clones that preferentially contribute to either lymphoid or myeloid lineages. The existence of such lymphoid-biased progenitors with long-term engraftment potential has recently been reported by Biasco et al in a clinical trial of HSPC gene therapy for WAS.[9] However, in contrast to Biasco's interpretation, Six et al suggest that such progenitors can be present for both lymphoid-dominant HSPCs and myeloid-dominant HSPCs, and that these lineage-biased progenitors can coexist within a bigger pool of unbiased multipotent HSPCs (see figure). The question of whether these lineage-dominant clones are the result of the actual transplant process or whether they also exist in an unperturbed human hematopoiesis as shown by Sun et al[7] in mice has been the topic of active discussions in the hematopoiesis field. Recent studies in the mouse by the Weissman laboratory suggest that some of these differences can indeed be the result of different transplantation conditions and conditioning regimens.[10] Thus, there remain plenty of unanswered questions in this rapidly evolving research area, but the findings by Six et al add to the growing body of studies challenging the originally proposed hierarchical lineage-relationships in human hematopoiesis and add valuable patient data to ongoing discussions.

Insights from this publication into human HSC biology aid to eventually close a long-standing gap between human hematopoiesis and the basic/preclinical HSPC research in animal models, such as the mouse or NHP, where lineage-dominant HSPCs have already been described. Findings in this publication significantly strengthen the translational potential of currently developed strategies in such animal models for the treatment of various hematological diseases and disorders. Although we are still not able to reliably identify such lymphoid- or myeloid-dominant human HSPC subsets using a defined set of cell surface markers as it is possible in the murine system, the potential targeting of these subsets for the treatment of specific diseases (such as immunodeficiencies or hemoglobinopathies) could be of clinical relevance.

Thus, with their improved processing and bioinformatics pipeline for vector-mediated ISA, Six et al have raised the bar and created a new standard for the analysis of such complex clonal tracking datasets in patients after HSPC gene therapy. Using their improved analysis technology, we can expect to further deepen our insights into human HSPC biology and hematopoiesis after transplantation.

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THROMBOSIS AND HEMOSTASIS

Comment on Johnston et al, page 1270

HIT: still stringing us along

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In this issue of *Blood*, Johnston and colleagues report that platelet factor 4 (PF4) binds to von Willebrand factor (VWF) strings, forming antigenic complexes that are recognized by heparin-induced thrombocytopenia (HIT) antibodies. Moreover, these PF4/VWF/immunoglobulin G complexes enhance platelet adhesion to injured endothelium via platelet FcγIIA receptors and GPIb-IX receptors, contributing to larger platelet-rich thrombi within the arterial circulation.[1]

HIT is among the most prothrombotic disorders in medicine. For patients diagnosed with HIT based upon a positive platelet activation assay with immunoassay corroboration, the frequency of thrombosis is at least 50%, representing a 12- to 15-fold increase over controls.[2] The spectrum of thrombotic complications is wide, with 15% to 20% of patients suffering arterial events, and 30% to 60% developing venous clots (some patients get both types of thrombi).[3] The strong association with arterial thrombosis is unusual, given that most hypercoagulability disorders (eg, factor V Leiden, protein C deficiency) evince venous rather than arterial thrombosis. Indeed, during the 1970s, prior to routine adoption of low-dose heparin thromboprophylaxis, when heparin was mainly given in therapeutic doses for treating acute thrombosis, HIT became recognized as a distinct entity through its frequent presentation with arterial thrombosis (its association with venous thrombosis was not established until the 1990s).[4] Moreover, the rank order of thrombotic events observed with HIT (limb artery > thrombotic stroke > myocardial infarction) is striking, as it is inverse to the relative frequency of (non-HIT-related) atherothrombosis (myocardial

infarction > stroke > limb artery thrombosis).[3] It has also been noted that HIT-associated arterial thromboses characteristically occur at sites of arterial injury.[5]

How can the strong association between HIT and arterial thrombosis be explained? Previously, Hayes and colleagues[6] showed that arterial injury causes PF4 to localize to unknown binding sites on peri-injury endothelial cells. These investigators have now extended their work in an intriguing new study that found that PF4 binds at multiple discrete sites along the surface of extended strings of VWF released from endothelium. A remarkable property of VWF molecules released from activated endothelial cells is their ability to self-associate into "strings" exceeding 100 μm in the direction of flow.[7] The new study shows that these VWF strings are capable of binding PF4 released from activated platelets, with the resulting PF4/VWF complexes forming antigen sites recognized by HIT antibodies. As a consequence of binding by HIT antibodies, there results greater accumulation of platelets to the injured endothelium. The independent contributing role of HIT antibodies as well as VWF itself was shown, respectively, by the attenuation of this process by an antibody that inhibits Fc receptor–mediated platelet activation, as well as by antibodies that bind to glycoprotein Ib/IX complexes (the platelet receptors for VWF).

The authors conducted these studies[6] using 2 HIT models of vascular injury. One model employed the "HIT mice" (with human PF4 and platelet FcγIIa receptors) well known to the HIT research community, and the other model used endothelialized microfluidics chambers. For both experimental settings, endothelial photochemical injury was created using hematoporphyrin. Data obtained from 1 model corroborated the results seen in the other.

What are the clinical implications of this research? First, these data help to explain the aforementioned proclivity of HIT to manifest arterial thrombosis. I encountered a dramatic example of this phenomenon 1 year ago, when our service was referred a 67-year-old female posttrauma patient receiving heparin thromboprophylaxis for ~1 week who developed a thrombotic stroke, with computed tomographic imaging showing at least 4 distinct regions of cerebral hypoperfusion; 2 days later, the patient developed thromboses in multiple arteries of both lower limbs, prompting diagnosis of HIT and hematology referral (further details including response to treatment with high-dose IV immunoglobulin are reported elsewhere[8]). Angiography revealed extensive calcified atherosclerotic disease of the abdominal aorta and its branches, and Doppler ultrasound showed calcified atheromatous plaque in both carotid arteries. The interaction of an acquired antibody-mediated platelet activation syndrome, HIT, together with preexisting severe vascular disease with presumed endothelial injury, viewed in the light of these novel findings by Johnston and coworkers, can explain this patient's clinical course. Indeed, this classic picture of multiple platelet-rich arterial thromboses in HIT is known as the "white clot syndrome."[9]

Second, their data could help explain ongoing risk for thrombosis in HIT even after heparin therapy has been discontinued. It is often presumed by clinicians that the thrombotic tendency of HIT rapidly de-escalates following discontinuation of heparin. However, this is not the case, as there are many patients where HIT persists, as shown by ongoing thrombocytopenia, persisting elevation in laboratory markers of hypercoagulability, and new onset or progression of thrombosis. To what extent ongoing thrombotic risk represents the consequences of typical heparin-dependent HIT antibodies that are able to recognize PF4 bound to multimolecular partners such as VWF or rather highly unusual "autoimmune" HIT antibodies that possess heparin-independent platelet-activating properties[10] in washed platelet HIT assays remains uncertain.

Third, the authors' work points to the prospect for novel treatment approaches, such as agents that decrease the size of VWF multimers, or their self-association into macromolecular strings, or that inhibit platelet/VWF interactions. These intriguing studies by Johnston et al reaffirm the remarkable utility of engineered HIT mice and investigator ingenuity in unraveling so much of the puzzling pathogenesis of HIT, including how physiological roles of VWF strings contribute to adverse pathophysiological consequences. It is amazing how views on HIT pathogenesis continue to evolve, as the fascinating story of HIT keeps stringing us along.

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