

avidly to PF4 displacing it from its binding to glycosaminoglycans, allowing the complex to enter the circulation where it forms a soluble circulating immunogen.

After the immune response is raised, the PF4-heparin complexes become decorated with immunoglobulin (Ig), and it is these immune complexes that induce the major clinical features of HIT. Particular platelets, through their receptor for IgG FcγRIIA, bind to and become activated by the immune complexes (illustrated in the figure). This causes consumption of platelets leading to thrombocytopenia in the majority of HIT patients, although the drop in platelet count is usually only mild to moderate, and only rarely leads to bleeding complications.<sup>3</sup> Thrombosis, however, is the major cause of clinical presentation, occurring in 30% to 60% of patients, with the majority of these associated with venous thrombosis. This may occur in various tissues of the body causing limb gangrene, skin necrosis, and other ischemic conditions. In this way, thrombotic complications contribute to significant morbidity and mortality associated with HIT.

Because of the thrombotic nature of HIT, with platelet activation being a principal early event, therapy and management of the disease has concentrated on anticoagulation. This needs to be done with agents that do not cross-react with circulating antibodies to the PF4-heparin complex, and therefore direct thrombin inhibitors have been a favored approach, including lepirudin, argatroban, and bivalirudin.<sup>2</sup>

Here, Reilly et al have exploited our knowledge of the underlying mechanisms of HIT, which include activation of platelets signaling through FcγRIIA, to focus on the use of a novel inhibitor of Syk tyrosine kinase called PRT-060318.<sup>1</sup> Syk is a gene with relatively restricted expression profile, limited substantially to B and T lymphocytes and platelets, although there is some expression in neuronal tissues and endothelial cells. Importantly, it had been shown that Syk is essential for signaling and activation of platelets by collagen, downstream of the glycoprotein VI (GPVI) receptor.<sup>4</sup> This receptor, a member of the Fcα receptor family, signals similarly to FcγRIIA in that they both recruit Syk through phosphorylation of an immunoreceptor tyrosine-based activation motif (ITAM). It was a highly reasonable hypothesis therefore that targeting Syk pharmacologically may ablate platelet

responses to stimulation of FcγRIIA. Because of the restricted expression of Syk, and its pivotal role in B-cell function and development, Syk has already been the subject of inhibitor development, and is currently successfully targeted in the treatment of B-cell lymphoma and immune-mediated disease such as immune thrombocytopenic purpura and rheumatoid arthritis. In the article by Reilly et al, the authors present evidence that their novel inhibitor of Syk, PRT-060318, is also highly effective in managing HIT in a mouse model system, both in vitro and in vivo. The mouse expresses a transgene for human FcγRIIA and also human PF4, to “humanize” the mouse platelets to allow them to respond to immune complexes. The data show clearly that PRT-060318 is highly selective for Syk and is substantially effective at selectively inhibiting Syk-dependent platelet function, such as aggregation in response to GPVI agonism or to a HIT-like antibody. This is the case whether the drug is administered to platelets in vitro or ex vivo after administration of PRT-060318 to mice orally. Importantly, in vivo administration of PRT-060318 was capable of preventing a thrombocytopenic episode in mice administered with a HIT-like antibody. The drug was also capable of blocking thrombotic events in vivo induced by the HIT-like antibody.

This report is therefore potentially a major development in the management of HIT and also in understanding its biology. The major effect of Syk inhibition is likely to be on HIT immune complex-induced platelet activation, but because PF4 needs to be released by plate-

lets in the first place, there may be an additional effect of PRT-060318 on early activation events leading to PF4 secretion. In addition, although the authors report no change in bleeding time with Syk inhibition, careful analysis of this, especially in the presence of residual heparin, will need to take place as one of the early steps in development of this approach in the treatment of HIT. Finally, although the effect is likely principally to be mediated by inhibition of platelet function, in the context of the control of thrombosis, it is possible that effects of Syk inhibition on immune cell function may also play a role in helping to dampen the response in HIT. These are points for further study and analysis, but in the first instance targeting platelet Syk would appear to be a promising novel way forward for management of this difficult clinical problem.

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

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## ● ● ● THROMBOSIS & HEMOSTASIS

Comment on Wootla et al, page 2257

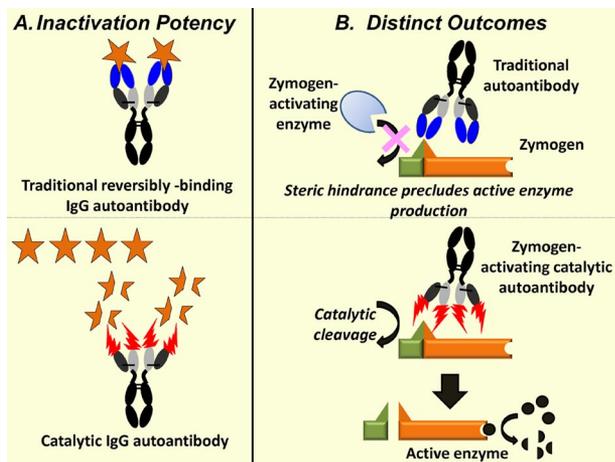
# Two-faced catalytic autoantibodies

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Factor IX (FIX)-activating catalytic antibodies in acquired hemophilia patients reported in this issue of *Blood* by Wootla et al<sup>1</sup> reveal the potential of the immune system to influence life processes in unexpected ways.

**C**atalytic antibodies were originally described as pathogenic mediators in autoimmune disease,<sup>2</sup> analogous to the traditional role of reversibly binding autoantibodies as mediators of Ehrlich's horror autotoxicus theory of autoimmunity. The FIX-activating antibodies, in contrast, are proposed to exert a

beneficial procoagulant effect that compensates for the anticoagulant effect of conventional autoantibodies to Factor VIII (FVIII) found in acquired hemophilia patients. The authors present biochemical evidence for generation of activated FIX and improved blood coagulation occurring upon the enzyme-like



Functional outcomes because of production of traditional and catalytic autoantibody. (A) The traditional IgG autoantibody binds 2 molecules of the autoantigen reversibly (top), whereas the catalytic autoantibody turns over repeatedly and can cleave thousands of autoantigen molecules over its lifetime in the blood (bottom). The inactivation of several autoantigens by catalytic autoantibodies has been described. (B) Reversibly binding autoantibodies can sterically mask the cleavage site in a zymogen, inhibiting its fragmentation by the zymogen-activating enzyme (top). In contrast, the catalytic autoantibodies described by Wootla et al themselves serve as the FIX zymogen-activating enzymes, resulting in the generation of the enzymatically active FIX fragment that in turn catalyzes FX activation (bottom).

cleavage of FIX by catalytic antibodies. Despite the heterogeneous clinical histories of the patients, the presence of FIX catalytic antibodies was widespread, and the authors claim a tendency toward improved survival. Only low-level FIX binding activity was detected. Because of the ability of a single catalytic antibody molecule to turnover multiple FIX molecules, catalysts can be detected more readily than traditional antibodies that bind autoantigens stoichiometrically by noncovalent means. For the same reason, catalysts exert more potent functional effects (see figure). The ability to activate the FIX zymogen by a cleavage reaction adds a new dimension, as binding of the target autoantigen by traditional antibodies only suppresses target autoantigen function. A previously published example of this type of zymogen activation is the generation of thrombin-like fragments on cleavage of prothrombin by autoantibodies from lupus patients.<sup>3,4</sup>

The immune genesis of catalytic antibodies is also distinctive. Mature antibodies are generated from approximately 100 variable region germ line genes along with the diversity, joining and constant region genes. Structural adaptation of the variable domains expands the repertoire, resulting in the capability of producing a very large number of antibodies with distinct antigen-binding specificity. Antibodies produced by healthy humans and animals display a promiscuous catalytic activity that cleaves peptide bonds by a nucleophilic mechanism, akin to the proteolytic pathway used by the serine protease family enzymes.

Catalysis, therefore, appears to be a homeostatic function of the innate immune system expressed without a requirement for antigen-driven adaptive maturation.<sup>5</sup> Indeed, decreased survival of septic shock patients is associated with reduced expression of promiscuous catalytic antibody activity,<sup>6</sup> and patients with autoimmune disease also display reduced promiscuous catalytic antibodies.<sup>5</sup>

Unlike the noncovalent binding function, stimulation of the immune system by exogenous antigens generally fails to induce rapid improvement of catalysis by immunoglobulin G (IgG) antibodies. The terminal step of the catalytic cycle is release of antigen fragments. Adaptive improvement of catalysis, therefore, militates against the B-cell clonal selection theory, which holds that continued antigen binding of antibody expressed as the B-cell receptor (BCR) drives immune selection. Autoantibodies, on the other hand, can frequently express antigen-specific catalytic activity, including IgGs that hydrolyze nucleic acids and polypeptide autoantigens such as vasoactive intestinal peptide, thyroglobulin, FVIII, myelin basic protein, amyloid  $\beta$  peptide, and now FIX. While the antigenic stimulus driving the formation of FIX-activating catalytic antibodies is not known, acquired hemophilia is generally idiopathic or arises in association with autoimmune disease, malignancy, and pregnancy, and treatment of this disorder with immunosuppressive drugs would not induce the autoantibodies. The FIX catalytic antibodies, therefore, are likely to be

true autoantibodies, and the frequent expression of catalysis by autoantibodies demands an explanation.

The answer may lie in electrophilic autoantigens that bind B cells and induce adaptive improvement of the germline-encoded catalytic function. Engineered antigens containing artificial electrophiles are capable of inducing proteolytic antibodies by binding covalently to nucleophilic BCR sites,<sup>7</sup> thereby rendering the nucleophilic reactivity underlying catalysis immunologically selectable over the course of B-cell differentiation. Autoimmune disease is associated with increased posttranslational generation of autoantigen adducts of lipid peroxidation metabolites and advanced glycation end-products.<sup>8</sup> Such adducts contain reactive electrophiles capable of stimulating adaptive immune selection of catalytic antibody nucleophilicity. Yet another possibility is that under the abnormal B-cell regulatory pathways found in autoimmune disease, peptide bond cleavage by catalytic BCRs is itself a selectable event. The cleavage reaction liberates a large amount of energy, and dysfunctional cellular signal transduction might permit productive use of the energy to drive B-cell division. In view of accumulating evidence for autoantigen-specific catalytic autoantibodies, further study of such nontraditional B-cell stimulatory mechanisms is warranted.

The functional outcome of catalytic autoantibody production depends on the biologic role of the target autoantigen. For instance, accumulation of amyloid  $\beta$  peptide aggregates in the brain is the hallmark of Alzheimer disease, and overproduction of amyloid  $\beta$  peptide has no known physiologic utility. Catalytic autoantibodies that hydrolyze amyloid  $\beta$  peptide are proposed to be beneficial by virtue of their autoantigen clearance function.<sup>9</sup> In acquired hemophilia, production of autoantibodies to FVIII is thought to underlie pathogenesis of the bleeding disorder, including a catalytic autoantibody subset that cleaves FVIII and renders it incapable of fulfilling its cofactor role in blood coagulation.<sup>10</sup> The FIX-activating catalytic antibodies found in acquired hemophilia are hypothesized by Wootla et al<sup>1</sup> to fulfill a beneficial compensatory role by accelerating the next step in coagulation, activated FIX-catalyzed FXa generation. The existence of diverse autoantibody specificities combined with distinctive B-cell regulatory pressures render credible the conception of catalytic autoimmune reactions as a competing

set of functionally harmful and beneficial interactions.

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## ● ● ● TRANSPLANTATION

Comment on Kuzmina et al, page 2265, and on Sarantopoulos et al, page 2275

# Chronic GVHD: B cells come of age

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The pathophysiology of chronic graft-versus-host disease (GVHD) in humans remains poorly understood. While donor T cells play a major role, B cells have emerged in the past few years as significant actors too. In this issue of *Blood*, 2 papers add important advances to our knowledge of how B-cell subsets are implicated in the protean forms of the disease and how these subsets are affected by treatment with Rituximab.

**B** cells are central players in the humoral immune response. They produce antibodies and provide a protective immune defense against bacterial and viral pathogens. Over the past decade this view of B cells solely acting in humoral immune responses has changed dramatically. Accumulating evidence suggests that apart from antibody production, B cells contribute to the immune response by antibody-independent mechanisms such as presentation of antigen, the production of cytokines and chemokines, as well as by acting as regulatory cells (reviewed in Shimabukuro-Vornhagen<sup>1</sup>).

The role of B cells in the pathogenesis of chronic GVHD has been underestimated for many years. However, early studies pointed out the clinical similarities, as a multi-organ systemic disease, between chronic GVHD and some autoimmune diseases such as systemic

lupus and scleroderma. Indeed, clinicians reported that some patients developed auto-antibodies. However, these auto-antibodies are neither constantly found nor always of the same type (eg, anti-nuclear, -nucleolus, -double strand DNA). Then, studies from Storek et al<sup>2</sup> and us<sup>3</sup> described an association between low total B-cell counts with high infection rate and chronic GVHD. Also at that time a fascinating paper by Glas et al<sup>4</sup> reported the relative lack of immunoglobulin (Ig)D negative peripheral B cells (considered as memory B cells for the time being), 1 year after grafting. In this study, neither healthy subject T cells nor transplant-recipient T cells were able to induce the accumulation of somatic mutation in stem cell transplant-recipient B cells, suggesting that B cells after transplantation had an intrinsic inability to be driven to accumulate somatic mutations.

In the 1990s multicolor flow cytometry became more easily available and knowledge of B-cell ontogeny improved, although mainly in the murine system. Several B-cell subsets were discovered (see figure) and their distortion in patients with autoimmune diseases reported. In particular, it was reported that CD21 negative B cells (immature/transitional cells) are increased in lupus and in primary immunodeficiency and that CD27 was a marker of memory B cells. Somewhat surprisingly, however, it was not until the late 2000s that B-cell reconstitution of these subsets after allogeneic stem cell transplantation was studied. Two groups were particularly involved, that of Ritz in Boston and Greinix in Vienna:

The Boston group reported that patients with chronic GVHD had increased B-cell activation factor (BAFF)/B cells ratio, delayed reconstitution of naive B cell and that BAFF serum level correlated with increased number of pre-germinal center B cells.<sup>5</sup>

The Vienna group reported that patients with active chronic GVHD had elevated numbers of CD21 negative transitional B cells and deficiency of memory CD27 positive B cells.<sup>6</sup> The association of a deficiency in memory B-cell reconstitution in patients with chronic GVHD was recently confirmed by our group in a large number of patients followed longitudinally for more than 2 years from transplantation.<sup>7</sup>

Thus, chronic GVHD is associated with perturbed B-cell homeostasis. Patients who develop chronic GVHD have a relative reduction in naive B cells and relatively higher numbers of activated memory type. Elevated levels of BAFF have been correlated with the development and severity of chronic GVHD. High levels of BAFF in the presence of lower numbers of naive B cells might thus foster the survival of activated alloreactive and auto-reactive B cells, resulting in immune pathology (reviewed in Shimabukuro-Vornhagen<sup>1</sup>). It was thus a logical step to introduce treatment with Rituximab in the chronic GVHD therapeutic arsenal.<sup>8</sup> Responses were reported in roughly 50% of patients failing first-line treatment with prednisone and cyclosporine.

In this issue of *Blood*, both Sarantopoulos et al<sup>9</sup> and Kuzmina et al<sup>10</sup> add significant pieces to the understanding of the role of B cells in chronic GVHD. Sarantopoulos et al<sup>9</sup> studied 20 patients more than 2 years after treatment with Rituximab. The authors convincingly show that naive B-cell reconstitution



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## **Two-faced catalytic autoantibodies**

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