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Review

Atomic features of an autoantigen in heparin-induced thrombocytopenia (HIT)☆

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ABSTRACT

Autoantigen development is poorly understood at the atomic level. Heparin-induced thrombocytopenia (HIT) is an autoimmune thrombotic disorder caused by antibodies to an antigen composed of platelet factor 4 (PF4) and heparin or cellular glycosaminoglycans (GAGs). In solution, PF4 exists as an equilibrium among monomers, dimers and tetramers. Structural studies of these interacting components helped delineate a multi-step process involved in the pathogenesis of HIT. First, heparin binds to the 'closed' end of the PF4 tetramer and stabilizes its conformation; exposing the 'open' end. Second, PF4 arrays along heparin/GAG chains, which approximate tetramers, form large antigenic complexes that enhance antibody avidity. Third, pathogenic HIT antibodies bind to the 'open' end of stabilized PF4 tetramers to form an IgG/PF4/heparin ternary immune complex and also to propagate the formation of 'ultralarge immune complexes' (ULCs) that contain multiple IgG antibodies. Fourth, ULCs signal through FcγRIIA receptors, activating platelets and monocytes directly and generating thrombin, which transactivates hematopoietic and endothelial cells. A non-pathogenic anti-PF4 antibody prevents tetramer formation, binding of pathogenic antibody, platelet activation and thrombosis, providing a new approach to manage HIT. An improved understanding of the pathogenesis of HIT may lead to novel diagnostics and therapeutics for this autoimmune disease.

Contents

1. Introduction

We are interested in the general process of autoantigen development and defining neoantigens at the atomic level. Heparin-induced thrombocytopenia (HIT) is the most common drug-induced, antibody-mediated autoimmune thrombotic disorder. HIT is caused by IgG antibodies that bind to a complex formed between platelet factor 4 (PF4), a host protein, and heparin or cellular glycosaminoglycans (GAGs)—host polysaccharides [1–3]. HIT may lead to recurrent thromboembolism, limb amputation and death in ~1% of patients receiving unfractionated heparin (UFH) for at least 5 days, and, less commonly, in patients who receive low-molecular-weight heparins (LMWH) and other anionic polysaccharides [4]. Circulating immune complexes composed of PF4/heparin and IgG antibodies bind to platelet and monocyte Fc receptors and promote cellular activation, leading to the generation of thrombin and downstream thromboembolic events [3,5]. Therapy is based on anticoagulants that directly or indirectly inhibit thrombin [6] with significant, but incomplete, reduction in recurrent thrombosis, no

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reduction in the rate of amputation or death and a significant risk of major bleeding [7].

Many if not most patients exposed to heparin develop anti-PF4 antibodies, yet few develop HIT. This raises both fundamental immunologic and clinical questions. First, how does an endogenous sugar convert a normal host protein into an autoantigen in such a high proportion of otherwise seemingly immunologically normal individuals? Second, is there a fundamental difference in epitope specificity that differentiates a small fraction of pathogenic antibodies from the almost ubiquitous anti-PF4 antibodies that form after heparin exposure but are not associated with HIT? Third, can this distinction be used to develop clinical diagnostic tools to identify pathogenic antibodies and even to intervene in formation of ULCs as a disease-specific non-anticoagulant approach to mitigate or prevent thrombosis?

To begin to address these questions, we studied a murine monoclonal antibody (KKO) to human PF4/heparin complexes that causes thrombocytopenia and thrombosis in a transgenic mouse that expresses human PF4 and platelet FcγRIIA receptors [8,9]. KKO provides a model antibody for the study of HIT because it competes with pathogenic human HIT antibodies for binding to PF4 in vitro [10], augments the formation of pathogenic ultralarge immune complexes (ULCs; see below) [11,12] and recapitulates the salient features of HIT in vivo. Therefore, we compared the properties of KKO with an isotype-matched anti-PF4 monoclonal antibody (RTO) that binds comparably to PF4 in vitro, but does not foster the formation of ULCs and is not pathogenic [10].

We solved the crystal structure of human PF4 in complex with a heparin-mimic pentasaccharide and crystal structures of PF4 complexed with Fab fragments derived from KKO and RTO [13]. These atomic level structural studies delineate the first three steps in a 4-step model of the pathogenesis of this autoimmune disease: *Step 1*: Heparin binds to the 'closed' end of PF4 tetramer. Heparin binding orients and stabilizes the 'open' end of PF4 tetramer, which contains an epitope recognized by KKO, thus increasing antibody affinity. *Step 2*: A heparin fragment can be 'shared' by multiple PF4 tetramers. This aligns and approximates the tetramers to form a large antigenic complex, with the potential to increase antibody avidity. *Step 3*: Binding of multiple pathogenic antibodies propagates oligomerization forming stable ultralarge immune complexes (ULCs). *Step 4*: ULCs engage FcγIIA receptors on the surface of platelets and monocytes, which activates the cells, leads to expression of tissue factor and generates thrombin, which back-activates hematopoietic and endothelial cells promoting thrombosis [5,9]. Binding of the non-HIT antibody RTO to PF4 monomer inhibits tetramerization, antigen formation and higher-order assembly into ULCs. As a consequence, RTO inhibits HIT antibody-induced platelet activation and aggregation in vitro and thrombus progression in vivo [13]. These studies provide insights into the process by which endogenous or exogenous GAGs interact with a normal human host protein, alter its structure and assembly and render the protein/GAG complex 'antigenic' to the mammalian immune system.

2. Pathogenesis of HIT: an autoimmune disease

2.1. Steps 1–2: formation of PF4/heparin antigenic complexes

PF4, a host protein, becomes antigenic after complexing with heparin or GAGs. Binding of pathogenic antibodies to PF4 is markedly enhanced by heparin. In the absence of polysaccharide, apo-human [14] and bovine PF4 [15] adopt an asymmetric tetrameric conformation at the high concentrations employed for crystallization. When the N-terminal fragment of PF4 was replaced by a fragment from interleukin-8, the conformation became symmetrical and binding of heparin decreased [16]. This lead to the inference that the asymmetry of the PF4 tetramer plays a role in binding heparin [16] and several alternative models [17,18] have been proposed to explain the formation of PF4/heparin complexes.

In order to study the formation of PF4/heparin antigenic complexes and to investigate how the polysaccharide might induce antigen formation, we solved the crystal structure of PF4 in complex with fondaparinux, a homogenous synthetic pentasaccharide heparin fragment (PDB ID: 4R9W) [13]. Fondaparinux forms complexes with PF4 as assessed by atomic force microscopy and photon correlation spectroscopy [19]. Fondaparinux induces anti-PF4/heparin autoantibodies [20–22] and occasionally causes HIT [23–26]. The crystal structure of PF4/fondaparinux reveals a potential mechanism underlying the first steps in the pathogenesis of HIT: formation of the PF4/heparin antigenic complex.

Both the apo-PF4 tetramer and PF4 in complex with fondaparinux displays a pseudosymmetry characterized by an 'open' end and a 'closed' end [13,14]. Due to the structural asymmetry, there are only two major positively charged grooves on the surface of the tetramer, each of which can accommodate one molecule of fondaparinux. These grooves are located at the 'closed' end of the tetramer and among monomers within each tetramer (Fig. 1, step 1). Binding of fondaparinux within the groove between monomers stabilizes their self-association.

A single fondaparinux molecule not only binds within the groove on the surface of one PF4 tetramer but it also has extensive potential interactions with a second adjacent tetramer, especially at the C-terminal helix. In this way, PF4 tetramers can cluster around a series of semi-rigid regions along the linear heparin chain (Fig. 1, step 2). We suggest that the alignment and clustering of PF4 tetramers along heparin chains represent the first two steps in pathogenesis by stabilizing the epitope recognized by pathogenic HIT antibodies.

2.2. Step 3: formation of IgG/PF4/heparin immune complexes

After the binary PF4/heparin complex is formed, both the affinity and avidity of pathogenic HIT antibodies is enhanced leading to the

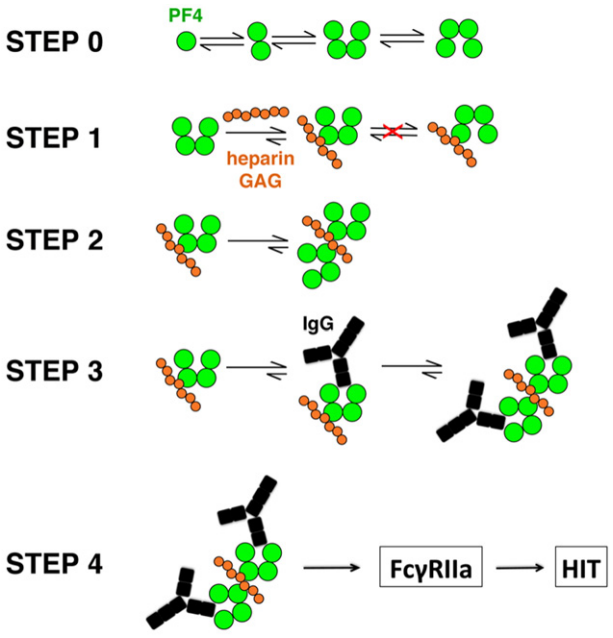

Fig. 1. Model of step-wise pathogenesis of HIT. Step 0: PF4 (green circles) released from activated platelets exists as an equilibrium among tetramers, dimers and monomers in plasma or whole blood. Tetramers may be transient and may switch between 'open–closed' and 'closed–open' conformations. Step 1: Heparin or GAGs (orange circles) bind to the 'closed' end of PF4 and stabilize the PF4 tetramers. Based on steric constraints, it is not possible for a similar switch in conformations to occur in heparin/GAG-bound tetramers. Step 2: Binding of the first tetramer enhances the binding and approximation of additional tetramers, which eventuates in the formation of large PF4/heparin antigenic complexes. Step 3: Binding of PF4 tetramers also enhances binding of pathogenic antibodies (black) to the 'open' end of the tetramer, forming ultralarge IgG/PF4/heparin immune complexes. Step 4: Immune complexes activate platelets and monocytes though FcγRIIAs and through expression of tissue factor, which leads to thrombin-dependent transactivation of platelets and endothelial cells.