Studies on coagulation-induced inflammation in mice
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Microvascular coagulopathy and disseminated intravascular coagulation

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Abstract:

Objective: To review the dual characteristics of disseminated intravascular coagulation (DIC), as both a contributor to multiple organ failure as well as a symptom of severe underlying disease associated with systemic vascular changes. Data Sources: Published literature data and unpublished results from the authors. Data Summary: Clinical and experimental studies strongly suggest that DIC contributes to multiple organ failure and death in patients with severe systemic disorders such as sepsis. DIC is evoked by systemic cytokine activity, and the inflammatory response aggravates vascular permeability, inflammation, and cell damage in tissues. In addition to intravascular fibrin formation, thrombin and fibrin generation in tissues is also an important aspect of DIC. An example of DIC at the organ level is adult respiratory distress syndrome, where fibrin in the lung is a characteristic feature. Intravascular fibrin formation and occlusion may elicit a hypoxic response with induction of hypoxia related transcription factors. The resulting ischemic preconditioning may offer protective effects to the involved organ(s).

Conclusions: Overall, the beneficial or harmful effects of activated coagulation and fibrin formation for organ pathology and recovery from DIC remain to be explored. This may be a critical element in the assessment of ischemia-reperfusion effects of specific anticoagulant therapy.

Introduction

Disseminated intravascular coagulation (DIC) is a systemic syndrome characterized by enhanced activation of coagulation with some intravascular fibrin formation and deposition, depending on the degree of activity.¹ DIC is thought to contribute to multi-organ failure and death in a variety of underlying conditions for several reasons. First, pathologic studies have repeatedly demonstrated the presence of intravascular fibrin in tissues of patients who had died from an illness associated with evidence of DIC, suggesting a causal relationship. Second, cohort studies have indicated an increased mortality in patients with DIC compared with those who have the same underlying disease but no evidence of DIC. And third, experimental studies of DIC associated with sepsis or low-grade activation of coagulation have repeatedly demonstrated that effective inhibition of DIC can indeed reduce mortality. In contrast, many investigators currently believe that it is not DIC, and particularly not fibrin formation itself that is harmful, but rather it is the generation of serine proteases and their potential interactions with pro-inflammatory mediators that contribute to organ failure and death.

The microvasculature is the critical interface for oxygen and energy delivery to the tissues. Thus, any damage to or obstruction of the microvasculature may have potentially harmful consequences. In diseases complicated by DIC, a systemic inflammatory response syndrome is a standard finding (Fig. 1). The generation of pro-inflammatory cytokines has several consequences for the microvasculature with relation to blood coagulation and DIC. Vascular endothelial cells may be
perturbed by the action of cytokines such as interleukins (IL)-1, -6, and -8, as well as tumor necrosis factor-α (TNF-α). These cytokines change the general anticoagulant phenotype of the endothelium into a procoagulant phenotype, at least under in vitro conditions, resulting in, among other features, reduced expression of thrombomodulin and heparan sulfates as well as potentially upregulated tissue factor (TF). Inducible TF is predominantly expressed by monocytes and macrophages. The expression of TF on monocytes is markedly stimulated by the presence of platelets and granulocytes in a P-selectin-dependent reaction. This effect may be the result of nuclear factor-κB activation induced by binding of activated platelets to neutrophils and mononuclear cells. This cellular interaction also markedly enhances the production of IL-1β, IL-8, monocyte chemoattractant protein-1 (MCP-1), and TNF-α.

Increased endothelial permeability facilitates the interaction of transmigrating leukocytes with the subendothelial space, such that extravascular inflammation and coagulation may occur. The generation of procoagulant pathways, as well as their interactions with platelets and leukocytes, in the microvasculature may lead to intravascular fibrin formation, which, in turn, may cause occlusion of the smaller vessels. We will now consider several aspects related to the generation of fibrin, the consequences that vascular occlusion might have, and the site of fibrin deposition in relation to inflammation.

**Generation of Fibrin and Its Deposition.**

From studies in human volunteers and in baboons challenged with lethal *Escherichia coli*, it is known that DIC can be distinguished in various stages, in relation to the degree of procoagulant derangement. Initially, thrombin generation and down-regulation of fibrinolysis occur, followed by intra-vascular fibrin formation and endothelial cell activation, as indicated by increased levels of fairly specific endothelial cell molecules. Subsequently, increased vascular permeability occurs, indicative of endothelial disruption and damage. In the latter stages, fibrin deposition occurs intravascularly as well as in extravascular spaces, for example, as seen in adult respiratory distress syndrome (ARDS). Because of depletion of clotting factors and platelets, bleeding may be seen in this stage of DIC.

The kinetics of fibrin deposition in organs was studied in a more systematic way by analyzing the lungs of mice with a mutation in the thrombomodulin gene that
were challenged with endotoxin. In these studies, thrombomodulin mutated mice with a mixed genetic background of Sv129 and C57Bl/6 were utilized, which might have contributed to part of the observed effects on fibrin formation and inflammation\textsuperscript{12} (R Franco, et al., unpublished observations). Nevertheless, intravascular fibrin formation occurred early after endotoxin challenge of mice (detectable after 30 mins), was associated with signs of inflammation, and disappeared, probably as a result of fibrinolysis, at 24 hrs. In plasma, evidence of DIC was provided by substantial elevations in the levels of thrombin-antithrombin complexes, which followed a time course that was approximately parallel to the pattern of fibrin deposition. This study illustrated that intravascular fibrin formation can be observed in a specific organ, and does not lead to overt organ damage except for transient evidence of inflammation. Furthermore, this process is reversible under certain conditions as a result of fibrinolytic clearance of the microvasculature.

**DIC and Vascular Occlusion**

Theoretically, when the trigger of DIC is stronger or more prolonged than anticoagulation, or when the anticoagulant or fibrinolytic mechanisms fail to protect, fibrin formation may persist and lead to prolonged vascular occlusion. The resulting hypoxia may contribute to organ ischemia and cell death (Fig. 2).

- Vascular occlusion by fibrin
- inflammatory response
- hypoxia \(\longrightarrow\) tissue ischemia \(\longrightarrow\) necrosis
- extravascular fibrin/inflammation

![Figure 2. Consequences of disseminated intravascular coagulation.](image)

Systemic hypoxia is known to cause fibrin formation as well. Several studies have indicated that, in the presence of either a defect in an anticoagulant pathway such as thrombomodulin\textsuperscript{13} (S Schoenmakers et al., unpublished observations), or a defect in the fibrinolytic system,\textsuperscript{14} hypoxia induced by keeping mice at 8% or less oxygen causes fibrin formation in the lungs. Although, at lower oxygen pressure (6%), fibrin may also accumulate in normal mice,\textsuperscript{15} these very low oxygen levels are not well tolerated in normal or other mice (data not shown). Furthermore, it is unknown to what extent fibrin formation occurs in tissues other than the lung. The proposed procoagulant mechanism is enhanced expression of TF by monocytes as a result of enhanced activity of transcription factors such as Egr-1\textsuperscript{16} but it may be possible that endothelial cell-induced TF also plays a role in this process. Nevertheless, it remains to be seen how the presence of fibrin influences the adjacent tissue and whether inflammation and clotting may facilitate local apoptosis and tissue damage. Local hypoxia also induces the expression of hypoxia-inducible transcription factors that, via ischemic preconditioning, may
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defend the organ against permanent damage. The relevance of these defense mechanisms in DIC remains to be investigated.

In addition to intravascular fibrin formation, fibrin may be generated in or transferred to extravascular areas, where it may, in turn, be deposited. For example, ARDS is frequently associated with intra-alveolar and intravascular fibrin formation, most likely a result of both systemic and local mediators of procoagulant reactions. Local production of TF, which is detectable in bronchoalveolar lavage fluid, may trigger the procoagulant reactions.

DIC and Inflammation

The inflammatory component involved in ARDS may be distinct from the pathway that leads to fibrin formation. In a rat model of E. coli induced pulmonary injury, a synthetic specific inhibitor of kallikrein prevented pulmonary vascular injury, but did not inhibit DIC. In contrast, the active site-blocked factor VIIa inhibited DIC but not pulmonary injury, suggesting that the inflammatory and coagulation reaction in the lungs to endotoxin are not intimately associated.

Several studies suggest a direct effect of fibrin on inflammatory activity: fibrinogen interacts with bacteria and modulates their activity, fibrin serves to encapsulate bacteria, or fibrin cleavage peptides may trigger the release of proinflammatory cytokines. Thus, in extravascular spaces such as the intraperitoneal cavity or pulmonary tissue, fibrin may be involved in the regulation of inflammatory activity and tissue damage. It remains unknown whether fibrin plays an important role in this regard, and it is entirely unknown whether fibrin has “good” or “bad” properties in localized inflammatory processes.

In an experimental ARDS model induced by installation of endotoxin endobronchially, a peribronchial inflammatory response occurs, with extravasation of leukocytes (mostly granulocytes). The granulocytes stain positive with specific antibodies against murine TF, possibly indicating that these cells may be involved in the local fibrin generating process. Indeed, local generation of thrombin is indicated by elevated thrombin-antithrombin complexes in bronchoalveolar lavage fluid (JJ Timmerman et al., unpublished observations) and fibrin appears to be localized at areas of inflammatory activity, suggesting a relationship between these elements.

Conclusion

Fibrin formation in the course of DIC may be an important determinant of organ morbidity. However, direct evidence to support this is essentially absent. All favorable studies suggesting a beneficial effect of anticoagulant treatment in humans or animals may be explained by the inhibition of intermediate proteases of the coagulation cascade that have proinflammatory activities. Whether preventing fibrin formation per se is helpful in limiting organ damage remains to be established. With the emergence of powerful anticoagulant strategies such as
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activated protein C.\(^\text{24}\) aspects involving ischemia-reperfusion damage and long-term organ recovery become important to investigate.

References:

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