



Published in final edited form as:

Crit Care Clin. 2020 April ; 36(2): 201–216. doi:10.1016/j.ccc.2019.12.003.

The Angiotensin-Tie2 Pathway in Critical Illness

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Abstract

Lethal features of sepsis and acute respiratory distress syndrome (ARDS) relate to the health of small blood vessels. For example, infiltration of the alveoli with proteinaceous fluid is often driven by breach of the microvascular barrier. Spontaneous thrombus formation within inflamed microvessels exacerbates organ ischemia, and in its final stages, erupts into overt disseminated intravascular coagulation (DIC). A signaling axis in the vascular endothelium, the Angiotensin-Tie2 pathway, may play a central role in the abrupt transition from microvascular integrity to pathological disruption. Tie2 signaling is highly active when vessels are in their quiescent state: stable junctions, anti-inflammatory, and anti-coagulant. During inflammation however, Tie2 signaling is rapidly toggled off as levels of its endogenous antagonist, Angiotensin-2, rise by 1–2 orders of magnitude. When Tie2 signaling drops, the microvascular barrier collapses, endothelium becomes adhesive to leukocytes, and the vessel wall no longer prevents spontaneous coagulation. This review will summarize a large body of preclinical and clinical results that implicate the Tie2 pathway in the pathogenesis of sepsis and ARDS. Results from groups worldwide now identify Angiotensins as dynamic circulating markers of the vasculature. Furthermore, Tie2 is emerging as a promising target to restore microvascular health in sepsis and ARDS. Translational pursuit of the Tie2 pathway may enable advances in the way critically ill patients are diagnosed, monitored, and treated.

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4-8 keywords to direct and optimize search results

Angiopoietin; Tie2; sepsis; ARDS; vascular leakage; coagulation; VE-PTP

INTRODUCTION

Homeostasis of small blood vessels exerts a powerful influence on the disease course in critically ill patients. In systemic inflammation, dysregulated microvessels throughout the body leak their contents into the surrounding tissue, potentiate the penetration of inflammatory cells into major organs, promote the formation of in situ thrombi, and disrupt the vascular tone necessary to deliver oxygen and nutrients where they are needed. Vascular dysfunction arising from systemic inflammation may impact clinically meaningful adverse outcomes in the ICU. The morbidity, mortality, and cost from these pathophysiological processes is vast while clinical care in the ICU remains largely supportive rather than targeted at the underlying pathophysiological mechanisms [1–3]. Mechanisms that regulate the transition between microvascular homeostasis and dysregulation are therefore of substantial fundamental and translational interest.

The vascular endothelium receives signals from the circulating inflammatory milieu and responds to these signals to control tone, barrier function, adhesiveness to leukocytes, and coagulation. When inflammation arises locally, the transition from homeostasis to the activated state is likely adaptive: in response to local tissue injury, the breakdown of the microvascular barrier enables humoral and cellular effectors of immunity to access the parenchyma; local vasorelaxation increases the flux of such effectors into the target region; and thrombus formation limits the dissemination of pathogens from the portal of entry. Yet, when the very same sequence proceeds unchecked or unfolds simultaneously across multiple vascular beds, the results look like the septic patient: edematous soft tissues, inflamed and swollen major organs, vasoplegia, and DIC.

Molecular pathways within the vascular endothelium collaborate to facilitate homeostasis of blood vessels. Involved pathways include, but are not limited to, the Tie2-Angiopoietin axis [4–6]; vascular endothelial growth factor (VEGF) interactions with its receptors, VEGFR-2 and VEGFR-1 [7–9]; sphingosine-1 kinase and its receptors [10]; and mechanical regulators such as shear stress generated by the flow of blood [11, 12]. Among vascular signaling pathways, the Tie2 receptor is unique in that its phosphorylation provides an active signal to promote quiescence and counteract the “activated” state of endothelium [13–15]. Perhaps not surprisingly, diverse critical illness syndromes including sepsis, DIC, shock, ARDS, myocardial infarction, and acute kidney injury have all been linked to suppression of Tie2 signaling [4, 6, 16–21].

BIOLOGY OF THE ANGIOPOIETIN-TIE2 AXIS

Overview:

As a transmembrane tyrosine kinase, Tie2 is the central molecule in this axis. An orthologous protein called Tie1 is also expressed in endothelium, but does not directly bind

Angiopoietins (Angpts) and remains an orphan receptor [22, 23]. A third transmembrane protein, vascular-endothelial protein tyrosine phosphatase (VE-PTP) is also expressed in the endothelium, where it closely associates with Tie2 to regulate its signaling.

In the quiescent state, Angiopoetin-1 (Angpt-1) binds to Tie2, an event that clusters individual Tie2 molecules into a cross-phosphorylating complex. Downstream of phosphorylated Tie2, the second messenger AKT is activated, preventing endothelial apoptosis and enhancing cellular quiescence [24]. During times of stress—e.g., inflammation signaled by cytokines such as TNF α —Angiopoietin-2 (Angpt-2) levels increase and competitively bind to the Tie2 receptor blocking Angpt-1 mediated phosphorylation [25, 26]. Because Angpt-2 is less efficient than Angpt-1 at clustering Tie2 monomers, the net effect of Angpt-2 induction in the context of inflammation is to suppress tonic Tie2 signaling. Loss of Tie2 signaling releases an important “brake” in the endothelium, enabling it rapidly shift its phenotype toward the activated state: weakened junctions [27, 28], expression of adhesion molecules for leukocytes including ICAM1 and VCAM1 [26], and enhancement of procoagulant proteins at the luminal surface [4, 29]. Each of these molecular-cellular events maps to major clinical manifestations in sepsis and ARDS. Weakened junctions lead to edema. Adhesion molecule induction promotes secondary inflammatory injury, and disruption of normal hemostatic mechanisms culminates in DIC. The subsequent sections summarize the molecular and cell biology of each major component in the Tie2 pathway.

Angiopoietin-1:

Angpt-1 is the canonical agonist ligand for the Tie2. Angpt-1 has a modular structure, starting at the amino terminus with a superclustering domain, then a coiled-coil domain, and finally, a fibrinogen-like domain. The N-terminal regions of Angpt-1 organize individual molecules into homotetramers and higher-order oligomers. The fibrinogen-like domain on the C-terminus is responsible for binding Tie2. Angpt-1 binds Tie2 very tightly at nanomolar affinity [25, 30]. The oligomeric status of Angpt-1 promotes the clustering of Tie2 monomers at the endothelial surface. The resulting molecular complex becomes an efficient signaling unit [31–33].

Angpt-1 knockout mice die in utero at ~E12.5 with a dilated, rudimentary vasculature that lacks branching and the support cells such as pericytes that are characteristic of a matured vasculature [34, 35]. The embryos exhibit edema and hemorrhage. Consistent with its role as an agonist, these features phenocopy global knockout of Tie2 [36]. Conversely, upregulation of Angpt-1 results in an increased quantity of branched vasculature that is no longer leaky [37–39].

Angpt-1 is expressed by platelets, mesenchymal cells, vascular smooth muscle cells, and pericytes [25, 34, 40, 41]. The last two cell types bathe the extracellular matrix surrounding endothelial cells in Angpt-1. Moreover, Angpt-1 is naturally adhesive to the matrix [42, 43]. This provides a constant stimulus for Tie2 activation in the quiescent state. As will be discussed below, Tie2 activation plummets during inflammation with the induction of Angpt-2. However, in this latter context of endothelial “dyshomeostasis,” Angpt-1 derived platelet granules may play an adaptive role. Platelets store large quantities of Angpt-1

protein, releasing it from granules under the influence of inflammatory modulators such as thrombin. Degranulation of platelets could thereby help restore vascular homeostasis [4, 29, 40, 44, 45].

Angiopoietin-2 :

In contrast to the stabilizing actions of Angpt-1, Angpt-2 is a context-dependent antagonist of the Tie2 receptor despite significant primary sequence homology to Angpt-1 [25]. Indeed, overexpression of Angpt-2 results in an analogous phenotype to the Angpt-1 knockout model. Specifically, the vasculature is discontinuous and the endothelial cells themselves appear to have poor connection to the underlying extracellular matrix as noted by their spherical appearance [25]. Angpt-2 knockout mice remain relatively normal during the embryonic period, but exhibit poor vasculature remodeling in the post embryonic period and do not survive past two weeks of age secondary to lymphatic defects [25, 46].

Although Angpt-1 has a slightly higher binding affinity than Angpt-2 for the Tie2 receptor, when mixed together, Angpt-2 antagonizes the Tie2 receptor [15, 47, 48]. The basis for this signaling difference may be that Angpt-2 exists as a dimer rather than the higher-order oligomers of Angpt-1 [31–33, 49]. Therefore, at molar excess, the fibrinogen like domain of Angpt-2 may compete Angpt-1 off the receptor, leaving Tie2 molecules in a more dispersed—i.e., less phosphorylated—state on the endothelial surface.

Endothelial cells of the small vasculature express Angpt-2 [46, 50]. Pre-formed Angpt-2 protein is stored in the Weibel-Palade bodies of these cells, making Angpt-2 readily available for secretion after endothelial cells are stressed by triggers including hypoxia, TNF α , turbulent flow and thrombin [50–56]. Therefore, noxious stimuli trigger the endothelium to release pre-formed Angpt-2 protein. This Angpt-2 acts in a local, or paracrine, fashion to unseat Angpt-1, thereby deactivating Tie2 signaling. The switch from quiescence to disruption is fortified because activated Tie2 tonically inhibits the transcription of the ANGPT2 gene by keeping the transcription factor FoxO1 sequestered in the endothelial cytoplasm [47, 50, 57]. Therefore, when Tie2 phosphorylation is switched off by the rapid release of pre-formed Angpt-2 protein, that endothelial cell then commences to synthesize more Angpt-2 protein through FoxO1-mediated gene transcription.

Tie2:

Tie2 knockout is embryonically lethal at E10.5 due to a poorly developed heart and dilated leaky vessels that lack an investment of support cells [13, 36, 58]. (See Figures 1–2). These features are similar to the Angpt-1 knockout model [34, 35]. Tie2 is expressed in both active and quiescent endothelium and is necessary for adult vasculature. The quantity of receptor is downregulated in sprouting and inflamed vasculature [14, 59, 60]. Loss of Tie2 *in vivo* and *in vitro* results in a dysfunctional endothelium with impaired barrier function [4, 50, 61].

In confluent or quiescent cells, Angpt-1 activates Tie2 at cell-cell borders in a *trans* configuration [62]. This signals PI3 kinase and Akt. Akt phosphorylates FoxO1 on key residues that inhibit its translocation to the nucleus and its binding of the ANGPT2 gene [50, 63–66]. Activated Akt also signals the molecular apparatus regulating the contractility of the endothelial cell and the essential junctional barrier protein VE-cadherin [6, 28,

67–69]. Expression of inflammatory adhesion molecules including ICAM1, VCAM1, and E-selectin is attenuated [70]. Finally, activated Akt suppresses key triggers of coagulation include phosphatidylserine and tissue factor [4]. The net output of these molecular events downstream of Tie2 activation is an endothelial layer that forms an effective barrier, suppresses the production of Angpt-2, inhibits leukocyte adhesion, and prevents the activation of coagulation.

Due to its critical function, Tie2 is regulated at multiple levels. Angpt-1 binding results in receptor ubiquitylation, which triggers receptor internalization and degradation [71]. Minimal receptor internalization occurs in the presence of Angpt-2. Tie2 releases both Angpt-1 and Angpt-2 after engagement, allowing for further activation of additional receptors [72]. Together, this indicates the system remains poised for recovery during critical insult as a quantity of Tie2 receptor remains available for phosphorylation during dysfunctional, Angpt-2 predominant states. Additional regulation of Tie2 is mediated by shedding of its extracellular domain. Matrix metalloproteinase cleavage facilitates both Angpt-1 and Angpt-2 induced shedding. Shed receptor binds additional ligand, preventing further receptor activation ultimately resulting in a smaller more directed pool of ligand and receptor [73, 74].

Tie1 and VE-PTP:

Further regulation of Tie2 receptor signaling occurs via transmembrane proteins that closely associate with Tie2. Although Tie1 does not directly bind Angpts, it is nonetheless critical for embryonic survival [75]. In *in vitro* and *in vivo*, Tie1 binding to Tie2 enhances the agonistic signaling of Angpt. Rapid cleavage of Tie1 occurs in response to inflammatory mediators such as TNF α and phorbol esters [22, 76].

VE-PTP enzymatically removes phosphates from proteins with which it associates. These include VEGFR-2, VE-cadherin, and Tie2 [77–79]. VE-PTP knockout mice die in utero of angiogenesis defects [80]. Inhibition of VE-PTP enhances Tie2 phosphorylation and strengthens the microvascular barrier against inflammatory triggers of permeability [81]. This barrier-enhancing effect of VE-PTP inhibition appears to require Tie2. Tie2 activation via VE-PTP inhibition is currently being explored in ocular conditions [82, 83].

PRE-CLINICAL EVIDENCE OF TIE2 REGULATING VESSELS DURING INFLAMMATION

Tie2 signaling appears to be a major determinant of the vascular response to a broad array of noxious stimuli. The earliest studies of the barrier-promoting effect of Tie2 stimulation demonstrated that Angpt-1 counteracts vascular leakage induced by unrelated triggers including VEGF, serotonin, and mustard oil [39, 84]. Since then, evidence from many laboratories testing diverse models of organ damage have coalesced around the central concept that Tie2 activation prevents vascular leakage, attenuates cellular inflammation, reduces spontaneous thrombus formation, and improves survival. As described next, this concept has been tested in preclinical models of sepsis, acute lung injury, and acute kidney injury. It has also been tested in experimental malaria, anthrax, and hemorrhagic

fevers including Ebola virus disease. The volume and diversity of the experimental settings convincingly propose that Tie2 signaling is a major determinant of outcomes across settings that acutely destabilize the vasculature.

Sepsis:

In the earliest report focusing on sepsis, Angpt-1 overexpression was shown to defend blood pressure, prevent leakage, attenuate inflammation, and improve survival in a model of endotoxic shock [85]. Angpt-1-mediated defense of vascular barrier function in the context of systemic endotoxin was thereafter shown to require p190RhoGap, a regulator of endothelial cell contraction [86], and p47phox, an upstream signal transducer [87]. Intravenous Angpt-1 administration improved multi-organ dysfunction and survival in a model of polymicrobial abdominal sepsis induced by cecal ligation and perforation (CLP) [88]. Acquired acute deficiency of Tie2 was found to be sufficient to weaken the vascular barrier in vivo [87]. Compared to wild-type littermates, loss of one Tie2 allele worsened vascular leakage and survival following either systemic endotoxin or CLP [87]. Tie1 expression must also be intact to counteract sepsis [22], and VE-PTP inhibition or genetic deletion has been shown to strengthen the vascular barrier against systemic inflammation [81].

Finally, Angpt-2 has been inhibited in several ways, all of which implicate this molecule as a mediator of adverse outcomes in experimental sepsis. First, partial genetic deletion of ANGPT2 was shown to protect CLP-induced mice from vascular leakage and to improve survival [89]. This result was verified by a second group, who also demonstrated protection against experimental sepsis with an Angpt-2 binding antibody [90]. The importance of the feed-forward loop that sustains Angpt-2 biosynthesis after the onset of inflammation was demonstrated in two studies that applied RNA interference to limit de novo production of Angpt-2 [66, 91]. Both reports demonstrated a positive impact on survival, suggesting that even a relatively late intervention—i.e., after pre-formed Angpt-2 protein has already been released—could still improve outcomes. The most recent work in this area has focused directly on the question of oligomerization by showing that a novel antibody that can “cluster” Angpt-2 also improves survival in two different models of sepsis [92]. Together, these studies propose that targeted manipulations to favor Tie2 signaling enhance organ function and survival whereas manipulations that weaken Tie2 signaling impair the organismic response to sepsis.

Lung injury/ARDS:

The Tie2 axis has been shown repeatedly to defend animals against disparate triggers of acute or chronic lung injury: this list includes intratracheal instillation of lipopolysaccharides [93]; parenteral lipopolysaccharides [81, 85, 86]; abdominal sepsis CLP [88, 91, 92, 94, 95]; hyperoxia in young animals [96]; the chemical warfare agent phosgene [97, 98]; and pulmonary hypertension following monocrotaline, serotonin or IL-6 [99, 100]. The salutary effect of Tie2 stimulation against unrelated noxious insults suggests a conserved role for Tie2 in the pathogenesis of lung injury.

Acute kidney injury:

The Tie2 axis has been tested in animal models of acute kidney injury (AKI) arising from sepsis. Angpt-2 deficiency was shown to protect against CLP-induced AKI [89]. Furthermore, intravenous Angpt-1 administration after the onset of CLP was shown to attenuate renal inflammation and expression of vascular adhesion molecules [88]. In models of acute injury that progress to fibrosis, administration of a recombinant Angpt-1 modified for better pharmacokinetic characteristics has been shown to ameliorate long-term outcomes [101, 102].

DIC:

Only two published studies have examined targeted manipulations of the Tie2 axis in the context of DIC. One study reported coagulation parameters in the CLP mouse model with and without treatment with the clustering antibody that converts Angpt-2 into a Tie2 agonist. That study demonstrated beneficial effects on sepsis-induced DIC with the use of the clustering antibody [92]. A second study examined the effects of partial Tie2 deletion and Angpt-1 addition in the early stages of endotoxemia or CLP [4]. Loss of one Tie2 allele was found to potentiate injury-induced fibrin deposition in vivo in the absence of inflammation. In the context of endotoxemia, the tendency of Tie2 heterozygotes to develop fibrin-rich clots was more severe than their wildtype littermates. Conversely, when Angpt-1 was administered in either the endotoxemia model or CLP, the tendency to clot formation was markedly attenuated.

Non-sepsis states of acute vascular destabilization:

Studies in models of acute vascular destabilization that do not involve sepsis collectively suggest that stimulation of Tie2 can defend or restore vascular quiescence in diverse contexts. These include hemorrhagic fevers (Puumala, hantavirus, Ebola, dengue), anthrax, malaria, influenza [95], chronic mycobacterial infection [50, 104], and systemic capillary leak syndrome (SCLS).

Hantavirus-infected endothelial cells are sensitized to triggers of permeability such as VEGF, an effect counteracted by Angpt-1 [105]. A study seeking to address causes of variation in the outcomes to Ebolavirus infection applied an experimental version of the virus to numerous outcrossed strains of mouse, then conducted genetics in the outliers. This study found that mice bearing hypomorphic alleles of TIE2 were more vulnerable to prolonged coagulation times, visceral hemorrhages and early death [106]. Certain TIE1 alleles were associated with increased weight loss, earlier death and increased overall mortality [106]. In a mouse model of dengue infection, treatment with Angpt-1 improved survival [107]. In a mouse model of malaria that develops cerebral edema, Angpt-1 was shown to restore vascular integrity, reduce edema, and improve survival [108].

Systemic administration of anthrax lethal toxin downregulated Tie2 signaling and increased vascular leakage in mice [103]. Partial deletion of Angpt-2 improved survival in this model. Partial loss of Angpt-2 attenuated survival, and administration of Angpt-1 by gene transfer improved survival in this model. Finally, in primates infected with an attenuated strain of *Bacillus anthracis* that nonetheless provokes vascular collapse, circulating Angpt-2

levels rose rapidly whereas Angpt-1 levels fell. The changes in circulating Angpts were proportional to the inoculum of bacteria [103].

CLINICAL EVIDENCE IMPLICATING THE TIE2 AXIS

Overview

Clinical studies of the Tie2 axis have primarily involved measurement of circulating levels of Angpt-1 and Angpt-2, although there are some reports on soluble levels of Tie2 and Tie1. Genetic polymorphisms influencing Angpt-2 and Tie2 expression have also been linked to risk of ARDS in the setting of both sepsis and non-sepsis.

Sepsis and ARDS

Early work in sepsis focused on the lung and showed that high circulating Angpt-2 was associated with impaired gas exchange, perhaps the result of vascular leak [109]. Subsequent studies in severe sepsis showed that Angpt-2 can increase dramatically in the circulation early in the course of disease and associated well with loss of vascular integrity in various organs [110, 111]. Angpt-2 levels even spike with infusion of low-dose endotoxin in healthy volunteers [112], leading many to propose that it could be a useful biomarker of infection. Furthermore, as Angpt-2 increases with systemic inflammation, Angpt-1 falls. Thus, the ratio of Angpt-2/Angpt-1 in the circulation may be a better indicator of Tie2 signaling impairment than either protein alone [111, 113]. However, “sterile inflammation” syndromes such as trauma or major surgery can also disrupt Tie2 signaling making it less specific for sepsis [114–116]. Finally, genetic polymorphisms linked to Angpt-2 over-secretion have shown an increased risk for ARDS [117].

Common variants reducing TIE2 gene expression itself have also been linked to the development of ARDS [95].

Sepsis and DIC

Disruption of the endothelial Tie2 axis also appears to be a sentinel event in septic DIC. We recently described proteomic analysis in septic DIC patients that revealed a network involving inflammation and coagulation with Angpt-2, occupying a central node [4]. Angpt-2 was strongly associated with traditional DIC markers including platelet counts, yet more accurately predicted mortality in 2 large independent cohorts (combined $N=1,077$). Similarly, in endotoxemic mice, reduced Tie2 signaling preceded signs of overt DIC. Intravital imaging of the microvascular revealed excessive fibrin accumulation in a pattern reminiscent of Tie2 deficiency. Conversely, Tie2 activation normalized prothrombotic responses by inhibiting endothelial tissue factor and phosphatidylserine exposure. Importantly, Tie2 activation in this model had no adverse effects on bleeding. Given that interventions targeting Tie2 can normalize coagulation without increasing bleeding risk this approach could prove superior to current DIC therapies.

Cardiopulmonary bypass and acute kidney injury

One study measured serum Angpt-2 from 25 adult patients before and after cardiopulmonary bypass CPB, and compared it with indices of organ dysfunction, duration of mechanical

ventilation, length of stay in the ICU, and hospital mortality. Angpt-2 levels steadily increased from 2.6 ± 2.4 ng/mL at 0 h up to 7.3 ± 4.6 ng/mL at 24 h following CPB ($P < 0.001$). The release of Angpt-2 correlated with the duration of CPB, aortic cross-clamp time, and post-CPB lactate levels. Changes in Angpt-2 during follow-up correlated with PaO₂/FiO₂ ratios, hemodynamics, fluid balance, and disease severity measures. Angpt-2 levels at 12 h predicted the durations of mechanical ventilation and ICU stay, and hospital mortality [118]. Another study of 21 patients who experienced a 50% or greater increase in serum creatinine after cardiac surgery compared circulating levels of Angpt-1, Angpt-2, and Tie2 to propensity matched controls. The investigators also measured kidney injury molecule-1 (KIM-1) and N-acetyl-beta-D-glucosaminidase (NAG) in the urine. Angpt-2 plasma levels increased over time in patients with AKI (from 4.2 to 11.6 ng/ml) but did so as well in control patients (from 3.0 to 6.7 ng/ml). Plasma levels of Tie2 and Angpt-1 also decreased in both AKI patients and controls. However, there was a positive correlation between plasma levels of Angpt-2 and urinary levels of NAG but not KIM-1 [21]. Although circulating Angpt-2 levels were predictive of AKI and renal failure in patients with acute pancreatitis the relationship may be non-specific [119]. Angpt-2 significantly correlated with acute phase proteins as well.

Other states of vascular destabilization

In patients with certain hemorrhagic fevers including hanta virus, dengue, and Crimean-Congo hemorrhagic fever the serum quantity of Angpt-2 increases in a similar fashion to that in septic patients [120–122]. In Clarkson disease (systemic capillary leak syndrome) patients have transient episodes of vascular leakage resulting in hemoconcentration and hypotension. Angpt-2 increases during disease flares and returns to baseline during remission [123]. Angpt-1 and Tie2 are both dysregulated in blood-outgrowth endothelial cells from affected humans suggesting a further role of this pathway in the systemic process [124]. Finally, *P. falciparum* cerebral malaria has also been associated with Angpt/Tie derangements. Once again as levels of Angpt-2 serum levels increased, the severity of *falciparum* malaria worsened [125].

CONCLUSIONS AND FUTURE DIRECTIONS

Vascular disruption underlies cardinal features of critical illness that culminate in death. A body of work spanning biochemistry, cell biology, rodent and primate models, human observational studies, and human genetics collectively propose that the Tie2 axis may be an important determinant of the vascular response to acute destabilizing stressors. While successful translation of preclinical observations to the bedside has been a major challenge in critical care medicine, the evidence emerging from laboratories around the world proposes several routes to clinical realization.

Genetic markers of the Tie2 axis and/or circulating Angpt measurements could one day inform clinicians regarding the susceptibility of a given patient to adverse vascular-related outcomes. In turn, such knowledge could be used to guide routine clinical care—e.g., fluid-conservative vs. - liberal resuscitation strategies or adjustment of ventilator settings to avoid secondary damage—or inform the selection of patients for trials of investigational

agents that target the vasculature. Because circulating Angpt-2 levels indirectly report Tie2 signaling, sequential Angpt-2 measurements could be used to monitor the response to investigational therapies targeting Tie2. Such a surrogate marker of target engagement amenable to evaluation in a Phase 1b setting can significantly impact clinical development. As a circulating protein, Angpt-2 is amenable to neutralization with an antibody. A clinical-stage Angpt-2 neutralizing antibody has already been developed for cancer testing [126]. Conversely, Angpt-1 could be delivered as a modified recombinant protein or by gene transfer or even cell-based therapy that is already being tested in ARDS [127]. VE-PTP inhibition is also being tested in patients with retinal edema [83, 128]. Therefore, multiple “shovel-ready” approaches exist to test this promising hypothesis in patients.

Translational pursuit of the Tie2 axis could result in new ways to diagnose, stratify, and treat ICU patients. Any such advance would constitute a breakthrough for the many patients affected by sepsis, ARDS, and related conditions.

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KEY POINTS

Cardinal clinical features of sepsis such as vascular leakage and coagulation relate to microvascular health. A vascular signaling pathway anchored by Tie2 actively promotes quiescence.

During sepsis, the endogenous Tie2 antagonist protein called Angiopoietin-2 is induced, leading to rapid reduction in Tie2 signaling. This renders microvessels leaky, proinflammatory, and prothrombotic.

Experimental models of sepsis, acute lung injury, and other acute stressors collectively demonstrate that restoration of Tie2 signaling exerts a protective effect and enhances survival.

ICU studies consistently demonstrate early and progressive elevation of circulating Angiopoietin-2 that is associated with worsening oxygenation, increased risk of DIC and other adverse outcomes.

Human genetic studies suggest that common variants in *ANGPT2* and *TIE2* genes may be linked to the risk of acute lung injury.

SYNOPSIS

Lethal features of sepsis and acute respiratory distress syndrome (ARDS) relate to the health of small blood vessels. For example, alveolar infiltration with proteinaceous fluid is often driven by breach of the microvascular barrier. Spontaneous thrombus formation within inflamed microvessels exacerbates organ ischemia, and in its final stages, erupts into overt disseminated intravascular coagulation (DIC). A vascular endothelial signaling axis, the Angiotensin-Tie2 pathway, may mediate the abrupt transition from microvascular integrity to pathological disruption. Tonic activation of Tie2 promotes vascular quiescence. During inflammation, however, Tie2 signaling is rapidly toggled off as levels of its endogenous antagonist, Angiotensin-2, rise. When Tie2 signaling drops, the microvascular barrier collapses, endothelium becomes adhesive to leukocytes, and the vessel wall no longer prevents spontaneous coagulation. This review will summarize a large body of preclinical and clinical results that implicate the Tie2 pathway as a promising target to restore microvascular health in sepsis and ARDS.

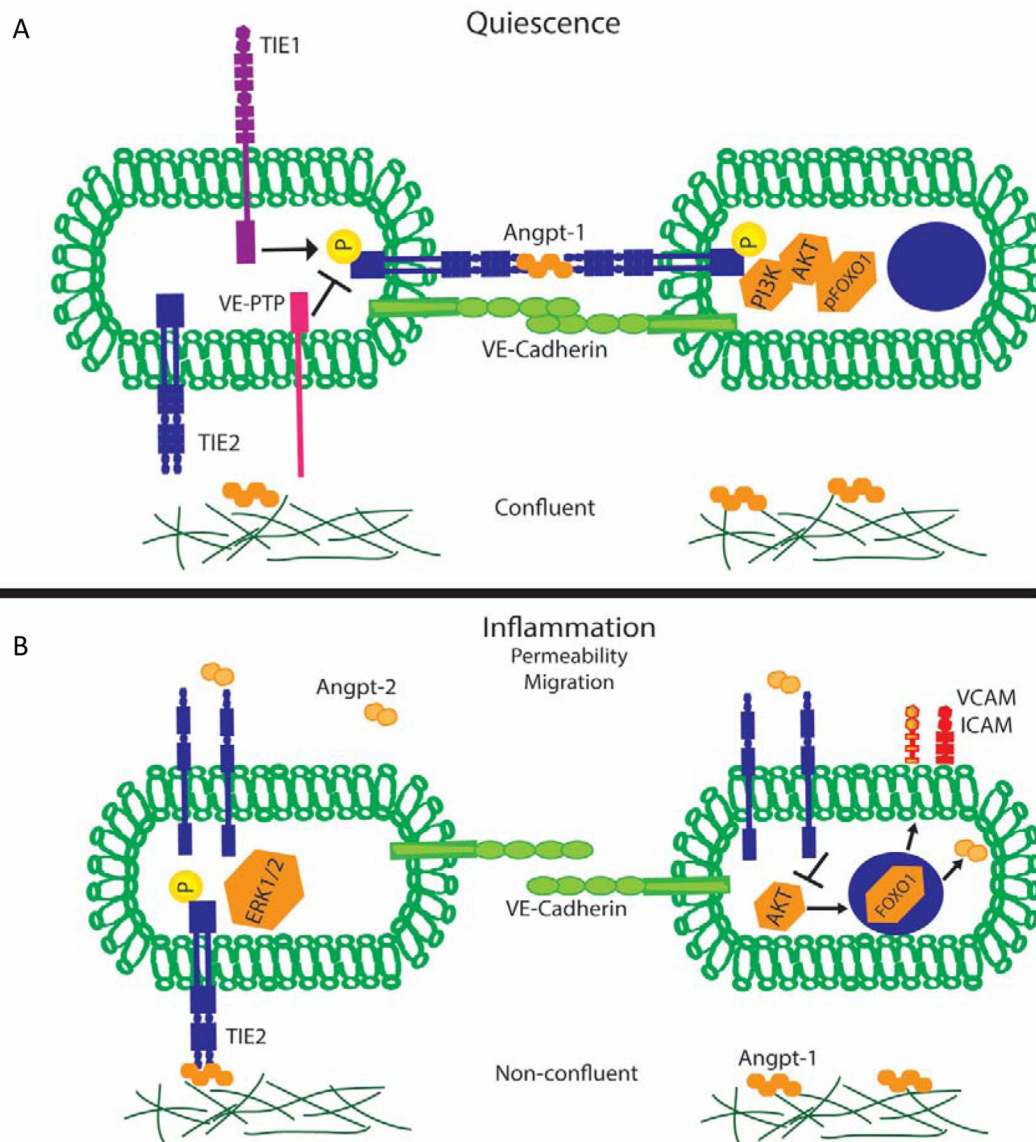


Figure 1:

A) Endothelial cell Tie2 signaling during quiescence. Tie2 receptors cluster together and engage tetramers of Angpt-1 which results in activation of the receptor in a trans configuration. Furthermore, Tie1 binding further potentiates Tie2 activation. Downstream activation of Tie2 results in increased barrier function. B) Endothelial cell Tie2 signaling during inflammation. Tie2 receptor binds Angpt-2 dimers resulting in dephosphorylation and FOXO1 migration to the nucleus resulting in translation of inflammatory mediations and additional Angpt-2.

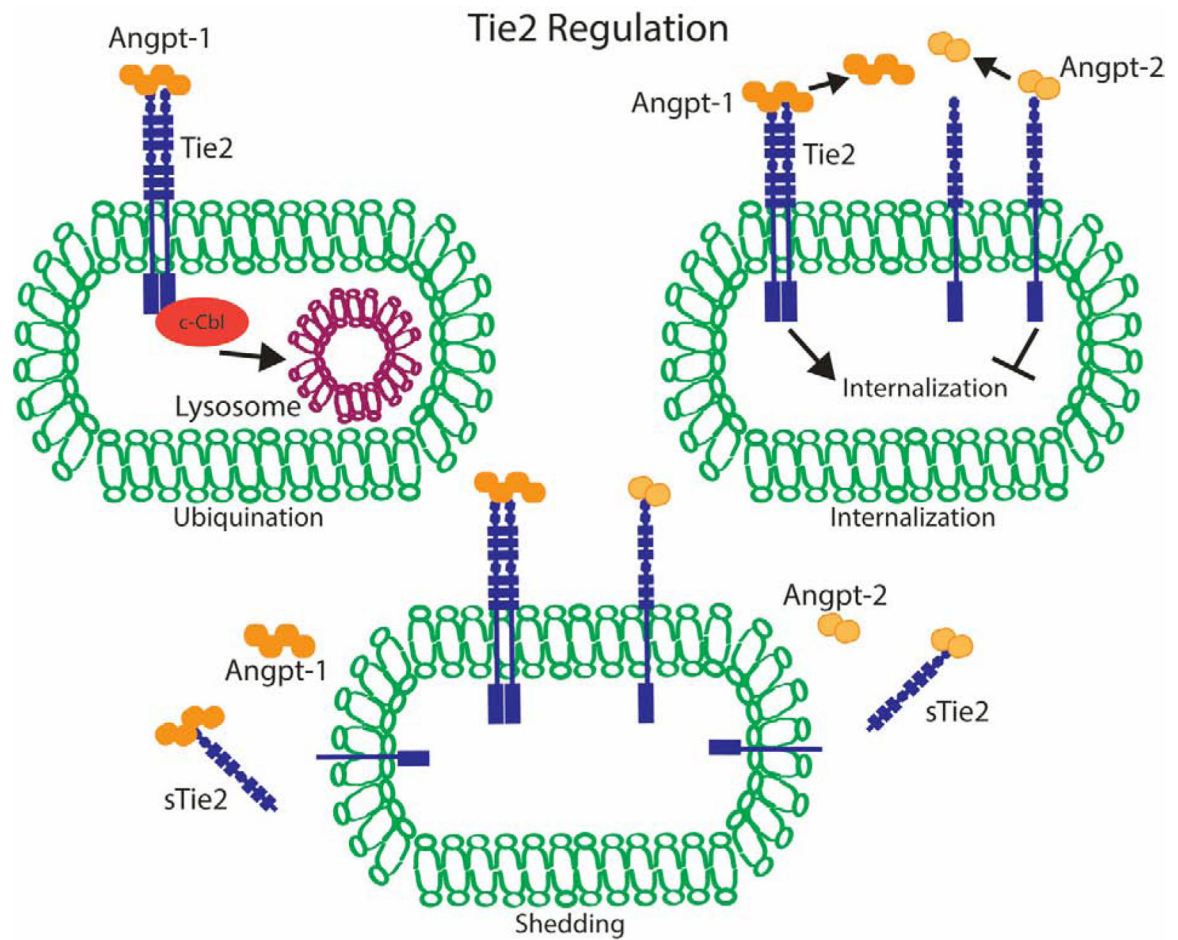


Figure 2: Tie2 regulation occurs through the above three mechanisms. Angpt-1 binding to Tie2 results in c-Cbl ubiquitination and resulting trafficking to lysosomes for destruction. Angpt-1 binding to Tie2 results in internalization whereas Angpt-2 binding is only able to minimally internalize receptor. Both Angpt-1 and Angpt-2 are released from receptor after engagement. Finally, Angpt-2 and Angpt-1 engaged receptors are shed from endothelial cells by metalloproteinase resulting in decreased receptor pool available for receptor engagement.

Table 1:

Factors modulating TIE2 signaling

Quiescence	Inflammation/Critical Illness
Angpt-1 (tetramers)	Angpt-2 (dimers)
TIE1	VE-PTP
Integrins	Cytokines
Confluence	Sub-confluence

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