Conflict-of-interest disclosure: P.D.M. declares no competing financial interests. ■

REFERENCES

1. Kim MG, Yu K, Yeh C-Y, et al. Low-intensity transcranial focused ultrasound suppresses pain by modulating pain-processing brain circuits. *Blood*. 2024;144(10):1101-1115.
2. Fry WJ. Ultrasound in neurology. *Neurology*. 1956;6(10):693-704.
3. Fry F, Ades H, Fry W. Production of reversible changes in the central nervous system by ultrasound. *Science*. 1958; 127(3289):83-84.
4. Tyler WJ, Tufail Y, Finsterwald M, Tauchmann ML, Olson EJ, Majestic C. Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound. *PLoS One*. 2008;3(10):e3511.
5. Spivak NM, Tyler WJ, Bari AA, Kuhn TP. Ultrasound as a neurotherapeutic: a circuit- and system-based interrogation. *Focus*. 2022; 20(1):32-35.
6. Riis TS, Feldman DA, Vonesh LC, et al. Durable effects of deep brain ultrasonic neuromodulation on major depression: a case report. *J Med Case Rep*. 2023;17(1): 449.
7. Legon W, Strohman A, In A, Payne B. Noninvasive neuromodulation of subregions of the human insula differentially affect pain processing and heart-rate variability: a within-subjects pseudo-randomized trial. *Pain*. 2024; 165(7):1625-1641.
8. Labrakakis C. The role of the insular cortex in pain. *Int J Mol Sci*. 2023;24(6): 5736.
9. Bobola MS, Chen L, Ezeokeke CK, et al. A review of recent advances in ultrasound, placed in the context of pain diagnosis and treatment. *Curr Pain Headache Rep*. 2018; 22(9):60.
10. Lo MC, Younk R, Widge AS. Paired electrical pulse trains for controlling connectivity in emotion-related brain circuitry. *IEEE Trans Neural Syst Rehabil Eng*. 2020;28(12): 2721-2730.

https://doi.org/10.1182/blood.2024025937

THROMBOSIS AND HEMOSTASIS

Comment on Ballard-Kordeliski et al, page 1116

Procoagulant membranes during hemostasis

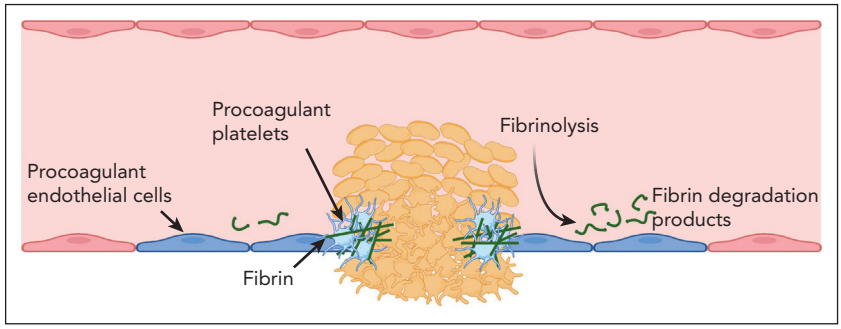
Timothy J. Stalker | Thomas Jefferson University

In this issue of *Blood*, Ballard-Kordeliski et al explore the intricate balance of procoagulant and fibrinolytic activities during hemostasis in vivo. Using 4-dimensional intravital imaging in a murine hemostasis model they show that, although both endothelial cells and platelets expose phosphatidylserine (PS) at sites of vascular injury, platelet procoagulant activity is most critical for fibrin formation within the nascent hemostatic plug. The authors also show that rapid fibrinolysis prevents fibrin accumulation from spreading along the vessel wall.[1]

Phospholipids have been known to be essential for coagulation since the earliest descriptions of the coagulation cascade. Rigorous biochemical studies have detailed the role of negatively charged membrane surfaces as binding sites for coagulation complex assembly, particularly for the intrinsic tenase and prothrombinase complexes.[2] These binding events localize coagulation reactions to injury sites and greatly enhance their catalytic activity. Their physiologic importance is highlighted by the utility of the anticoagulant warfarin, which acts by disrupting γ-carboxylation of vitamin K–dependent coagulation factors, thus preventing their interaction with membranes. Historically, platelets were considered the primary source of procoagulant membranes during clotting in vivo. Platelets exhibit procoagulant activity upon stimulation in vitro, and impaired platelet procoagulant activity is associated with a bleeding disorder (Scott syndrome).[3] However, in vivo studies over the past 15 to 20 years have questioned the predominant role for platelets in supporting coagulation. Alternative procoagulant membranes, particularly from endothelial cells, are seen at sites of vascular injury in multiple vascular beds.[4-6] Moreover, several studies have reported normal fibrin formation in contexts of impaired platelet activation and adhesion,[4,6,7] suggesting that alternative sources of procoagulant membranes can support coagulation in vivo.

Ballard-Kordeliski et al provide new insights into the roles of different cellular sources of procoagulant membranes during hemostasis. Using 4-dimensional intravital imaging, they first document the spatial distribution of platelets, PS-positive membranes, and fibrin after a penetrating injury of the mouse saphenous vein. Consistent with prior studies, PS exposure initially appears at the interface of the platelet plug and endothelium, and it then extends across the surrounding endothelium (see figure). Fibrin forms a ring around the injury site, primarily at the interface of the platelet plug and damaged endothelium. Two questions arising from these observations are: (1) What are the relative contributions of procoagulant activity from platelets vs endothelial cells to fibrin spatial localization? (2) How is fibrin deposition constrained to the boundary of the injury site? To address the latter question, the authors used both genetic (plasminogen-deficient mice) and pharmacologic (tranexamic acid) approaches to demonstrate that the lack of fibrin extension across the surface of procoagulant endothelial cells is due to ongoing fibrinolysis that prevents fibrin accumulation in that region. Inhibition of fibrinolysis also increased fibrin formation in the ring around the injury site. A particularly interesting aspect of these results is the timing of fibrinolytic processes during the hemostatic response. Rather than fibrinolysis as a relatively late event that acts to break down a formed fibrin clot, the results show that fibrinolysis can occur concurrently with fibrin formation. Thus, fibrin deposition during hemostasis represents a balance of fibrin formation and lysis that may be spatially regulated within local microenvironments present at sites of injury. How this balance is regulated on the endothelial cell surface as compared with the boundary of the platelet plug requires further study.

The role of different cellular sources of procoagulant activity was explored using



Penetrating vascular injury results in the formation of a platelet plug at the injury site, with phosphatidylserine exposure on both platelets and endothelial cells (blue cells). Platelet procoagulant activity is necessary for fibrin formation at the platelet-endothelial cell interface (green fibers), whereas fibrinolysis prevents fibrin accumulation from spreading across the surface of the endothelium.

cyclophilin D–deficient mice (*CypD*$^{-/-}$). Cyclophilin D is a regulator of the mitochondrial permeability transition pore that controls PS exposure in platelets.[8] The authors showed that PS exposure at the injury site (both platelet and endothelial cell) is significantly attenuated in *CypD*$^{-/-}$ mice, resulting in decreased fibrin formation. They then performed reciprocal adoptive transfer experiments to isolate the contribution of platelet vs endothelial cell procoagulant activity. Fibrin formation was significantly attenuated in mice lacking platelet PS exposure (*CypD*$^{-/-}$ platelets), but not in mice deficient in endothelial cell *CypD* (wild-type platelets). These results are consistent with a significant role for platelet, rather than endothelial cell, procoagulant activity in the generation of thrombin and fibrin during hemostasis in vivo.

The genetic approach used by the authors to demonstrate the cell-type–specific contribution of procoagulant activity to fibrin formation provides convincing results, but some questions remain. One limitation acknowledged by the authors is that they assessed a single vascular injury model, and it is unclear whether the source of procoagulant membranes would be the same in all vascular beds or types of vascular injury. Indeed, prior studies showing normal fibrin formation in the absence of platelet accumulation were performed in the microcirculation, and they did not involve a penetrating vascular injury with bleeding.[4,6,7] Contextual variables, including vascular bed, vessel wall composition, local hemodynamics, and injury type, can influence the spatiotemporal regulation of the hemostatic response. In addition, other cells, such as red cells, leukocytes, and microvesicles from a variety of cell sources, may contribute procoagulant activity in different contexts. More thorough examination of the differential contribution of specific components of the hemostatic system, including procoagulant membranes, to tissue-specific hemostasis is clearly warranted. Also, although Ballard-Kordeliski and colleagues have pushed the limits of intravital imaging by acquiring 3-dimensional image stacks over time, the spatial resolution of the imaging is insufficient to definitively localize PS exposure on specific cell surfaces. Alternative imaging approaches are available that can better resolve single platelets and adjacent cellular membranes, but performing such high-resolution imaging in live animals remains challenging.

Overall, this study brings platelets back into the limelight as an important source of procoagulant membranes in vivo, at least in certain physiologic contexts. It also highlights the continued need for investigations into the integration of cellular and biochemical events occurring during hemostasis that cannot be completely appreciated using isolated systems in vitro. Further studies are required to enhance our understanding of context-dependent variables that regulate the hemostatic response, and how they may go awry in both pathologic bleeding and thrombotic scenarios.

Conflict-of-interest disclosure: T.J.S. declares no competing financial interests. ■

REFERENCES

1. Ballard-Kordeliski A, Lee RH, O'Shaughnessy EC, et al. 4D intravital imaging studies identify platelets as the predominant cellular procoagulant surface in a mouse hemostasis model. *Blood*. 2024;144(10):1116-1126.
2. Mann KG, Nesheim ME, Church WR, Haley P, Krishnaswamy S. Surface-dependent reactions of the vitamin K-dependent enzyme complexes. *Blood*. 1990;76(1):1-16.
3. Weiss HJ. Scott syndrome: a disorder of platelet coagulant activity. *Semin Hematol*. 1994;31(4):312-319.
4. Ivanciu L, Krishnaswamy S, Camire RM. New insights into the spatiotemporal localization of prothrombinase in vivo. *Blood*. 2014;124(11):1705-1714.
5. Kaplan ZS, Zarpellon A, Alwis I, et al. Thrombin-dependent intravascular leukocyte trafficking regulated by fibrin and the platelet receptors GPIb and PAR4. *Nat Commun*. 2015;6:7835.
6. Schmaier AA, Anderson PF, Chen SM, et al. TMEM16E regulates endothelial cell procoagulant activity and thrombosis. *J Clin Invest*. 2023;133(11):e163808.
7. Vandendries ER, Hamilton JR, Coughlin SR, Furie B, Furie BC. Par4 is required for platelet thrombus propagation but not fibrin generation in a mouse model of thrombosis. *Proc Natl Acad Sci U S A*. 2007;104(1):288-292.
8. Jobe SM, Wilson KM, Leo L, et al. Critical role for the mitochondrial permeability transition pore and cyclophilin D in platelet activation and thrombosis. *Blood*. 2008;111(3):1257-1265.

https://doi.org/10.1182/blood.2024025430