Review

Bench-to-bedside review: Angiopoietin signalling in critical illness – a future target?

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Abstract

Multiple organ dysfunction syndrome (MODS) occurs in response to major insults such as sepsis, severe haemorrhage, trauma, major surgery and pancreatitis. The mortality rate is high despite intensive supportive care. The pathophysiological mechanism underlying MODS are not entirely clear, although several have been proposed. Overwhelming inflammation, immunoparesis, occult oxygen debt and other mechanisms have been investigated, and - despite many unanswered questions - therapies targeting these mechanisms have been developed. Unfortunately, only a few interventions, usually those targeting multiple mechanisms at the same time, have appeared to be beneficial. We clearly need to understand better the mechanisms that underlie MODS. The endothelium certainly plays an active role in MODS. It functions at the intersection of several systems, including inflammation, coagulation, haemodynamics, fluid and electrolyte balance, and cell migration. An important regulator of these systems is the angiopoietin/Tie2 signalling system. In this review we describe this signalling system, giving special attention to what is known about it in critically ill patients and its potential as a target for therapy.

Introduction

Critical illness is a life-threatening disease by definition. Patients treated for critical illness in the intensive care unit have underlying causes such as infection, trauma, major surgery, hemorrhagic shock, pancreatitis and other major insults. Despite maximal supportive care, severely ill patients treated in intensive care units are still likely to die, usually after an episode of increasing failure of multiple organs [1].

The mechanisms that underlie multiple organ dysfunction syndrome (MODS) are not known [2], although several have been proposed, including overwhelming infection or immune response, immune paralysis, occult oxygen debt and mitochondrial dysfunction [3-5]. Although these potential mechanisms have features in common, it is not clear whether MODS is a final common pathway or when it is engaged. The innate and adaptive immune systems, coagulation, and hormonal and neuronal signalling are undoubtedly involved and are all connected. For example, the hypoxic response is linked to innate immunity and inflammation by the transcription factor nuclear factor-κB (NF-κB) [6]. It is no coincidence that the few interventions that appear to be of benefit, although this is still under debate, have pleiotropic mechanisms of action [7-9]. Thus, it seems reasonable to study the intersections between and within cellular and molecular systems to elucidate the interactions and to develop therapeutic options.

One of the central cellular players in this system is the endothelial cell (EC). Once thought to serve as an inert vascular lining, ECs are highly heterogeneous and constitute an active disseminated organ throughout the circulatory system. ECs form the border between every organ and the bloodstream and thus with the rest of the body. The EC receives and gives signals, stores active substances of multiple systems, and regulates the passage of fluids, electrolytes, proteins and cells. The EC has a time and place dependent phenotype that is dynamically controlled, and its reactions to stimuli are specific to organ and vascular bed [10-13]. The EC merits robust investigation in critical illness, as in vascular medicine [14].

ECs fulfil three functions. First, they participate in the formation of new blood vessels. This is important in embryo-

Ang = angiopoietin; Ang/Tie system = angiopoietin/Tie2 signalling system; EC = endothelial cell; HUVEC = human umbilical vein endothelial cell; LPS = lipopolysaccharide; MODS = multiple organ dysfunction syndrome; NF-κB = nuclear factor-κB; PI3K = phosphoinositide-3 kinase; TNF = tumour necrosis factor; VEGF = vascular endothelial growth factor; WPB = Weibel-Palade body.

genesis and organogenesis in normal physiology and in wound repair, but it is considered pathologic in tumour growth and diabetes [15]. Second, in the adult organism, ECs help to maintain homeostasis, including fluid, electrolyte and protein transport, and cell migration into and out of the vessel, and to regulate blood flow. Third, ECs react and respond to disturbances of homeostasis (for example, in inflammation, coagulation and hypoxia/reperfusion).

All three functions are involved in MODS, in which ECs are shed, blood flow regulation is hampered, vessels become leaky, cells migrate out of the vessel and into the surrounding tissue, and coagulation and inflammation pathways are activated [16]. The machinery involved - receptors, signalling pathways and effectors - is largely the same in each function, but the net effect is determined by the balance between the parts of the machinery and the context [15].

The angiopoietin/Tie2 signalling system (Ang/Tie system) appears to be crucial in all three functions [17,18]. The Ang/Tie system, which was discovered after vascular endothelial growth factor (VEGF) and its receptors, is mainly restricted to EC regulation and is the focus of this review. Accumulating evidence suggests that this system is non-redundant and is involved in multiple MODS-related pathways. All components of potential pathophysiological mechanisms in MODS should be viewed within their own context, because all systems are mutually dependent. Thus, examination of the Ang/Tie system might offer insight into the mechanisms underlying MODS and provide opportunities for therapeutic intervention.

Is the Ang/Tie system involved in critical illness?

The notion that the Ang/Tie system contributes to disease pathogenesis is supported by clinical studies and studies in animal models, and by the relation between symptoms of critical illness and disturbances in this system. In mice, Ang-2 over-expression in glomeruli causes proteinuria and apoptosis of glomerular ECs [19]. In a rat model of glomerulonephritis, Tie2 is over-expressed by ECs, and Ang-1 and Ang-2 are over-expressed by podocytes in a time-dependent manner during the repair phase [20]. Therefore, Ang/Tie might be involved in renal failure and repair.

Lung dysfunction is common in critical illness, and evidence of Ang/Tie involvement has been found in animal models. In a rat model of acute respiratory distress syndrome, Ang-1 reduces permeability and inflammation, whereas Tie2 deficiency increases damage [21]. In an experimental model of asthma, Ang-1 mRNA was decreased, and Ang-1 supplementation decreased alveolar leakage and NF-κB-dependent inflammation [22]. In hypoxia-induced pulmonary hypertension in rats, decreased activity of the Tie2 pathway contributed to right ventricular load, and this effect was antagonized by Ang-1 [23]. On the other hand, a causative role for Ang-1 in pulmonary hypertension has also been suggested [24]. In hyperoxic lung injury, Ang-2 is involved in lung permeability and inflammation [25].

Ang/Tie also may contribute to critical illness in patients with pulmonary conditions. Ang-1 and Ang-2 concentrations in sputum from asthma patients correlated with airway microvascular permeability [26]. In patients with exudative pleural effusion, the Ang-2 level was increased whereas Ang-1 was unchanged [27]. Ang-2 levels are associated with pulmonary vascular leakage and the severity of acute lung injury. Plasma from patients with acute lung injury and high Ang-2 concentrations disrupts junctional architecture *in vitro* in human microvascular ECs [28,29].

Patients with cardiovascular disorders also exhibit changes in the Ang/Tie system. Circulating Ang-1 concentrations are stable in patients with atrial fibrillation, but Ang-2 concentrations are increased, along with markers of platelet activation, angiogenesis and inflammation [30]. Patients with hypertension resulting in end-organ damage have increased levels of circulating Ang-1, Ang-2, Tie2 and VEGF [31]. Congestive heart failure is associated with elevated plasma levels of Ang-2, Tie2 and VEGF, but normal levels of Ang-1 [32]. A similar pattern is seen in acute coronary syndrome [33].

Circulating levels of components of the Ang/Tie system have been measured in patients admitted to the critical care unit. In trauma patients plasma Ang-2, but not plasma Ang-1 or VEGF, was increased early after trauma, and the level correlated with disease severity and outcome [34]. In children with sepsis and septic shock, Ang-2 levels in plasma were increased and once again correlated with disease severity, whereas Ang-1 levels were decreased [35]. The same Ang-1/Ang-2 pattern is seen in adults with sepsis [28,29,36-39]. The results of studies of the Ang/Tie system in humans are summarized in Table 1. In sepsis, VEGF and its soluble receptor sFLT-1 (soluble VEGFR-1) are also increased in a disease severity-dependent manner [40-42].The picture that emerges from these studies is that the Ang/Tie signalling system appears to play a crucial role in the symptoms of MODS. Findings in animal models and in patients suggest that Ang-1 stabilizes ECs and Ang-2 prepares them for action. The close relation with VEGF is also apparent.

The angiopoietin signalling system

Ligands and receptors

The angiopoietin signalling system consists of four ligands and two receptors (Figure 1). The ligands are Ang-1 to Ang-4, the best studied being Ang-1 and Ang-2 [17,43-45]. The roles of Ang-3 (the murine orthologue of Ang-4) and Ang-4 are much less clear [18]. Angiopoietins are 70-kDa glycoproteins that contain an amino-terminal angiopoietin-specific domain, a coiled-coil domain, a linker peptide and a carboxyl-terminal fibrinogen homology domain [17,44,46,47]. Ang-1 and Ang-2 bind to Tie2 after polymerization of at least

Table 1

Clinical studies of Ang-1, Ang-2 and soluble Tie2 in critically ill patients

Study	Patients	Ang-1	Ang-2	Soluble Tie2	Clinical effects
Lee *et al.* [33]	ACS: 82 AMI, 44 unstable angina, 40 stable CAD 40 HCs	NS	Higher in AMI versus HCs, SA, unstable angina	Higher in AMI versus HCs, SA, unstable angina	ND
Kugathasan *et al.* [23]	PAH: 6 idiopathic, 7 with other disease 8 HCs	NS	NS	Tie2 mRNA higher in lung of PAH patients versus HCs	ND
Parikh *et al.* [28]	ICU patients: 17 severe sepsis, 5 mild sepsis 29 HCs	NS	Higher in severe sepsis versus HCs	ND	Ang-2 levels correlate with low Pa_{O_2}/Fi_{O_2}
Gallagher *et al.* [180]	Vascular leakage: 14 IL-2, 14 IL-2+bevacizumab	ND	Higher during therapy	ND	Ang-2 levels rise on days of IL-2 therapy; high levels on day 3 predict vascular leakage (stop therapy)
Gallagher *et al.* [29]	ARDS: 45 ICU, 18 ARDS	ND	Higher in ARDS versus ICU patients; in ARDS, higher in nonsurvivors	ND	High levels of Ang-2 on day patient meets ARDS criteria. Ang-2 levels in ARDS patients correlate with mortality
Giuliano *et al.* [35]	Septic shock children: 20 SIRS 20 sepsis, 61 septic shock 15 HCs	Ang-1 lower in septic shock versus sepsis and SIRS	Higher in septic shock versus HCs, SIRS and sepsis	ND	ND
Orfanos *et al.* [37]	ICU patients: 6 no SIRS, 8 SIRS, 16 sepsis, 18 severe sepsis, 13 septic shock	ND	Higher in severe sepsis versus no SIRS and sepsis	ND	Ang-2 levels related to severe sepsis and TNF-α levels
Scholz *et al.* [181]	180 liver cirrhosis patients 40 HCs	ND	Higher in cirrhosis versus HCs	ND	ND
Ganter *et al.* [34]	208 trauma patients in ER	Unchanged	Higher within 30 minutes after ER admission	ND	Ang-2 levels correlate with met ISS and mortality. Ang-2 higher in nonsurvivors
van der Heijden *et al.* [39]	24 sepsis, 88 nonseptic critically ill 15 HCs	Ang-1 lower in sepsis and critical illness versus HCs	Higher in critically ill patients; higher in septic than nonseptic patients	ND	Ang-2 levels associated with pulmonary permeability oedema and severity of ALI in septic and nonseptic critically ill patients
Lukasz *et al.* [36]	94 critically ill medical ICU patients 30 HCs	Ang-1 correlated negatively with SOFA score	Ang-2 correlates positively with SOFA score	ND	Ang-1 correlates negatively and Ang-2 positively with SOFA score
Siner *et al.* [38]	Critically ill patients: 20 nonseptic (ICU), 10 sepsis, 12 severe sepsis, 24 septic shock	ND	Ang-2 increases with severity of sepsis	ND	Increase in Ang-2 associated with severity of illness and hospital mortality

ACS, acute coronary syndrome; AMI, acute myocardial infarction; ARDS, acute respiratory distress syndrome; CAD, coronary artery disease; ER, emergency room; Fi_{O_2}, fractional inspired oxygen; HC, healthy control; ICU, intensive care unit; IL, interleukin; ISS, International Severity Score; ND, not determined; NS, not significant; PAH, pulmonary arterial hypertension; Pa_{O_2}, pulmonary artery oxygen tension; SA, stable angina; SOFA, Sequential Organ Failure Assessment score; TNF, tumour necrosis factor.

four (Ang-1) and two (Ang-2) subunits [48,49]. The dissimilarity between Ang-1 and Ang-2 signalling lies in subtle differences in the receptor binding domain that lead to distinct intracellular actions of the receptor; differential cellular handling of both receptor and ligands after binding and signalling initiation may also play a role [49,50].

The receptors are Tie1 and Tie2 [51]. Tie2 is a 140-kDa tyrosine kinase receptor with homology to immunoglobulin and epidermal growth factor [47,52]. Tie receptors have an amino-terminal ligand binding domain, a single transmembrane domain and an intracellular tyrosine kinase domain [51]. Ligand binding to the extracellular domain of Tie2 results in receptor dimerization, autophosphorylation and docking of adaptors, and coupling to intracellular signalling pathways [47,53-55]. Tie2 is shed from the EC and can be detected in soluble form in normal human serum and plasma; soluble Tie2 may be involved in ligand scavenging without signalling [56]. Tie2 shedding is both constitutive and induced; the latter can be controlled by VEGF via a pathway that is dependent on phosphoinositide-3 kinase (PI3K) and Akt [57]. Shed soluble Tie2 can scavenge Ang-1 and Ang-2 [56]. Tie1 does not act as a transmembrane kinase; rather, it regulates the binding of ligands to Tie2 and modulates its signalling [58-60].

Origin of ligands and distribution of receptors

Ang-1 is produced by pericytes and smooth muscle cells (Figure 1). In the glomerulus, which lacks pericytes, Ang-1 is produced by podocytes [61]. Ang-1 has a high affinity for the extracellular matrix, and so circulating levels do not reflect tissue levels, which in part is probably responsible for the constitutive phosphorylation of Tie2 in quiescent endothelium [62-65]. Ang-2 is produced in ECs and stored in Weibel-Palade bodies (WPBs) [66,67]. The release of Ang-2 from WPBs by exocytosis can be regulated independently of the release of other stored proteins [68]. Tie2 is expressed predominantly by ECs, although some subsets of macrophages and multiple other cell types express Tie2 at low levels [69,70]. In ECs, Tie2 is most abundant in the endothelial caveolae [71].

Genetics and transcriptional regulation of components of the Ang/Tie system

The Ang-1 and Ang-2 genes are located on chromosome 8. Functional polymorphisms have not been identified in the Ang-1 gene, but three have been identified in the coding region of Ang-2 [72]. In ECs under stress, Ang-2 mRNA expression is induced by VEGF, fibroblast growth factor 2 and hypoxia [44,73]. Upregulation of Ang-2 induced by VEGF and hypoxia can be abolished by inhibiting tyrosine kinase or mitogen-activated protein kinase [73]. Ang-2 mRNA expression can be downregulated by Ang-1, Ang-2, or transforming growth factor [74]. After inhibition of PI3K by wortmannin, Ang-2 mRNA production is induced by the transcription factor FOXO1 (forkhead box O1) [75]. EC-specific Ang-2 promoter activity is regulated by Ets-1 and the Ets family member Elf-1 [76,77]. Because Tie2 signalling is required under circumstances that usually hamper cell metabolism, its promoter contains repeats that ensure transcription under difficult circumstances, including hypoxia [78].

The Tie2 downstream signalling pathway

Tie2 is present in phosphorylated form in quiescent and activated ECs throughout the body [62]. Signalling is initiated by autophosphorylation of Tie2 after Ang-1 binding and is conducted by several distinct pathways [54,71,79,80]. Tie2 can also be activated at cell-cell contacts when Ang-1 induces Tie2/Tie2 homotypic intercellular bridges [65]. In human umbilical vein endothelial cells (HUVECs), Ang/Tie signalling resulted in 86 upregulated genes and 49 downregulated genes [81,82]. Akt phosphorylation by PI3K with interaction of nitric oxide is the most important intracellular pathway [51,83-86]; however, ERK1/2, p38MAPK, and SAPK/JNK can also participate in Ang/Tie downstream signalling [71,81,84,87-90]. Endothelial barrier control by Ang-1 requires p190RhoGAP, a GTPase regulator that can modify the cytoskeleton [80]. The transcription factors FOXO1, activator protein-1, and NF-κ B are involved in Ang/Tie-regulated gene transcription [75,91-93]. Ang-1-induced signalling is has also been implicated in cell migration induced by reactive oxygen species [94]. ABIN-2 (A20-binding inhibitor of NF-κB 2), an inhibitor of NF-κB, is involved in Ang-1-regulated inhibition of endothelial apoptosis and inflammation in HUVECs [93]. However, the downstream signalling of Tie2 varies depending on cell type and localization and whether a cell-cell or cell-matrix interaction in involved, which results in spatiotemporally different patterns of gene expression. For example, Ang-1/Tie2 signalling leads to Akt activation within the context of cell-cell interaction, but it leads to ERK activation in the context of cell-matrix interaction. The microenvironment of the receptor in the cell membrane plays a central role in this signal differentiation. Adaptor molecules such as DOK and SHP2 and the availability of substrate determine which protein is phosphorylated [95].

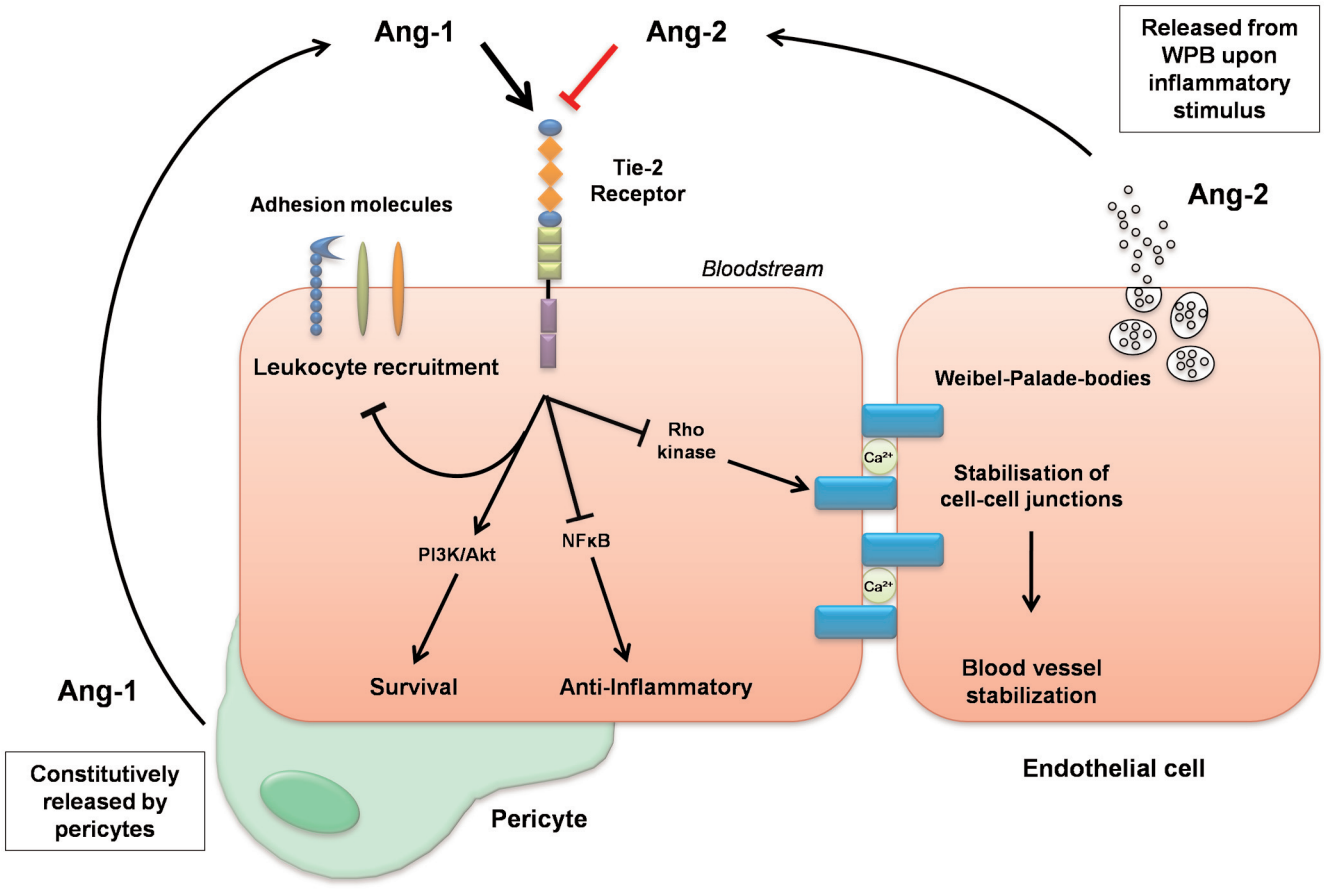
Signal regulation

After binding of Ang-1, and to a lesser extent Ang-2, Tie2 is internalized and degraded, and Ang-1 is shed in a reusable form [50]. VEGF is an important co-factor that can exert different effects on Ang-1 and Ang-2 signalling [88]. Ang-2 is anti-apoptotic in the presence of VEGF but induces EC apoptosis in its absence [96]. Autophosphorylation and subsequent signalling are inhibited by heteropolymerization of Tie1 and Tie2 [59]. Although the Ang/Tie system appears to play its role mainly in paracrine and autocrine processes, its circulating components have been found in plasma. The significance of this finding in health and disease has yet to be determined.

Summary

The Ang/Tie system is an integrated, highly complex system of checks and balances (Figure 1) [45,54]. The response of

Figure 1



A schematic model of the angiopoietin-Tie2 ligand-receptor system. Quiescent endothelial cells are attached to pericytes that constitutively produce Ang-1. As a vascular maintenance factor, Ang-1 reacts with the endothelial tyrosine kinase receptor Tie2. Ligand binding to the extracellular domain of Tie2 results in receptor dimerization, autophosphorylation, docking of adaptors and coupling to intracellular signalling pathways. Signal transduction by Tie2 activates the PI3K/Akt cell survival signalling pathway, thereby leading to vascular stabilization. Tie2 activation also inhibits the NF-κB-dependent expression of inflammatory genes, such as those encoding luminal adhesion molecules (for example, intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin). Ang-2 is stored and rapidly released from WPBs in an autocrine and paracrine fashion upon stimulation by various inflammatory agents. Ang-2 acts as an antagonist of Ang-1, stops Tie2 signalling, and sensitizes endothelium to inflammatory mediators (for example, tumour necrosis factor-α) or facilitates vascular endothelial growth factor-induced angiogenesis. Ang-2-mediated disruption of protective Ang-1/Tie2 signalling causes disassembly of cell-cell junctions via the Rho kinase pathway. In inflammation, this process causes capillary leakage and facilitates transmigration of leucocytes. In angiogenesis, loss of cell-cell contacts is a prerequisite for endothelial cell migration and new vessel formation. Ang, angiopoietin; NF-κB, nuclear factor-κB; PI3K, phosphoinositide-3 kinase; WPB, Weibel-Palade body.

ECs to Ang-1 and Ang-2 depends on the location of the cells and the biological and biomechanical context [97,98]. It is believed that PI3K/Akt is among the most important downstream signalling pathways and that VEGF is one of the most important modulators of effects. Below we describe in more detail how this system responds to changes in homeostatic balances under various conditions of damage and repair.

Ang/Tie signalling system in health and disease

Angiogenesis, inflammation and homeostasis are highly related, and the Ang/Tie system lies at the intersection of all three processes [99,100]. The Ang/Tie system is critically important for angiogenesis during embryogenesis, but in healthy adults its function shifts toward maintenance of homeostasis and reaction to insults. Except for follicle formation, menstruation and pregnancy, angiogenesis in adults is disease related. Neoplasia-associated neoangiogenesis and neovascularization in diabetes and rheumatoid arthritis are unfavourable events, and improper angiogenesis is the subject of research in ischaemic disorders and atherosclerosis. Finally, failure to maintain homeostasis and an inappropriate reaction to injury are detrimental features in critical illness.

Angiogenesis

Angiogenesis is dependent on multiple growth factors and receptors and their signalling systems and transcriptional regulators [101]. The process is complex and encompasses the recruitment of mobile ECs and endothelial progenitor cells, the proliferation and apoptosis of these cells, and reorganization of the surroundings [102]. To form stable new blood vessels, the response must be coordinated in time and space, and the Ang/Tie system is involved from beginning to end. To prepare for angiogenesis, Ang-2 destabilizes quiescent endothelium through an internal autocrine loop mechanism [44,103]. Before vascular sprouting starts, focal adhesion kinase and proteinases such as plasmin and metalloproteinases are excreted [85]. Often, this stage is preceded by activation of innate immunity and inflammation [104]. Apparently, the machinery to clean up after the work has been finished is installed before the work is commenced, again illustrating the close relations among the different processes [104].

Ang-1 maintains and, when required, restores the higher order architecture of growing blood vessels [43,44,105,106]. This is achieved by inhibiting apoptosis of ECs by Tie2-mediated activation of PI3K/Akt signalling [107-109]. Ang-1/Tie2 signalling is involved in angiogenesis induced by cyclic strain and hypoxia [110,111]. Although its role is less clear, Tie1 might be involved in EC reactions to shear stress [112]. Ang-1 is a chemoattractant for ECs [83-85], and both Ang-1 and Ang-2 have proliferative effects on those cells [98,113]. At the end of a vascular remodelling phase, Ang-2 induces apoptosis of ECs for vessel regression in competition with the survival signal of Ang-1 [106]. This apoptotic process requires macrophages, which are recruited by Ang-2 [70,114].

ECs require support from surrounding cells such as pericytes, podocytes, and smooth muscle cells [63]. These cells actively control vascular behaviour by producing signalling compounds (for instance, Ang-1 and VEGF) that govern the activity and response of ECs [61]. To attract ECs, Ang-1 secreted by support cells binds to the extracellular matrix. In quiescent ECs, this binding results in Tie2 movement to the site of cell-cell interaction. In mobile ECs, Ang-1 polarizes the cell with Tie2 movement abluminal site [65]. In tumour angiogenesis and in inflammation, Ang-2 recruits Tie2-positive monocytes and causes them to release cytokines and adopt a pro-angiogenic phenotype [111].

Homeostasis

The Ang/Tie system provides vascular wall stability by inducing EC survival and vascular integrity. However, this stability can be disrupted by Ang-2 injection, which in healthy mice causes oedema [28,79,115,116] that can be blocked by systemic administration of soluble Tie2 [115]. Ang-2 can impair homeostatic capacity by disrupting cell-cell adhesion through E-cadherin discharge and EC contraction [28,117]. In contrast, through effects on intracellular signalling, the cytoskeleton and junction-related molecules, Ang-1 reduces leakage from inflamed venules by restricting the number and size of gaps that form at endothelial cell junctions [80,118,119]. Ang-1 also suppresses expression of tissue factor induced by VEGF and tumour necrosis factor (TNF)-α, as well as expression of vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and E-selectin. As a result, endothelial inflammation is suppressed [120-123].

In primary human glomerular ECs *in vitro*, Ang-1 stabilizes the endothelium by inhibiting angiogenesis, and VEGF increases water permeability [124]. Similar observations were made in bovine lung ECs and immortalized HUVECs, in which Ang-1 decreased permeability, adherence of polymorphonuclear leucocytes and interleukin-8 production [123].

Injury

Reaction to injury can be seen as an attempt to maintain homeostasis under exceptional conditions. ECs can be affected by several noxious mechanisms. The Ang/Tie system is considered crucial in fine-tuning their reaction to injury and in containing that reaction. Ang-2-deficient mice cannot mount an inflammatory response to peritonitis induced chemically or with *Staphylococcus aureus* [125], but they can mount a response to pneumonia, suggesting the existence of inflammatory reactions for which Ang-2 is not mandatory. Ang-2 sensitizes ECs to activation by inflammatory cytokines. In Ang-2-deficient mice, leucocytes do roll on activated endothelium but they are not firmly attached, owing to the lack of Ang-2-dependent upregulation of adhesion molecules and the dominance of Ang-1-regulated suppression of adhesion molecules [120-123,125].

In bovine retinal pericytes, hypoxia and VEGF induce Ang-1 and Tie2 gene expression acutely without altering Ang-2 mRNA levels. The opposite occurs in bovine aortic ECs and microvascular ECs, underscoring the heterogeneity of ECs from different microvascular beds [73,126,127].

Lipopolysaccharide (LPS) and pro-inflammatory cytokines can shift the Ang/Tie balance, rouse ECs from quiescence and provoke an inflammatory response. In rodents LPS injection induces expression of Ang-2 mRNA and protein and reduces the levels of Ang-1, Tie2 and Tie2 phosphorylation in lung, liver and diaphragm within 24 hours, which may promote or maintain vascular leakage. The initial increase in permeability is probably due to release of Ang-2 stored in WPBs [39,128]. In a mouse model of LPS-induced lung injury, pulmonary oedema was found to be related to the balance between VEGF, Ang-1 and Ang-4 [129]. In a comparable model, Ang-1-producing transfected cells reduced alveolar inflammation and leakage [130].

In choroidal ECs, TNF induces Ang-2 mRNA and protein before affecting Ang-1 and VEGF levels [131]. In HUVECs, TNF-induced upregulation of Ang-2 is mediated by the NF-κB

pathway [132], and TNF-induced Tie2 expression can be attenuated by both Ang-1 and Ang-2. Without TNF stimulation, only Ang-1 can reduce Tie2 expression [133]. Ang-2 sensitizes ECs to TNF, resulting in enhanced expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin [74,125,134]. By inhibiting those endothelial adhesion molecules, Ang-1 decreases leucocyte adhesion [122].

Angiopoietins can mediate the synthesis of platelet-activating factor by ECs to stimulate inflammation [90]. Moreover, both Ang-1 and Ang-2 can translocate P-selectin from WPBs to the surface of the EC [135], and both can also increase neutrophil adhesion and chemotaxis and enhance those processes when they are induced by interleukin-8 [86,136,137].

In a rat model of haemorrhagic shock, Ang-1 reduced vascular leakage, and it inhibited microvascular endothelial cell apoptosis *in vitro* and *in vivo* [107,138]. In this model, Ang-1-promoted cell survival was partly controlled through integrin adhesion [139]. It has been suggested that EC apoptosis in haemorrhagic shock contributes to endothelial hyperpermeability [140-142]. Apoptosis is one of the reactions to MODS-related injury as demonstrated in hypoxia/reperfusion [143].

Cell adhesion

Ang-1 and Ang-2 are involved in cell-cell and cell-matrix binding [139,144-146]. Endothelial permeability is greatly dependent on cell-cell adhesion. The major adherens junction is largely composed of vascular-endothelial cadherin. This complex can be disrupted by VEGF, leading to increased vascular permeability [147,148], which can be antagonized by Ang-1 [149,150]. ECs can also bind to the matrix through the binding of Ang-1 to integrins, which can mediate some of the effects of Ang-1 without Tie2 phosphorylation [146,151]. At low Ang-1 concentrations, integrin and Tie2 can cooperate to stabilize ECs [151]. Ang-2 might play a role in inflammatory diseases such as vasculitis by disrupting the cell-cell junction and inducing denudation of the basal membrane [152]. Ang-1 can mediate the translocation of Tie2 to endothelial cell-cell contacts and induce Tie2-Tie2 bridges with signal pathway activation, leading to diminished paracellular permeability [65].

Summary

In the mature vessel, Ang-1 acts as a paracrine signal to maintain a quiescent *status quo*, whereas Ang-2 induces or facilitates an autocrine EC response [74,153]. In general, Ang-1 can be viewed as a stabilizing messenger, causing continuous Tie2 phosphorylation, and Ang-2 as a destabilizing messenger preparing for action [17]. Attempts to unravel the exact molecular mechanisms that control the system are complicated by microenvironment-dependent endothelial phenotypes and reactivity and by flow type-dependent reactions to dynamic changes [13,154,155]. Hence, the EC must be viewed in the context of its surroundings - the pericyte at the abluminal site, and the blood and its constituents on the luminal site [64]. The Ang/Tie system certainly functions as one of the junctions in signal transduction and plays a key role in multiple cellular processes, many of which have been linked to MODS.

Targeting the Ang/Tie system in critical illness

A therapy should intervene in the right place and at the right time, with the proper duration of action and without collateral damage [156,157]. The Ang/Tie system is involved in many processes and lies at the intersection of molecular mechanisms of disease. Thus, interventions targeting this system might have benefits. As in other pleiotropic systems, however, unexpected and unwanted side effects are a serious risk. The absence of redundant systems to take over the function of Ang/Tie2 has the advantage that the effect of therapeutic intervention cannot easily be bypassed by the cell. On the other hand, because the cell has no escape, the effect may become uncontrolled and irreversible. Moreover, the exact function of the Ang/Tie system in the pathological cascade is not fully established. What we see in animal models and in patients is most probably the systemic reflection of a local process. We do not know whether this systemic reflection is just a marker of organ injury or even a mediator of distant organ involvement.

Of the three main functions of the Ang/Tie system, it is mainly angiogenesis that has been evaluated as a therapeutic target. So far, the focus of Ang/Tie modulation has been on inhibiting angiogenesis related to malignant and ophthalmological diseases and to complications of diabetes [158,159]. In peripheral arterial occlusive disease, stimulation of angiogenesis seems a logical strategy to attenuate the consequences of ongoing tissue ischaemia. In a rat model of hind limb ischaemia, combined delivery of Ang-1 and VEGF genes stimulated collateral vessel development to the greatest extent [160,161]. Thus far, therapy directed at VEGF has reached the clinic, but not therapy directed at Ang/Tie [162].

Targeting homeostasis and repair/inflammation in critically ill patients is an attractive option and has already led to the development of new drugs [45,158,163]. From current knowledge, one can speculate about the best options for therapy aimed at the Ang/Tie system. In critical illness, Ang-1 is considered to be the 'good guy' because it can create vascular stability and thus its activity should be supported. In contrast, Ang-2 appears to be a 'bad guy' that induces vascular leakage, so its activity should be inhibited [164].

Production of recombinant Ang-1 is technically challenging as Ang-1 is 'sticky' because of its high affinity for the extracellular matrix [165]. However, stable Ang-1 variants with improved receptor affinity have been engineered. A stable soluble Ang-1 variant has anti-permeability activity [165]. When injected intraperitoneally in mice, human

recombinant Ang-1 can prevent LPS-induced lung hyperpermeability [80]. In diabetic mice, a stable Ang-1 derivative attenuated proteinuria and delayed renal failure [166], and manipulating the Ang-1/Ang-2 ratio changed infarct size [167]. A more profound Ang-1 effect can be achieved by locally stimulating Ang-1 production. In experimental acute respiratory distress syndrome, transfected cells expressing Ang-1 reduced alveolar inflammation and leakage [130]. An adenovirus construct encoding Ang-1 protected mice from death in an LPS model, and Ang-1 gene therapy reduced acute lung injury in a rat model [21,168,169]. In hypertensive rats, a plasmid expressing a stable Ang-1 protein reduced blood pressure and end-organ damage [170]. If used in a disease with a limited duration, as critical illness should be, virus/plasmid-driven production of Ang-1 could easily be shut down when it is no longer needed.

Manipulating Ang-2 activity is also difficult. Ang-2 stored in WPBs is rapidly released and must be captured immediately to prevent autocrine/paracrine disruption of protective Ang-1/Tie signalling. Soluble Tie2 or Ang-2 inhibitors should be effective [26,171]. Neutralizing antibodies against Ang-2 might also be an option. Replenishment of Ang-2 stores could be abolished by small interfering RNA techniques or spiegelmer/aptamer approaches [25,172,173].

However, no bad guy is all bad, and no good guy is all good. For example, Ang-1 has been linked to the development of pulmonary hypertension [174]. Also, under certain circumstances Ang-2 can act as a Tie2 agonist and exert effects similar to those of Ang-1 - an unexplained finding that illustrates our limited understanding of the Ang/Tie system [75]. Complete blockade of Ang-2 might also hamper innate immunity and revascularization.

Finding the right balance and timing will be the major challenge when developing therapies to target the Ang/Tie system. In the meantime, we might have already used Ang/Tie-directed therapy with the most pleiotropic of all drugs - corticosteroids. In the airways, steroids suppressed Ang-2 and increased Ang-1 expression [26,171,175]. Interventions further downstream targeting specific adaptor molecules, signalling pathways, or transcription factors have yet to be explored.

Diagnostic and prognostic opportunities

In patients with malignant disease, the Ang/Tie system might serve as a tumour or response marker. In patients with multiple myeloma, normalization of the Ang-1/Ang-2 ratio reflects a response to treatment with anti-angiogenesis medication [176]. In patients with non-small-cell lung cancer, Ang-2 is increased in serum and indicates tumour progression [177]. After allogeneic stem cell transplantation in patients with high-risk myeloid malignancies, the serum Ang-2 concentration predicts disease-free survival [178], possibly reflecting a relation between cancer-driven angiogenesis and Ang-2 serum level.

In nonmalignant disease, the levels of Ang/Tie system components correlate with disease severity [28,29,34-37,39]. However, current data are insufficient to justify the use of serum soluble Tie2/Ang levels for diagnostic and prognostic purposes. In critical illness, assessment of the Ang/Tie system in patients with different severities of disease and with involvement of different organ systems might help to define our patient population and allow us to rethink our concepts of MODS. In this way, such work may lead to enhanced diagnosis and prognostication in the future [2].

Conclusions

Accumulating evidence from animal and human studies points to the involvement of the Ang/Tie system in vascular barrier dysfunction during critical illness. Many processes in injury and in repair act through this nonredundant system. Thus far, only preliminary studies in critically ill patients have been reported. Methods to manipulate this system are available but have not been tested in such patients. The response to treatment is difficult to predict because of the pleiotropic functions of the Ang/Tie system, because the balance among its components appears to be more important than the absolute levels, and because the sensitivity of the endothelium to disease-related stimuli varies, depending on the environment and the organ involved. To avoid disappointment, further experimental and translational research must be carried out, and Ang/Tie modulation must not be introduced into the clinic prematurely. Implementing the results of this research in critical care represents an opportunity to show what we have learned [2]. Ang/Tie signalling is a very promising target and must not be allowed to become lost in translation [179].

Competing interests

The author(s) declare that they have no competing interests.

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