Microvascular thrombosis and multiple organ dysfunction syndrome

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This paper will review the involvement of disseminated intravascular coagulation-induced microvascular thrombosis in the pathogenesis of multiple organ dysfunction syndrome and the interaction between disseminated intravascular coagulation and systemic inflammatory response syndrome in critically ill patients. The published literature on clinical and experimental studies are the data sources of the study. Histologic evidence of microvascular thrombosis and tissue injury in disseminated intravascular coagulation has been reported in clinical, experimental, and autopsy findings. Prolinflammatory cytokine-evoked neutrophil–endothelial activation and interplay between inflammation and coagulation through protease-activated receptors contribute to enhanced microvascular fibrin deposition in organs. In a clinical setting, systemic inflammatory response syndrome and disseminated intravascular coagulation synergistically play pivotal roles in the development of multiple organ dysfunction syndrome and in the poor prognosis of critical illness. Disseminated intravascular coagulation contributes to microvascular thrombosis and subsequent multiple organ dysfunction syndrome. Recent knowledge on the relationship between disseminated intravascular coagulation and systemic inflammatory response syndrome gives further insight into the pathogenic mechanisms of multiple organ dysfunction syndrome in critically ill patients. (Crit Care Med 2010; 38[Suppl.]:S35–S42)

Key Words: thrombosis; disseminated intravascular coagulation; multiple organ dysfunction syndrome; systemic inflammatory response syndrome; coagulation; inflammation; cytokine; neutrophil; endothelium; protease-activated receptor

A variety of disorders in critically ill patients lead to disseminated microvascular thrombosis. The lesions, which are composed of fibrin and/or platelets, may occlude arterioles and capillaries, and thus give rise to multiple organ dysfunction syndrome (MODS). The two major syndromes associated with microvascular thrombosis and MODS in critically ill patients are thrombotic microangiopathies (TMA) and disseminated intravascular coagulation (DIC) (1, 2). Although TMA and DIC share pathologic features, they are distinct entities (Fig. 1). In this review, I briefly discuss TMA, and then turn the attention to the pathogenesis and clinicopathological manifestations of DIC. For additional information, particularly as it relates to the link between inflammation and coagulation in DIC, the reader is referred to the review by Levi and van der Poll in this issue.

Thrombotic Microangiopathy

TMA are disorders characterized by the formation of microvascular platelet aggregates; in some cases, this is accompanied by fibrin formation (1). Leading causes of TMA include thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TMA) and disseminated intravascular coagulation (DIC) (1, 2). Although TMA and DIC share pathologic features, they are distinct entities (Fig. 1). In this review, I briefly discuss TMA, and then turn the attention to the pathogenesis and clinicopathological manifestations of DIC. For additional information, particularly as it relates to the link between inflammation and coagulation in DIC, the reader is referred to the review by Levi and van der Poll in this issue.

Disseminated Intravascular Coagulation

DIC is characterized by the widespread activation of tissue-factor dependent coagulation, insufficient control of coagulation by physiologic anticoagulation pathways, and plasminogen activator inhibitor-1–mediated attenuation of fibrinolysis (2). Collectively, these changes result in the widespread formation of fibrin clots, microvascular occlusion, and reduced oxygen delivery to cells and tissues. When severe and/or sustained, such changes may lead to organ dysfunction (7). DIC is most commonly precipitated by sepsis or trauma. In each condition, activation of coagulation begins as an adaptive host response, serving to prevent spread of microorganisms into the systemic circulation, limit exsanguination, and/or promote wound healing (8). DIC represents an exaggeration of this response.

Disseminated intravascular coagulation also leads to a consumptive coagulopathy, giving rise to hemorrhagic diathesis attributable to the consumption of platelets and coagulation proteins. Therefore, DIC has been recognized as a consumptive thrombohemorrhagic disorder
that can be manifested by a hemorrhagic or thrombotic pathology. The spectrum of DIC is also affected by the existence of fibrin/fibrinogenolysis. Based on these pathologic mechanisms, DIC can be subdivided into two types. DIC associated with sepsis and a late-stage of trauma is classified as DIC with a thrombotic phenotype as manifested by microvascular thrombosis and organ dysfunction (MODS). DIC with fibrinolytic (hemorrhagic) phenotype is associated with excessive hemorrhage. This type is often seen in hematologic malignancies (9), and it is associated with tissue-type plasminogen activator-induced fibrino/fibrinogenolysis and consumptive coagulopathy (10). Another fibrinolytic phenotype of DIC is a coagulation disorder associated with early trauma. In this setting, the presence of excessive fibrino/fibrinogenolysis, hypothermia, acidosis, and dilutional coagulopathy may lead to an accentuation of blood loss. These hemorrhagic phenotypes are not discussed further in the current review.

**Microvascular Thrombosis**

Histologic evidence of microvascular thrombosis and tissue injury in DIC patients has been reported in clinical, experimental, and autopsy findings. The extent of necrotic tissue injury was shown to be dependent on the quantity and duration of thrombosis, and a clear correlation was also demonstrated between thrombi and ischemic tissue damage (11, 12). Shimamura et al (13) studied the distribution pattern of microthrombi in 37 autopsy cases of DIC. They reported that the incidence of microthrombi differed between organs: lung (100%), liver (94.6%), kidney (75.5%), heart (56.8%), pancreas (48.7%), adrenal gland (32.4%), and gastrointestinal tracts (18.9%). The thrombotic involvement of the brain and skin were also confirmed in DIC patients (14, 15). Dermal ischemia and hemorrhagic necrosis in the skin and large vessel obstruction, in addition to multiple cortical and brain stem infarctions, were observed in patients with DIC. In addition, the time course of thrombus formation in the microvasculature of various organs was studied in an animal model of DIC, in which rabbits received two intravenous injections (24 hrs apart) of lipopolysaccharide (LPS) derived from Escherichia coli (200 μg/kg) (16). Microthrombi first appeared in the liver, lung, and spleen (2 hrs after the second injection) and only subsequently appeared in other organs. The lesions disappeared rapidly from the hepatic sinusoids while persisting in other organs, such as the kidney and lung. Together, these studies suggest a pathogenic role of microvascular thrombosis in MODS observed in DIC.

Nevertheless, the acceptance of this concept does not mean that only the reduction of oxygen delivery to the cells and tissues associated with microvascular thrombosis is a lethal chain of events. Taylor et al (17) demonstrated that a complete inhibition of thrombosis with an active site inhibited factor Xa did not protect the baboons with E. coli-induced sepsis against organ failure and death. However, the inhibition of microvascular coagulation with active site inhibited factor VIIa with anti-tissue factor antibody, or with the early treatment of tissue factor pathway inhibitor, protected approximately 70% of these animals against organ failure and death (18–20). These findings led us to suspect that other factors (i.e., inflammation) as well as microthrombosis therefore may contribute to the poor prognosis observed in the patients associated with DIC.

**Cytokines**

DIC is associated with concomitant activation of coagulation and inflammatory cascades (2). The major links between inflammatory cytokines and microvascular thrombosis involve the activation of coagulation, inhibition of anticoagulation pathways, and depression of fibrinolysis. However, the nature of these links is complicated and differs depending on the experimental design and animal species used (21, 22). Therefore, conclusions drawn from one species are not easily extrapolated to others, or to the clinical situation. Current evidence suggests that proinflammatory cytokines (e.g., tumor necrosis factor-alpha [TNF-α], interleukin-1β [IL-1β], and IL-6) are the most important inflammatory mediators that regulate the process of microvascular thrombosis (2, 21, 22). The interactions of proinflammatory cytokines with regulators of thrombosis, such as platelets, leukocytes, and endothelium, are shown in Figure 2.

**Neutrophils and Endothelium**

Emerging evidence suggests that leukocyte–endothelial cell interactions have pivotal roles in the endothelial damage resulting from inflammatory responses (23). Adhesion molecules, such as the selectins, the integrins, and the immunoglobulin superfamily, mediate the interaction between neutrophils and the endothelium, and between platelets and the endothelium. TNF, IL-1, and thrombin can initiate the synthesis and expression of E-selectin or lead to rapid expression of P-selectin on the endothelial cells, thus leading to adherence of neutrophils and activation of the endothelium (24, 25). After adherence, neutrophils secrete several enzymes such as myeloperoxidase, cathepsin G, and elastase from azurophilic granules, and produce reactive oxygen species, which cause endothelial activation or injury (23, 26). Frank denudation of the endothelium leads to the exposure of blood to procoagulant subendothelial elements, such as collagen, von Willebrand factor (VWF), and fibronectin (27). Activation of endothelial cells results in a thrombogenic phenotype. For example, expression of thrombomodulin and endothelial protein C receptor is downregulated, whereas expression and/or secretion of plasminogen activator inhibitor-1 and VWF are induced. In addition, P-selectin accelerates thrombosis by platelet–leukocyte activation and their interaction and expression of tissue factor on monocytes (28). These processes, inflammation, and neutrophil–endothelial activation, followed by endothelial injury, are key elements in the microvascular thrombosis, endothelial permeability, and MODS occurring during DIC associated with sepsis (29, 30).
Protease-Activated Receptors

Protease-activated receptors (PAR) play important roles in the interplay between inflammation and coagulation (31, 32). To date, four distinct PAR—PAR-1, PAR-2, PAR-3, and PAR-4—have been described. Thrombin (factor IIa) activates PAR-1, PAR-3, and PAR-4, whereas tissue factor/factor VIIa complex and tissue factor/factor VIIa/factor Xa ternary complex are selective ligands for PAR-2. In addition, tissue factor/factor VIIa/factor Xa ternary complex activates PAR-1 (33, 34). There is conflicting evidence regarding the involvement of PAR in both inflammation and microvascular thrombosis in endotoxemia and sepsis (35, 36). Using genetically modified mice, Camerer et al (37) found that no single or combined PAR deficiency protected the mice from inflammatory or lethal responses to endotoxin, thus arguing against PAR playing an important role in inflammation and microthrombosis. In contrast, Pawlinski et al (36, 38) showed that combining hirudin treatment to inhibit thrombin signaling through PAR-1 and PAR-4 with PAR-2 deficiency confers a survival advantage in a mouse model of endotoxemia, an effect that was associated with reduced circulating levels of IL-6. However, a deficiency in either PAR-1 or PAR-2 alone did not affect either the IL-6 expression or mortality (36, 38). A cell-penetrating pepducin antagonist of PAR-4 dose-dependently diminished inflammation and preserved multiple organ function in a neutrophil-mediated manner in a mouse model of systemic inflammation and DIC (39). Furthermore, PAR-2 blocking peptide ameliorates liver injury with suppression of TNF elevation and normalization of coagulation and fibrinolysis (40). Taken together, these studies suggest that PAR-1, PAR-2, and PAR-4 may play an important role in LPS-induced organ dysfunction through the activation of both inflammation and coagulation. The relationship between PAR, inflammation, and DIC are summarized in Figure 3. Table 1 shows the role of PAR in the cross-talk between coagulation and inflammation. Coagulation factors activate PAR, followed by inflammation, platelet and coagulation activation, a dysfunction of anticoagulation pathway, and the suppression of fibrinolysis.

High-Mobility Group Box 1 Protein and ADMS13

High-mobility group box 1 protein, an abundant intranuclear protein, was recently identified as a potent lethal mediator of endotoxemia (41). High-mobility group box 1 protein inhibits the anticoagulant protein C pathway mediated by the thrombin–thrombomodulin complex.

Figure 2. Effects of proinflammatory cytokines on the regulator of thrombosis such as platelets, neutrophils, monocytes/macrophages, and endothelium. TNF, tumor necrosis factor-α; IL-1, interleukin-1; IL-6, interleukin-6; ICAM-1, intracellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; PAF, platelet-activating factor; NO, nitric oxide; EPCR, endothelial protein C receptor; t-PA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor-1; 5-HT, 5-hydroxytryptamine; VWF, von Willebrand factor; TFPI, tissue factor pathway inhibitor; MMP, matrix metalloprotease; DIC, disseminated intravascular coagulation; MODS, multiple organ dysfunction syndrome; SLeX, sialyl Lewis (X).
and stimulates tissue factor expression in vitro, thus resulting in fibrin deposition in glomeruli associated with increased mortality in a rat model of sepsis (42). The results suggest that high-mobility group box 1 protein may be among the molecules that promote the development of microvascular thrombosis in DIC.

ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 motif, number 13) plays an important role in VWF processing by cleaving the unusually large multimers of VWF originally secreted by endothelial cells and those found contained within platelets (43). A deficiency of ADAMTS13 allows usually large VWF multimers to persist in circulation, resulting in intravascular VWF–platelet aggregation and widespread microvascular thrombosis (44). Recent clinical and experimental studies demonstrated that ADAMTS13 activity declined stepwise according to the extent of inflammation, followed by the appearance of tissue factor expression in vitro, thus resulting in fibrin deposition in glomeruli associated with increased mortality in a rat model of sepsis (42). The results suggest that high-mobility group box 1 protein may be among the molecules that promote the development of microvascular thrombosis in DIC.

Table 1. PAR and their actions on platelets, leukocytes, and endothelium

<table>
<thead>
<tr>
<th>Induction of Expression, Release, etc</th>
<th>Platelets</th>
<th>Leukocytes</th>
<th>Endothelial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT, ADP, TXA2, P-selectin GPⅠb/Ⅲa</td>
<td>5-Hydroxytryptamine</td>
<td>TNF-α, IL-6, IL-8, GD11b Tissue factor</td>
<td>VWF, PAF IL-1, IL-6, IL-1 P-selectin, E-selectin, ICAM-1, VCAM-1, MCP-1 MMP-7, MMP-9, apoptosis Tissue factor, PAI-1, TM down-regulation</td>
</tr>
<tr>
<td>5-HT, ADP, TXA2, P-selectin GPⅠb/Ⅲa</td>
<td>Platelet activation, adhesion, aggregation</td>
<td>Proinflammatory</td>
<td>Platelet activation and consumption Proinflammatory</td>
</tr>
<tr>
<td>5-HT, ADP, TXA2, P-selectin GPⅠb/Ⅲa</td>
<td></td>
<td>Activation of coagulation</td>
<td>Endothelial cells activation</td>
</tr>
<tr>
<td>5-HT, ADP, TXA2, P-selectin GPⅠb/Ⅲa</td>
<td></td>
<td>Endothelial cells injury</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>5-HT, ADP, TXA2, P-selectin GPⅠb/Ⅲa</td>
<td></td>
<td></td>
<td>Activation of coagulation, suppression of fibrinolysis, impairment of anticoagulation</td>
</tr>
<tr>
<td>5-HT, ADP, TXA2, P-selectin GPⅠb/Ⅲa</td>
<td></td>
<td></td>
<td>Vessels dilation, increased permeability</td>
</tr>
<tr>
<td>5-HT, ADP, TXA2, P-selectin GPⅠb/Ⅲa</td>
<td></td>
<td></td>
<td>Vessel constriction</td>
</tr>
</tbody>
</table>

PAR, protease-activated receptors; 5-HT, 5-hydroxytryptamine; ADP, adenosine diphosphate; TXA2, thromboxane A2; GP, glycoprotein; TNF, tumor necrosis factor; IL, interleukin; VWF, von Willebrand factor; PAF, platelet-activating factor; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; MCP-1, monocyte chemotactic protein-1; MMP, metalloprotease; PAI-1, plasminogen activator inhibitor-1; TM, thrombomodulin; EDHF, endothelial hyperpolarizing factor.
ance of usually large multimer VWF in plasma, with increases in DIC scores, low platelet counts, endothelial dysregulation, organ dysfunction, and lethality in sepsis-induced DIC patients (45, 46, 47). Additionally, a recent study in children with sepsis-associated multiple organ dysfunction and reduced plasma ADAMTS13 activity demonstrated improved organ function and survival after plasma exchange and restoration of ADAMTS13 activity (48). These studies suggest that ADAMTS13 thus plays a pivotal role in the microvascular thrombosis and organ dysfunction associated with DIC.

**Microvascular Thrombosis and Organ Dysfunction**

**Brain.** Sepsis associated with brain dysfunction, termed *sepsis-associated encephalopathy*, varies from a transient, reversible encephalopathy to irreversible damage (49). The pathogenesis of sepsis-associated encephalopathy is likely to be multifactorial, involving microvascular disorders, endothelial activation, breakdown of the blood–brain barrier, brain inflammation, neuronal degeneration, and apoptosis (49). DIC has been observed in a septic patient with encephalopathy (14, 50). Furthermore, a recent study using magnetic resonance imaging demonstrated abnormalities in the brain in accordance with the infarct areas associated with the occlusion of small vessels with acute inflammation and fibrinoid necrosis in patients with septic encephalopathy (51). In a postmortem study of 23 patients who died of septic shock, 14 (61%) had DIC, three of whom had cerebral hemorrhage and one had multiple fibrinous microthrombi and diffuse small microinfarcts in the brain (52). In an experimental model of sepsis, LPS induced tissue factor protein and messenger RNA expression in astrocytes of the rabbit brain (53). In addition to tissue factor, expression of TNF, a key mediator of sepsis-associated encephalopathy, and fibrin were observed in the frontocortical tissues of the brain (54). These changes are associated with the expression of PAR-1 to PAR-4 protein and messenger RNA. These results indicate that intravascular fibrin thrombosis attributable to systemic DIC and extravascular inflammation and coagulation abnormalities in the brain thus may play a role in sepsis-associated encephalopathy.

**Lung.** Intravascular and intra-alveolar fibrin deposition is common in acute lung injury/acute respiratory distress syndrome (55). There is ample evidence that coagulation and inflammation play pathogenic roles in acute lung injury/acute respiratory distress syndrome. Fibrin deposition in the alveolar space and lung vasculature likely result from activation of coagulation, insufficient anticoagulation, and impaired fibrinolysis, triggered by inflammation, which resembles the aforementioned pathophysiology of DIC (55). Pulmonary microvascular thrombosis was described in septic patients three decade ago (56). Malik et al (57–60) clearly demonstrated that intravascular thrombin, fibrin, and neutrophils interacted synergistically to increase lung endothelial permeability to protein. It is now accepted that DIC-associated microvascular thrombosis, together with neutrophil–endothelial activation, and secondary endothelial injury contribute to the initiation, course, and prognosis of acute lung injury/acute respiratory distress syndrome (61). Furthermore, the interaction between inflammation, coagulation, and fibrinolysis through PAR is implicated in LPS-induced acute lung injury (62).

The liver can be injured and its function altered by the activation of coagulation and inflammatory processes in sepsis (63). In patients with DIC associated with sepsis, hepatocellular necrosis and formation of fibrin thrombi in the sinusoids around the necrotic area are frequently demonstrated at autopsy (12, 13, 16). Using experimental models, Shibayama (64) confirmed that LPS administration induces fibrin deposition and infiltration of neutrophils in the hepatic sinusoid, leading to coagulative hepatocellular necrosis and liver injury. These findings implicate a pathogenic role for sinusoidal thrombosis and microvascular occlusion in LPS-induced liver injury. LPS stimulates Kupffer cells to secrete TNF and IL-1, which contribute to the induction of plasminogen activator inhibitor-1 in hepatocytes and endothelial cells, and neutrophil-dependent liver injury (65, 66, 67). In hepatectomized rats, LPS was shown to induce tissue factor activity in Kupffer cells, an effect that was associated with microvascular fibrin deposition, followed by severe injury to hepatocytes and endothelial cells (68). The messenger RNA expression of tissue factor pathway inhibitor was almost undetectable and the unaltered levels of tissue factor pathway inhibitor protein were observed after LPS administration, thus indicating that the tissue factor pathway inhibitor-related anticoagulation pathway does not match the increased levels of tissue factor expression (40, 69). Antithrombin treatment attenuates LPS-mediated hepatic necrosis caused by sinusoidal fibrin thrombosis (69). Together, these data suggest that LPS-induced liver injury is mediated by a combination of inflammation, the activation of coagulation, and impaired fibrinolysis, whereas the physiologic anticoagulation pathways may also play a role in this injury through sinusoidal microcirculatory disturbances attributable to microvascular thrombosis.

**Kidney.** DIC has been associated with glomerular microthrombosis and acute renal failure (13, 70). In the presence of inflammation, monocytes/macrophages are induced to migrate into and become activated in glomeruli, where they produce tissue factor or procoagulant activity leading to fibrin deposition (71, 72). Mesangial cells also participate in glomerular fibrin deposition. Under *in vitro* conditions, incubation of mesangial cells with LPS and TNF results in increased tissue factor production, suggesting that these cells may contribute to fibrin formation during inflammatory glomerular injury (73). Welty-Wolf et al (74) showed that the tissue factor/factor VIIa complex, which is a selective ligand for PAR-2, contributes to renal failure in *E. coli* bacteremia through the release of proinflammatory cytokines, tissue factor expression, and fibrin deposition in glomeruli and small vessels. In a rat model of LPS-induced renal failure, time-dependent PAR-2 expression was observed in accordance with elevation of TNF, activation of coagulation, unchanged levels of tissue factor pathway inhibitor, and increases in plasminogen activator inhibitor-1 associated with glomerular fibrin deposition (75). Active site-inactivated factor VIIa and PAR-2 blocking peptide preserve or ameliorate renal function, inflammation, coagulation, and fibrinolysis (74, 75). Therefore, glomerular and microvascular thrombosis contribute to the occurrence of acute renal failure in sepsis.

**Systemic Inflammatory Response Syndrome, DIC, and MODS**

Both infectious (sepsis) and noninfectious insults (trauma) can produce systemic inflammatory response syndrome (SIRS), characterized by systemic proinflammatory cytokine release and generalized activation of leukocytes and endothelium, leading to MODS (76). Stepwise
increases in the prevalence of DIC are observed in the hierarchy from SIRS to sepsis, and DIC is a frequent complication of SIRS (77–80). Both results suggest that irrespective of infectious or noninfectious insults, the clinical combination of SIRS and DIC can synergistically give rise to MODS (Fig. 4). DIC associated with significant dysfunction of anticoagulant mechanisms is a strong predictor of MODS and mortality in septic shock (81). DIC in patients with sepsis and trauma has been reported to show higher SIRS criteria, and a higher prevalence of MODS in comparison to those without DIC (79, 80). Similar to these results, Dhainaut et al (82) pointed out that coagulopathy precedes MODS and that a continued abnormality in coagulation and fibrinolysis during the first day of severe sepsis increases the risk of new organ dysfunction and death. In the same manner, DIC scores, Sequential Organ Failure Assessment (SOFA) scores, and mortality increase in accordance with a stepwise increase in SIRS criteria in critically ill patients (83). All of these studies support a clinical bidirectional interaction between inflammation (SIRS) and coagulation (DIC) that plays a pivotal role in the development of MODS and poor prognosis in critically ill patients. Furthermore, these results suggest that DIC is not an epiphenomenon associated with serious illnesses at their terminal stage, but it is instead a primary cause of MODS, thus leading to death of the critically ill patients (Fig. 5).

CONCLUSION

There is ample evidence that DIC contributes to microvascular thrombosis and consequent MODS in critically ill patients. Recent knowledge on the bidirectional interplay of DIC (coagulation) and SIRS (inflammation) provides further valuable insight into the pathogenesis of MODS in critically ill patients.

REFERENCES


