palmitoylation, a mutation in an alternative pathway that reactivates MAPK signaling, or by the use of alternative enzymes and/or lipid moieties, as was found when using farnesylation inhibitors.

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Sparkenbaugh et al, page 1783

Sickle cell vaso-occlusion: the clot thickens

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In this issue of *Blood*, Sparkenbaugh et al reported that inhibition of tissue factor, the initiator of the extrinsic coagulation pathway, or of protease-activated receptor-1 (PAR-1), the endothelial receptor for thrombin, attenuates microvascular occlusion in murine sickle cell disease, suggesting anticoagulation should be explored for the prevention of vaso-occlusive events.[1]

Painful vaso-occlusive events that frequently require emergency department visits and hospitalization are the most pressing concern for patients with sickle cell disease. The exact cause of these events and how to prevent them has been the subject of intensive research for decades. Progress is being made, but our knowledge is incomplete and there is much room for new discoveries.

Deformed erythrocytes that result from polymerization of deoxygenated hemoglobin S initiate the obstruction of microvessels of the bone marrow and other organs.[2] Adherence to the microvascular endothelium by these misshaped erythrocytes as well as by neutrophils and platelets appears to be fundamental to these painful events.[3] The pharmacologic agents that have been approved by the US Food and Drug Administration to prevent vaso-occlusive events in sickle cell disease provide potential insights into the pathogenesis of these events.

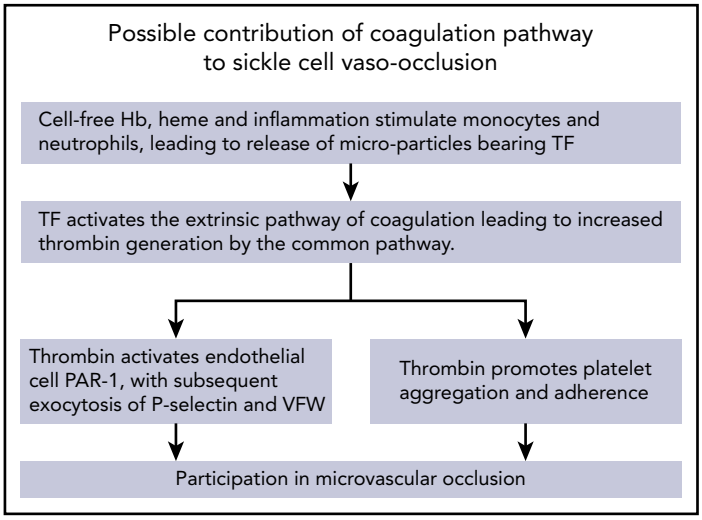
Hydroxyurea prevents hemoglobin S polymerization by raising the concentration of hemoglobin F within erythrocytes; it also reduces inflammation and promotes the production of nitric oxide.[4] Endari is a pharmacologic grade of L-glutamine, an amino acid important for the production of the antioxidant, glutathione.[5] Crizanlizumab inhibits endothelial expression of the adhesion molecule, P-selectin.[6]

Thus, sickling-induced changes in the erythrocyte membrane, inflammation, deficient nitric oxide signaling, oxidative stress, and abnormal expression of cellular adhesion molecules seem to be part of a complex process that leads to painful vaso-occlusive events. None of these approved pharmacologic agents provide full protection from painful events, and there must be other processes involved in the pathogenesis of vaso-occlusion.

The findings by Sparkenbaugh et al provide a potential path forward. Activation of coagulation is an important feature of sickle cell disease.[7] The Sparkenbaugh et al paper builds on a series of publications over the past 7 years that explored the role of the coagulation system in vaso-occlusive complications of sickle cell disease, mostly in murine models. The present paper shows that inhibition of tissue factor, at the initiation of the extrinsic pathway of coagulation, and inhibition of nonhematopoietic PAR-1, which mediates some of the responses of endothelial cells to thrombin, has effectiveness in preventing microvascular vaso-occlusion in murine models of sickle cell disease.

Specifically, the investigators explored the contributions of thrombin-dependent endothelial PAR-1 activation on microvascular stasis in murine models of sickle cell disease. Using intravital microscopy of dorsal skinfold chambers and real-time quantitative fluorescence intravital lung microscopy, they visualized the amount of microvascular stasis induced by stroma-free hemoglobin when mice were pretreated with various interventions. These modalities included anti-tissue factor antibody, anticoagulants targeting factor Xa (rivaroxaban) and thrombin (dabigatran), and an inhibitor of PAR-1 (vorapaxar). The investigators observed a marked attenuation of microvascular stasis with each of the interventions tested. PAR-1 null sickle mice were also resistant to microvascular stasis induced by stroma-free hemoglobin. In PAR-1 null sickle cell mice, the investigators noted decreased von Willebrand factor and P-selectin expression in lung tissue, in addition to decreased arteriolar neutrophil-platelet microemboli.[1]

The finding that antithrombotic agents currently used in the clinic induced a marked reduction in microvascular stasis



Potential involvement of tissue factor, thrombin and endothelial PAR-1 in vaso-occlusion in sickle cell disease. Hb, hemoglobin; TF, tissue factor; VWF, von Willebrand factor.

in murine models of sickle cell disease is potentially important. It provides a strong rationale for considering the evaluation of these agents to prevent vaso-occlusive events in patients with sickle cell disease. Vorapaxar is an antiplatelet agent approved for the treatment of myocardial infarction and peripheral vascular disease but is contraindicated in patients with a history of stroke[8]; therefore, it is not ideal for studying in sickle cell disease patients. Rivaroxaban appears to be safe and effective to treat venous thromboembolism in patients with sickle cell disease.[9] The investigators previously reported that both rivaroxaban and dabigatran efficiently anticoagulated sickle cell mice without spontaneous bleeding.[10]

Informed by the work of Sparkenbaugh et al,[1] the figure presents a conceptual scheme of how increased tissue factor expression, thrombin generation, and activation of PAR-1 might contribute to sickle vaso-occlusive phenomena. It is intriguing to consider that oral anticoagulants currently in use might find a niche in preventing the vaso-occlusive events that are such a burden to citizens with sickle cell disease.

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THROMBOSIS AND HEMOSTASIS

Comment on Darzi et al, page 1788

A Magic 8-Ball for inpatient VTE?

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In this issue of *Blood*, Darzi et al have reported the results of a systematic review of the literature for prognostic factors for venous thromboembolism (VTE) and bleeding in acutely ill, critically ill, and chronically ill hospitalized medical patients.[1]

The authors identified 14 studies of prognostic factors for VTE and 3 studies of prognostic factors for major or clinically relevant nonmajor bleeding in hospitalized patients. The authors report finding moderate-certainty evidence of association among 18 characteristics and increased risk of VTE, with variable strengths of association. Only 8 prognostic factors, including male sex, elevated D-dimer, elevated C-reactive protein (CRP), elevated heart rate, thrombocytosis, Barthel Index (a measure of functional independence) ≤9, immobility, paresis, previous VTE, thrombophilia, and active or past history of cancer had calculated odds ratios (ORs) of 2 or more.

There was moderate certainty of association between 15 characteristics and increased rates of bleeding, with similar variability in strength of association. Only 6 prognostic factors were calculated to increase the OR to 2 or greater, including