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Mending Leaky Blood Vessels: The Angiopoietin-Tie2 Pathway in Sepsis

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ABSTRACT

Sepsis is a systemic inflammatory response to infection. A common end-feature, these patients regularly suffer from is the so-called multiple organ dysfunction syndrome, an often fatal consequence of organ hypoperfusion, coagulopathy, immune dysregulation, and mitochondrial dysfunction. Microvascular dysfunction critically contributes to the morbidity and mortality of this disease. The angiopoietin (Angpt)/Tie2 system consists of the transmembrane endothelial tyrosine kinase Tie2 and its circulating ligands (Angpt-1, -2, and -3/4). The balance between the canonical agonist Angpt-1 and its competitive inhibitor, Angpt-2, regulates basal endothelial barrier function and the leakage and vascular inflammation that develop in response to pathogens and cytokines. Here we summarize recent work in mice and men to highlight the therapeutic potential in this pathway to prevent or even reverse microvascular dysfunction in this deadly disease.

Introduction

Sepsis is a systemic inflammatory response of the organism to an often local infection. Most serious manifestations of the disease are severe sepsis and septic shock with organ dysfunction and hypotension. Sepsis is a major health care problem, affecting millions of individuals around the world each year (Martin et al., 2003). Unfortunately, its incidence appears to be rising, and the mortality caused by this syndrome remains between 38 and 59% (Dombrovskiy et al., 2007). Despite the ability to mechanically “replace” the function of the lungs and kidney, mortality rates when acute lung injury (ALI) and/or acute kidney injury (AKI) complicate sepsis range between 40 and 80% (Ware and Matthay, 2000; Schrier and Wang, 2004).

The pathophysiology of the disease is highly complex and includes diverse facets from an altered immune system, including coagulopathy and endothelial dysfunction. Of numerous investigated pathways, only five randomized controlled clinical trials improved survival in septic patients about a decade ago (Brower et al., 2000; Bernard et al., 2001; Rivers et al., 2001; van den Berghe et al., 2001; Annane et al., 2002). Three of those have been disproven in larger studies, with activated protein C being the latest and most prominent candidate that was removed from the market in late 2011 (Ranieri et al., 2012). The lack of targeted therapies is not for want of effort, but is probably a result of focusing on elucidating the humoral and immune pathways over the last decades. The immune response in septic patients is at least bimodal, with an early hyper- and late hypoinflammatory response, a fact that alone would require tailored patient-specific treatment regimens. Particularly hard to achieve, however, is the monitoring of the highly complex and redundant immune system. As an illustrating example, the efficacy of (later failed) anti–tumor necrosis factor therapeutics is highly dependent on the immune status the septic organism is confronted with at the actual time of treatment. What might help one patient might actually harm another. Therefore, optimal stratification of study populations for randomized clinical trials is particularly challenging for this disease.

The observation that clinical manifestations of sepsis (shock, adult respiratory distress syndrome, and disseminated intravascular coagulopathy) have a strong vascular component (permeability, loss of vascular tone, disturbed endothelial–platelet interaction) suggests that further study of sepsis as a state of profound vascular dysregulation may yield additional insights. Within the vasculature, it is the endothelium that is in direct contact with the circulating blood, thereby being involved in an adaptive response to various environmental stimuli. The endothelium controls vasomotor tone, regulates cellular oxygen and nutrients...
trafficking, maintains blood fluidity, contributes to the local balance in proinflammatory and anti-inflammatory mediators, and participates in generation of new blood vessels. All of these physiologic functions can be altered in sepsis. As a net result, the endothelial phenotype is changed from a more or less quiescent state to an overactivated state characterized by procoagulant, proinflammatory, and hyperpermeable properties. Hyperpermeability mechanistically contributes to the impaired gas exchange characteristic of acute lung injury and adult respiratory distress syndrome by increasing the distance impaired gas exchange characteristic of acute lung injury and adult respiratory distress syndrome by increasing the distance essential nutrients and waste products must diffuse. Moreover, it gives rise to hypovolemia aggravating hemoconcentration, stasis of blood flow, and shock.

In short, it is clear that several of the most critical clinical manifestations of sepsis arise from dysfunction and injury to blood vessels. Thus, pharmacological strategies that address the septic circulation have the potential to ameliorate sepsis-blood vessels. Thus, pharmacological strategies that address the septic circulation have the potential to ameliorate sepsis-blo

The Angiopoietin/Tie2 System

The angiopoietin (Angpt)/Tie2 system consists of the transmembrane endothelial tyrosine kinase Tie2 and its four circulating ligands; of those, Angpt-1 and Angpt-2 have been studied most comprehensively. The activation (i.e., phosphorylation) state of Tie2 regulates baseline endothelial quiescence and its response to an injurious stimulus. In endothelial cells, Tie2 phosphorylation is largely controlled by the ratio that exists between the agonistic ligand Angpt-1 and competitive inhibitor Angpt-2 (Maisonpierre et al., 1997; Yuan et al., 2009). Their mutually antagonistic properties are supported by functional data. For example, during embryonic development, Angpt-1 and Tie2 global knockout phenotypes are indistinguishable from each other and phenocopy Angpt-2 transgenics. In inflammatory diseases, Angpt-1 and Angpt-2 appear to have opposing functions as well; the former mitigates vascular inflammation and leakage, whereas the latter sensitizes the endothelium to inflammatory cytokines. From the signaling point of view, Angpt-1 ligation to Tie2 results in tyrosine phosphorylation that maintains vessel integrity, inhibits vascular leakage, suppresses inflammatory gene expression, and prevents recruitment and transmigration of leukocytes mainly by activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and by regulating small GTPase proteins (e.g., RhoA, Rac1). Together, the endothelial effects of Angpt-1/Tie2 signaling can be summarized as anti-inflammatory, antiapoptotic, antipermeable and prosurvival (Fig. 1).

As Angpt-1 and Angpt-2 are circulating proteins, they are accessible for detection in patients’ blood. We and others found that, in septic individuals, circulating levels of Angpt-2 can increase up to ~50 times, whereas Angpt-1 remains more or less unchanged (Kumpers et al., 2008; Lukasz et al., 2008). It is important to understand that Tie2 is a growth factor receptor that, in contrast to other family members, is constitutively activated in quiescent mature vasculature. It is widely believed that tonic Tie2 signaling may mediate the quiescent, healthy state of blood vessels, although this has yet to be formally demonstrated. In sepsis, the disturbed Angpt-1/2 homeostasis may deactivate baseline Tie2 signaling, thereby actively contributing to the devastating overactivated endothelial phenotype. Consistent with this hypothesis, circulating Angpt-2 levels of septic individuals are strongly associated with severity of illness, markers of endothelial inflammation, and most importantly, adverse outcome (Kumpers et al., 2008, 2010).

Based on these observations, several groups have investigated whether modulation of Tie2 signaling might be beneficial in experimental sepsis (Table 1). Two major approaches have been used.

The Tie2 Agonist Perspective. In 2005, Witzenbichler et al. showed that excess Angpt-1 was protective in endotoxic shock (Witzenbichler et al., 2005). To achieve high levels of circulating Angpt-1, they used an adenoviral gene transfer approach. They showed improved hemodynamic function, reduced lung injury, and less inflammatory response accompanied by improved survival. Although adenoviral transduction is generally not feasible in humans and leads to a very high expression of Angpt-1 (up to 1 μg/ml), Witzenbichler et al. (2005) demonstrated here a potential role of Angpt-1 as an adjunctive agent for the treatment of septic shock, and opened a new avenue for specific sepsis therapeutics. Remembering Paracelsus’ assumption, one might wonder if excess Angpt-1 could have negative side effects. The available data are controversial and mostly limited to the fine print. Thurston et al. (2000) reported parenthetically that up to 1 month of transgenic Angpt-1 overexpression had no adverse effect on vessel morphology. On the other hand, Ward et al. (2004) showed that prolonged transgenic Angpt-1 overexpression, even postdevelopmentally, was locally injurious in the liver.

Mei et al. (2007) provided further evidence for the protective potential of Angpt-1. They showed, in a set of in vivo experiments, the effect of mesenchymal stem cells overexpressing Angpt-1 for the prevention of lipopolysaccharide
TABLE 1
Tie2-modulating therapeutic strategies in different systemic inflammatory (infl) models from different groups using different models

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Treatment</th>
<th>Injury Model</th>
<th>Results</th>
<th>Supporting Hypothesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>Thurston et al., 1999</td>
<td>Ad Angpt-1</td>
<td>VEGF</td>
<td>Leakage-resistant vessels</td>
<td>Standard</td>
</tr>
<tr>
<td>2000</td>
<td>Thurston et al., 2000</td>
<td>rhAngpt-1</td>
<td>VEGF</td>
<td>Leakage-resistant vessels</td>
<td>Standard</td>
</tr>
<tr>
<td>2005</td>
<td>Witzenbichler et al., 2005</td>
<td>Ad Angpt-1</td>
<td>LPS</td>
<td>Improved survival and cardiac function</td>
<td>Standard</td>
</tr>
<tr>
<td>2005</td>
<td>Hall and Brookes, 2005</td>
<td>rhAngpt-1</td>
<td>LPS</td>
<td>Increased arteriolar vasoconstriction</td>
<td>Standard</td>
</tr>
<tr>
<td>2006</td>
<td>Parikh et al., 2006</td>
<td>rhAngpt-2</td>
<td></td>
<td>Spontaneous leak</td>
<td>Standard</td>
</tr>
<tr>
<td>2006</td>
<td>Fiedler et al., 2006</td>
<td>Angpt-2 (-/-) KO</td>
<td>Peritonitis</td>
<td>Ameliorated infl. response to TNFα</td>
<td>Standard</td>
</tr>
<tr>
<td>2007</td>
<td>Mammoto et al., 2007</td>
<td>Ad Angpt-1</td>
<td>LPS</td>
<td>Reduced vascular leakage</td>
<td>Standard</td>
</tr>
<tr>
<td>2009</td>
<td>Hwang et al., 2009</td>
<td>COMP-Angpt-1</td>
<td>LPS</td>
<td>Ameliorated leukocyte adhesion</td>
<td>Standard</td>
</tr>
<tr>
<td>2009</td>
<td>Kim et al., 2009</td>
<td>COMP-Angpt-1</td>
<td>LPS</td>
<td>Protects against acute kidney injury</td>
<td>Standard</td>
</tr>
<tr>
<td>2010</td>
<td>Tabranyi et al., 2010</td>
<td>Angpt-2 inhibition</td>
<td>M. pulmonis</td>
<td>Reduced local inflammation</td>
<td>Standard</td>
</tr>
<tr>
<td>2011</td>
<td>David et al., 2011b</td>
<td>rhAngpt-1 i.v.</td>
<td></td>
<td>Improved survival, better organ function</td>
<td>Standard</td>
</tr>
<tr>
<td>2011</td>
<td>Kumpers et al., 2011</td>
<td>Tie2 agonist</td>
<td>CLP</td>
<td>Improved survival, better organ function</td>
<td>Standard</td>
</tr>
<tr>
<td>2011</td>
<td>David et al., 2011a</td>
<td>Tie2 agonist</td>
<td>CLP</td>
<td>Improved leakage, organ function, survival</td>
<td>Standard</td>
</tr>
<tr>
<td>2012</td>
<td>Ghosh et al., 2012</td>
<td>Angpt-2+/- Ad Angpt-1</td>
<td>Anthrax</td>
<td>Improved leakage, organ function, survival</td>
<td>Standard</td>
</tr>
<tr>
<td>2012</td>
<td>Kurniati et al., 2012</td>
<td>Angpt-2(-/-)</td>
<td>LPS</td>
<td>Worse kidney function</td>
<td>Alternative</td>
</tr>
<tr>
<td>2012</td>
<td>Tzepi et al., 2012</td>
<td>rhAngpt-2</td>
<td>PSA sepsis</td>
<td>Improved survival</td>
<td>Alternative</td>
</tr>
<tr>
<td>2012</td>
<td>David et al., 2012</td>
<td>Angpt-2(+/-)</td>
<td>CLP/LPS</td>
<td>Improved organ function and survival</td>
<td>Standard</td>
</tr>
</tbody>
</table>

Ad, adenovirus; Angt, angiopoietin; CLP, cecal ligation and puncture; COMP, cartilage oligomeric matrix protein; KO, knockout; LPS, lipopolysaccharide; M. pulmonis, Mycoplasma pulmonis; PSA, pseudomonas aeruginosa; rhAngt, recombinant human angiopoietin; TNFα, tumor necrosis factor α; VEGF, vascular endothelial growth factor.

(LPS)-induced ALI. Administration of mesenchymal stem cells transfected with Angpt-1 resulted in a nearly complete reversal of LPS-induced pulmonary hyperpermeability, as reflected by reductions in IgM and albumin levels in bronchoalveolar lavage (Mei et al., 2007). Unfortunately, neither adenoviral nor modified stem cell delivery is a feasible technique to translate these findings from mice to men.

Kim et al. (2009) determined the positive effect of increased Angpt-1/Tie2 signaling in LPS-induced AKI using a slightly different approach. Mice were pretreated with an engineered variant of native Angpt-1, the so-called “Angpt-1 with cartilage oligomeric matrix protein,” which is more potent in phosphorylating Tie2 than native Angpt-1 (Kim et al., 2009). It is important to note that the delivery form of this engineered Angpt-1 variant was again an adenoviral one. Their findings demonstrate a protective effect of Angpt-1 with cartilage oligomeric matrix protein pretreatment against endotoxic AKI. A comparable experiment with a rescue application to simulate a more realistic setting was not provided in this work (probably due to feasibility reasons using viral transfer), but would have been highly desirable.

Another way to augment Tie2 phosphorylation is the administration of vasculotide (VT), a PEG(polyethylene glycolylated 7-mer peptide (HHHRRHF)) which was recently identified by screening a phage display library for binding to the Tie2 receptor (Tournaire et al., 2004). In human microvascular endothelial cells, VT completely prevents characteristic morphologic and functional changes induced by LPS. From a pharmaceutical point of view, VT may hold promise as a drug-like compound. We therefore assessed VT in vivo in a model of endotoxemic ALI. Indeed, VT treatment was sufficient to prevent the LPS-induced development of lung vascular leakage, and improved survival of endotoxemia by 41%, an effect that was completely abolished in Tie2 heterozygous knockout mice, indicating the high specificity of VT for Tie2 (David et al., 2011a). In an additional study, Kümpers et al. (2011) showed that prophylactic as well as therapeutic (i.e., rescue) administration of VT was sufficient to ameliorate AKI and reduce mortality in a clinically relevant surgical model of sepsis, cecal ligation, and perforation.

The Tie2 Antagonist Perspective. Studies from independent laboratories in models of in vivo inflammation, tissue injury, and bacterial infections (including sepsis) suggest that Angpt-2, a context-dependent antagonist of the endothelial receptor Tie2, exacerbates a wide spectrum of pernicious manifestations (Table 1). In 2006, Parikh et al. reported that circulating Angpt-2 was elevated in subjects with severe sepsis, and showed that Angpt-2 administration in otherwise healthy adult mice resulted in lung injury and vascular leakage (Parikh et al., 2006). These results provided the first direct evidence suggesting that Angpt-2 was both a marker and mediator of vascular injury in sepsis. This study also showed that septic human serum disrupted the integrity of microvascular endothelium in a fashion reversible by Angpt-1, suggesting a mechanism by which excess Angpt-2 could be pathogenic.

In the same year, Fiedler et al. (2006) used intravital microscopy in models of sterile chemical peritonitis to show that gene deletion of Angpt-2 dose-proportionally attenuated leukocyte-endothelial interactions. They found that tumor necrosis factor-α–induced upregulation of inflammatory adhesion molecules by endothelial cells required Angpt-2 expression by those cells (Fiedler et al., 2006). Shortly thereafter, Bhandari et al. (2012) reported that hyperoxia-induced acute lung injury in mice was ameliorated by genetic or small interfering RNA depletion of Angpt-2, and showed that bronchoalveolar lavage fluid from individuals with acute lung injury contained high levels of Angpt-2. Of note, the authors argued for an injurious role of Angpt-2 in the alveolar epithelial necrosis characteristic of this model.

In contrast to these findings, Tzepi et al. (2012) recently reported in The Journal of Pharmacology and Experimental Therapeutics that systemic delivery of recombinant human angiopoietin-2 (rhAngpt-2), but not rhAngpt-1, improved survival in mice challenged with live pseudomonas. How are these findings to be reconciled with the existing literature and our current understanding of Angpt/Tie2 signaling? First, the use of this particular bacterium is new. To mitigate concerns of unique model-specific factors, the authors also demonstrated a protective effect of rhAngpt-2 in Escherichia coli
bacteremia. Curiously, however, they did not observe an Angpt-2-dependent survival benefit in mice injected with E. coli LPS. Second, from our point of view, the experimental approach may be critical. The applied dose of rhAngpt-2 results in a circulating concentration exceeding those known from humans with septic shock by 5–10-fold. Supraphysiological concentrations of Angpt-2 appear to activate Tie2 in mice, as shown by Daly et al. (2006). Measurement of Tie2 phosphorylation in the work by Tzepi et al. (2012) would have added to our understanding of the molecular pathogenesis of pseudomonal bacteremia and to the underlying mechanism driving the benefit of rhAngpt-2 in this model.

Our own results with vasculotide in cecal ligation and puncture and E. coli LPS clearly demonstrate the protection conferred in experimental sepsis by enhancing Tie2 phosphorylation. The intriguing results of Tzepi et al. (2012) could also reflect other putative mechanisms—e.g., stimulation of nonendothelial cells and/or signaling through non-Tie2 receptors (Felcht et al., 2012). In our opinion, the simplest test of whether Angpt-2 induction is pathogenic (or protective) in sepsis would target the endogenous protein by inhibiting its production, blocking its release, or neutralizing its effects. Such a study should also characterize the signaling consequences, ideally in multiple organ beds and hematopoietic cells and at sequential times after model induction.

Kurniati et al. (2012) recently used Angpt-2 (−/−) mice and their wild-type (+/+) littermates to analyze the role of Angpt-2 in the development of endothelial AKI. Surprisingly, they found that Angpt-2–deficient mice were not protected from the development of septic renal dysfunction. Moreover, this group found that lack of Angpt-2 was associated with higher circulating and tissue cytokine levels. The authors speculate that early excess Angpt-2 could actually represent a compensatory attempt in suppressing cytokine production. From our point of view, another explanation might be worth mentioning here. As the authors note in their discussion, homozygote Angpt-2–null mice have a phenotype that is dominated by severe lymphatic defects (Gale et al., 2002). Given the contribution of the lymphatics in the complex physiology of the immune response, one should keep in mind that this could dramatically influence the hosts LPS response. In addition, from our experience (Tran et al., 2011), sustained overt renal failure is difficult to achieve in endotoxemia because a strong dose of LPS results in early lethality, whereas a weak dose produces mostly a "prerenal" response that reflects renal ischemia without substantial structural injury to the kidney. Furthermore, traditional assays for serum creatinine become falsely positive from the ketosis that accompanies sepsis. As a result, the authors may have been compelled to use markers of AKI that are not the gold standard.

We recently applied models of sepsis in mice lacking just one allele of Angpt-2 (+/−). Compared with wild-type littermates (+/+), these mice exhibited less tissue inflammation, less renal failure, less lung injury, and better survival (David et al., 2012). Moreover, severe morphologic changes induced in endothelial cell culture by coinubcation with serum from septic patients were completely abrogated by treatment with an Angpt-2 function-blocking antibody. Finally, we studied patients presenting to the emergency room with suspicion of infection. We found that circulating Angpt-2 measured within the first hour of emergency room admission was proportionally elevated to the future severity of sepsis, and was predictive of septic shock and death with cutoff values that performed equally well in two independent cohorts of similar subjects. These in vivo and in vitro experiments provide evidence that Angpt-2 might directly contribute to the adverse outcomes in sepsis, and might therefore be a promising candidate for future evaluation.

As the work of Tzepi et al. (2012) and Kurniati et al. (2012) we should not view Angpt-2 as purely harmful. Indeed, it may be necessary for the early adaptive response to infection—e.g., by facilitating the egress of cellular and humoral mediators of immunity from blood vessels to neutralize pathogens at the portal of entry. However, when this locally adaptive response is applied throughout the circulatory system, the resulting vascular leak and systemic inflammation may well be detrimental to the host.

Together, data from one decade of in vitro and in vivo research suggest that both an Angpt-1 mimetic as well as an Angpt-2—depleting or—inhibiting strategy might be a useful and specific pharmaceutical tool to treat sepsis-induced vascular barrier breakdown.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: David, Kümper, von Slyke, Parikh.

References


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