

synthesized in other organs. For example, does hepcidin synthesized in the heart play an essential role in baseline cardiac physiology or the response of mice to cardiac iron overload, hypoxia, and inflammation? What effect does hepcidin deficiency in neurons and other resident cell types have on iron transport across the blood-brain barrier and the choroid plexus–cerebrospinal fluid barrier or on distribution of iron to specific regions within the central nervous system?

Zumerle et al’s report also has therapeutic implications. If the liver is the origin of systemically active hepcidin, then targeting hepcidin expression by the liver should be sufficient to impact systemic iron homeostasis. Indeed, pharmacologic antagonism of TMPRSS6, a physiological inhibitor of hepcidin expression present mainly in the liver, markedly attenuates the disease characteristics of mouse models of hereditary hemochromatosis and β -thalassemia.^{5,6} Other therapeutic means of modulating hepcidin expression are actively being explored, including hepcidin agonists and antagonists. Results of their application to models of diseases of systemic hepcidin deficiency or excess are encouraging.⁷ Although these novel potential treatments for iron-related diseases are tremendously exciting, such advances all originated when fundamental, mechanism-based questions about physiological processes underlying iron biology were asked and addressed by researchers such as Zumerle et al.

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

REFERENCES

- Zumerle S, Mathieu JRR, Delga S, et al. Targeted disruption of hepcidin in the liver recapitulates the hemochromatotic phenotype. *Blood*. 2014;123(23):3646–3650.
- Ganz T. Systemic iron homeostasis. *Physiol Rev*. 2013; 93(4):1721–1741.
- Walker JM. Thalassaemia major and the heart: a toxic cardiomyopathy tamed? *Heart*. 2013;99(12): 827–834.
- Crichton RR, Dexter DT, Ward RJ. Brain iron metabolism and its perturbation in neurological diseases. *J Neural Transm*. 2011;118(3):301–314.
- Guo S, Casu C, Gardenghi S, et al. Reducing TMPRSS6 ameliorates hemochromatosis and β -thalassemia in mice. *J Clin Invest*. 2013;123(4): 1531–1541.
- Schmidt PJ, Toudjarska I, Sendamarai AK, et al. An RNAi therapeutic targeting *Tmprss6* decreases iron overload in *Hfe(-/-)* mice and

ameliorates anemia and iron overload in murine β -thalassemia intermedia. *Blood*. 2013;121(7): 1200–1208.

7. Schmidt PJ, Fleming MD. Modulation of hepcidin as therapy for primary and secondary

iron overload disorders: preclinical models and approaches. *Hematol Oncol Clin North Am*. 2014;28(2): 387–401.

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● ● ● **THROMBOSIS & HEMOSTASIS**

Comment on Warkentin et al, page 3651

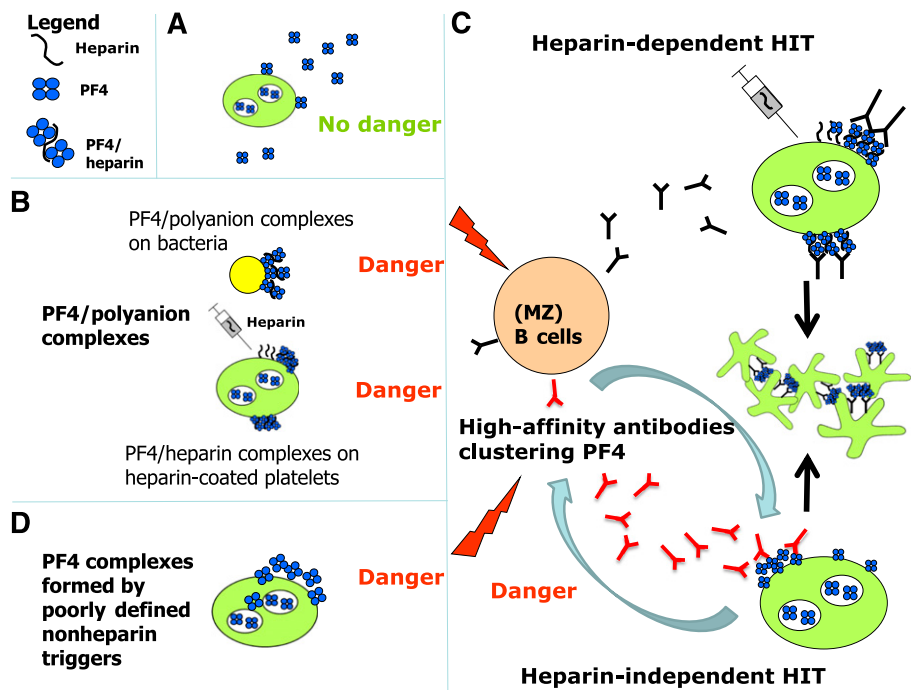
Me or not me? The danger of spontaneity

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In this issue of *Blood*, Warkentin et al provide insight into certain rare but often catastrophic cases of thrombotic complications, termed “spontaneous” (or “autoimmune”) heparin-induced thrombocytopenia (HIT).¹

Some time ago, I consulted on the case of a 31-year-old woman admitted with severe headache and thrombocytopenia. Except for an upper respiratory tract infection that began 10 days earlier, she was healthy and taking no medications. Laboratory studies showed international normalized ratio 1.4, activated partial thromboplastin time 34 s,

fibrinogen 0.6 g/L, D-dimer > 35 mg/L (<0.5), platelets $31 \times 10^9/L$, no bleeding, no signs of infection, and normal computed tomography (CT) head scan (to exclude sinus vein thrombosis). She received 4 g of fibrinogen and low molecular weight heparin (LMWH) for thrombosis prophylaxis. The next day, the platelet count was 15, she developed deep



Mechanisms of induction of anti-PF4/polyanion antibodies. (A) Noncomplexed PF4 is not immunogenic. (B) However, when PF4 forms complexes with polyanions it undergoes a conformational change exposing epitopes to which PF4/heparin antibodies bind. These polyanions can be structures on bacterial surfaces or heparin bound to platelets. (C) The PF4/polyanion complexes induce antibody production of (most likely) MZ B cells (black antibodies, upper portion of panel C), which can induce platelet activation in the presence of heparin, that is, they are typically heparin-dependent. In delayed onset HIT and spontaneous HIT, the B cells produce antibodies which bind to, and potentially thereby cluster, PF4 and then activate platelets, typically in the absence of heparin (red antibodies, lower portion of panel C). It is unresolved which factors trigger these antibodies (D): potential candidates are PF4/bacteria clusters or PF4/nucleic acid clusters; it is also unclear whether heparin-independent platelet activation is a quantitative or a qualitative feature of these antibodies.

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vein thrombosis, and her headache persisted. Although HIT seemed implausible, the coincidence of worsening thrombocytopenia and new thrombosis during LMWH prompted HIT testing, which showed, to my surprise, high-titer platelet-activating anti-PF4/heparin immunoglobulin G (IgG) antibodies. The functional heparin-induced platelet activation test (HIPA) was also strongly positive in the sample without addition of heparin. Even when testing was repeated using a pre-LMWH admission sample, the same result was obtained. Despite the immediate start of therapeutic-dose danaparoid anticoagulation, she deteriorated neurologically the same day, and massive sinus vein thrombosis associated with intracerebral bleeding was demonstrated by repeat CT imaging. Despite eventual platelet count normalization over the next week, cerebral function remained impaired, and she died.

Although the nature and pathogenesis of spontaneous HIT is still a matter of debate, this phenomenon exists, and in my opinion reflects the autoimmune-extreme of the anti-platelet factor 4 (PF4)/heparin immune response. This immune response ranges from nonplatelet-activating antibodies (enzyme-linked immunosorbent assay positive; even found in the general population and in up to 50% of non-HIT patients after cardiac surgery), to heparin-dependent platelet-activating IgG antibodies (typical for clinical HIT; but also found in up to 20% of non-HIT patients when screened after major surgery), to IgG antibodies that strongly activate platelets even in the absence of heparin. The latter pattern is typical for delayed onset and spontaneous HIT; but also, ~30% of patients with typical HIT show this pattern during the first days after onset of HIT.

How could one understand and potentially explain this unusual spectrum? The immune response toward PF4/polyanion complexes is likely an ancient immune response pattern. PF4 and its receptor CXCR3B are preserved during evolution² and the B cells producing these antibodies are marginal zone (MZ) B cells³ (at least in mice), which are also considered primitive B cells from an evolutionary viewpoint. MZ B cells can produce IgG antibodies without T-cell⁴ involvement but typically require additional danger signals to become activated. In addition, even normal individuals harbor B cells which, after in vitro stimulation, produce anti-PF4/

heparin antibodies,⁵ and patients post liver transplant can produce anti-PF4/heparin IgG despite pharmacologic T-cell suppression.⁶ Both observations imply that, also in humans, MZ B cells are likely involved in producing anti-PF4/heparin IgG.

Several years ago, we showed that PF4 binds to polyanions on bacteria and that PF4-coated bacteria induce anti-PF4/heparin antibodies in mice, which facilitate opsonization of PF4-coated bacteria. We postulated that anti-PF4/polyanion antibodies are an ancient bacterial host defense mechanism, and that HIT results when this defense mechanism becomes misdirected⁷ toward PF4 on heparin-coated platelets (see figure, panels B-C).

In 2001, the phenomenon of “delayed onset HIT” was described by Warkentin and Kelton.⁸ Here, HIT begins (or worsens) after all heparin has been stopped; although the immune response is triggered by heparin, the B cells produce antibodies that bind to platelet-bound PF4 and induce platelet activation even in the absence of heparin, as shown by strong buffer reactivity in functional assays. Similarly-reacting antibodies are found in ~30% of patients with acute HIT and, as shown by Warkentin et al,¹ in the serum of patients with spontaneous HIT.

Sachais et al provided one potential explanation for these observations.⁹ The monoclonal antibody KKO, which recognizes PF4/heparin complexes, is able to cluster PF4 itself. In other words, this antibody creates its own (HIT) antigen(s). Antibodies in spontaneous HIT also recognize PF4 in the absence of heparin and, potentially, they are also able to cluster PF4, creating the antigen(s) to which HIT antibodies bind (although this needs to be shown experimentally).

It is unresolved whether this phenomenon just depends on the quantity of circulating anti-PF4/heparin antibodies or whether it is a qualitative characteristic of a subgroup of anti-PF4/heparin antibodies that are produced as a consequence of epitope spreading of B cells. A typical example of epitope spreading is posttransfusion purpura. Here, susceptible women, previously exposed to a platelet alloantigen (usually HPA-1a), develop high-titer anti-HPA-1a alloantibodies when they are boosted many years later by a blood transfusion containing HPA-1a platelets. These newly formed high-titer

antibodies paradoxically do not follow “textbook immunology” but bind both HPA-1a–positive platelets as well as autologous HPA-1bb platelets, causing severe thrombocytopenia. Just as with spontaneous HIT and delayed onset HIT, these antibodies “broaden their specificity” but fortunately also disappear within a few weeks.

How is spontaneous HIT triggered? The frequently observed association between spontaneous HIT and recent infections or major surgery suggests the following concept: bacteria can bind PF4 and expose PF4 clusters²; PF4 also binds negatively charged nucleic acids, which are released during major surgery, forming PF4/nucleic acid complexes.¹⁰ Both mechanisms can thus generate “HIT antigens,” which in the setting of infection or major surgery induce “danger signals,” triggering B cells to produce the pathogenic anti-PF4/polyanion antibodies (see figure).

There are 3 major implications from spontaneous HIT.

First, should we test any patient with thrombocytopenia and thrombosis for anti-PF4/heparin antibodies? No! Such an approach would produce an avalanche of misdiagnoses and potentially dangerous overtreatment. However, in patients presenting with inexplicable thrombosis and thrombocytopenia, spontaneous HIT should be considered as a rare cause. As per the recommendations of Warkentin and colleagues,¹ the diagnosis should only be made if there are high-titer anti-PF4/heparin IgG antibodies and a positive functional assay for HIT antibodies with the additional feature of a positive buffer control reaction. Second, in a patient who develops moderate thrombocytopenia during post surgical thrombosis prophylaxis with one of the new anticoagulants, spontaneous HIT should be excluded before the anticoagulant is stopped.

Third, systematic studies are needed focusing on the clinical course of HIT in patients showing a positive functional assay in the absence of heparin. Potentially, these patients could have more severe HIT.

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

REFERENCES

1. Warkentin TE, Basciano PA, Knopman J, Bernstein RA. Spontaneous heparin-induced thrombocytopenia syndrome: two new cases and a proposal for defining this disorder. *Blood*. 2014;123(23):3651-3654.

2. Krauel K, Weber C, Brandt S, et al. Platelet factor 4 binding to lipid A of Gram-negative bacteria exposes PF4/heparin-like epitopes. *Blood*. 2012;120(16):3345-3352.
3. Zheng Y, Yu M, Podd A, et al. Critical role for mouse marginal zone B cells in PF4/heparin antibody production. *Blood*. 2013;121(17):3484-3492.
4. Fagarasan S, Honjo T. T-Independent immune response: new aspects of B cell biology. *Science*. 2000; 290(5489):89-92.
5. Zheng Y, Wang AW, Yu M, et al. B-cell tolerance regulates production of antibodies causing heparin-induced thrombocytopenia. *Blood*. 2014;123(6):931-934.
6. Bakchoul T, Assfalg V, Zöllner H, et al. Anti-platelet factor 4/heparin antibodies in patients with impaired graft function after liver transplantation [published online ahead of print March 24, 2014]. *J Thromb Haemost*. doi:10.1111/jth.12569.
7. Krauel K, Pötschke C, Weber C, et al. Platelet factor 4 binds to bacteria, [corrected] inducing antibodies cross-reacting with the major antigen in heparin-induced thrombocytopenia [published correction appears in *Blood*. 2011;117(21):5783]. *Blood*. 2011;117(4):1370-1378.
8. Warkentin TE, Kelton JG. Delayed-onset heparin-induced thrombocytopenia and thrombosis. *Ann Intern Med*. 2001;135(7):502-506.
9. Sachais BS, Rux AH, Cines DB, et al. Rational design and characterization of platelet factor 4 antagonists for the study of heparin-induced thrombocytopenia. *Blood*. 2012; 119(25):5955-5962.
10. Jaax ME, Krauel K, Marschall T, et al. Complex formation with nucleic acids and aptamers alters the antigenic properties of platelet factor 4. *Blood*. 2013;122(2):272-281.

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● ● ● TRANSFUSION MEDICINE

Comment on Pulsipher et al, page 3655

The donor's dilemma

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In this issue of *Blood*, Pulsipher et al identify key differences in adverse events related to donation for hematopoietic cell transplantation (HCT) from a prospective cohort of nearly 9500 unrelated allogeneic donors recruited through the National Marrow Donor Program.¹ Although the rates of life-threatening complications were very low overall (<0.3%) for donors of both peripheral blood and bone marrow, those donating bone marrow had a 4-fold increased risk of experiencing a serious adverse event. Most adverse events were acute in nature, resolving within a matter of days to weeks, and importantly, no deaths as a result of donation procedures occurred. This prospective study, with more than 20 000 donor-years of follow-up after granulocyte colony-stimulating factor administration, also showed no increased risk for hematologic or other cancers, autoimmune diseases, or thrombosis associated with growth factor mobilization. In fact, donors in this cohort had a significantly lower incidence of cancer compared with the general population, regardless of granulocyte colony-stimulating factor exposure.

Will these results affect the rates of peripheral blood stem cell (PBSC) vs marrow harvest for our current and future donors? Several potential risks and benefits are inherent with each allograft source (see figure). PBSC grafts may be viewed as more convenient and not requiring general anesthesia or recovery from an invasive surgical procedure, yet they may require several days of filgrastim and 1 to 3 days of apheresis. However, central venous access is not needed during marrow harvesting, sparing the rare but serious complications of central venous access, including pneumothorax, thrombosis, and infections. Donor safety and the preferred product for the intended recipient must both

be considered in any debate of an optimal stem cell source.² The report by Pulsipher et al demonstrates that donor safety has improved over time, and it is indeed reasonable to consider both stem cell donation methods very safe. Interestingly, a recent study of donor quality of life revealed a greater sense of meaning and emotional benefit from bone marrow over peripheral blood stem cell donation.³

Another issue is the optimal source of hematopoietic cells for the recipient. This subject has been a matter of ongoing debate since the development and refinement of apheresis procedures for the harvesting of PBSC in the 1980s and 1990s.⁴ Differences in

hematopoietic and immune reconstitution, rates of acute and chronic graft-versus-host disease (GVHD), relapse risk, and cost have led to a marked increase in the use of PBSC over marrow.^{5,6} Rates of PBSC donation (approximately 75% of collections) dominated over marrow donation (approximately 15% of collections) for adult recipients of HCT from 2002 to 2011, with cord blood HCT making up the remainder.⁷ The preferred graft source for the recently growing experience in haploidentical grafting is even less certain. Thus, it remains unclear whether recipient outcomes manifest mostly as greater risks for chronic GVHD with PBSC grafts⁸ vs the ease of collection, or whether these small but recognized differences in donor safety will alter this trend, at least for unrelated donor transplantation. Bone marrow is generally preferred for most nonmalignant diseases and for children in whom any extra risks for chronic GVHD are unwarranted. The growing experience with cord blood transplants as an acceptable hematopoietic stem cell source raises new options that pose no risk to the donor, in contrast to the risks inherent in a marrow harvest or apheresis. However, a suitable graft, collectable in many ways, may not be the ideal graft for any cohort of recipients. The cellular composition varies substantially with different hematopoietic stem cell sources, and as such, the optimal cellular content of T cells, myeloid cells, and other subsets that result in the best outcomes, reproducible across the different graft sources, remains to be defined.⁹

Because it is unlikely that unrelated donor HCT is going to become outmoded in the near future, how can we best serve our unrelated donors, who make possible thousands of potentially life-saving HCT procedures each year? Continued studies on donor safety¹⁰ and quality of life³ should remain a priority, but novel methods of hematopoietic allograft engineering and improved pharmacologic approaches to stem cell mobilization, such as the upcoming Center for International Blood and Marrow Transplant Research-sponsored study of plerixafor for related donor HCT, should also be a priority. Such new techniques could one day obviate the need for surgical procedures or prolonged cytokine administration, thus avoiding the present dilemma of marrow vs PBSC donation for future donors.