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THROMBOSIS AND HEMOSTASIS

Comment on Keshari et al, page 2678

# Complement and coagulation: so close, yet so far

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In this issue of *Blood*, Keshari et al report on the striking absence of substantial complement system activation in a model of generalized experimental coagulation and fibrinolysis in baboons.[1]

Their observation appears in sharp contrast with earlier studies that did identify a potential for thrombin and plasmin to activate the complement system.[2,3] The apparent contradiction thus raises a fundamental question: Is this a case of "in vivo veritas," or are there experimental or physiological grounds that may explain the differences?

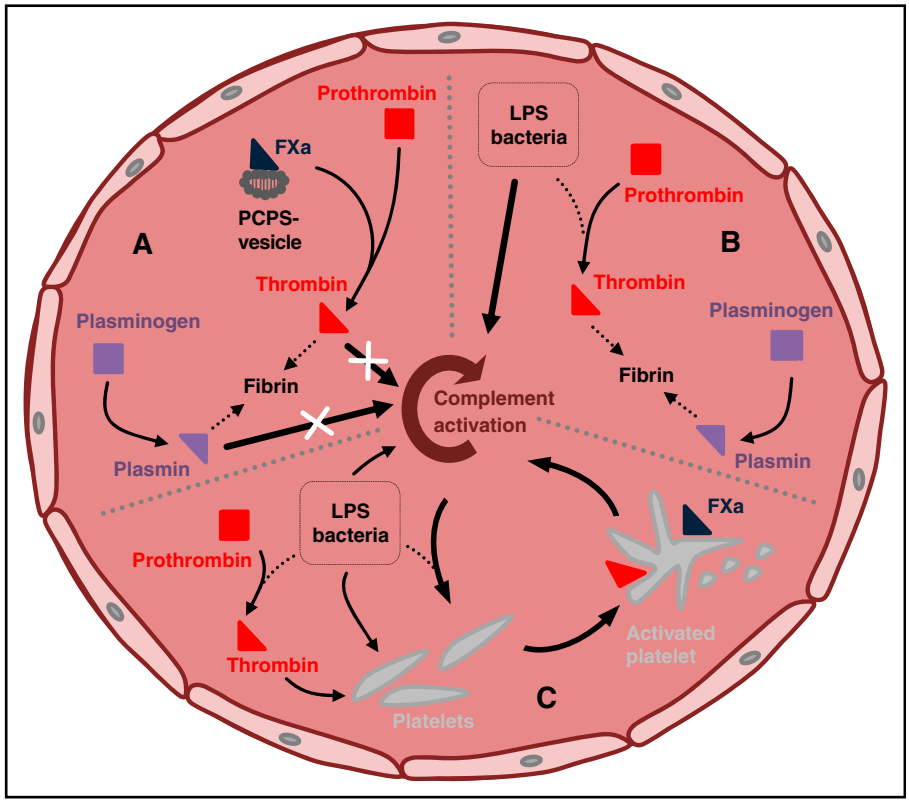
One infamous setting where activation of complement, coagulation, and fibrinolysis coincide is sepsis. Most typically triggered by bacterial infections that disseminate to the systemic circulation, sepsis sets in motion a vicious cycle of generalized inflammation, inappropriate clotting, and uncontrollable bleeding that can lead to multiple organ failure. The overall incidence of sepsis is on the rise, owing to a combination of an aging population, more frequent surgical interventions, and growing antibiotic resistance. Thus, Keshari et al probe a critical topic here, and a detailed understanding of the mechanisms underlying sepsis, including the identification of harmful cross connections between contributing systems, likely holds the key to more effective treatment options.

One of the main challenges to effectively combat bacterial sepsis is to control the generalized and simultaneous triggering of multiple systems. These systems drive essentially defensive mechanisms but in sepsis operate in an uncontrolled, overextended manner and so contribute to (immune) pathology. In contrast, a more controlled activation of complement, coagulation, fibrinolysis, but also platelets, can help limit systemic infection, both physically and immunologically, by binding and arresting bacteria, restricting their unhindered hematogenous spread, and promoting their direct lysis and uptake by phagocytes. It is becoming increasingly clear that to achieve these infection-limiting effects, interactions extend well beyond the familiar collaboration between platelets, coagulation, and fibrinolysis in hemostasis. For instance, detailed in vivo and in vitro studies documented how collaboration between platelets and complement[4] or von Willebrand factor[5] helps target intravascular bacteria to phagocytes, that complement activates platelets and vice versa,[6] and that plasmin[2] and thrombin[3] can in principle activate complement.

In their current study, Keshari et al put the latter concept to the test in baboons, examining its physiological and pathological relevance in a setting as close to humans as we can get. To do so, they take an elegant "pure coagulopathy" approach, infusing the animals with preactivated factor X (FXa) and a surrogate procoagulant surface of phosphatidylcholine-phosphatidylserine (PCPS) vesicles (see figure panel A). Normally, thrombin is generated from prothrombin through catalytic conversion by the prothrombinase complex, which consists of FXa and FVa and assembles in the presence of calcium ions on phosphatidylserine-rich cell surfaces, such as activated platelets or apoptotic or damaged cells. Thus, the "pure coagulopathy" method allowed the authors to eliminate tissue damage or inflammation as confounding factors and to concentrate on thrombin and plasmin instead. The method induced a sharp spike in thrombin and plasmin that resulted in near-full fibrinogen consumption within merely 15 minutes. However, strikingly, the drastic mobilization of thrombin and plasmin did not lead to substantial complement activation, as evidenced by a near absence of C3b, C5a, and sC5b-9 generation in the circulation. In contrast, infusion of a lethal dose of *E coli* did induce robust complement activation, and its less immediate induction mirrored the protracted course of bacteremia, serum LPS, coagulation, and fibrinolysis. As the "pure coagulopathy" experiment indicated that plasmin and thrombin are dispensable for complement activation, the *E coli* sepsis experiment leads the authors to conclude that bacteria and LPS are the main drivers of complement activation (see figure panel B).

This may indeed be the case. Still, there are additional contributing factors that deserve closer scrutiny. One aspect that was not addressed in the current study is the role of platelets. When examining the potential of complement C3 and C5 inhibition in previous sepsis studies, the authors noticed a drastic (up to 80%) reduction of platelets in the circulation of untreated control baboons upon *E coli* infusion.[7,8] This observation may be equally relevant to the current study.

Activated platelets provide a productive procoagulant surface for the prothrombinase complex that not only generates thrombin but equally promotes complement activation (see figure panel C).[9] In addition, activated platelets propagate classical complement activation through C1q affinity for qC1qR, chondroitin sulfate, and phosphatidylserine, whereas C3b and properdin binding to P-selectin drive the alternative pathway.[10] It is therefore conceivable that PCPS vesicles, used for the "pure coagulopathy" method, deprive the complement and coagulation systems of their natural common surface, needed for close interaction. Surface, location, local concentration, and timing are key factors to the short-lived enzymatic complexes that




Schematic representation of results by Keshari et al and extended interpretation integrating platelets. (A) "Pure coagulopathy" model: infusion of preactivated FXa and surrogate procoagulant PCPS vesicles led to robust induction of coagulation and fibrinolytic pathways, with fulminant turnover of prothrombin, plasminogen, and fibrinogen, but did not drive measurable complement activation in the circulation. (B) *Escherichia coli* sepsis model: infusion of a lethal dose of *E coli* induced protracted activation of coagulation and fibrinolytic pathways when compared with the "pure coagulopathy" model, as evidenced by delayed but strong consumption of prothrombin, plasminogen, and fibrinogen. In addition, the sepsis model also fueled strong complement activation. Given the observed lack of complement activation in the "pure coagulopathy" model (see panel A), Keshari et al propose that lipopolysaccharide (LPS) and bacteria drive complement independently from coagulation and fibrinolysis. (C) Extended interpretation integrating platelets: bacteria, thrombin, and complement can all contribute to platelet activation, and platelet activation is strong during sepsis. The activated platelet surface provides a procoagulant surface for the prothrombinase complex (represented by FXa) and equally promotes complement activation. Thus, the activated platelet surface offers a microenvironment where factors of the coagulation and complement systems can closely and simultaneously interact. In turn, complement activation promotes platelet activation, creating a positive feedback cycle that matches the pathological exacerbations observed during sepsis.

characterize the complement, coagulation, and fibrinolytic cascades. For instance, systemic treatment of animals with cobra venom factor potently activates circulating complement in the fluid phase to the point of full depletion, without ill effect to the animal. On the other hand, even limited local, unregulated complement activation on tissues or cells can be the cause of severe autoimmune disease.

Keshari et al have advanced our insight by convincingly demonstrating in a relevant in vivo model that robust activation of the clotting cascade per se does not have to result in exhaustive complement activation. An important question that remains for future exploration is how the microenvironment, in particular cellular surfaces, fosters interaction between the complement and coagulation systems and influences their cross talk. This can be particularly worthwhile because such areas of cross talk resemble a biological switchboard that controls multiple downstream effects. Therefore, identifying these key switches in sepsis may someday allow us to silence multiple pathogenic lines by pulling 1 therapeutic plug.

*Conflict-of-interest disclosure: C.Q.S. is an inventor of patent applications that describe the use of complement inhibitors for potential therapeutic applications. A.V. declares no competing financial interests.* ■

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● ● ● TRANSPLANTATION

Comment on Hashem et al, page 2682

# HSCT cures ADA2 deficiency

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In this issue of *Blood*, Hashem et al report a remarkable therapeutic result: all facets of the diverse spectrum of the rare disease adenosine deaminase 2 (ADA2) deficiency, which range from vasculopathy including stroke to severe anemia and immunodeficiency, can be corrected by hematopoietic stem cell transplantation (HSCT).[1] What unites these diverse