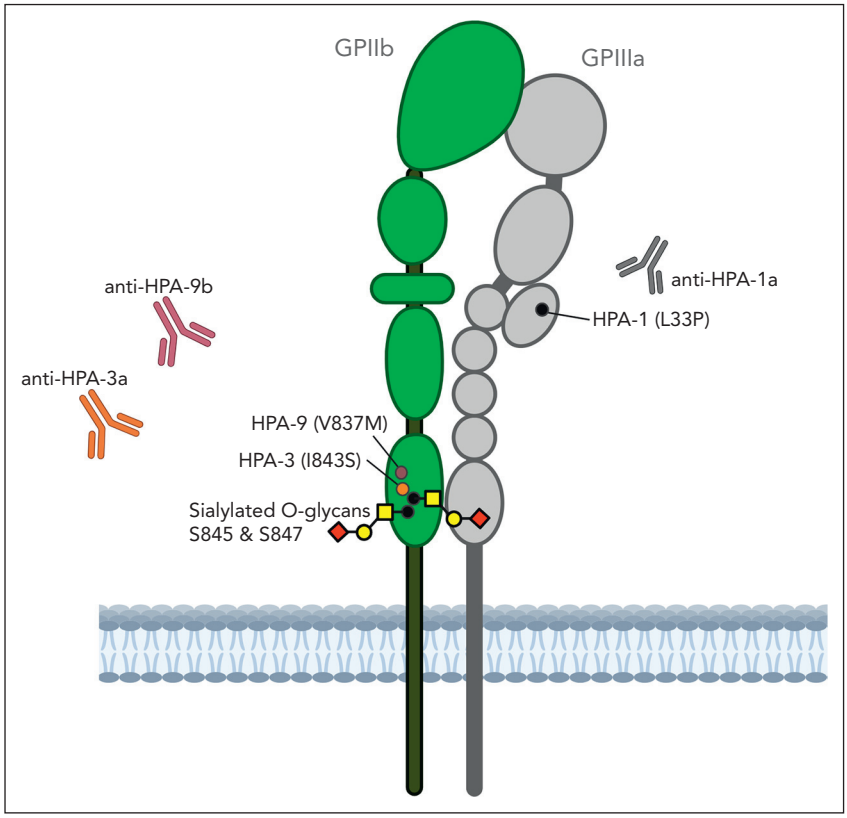

The reactivity of some alloantibodies to HPA-3a and HPA-9b may be sensitive to the sialylation status of the O-linked glycans at nearby serine residues on GPIIb. Figure created with Biorender.com.

cells and thus potentially being a target of anti–HPA-1a. However, FNAIT cases with antibodies to HPA-9b, HPA-3a, or HPA-3b (all on GPIIB) have also been implicated in FNAIT complicated by ICH, suggesting that the "promiscuity hypothesis" is not the sole explanation for ICH. Also, why these 2 antigens close together on GPIIB (figure) are linked in both having hard-to-detect alloantibodies that may become easier to identify with desiayalation is unclear. Finally, it is not known how many other platelet antigens are similar.

Research is essential for moving FNAIT forward despite limited sample sizes available due to the rarity of the condition. Expanding studies on the use of cell lines, including those with altered glycosylation, may improve antibody testing well beyond the rare cases of HPA-9b incompatibility (HPA-3a and HPA-3b incompatibilities occur in 3%-10% of pregnancies) and may help to delineate the extent to which certain antibodies tend to induce ICH in FNAIT-affected thrombocytopenic neonates. The proof of principle reported here opens up the FNAIT field, and we may anticipate with this and other techniques further sophisticated antigen characterization and improved antibody detection in the future.

Conflict-of-interest disclosure: J.B.B. is a consultant for RallyBio, Janssen, UCB, and Argenx. M.T.A. declares no competing financial interests. ■

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https://doi.org/10.1182/blood.2023022495

VASCULAR BIOLOGY

Comment on Ivy et al, page 1918

Cold comfort in sickle cell disease

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In this issue of *Blood*, Ivy et al[1] show that the alternative complement pathway drives vaso-occlusion and pain after cold exposure in a mouse model of sickle cell disease (SCD).

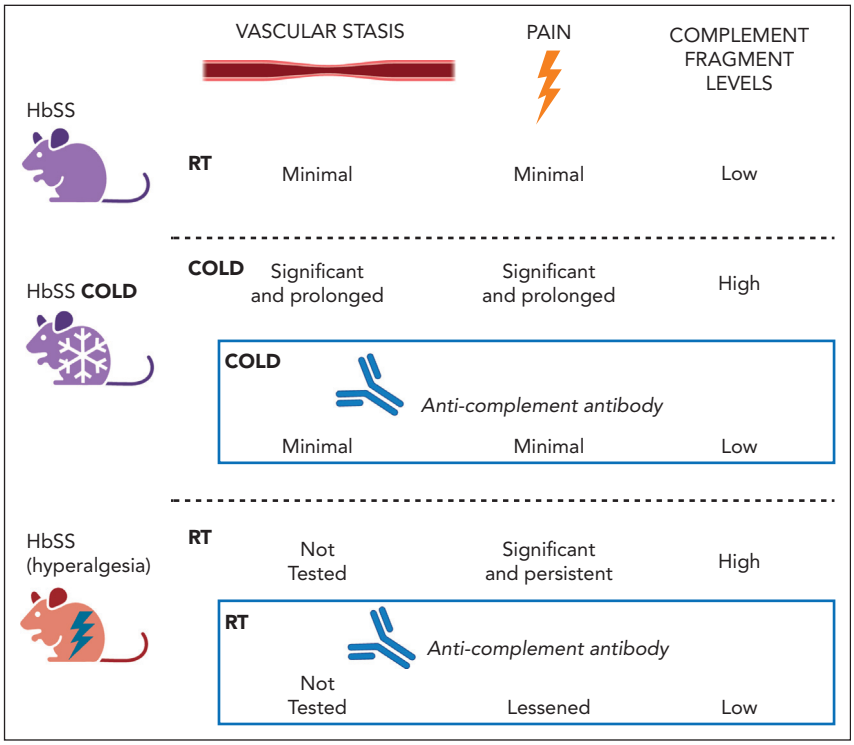
In Minnesota, they must think about cold a lot. Kofi Annan said that, as a young Ghanaian student in St. Paul, he found earmuffs "inelegant"—until it was −23°F. "I learned a precious lesson—that you don't walk into a situation . . . and pretend you know better than the locals. . . . You better listen to them and look at what they do."[2] Anecdotally, people with SCD have told providers that their symptoms are worse in the cold, in the heat, when the weather changes, and after getting out of the pool; epidemiological and pain testing suggests this as well.[3,4] In this work, Ivy et al have used a murine model of pain in SCD that listens to the locals—that is, may more closely mimic what people with SCD actually experience in their lives. The very interesting results are revealing about microvascular stasis, complement, and the systemic effects of cold exposure on pain and inflammation and may help us think about new approaches to sickle cell that extend beyond buttoning up one's overcoat.

Sickle hemoglobin (HbS) is a gain-of-function mutation in the β chain of Hb, in which Hb molecules acquire the ability to polymerize when deoxygenated. This remarkable mutation has been described molecularly since at least 1949,[5] but our knowledge about the relationship of this "simple" single base pair mutation of β-globin gene and its profound impact on quality of life for individuals living with SCD has been slow to come and is incomplete. Ivy et al's work is a step in the direction of understanding this complicated relationship.

Based on earlier work,[6,7] this group was interested in the role of complement in SCD, and here, rather than using biochemical triggers of vaso-occlusive episodes (complement fragments, free Hb, hypoxia), they instead used exposure to cold (50°F). The Townes humanized mouse model of sickle Hb will, with time alone, develop hyperalgesia, and the investigators selected only non-hyperalgesic (low pain) mice for comprehensive studies of microvascular stasis in dorsal skin fold chambers, complement levels, quantitative pain thresholds, and hepatic inflammation. Cold exposure induced prolonged pain and vascular stasis in HbSS-containing mice; strikingly, this was associated with a marked increase in detectable plasma complement factor B's active subunit (Bb) and complement component 5a (C5a). (see figure). Pre–cold exposure treatment with anti-C5a and C5a receptor antibodies decreased levels of plasma C5a and also, somewhat mysteriously (as is the way with complement), Bb, while abrogating cold-induced vascular stasis and hyperalgesia in these mice.

A few additional points: these authors had earlier described endothelial upregulation of P-selectin (via Weibel-Palade bodies) after infusion of purified C5a. In the current, more physiological, studies, they showed that cold-induced microvascular stasis and pain were also inhibited by pretreatment with anti-P-selectin antibodies. Notably, P-selectin involvement in mechanical hyperalgesia was not seen in a different pain model using non-HbSS mice. This suggests that P-selectin selectively influences pain downstream of vaso-occlusion, rather than having a broad impact on pain sensation itself. Finally, the supplementary figures from Ivy et al showing nuclear factor κB phosphorylation and upregulation of vascular endothelial adhesion markers in the liver at least 4 hours after cold exposure remind us how systemic and long-lasting the aftereffects of temperature change may be in SCD.

This is a great thought-provoking study, and one that resists the clean arrows of mechanistic figures. So now, a paragraph of questions: What comes first following cold exposure: Hb polymerization and hemolysis, complement activation, vascular stasis, or pain?[8] These authors showed that blocking complement affects vascular stasis and pain, but does primarily blocking Hb polymerization or hemolysis, vascular stasis, or pain affect the other phenotypes? Does cold exposure induce hemolysis? And how many circles are there within these circles—for instance, between inflammation and dysautonomia in HbSS, which may exacerbate aberrant responses to cold?[9] This relationship is hinted at in supplementary figure 4 in Ivy et al, in which acetylcholine abrogates microvascular stasis following cold exposure in HbSS; it would be good to know whether complement levels or pain are lessened there as well. Also unclear is how these data apply to variant disease hemoglobin SC (HbSC) or hemoglobin SBeta+thalassemia (HbSβ$^+$ thalassemia), which was not tested here.



Vascular stasis, pain, and complement in a mouse model of HbSS. Shown is a summary of key results from Ivy et al,[1] in which non-hyperalgesic mice were examined at room temperature (RT) and then following cold exposure. Hyperalgesic mice were also studied at RT. Figure prepared with BioRender.com.

The hyperalgesic HbSS mice are also very interesting. Without cold exposure, these mice have pain and elevated complement fragments, both of which are lessened by treatment with anti-complement antibodies. Nothing is reported yet about vascular stasis or response to cold in these mice. Can these mice tell us something about the pathophysiology of chronic pain in HbSS?

This work reminds us how profoundly the environment can affect people with SCD; it goes without saying that comfort and warmth benefit all chronically ill people, and in those with SCD, the latter may literally be true. However, as eager as one is to make meaningful change in symptom burden for people with SCD, the specter of preemptive and chronic complement blockade in patients who may have hyposplenism is daunting (NCT 05075824 and NCT 05565092[10]) and will need to be monitored very closely, so that infectious risks can be identified and minimized.

Conflict-of-interest disclosure: J.L. receives research support from Pfizer, Novo-Nordisk, and the American Society of Hematology. E.S. receives research support from CSL Behring and National Institutes of Health. ■

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https://doi.org/10.1182/blood.2023022621

RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Denton et al, page 1932

Deironing the spleen with luspatercept

Domenico Girelli[1,2] and Giacomo Marchi[1,2] | [1]University of Verona and [2]Azienda Ospedaliera Universitaria Integrata Verona

In this issue of *Blood*, Denton et al[1] show that luspatercept induces iron redistribution by reducing splenic iron without changing liver iron content in patients with β-thalassemia (β-thal). This challenges the understanding that the iron content of the liver reflects total body iron and suggests that magnetic resonance imaging (MRI) scans should ideally include the liver and spleen to adequately monitor iron status in patients with iron-loading anemias treated with an erythroid maturation agent.

Ineffective erythropoiesis (IE) implies an expansion of early-stage erythroid progenitors and apoptosis of their late-stage counterparts. IE is the key defect in the so-called iron-loading anemias, which include both inherited (eg, β-thal) and acquired (eg, low-risk myelodysplastic syndromes [MDSs]) disorders.[2] In iron-loading anemias, the iron overload is related to chronic transfusions and/or increased iron absorption due to hepcidin suppression by factors released by disturbed erythroid progenitors, and substantially contributes to the disease's burden. Targeted therapy for IE has been a long-standing goal, recently addressed by the development of luspatercept, a first-in-class erythroid maturation agent. Luspatercept is a recombinant fusion protein that binds transforming growth factor-β (TGF-β) superfamily ligands, thereby preventing their interaction with type II TGF-β receptors. This counteracts the constitutive SMAD2/3 signaling, which inhibits red blood cell maturation in IE, ultimately promoting erythropoiesis. Unexpectedly, despite successes in improving anemia and reducing transfusion burden in either β-thal or low-risk MDS,[3,4] luspatercept did not reduce the liver iron concentration (LIC) estimated by MRI, which is now considered the best available proxy of total body iron. Using an internal protocol, Denton et al measured spleen iron concentration and content (ie, concentration × volume) in addition to usual liver iron parameters in patients with β-thal before and after luspatercept therapy (median time of drug exposure, 30.9 months). Although confirming that LIC remained unchanged, the authors observed a substantial decrease in spleen iron content, with a Cohen *d* coefficient (the difference between 2 means divided by the SD, which estimates the effect size) ≥1.0, which means a large to very large effect. This suggests that luspatercept may induce a redistribution of iron, ultimately leading to a decrease of total body content, which is not captured by the current measurement of LIC. This study has important limitations. First, the number of patients with β-thal examined (11 transfusion dependent and 4 non–transfusion dependent) was small. Second, the MRI-based estimate of spleen iron might be not as precise as liver iron, because of the obvious inability to validate the method through biopsy and direct iron quantification. Nevertheless, if confirmed, the observation by Denton et al implies a key practical message about the