INFLAMMATION

Inflammatory platelet death drives sepsis

Thrombocytopenia is common in severe sepsis and is associated with an increased risk of mortality. A new study shows that platelet pyroptosis initiated during infection promotes a feedforward loop of neutrophil-mediated inflammation that worsens outcomes during sepsis.

Luke Brown and Bryan G. Yipp

Sir William Osler noted that "Except on few occasions, the patient appears to die from the body's response to infection rather than from it", highlighting that the inflammatory response is the main driver of sepsis immunopathology. During sepsis, neutrophils, the major inflammatory leukocyte, release host-defense molecules that inadvertently contribute to bystander tissue injury and organ damage. Simultaneously, sepsis leads to a hypercoagulable state with platelet-mediated microvascular impairments, tissue hypoxia and thrombocytopenia (low platelets), which result in worse outcomes[1]. Emerging research has shown essential connections that link host defense, inflammation, platelet function and coagulation; however, more mechanistic evidence is needed to develop therapeutic agents that could exploit these connections and improve conditions such as sepsis. Research now reveals that a specialized type of platelet death is a major driver of a perpetual neutrophil-dependent inflammatory loop, which boosts the severity of sepsis and increases death.

Neutrophils use a potent host-defense mechanism in which they extrude nuclear DNA loaded with toxic granules, termed neutrophil extracellular traps (NETs), in response to infection and during sepsis. Unfortunately, these structures can intensify inflammation[2] and lead to tissue and organ injury. Although platelets are primarily involved in clotting and hemostasis, surprising functions beyond clotting continue to be discovered. Indeed, collaborations between neutrophils and platelets have been previously described during severe infections and sepsis. For example, during human and mouse models of sepsis, platelets activated via Toll-like receptor 4 (TLR4) stimulate rapid NET release from neutrophils, which helps to capture bloodstream pathogens[3]. Subsequently, it was shown that neutrophil proteases induce coagulation and thrombosis as an intravascular host defense, termed immunothrombosis, to limit bacterial dissemination[4]. Despite a growing body of evidence that implicates an axis involving neutrophils, NETs and platelets during host defense and bystander tissue injury, the full scope of this complex problem is not well defined.

In this issue of *Nature Cardiovascular Research*, Su et al.[5] report that during

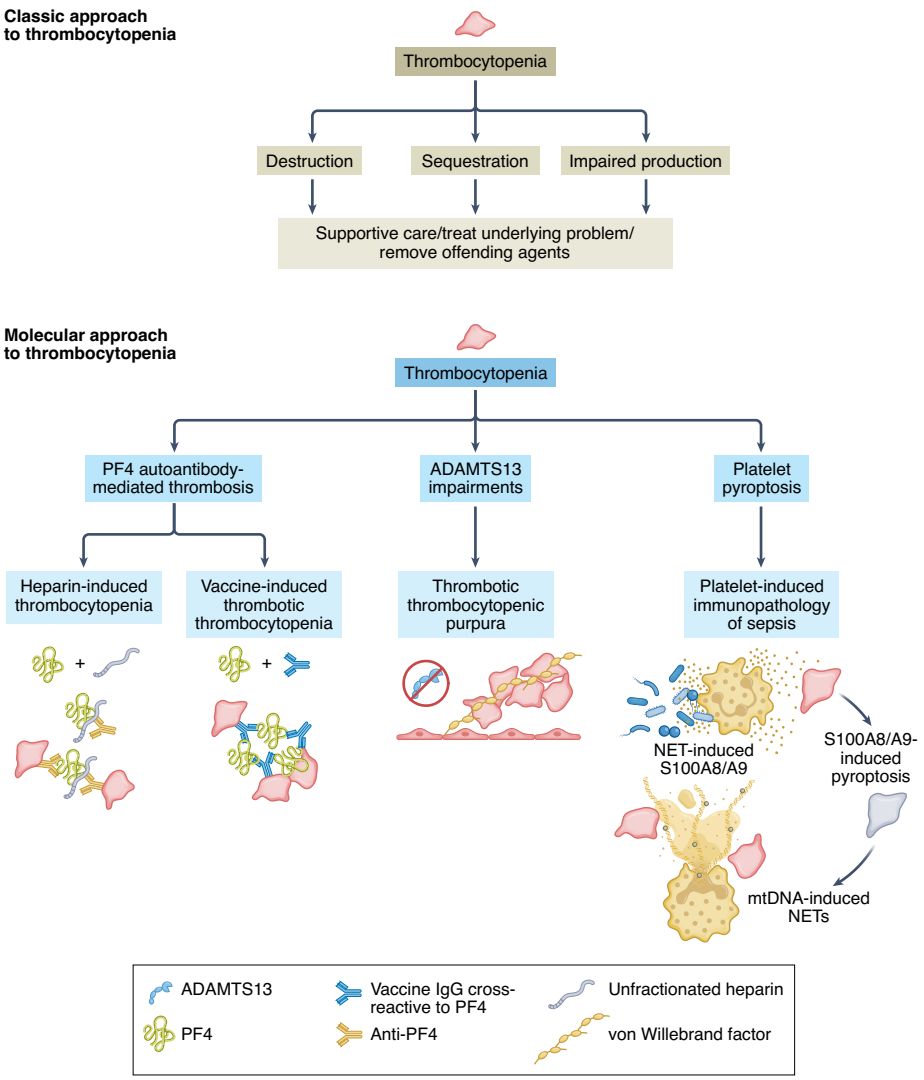

Fig. 1 | Approaches to thrombocytopenia. A classic clinical approach to thrombocytopenia is shown in the top panel. The bottom panel shows emerging examples of pathophysiological and mechanistic approaches to clinical problems. During sepsis, neutrophils release S100A8/A9, which drives GSDMD-induced platelet pyroptosis and leads to further neutrophil activation via the release of oxidized mitochondrial DNA (mtDNA) and increased NETs and S100A8/A9.

life-threatening infectious conditions, including sepsis, an inflammatory form of platelet cell death fuels the activation of neutrophils and exacerbates immunopathology. They found that during infection, the host defensive and proinflammatory molecule S100A8/A9 (also known as calprotectin) causes inflammatory cell death, known as pyroptosis, in platelets. This requires the activation of gasdermin D (GSDMD) via platelet TLR4. In turn, dying platelets release mitochondrial DNA, which promotes NET release and augments the further release of S100A8/A9, thereby creating a positive-feedforward loop that exacerbates and perpetuates inflammation during sepsis.

Su et al.[5] began investigating retrospective pediatric electronic medical records and found that children with severe sepsis or septic shock had moderate and high mortality on admission. In addition, patients with severe sepsis had increased pro-inflammatory cytokine profiles and evidence of thrombocytopathy and thrombocytopenia. Focusing on this cohort of severe sepsis or septic shock, the investigators performed proteomic analysis of platelets and found differential expression of apoptosis and pyroptosis-associated proteins, particularly GSDMD, which when cleaved forms membrane pores that cause cell lysis. Notably, more than half of patient platelets were positive for the pyroptosis marker caspase 1, which indicates that pyroptosis is the predominant form of cell death in patients with severe sepsis (with or without septic shock). To directly test whether platelet-specific GSDMD-induced pyroptosis was responsible for promoting further NET release, persistent inflammation and mortality during sepsis, the authors generated a platelet-specific GSDMD-deficient mouse (*Gsdmd*$^{flox/flox}$ *Pf4-cre*). During a cecal-ligation and puncture model of sepsis, the *Gsdmd*$^{flox/flox}$ *Pf4-cre* mice had a significant reduction in NETs and decreased proinflammatory cytokines, which were associated with improved survival.

Moreover, the authors found that the percentage of pyroptotic platelets correlated with levels of S100A8/A9 in patients with sepsis, and that recombinant S100A8/A9 could induce platelet pyroptosis both in vitro and in animal models. By genetic depletion, they confirmed that S100A8/A9 is secreted by neutrophils, and using blocking antibodies against the three main S100A8/A9 receptors on platelets (CD36, RAGE or TLR4), they found that pyroptosis was mediated by TLR4. Pharmacological inhibition of S100A8/A9 binding and signaling through TLR4 with the immunomodulatory compound paquinimod reduced inflammatory platelet death, decreased proinflammatory cytokines, and improved survival in septic mice[5].

This study adds new pathophysiological data to show that platelets are the primary drivers of inflammation and mortality during sepsis, and are therefore a primary therapeutic target. Improved molecular and cellular understanding of disease processes are important steps forward in clinical medicine. For example, for critically ill patients with sepsis in intensive care units, a common question posed to clinicians is how to manage thrombocytopenia[6], which is often caused by destruction of platelets as a result of increased consumption or sequestration, or decreased platelet production due to marrow suppression (Fig. 1). In general, clinical approaches to medical problems lack mechanistic pathophysiological considerations. However, defining pathological mechanisms improves clinical care and results in improved diagnostic and therapeutic targeting. For example, heparin-induced thrombocytopenia is caused by autoantibodies to PF4 that induce platelet activation, and lead to thrombotic complications. Understanding this has led to better diagnostic tests, and changes to clinical practice such as using less problematic anticoagulants. Importantly, the mechanistic understanding of heparin-induced thrombocytopenia allowed scientists to rapidly understand the emerging problem of vaccine-induced immune thrombotic thrombocytopenia, which also results from PF4 autoantibody activation of platelets[7]. Acquired thrombotic thrombocytopenic purpura (TTP) is a life-threatening condition with excessive mortality if left untreated. Mechanistic research has shown that TTP is due to impairment in ADAMTS13, an enzyme essential for regulating intravascular platelet activation and clot formation via cleavage of von Willebrand factor. This mechanism has been exploited both for diagnostic tests and for the development of therapeutic antibodies (such as Caplacizumab, a humanized anti-von Willebrand factor antibody fragment) to improve TTP outcomes in humans[8]. Now, GSDMD-induced platelet pyroptosis could be added to the molecular understanding of thrombocytopenia involved in sepsis.

Another important discovery made by Su et al.[5] was that blocking platelet pyroptosis via paquinimod improved mouse sepsis. Although a justified pharmacological approach, it remains to be seen whether this can be translated to humans. Notably, paquinimod is thought to act by blocking S100A8/A9 activation of TLR4, yet several highly specific TLR4 inhibitors, including Eritoran, have been tested in human sepsis in randomized trials with disappointing results[9].

Finally, it will be important to apply these results to the current lung inflammation and hypercoagulable state observed in severely ill patients with COVID-19. Neutrophils, platelets, NETs and thrombosis have been implicated in driving lung failure; however, thrombocytopenia tends to be mild when directly compared with bacterial sepsis[10]. Nonetheless, the parallels and similarities that exist between platelet-mediated pathology in both sepsis and COVID-19 suggest shared pathophysiology, and potentially overlapping therapeutic strategies. ❐

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Competing interests

The authors declare no competing interests.