bind to precisely the same amino acids on PF4 as heparin.[3] Danaparoid, a low-molecular-weight heparinoid that consists predominantly of dermatan sulfate and low-sulfated heparan sulfate, presumably also binds to positively charged PF4 and thus can displace VITT antibodies in a similar manner, because it has been shown to inhibit the formation of PF4-heparin complexes.[4]

These basic mechanistic data provide important biological information about VITT-induced thrombosis; however, the safety of heparin as a treatment for VITT remains uncertain. Evidence in favor of its use include in vitro studies like the one by Singh and colleagues showing that platelet activation induced by VITT sera can be inhibited by the addition of pharmacological concentrations of heparin,[5] as opposed to HIT where the addition of pharmacological heparin enhances platelet activation in vitro. In addition, there have been several reports of patients with VITT who were treated with unfractionated heparin without evidence of worsening outcomes.[6] On the other hand, recent data have shown that some VITT antibodies can bind to sites on PF4 that are distinct from the heparin binding site, as described in a report of VITT following the Ad26.COV2.S (Johnson & Johnson/Janssen) vaccine.[7] Thus, it is possible that heparin can enhance platelet activation in some VITT patients, similar to HIT. Furthermore, in a large clinical study of patients with VITT (n = 220), mortality was higher among patients who received unfractionated heparin compared with nonheparin alternatives (10/50 [20%] vs 27/170 [16%]).[8]

Despite the rapid decline in the incidence of VITT and the discontinuation of adenoviral vector vaccines for COVID-19 in many countries, there is an ongoing need for further clinical and basic research in this highly prothrombotic disorder. Adenoviral vector vaccines will continue to be used for COVID-19 in many countries; thus, there is a global responsibility to optimize their safety. In addition, some patients with VITT continue to show serological evidence of anti-PF4 antibodies and clinical morbidities even 1 year later ("long VITT"[9]), and optimizing their treatment is a priority. Finally, understanding the mechanisms by which anti-PF4 antibodies alone can cause such severe thrombosis provides a unique opportunity to advance our understanding of the intersection between immunity and thrombosis.[10]

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Doty et al, page 3439

Defending the island against excess heme

Lionel Blanc[1,2] and Jeffrey M. Lipton[1,2] | [1]The Feinstein Institutes for Medical Research; [2]Cohen Children's Medical Center of New York

In this issue of *Blood*, Doty et al[1] present evidence that excess heme generated in the Diamond Blackfan anemia (DBA) erythron has the unique ability to affect normal erythropoiesis. This DBA extrinsic mechanism suggests to the investigators that the level of chimerism achieved by genetic manipulations must be considered in designing gene therapy/editing approaches.

First described in 1936 by Josephs and well characterized in 1938 by Diamond and Blackfan, DBA is a rare inherited bone marrow failure syndrome and the founding member of a novel class of disorders, the ribosomopathies.[2] Patients with DBA classically present with moderate to severe macrocytic anemia, reticulocytopenia, short stature, and a predisposition to cancer.[3] Mutations in >20 ribosomal proteins as well as some nonribosomal proteins account for ~75% of the cases; 25% remain unexplained. *RPS19* was the first gene identified to be mutated in DBA[4] and represents ~25% of the cases.

The heterogeneity of mutations and the variable expression and penetrance of these mutations suggest multiple mechanisms for the clinical manifestations of DBA. It is likely that all of the mechanisms described thus far are operational to a greater or lesser extent, perhaps the consequence of the specific haploinsufficient ribosomal protein. They include global and specific translation defects, cell-cycle perturbation, p53 activation, abnormal GATA1 expression, and the toxicity of excess heme caused by dyssynchronous globin synthesis.[5] Thus, which mechanism to address in order to

permit the best therapeutic option is the subject of considerable thought.

Studies demonstrating increased heme toxicity in the erythron suggest that mitigating toxic heme levels could be beneficial to patients with DBA. Eltrombopag (EPAG), a thrombopoietin-mimetic agent approved for the treatment of immune and other causes of thrombocytopenia, has recently been shown to be a potent intracellular iron chelator.[6] In accordance with these findings and consistent with a toxic role for intracellular heme in the DBA erythron, a recent study showed that EPAG was effective in rescuing the anemia in a patient with DBA.[7] However, the mutation involved as well as the precise mechanism by which the patient responded to EPAG remained unknown.

To address this, Doty et al first undertook a genetic characterization of the mutation in this patient with DBA. Surprisingly, they found that the patient was mosaic for a novel pathogenic mutation in RPS19. Furthermore, although the patient responded to EPAG, the anemia returned when EPAG was discontinued. Because EPAG is a TPO agonist, and TPO receptors are absent from the surface of erythroid progenitors, they hypothesized that EPAG acts through chelating iron. They observed that even the normal erythron in this mosaic patient failed to support erythropoiesis. It was thus surmised that a cell extrinsic mechanism was responsible for the erythroid failure in the normal erythron.

To test this hypothesis, Doty et al used Rpl11 and Flvcr mutant mice, which present with ineffective erythropoiesis, macrocytosis, and slight reticulocytopenia. They performed competitive transplantation assays and observed significant differences between the 2 models. Indeed, even with 50% wild-type hematopoietic progenitor cells, the Rpl11-chimeras only presented with partial improvement of anemia and macrocytosis, whereas as few as 5% normal hematopoietic progenitor cells was enough to rescue the Flvcr-chimera. The Flvcr-mutant mice cannot export heme, restricting the defect to the mutant cells, whereas Rpl11 mutants have increased heme export to attempt to compensate for slowed globin synthesis.[8,9]

Key to their hypothesis is the functional unit of the erythroid niche, the erythroblastic island, composed of a central macrophage surrounded by maturing erythroid cells.[10] The authors demonstrate that in chimeric mice Rpl11 haploinsufficient erythroid precursors and wild type share a central macrophage in close enough proximity to each other to expose wild-type precursors to toxic excess heme. Thus, the investigators show that by either blocking heme egress from Rpl11-mutated cells or more practically chelating iron to reduce intrinsic cell heme, the toxicity of excess heme escaping to the erythroblastic island niche can be ameliorated.

Finally, the authors attempted to rescue the anemia in their mouse models by giving EPAG orally. There was no significant improvement in the anemia in the models, leading the authors to suggest that higher doses of EPAG are needed. Nevertheless, another hypothesis can be offered. Because EPAG is known to increase hematopoietic stem cell production in mice, it would be interesting to measure their numbers in the different models used by Doty et al and determine if the failure to rescue anemia in the Rpl11- or Flvcr-deficient mice is due to mechanisms in addition to those proposed by the authors.

Still, the findings from Doty et al offer a novel perspective in DBA research. For example, although anemia owing to a block in the differentiation of erythroid progenitors is undeniable, one must consider the role of heme toxicity and its consequences in the erythroblastic islands when designing therapies.

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