The Pathophysiology of Heparin-Induced Thrombocytopenia*

Biological Basis for Treatment

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Abbreviations: FcR = Fc receptor; HIT = heparin-induced thrombocytopenia; HITTS = heparin-induced thrombocytopenia with thrombosis; HR = high responder; LR = low responder; PF4 = platelet factor 4

Heparin was discovered > 80 years ago,[1] and within a short interval it was used as an anticoagulant.[2] Heparin has advantages that led to its widespread use, including its immediate onset of action, its relatively short half-life (3 h), and its ability to be reversed using protamine.

In 1958, Weismann and Tobin[3] described paradoxical thrombi during heparin therapy. Ten patients developed severe and sometimes catastrophic arterial occlusion while receiving heparin. Six of the 10 patients died as a result of the thrombosis.[3] The clots were described as being a pale salmon color in appearance and platelet-rich when examined microscopically. An additional 11 patients were described 5 years later by another group.[4] Once again, pale, platelet-rich arterial thrombi were described. And again, no mention of thrombocytopenia was made.[4] We now recognize that although platelet-rich "white clots" can occur, they are uncommon, and the majority of thrombi complicating heparin-induced thrombocytopenia (HIT) are RBC-rich fibrin clots.

In 1973, Rhodes et al[5] described thrombocytopenia as a component of the syndrome. The pathogenic role of heparin was confirmed by the recurrence of thrombocytopenia when a patient was reexposed to heparin.[5] A potential immunologic component was postulated based on the ability of the plasma from these patients to induce platelet aggregation in the presence of heparin.

Our understanding of HIT has evolved dramatically over the past 30 years. In this report, these changing concepts are summarized, with a particular focus on how a better understanding of the pathophysiology of HIT has translated into better treatments.

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Changing Concepts of the Clinical Expression of HIT

HIT was first described as an arterial thrombotic disorder with the emboli being pale-colored because they were platelet-rich.[2,3] As the recognition of HIT increased across the decades of the 1980s and 1990s, some investigators began to question whether venous thrombi could also be part of the thrombotic syndrome complex. The problem with this hypothesis was that those disorders in which venous thrombi were implicated in HIT, such as in orthopedic surgery, could, by themselves, cause venous thrombi. Boshkov and colleagues[6] addressed this question by relating the type of thrombotic event to the associated medical or surgical situation or surgical procedure. The results of this study suggested that the vascular location of the thrombotic complications (arterial or venous) was related to the underlying vascular damage or associated surgical risk factors. For example, recent arterial surgery or severe atherosclerosis were associated with arterial thromboembolism. Venous thrombi complicating HIT were likely to occur when there were additional risk factors for venous thromboembolism such as recent surgery.[6]

The demonstration that HIT triggered both arterial and venous thrombi was unexpected, since with few exceptions (*eg*, the antiphospholipid syndrome) thrombi are typically restricted to either the venous or arterial circulations, but not both. Based on these observations, it was postulated that HIT was a panvascular, prothrombotic disorder with localization to the venous or arterial circulation depending on the risk factors present in each circulation. Today, it is acknowledged[7,8] that venous thromboembolic events dominate over arterial thromboembolic events at a ratio of approximately 4:1.

Recognition of HIT as an Immunologic Disorder

HIT was initially termed *heparin-associated thrombocytopenia* because it was assumed that the thrombocytopenia was associated, and was not necessarily causally related. Some investigators, including our group, thought that a contaminant within the heparin preparation could be causing the reaction. Indirect support for this hypothesis was provided by the demonstration that different heparin preparations carried different risks for HIT.[9] Heparin is a purified preparation of glycosaminoglycans (*ie*, long chains of highly charged sugars) that are isolated from beef lung or pork intestine. Unlike most other drugs, heparin is a complex mixture of a large number of related compounds with molecular weights ranging from 1,000 to $>$ 40,000.

Early evidence that HIT was an immunologic disorder included the report of Rhodes et al,[5] which proposed that HIT was caused by a non-complement-fixing, heparin-dependent antibody. Other investigators[10–13] also provided evidence of an immunologic basis for HIT using a variety of assays.

The mechanism by which the heparin and IgG interacted with the platelet soon became a focus for research in many laboratories. Our group showed[14] that standard platelet aggregation was neither sufficiently sensitive nor specific enough to serve as a diagnostic test for HIT. Based on these results, Sheridan et al[15] developed a sensitive and specific test for HIT. By using platelets from healthy donors who had been radiolabeled with a platelet granular component, serotonin, Sheridan et al[15] were able to dramatically increase the sensitivity of the test. The addition of increasing concentrations of heparin to this mixture of the radiolabeled platelets and the test sera from HIT patients resulted in an unusual pattern of platelet activation. A unimodal heparin-dependent pattern of platelet activation was observed, which suggested the possibility of an immune complex disorder.[15] Together, these observations formed the basis of a test for HIT, which is used today.

In 1988, we reported that platelets from different patients with Glanzmann thrombasthenia and Bernard Soulier syndrome, who together lacked glycoproteins Ib, IX, V, IIb, and IIIa, were all capable of being activated by HIT serum and heparin.[16] This indicated that these glycoproteins did not directly participate in the HIT reaction. We observed that purified Fc from nonimmune IgG was capable of blocking the reaction. This observation led us to propose that HIT was caused by heparin-IgG immune complexes that activated platelets through their Fc receptors (FcRs). We were not able to demonstrate that the heparin bound to the patient IgG. This suggested that an unidentified factor was also involved in the formation of the immune complex.

The next focus for research was the identification of the antigenic target of HIT. In 1992, in a significant advance for HIT-related research, Amiral et al[17] demonstrated that it was platelet factor 4 (PF4) that bound HIT IgG. These observations were confirmed by other groups.[18–20] The next focus of research was the clarification of the role of PF4 as the target antigen. It was demonstrated that the optimal target ratio range of PF4 to heparin was 4:1 to 8:1.[19,21,22] The small size of PF4 (70 amino acids) allowed Horsewood et al[21] to synthesize a series of peptides that spanned the entire length of the PF4 molecule. These investigators found that a minimal length of PF4 (specifically, 19 amino acids encompassing the carboxy-terminal peptide including the lysines, which bind heparin) was required for reactivity with the HIT-IgG.[21] But, it was not possible to identify a linear epitope on the PF4 molecule that served as the target antigen. Rather, the results of these studies were consistent with the hypothesis that heparin molecules bundle the PF4, resulting in conformational changes to the molecule, which in turn, become the binding sites for the HIT-IgG.[21] Visentin[23] has reviewed the interaction of heparin with PF4.

Characterization of the Antibodies That Cause HIT

The demonstration that HIT was an immune complex that activated platelets through the FcR confirmed the central role of IgG in the pathophysiology of this disorder. The majority (about 90%) of the HIT-IgG is polyclonal, IgG_1, which is expressed alone or with IgG_2.[24] IgM and IgA also have been found in patients with HIT, and some of the enzyme immunoassays measure the levels of these antibodies.[22,25] However, their biological relevance remains uncertain. Amiral et al[26] have also documented a variety of other autoantibodies in patients with HIT, particularly those acting against certain cytokines such as interleukin-8 and neutrophil-activating peptide 2. Additionally, antiphospholipid antibodies are sometimes found in these patients.[27–30] However, the clinical relevance of all of these antibodies remains unknown.

Certain aspects about the antibody (HIT-IgG) that binds to PF4 remain not well understood. It is surprising that this antibody develops so frequently in individuals who are exposed to heparin because heparan sulfate, a glycosaminoglycan that is very