the development of antibodies to PF4 in patients with sepsis, and this possibility has to be investigated.

Finally, Krauel et al concluded that HIT can be considered as a misdirected host-defense immune response that occurs in some patients exposed to heparin who have been previously immunized with PF4-coated bacteria. This new concept is also supported by the relatively high prevalence of IgM and IgG anti-PF4/heparin antibodies evidenced in a large population of German individuals who had not received any heparin treatment in the 12 months preceding blood sampling. However, it is noteworthy that the same team failed to detect memory B cells in cardiac surgery patients who frequently develop anti-PF4/heparin antibodies postoperatively.3 To explain this apparent discrepancy, Krauel et al suggest that the immune response associated with HIT could mainly involve marginal zone (MZ) B cells that are major players at the interface between the initial innate immune response and the delayed adaptive response. Indeed, the ability of MZ B cells to respond rapidly to encapsulated bacteria by differentiating into antigen-specific plasma cells helps keep such infections under control. MZ B cells might thus also contribute to the rapid synthesis of IgG, IgM, and IgA PF4-specific antibodies in HIT and explain why there is a rapid decline in antibody titers, which usually disappear within 100 days, whereas a normal memory B-cell response maintains these antibodies for a longer time. The role of T cells in the HIT immune response remains uncertain in this context, although it was supported by a study based on a murine immunization model.4

As illustrated in their article (see figure), Krauel et al also deduced from their experiments that antibodies specific to modified PF4 and synthesized in infected patients could contribute to host defenses by allowing the binding of PF4-coated bacteria to granulocytes and their subsequent phagocytosis. Their findings thus support the concept that PF4 has a significant role in bacterial defense. This process could be viewed as a “generic” host-defense mechanism because PF4 was shown to bind to several different strains of bacteria and anti-PF4/polyanion antibodies might therefore react with bacteria not previously encountered by the host-immune system. Whether it is an “ancient” host-defense mechanism, as suggested by Krauel et al, remains to be established. Their findings are also reminiscent of what was observed more than 20 years ago when PF4 was shown to modulate the antibody response to pneumococcal polysaccharides.5 Moreover, the binding of PF4 to bacteria could also result in direct bactericidal activity dependent on peptides derived from PF4 and containing heparin-binding motifs.6

Whether the phenomenon observed by Krauel et al can also occur with other endogenous proteins warrants further study. Intriguingly, peptides derived from another autoantigen, beta-2 glycoprotein I (β2GPI), were recently shown to exhibit antibacterial activities against Gram-positive and Gram-negative bacteria,7 but it is not known whether the binding of β2GPI to bacteria elicits conformational changes and subsequent anti-β2GPI immune response. However, this protein is the main target of antibodies associated with the “antiphospholipid syndrome” which exhibits several similarities to HIT8 and can also occur in the context of bacterial infections.

In conclusion, this study is an important contribution that significantly adds to our understanding of the mechanisms potentially responsible for HIT, and opens new areas of research concerning host-defense mechanisms and their links with autoimmunity.

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Conflict-of-interest disclosure: The authors declare no competing financial interests.

THROMBOSIS & HEMOSTASIS

Comment on Brill et al, page 1400

Clues to DVT pathogenesis

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In this issue of *Blood*, Brill and colleagues demonstrate that von Willebrand factor (VWF) release and subsequent platelet adhesion to endothelial cells are required for thrombus formation in a mouse model of deep venous thrombosis (DVT).

It is probably a good thing that laboratory mice generally do not smoke, use oral contraceptives, become obese, or spend extended periods of time immobilized on long trips, because it turns out that they can indeed develop venous thrombosis that greatly resemble human DVTs. The use of laboratory animals to model human disease has been an important component of medical research since at least the mid-19th century when Rudolf Virchow investigated the pathogenesis of pulmonary embolism (PE) using dogs.1 Interestingly, it was through these studies that Virchow determined that the thrombi found in the lungs actually formed elsewhere in the circulation and somehow traveled to, and became lodged in, the lungs— a process he termed “embolia.”2

Now more than 150 years later, it is well understood that PEs arise within the deep veins of the lower extremities, and often embolize to the pulmonary circulation with dire physiologic consequences. Despite this process being understood by nearly all physicians worldwide, surprisingly little is known regarding the molecular mechanisms which underlie DVT pathogenesis. Classically, Virchow’s triad comprises 3 factors that contribute to venous thrombosis: hypercoagulability,
restriction of blood flow, and prothrombotic changes to the vessel wall.

The contribution of hypercoagulability to DVT is borne out by clinical experience with hereditary procoagulant conditions such as factor V Leiden and the prothrombin G20210A mutation. However, the mechanism by which flow disturbance results in endothelial prothrombotic changes and subsequent development of venous thrombosis remains unknown.

Now, in this issue, Brill and colleagues provide important new insight into the pathogenesis of DVT. Using 2 models of venous thrombosis in mice (the widely used inferior vena cava [IVC] stasis model, and, importantly, a recently developed IVC stenosis model), these authors demonstrate a clear role for VWF and platelet adhesion in venous thrombosis.

First, using VWF “knockout” mice, the authors demonstrate that VWF deficiency protects against thrombosis induced both by complete (stasis model) and partial (stenosis model) flow restriction in the IVC, although the effect was much more pronounced in the stenosis model. Similarly, mice with a 50% decrease in plasma VWF (VWF+/− heterozygotes) were also protected from thrombosis in the stenosis model. Importantly, the thrombi induced by stenosis were found to be composed of 2 clearly morphologically distinguishable components: (1) a platelet-rich white section, and (2) a red section with considerably fewer platelets. Fibrin was also demonstrated to be present throughout the thrombus. Thus, IVC flow restriction in mice resulted in thrombi very similar in appearance and composition to human DVTs.

In addition to mediating platelet recruitment to the endothelial surface, VWF also functions as a carrier for coagulation factor VIII (FVIII), significantly increasing its half-life and plasma concentration. However, restoration of normal circulating levels of FVIII (via infusion of recombinant FVIII) failed to restore thrombus formation secondary to IVC stenosis, thus demonstrating that the protection from venous thrombosis/DVT observed with VWF deficiency most likely was due to an effect of VWF apart from its role in stabilizing FVIII.

Consistent with this observation, the authors next demonstrated that blocking the association of platelets with VWF (with an agent that interferes with the VWF-platelet glycoprotein Ib-α interaction) also strongly protected from thrombosis in the IVC stenosis model. Finally, using immunofluorescence and sophisticated intravital microscopy techniques, the authors showed that IVC stenosis results in endothelial activation, release of VWF (and other Weibel-Palade body contents such as P-selectin), and subsequent adhesion of platelets to the endothelial surface. Together, these observations strongly suggested that VWF is functioning in venous thrombosis/DVT by promoting platelet adhesion to the endothelial surface, which became activated as a result of stenosis-induced disturbances of blood flow.

Although these findings provide significant insight into the roles of VWF and platelets in the pathogenesis of venous thrombosis, the authors are clear to point out that the mouse models used in this study may not recapitulate all of the key aspects of DVT in humans. That said, the potential implication of VWF-mediated platelet adhesion in human DVT pathogenesis provides both a mechanistic explanation for the long-held (and in some studies, clinically supported) notion that aspirin therapy reduces the risk of DVT and PE, and opens the door to rational targeting of VWF for the prophylaxis and therapy for these serious clinical conditions which, in the United States alone, affect close to 1 million patients per year.

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

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