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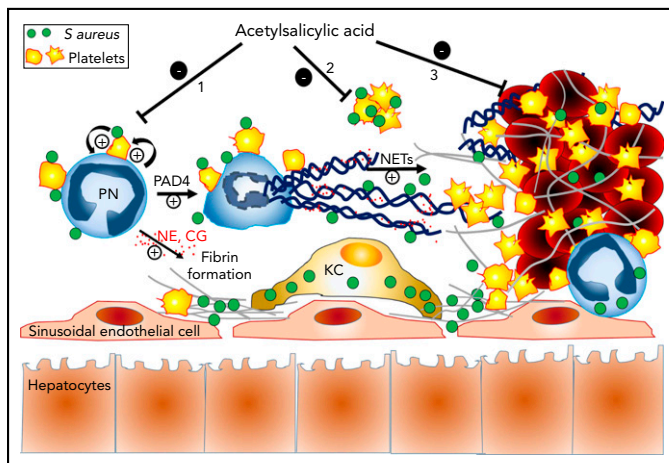
Acetylsalicylic acid to fight thrombosis in sepsis

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In this issue of *Blood*, Carestia et al report that acetylsalicylic acid (ASA) helps prevent *Staphylococcus aureus* sepsis-induced intravascular coagulation and liver dysfunction by limiting neutrophil-mediated microvascular thrombosis.[1] The authors show that ASA reduces neutrophil and platelet accumulation as well as the formation of procoagulant neutrophil extracellular traps (NETs) in the liver microvasculature of infected mice. Thus, ASA inhibits thrombin formation and platelet consumption in this model. Remarkably, the beneficial effects of ASA occur without impairing bacterial clearance when ASA was administered 1 hour before or 3 hours after infection. Importantly, no bleeding complications were reported in ASA-treated mice.

The current study by Carestia et al is one of several studies implicating platelet/neutrophil interactions in sepsis-induced intravascular coagulation and organ dysfunction. In 2007, 3 years after the discovery of NETs and of their antibacterial activity,[2] Kubes' group showed that sepsis-induced platelet/neutrophil interactions stimulated the formation of NETs, and provided the first in vitro evidence that NETs could form and trap bacteria under flow conditions.[3] The ability of neutrophils and NETs to capture circulating bacteria was later confirmed in vivo in mouse models of sepsis.[4,5] Results by Massberg et al further suggested that the procoagulant activity of neutrophils could also help limit bacterial dissemination by immobilization of bacteria onto intravascular fibrin deposits in liver sinusoids.[4] Although these milestone studies pointed out the potential protective action of neutrophils and NETs during sepsis, several important studies that followed stressed the duality of NETs in this thromboinflammatory setting by demonstrating that NETs also contributed to sepsis-induced tissue injury and organ dysfunction.[5-7] In fact, peptidylarginine deiminase-4-deficient mice (PAD4$^{-/-}$ mice), which are defective for NETosis, as well as mice treated with DNAse to eliminate intravascular NETs, showed reduced sepsis-induced thrombin formation and platelet aggregation, thus leading to improved microvascular perfusion and reduced organ damage.[5-7] Strikingly, neither PAD4 deficiency nor DNase treatment affected bacteremia. Thus, although NETs can capture circulating bacteria in experimental sepsis, specific targeting of this component of neutrophil antimicrobial activity can be overcome, possibly due to redundancy with and/or compensation by other antibacterial host defense mechanisms. These experimental findings showing that targeting NETs can prevent thrombotic complications of sepsis support the hypothesis forwarded in 2006 by Brown et al, who, noting that accumulation and activation of neutrophils in the microvasculature were key features of sepsis-induced multiple organ failure, proposed that "targeting neutrophils and their interactions with blood vessel walls could be a worthwhile therapeutic strategy for sepsis."[8] Moreover, the deleterious role of NETs may contribute to the life-threatening syndrome of sepsis, with organ dysfunction caused by an exaggerated and dysregulated immune response to infection (see figure).

By targeting platelets with ASA, Carestia et al considered the central role of platelets in inflammation and thrombosis and thus chose to tackle the immunothrombotic cascade upstream of NETs. This strategy offers a prophylactic action to prevent intravascular coagulation, before NETs are formed. It is also worth noting that, despite clinical evidence that ASA, or antiplatelet therapy in general, could improve sepsis outcome,[9] there is a dearth of preclinical and mechanistic data that explore and validate this beneficial effect. The results of Carestia et al provide important insights into the possible mechanisms underlying the protective action of ASA in sepsis.

Proposed mechanisms of the protective action of ASA against *S aureus* sepsis-induced hepatic thrombosis. Once in the blood, circulating *S aureus* triggers an immunothrombotic cascade resulting in intravascular coagulation and occlusion of liver sinusoids. Circulating *S aureus* activates neutrophils and platelets through direct physical interactions (eg, via toll-like receptors and other pattern-recognition receptors) and secretion of immuno-modulatory proteins, thus stimulating neutrophil-platelet reciprocal activating interactions. These interactions lead to the release of neutrophil elastase (NE) and cathepsin-G (CG) by neutrophils (PN), as well as to PAD4-dependent formation of procoagulant NETs. Together with nucleosomes, free and NETs-associated NE and CG enhance tissue factor- and factor XII-dependent coagulation, by degrading the tissue factor pathway inhibitor. Intravascular fibrin deposits and NETs help to capture and kill circulating *S aureus*, while at the same time, they promote liver injury and dysfunction by occluding sinusoids. Depending on the timing of administration, treatment with ASA can prevent thrombosis in sinusoids and associated liver injury by acting on at least 3 possible levels: (1) prevention of NETosis through inhibition of neutrophil-platelet interactions and reciprocal activation, (2) inhibition of *S aureus*–induced platelet aggregation, and (3) inhibition of platelet incorporation and aggregation on preformed prothrombotic NETs. The antibacterial action of "non-NETotic" neutrophils and other immune cells like Kupffer cells (KC) helps minimize the impact of ASA treatment on bacterial elimination.

Given the high mortality rate of sepsis and increasing antimicrobial resistance, there is a great need for new therapeutic options for sepsis. The work of Carestia et al could have clinical implications. Future studies may determine whether the efficacy and safety of ASA the authors describe here in models of sepsis induced by IV or intraperitoneal injection of *S aureus* also apply to other models and with different bacterial species. Platelets have indeed been shown to continuously prevent bleeding in various inflammatory situations, where they intervene to both inhibit bacterial growth and prevent bleeding at the primary site of infection.[10]

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

REFERENCES

1. Carestia A, Davis RP, Grosjean H, Lau MW, Jenne CN. Acetylsalicylic acid inhibits intravascular coagulation during *Staphylococcus aureus*–induced sepsis in mice. *Blood*. 2020; 135(15):1281-1286.

2. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science*. 2004;303(5663):1532-1535.

3. Clark SR, Ma AC, Tavener SA, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med*. 2007;13(4):463-469.

4. Massberg S, Grahl L, von Bruehl ML, et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat Med*. 2010;16(8):887-896.

5. McDonald B, Urrutia R, Yipp BG, Jenne CN, Kubes P. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe*. 2012;12(3): 324-333.

6. McDonald B, Davis RP, Kim SJ, et al. Platelets and neutrophil extracellular traps collaborate to promote intravascular coagulation during sepsis in mice. *Blood*. 2017;129(10): 1357-1367.

7. Martinod K, Fuchs TA, Zitomersky NL, et al. PAD4-deficiency does not affect bacteremia in polymicrobial sepsis and ameliorates endotoxemic shock. *Blood*. 2015;125(12):1948-1956.

8. Brown KA, Brain SD, Pearson JD, Edgeworth JD, Lewis SM, Treacher DF. Neutrophils in development of multiple organ failure in sepsis. *Lancet*. 2006;368(9530):157-169.

9. Ouyang Y, Wang Y, Liu B, Ma X, Ding R. Effects of antiplatelet therapy on the mortality rate of patients with sepsis: A meta-analysis. *J Crit Care*. 2019;50:162-168.

10. de Stoppelaar SF, Van 't Veer C, Claushuis TAM, et al. Thrombocytopenia impairs host defense in gram-negative pneumonia-derived sepsis in mice. *Blood*. 2014;124(25):3781-3790.

TRANSPLANTATION

Comment on Schultz et al, page 1287

A few steps on the long road toward biomarkers in GVHD

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In this issue of *Blood*, Schultz et al report the biomarker profiles of 241 patients 3 months after allogeneic hematopoietic stem cell transplantation (alloHSCT) who were followed for the development of chronic graft-versus-host disease (cGVHD) or late acute GVHD. The patients were part of the prospective ABLE (Applied Biomarker of Late Effects of Childhood Cancer) trial, which was the first prospective multicenter biomarker trial stringently applying the National Institutes of Health (NIH) criteria for diagnosis of cGVHD in pediatric and adolescent patients.[1]

The authors report multiple provocative findings. First, the biomarker profiles of patients who later developed signs of immune-mediated damage with distinctive but not diagnostic features of cGVHD by NIH criteria and required immunosuppressive treatment did not differ significantly from those of patients with NIH-defined cGVHD. This suggests that cGVHD may involve more targets than currently included in the NIH consensus criteria, which classify cGVHD based on the most frequently involved organs.[2] Fifteen percent of the patients in the analyzed cohort showed immune-mediated damage that did not fulfill the NIH definition, likely similar to what has been labeled by a European Society for Blood and Marrow Transplantation/NIH/Center for International Blood and Marrow Transplant Research taskforce as "undefined other cGvHD."[3(pp1401-1415)] Patients with signs of immune-mediated damage lacking NIH-defined manifestations may eventually be classified as having cGVHD in future trials or at least be included in further analysis of biomarkers, assuming the finding can be confirmed in other study cohorts.

Second, although the NIH consensus criteria provide clinical definitions for the distinction of late acute GVHD from classic cGVHD,[2] the data provide hints of a biological distinction, where patients with both entities have B-cell abnormalities, but the expansion of naïve CD4^{+} T cells seems to be restricted to patients developing cGVHD. Interestingly, metalloproteinase 3 (MMP3), ST2, and soluble CD13 (sCD13) were confirmed as prognostic biomarkers of cGVHD, whereas the previously proposed plasma biomarkers CXCL9 and osteopontin were not significant in either entity for unknown reasons.[4] The frequent identification of MMP3 suggests that remodeling is a distinctive feature of cGVHD. Heterogeneity in biomarkers was observed in a multicenter analysis that included adult patients, with significant differences found between different cohorts.[5] This indicates the need for large verification and qualification cohorts.[6]

Third, the analysis indicates in a small subset of 14 patients that the day-100 biomarker profile (plasma biomarkers MMP3, sCD13, and ST2 and alterations of B-cell subpopulations) is not affected by the presence of active GVHD. Previous studies of sBAFF showed a significant impact of corticosteroids.[7] If this is confirmed in larger studies, trials evaluating biomarkers at day 100 after alloHSCT may include patients with active GVHD, which will help with accrual and better represent a high-risk group.

Fourth, age has an impact on biomarkers, indicating the need for age-specific pediatric cohorts in the identification of biomarkers for acute GVHD and cGVHD. For example, children age <12 years rarely develop de novo onset of cGVHD.[8]

Finally, the analysis reveals indirectly a continuing pathophysiology from acute