New insights into pathways that determine the link between infection and thrombosis

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ABSTRACT

Severe infection is often linked to prothrombotic events. Indeed, haemostatic abnormalities are encountered in most cases of infection, ranging from an increase in sensitive markers for coagulation activation or insignificant laboratory changes to gross activation of coagulation that may result in localised thrombotic complications or disseminated intravascular coagulation. Systemic inflammation as a consequence of infection results in activation of coagulation, due to tissue factor-mediated thrombin generation, down-regulation of physiological anticoagulant mechanisms, and inhibition of fibrinolysis. Pro-inflammatory cytokines, immune cells and the endothelium form the interface on which differential effects on the coagulation and fibrinolysis pathways may ensue. Conversely, activation of the coagulation system may importantly affect inflammatory responses by direct and indirect mechanisms. Apart from the general coagulation response to inflammation associated with severe infection, specific infections may cause distinct features, such as haemorrhagic fever or thrombotic microangiopathy.

KEYWORDS

Infection, inflammation, thrombosis, coagulation, endothelium, cytokines

INTRODUCTION

Increasing evidence points to a tight interaction between coagulation on the one hand and inflammation as a response to severe infection or chronic inflammatory states on the other hand.¹² In recent years the various mechanisms that play an important role in this interaction have been elucidated and this knowledge has indeed been demonstrated to be applicable for the improvement of our understanding of the pathogenesis of severe infection or chronic inflammatory states and, even more importantly, the clinical management of these patients.⁴⁵ In this article the mechanisms that play a role in the interaction between infection, inflammation and coagulation will be reviewed. Specific features of infectious disease-mediated effects on the coagulation system will be highlighted and the relevance for clinically relevant thrombotic manifestations is discussed.

INFECTION AND INFLAMMATION RESULT IN ACTIVATION OF COAGULATION

Acute inflammation, as a response to severe infection or trauma, results in a systemic activation of the coagulation system.⁴⁵ It was initially thought that this systemic activation of coagulation was a result of direct activation of the contact system of coagulation by microorganisms or endotoxin. However, in the 1990s it became apparent that cytokines played a mediatory role in the activation of coagulation and subsequent fibrin deposition and that the point of impact on the coagulation system was rather the tissue factor-factor VIIa (‘extrinsic’) pathway than the contact system (‘intrinsic pathway’).⁷⁸ Furthermore, the significance of impaired physiological anticoagulant pathways became increasingly clear.⁹ Lastly, it was shown that impaired fibrin removal by a suppressed fibrinolytic system contributed importantly to the microvascular deposition of fibrin.

Vascular endothelial cells play a central role in all mechanisms that contribute to inflammation-induced activation of coagulation (figure 1). Endothelial cells respond
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Inflammation-induced coagulation activation is characterised by widespread intravascular fibrin deposition, which appears to be a result of enhanced fibrin formation and impaired fibrin degradation. Enhanced fibrin formation is caused by tissue factor-mediated thrombin generation and simultaneously occurring depression of inhibitory mechanisms, such as the protein C and S system. The impairment of endogenous thrombolysis is mainly due to high circulating levels of plasminogen activator inhibitor type 1 (PAI-1), the principal inhibitor of plasminogen activation. These derangements in coagulation and fibrinolysis are mediated by differential effects of various pro-inflammatory cytokines.

Tissue factor plays a central role in the initiation of inflammation-induced coagulation. Blocking tissue factor activity completely inhibits inflammation-induced thrombin generation in models of experimental endotoxaemia or bacteraemia. The vast majority of cells constitutively expressing tissue factor are found in tissues not in direct contact with blood, such as the adventitial layer of larger blood vessels. However, tissue factor comes into contact with blood when the integrity of the vessel wall is disrupted or when endothelial cells and/or circulating blood cells start expressing tissue factor. The in vivo expression of tissue factor seems mostly dependent on interleukin (IL)-6, as demonstrated in studies showing that inhibition of IL-6 completely abrogates tissue factor-dependent thrombin generation in experimental endotoxaemia, whereas specific inhibition of other pro-inflammatory cytokines had less or no effect. Inflammatory cells in atherosclerotic plaques produce abundant tissue factor and upon plaque rupture there is extensive tissue factor exposure to blood. In severe sepsis, mononuclear cells, stimulated by pro-inflammatory cytokines, express tissue factor, which leads to systemic activation of coagulation. Even in experimental low-dose endotoxaemia in healthy subjects, a 125-fold increase in tissue factor mRNA levels in blood monocytes can be detected. A potential alternative source of tissue factor may be endothelial cells, polymorphonuclear cells, and other cell types. It is hypothesised that tissue factor from these sources is shuttled between cells through microparticles derived from activated mononuclear cells. It is, however, unlikely that these cells actually synthesise tissue factor in substantial quantities.

Upon exposure to blood, tissue factor binds to factor VIIa. The complex of tissue factor-factor VIIa catalyses the conversion of factor X to Xa, which will form the prothrombinase complex with factor Va, prothrombin (factor II) and calcium, thereby generating thrombin (factor IIa). One of the key functions of thrombin is to convert fibrinogen into fibrin. The tissue factor-factor VIIa complex can also activate factor IX, forming a tenase complex with activated factor IX and factor X, generating additional factor Xa, thereby forming an essential amplification loop. The assembly of the prothrombinase and tenase complex is markedly facilitated if a suitable phospholipid surface is available, ideally presented by activated platelets. In the setting of inflammation-induced activation of coagulation, platelets can be activated directly by endotoxin or by pro-inflammatory mediators, such as platelet activating factor. Thrombin itself is one of the strongest platelet activators in vivo. Activation of platelets may also accelerate fibrin formation by another mechanism. The expression of tissue factor

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**Figure 1. Schematic representation of the link between infection/inflammation and coagulation**

Activated mononuclear cells and endothelial cells induce expression of tissue factor that activates platelets and the coagulation system. Activated coagulation proteases bind to protease-activated receptors (PARs), which may induce additional pro-inflammatory stimuli by releasing cytokines that target endothelial cells and mononuclear cells.

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on monocytes is markedly stimulated by the presence of platelets and granulocytes in a P-selectin dependent reaction.\textsuperscript{44} This effect may be the result of nuclear factor kappa B (NF-κB) activation induced by binding of activated platelets to neutrophils and mononuclear cells.\textsuperscript{44} This cellular interaction also markedly enhances the production of IL-1β, IL-8, macrophage chemoattractant protein (MCP)-1, and tumour necrosis factor (TNF)-α.\textsuperscript{46} The expression of P-selectin on the activated platelet membrane will mediate the adherence of platelets to endothelial cells and leukocytes.

**Impaired Regulatory Pathways in Infection and Inflammation**

Procoagulant activity is regulated by three important anticoagulant pathways: antithrombin (AT), the protein C system and tissue factor pathway inhibitor (TFPI). During inflammation-induced activation of coagulation, the function of all three pathways can be impaired.\textsuperscript{47} The serine protease inhibitor antithrombin is the main inhibitor of thrombin and factor Xa. Without heparin, AT neutralises coagulation enzymes in a slow, progressive manner.\textsuperscript{48} Heparin induces conformational changes in AT that result in at least a 1000-fold enhancement of AT activity. Thus, the clinical efficacy of heparin is attributed to its interaction with AT. Endogenous glycosaminoglycans, such as heparan sulphates, on the vessel wall also promote AT-mediated inhibition of thrombin and other coagulation enzymes. During severe inflammatory responses, AT levels are markedly decreased owing to impaired synthesis (as a result of a negative acute phase response), degradation by elastase from activated neutrophils, and – quantitatively most importantly – consumption as a consequence of ongoing thrombin generation.\textsuperscript{49} Pro-inflammatory cytokines can also cause reduced synthesis of glycosaminoglycans on the endothelial surface, which will also contribute to reduced AT function, since these glycosaminoglycans can act as physiological heparin-like cofactors of AT.\textsuperscript{49} Activated protein C (APC) appears to play a central role in the pathogenesis of sepsis and associated organ dysfunction.\textsuperscript{50} There is ample evidence that an insufficient functioning of the protein C pathway contributes to the derangement of coagulation in sepsis.\textsuperscript{38,43,53} The circulating zymogen protein C is activated by the endothelial cell-bound thrombomodulin once this is activated by thrombin.\textsuperscript{44} APC acts in concert with its co-factor protein S to proteolytically degrade the essential coagulation co-factors Va and VIIIa, and in that manner functions as an effective anticoagulant. The endothelial protein C receptor (EPCR) not only accelerates the activation of protein C several-fold, but also serves as a receptor for APC, and binding of APC to this receptor may amplify its anticoagulant and anti-inflammatory effects.\textsuperscript{35} A recent study has demonstrated that exposure of cultured endothelial cells to APC results in the release of microparticles that contain EPCR.\textsuperscript{36} but the relevance of that observation for coagulation or inflammation is not yet clear. In patients with severe inflammation, the protein C system is malfunctioning at virtually all levels. First, plasma levels of the zymogen protein C are low or very low, due to impaired synthesis, consumption, and degradation by proteolytic enzymes, such as neutrophil elastase.\textsuperscript{37,39} Furthermore, a significant down-regulation of thrombomodulin, caused by pro-inflammatory cytokines such as TNF-α and IL-1, has been demonstrated, resulting in diminished protein C activation.\textsuperscript{40,41} Low levels of free protein S may further compromise an adequate function of the protein C system. In plasma, 60% of the co-factor protein S is complexed to a complement regulatory protein, C4b binding protein (C4bBP). Increased plasma levels of C4bBP as a consequence of the acute phase reaction in inflammatory diseases may result in a relative protein S deficiency, which further contributes to a procoagulant state during sepsis. Although it has been shown that the β-chain of C4bBP (which mainly governs the binding to protein S) is largely unaffected during the acute phase response,\textsuperscript{42} support for this hypothesis comes from studies showing that the infusion of C4bBP in combination with a sublethal dose of *Escherichia coli* (E. coli) into baboons resulted in a lethal response with severe organ damage due to disseminated intravascular coagulation (DIC).\textsuperscript{43} Finally, but importantly, in sepsis the EPCR has shown to be down-regulated, which may further negatively affect the function of the protein C system. Apart from these effects, sepsis may cause a resistance toward APC by other mechanisms, which are partly dependent on a sharp increase in factor VIII levels (released from endothelial cells), but partly occur by yet unidentified mechanisms.\textsuperscript{44} In experimental models of severe infection fibrinolysis is activated, demonstrated by an initial activation of plasminogen activation, followed by a marked impairment caused by the release in blood of PAI-1.\textsuperscript{46,47} The latter inhibitor strongly inhibits fibrinolysis causing a net procoagulant situation. The molecular basis is cytokine-mediated activation of vascular endothelial cells; TFPI and IL-1 decreased free tissue plasminogen activator (tPA) and increased PAI-1 production, TFPI increased total urokinase type plasminogen activator (uPA) production in endothelial cells,\textsuperscript{48-50} Endotoxin and TFPI stimulated PAI-1 production in liver, kidney, lung and adrenals of mice. The net procoagulant state is illustrated by a late rise in fibrin breakdown fragments after *E. coli* challenge of baboons. Experimental data also indicate that the fibrinolytic mechanism is active in clearing fibrin from organs and circulation. Endotoxin-induced fibrin
Hepatitis B and C infection may cause thrombosis or vasculitis. Symptoms and signs result in thrombohaemorrhagic syndromes, haemolytic inflammation as discussed above, specific infections may initiate thrombosis.

Apart from the generalised response upon systemic infection-induc ed thrombosis and vascular complications, the fibrinolytic shut-off. May further increase the levels of PAI-1 and contribute to mortality. These data suggest that the enhanced thrombotic risk may be related to inflammation, either occurring on itself (e.g. as a consequence of an autoimmune disorder) or related to infection.

Viral haemorrhagic fever is complicated by DIC in the most severe cases. DIC is not frequently encountered in other viral infections but has been reported in cases of infection with rotavirus, varicella, rubella, rubeola and influenza. TTP and HUS, triggered by a viral or bacterial infection, frequently lead to bleeding symptoms, but also platelet and fibrin thrombi may be generated in various organs, leading to prominent symptoms with organ dysfunction. In specific infections, such as viral haemorrhagic fever, bleeding complications are prominent. In other viral and bacterial infections associated with TTP or HUS, bleeding is also often the prominent and presenting symptom. Bacterial and viral infections may result in a vasculitis-like syndrome with either bleeding manifestations or ischaemic injury. Vasculitis is a well-documented phenomenon in CMV infection, occurring predominantly in the vasculature of the gastrointestinal tract where it causes colitis, the central nervous system where it causes cerebral infarction, and the skin where it results in petechiae, purpura papules, localised ulcers or a diffuse maculopapular eruption. HIV infection may be accompanied by vasculitis syndromes, e.g. polyarteritis nodosa, Henoch-Schönlein purpura and leucocytoclastic vasculitis. Hepatitis B and C infection may cause
polyarteritis-like vasculitis.\textsuperscript{99,100} Parvovirus B19 has been suggested to be associated with vasculitis-like syndromes including Kawasaki disease, polyarteritis nodosa and Wegener’s granulomatosis.\textsuperscript{101-103}

**THERAPEUTIC IMPLICATIONS**

Anticoagulant therapy in patients with severe infection remains controversial. Experimental studies have shown that heparin can at least partly inhibit the activation of coagulation in severe sepsis and other infections. However, a beneficial effect of heparin on clinically important outcome events in patients with DIC has not been demonstrated in controlled clinical trials. Also, the safety of heparin treatment is debatable in patients with haemorrhagic complications of infection, such as in some viral diseases or in DIC, who are prone to bleeding.\textsuperscript{104} A large trial in patients with severe sepsis showed a slight but non-significant benefit of low-dose heparin on 28-day mortality in patients with severe sepsis and no major safety concerns.\textsuperscript{105} There is general consensus that administration of heparin is beneficial in some categories of infection-related procoagulant states. Heparin is obviously indicated for treating thromboembolic complications in large vessels in patients with inflammation and infection. Heparin administration may be helpful in patients with acute DIC when intensive blood component replacement fails to improve excessive bleeding or when thrombosis threatens to cause irreversible tissue injury (e.g., acute cortical necrosis of the kidney or digital gangrene).

Theoretically, the most logical anticoagulant agent to use in the setting of hypercoagulability in the setting of infection or inflammation is directed against tissue factor activity. Potential agents include recombinant TFPI, inactivated factor VIIa, and recombinant nematode anticoagulant protein c2 (NAPc2), a potent and specific inhibitor of the ternary complex of TF/factor VIIa and factor Xa. Phase II trials of recombinant TFPI in patients with sepsis showed promising results but phase III trials in patients with severe sepsis or severe pneumonia and organ failure did not show an overall survival benefit in patients who were treated with TFPI.\textsuperscript{106} Recombinant human soluble thrombomodulin binds to thrombin to form a complex that inactivates thrombin’s coagulant activity and activates protein C and, thus, is a potential drug for the treatment of patients with DIC. In a phase III randomised double-blind clinical trial in patients with DIC, administration of the soluble thrombomodulin had a significantly better effect on bleeding manifestations and coagulation parameters than heparin. Currently ongoing trials with soluble thrombomodulin focus on DIC, organ failure and mortality rate.

**CONCLUSION**

There is a tight link between infection and inflammation on the one hand and activation of coagulation and venous and arterial thrombosis on the other hand. Pro-inflammatory cytokines are crucial in mediating these effects. The interaction between inflammation and coagulation involves significant cross-talk between the systems and seems to occur at the interface formed by endothelial cells. Several mechanisms contribute to an enhanced risk of both venous thromboembolism and accelerated atherothrombosis in patients with infections and (chronic) inflammation. Although it is likely that anticoagulant treatment is important to prevent infection- and inflammation-associated thrombotic complications, clinical evidence of efficacy and safety of this approach is still limited.

**REFERENCES**


