality, with subsequent improvement in end-organ function, when administered in either a prophylactic or therapeutic regimen in a murine model of polymicrobial sepsis.[68] VT has also been shown to prevent lung vascular leak and improve survival by 30–40% in a murine model of LPS-induced lung injury (intraperitoneal LPS administration).[69] Another study by Alfieri et al.[70] evaluated the effects of an Ang-1 mimetic, MAT.Ang-1, in experimental LPS-induced sepsis in mice. Therapeutic administration of a single dose of MAT.Ang.1, 20 h after LPS administration, decreased vascular leak without altering vascular resistance. A growing number of Ang-1/Tie2 agonists represent promising agents for the prevention or amelioration of infection-induced endothelial activation/dysfunction and consequential microvascular leak.

Angiopoietin-2 Antagonists

Angiopoietin-2 (Ang-2), a functional Ang-1 antagonist that binds to Tie2, counterbalances the vascular stabilizing effects of Ang-1 by promoting proinflammatory endothelial responses and disrupting endothelial barrier function.[71,72] Current evidence suggests that the overall status of Tie2 phosphorylation is largely controlled by the relative ratio of Ang-1:Ang-2. Elevated Ang-2 levels have been reported in septic patients and have been associated with increased vascular leak, severity of illness and adverse outcomes.[71,73-75]

In both cecal ligation and puncture (CLP)- and LPS-induced murine models of sepsis, *Ang-2+/-* heterozygous mice were relatively protected against vascular leak, acute lung injury, acute kidney injury and death compared with wild-type littermate controls.[75] Notably, this study also reported elevated circulating levels of Ang-2 in a cohort of patients with sepsis within one hour of their presentation to the emergency department. The level of Ang-2 elevation correlated with disease severity and predicted subsequent development of shock or death. Serum from affected patients disrupted endothelial barrier function in vitro, via a mechanism inhibited by treatment with an antagonistic Ang-2 monoclonal antibody. These findings suggest that Ang-2 upregulation occurs early in the course of sepsis and precedes the development of end-organ dysfunction. In contrast, recombinant Ang-2 increased survival when administered two hours prior to *Escherichia coli*-induced murine experimental sepsis or 30 min after *Pseudomonas aeruginosa*-induced murine experimental sepsis.[76] However, it is possible that circulating Ang-2 levels in

experimental mice exceeded the physiological concentration of Ang-2 in patients with septic shock, a finding which has previously been associated with increased Tie2 phosphorylation.[77] Overall, these studies suggest that Ang-2 inhibition strategies merit further investigation for the treatment of sepsis.

Statins

HMG-CoA reductase inhibitors, commonly known as statins, are widely used for the treatment of hyperlipidemia and ischemic heart disease.[78] Based on recently recognized anti-inflammatory properties, statins have received increasing interest for their potential role(s) in the treatment of inflammation-related diseases.[79-81] These effects include modulation of endothelial cell adhesion molecule expression (including downregulation of P-selectin[82] and ICAM-1[83]), downregulation of NFκB-dependent gene expression[84] and decreased mononuclear inflammatory cell infiltrates in target tissues.[85]

Preliminary evidence suggests that prolonged statin use may decrease endothelial activation and improve outcomes in experimental bacterial pneumonia.[85] Mice receiving high dose simvastatin for several weeks prior to infection developed less bacteremia and inflammatory cell infiltrates in the lung, which were associated with decreased expression of MCP-1 and ICAM-1.[85] However, overall mortality was similar between simvastatin-treated animals and controls.

Atrial Natriuretic Peptide

Atrial natriuretic peptide (ANP) plays an important physiological role in the maintenance of arterial blood pressure and volume. These effects are mediated by binding to natriuretic peptide receptors (NPR) A–C. NPRs are guanylyl cyclase-linked, regulated by cGMP synthesis, and are highly expressed in the vascular endothelium. Vascular endothelial-specific NPR-A knockout mice developed systemic hypertension and cardiac hypertrophy, yet maintained a direct vasodilatory response to ANP.[86] Pre-treatment with ANP reduced paracellular endothelial gaps and stabilized VE-cadherin in TNF-activated endothelial cells.[87] These observations suggest that ANP moderates arterial blood pressure and volume via its effects on vascular permeability.

ANP modulates signaling pathways important for the production of proinflammatory cytokines and remodeling of the endothelial cell cytoskeleton. In vitro, endothelial cells pre-treated with ANP followed by LPS stimulation displayed significantly attenuated p38 MAPK, NFκB, and Rho-dependent signaling 6 h post-LPS stimulation.[88] In a murine model of LPS-induced lung injury, ANP modulated vasculature stability via increased Rac-dependent p21-activated kinase (PAK1) phosphorylation, resulting in increased endothelial cell barrier integrity.[87] PAK1 is a cytoskeletal Rac effector protein that initiates peripheral actin polymerization.[89]

Increased levels of ANP have been observed in patients with septic shock,[90] and in murine models of lung injury.[87] Moreover, ANP⁻/⁻ mice have been reported to develop more severe LPS-induced lung injury and vascular leak compared with wild-type mice,[87] suggesting a potential mechanistic role for ANP in sepsis and acute lung injury. Experimental acute lung injury-associated pulmonary edema induced by multiple stimuli—including thrombin, VEGF, LPS, peptidoglycan, and lipoteichoic acid—is reduced by concomitant or prophylactic ANP administration.[87,88,91-94]

Evaluation of therapeutic ANP administration in patients with lung injury is limited. In a cohort of 40 individuals with acute lung injury requiring mechanical ventilation with positive end-expiratory pressure (PEEP), ANP administration improved lung injury and oxygenation.[95] In contrast, Bindels et al.[96] observed no difference in pulmonary gas exchange in ten patients with acute respiratory distress syndrome who received ANP. Large, randomized controlled trials are warranted to evaluate the use of exogenous ANP as a potential therapeutic agent for the treatment of infection-induced lung injury.

Mesenchymal Stromal (Stem) Cells

Mesenchymal stromal (stem) cells (MSCs) represent a heterogenous subset of non-hematopoeitic pluripotent stromal cells with multi-lineage potential that can be isolated from various tissues (e.g., adult bone marrow).[97] While initial interest in MSCs was focused on their potential in regenerative medicine, a growing interest in their potential in immunomodulatory therapy has evolved based on their capacity to modulate the host response in diseases and syndromes associated with inflammation. It has been recently recognized that MSCs secrete an array of growth factors, cytokines, and lipid mediators that modulate host inflammation, improve pulmonary alveolar fluid clearance, and strengthen endothelial integrity thereby improving organ function and decreasing mortality in pre-clinical models of infectious diseases including LPS-induced acute lung injury and sepsis following cecal ligation and puncture.[2,98-106]

MSC-mediated effects on the vascular endothelium have been investigated in vitro and in vivo. VEGF-treated pulmonary endothelial cells exposed to MSC-conditioned media preserved adherens junctions (β-catenin and VE-cadherin) leading to increased endothelial barrier stability.[106] This finding was confirmed in a rat model of mild hemorrhagic shock (removal of 2 ml/100 g of blood over 10 min followed by resuscitation 1 h post-shock), where MSC administration decreased pulmonary edema, in part, by increasing adherens junction and tight junction protein expression, including VE-cadherin, claudin-1, and occludin-1.[106] A network analysis of experimental sepsis-induced MSC-mediated effects on common transcriptional responses in major target organs identified coordinated expression of transcriptional programs involved in preserving endothelial/vascular integrity, including upregulation of genes associated with gap junction tightening, calcium signaling, focal adhesion, and the Ang-1/Tie-2 pathway.[107]

Ang-1 and keratinocyte growth factor (KGF) are thought to play important roles in the induction of MSC-mediated therapeutic effects.[2,102,108] In vitro, MSC-mediated alveolar cell permeability was attributed to Ang-1 production.[108] Moreover, in

a murine model of LPS-induced lung injury, Ang-1 transfected MSCs were more effective at restoring lung/vascular integrity than non-transfected MSCs.[2] KGF production by MSCs was implicated in the restoration of alveolar fluid clearance in LPS-treated ex vivo-perfused human lungs.[102]

Future Directions and Limitations

Emerging pre-clinical therapeutic strategies that target microvascular endothelial barrier activation/dysfunction represent promising approaches to prevent and/or limit end-organ dysfunction/injury in infectious diseases (summarized in **Table 2**). In addition to the strategies discussed in this review, $\alpha_v\beta_3$ integrin regulators such as Fibulen5, NRP1, and Syndecan1 warrant pre-clinical evaluation for possible applications to limit infection-related microvascular leak.[109-111]

Given that a variety of viral infections may mediate endothelial dysregulation, therapeutic agents that target the host endothelium could potentially have relatively broad utility in the management of serious viral infections. It will also be important to assess new barrier-enhancing agents in combination with established therapeutic modalities (e.g., in combination with oseltamivir for the specific treatment of influenza). This complementary and robust treatment approach should allow for optimal therapeutic efficacy by targeting the infectious agent and the deleterious host responses in concert. For example, endothelial-stabilizing agents may be more effective when antimicrobial replication is diminished.

Given the failure of multiple therapeutic strategies showing promise in pre-clinical testing to yield positive results in clinical trials for the treatment of sepsis,[35,36,112] one must remain cautious when considering the therapeutic potential of endothelial stabilizing agents. It is also important to recognize that murine models, such as experimental sepsis, may fail to replicate important pathophysiological features of human disease.[113,114] Well-designed, controlled clinical trials will be necessary to determine whether endothelial stabilization strategies will be effective in reducing infection-related morbidity and mortality associated with endothelial activation/dysfunction and associated microvascular leak.

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Table 2. Experimental results of selected pharmacological agents that have been investigated for their ability to enhance endothelial barrier integrity and reduce vascular leak

Infectious disease/ infectious agent/ model of vascular leak	Agent	Results
Sepsis	Bevacizumab (VEGF antagonist)	Ongoing clinical trial[18]
	APC	Failed to demonstrate therapeutic benefit in several clinical trials[32-36]
	5A-APC (APC variant)	~40% reduction in mortality in murine LPS-induced endotoxemia model of sepsis and *S. aureus* or *E. coli* infection model of sepsis[37]
	Bβ$_{15-42}$ (FX06)	Attenuated capillary leak in the lungs in murine model of sepsis (I.V. LPS administration),[51] reduced leukocyte infiltration and proinflammatory cytokines in the lung, liver and blood in a murine CLP model of sepsis[52]
	Slit2N	Enhanced microvasculature integrity and improved survival in a murine CLP sepsis model[55]
	Angiopoietin-1	Decreased vascular leak in murine model of LPS-induced sepsis[70]
	Vasculotide	Protected against vascular leak, improved end-organ function and increased survival (~40%) in a murine CLP model of sepsis[68]
	Mesenchymal stromal (stem) cells	Improved organ function and decreased mortality in murine CLP model of sepsis[104,105]
Acute lung injury	1. SIP agonist	Decreased pulmonary edema and attenuated vascular barrier dysfunction in murine and beagle dog lung injury models induced by LPS and high tidal volume mechanical ventilation[38]
	Bβ$_{15-42}$ (FX06)	Attenuated capillary leak in the lungs in a murine pneumonitis model (intranasal LPS-administration)[51]
	Slit2N	Enhanced microvasculature integrity and improved survival in a murine LPS model of ALI[55]
	Angiopoietin-1	Decreased microvascular leak in murine models of ALI[2,66,67]
	Vasculotide	Prevented lung vascular leak and improved survival by ~30–40% in a murine LPS-induced (I.P. administration) model of ALI[69]
	Statins	Decreased ICAM-1 and no effect on survival in a murine model of bacterial pneumonia[85]
	ANP	Improved endothelial cell barrier integrity in murine LPS-induced lung injury model[87]
	Mesenchymal stromal (stem) cells	Improved pulmonary alveolar fluid clearance in ex vivo perfused lung[102] and strengthened endothelial integrity, resulting in improved organ function and decreased mortality in murine models of ALI[2,98,99,101]
Influenza	S1P agonist ([R]-AAL)	Decreased pulmonary edema and inflammation in murine model of influenza, effective as adjunctive therapy in combination with oseltamivir[45]
	Slit2N	Enhanced microvasculature integrity and improved survival in murine model of avian influenza (H5N1)[55]
Dengue shock syndrome	Bβ$_{15-42}$ (FX06)	Improved survival by ~40% in a murine model of dengue shock syndrome[51]
Malaria	S1P agonist (FTY720)	Preserved blood brain barrier integrity and enhanced endothelial stability in murine malaria model, effective as adjunctive therapy in combination with artesunate[29]
Hantavirus	Pazopanib and dasatinib (VEGFR2 and Src family kinase inhibitors)	Increased endothelial integrity in vitro[5,19]
	S1P	Increased endothelial integrity in vitro[20]

Agents are categorized by infectious disease/infectious agent/model of vascular leak. Abbreviations: VEGF, vascular endothelial growth factor; APC, activated protein C; S1P, sphingosine-1-phosphate; LPS, lipopolysaccharide; I.V., intravenous; CLP, cecal ligation and puncture; ALI, acute lung injury; I.P., intraperitoneal; ICAM-1, intercellular adhesion molecule 1.

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