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DOI 10.1182/blood.2020008466
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TRANSFUSION MEDICINE

Comment on Pal et al, page 269

Heme control to major B (cell): are you listening?

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In this issue of *Blood*, Pal et al demonstrate that heme released during hemolysis inhibits human plasma B-cell differentiation, but this pathway was inhibited in B cells from alloimmunized patients with sickle cell disease (SCD). Thus, insensitivity to heme inhibition in B cells may be a potential alloimmunization risk factor.¹

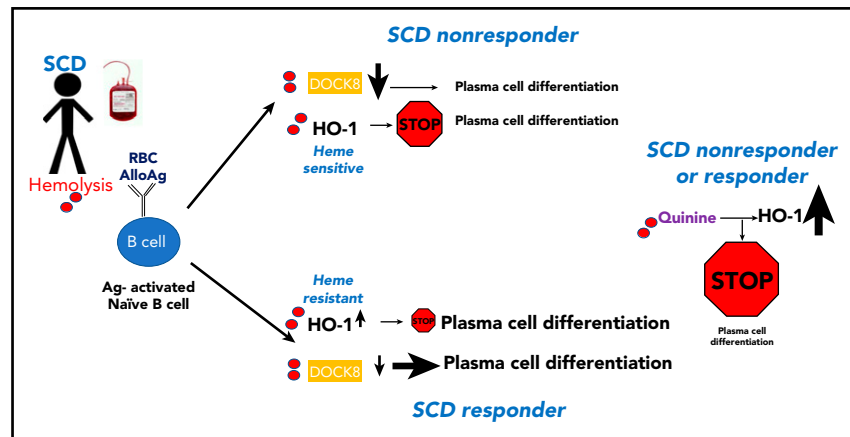
SCD patients are at high risk of alloimmunization, and some patients, referred to as responders, develop alloantibodies after only a few transfusions. Alloimmunization can be life-threatening when it causes delayed hemolytic transfusion reactions (DHTRs). In the severest cases of DHTRs, both transfused and autologous red blood cells (RBCs) are destroyed,

releasing free hemoglobin and heme that can damage the underlying vasculature. The occurrence and clinical progression of DHTRs is unpredictable. In about one-third of these reactions, no antibodies are detected, which further confounds the enigmatic nature and underestimated potential of DHTRs.²⁻⁴ Blood group matching, either phenotypically or genetically, is

effective but because of its availability and/or cost, is not universally used, which increases the incidence of DHTRs. Our group has developed a transfusion strategy to minimize the occurrence of DHTRs that is based on clinical parameters, frequency of transfusions (eg, chronic vs episodic), alloimmunization status, and history of DHTRs. A nomogram to diagnose DHTRs has also been developed.^{3,5,6}

A parallel tactic for prevention has been to understand the mechanisms of alloimmunization in SCD, including genetic approaches, which have identified several candidate gene alleles associated with the responder or nonresponder traits in patients with SCD.⁷ In vitro assays with samples from patients with SCD and in vivo animal models have been equally informative in identifying the immune cell types involved in the response to transfusion.⁸ It is likely that some mechanisms that govern RBC alloimmunization will be common across diverse at-risk patient populations such as pregnant women, patients with thalassemia, and patients with neoplastic diseases who require repeat transfusions. However, it is also possible that some responder and nonresponder mechanisms are completely unique to SCD. Patients with SCD suffer from chronic hemolysis with an estimated one-third of RBC destruction occurring intravascularly. The released hemoglobin and specifically its oxidized byproduct, heme, can induce cellular damage and inflammation. However, cell-free heme also causes the upregulation of heme detoxifying enzyme heme oxygenase 1 (HO-1) which, through its enzymatic byproducts, carbon monoxide and biliverdin, induces anti-inflammatory properties in human immune cells. A differential response to heme has been described in monocytes of SCD responders and nonresponders, in part because of differences in monocyte levels of HO-1.⁹ Little is known regarding the direct effects of hemolysis on the humoral immune cell response and alloimmunization.

In the study by Pal et al, the authors have uncovered heme sensing pathways that are crucial checkpoints that control B-cell differentiation in patients with SCD. They demonstrate in vitro that exogenous heme inhibits differentiation of B cells into plasma cells in healthy controls and that heme-mediated inhibition occurs through blocking the DOCK8/STAT3 signaling pathway, which is critical for B-cell activation and upregulation of HO-1.



Proposed model of heme pathways in SCD B cells and response to allogeneic transfusion. Upon engagement with RBC alloantigens, activated naïve B cells from nonresponder SCD patients do not differentiate into plasma cells because of heme-mediated downregulation of DOCK8 and HO-1-mediated signaling. In responder SCD patients, these heme pathways are impaired, enabling plasma cell differentiation to proceed. The combination of heme and the heme-binding molecule quinine causes induction of very high levels of HO-1 and suppression of plasma cell differentiation in both responder and nonresponder SCD patients. Ag, antigen.

Differentiation of B cells from non-alloimmunized SCD patients was also inhibited by cell-free heme through the same pathways, but B cells from responders did not respond to heme or HO-1 and readily differentiated into plasma cells. These results imply that heme pathways may have a unique impact on B-cell differentiation and possibly alloimmunization in patients with SCD. Hemolytic indices such as lactate dehydrogenase, hemopexin, and haptoglobin levels or reticulocyte percent did not differ between responder and non-responder SCD patients. When added to B cells from healthy non-SCD controls, the effects of sera from either group on plasma cell differentiation were similar, arguing against possible differences in the hemolytic milieu between responders and nonresponders. Instead, the results point to intrinsic B-cell differences in the response to heme and/or sensitivity to heme and the development of antibody-producing plasma cells. Besides B cells, an impaired heme response has been detected in various steps involved in the initiation of a humoral response that includes monocytes and dendritic cells of responders with SCD, further supporting a role for heme-sensing pathways in the control of alloimmunization in SCD.^{9,10} The authors speculate that the effects of heme on B cells and humoral responses would be limited to organs and sites with elevated local heme levels such as the spleen in which RBC destruction occurs. RBC alloimmunization which primarily develops in the spleen would thus be impacted by heme, but not by vaccine responses which develop in lymph nodes or at the sites of vaccine delivery such as the skin or muscle where little or no RBC destruction occurs.

The study has some implications for our understanding of DHTRs in terms of antibody development. In patients who

have severe DHTRs and hyperhemolysis, the reason why some patients have no detectable antibodies may be that their B cells have intact heme sensors with the ability to block B-cell differentiation programs, whereas those in whom new antibodies are identified have impaired B-cell heme pathways. Studies of human SCD patients with DHTRs are needed to confirm this. Mouse experiments cannot be pursued because mouse B cells do not seem to use the same B-cell heme pathways as human B cells.

An interesting translational aspect of the study was the identification of quinine, an antimalarial US Food and Drug Administration–approved drug that targets heme, for its ability to reverse B-cell resistance to heme inhibition in SCD responders. By complexing with heme, quinine induced a fivefold increase in HO-1 levels in stimulated B cells and exhibited potent immunomodulatory activity. Quinine inhibited plasma B-cell differentiation in both responders and nonresponders. Heme scavengers such as hemopexin were not tested. The potential for the novel therapeutic use of quinine to prevent alloimmunization in patients with SCD is exciting. SCD mice were not affected by quinine treatment, most likely because of species differences in alloimmune and B-cell heme responses between human and mouse SCD. So human clinical studies will be needed to test this further.

In summary, the study by Pal et al highlights the unique role of hemolysis and differential heme response pathways in patients with SCD. This may help identify novel biomarkers and therapeutic interventions against RBC alloimmunization and possibly other SCD complications.

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

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DOI 10.1182/blood.2020009593

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