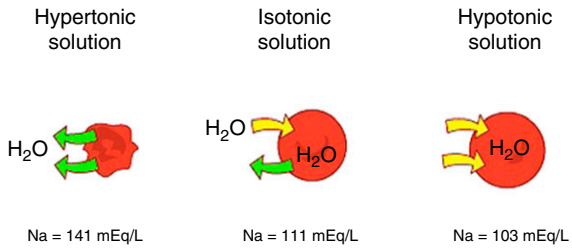
A Intravenous solutions commonly used in the management of vaso-occlusive crises

Solution	NaCl (mEq/L)	Glucose (g/L)	mOsm/L
5% DW	0	50	252
0.9% NaCl	154	0	308
0.45% NaCl	77	0	154
10% DW	0	100	505
5%DW/0.45%NaCl	77	50	406
5%DW/0.9%NaCl	154	50	560
Lactated Ringer	130 Na^+, 109 Cl^-	0	275

D5W = 5% dextrose in water; D10W = 10% dextrose in water

B



Intravenous solutions and the effect of tonicity of extracellular fluids on RBC. (A) IV fluids that are usually used to treat hydration in patients with SCD. (B) RBCs suspended in hypertonic solution lose water, shrink, and are transformed to dehydrated xerocytes. Suspended in hypotonic solution, RBCs gain water, swell, and are transformed to spherocytes. Panel B adapted from OpenStax[11] (source: Mariana Ruiz Villareal; licensed under a Creative Commons Attribution License 4.0). Download for free at http://cnx.org/contents/185cbf87-c72e-48f5-b51e-f14f21b5eabd@10.118.

vaso-occlusion, the authors proceeded to show the effects of the tonicity of IV fluids on the adhesion of sRBCs to the vascular endothelium and laminin. Admixtures of sRBCs with a sodium concentration of 141 mEq/L demonstrated increased adhesion to both human endothelium and laminin. On the other hand, admixtures of sRBCs with a sodium concentration of 103 mEq/L showed decreased adhesion to the endothelium and subendothelium matrix, probably because the hydrated swollen spherocytes may alter cell membrane contact points with the endothelium and subendothelium.[9] This effect, however, seems to be offset by the prolongation of the transit time of swollen spherocytes in capillary-sized microchannels.

This study presents a seminal translational approach to determine the appropriate IV fluids to be used in the management of patients with SCD during VOCs. The authors are aware that their study did not specify the type of sRBCs, such as reticulocytes, dense cells, etc., and hope to overcome these limitations in future projects. They are also aware that plasma components affect the rheology of sRBCs beyond their effect on tonicity.[10] The authors did specify the IV route of fluid administration and the tonicity of the fluids used. They did not, however, address the quantity to be used and followed during hospitalization. Patients with sickle cell anemia are known to have an inability to concentrate urine. Urinary osmolality of 400 to 450 mOsm/kg is often seen in adult patients with sickle cell anemia after water deprivation conditions. Perhaps this should be considered in choosing the tonicity of the fluids to be used for hydration.

This well-designed study indicates the need for clinical randomized trials to determine the safety and efficacy of different routes, types, and quantities of fluids administered to patients with different types of SCD during VOCs.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● THROMBOSIS AND HEMOSTASIS

Comment on Nazir et al, page 2664

On PAR with aPC to target inflammasomes

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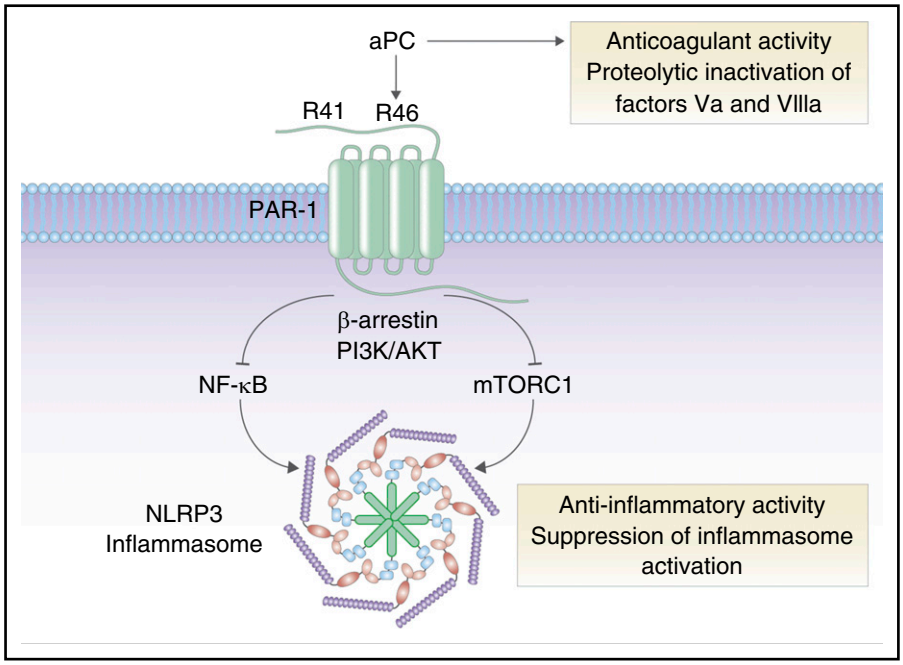
In this issue of *Blood*, Nazir et al demonstrate that activated protein C (aPC) protects from ischemia-reperfusion injury by suppressing the activation of NLRP3 inflammasomes, revealing a novel target of the anti-inflammatory pathway triggered by biased agonism of proteinase-activated receptor 1 (PAR-1).[1]

As a postdoctoral fellow in 1991, I remember being amazed by the discovery of PAR-1 by Shaun Coughlin and colleagues.[2] Coughlin's work was a scientific tour de force, identifying PAR-1 as the long-sought thrombin receptor and demonstrating a novel tethered ligand mechanism of proteolytic activation of a G-protein–coupled receptor. PAR-1 was the first identified member of the PAR family, which also includes PAR-2,



aPC is an anticoagulant serine protease that inactivates coagulation factors Va and VIIIa. In addition to its anticoagulant activity, aPC can induce anti-inflammatory cell signaling via cleavage of PAR-1 at arginine 46 (R46). Activation of PAR-1 by aPC triggers "biased" signaling via β-arrestin, phosphoinositide 3-kinase (PI3K), and AKT, leading to inhibition of NF-κB and mTORC1, and suppression of NLRP3 inflammasomes. In contrast, thrombin cleaves PAR-1 at arginine 41 (R41) and stimulates proinflammatory signaling pathways (not shown). Professional illustration by Somersault18:24.

PAR-3, and PAR-4. We know now that PARs can be activated or inactivated by a myriad of different serine proteases and matrix metalloproteinases, and that PARs can orchestrate differential "biased" cellular signaling responses to different proteases.[3] For example, thrombin cleaves PAR-1 at arginine 41 and stimulates proinflammatory signaling pathways, whereas aPC cleaves at arginine 46 and directs anti-inflammatory responses[4] (see figure).

Biased PAR-1 signaling by aPC or its analog 3K3A-aPC (which has reduced anticoagulant activity) protects from cerebral ischemia-reperfusion injury in animal models, and 3K3A-aPC is currently being evaluated in a phase 2 clinical trial in patients with ischemic stroke.[4] aPC-mediated anti-inflammatory PAR-1 signaling also protects from myocardial reperfusion injury, but the mechanisms are incompletely understood. Nazir et al now demonstrate that aPC signaling via PAR-1 suppresses activation of NLRP3 inflammasomes in a murine model of myocardial reperfusion injury.

Inflammasomes are multimeric protein complexes that assemble in response to danger-associated molecular patterns, functioning as innate immune sensors that trigger caspase-mediated activation of interleukin-1β (IL-1β) and IL-18 and induce pyroptosis.[5] Nazir et al found that treating mice with aPC decreased infarct size and inhibited expression of NLRP3, activation of caspase-1, and production of IL-1β and IL-18 after reperfusion injury in the heart or kidney. The protective effect of aPC in the heart was mimicked by 3K3A-aPC or parmodulin-2, a biased PAR-1 modulator, and lost in mice expressing a hyperactive NLRP3 variant. These findings are entirely consistent with prior work showing that NLRP3 inflammasome inhibition decreases infarct size and preserves cardiac function in animal models of myocardial infarction.[6] Importantly, Nazir et al found inhibition of inflammasome activation by aPC was completely independent of its anticoagulant activity but required PAR-1-dependent signaling, suggesting that aPC-dependent biased signaling via PAR-1 restricts inflammasome assembly following reperfusion injury. Interestingly, aPC-mediated PAR-1 signaling in this setting was independent of the endothelial cell protein C receptor, a coreceptor required for biased PAR-1 activation by aPC in the brain and other tissues.[4]

The mechanism by which aPC suppresses inflammasome activation appears to involve inhibition of both NF-κB and the mechanistic target of rapamycin complex 1 (mTORC1) (see figure). Nazir et al found that deficiency of the endogenous mTORC1 inhibitor TSC1 blocked the ability of aPC to suppress NLRP3 inflammasomes in cultured cells, which suggests that biased aPC-PAR-1 agonism may limit mTORC1 signaling, possibly via effects on adenosine 5′-monophosphate–activated protein kinase.[7] It remains to be determined whether the suppressive effect of aPC is limited to the priming step of inducing NLRP3 expression (which is known to be mediated by NF-κB) or also involves assembly of the inflammasome complex and/or inhibition of noncanonical inflammasome activation pathways.[5]

The new findings reported by Nazir et al expand our understanding of how an anticoagulant protease (aPC) and a facile, nuanced protease-sensitive G-protein–coupled receptor (PAR-1) can trigger potent anti-inflammatory and cytoprotective responses in the setting of reperfusion injury. The identification of the NLRP3 inflammasome as a key target of aPC-PAR-1 signaling suggests several potential therapeutic strategies, including aPC analogs, parmodulins, NLRP3 inhibitors, and perhaps even mTOR inhibitors, for the prevention of myocardial reperfusion injury. In this regard, it is encouraging that aPC was protective even when administered 30 minutes after the onset of reperfusion. Finally, these findings imply the potential to target biased PAR-1 signaling in other inflammatory conditions in which inflammasome activation is a prominent driver, such as tumor progression and metastasis, colitis, fibrosis, and aberrant wound healing.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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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