Shedding New Light on Platelet Extracellular Vesicles in Sickle Cell Disease

In this issue of the *Journal* (pp. 33–46), Vats and colleagues demonstrate an important, previously unrecognized role of platelet extracellular vesicles (EVs) in driving IL-1β–dependent acute lung injury in sickle cell disease (SCD) (1). Platelets are anucleate cells released into the circulation through fragmentation from their parent cell, the megakaryocyte. Platelets are well known to trigger hemostasis through adhesion to subendothelial matrix substrates or activated endothelium (2, 3). Established and emerging evidence, however, also indicates that platelets are dynamic effector cells across immune and inflammatory continua, including roles during acute lung injury (4, 5). Infectious and noninfectious agents can trigger noncanonical platelet responses, as may occur in chronic inflammatory diseases, such as systemic lupus erythematosus and rheumatoid arthritis, and may contribute to lung injury.

SCD is a monogenic disorder affecting millions worldwide and is characterized by the prevalence of hemoglobin S (Hgb S) rather than Hgb A. Hgb S can polymerize and cause "sickling" of the red blood cells under physiologic stressors such as infection, trauma, or hypoxemia. Red blood cell sickling can precipitate a vaso-occlusive crisis hallmarked by ischemia/infarction, pain, and hemolysis (6). Platelets have been implicated in the pathogenesis of SCD and its most serious pulmonary manifestation: acute chest syndrome (ACS). Histologic studies have found platelet thrombi and microthrombi occluding pulmonary arterioles in patients with SCD with ACS (7). Patients with SCD have higher numbers of circulating platelet–neutrophil aggregates under steady-state conditions and in response to LPS stimulation *in vitro* (8). Moreover, these heterotypic aggregates have been shown to cause arteriolar microthrombi in a murine model of SCD, mimicking features of vaso-occlusive crisis (8).

Platelets possess receptors that aid in recognition of danger-associated molecular pattern receptors and pathogen-associated molecular pattern receptors, including the TLRs (Toll-like receptors) 2, 4, and 9. TLR4 and its ligand LPS have been the best studied in platelets, although controversy remains with regard to LPS activation of classical platelet hemostatic responses (9, 10). However, LPS is known to stimulate noncanonical platelet function responses, including platelet mitochondrial respiration (10), the splicing of endogenous mRNAs (11), and the generation of platelet–leukocyte aggregates (12). Recently, Bennewitz and colleagues hypothesized that gut translocation of LPS triggers platelets "primed" by endogenous ligands (i.e., heme) to form platelet–neutrophil aggregates and induce vaso-occlusive crisis (8). Nevertheless, innate immune mechanisms underpinning these increased platelet–neutrophil aggregates and how they mediate sickle cell pathogenesis and outcomes remain largely unknown.

In a series of elegant and complementary clinical *in vitro* and *in vivo* studies, Vats and colleagues (1) investigated whether SCD induces platelet inflammasome activation and shedding of EVs carrying caspase 1 and IL-1β, as well as if these shed EVs trigger platelet–neutrophil aggregate formation. The authors identified by scanning electron microscopy that when whole blood from patients with SCD (or control subjects) was perfused under physiological shear stress, SCD platelets appeared to be activated (and thus primed for EV shedding) with large aggregates of platelets adhered to neutrophils. Remarkably, treating blood from patients with SCD with a TL4 antagonist restored platelets to a more quiescent appearance.

The authors next found that LPS pretreatment of platelets from patients with SCD induced caspase 1 cleavage and colocalization of ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) and NLRP3 (NACHT, LRR, and PYD domains–containing protein 3), two components of the inflammasome. This coincided with increased platelet–neutrophil aggregates, suggesting an intriguing—and novel—link between inflammasome activation and platelet–neutrophil aggregate formation in SCD. In blood from patients with SCD, scavenging mitochondrial reactive oxygen species or inhibiting the inflammasome reduced formation of platelet–neutrophil aggregates *ex vivo*. Using nanogram quantities of LPS to activate platelets from patients with SCD or matched healthy donors, the authors observed that patients with SCD generated significantly more and larger platelet EVs in response to LPS. They found that LPS-induced platelet EVs are richly packaged with IL-1β. EV formation in patients with SCD appeared to be, in part, IL-1B dependent because inhibiting caspase 1, necessary for the generation of active IL-1B, reduced EV formation.

Using a mouse model of SCD and intravital microscopy, the authors observed increased platelet–neutrophil aggregates in the pulmonary circulation after LPS injection; formation of these aggregates was reduced when the inflammasome was inhibited. Next, platelet EVs from SCD or control mice were generated by LPS stimulation, isolated, and used in adoptive transfer experiments. Injection of donor SCD EVs was sufficient to induce pulmonary vaso-occlusion in untreated recipient SCD mice. Pulmonary vaso-occlusion was reduced in the presence of an IL-1 receptor antagonist or caspase 1 inhibitor. LPS-generated EVs from control mice did not form aggregates with neutrophils. These data suggest that platelet EVs shed in a mouse model of SCD aggregate with

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neutrophils and occlude the pulmonary vasculature in a caspase 1– and IL-1–dependent manner.

These findings are intriguing because they shed new light on platelet-derived EVs in SCD and link platelet-derived EVs to previously described roles of platelet–neutrophil aggregates in ACS. The authors postulate that platelet EVs may potentiate the formation of heterotypic platelet–neutrophil aggregates, resulting in additional EV shedding in a positive feedback loop. These investigations also bring to light the potential that additional TLRs may contribute to activation of the inflammasome, platelet EV shedding, and the formation of platelet–neutrophil aggregates in SCD, an important area for further investigation. For example, TLR9 is known to bind free heme (generated during hemolysis, a prominent feature of SCD). Gram-positive bacteria and porins (components of encapsulated organism cell walls) are ligands for TLR2. Thus, additional TLRs could be targets for future investigations. In addition, the role of EVs derived from red blood cells and endothelial cells in driving vaso-occlusive crisis remains incompletely understood. Finally, these findings indicate that inhibiting the shedding of platelet EVs may be of therapeutic benefit in SCD, particularly with regard to prevention or treatment of ACS. Indeed, these novel data may function as a catalyst to employ pharmacologically available inhibitors of IL-1B (anakinra), TLR4 (eritoran), and caspase 1 (VX-765) in clinical trials of vaso-occlusive crisis in patients with SCD. ■

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Discovering Causal Mechanistic Pathways in Sepsis-associated Acute Respiratory Distress Syndrome

In this issue of the *Journal*, Jones and colleagues (pp. 47–56) find that sRAGE (soluble receptor for advanced glycation end products) blood levels are related to sepsis-associated acute respiratory distress syndrome (ARDS) (1). Moreover, they make the bold claim that sRAGE causally contributes to sepsis-associated ARDS. This is an important step to take, for multiple reasons. First, the sepsis and ARDS fields are littered with hundreds of reports of an association between inflammatory pathway molecule measurements and clinical outcomes (2). Although they are interesting, these associations have not led to the introduction of new therapies to prevent or ameliorate sepsis-induced lung injury/ARDS or any other clinically important outcome (3). Second, identification of a true causal pathway points the field toward a plausible intervention strategy and relevant biomarkers,

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