Editorial

Heparin-induced thrombocytopenia: new insights into the immune response

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Heparin induced thrombocytopenia (HIT) is an immune-mediated adverse reaction to heparin (both unfractionated and low molecular weight) due to the development of IgG antibodies against a self antigen, that is the complex of platelet factor 4 (PF4) and heparin or other polyanions. The IgG/heparin-PF4 immunocomplexes can cross-link with the Fcγ receptor IIa (FcγRIIa) on platelet surface. As a consequence, platelets are activated with intravascular aggregation and consumption, leading to thrombocytopenia. However, a paradoxical prothrombotic state ensues with coagulation activation and life-threatening venous and/or arterial thrombotic complications (1).

HIT is a clinicopathological syndrome associated with (I) one or more clinical events (primarily thrombocytopenia with or without thrombosis); and (II) laboratory evidence for a heparin-dependent immunoglobulin (usually IgG) using a sensitive and specific assay (2).

Heparin is still widely used both in surgical and medical patients and thrombocytopenia is common in these patients. Thrombocytopenia is also common in cancer patients in whom a high frequency of venous thromboembolic complications is observed and for which heparin, in particular low molecular weight heparin (LMWH), is still the prophylactic and therapeutic agent of choice. The numerous alternative causes of thrombocytopenia in surgical, medical and cancer patients make HIT diagnosis and treatment a challenging clinical problem, with high potential risk of malpractice claims.

HIT was first described almost 60 years ago in 1958 (3), but its immunological pathogenesis is still not completely understood (4) for its atypical pattern as IgG antibodies are generated rapidly (within 4 to 14 days from exposure, with detectability at a median of 4 days) and without preceding IgM expression, suggesting a secondary immune reaction (5,6).

PF4 is a chemokine of the CXC subfamily expressed by megakaryocytes and stored in and released from platelet α-granules upon platelet activation. PF4 then binds to glycosaminoglycans on vascular cell surfaces for its high positive charge with high affinity for heparin and other large, anionic molecules (6,7). PF4 monomer is a 70 amino-acid protein of 7.7 kDa. PF4 molecules are strongly cationic and repel each other while longer heparin chains can neutralize these repulsive forces for their high negative charge, allowing PF4 molecules to approximate and form dimers and tetramers of approximately 31 kDa. Binding of heparin stabilises tetramer configuration with the formation of ultra large complexes (ULC) (4). As a result, the open end of the PF4 tetramer is oriented, revealing neo-epitopes necessary for antibody binding (8). Optimal complex formation can occur at prophylactic-dose unfractionated heparin (UFH) and high PF4 levels, while therapeutic-dose LMWH concentrations are too high for optimal complex formation; concentrations of fondaparinux are usually below the optimal stoichiometric range. Immunization occurs more frequently after strong rather than weak minor

platelet activation such as in surgical rather than medical patients, and-for a given degree of PF4 availability—with decreasing frequency with: prophylactic-dose UFH > therapeutic-dose UFH > prophylactic-dose LMWH, therapeutic-dose LMWH > fondaparinux (9).

Several hypotheses have been proposed to explain why the PF4 self-antigen is immunogenic. Greinacher *et al.* suggested a bacterial immunization model as a primer for immunogenicity to PF4 with the involvement of the innate immune response which is designed to recognise patterned structures on microbial surfaces (5). PF4 serves as a host defense peptide with antimicrobial functions as it can bind to anionic surfaces, such as nucleic acids and the lipid A component of lipopolysaccharide, and disrupts microbial cell membranes. Infections associated with platelet activation and PF4 release generate circulating PF4/bacterial polyanions complexes that activate and prime the innate immune system to respond to subsequent heparin exposure with high titer IgG antibodies within 5 days against new pathogens or other polyanions such as heparin, as long as they bind PF4 (10).

The contribution of the complement system in the initiation of the PF4/heparin immune response is not well investigated. The complement system is an innate host defense mechanism rapidly activated by molecules found on invading pathogens with the aim of microorganisms destruction (11). The complement system has also key functions in adaptive immunity through complement receptors (CRs) expressed on B cells (11). Binding of complement-coated antigen to B-cell CR2 (CD21) receptor enhances the immunogenicity of some antigens 1,000 to 10,000-fold (12). Khandelwal *et al.* examined the role of complement in the immune response to PF4/heparin complexes (13). Unlabeled PF4/heparin complexes were added to whole blood from healthy volunteers and binding of PF4/heparin complexes was measured by using fluorescently labeled KKO, a murine monoclonal antibody to human PF4/heparin complexes and cell-specific markers (13). PF4/heparin complexes bound to >90% of B cells as measured by KKO binding but to <1% of T cells, ~2% of monocytes, and <1% of neutrophils. Binding of PF4/heparin to B cells showed marked dependence on heparin concentration. No binding was detected when PF4 was added alone or in the presence of low concentrations of heparin (<0.005 U/mL) in whole blood, but as the concentration of heparin was increased (0.005 to 1 U/mL), binding of KKO (PF4/heparin complexes) was markedly enhanced. Cell-surface binding of KKO was lost at heparin concentrations ≥5 U/mL. Whole blood from patients treated with heparin was also analyzed in the absence of exogenous PF4 or heparin. Binding of KKO to patient B cells and indicative of endogenous PF4/heparin complexes ranged from 0.3% to 82%. Overall, B cells from 6 (37%) of 16 patients studied bound KKO. As with healthy donors, *in vivo* binding of PF4/heparin was essentially confined to the B-cell population. Binding of PF4 to B cells *in vivo* was also heparin induced, as PF4/heparin-positive B cells were not detected before UFH therapy (–2 hours), while UFH at a dose of 13 to 18 U/kg per hour was associated with a steady rise in the percentage of PF4/heparin positive B cells over time (13–45 hours). At supratherapeutic levels of heparin, as indicated by activated partial thromboplastin time >300 seconds, there was complete loss of antigen binding. The observation that a high proportion of circulating B cells in healthy donors are capable of binding PF4/heparin complexes suggests that binding might be mediated by antigen-independent binding to B cells through complement binding.

To investigate the role of complement in binding of PF4/heparin complexes to B cells, whole blood was incubated with PF4/heparin under experimental conditions known to inhibit complement activation (heat inactivated sera/ice/EDTA) but not interfering with formation of PF4/heparin complexes. Binding of complement fragments C3c/C4c to B cells was assessed by flow cytometry with significant amounts of complement fragments C3c and C4c deposited on B cells pre-incubated with PF4/heparin, but not with buffer or PF4 alone. PF4/heparin complexes bound to B cells in the presence of buffer or control IgG, but did not bind to B cells preincubated with polyclonal anti-CD21. Together, these results establish that complement mediates binding of PF4/heparin complexes to human B lymphocytes via CD21. Preferential binding of PF4/heparin to B cells may be attributable to the use of whole blood, which provides a source of complement, rather than use of isolated cell populations as described in previous studies.

The authors propose the following model of immune activation leading to HIT. Heparin displaces PF4 from cell surfaces into the circulation, generating ULCs that vary in size and charge. By as yet uncharacterized mechanisms, complexes of PF4 and heparin formed at optimal ratios of reactants in solution activate complement, which leads to incorporation of complement activation fragments (C3/C4) into the PF4/heparin complexes. Complement-coated

PF4/heparin complexes then bind to circulating B cells via CD21. Binding of antigen to CD21 facilitates either direct activation of B cells and/or antigen transport to secondary lymphoid follicles and antigen transfer culminating in an immune response to the heparin-containing complex. These findings suggest that complement and/or CD21 may serve as potential therapeutic targets for reducing the immune-mediated complications of heparin therapy.

The paper by Khandelwal *et al.* (13) is the first observation showing complement involvement in the immune response to heparin/PF4 complexes *in vitro* and *ex vivo*. However, it does not address the mechanisms by which heparin-PF4 complexes activate complement nor how complement incorporation and CD21 engagement by PF4/heparin complexes leads to an immune response and then to HIT. Further studies are needed to unravel the basis of HIT in terms of pathogenesis of immunization to heparin/PF4 complexes, interindividual variation in the formation of immunocomplexes and platelet activation with the objective to identify improved laboratory tests and treatments. In addition, understanding the immunological basis of HIT can lead to discovery of pathways that could be controlled in autoimmune or allergic disease.

Moreover, in the foreseeable future, heparin will remain widely employed for thromboprophylaxis in both medical and surgical patients. Direct oral anticoagulants (DOACs) have only been approved for thromboprophylaxis in elective major orthopedic surgery but not in medical or other types surgical patients or cancer patients. The single oral drug approach in the treatment of VTE with DOACs, such as rivaroxaban and apixaban, could replace UFH/LMWH initial treatment of VTE in many patients and thus limit the use of heparin products. However LMWH is still the anticoagulant of choice in cancer patients for prophylaxis and treatment of venous thromboembolism, although clinical trials of DOACs for venous thromboembolism in cancer patients are ongoing and their results, if positive, may further reduce the use of heparin. However, heparin has the advantage of low cost, ease of monitoring, very limited interference with other drugs and the availability of a low cost antidote, protamine sulphate. As a result, it will remain the anticoagulant of choice for use in extracorporeal circuits such as in cardiac surgery and hemodialysis, and also in vascular surgery and percutaneous coronary revascularization procedures. With the use of heparin, HIT will remain a clinical challenge for both physicians and surgeons.

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