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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Allali et al, page 1972

Sickle cell inflammation: is HbS the answer?

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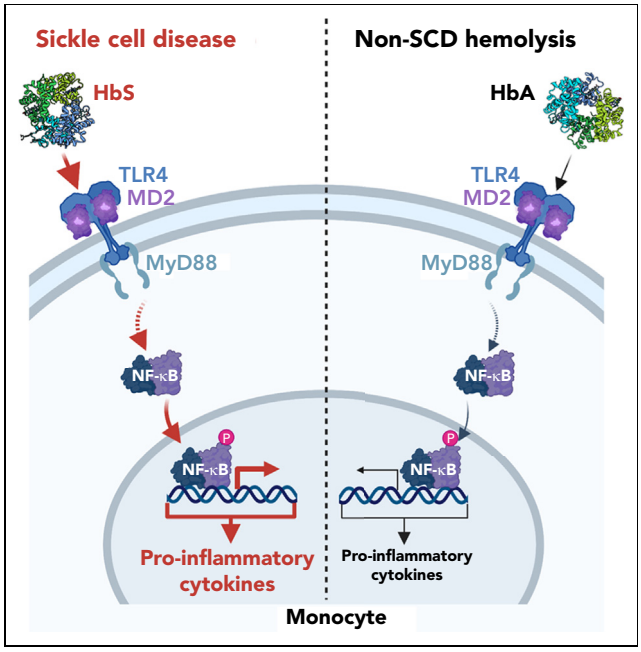
In this issue of *Blood*, Allali et al report that free hemoglobin S (HbS) released by intravascular hemolysis leads to monocyte activation and inflammatory cytokine production in patients with sickle cell disease (SCD).[1] Strikingly, the effects of HbS are reported to be significantly and profoundly greater than the effects of hemoglobin A (HbA), despite the difference of only one amino acid between the hemoglobins.

Allali et al address an old and perplexing issue: Why do patients with SCD have elevated baseline inflammatory cell activation and elevated levels of nearly all proinflammatory cytokines, compared with patients with β-thalassemia or patients without SCD with intravascular hemolysis? In patients with SCD, proinflammatory cytokines induced by NF-κB signaling (ie, tumor necrosis factor [TNF], interleukin 6 [IL-6], and IL-1β) are elevated at baseline and further increase during vaso-occlusive crises, during which they activate inflammatory cells, promote endothelial cell damage, and ultimately potentiate vaso-occlusion.[2] In addition, elevated levels of type 1 interferons (IFN-α/β) have been recently identified in patients with SCD,[3] with these inflammatory interferons having implications during red blood cell (RBC) transfusion and viral infection.[4] However, triggers for cytokine production in SCD represent a gap in knowledge in the field.

One described mechanism is that patients with hemolytic anemia have elevated levels of free heme, which has been shown to activate endothelial cells during vaso-occlusive crises.[5] Hemin, the oxidized form of heme, binds a complex of Toll-like receptor 4 and myeloid differentiation 2 (TLR4/MD-2), resulting in downstream production of NF-κB–induced cytokines. Although activated monocytes producing these proinflammatory cytokines are elevated in patients with SCD,[2] hemin fails to induce IL-6 production by monocytes of patients with SCD, despite elevated expression of TLR4.[6]

Allali et al hypothesized that HbS may contribute to the enhanced inflammation in SCD, compared with patients without SCD who had hemolytic anemia. They illustrated that although hemin and HbA had minimal effects, addition of HbS from patients with SCD to monocytes from healthy controls profoundly stimulated IL-6 and other NF-κB–induced cytokine production (see figure). They also demonstrated that HbS induced expression of IFN-α/β–stimulated genes to a lesser degree. HbS, but not HbA, promoted chemotaxis of monocytes. These effects were abrogated when adding a TLR4 inhibitor or when studying monocytes lacking TLR4. The authors also used surface plasma resonance to illustrate that HbS has strong affinity for TLR4/MD-2, whereas HbA has significantly lower affinity. Finally, treatment of humanized sickle cell mice with HbS led to increased serum TNF and IL-6, whereas HbA had a minimal effect. Interestingly, treatment of control mice with either hemoglobin had no effect on cytokine production, which may be due to hemoglobin binding to haptoglobin, which is absent in sickle cell mice.

One caveat to these findings is that lipopolysaccharide (LPS; ie, endotoxin) also binds the TLR4/MD-2 complex on monocytes and strongly induces NF-κB–induced cytokines and IFN-α/β.[7] The authors excluded hemoglobin preparations containing detectable endotoxin, and by utilizing an antibiotic that inactivates LPS, they showed that LPS inactivation had no effect on HbS-induced TNF and IL-6 production. Although this indicates that HbS preparations did not contain LPS, HbS content, measured by an oxidizing reagent, ranged from 56% to 88% of total protein in HbS preparations. Thus, the possibility that undetectable levels of LPS or other components in the hemoglobin



Patients with SCD have high levels of proinflammatory cytokines, which can potentiate SCD sequalae. The work by Allali et al demonstrates that cell-free HbS induces high levels of proinflammatory cytokine production by monocytes. The authors further elucidate the pathway by showing that HbS, although differing by only one amino acid from HbA, has high affinity for the TLR4/MD-2 complex. On ligation of HbS to the TLR4/MD-2 complex, downstream signaling induces NF-κB activation and subsequent proinflammatory cytokine production.

preparations influenced cytokine production cannot fully be excluded.

Nevertheless, the elevated responses to HbS beg the question: Why is the affinity of HbS for TLR4/MD-2 so much greater than that of HbA? This question requires further investigation. LPS and other endogenous hydrophobic molecules bind a large hydrophobic pocket within MD-2.[7] Given that HbS has a hydrophobic valine instead of hydrophilic glutamic acid in HbA, the authors postulate that the hydrophobicity of HbS may lead to increased affinity for TLR4/MD-2. The testing of this hypothesis is eagerly awaited.

Although the data presented are striking and surprising, we must ask: What is the potential implication of identifying HbS-induced inflammation? The authors hypothesize that inhibiting the interaction between HbS and TLR4/MD-2 with novel small molecules could interrupt the vicious cycle of inflammation during vaso-occlusion. Such an intervention has the potential to improve vaso-occlusive sequelae, including acute chest syndrome, cerebrovascular accidents, and pain crises. The authors surmise that disrupting HbS/TLR4 interactions may be superior to general TLR4 inhibition, which would predispose patients to infection.

The induction of IFN-α/β by HbS may also have clinical implications. IFN-α/β were discovered for their role in antiviral immunity, and some reports have indicated patients with SCD are more susceptible to severe complications of viral infection.[8] In addition, IFN-α/β promote deleterious anti-RBC antibody production in murine transfusion models.[9] Given that patients with SCD have a high prevalence rate of anti-RBC antibodies, one wonders whether HbS-induced IFN-α/β production may contribute to alloantibody formation.

As the identification of elevated levels of IFN-α/β in patients with SCD is a relatively new finding, recent studies have addressed mechanisms underlying IFN-α/β production. Mitochondria from SCD RBCs were reported to induce IFN-α/β in neutrophils.[10] Intravascular hemolysis in mice with SCD induces IFN-α/β production by hepatic monocytes, which enhances erythrophagocytosis of transfused RBCs. Interestingly, hemolysis-induced IFN-α/β production was shown to be independent of TLR4 activation.[3] Thus, multiple mechanisms, including HbS/TLR4 interactions, contribute to IFN-α/β production in SCD, and more are likely to emerge.

In conclusion, the authors are congratulated on significant progress toward identifying mechanisms that promote inflammation in patients with SCD. The striking difference in HbS- and HbA-induced inflammatory cytokine production and monocyte activation is highly novel and will lead to studies that shed light on an underlying mechanism. Although other sources also contribute to inflammation in SCD, identification of HbS-induced inflammation by monocytes, and possibly other inflammatory cells, has the potential to lead to interventions that combat severe sequelae of inflammation in patients with SCD.

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

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THROMBOSIS AND HEMOSTASIS

Comment on Simon et al, page 1983

Safer steps on a narrow path

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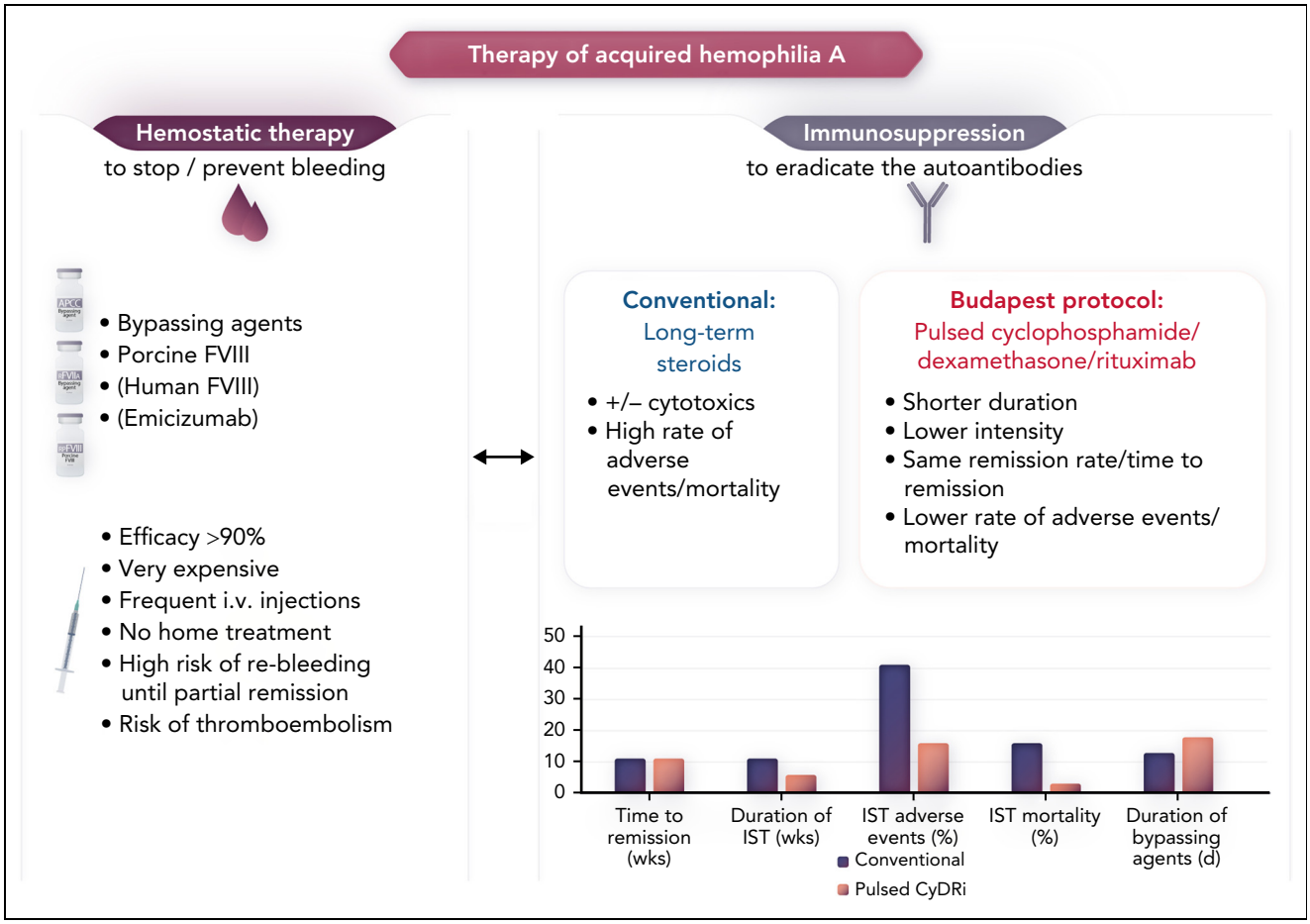
Choosing the best immunosuppression for acquired hemophilia A (AHA) is a narrow path between shortening the time to remission and avoiding deleterious adverse effects. The article in this issue of *Blood* by Simon et al[1] provides some safer steps to accomplish this.

In AHA, autoantibodies block coagulation factor VIII (FVIII), which leads to severe bleeding. This requires very expensive hemostatic therapy with bypassing agents (recombinant FVIIa, activated prothrombin complex), or recombinant porcine FVIII.[2] For immunosuppression (the causal therapy for eradicating the autoantibodies), daily doses of steroids with or without other cytostatic or immunosuppressive drugs, especially cyclophosphamide, for several weeks have long been used, but they cause an unacceptable high rate of adverse events (up to 46%) and mortality (16%) (see figure). These high rates have been demonstrated in the large European Acquired Haemophilia Registry (EACH2)[3] and the prospective German, Austrian and Swiss Society of Thrombosis and Hemostasis Research (GTH)-AH 01/2010 trial[4] in which half the affected patients were older than age 74 years and were often in frail condition.[5]

Simon et al report on using a different strategy for immunosuppression, which seems to be equally effective in eradicating the autoantibodies but is better tolerated with fewer adverse events. Their strategy is quite similar to some myeloma protocols: pulses of 1000 mg of cyclophosphamide (a moderate dose) on days 1 and 22 and 40 mg of dexamethasone (a high dose) plus 100 mg of rituximab (a low dose) on days 1, 8, 15,



The figure depicts the interdependence between the expensive hemostatic therapy and the immunosuppression with a high rate of adverse events in patients with AHA. The pulsed CyDRi protocol may shift the scales, offering a safer, less toxic pathway for this largely older patient population. IST, immunosuppressive therapy. Professional illustration by Somersault18:24.