

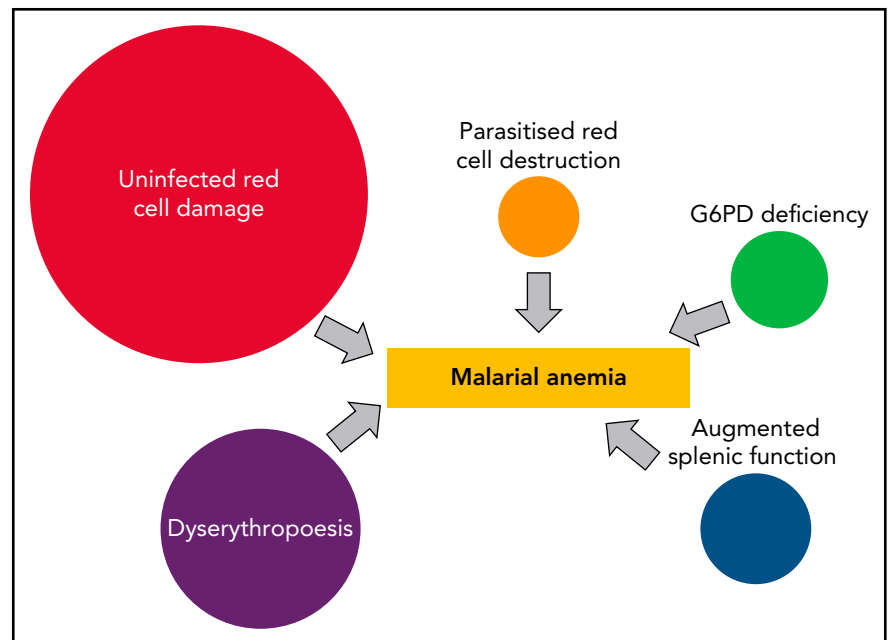
settings, the hemoglobin usually starts to rise as soon as the infection is controlled by antimalarial treatment. In lower transmission settings, there is a delay of several days before dyserythropoiesis resolves sufficiently for reticulocytes to increase, and for the hemoglobin to rise.

Mahamar et al conducted a pilot quantitative proteomic screen of a very large number of potential blood markers in 9 Malian children with different hematological responses to *P falciparum* infection to try to understand why these different hematologic responses occur. The lead candidates were then evaluated in a prospective series of more than 70 children from the same cohort. The study excluded children with hemoglobin AS or AC, but did not exclude G6PD deficiency, which may exacerbate malaria-associated hemolysis.<sup>8</sup> Higher plasma levels of circulating 20S proteasome and lower levels of insulin-like growth factor-1 were confirmed in children with reduced hemoglobin. The investigators speculated that circulating 20S proteasome may contribute to hemolysis by digesting erythrocyte membrane proteins modified by oxidative stress, whereas decreased insulin-like growth factor-1, which is important for erythroid maturation, might contribute to inadequate erythropoiesis. Of course, association does not necessarily mean causation, so further studies are warranted to elucidate these very interesting findings. These should focus on quantitating the preceding malaria parasite biomass, which is reflected poorly by the parasite density at the time of anemia, and characterizing the sequence of events that preceded the reduction in hemoglobin.

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

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Direct contributors to malarial anemia. The proportions vary but, in general, uninfected erythrocyte hemolysis is the major contributor to acute anemia, whereas dyserythropoiesis is a major contributor to subacute anemia in children in high transmission settings.

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## THROMBOSIS AND HEMOSTASIS

Comment on Cointe et al, page 2377

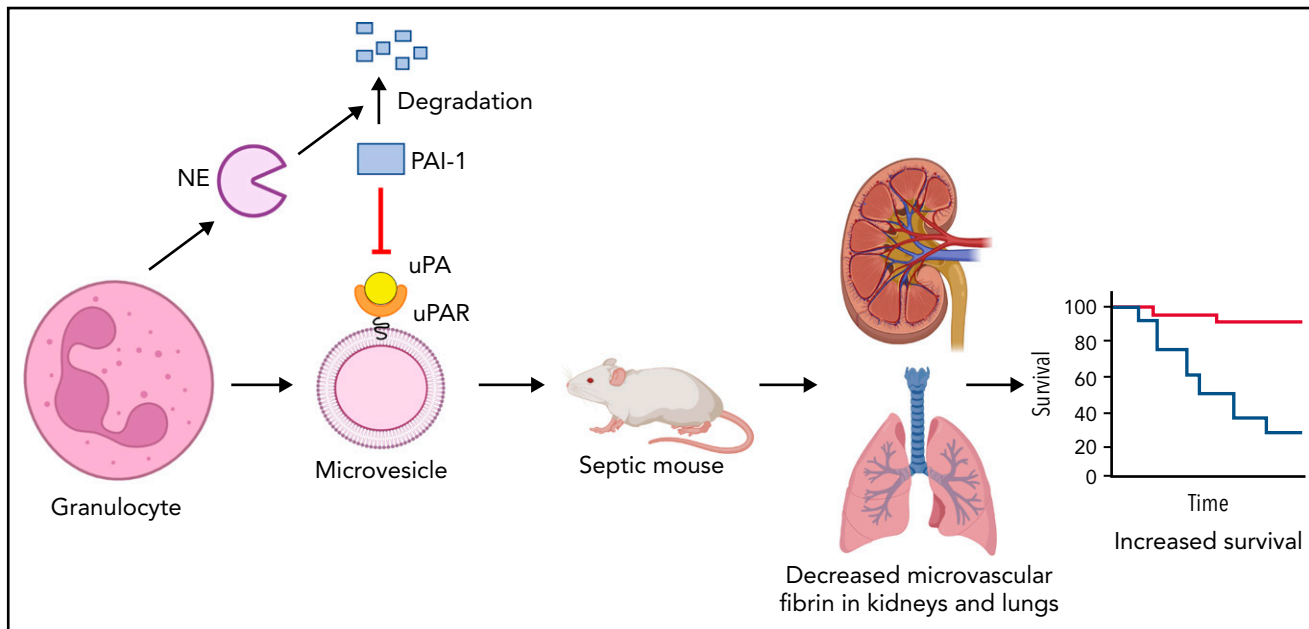
# Therapeutic potential of granulocyte microvesicles in sepsis

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**The study by Cointe et al<sup>1</sup> in this issue of *Blood* shows that microvesicles (MVs) from human granulocytes express urokinase plasminogen activator receptor (uPAR) and when loaded with urokinase (uPA) lyse clots in vitro and reduce microthrombi in kidneys and lungs of septic mice and increase their survival. In addition, neutrophil elastase (NE) increased fibrinolysis by degrading plasminogen activator inhibitor 1 (PAI-1).**

Extracellular vesicles are small membrane vesicles (0.1-1  $\mu\text{m}$ ), which includes MVs and exosomes, that are highly abundant in blood.<sup>2</sup> The study of extracellular vesicles has exploded in recent years, with

the therapeutic potential of extracellular vesicles being particularly exciting. Extracellular vesicles can be considered minicells that express cell-surface receptors from their parent cell and contribute to



Human granulocytes increase fibrinolysis by releasing NE and MVs expressing uPAR. NE degrades PAI-1. Human granulocyte release MVs that express uPAR and bind uPA. Injection of granulocyte MVs and uPA into septic mice reduced microvascular thrombosis and increased survival. The figure was created using BioRender.com.

vascular homeostasis and cell-cell communication.<sup>2</sup> The major source of MVs in blood is platelets, with these particles being originally referred to as platelet dust. It is now known that many cells release MVs into the blood, including endothelial cells, erythrocytes, granulocytes, and monocytes.<sup>2</sup> Much of the early work on MVs focused on their procoagulant properties. For instance, the presence of tissue factor and negatively charged phospholipids, such as phosphatidylserine, make MVs highly procoagulant, and many studies have shown that they contribute to thrombosis.<sup>3</sup> More recently, it was discovered that MVs derived from endothelial cells and leukocytes possess fibrinolytic activity.<sup>4</sup> Interestingly, endothelial cell-derived MVs bound tissue plasminogen activator, whereas leukocyte-derived MVs expressed uPAR and bound uPA.<sup>4</sup> MVs derived from neutrophils and monocytes had similar fibrinolytic activity but monocyte-derived MVs have much higher procoagulant activity because of the presence of tissue factor.<sup>1</sup> An early study reported that human neutrophils express uPAR; therefore, MVs derived from these cells would be expected to also express uPAR.<sup>5</sup>

Septic shock is a systemic response to infection with a high mortality rate, and approved treatments are generally focused on supportive care and eradicating the source of the infection. Patients

with sepsis have systemic inflammation, microvascular thrombosis, decreased fibrinolysis because of increased levels of PAI-1, and often have disseminated intravascular coagulation (DIC) and multiorgan failure.<sup>6</sup> Unfortunately, despite considerable efforts targeting inflammation and coagulation has not improved outcomes in patients with sepsis. In terms of fibrinolytic capacity, 1 study found that patients with sepsis who developed DIC had higher levels of PAI-1 compared with those without DIC, and higher levels of PAI-1 were associated with decreased survival.<sup>7</sup> Recently, Cointe and colleagues<sup>8</sup> extended the analysis of fibrinolysis in patients with septic shock and found that lower levels of MV-plasmin generating capacity (MV-PGC) were associated with decreased survival. These studies suggest that reduced fibrinolytic capacity from either high PAI-1 or low MV-PGC increases the risk of microvascular thrombosis, DIC, organ failure, and death in patients with septic shock.

In this study, Cointe et al extended their work on the analysis of MV-PGC in patients with septic shock. Importantly, they found that granulocyte-MVs (Gran-MVs) from patients with high levels of MV-PGC had higher levels of both uPA and uPAR compared with patients with low MV-PGC, which suggested that granulocytes were the primary source of the MV-PGC. Gran-MVs from patients

with septic shock lysed clots *in vitro* in a uPA- and uPAR-dependent manner. Addition of exogenous uPA increased the clot lysis activity of Gran-MVs. Taken together, these studies suggest that the balance between the coagulation system and the fibrinolytic system appears to play an important role in the outcome of patients with sepsis.

Next, the authors determined the effect of IV injection of MVs derived from human granulocytes from healthy individuals with or without exogenous uPA or a supernatant control on septic mice. Gran-MVs were injected daily for 4 days, and survival of the mice was evaluated at day 5. Levels of plasmin- $\alpha$ 2 antiplasmin were increased in mice receiving Gran-MVs + uPA compared with the other 2 groups of mice at day 2, indicating increased plasmin generation. In addition, Gran-MVs + uPA prevented the increase in D-dimer at day 5 in mice, suggesting reduced fibrin generation. Importantly, injection of Gran-MVs + uPA reduced microvascular thrombi in the kidneys and lungs and increased survival of the septic mice compared with the other 2 groups (see figure). Injection of uPA without Gran-MVs did not protect the mice. There is long road between studies with septic mice and treatment of patients with sepsis. Nevertheless, the possibility of using Gran-MVs with PGC in the treatment of patients with sepsis,

particularly those with low MV-PGC and/or high PAI-1, is exciting.

Finally, Cointe et al analyzed plasma factors from patients with sepsis that affected fibrinolysis. Plasma from patients with sepsis was analyzed using a multiplex array for 23 molecules that are known to modulate the uPA/uPAR system. Levels of NE correlated with Gran-MV-PGC. NE has also been shown to degrade PAI-1.<sup>9</sup> Indeed, addition of NE increases the fibrinolytic activity of Gran-MVs because of degradation of PAI-1 (see figure). Therefore, neutrophils increase fibrinolysis in 2 ways: they release uPAR-expressing MVs that bind uPA and increase plasmin generation, and also via release of NE that degrades PAI-1.

The study has some limitations. Although the data supporting the notion that Gran-MVs improve survival in septic mice by rebalancing the coagulation and fibrinolytic systems is compelling, one cannot exclude the possibility that Gran-MVs have other beneficial activities that increase survival of the septic mice. For instance, neutrophil MVs contain annexin 1, which has anti-inflammatory activity. For the mouse experiments, MVs were derived from unstimulated human granulocytes rather than from murine neutrophils. MVs released from human granulocytes cultured under different conditions may have different fibrinolytic activities. One study prepared 2 types of MVs from stimulated human neutrophils and found that only 1 type of MV protected septic mice.<sup>10</sup> Another study found that neutrophil-derived MVs isolated from the peritoneal cavity of septic mice reduced survival of septic mice.<sup>11</sup> Clearly, further work is needed but the current study suggests that MVs derived from human granulocytes may be useful in treating disorders associated with microvascular thrombosis, such as sepsis and COVID-19.

*Conflict-of-interest disclosure:* The authors declare no competing financial interests. ■

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## TRANSPLANTATION

Comment on Docampo et al, page 2392

# GPR109A in GVHD: friend or foe?

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**In this issue of *Blood*, Docampo et al<sup>1</sup> explore the role of G protein-coupled receptor (GPR)109A, a receptor for the short chain fatty acid (SCFA) butyrate and the B vitamin niacin, in the pathophysiology of experimental graft-versus-host disease (GVHD).**

Previous work has demonstrated that activation of GPR109A in colonic antigen-presenting cells (APCs), such as macrophages and dendritic cells (DCs), promotes anti-inflammatory responses by inducing regulatory T cell (Treg) differentiation and interleukin-10 (IL-10) production by T cells.<sup>2</sup> In addition, GPR109A was previously shown to be essential for butyrate-mediated induction of IL-18 and tissue repair in the intestinal epithelium.<sup>2</sup> These data suggested that GPR109A may mitigate GVHD. Docampo et al tested the role of GPR109A in murine models of GVHD and found that its absence in recipients did not alter GVHD severity or mortality. Surprisingly, however, GPR109A-deficient (GPR109A<sup>-/-</sup>) donor T cells caused dramatically less GVHD. Recipients of GPR109A<sup>-/-</sup> donor T cells had reduced GVHD histopathological scores, decreased proliferation of alloreactive T cells in target organs, and reduced accumulation of alloreactive T cells in target tissues.

Functional analyses demonstrated that GPR109A<sup>-/-</sup> T cells were more prone to apoptosis and had diminished mitochondrial oxidative phosphorylation capabilities. Hence, alloreactive GPR109A<sup>-/-</sup> T cells were metabolically dysregulated. However, antioxidant treatment with N-acetyl cysteine restored their alloreactivity and ability to drive GVHD. At steady-state, GPR109A deficiency did not alter T-cell subtypes, activation/exhaustion markers, differentiation, or Treg suppressive function, which suggests that GPR109A alters T-cell function under inflammatory conditions. Importantly, the graft-versus-tumor (GVT) and antiviral activities of GPR109A<sup>-/-</sup> donor T cells were maintained. These data collectively suggest that GPR109A in donor T cells is indispensable for expansion and metabolic homeostasis following allogeneic hematopoietic cell transplantation (allo-HCT). Thus, GPR109A in allogeneic T cells enhances their inflammatory capacity, whereas in gut epithelial