Heparin-Induced Thrombocytopenia: Molecular Pathogenesis

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Introduction

Heparin-induced thrombocytopenia (HIT), a relatively common complication of heparin therapy, is characterized by an unexpected fall in platelet count occurring 5 days or more after the initiation of treatment.1,2 Patients who manifest only the thrombocytopenia rarely experience severe bleeding or other major adverse effects.3 Often, however, the thrombocytopenia is accompanied by arterial and/or venous thrombosis and thromboembolism, a complication which can be devastating.4 HIT, with or without thrombosis, is probably the most common cause of immunologically-mediated, drug-induced thrombocytopenia. HIT usually occurs in patients given standard therapeutic doses of heparin, especially of bovine origin.5 However, it has been reported following low-dose, subcutaneous heparin6 and in patients exposed to heparin “flushes” used to maintain the patency of intravenous lines.7 Even minute quantities of heparin released from heparin-bonded catheters appear to be capable of causing the disorder in previously sensitized patients.8 HIT has been induced by low molecular weight heparin (LMWH)9,10 and other heparin-like polysaccharides.11,12

HIT differs from most other forms of drug-induced immune thrombocytopenia in that the responsible antibodies activate platelets in the presence of pharmacologic doses of heparin (0.1-1 U/ml), rather than merely binding to platelets to promote their destruction in the reticuloendothelial system.3,13,14 The observation that heparin-induced platelet activation can be blocked in vitro by monoclonal antibodies specific for the platelet Fc receptor (FcγRIIA)15 suggests that antibodies associated with HIT somehow interact with heparin to form platelet-activating immune complexes. Attempts to demonstrate such complexes and their binding to intact platelets, however, have generally yielded negative or equivocal results.14,16,17 The association of HIT with thrombosis and disseminated intravascular coagulation (DIC) suggests that heparin-induced antibodies might react with endothelial cells (EC). Binding of IgG from the serum of patients with HIT to cultured human umbilical vein endothelial cells (HUVEC) observed in one study,18 but not confirmed in another,19 left open the question of whether immune-mediated vascular damage is important in pathogenesis.

In 1992, Amiral and coworkers obtained evidence that antibodies associated with HIT are specific for complexes of heparin and platelet factor 4 (PF4), a heparin-binding protein stored in platelet α granules.20 These findings were subsequently confirmed by several groups, including ours.21,23 We also provided the first direct evidence that HIT antibodies bind to HUVEC in the presence of PF4, but not in its absence, thus, suggesting that the antibodies also recognize PF4 complexed with the heparan sulfate molecules displayed on HUVEC.21 Furthermore, Greinacher et al22 showed, by adsorption/elution of total IgG derived from HIT patients on PF4-coated EC, that the eluted antibodies also caused heparin-dependent platelet activation, thus, indicating that they recognize epitope(s) present on both PF4-heparin and PF4-heparan complexes.

The Antigen

Studies from our group21,24,25 and others26,27 have shown that antibodies reactive with PF4-heparin complexes are nearly always present in patients with HIT, suggesting that the PF4-heparin complex is, indeed, the major antigenic target of this drug-induced, immunoglobulin-mediated disorder. PF4 is a basic chemokine with high affinity for heparin that is stored in platelet α granules and is secreted upon platelet activation.28 The PF4 monomer, containing seventy amino acids and two intrachain disulfide bonds,29 consists of an extended N-terminal loop followed by three strands of anti-parallel β-sheet and a C-terminal α-helix30 (Fig. 1). PF4 monomers spontaneously organize into tetramers comprised of two, six-stranded β-sheets (formed by two monomers) arranged back-to-back to form a β “bilayer” structure. The carboxy-terminal α-helices are arranged as anti-parallel pairs on opposite sides of the β-sheet bilayer.30 Lysine residues on the exterior faces of the four α helices are critical for heparin binding.31 Additional residues located elsewhere on the tetramer have also been proposed to be involved in the PF4-heparin interaction32,33 (Fig. 2).

Heparin, a polyanionic molecule, belongs to the glycosaminoglycans (GAGs), a family of unbranched polysaccharides of alternating, variously sulfated, and therefore negatively charged, residues of uronic acid and hexosamine. Commercially available, unfractionated heparin is heterogeneous and polydisperse, ranging in length from ten to eighty or more saccharide units. Other GAGs, such as heparan sulfate and chondroitin sulfate, are expressed on the surface of adherent mammalian cells (e.g. endothelial cells) in the form of proteoglycans and consist of oligosaccharides covalently linked to a core protein (e.g. syndecan).34
The Role of Heparin: The Polyanion Player

Antibodies associated with HIT are specific for complexes of heparin and PF4. The production of these antibodies is surprising because both heparin and PF4 are normal body constituents. HIT antibodies fail to recognize heparin alone or PF4 alone but bind avidly to the PF4-heparin complex. One proposed mechanism is that these antibodies recognize combinatorial epitopes consisting partly of heparin and partly of PF4 at one or more positions where the molecules contact each other when the complex is formed. With each PF4 monomer containing seventy amino acid residues, many such combinatorial epitopes are possible. An alternative mechanism is that HIT antibodies recognize a conformational change elsewhere on the PF4 molecule that is created when the PF4-heparin complex forms.

The structural requirements of heparin necessary for the generation of epitopes recognized by HIT antibodies have been evaluated in several studies. Kelton et al. provided the first evidence that a variety of GAG molecules interact with PF4 to form complexes recognized by HIT antibodies. The optimal ratio of GAG to PF4 for the formation of antibody binding complexes differed among the various polyanions. Greinacher and colleagues showed that a minimum length of ten saccharide residues of the heparin molecule was necessary to promote platelet serotonin release by HIT antibodies. Moreover, they determined that the degree of sulfation and three-dimensional structure of sulfated polysaccharides, but not the type of glycosidic linkage between disaccharides, affects epitope formation. Positive correlation between polysaccharide sulfation and epitope generation was also confirmed in studies by Amiral et al.

Not surprisingly, the heparinoid anticoagulant, danaparoid (Lomoparan®, Orgaran®), a mixture of non-heparin, low molecular weight GAG having a low degree of sulfation, formed complexes that reacted with less than one-third of patient samples tested. Furthermore, Maccaranna and Lindahl have shown that, in contrast to the specific interaction between heparin and antithrombin, heparin binding to PF4 occurred by relatively non-specific electrostatic interactions increasing continuously with the increasing size of the oligosaccharides and the increasing overall charge (hence the relationship with the degree of sulfation).

Studies from several laboratories have shown that a narrow range of heparin to PF4 ratios is required for optimal HIT antibody binding. However, the ratio of heparin to PF4 is critical only for PF4-heparin complexes prepared in solution. When PF4 is added to heparin immobilized by end-linkage, the ratio of PF4 to heparin is of no consequence, since it is only necessary to saturate the heparin with PF4. The narrow range of concentrations over which complexes of PF4-heparin suitable for antibody binding are formed is probably related to the size heterogeneity of the molecules contained in unfractionated heparin. For example, the ratio is not critical when low molecular weight heparin, which is relatively uniform in size, is used.

In studies performed in our laboratory using a polyanion-PF4 enzyme-linked immunosorbent assay (ELISA), we determined that the minimum length needed for a polyanion to be able to form epitopes recognized by HIT antibodies when complexed to PF4 was approximately 42 Å. This is equivalent to five disaccharide subunits, with each disaccharide measuring approximately 8.4 Å in length. In addition, a heparin fragment of twelve saccharide residues (about 50 Å in length) interacted...
with PF4, inducing epitope formation recognized by HIT antibodies. The antibody reactivity seen with this heparin fragment was indistinguishable from that obtained using unfractionated PF4-heparin as the target.

Using the same ELISA methodology, we showed that several polyanions, including unfractionated heparin, LMWH, heparan sulfate, dextran sulfate, and fucoidan, all interacted with PF4 in such a way as to create sites for HIT antibody binding. Furthermore, we found that neither saccharide chain nor sulfate side groups were essential for a polyanion to react with PF4 to create sites for antibody binding, since polyanionic compounds such as polyvinyl-sulfate, polyvinyl-sulfonate, polyvinyl-phosphate, polyvinyl-phosphonate, and polyvinyl-polyanethole sulfonate were all suitable substitutes for heparin in the detection of HIT antibodies.

These observations suggest that polyanions suitable for an immunogenic interaction with PF4 have a strong negative charge, comparable to that of a polymer substituted with multiple sulfate ($\text{ROS}_3^-$), sulfonate ($\text{RSO}_3^-$), phosphate ($\text{ROPO}_3^-$), or phosphonate ($\text{RPO}_3^-$) groups. In addition, no particular subunit structure is required. The finding that any one of many polymers containing a linear array of negative charges complexed to PF4 is sufficient for antibody binding suggests that antibodies associated with HIT recognize conformational,

Figure 2. Molecular model (WebLab ViewerPro™, Molecular Simulation Inc., San Diego, CA) of the human PF4 tetramer, based on the crystallographic coordinates. Each monomer is shown as a ribbon diagram: A (purple), B (orange), C (black), D (fuchsia). The amino acid residues crucial for heparin binding [C-terminal $\alpha$-helix residues encompassing lysines 61-62 and 65-66 (cyan), and arginines 20, 22, and 49 (green)] are displayed.
rather than combinatorial, determinants induced on PF4. Studies by Greinacher and colleagues \(^{35}\) have suggested that a carbohydrate-based anticoagulant with a reduced risk of forming the antigenic determinant recognized in HIT should consist of a linear molecule with a degree of sulfation of less than 0.6 or a molecular weight of less than 2.4 kDa.

Molecular details concerning the association of heparin with PF4 can be derived, in part, from several structural studies, including the crystallographic definition of both bovine\(^{39}\) and human PF4.\(^{30}\) Lysine residues on the exterior faces of the four \(\alpha\)-helices have been identified as critical for heparin binding.\(^{31}\) In addition, residues located elsewhere on the tetramer have also been shown to play a role in this interaction\(^{33,40}\) (Figs. 1-2).

According to one model, the heparin molecule may interact with the PF4 tetramer by binding to a series of positive charges running perpendicular to the four \(\alpha\)-helices.\(^{41,42}\) Experimental evidence suggests that a heparin molecule containing 18 saccharide residues (molecular weight approximately 5.4 kDa) could extend halfway around the PF4 tetramer and interact with both anti-parallel, \(\alpha\)-helical domains.\(^{41}\)

Based on their research, Stringer and Gallagher\(^{43}\) proposed a model in which a “heparan sulfate fragment wraps around a ring of positive charges on PF4, with the iduronate 2-\(O\)-sulfates within the sulfated domains binding strongly to lysine clusters on opposite faces of the tetramer.” Mayo and colleagues\(^{33,40}\) investigated the interaction of heparin with an N-terminal

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Figure 3. Molecular model (WebLab ViewerPro\textsuperscript{TM}, Molecular Simulation Inc., San Diego, CA) of the human PF4 tetramer, based on the crystallographic coordinates.\(^{30}\) Each monomer is shown as a ribbon diagram: A (purple), B (orange), C (black), D (fuchsia). The amino acid residues, P37 (red), R49 (yellow), L55 (gray), and K61 (purple), crucial for HIT antibody recognition\(^ {46,48}\) are displayed.
chimeric mutant of PF4 (PF4-M2) using structural analysis by nuclear magnetic resonance (NMR) imaging. Their data, contrary to PF4-heparin binding models centering around C-terminal α-helix lysines, indicate that arginines 20, 22, and 49, and to a lesser extent histidine 23, threonine 25, and lysine 46, are crucial to the heparin binding process (Figs. 1-2). Whenever amino acids in PF4 participate in this interaction, it appears that a polyanion displaying a string of strong negative charges along its length is required to form PF4-polymer complexes that are recognized by antibodies associated with HIT.\(^{36}\)

Physical measurements of heparin have shown that, in aqueous solution under isocotic conditions, the chain configuration is that of a “free-draining” coil (as opposed to a compact coil or a rigid rod).\(^{44}\) Indeed, another requirement for the formation of PF4-heparin complexes, for which HIT antibodies are specific, appears to be that the saccharide chain making up the heparin molecule must be in a flexible, relatively unconstrained state. This is because heparin-induced antibodies associated with HIT recognize complexes formed between PF4 and heparin fragments attached by end linkage to agarose beads but fail to recognize PF4 complexed with heparin molecules immobilized by multiple crosslinkages.\(^{38}\) Taken together, these findings suggest that antibodies associated with HIT recognize conformational changes on the PF4 molecule created by the electrostatic binding of a linear polyanion of a length equal to at least a ten residue heparin molecule (approximately 42 Å), sufficient to span one-third of the circumference of the PF4 tetramer.

The Role of Platelet Factor 4: The Protein Player

Several approaches have been used to identify regions on the PF4 molecule involved in creating HIT antibody epitopes when complexed with heparin. Horsewood et al studied the epitope specificity of 29 HIT patient antisera that were reactive in the serotonin release assay. An ELISA format was used in which either PF4-heparin, reduced/alkylated PF4, heparin-reduced/alkylated PF4, PF4 peptides, or PF4-heparin peptides were coated as test antigen.\(^{35}\) They found that, while all 29 antisera reacted with PF4-heparin complexes, only a subset of five antisera were able to react with reduced PF4 or a peptide corresponding to amino acids 52-70 of the PF4 molecule only in the presence of heparin. Neither reduced PF4 nor the C-terminal peptide could inhibit binding of HIT antibodies to PF4-heparin complexes, even at high concentrations. The authors were, therefore, unable to mimic a heparin-induced, potentially immunogenic, conformational change in PF4 by artificially altering PF4 structure through reduction or synthesis of linear peptides.

Potential targets for HIT antibodies, other than PF4-heparin, have been reported by Amiral et al. These investigators studied a subgroup of 15 HIT patients whose antibodies were characterized as positive in a platelet aggregation assay but negative in a PF4-heparin ELISA.\(^{45}\) Antibodies from nine of the patients involved in this study were characterized as reactive to neutrophil-activating peptide (NAP-2) and/or interleukin-8 (IL-8), two members of the chemokine family that are homologous to PF4. These findings are of interest because five of the nine patients manifested thrombotic episodes. The reactions of these antibodies against NAP-2 or IL-8 in solution, rather than denatured on a plastic surface, were not described, and their relationship to antibodies that recognize PF4-heparin complexes is uncertain.

Ziporen et al used a molecular biology approach to directly identify amino acids in the PF4 molecule necessary for the creation of epitopes to antibodies from 50 HIT patients.\(^{46}\) Alanine mutagenesis of PF4 at residues K62, K65, and K66 (three of four C-terminal lysines critical for heparin binding)\(^{31}\) had only a minimal effect on HIT antibody binding. Mutation of the fourth lysine, K61, to alanine reduced antibody binding by only about 50%. These data suggest minimal participation by the C-terminal lysines in HIT antibody epitope formation. Ziporen et al also used NAP-2/PF4 chimeric constructs to investigate HIT antibody epitope formation and found that only the NAP-2 substitution of PF4 amino acids 37-47 (between the third and fourth cysteine residues) abolished HIT antibody binding.\(^{46}\) A single amino acid substitution in PF4: P37→N also abolished antibody binding (Figs. 1-2).\(^{46}\)

We used a similar approach of making rat/human PF4 chimeric constructs to investigate HIT antibody epitope requirements. Rat PF4 has 74% sequence identity to human PF4\(^{47,29}\) but is unreactive with HIT antibodies when tested either in the presence or absence of heparin.\(^{46}\) Furthermore, only six of the 47 C-terminal amino acids differ between rat and human PF4 (Fig. 1). This information was then used to create constructs in which nonreactive rat amino acids were substituted at the corresponding positions in the human molecule. Each of fifteen antibodies from HIT patients recognized PF4-heparin complexes containing PF4 constructs bearing mutations: E4→S, L11→V, and T16→S at the N-terminus, or A57→V at the C-terminus equally well to wild-type PF4-heparin complexes. In contrast, complexes containing other C-terminal mutants, namely P37→A/T38→V/A39→P/R49→S, or L55→R exhibited varying degrees of reduced binding.\(^{48}\) The HIT antibodies tested recognized PF4 mutated at position 49 or 55 only at a higher concentration of heparin (0.8 U/ml versus 0.5 U/ml). None of the fifteen antibodies recognized peptides comprising the 26 or 15 C-terminal amino acid residues of the PF4 monomer or reduced/alkylated human PF4, either in the presence or absence of heparin.\(^{48}\)

Although identification of the minimum modifications of human PF4 that lead to loss of HIT antibody binding does not necessarily localize the epitope at which an antibody attaches, i.e., the actual binding site could be a conformational change elsewhere in the PF4 tetramer, our studies,\(^{48}\) together with those of Ziporen,\(^{46}\) pinpoint the region between the third and fourth cysteine residue and, in particular, amino acid P37 (Fig. 3), as a major antigenic determinant for HIT antibody binding. The impaired reactivity of HIT antibodies versus complexes of heparin with PF4 mutated at positions 49, 55, and 61\(^{46,48}\) suggests the existence of multiple epitopes on the PF4-heparin complex (Fig. 3). Indeed, using biotin-labeled affinity-purified HIT antibodies in a competitive inhibition assay,\(^{48}\) we observed that at least three dominant HIT antibody recognition sites can be distinguished on PF4 when it binds to heparin.
The Immune Response

The immune response in HIT is peculiar in that antigen formation results from the noncovalent interaction of a specific protein with a specific drug. In HIT, heparin binds to the target protein, PF4. As mentioned, we speculated that the binding of heparin to PF4 induces either a conformational change(s) in PF4 (neoepitope) or creates combinatorial epitope(s) at one or more site(s) where heparin contacts the PF4 molecule. In either case, the resulting “neoantigens” are recognized as foreign, eliciting an immune response. Viewed in this light, the HIT response can be postulated as a variant mechanism of autoimmunerecognition. In fact, in this particular immune response, the drug is not recognized as antigen by itself, nor is PF4 as part of the “self.” However, the PF4-heparin complex triggers the immune system in certain individuals.

The immune response to PF4-heparin complexes is presumed to involve the following steps: a) recognition by B cells of “neoantigens” created on the PF4 molecule following its interaction with heparin, b) internalization of these complexes by B cells and presentation of peptides derived from PF4 to helper T cells in association with HLA class II molecules, and c) stimulation of these T cells, leading to lymphokine secretion, expansion of relevant B cell clones, and antibody production.

It is known that autoreactive T and B cells can be detected even in healthy individuals. Efficient regulatory mechanisms normally keep autoreactive cells in check. When immune control fails, autoimmune disease ensues. Loss of “self-tolerance” in individuals with autoimmune diseases is not simply an inherent failure of their immune systems to discriminate between self and non self antigens; rather, it appears to result from environmental factors that render a genetically susceptible immune system temporarily blind to the differences between self and nonself. Basic immunological studies have confirmed that the normal peripheral immune system can either gain or lose reactivity to specific antigens (either self or non-self), depending on the accessibility of the antigen to antigen presenting cells (APC), the amount of antigen available, and the density of costimulatory ligands expressed by APC.

Our group and others have shown that plasma from HIT patients contains antibodies reactive with complexes of PF4-heparin from several immunoglobulin isotypes including IgG, IgM, and IgA, which suggests class-switching associated with primary and secondary humoral immunity and the likely involvement of T helper cells.

Using the methods of T cell receptor (TCR) spectratyping,52 also called immunoscope53,54 and clonotyping,55 we identified a number of PF4-heparin-responsive spectratypes and PF4-heparin-specific clonotypes from patients with severe HIT. TCR spectratyping is a polymerase chain reaction or PCR-based approach that provides a readout of the TCR β-chain diversity and usage for a given T cell population. Accordingly, this provides a representation of the cell population’s T cell repertoire since only one TCR β-chain is typically expressed per T cell. TCR clonotyping is a refinement of spectratyping whereby oligonucleotide probes specific to a given CDR3 loop region from a particular TCR β-chain (thus a “clonotype”) are used to detect the presence or absence of the clonotype in a given T cell population. The TCR β-chain, CDR3 loop region is one of the key points of molecular interaction between CD4+ T cells and B cells in a secondary immune response. Analogous to the immunoglobulin genes, the TCR β-chain locus spawns a plethora of unique TCRs necessary for a healthy T cell repertoire through recombination of 26 variable, 2 diversity, 13 joining, and 2 constant regions.56 Clonotyping permits a precise determination as to whether a given clonotype has responded to a stimulus through T cell maintenance or expansion during in vitro culturing.

We have analyzed several HIT patients’ T cell repertoires to further characterize the immune response in HIT at the level of T cell activation. Our earliest findings showed that in vitro culturing of peripheral blood mononuclear cells (PBMC) from patients experiencing HIT, but not from normal donors in the presence of PF4-heparin (but not heparin or PF4 alone), leads to selective activation of subsets of T cells with apparent PF4-heparin specificity.57,58 Additionally, through TCR spectratyping and clonotyping, we identified several clonotypes that, when cultured in vitro, showed preferential maintenance or expansion in response to complexes of PF4-heparin.59 Since the autoreactive antibodies associated with HIT are specific for complexes of PF4-heparin, our findings imply that subsets of T helper cells in the peripheral blood of HIT-affected individuals may facilitate T helper cell memory responses, which could lead to B cell stimulation and autoreactive antibody production.

In one of our studies, we found that two HIT patients had predominant and similar β-chain variable (Vβ) family 5.1 TCR clonotypes with shared CDR3 region amino acid motifs that were PF4-heparin stimulation-specific upon in vitro culturing.58 In a study of a subsequent HIT patient, Bacsi et al found PF4-heparin-specific expansion of several Vβ family 17 TCR clonotypes with yet another shared CDR3 region amino acid motif upon in vitro culturing.59 Given the complex nature of our analysis and the limited number of HIT patient samples examined, we could not determine a trend in the β-chain variable region uses of PF4-heparin-specific clonotypes among patients. This lack of a common Vβ family usage might be expected for HIT, as in cases where the immune system functions to generate required populations of T cells through private, non-HLA-restricted mechanisms rather than public ones. Such cases could result in diverse repertoires of T cells serving the same immune function in individuals. The small study that did not find evidence for an HLA restriction in HIT supports this idea.50

The key to autoreactive T cell stimulation in HIT lies in the antigen processing and presentation of peptide fragments of PF4. An interesting, and perhaps unique, feature of this process is the apparent critical role of heparin. Our experiments show the existence of T cells that require a combination of both components to respond. Because we defined specificity on this basis, we cannot rule out that other T cells exist in HIT that can respond to PF4 in the absence of heparin. However, PF4 must be complexed with heparin for HIT antibody recognition and heparin could play an indirect but key role in the uptake and/or antigen processing of PF4 by antigen processing cells. The role of exogenous heparin is not understood, as both GAGs and PF4 are natural body constituents, and HIT does not result normally. Either HIT represents a breaking of tolerance in sensitive
individuals, or there are some further modifications in the exogenous heparin, which in combination with PF4, generate a neoantigen. Resolution of this issue may teach us about new facets of antigen processing and presentation.

These studies support a hypothesis whereby pharmacologic doses of heparin result in unusual processing of PF4-heparin complexes by the cellular immune system in a T cell or B cell-mediated fashion. We next need to dissect the drug-induced phenomenon of errant or altered processing of PF4 and perhaps develop strategies for identification of immune system risk factors relating to heparin therapy.

**Implications**

On the basis of experimental evidence, a new model for the pathogenesis of HIT has been proposed. Under normal conditions, only minute quantities (3 ng/ml) of PF4 are found in human plasma. However, significant amounts of PF4 are normally associated with endothelial cell proteoglycans. After intravenous injection of heparin, the plasma level of PF4 increases 15- to 30-fold and remains elevated for several hours, apparently because PF4 is mobilized from endothelial cells by the injected heparin to form circulating PF4-heparin complexes. In a patient with IgG antibodies specific for these complexes who is treated with heparin, the following sequence of events can be envisioned (Fig. 4): 1) Injected heparin scavenges the PF4 released from platelets or normally associated with GAG on the surface of endothelial cells (EC); 2) PF4-heparin complexes are formed; 3) Antibodies are generated that bind to PF4-heparin to form immune complexes (IC); 4) IC react with platelet FcRIIA receptors and activate them, leading to more PF4 release and thrombocytopenia; 5) The additional PF4 released reacts with heparin and IgG to form new IC, promoting further platelet activation, thrombocytopenia, and generation of procoagulant platelet-derived microparticles, leading to thrombin generation; 6) PF4 released in excess of the amount that can be neutralized by available heparin binds to glycosaminoglycans (GAG) on endothelial cells (EC); 7) These PF4-GAG complexes provide targets for antibody, leading to antibody-mediated EC injury and/or activation, expression of endothelial tissue factor, and consequently, more thrombin generation with predilection to thrombosis or disseminated intravascular coagulation. IgM antibodies may be more destructive to EC than IgG because of their greater capacity for complement activation.

![Figure 4. Proposed model of pathogenesis in heparin-induced thrombocytopenia.](image-url)
Acknowledgements

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References


