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Crosstalk between the coagulation and complement systems in sepsis

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ABSTRACT

Sepsis is a potent activator of the hemostatic and complement systems. While local activation of these proteolytic cascades contributes to the host defense, their uncontrolled systemic activation has major tissue damaging effects that lead to multiple organ failure and death. We have extensively studied the activation of complement and coagulation cascades in experimental sepsis using baboons challenged with live bacteria, such as Gram-negative *Escherichia coli* or Gram-positive *Staphylococcus aureus* and *Bacillus anthracis*, or with the bacterial product peptidoglycan. We observed that these challenges rapidly induce disseminated intravascular coagulation and robust complement activation. We applied a potent C3 convertase inhibitor, compstatin, which prevented sepsis-induced complement activation, reduced thrombocytopenia, decreased the coagulopathic responses, and preserving the endothelial anticoagulant properties. Overall, our work demonstrates that live bacteria and bacterial products activate the complement and coagulation cascades, and that blocking formation of complement activation products, especially during the organ failure stage of severe sepsis could be a potentially important therapeutic strategy.

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Introduction

Sepsis is multistage, multi-factorial disease and a major cause of morbidity and mortality worldwide [1,2]. Sepsis progression results in the aberrant breakdown of the blood/tissue barrier due to an exaggerated and systemic host response to bacterial pathogen-associated molecular patterns (PAMPs) [2]. The excessive activation of inflammation, complement and coagulation systems damage the host's own tissues and organs leading to multiple organ failure and death [3]. Moreover, sepsis survivors can have life-long impairments, ranging from limb amputations to diffuse organ fibrosis [4] that affect the quality of life and increase the risk of death from subsequent challenges. Despite the clinical importance of the disease and extensive research, no specific treatment is available for sepsis.

In this review we present recent advancements in the field, with particular focus on the pathophysiology of sepsis and the inter-talk between complement, coagulation and innate immunity during sepsis progression, as revealed by the experimental models in baboons.

Models of Sepsis Progression in Baboons

As illustrated in Fig. 1, live bacteria or bacterial-derived PAMPs, such as lipopolysaccharide (LPS or endotoxin) from Gram negative (G-) or peptidoglycan (PGN) from Gram positive (G+) bacteria, bind pattern-recognition receptors (PRR) such as Toll-like receptor (TLR) 4, or nucleotide oligomerization domain (NOD) receptors [5], respectively. Recognition of PAMPs by PRRs triggers a cascade of cellular signals that activate the transcription factor nuclear factor kappa B (NFkB) leading to rapid and massive production of inflammatory mediators and induction of procoagulant activities such as tissue factor expression [6]. Non-human primate models of *E. coli* sepsis developed by our group demonstrated that the events controlling sepsis progression are spatially and temporarily defined and have specific inducers [2]. Early events are direct intravascular responses to the PAMPs of invading pathogens, while late events are associated with extravascular ischemia-reperfusion (IR) injury and oxidative stress (OS) [2].

Activation of Coagulation in Sepsis

Like inflammation, activation of blood clotting cascade during sepsis is a host-defense mechanism that facilitate the containment and destruction of pathogens to protect against bacterial spreading within the body.
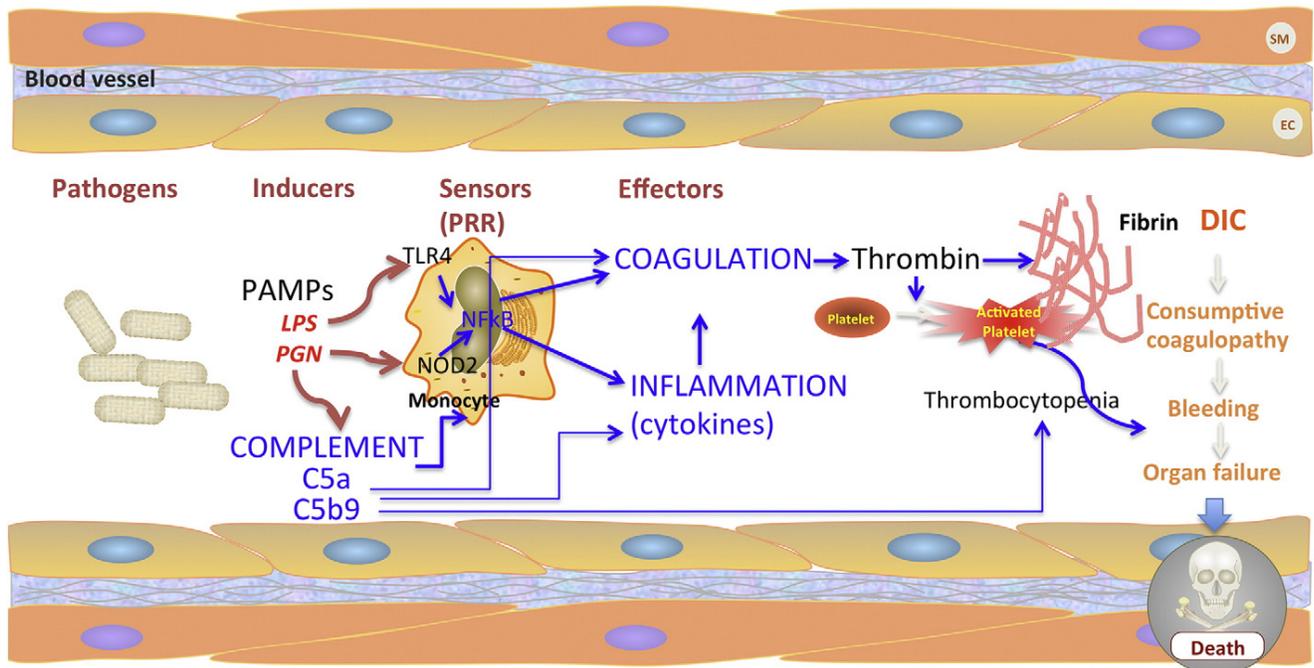
Fig. 1. Interactions between inflammation, coagulation and complement activation during sepsis progression.

Inflammation and coagulation are tightly inter-connected. Uncontrolled inflammation can promote disseminated intravascular coagulopathy (DIC), a central event in the pathophysiology of sepsis and probably the most important marker of poor prognosis. DIC is characterized by massive thrombin production and platelet activation/consumption, coupled with impaired fibrinolysis and microvascular thrombosis.

Sepsis-induced DIC is driven by: (i) tissue factor (TF)-mediated thrombin generation [6]; (ii) depression of natural anticoagulant mechanisms (antithrombin, protein C and TFPI) and impaired fibrinolysis which cannot balance the overwhelming procoagulant activity [7]; (iii) activation of the complement system, that can further amplify the inflammation and coagulation responses and promote tissue damage [8].

Induction of Procoagulant Factors

There are strong evidences that coagulation in sepsis is primarily TF-driven [6]. TF activates coagulation via the extrinsic pathway, involving factor VIIa. The TF-VIIa complex activates thrombin, which cleaves fibrinogen to fibrin while simultaneously causing platelet aggregation. The actual source of the TF is not fully established. While TF expression by monocytes is well established, TF was also detected on polymorphonuclear leukocytes, platelets and endothelial cells, although is not clear if is synthesized or transferred to these cells via monocyte-derived microparticles [6]. Focal TF increases at branches of large vessels and within the subendothelial space and this is associated with fibrin deposition and increased endothelial permeability [9]. Targeting of the extrinsic pathway with monoclonal antibodies or inhibitors specifically directed against TF [10] or factor VIIa activity [11] prevented the occurrence of DIC organ failure and mortality in baboons that were infused with *E. coli* [12].

Intrinsic pathway of coagulation, also known as contact activation or kallikrein/kinin system is located at the interface between coagulation, fibrinolysis and complement activation. Moreover, contact activation leads to the release of Bradykinin, a highly potent proinflammatory, vasoactive peptide. Systemic activation of the contact system was reported both in animal models [13] and patients suffering from sepsis. Activation of this pathway may contribute not only to DIC but also to other serious complications such as hypotension and vascular leakage

[13]. Inhibition of factor XI activation was reported to attenuate inflammation and coagulopathy and to improve survival in a mouse model of polymicrobial sepsis [14]. Otherwise, upstream inhibition at factor XII level did not prevent DIC but alleviated sepsis induced hemodynamic instability and hypotension in the baboon model of E. coli sepsis [15]. These discordances may reflect differences in the animal model and/or bacterial challenge.

Depression of Anticoagulant Mechanisms

Several anticoagulant proteins, including Protein C, antithrombin, thrombomodulin and TFPI are markedly decreased in septic baboons and in patients with DIC [7]. This reduction is caused by decreased synthesis, increased consumption, degradation by proteases, such as plasmin [16,17], supporting a role for plasmin in proteolytical degradation of TFPI during sepsis. Moreover, acute thrombin generation can contribute to the depletion of the endothelial pool of TFPI [18].

While most of functionally relevant TFPI is associated with endothelial cells and platelets, pharmacologic doses of TFPI delivered in plasma, prevented mortality, suggesting that high concentrations of TFPI can control TF-mediated coagulation during systemic inflammation in baboons [19].

The damaging effects of DIC prompted the use of anticoagulants as sepsis therapy. This had mixed results because of the duality of DIC as both clotting and bleeding disorder, where the consumption of clotting factors and platelets can lead to severe bleeding that also contribute to organ failure and death. Anticoagulant therapies have failed in clinical trials, because of bleeding adverse effects [15].

Activation of Complement in Sepsis

Similar to coagulation, complement is a critical component of the innate immune defense against pathogens but uncontrolled complement activation can contribute to the pathology of sepsis [20]. The immunoglobulin-initiated classical pathway or the mannose binding lectin-initiated pathway converge on C3 activation before assembly of the C5b-9 terminal attack complex. The small activation fragments that are released during the activation of complement, C3a and C5a (also called anaphylatoxins) have potent proinflammatory effect by

increasing the permeability of blood vessels, and by attracting leukocytes [8,21,22]. When complement activation gets uncontrolled, overwhelming inflammation and host tissue damage can occur [23].

Complement activation during the early bacteremic stage of sepsis is beneficial to the host defense. Persons and mice that lack C3 and cannot generate C5b-9 are vulnerable to bacterial infections. However, complement activation during the late stages of sepsis can promote tissue damage and contribute to multiple organ failure and death. Hence, clinical studies have consistently shown an association between the extent of complement activation and poor prognosis, suggesting that complement inhibition during the organ failure stage of sepsis may offer protection. Indeed, we showed that delayed inhibition of complement effectively attenuates sepsis-induced inflammation and microvascular thrombosis and provides organ protection [8].

Crosstalk Between Complement and Coagulation

The functions of complement and coagulation pathways in sepsis are closely intertwined [24]. Complement end products can increase the thrombogenicity of the blood by simultaneous induction of procoagulant [25] and antifibrinolytic proteins and inhibition of natural anticoagulants [24]. C5b-9 terminal complex can induce TF [24] and C5b-7 initiation complex can decrypt TF via a mechanism dependent on protein disulfide isomerase [26]. Additionally, C5b-9 can induce phosphatidylserine on the platelet surfaces to provide catalytic surface for prothrombinase assembly [27]. Recently we showed that PGN, a G + PAMP, can induce complement activation via the classical pathway, C5b-9 deposition on the platelet surface and subsequent platelet aggregation and exposure of PS-rich procoagulant activity [28]. These changes were mediated by PGN–anti-PGN immune complexes activating complement consumption.

Inhibition of complement in *E. coli* challenged baboons using compstatin, a C3 convertase inhibitor, reduced thrombocytopenia and microvascular thrombosis, and preserved the endothelial anticoagulant properties [8]. Compstatin treatment also improved vascular barrier function and attenuated organ injury. These data reveal the complement-coagulation interplay that contributes to the progression of severe sepsis. Hence, blocking the harmful effects of complement activation products, especially during the organ failure stage of severe sepsis, is a potentially important therapeutic strategy.

The crosstalk between coagulation and complement occurs in both directions. Not only complement proteins can turn on the coagulation cascade, certain coagulation enzymes, such as thrombin and factor Xa can directly activate components of the complement cascade [29], while the anticoagulant thrombomodulin-Protein C pathway can inhibit complement activation [30].

Furthermore, there are shared factors that function both in the complement and coagulation pathways. Thus, Factor XIIa acts both in the contact activation of coagulation and in the activation of complement via the classical pathway [31] and C1 inhibitor is a potent neutralizer both of factor XIa of the coagulation system and C1 component of the classical complement pathway [32].

Conclusions

Coagulation and complement systems are tightly interconnected and cross-regulated to achieve effective protection of the host. Though, uncontrolled activation of these enzymatic cascades during severe sepsis has major contribution to organ failure and death. Inhibition of coagulation during the late stage of sepsis is not beneficial due to increased bleeding risk while complement inhibition during the early bacteremic stage can interfere with bacterial clearance. Identification of the appropriate therapeutic windows and development of combination therapies targeting coagulation and complement hold the promise to prevent the harmful effects that promote tissue damage and organ dysfunction without obliterating the protective role of each system.

Authors' Contribution

All authors contributed to the paper.

Conflict of Interest Disclosures

The authors do not have financial interests to disclose.

Acknowledgments

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