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Fc receptors in immune thrombocytopenias: a target for immunomodulation?

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Commentary

In autoimmune disease, Fc receptors (FcRs) form the interface between immune effector cells and their antibody-coated targets, and as such are attractive targets for immunomodulatory therapy. In this issue of the *JCI*, two highly novel studies of Fc–FcR interactions provide new insights into the role of FcRs in immune thrombocytopenia. Asahi et al. utilized a comprehensive platform of immunological assays to examine the mechanism underlying *Helicobacter pylori*–associated immune thrombocytopenic purpura, and Ghevaert et al. describe a specially designed antibody that saturates binding sites on fetal platelets without initiating FcγR-mediated platelet phagocytosis, preventing the binding of pathological maternal anti-HLA antibodies that cause fetomaternal alloimmune thrombocytopenia (see the related articles beginning on pages 2939 and 2929, respectively). These reports illustrate how a remarkably detailed molecular understanding of the FcR network may translate into new therapeutic strategies with high clinical impact.

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Fc receptors in immune thrombocytopenias: a target for immunomodulation?

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In autoimmune disease, Fc receptors (FcRs) form the interface between immune effector cells and their antibody-coated targets, and as such are attractive targets for immunomodulatory therapy. In this issue of the *JCI*, two highly novel studies of Fc–FcR interactions provide new insights into the role of FcRs in immune thrombocytopenia. Asahi et al. utilized a comprehensive platform of immunological assays to examine the mechanism underlying *Helicobacter pylori*–associated immune thrombocytopenic purpura, and Ghevaert et al. describe a specially designed antibody that saturates binding sites on fetal platelets without initiating FcγR-mediated platelet phagocytosis, preventing the binding of pathological maternal anti-HLA antibodies that cause fetomaternal alloimmune thrombocytopenia (see the related articles beginning on pages 2939 and 2929, respectively). These reports illustrate how a remarkably detailed molecular understanding of the FcR network may translate into new therapeutic strategies with high clinical impact.

A focus on the Fc receptor network present on macrophages

The interactions between immune cells and their target cells in autoimmune diseases have been the focus of much attention, and intense efforts have been made to manipulate the signaling pathways involved. The great majority of studies have examined T and B cells, and recently there has been increased interest in the role of Tregs as deft orchestrators of the immune response (1, 2). In contrast, macrophages have largely been investigated for their ability to execute intracellular killing and are considered to be the mobile but passive "clean-up men" of the host defense system. Part of their weaponry, which only devotees care to distinguish into subgroups, comprises the Fc receptors (FcRs). Seminal studies, initially from Ravetch's group and subsequently from the Lazarus laboratory, developed key insights into the network of FcRs expressed on macrophages, and the interactions between these phagocytic cells and antibodies emerged as attractive targets for immunomodulatory therapy (3, 4). Two articles in this issue of the *JCI* involve very different manipulations of the Fc-FcR interaction in order to increase our understanding of the pivotal role played by the FcR network in the pathogenesis of immune thrombocytopenia. Both reports are highly clinically relevant. In the first study, Asahi et al. examined the changes in the balance of FcRs expressed by patients with immune thrombocytopenia purpura (ITP) and *Helicobacter pylori* infection in order to explore the mechanism of platelet recovery that has been observed in these individuals following treatment to eradicate *H. pylori* (5). In the second study, Ghevaert et al. report the development and preclinical testing of a recombinant antibody designed to prevent FcR-mediated alloimmune destruction of platelets, which may have potential as a treatment approach for fetomaternal alloimmune thrombocytopenia (FMAIT) (6).

Inhibiting FcγR-mediated platelet clearance in ITP: a historical context

The central immunopathological disturbance in immune thrombocytopenia is the destruction of antibody-coated platelets by phagocytic cells in the reticuloendothelial system (7). Circulating monocytes and resident macrophages in the spleen and liver

Nonstandard abbreviations used: FcR, Fc receptor; FMAIT, fetomaternal alloimmune thrombocytopenia; HPA, human platelet antigen; ITP, immune thrombocytopenia purpura; IVIG, i.v. immunoglobulin.

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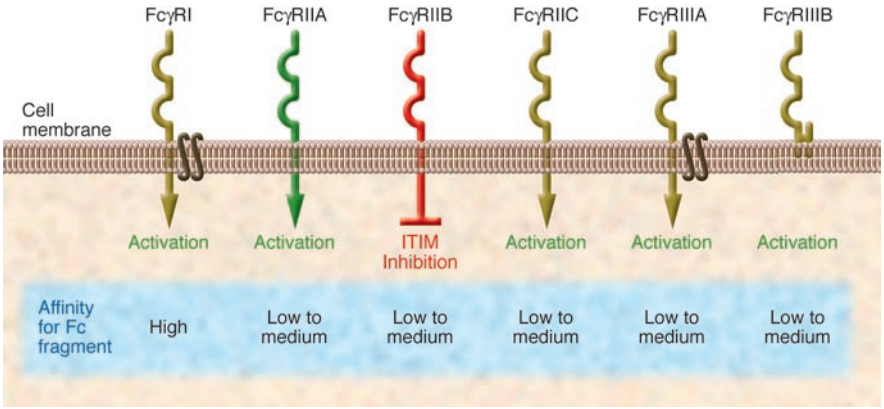

Figure 1
The activating and inhibitory human FcγRs. Humans have one inhibitory FcγR, FcγRIIB, which contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) as its intracellular signaling domain. Upon binding to Fc fragments, the ITIM recruits negative regulatory signaling proteins. Fc binding to the other FcγRs, including FcγRIIA, induces recruitment of proteins that are involved in activation signaling, via immunoreceptor tyrosine-based activation motifs (ITAMs), which typically consist of a ligand-binding α-chain. FcγRI and FcγRIIIA have signal-transducing γ-chain dimers (indicated by SS). As reported by Asahi et al. in this issue of the *JCI* (5), the balance between the numbers of inhibitory (FcγRIIB) versus activating (FcγRIIA) FcγRs is disturbed in patients with ITP and *H. pylori* infection, with downregulation of the inhibitory receptor FcγRIIB. Eradication of *H. pylori* was found to normalize the FcγR balance and reduce opsonophagocytosis of platelets by macrophages of the reticuloendothelial system. There is one high-affinity receptor, FcγRI. The other FcγRs have low to medium affinity for the Fc portion. Unlike the other transmembrane receptors, FcγRIII is a glycosylphosphatidylinositol-linked protein.

bind to the exposed Fc portion of platelet-associated IgG molecules via Fc receptors for IgG, namely FcγR. This system first entered the limelight when it became clear that a primary mechanism of action of two first-line therapies for ITP, steroids and i.v. immunoglobulin (IVIG), occurred via interference with FcγR-mediated platelet clearance. The earliest theories for the mechanism of action of IVIG were built on the observation by Fehr et al. in 1982, and ourselves a year later, that treatment with IVIG in non-splenectomized patients with ITP prolonged the clearance of radiolabeled, antibody-coated red blood cells, suggesting competitive inhibition of FcγR-bearing phagocytes in the spleen (8, 9). This development set the stage for the design of more targeted therapies against the FcγR system, with the goal of improving efficacy and avoiding the therapeutic use of human blood products.

Manipulating the immunoglobulin Fc fragment

Soon thereafter, more specific FcγR-blocking treatments were explored, including i.v. infusions of the immunoglobulin anti-D (which binds specifically to the erythrocyte D antigen) (10) and infusion of a monoclonal anti-FcγRIII antibody (11). Another approach was to modify the IgG in IVIG by digesting the Fc portion in order to change its interaction with the FcγR system. The partially digested product was less effective than intact IVIG in children with ITP (12). However, infusions of isolated Fc fragments of IgG were shown to have similar effects to intact IgG on platelet counts in children with ITP, confirming that Fc-FcγR interactions were important in mediating the therapeutic effects of IVIG (13). These crude manipulations of IgG were abandoned, and among these agents only i.v. anti-D continued forth into routine clinical usage. Nonetheless, these studies illustrated the potential of modulating the interactions between circulating antibodies and FcRs.

Inhibitory and activating FcγRs: a fine balance

Over time, as additional FcRs were identified, the complexity of the FcR system was revealed (Figure 1). As early as 1964, Brambell hypothesized that there existed a process to recycle IgG, later shown to involve the neonatal FcR, FcRn. This FcR is unique among FcRs in that it is a heterodimer consisting of an MHC-1–related glycoprotein bound to a β2 microglobulin protein and has been studied as a therapeutic target to prevent the recycling of autoantibodies in autoimmune disease and, in doing so, shorten autoantibody half-lives (14, 15). Importantly, FcRn also mediates transplacental passage of maternal IgG into the fetus (16, 17). Subsequent exploration of the FcγR system resulted in the remarkable discovery of distinct inhibitory and activating FcγRs; in particular, description of the inhibitory receptor FcγRIIB initiated a new era in studies of FcR manipulation (18, 19).

The realization that the in vivo action of an IgG antibody binding to an FcγR-bearing cell depended on the net balance of activating versus inhibitory FcγR signaling led to the next major clinically related breakthrough, which provided insight into the mechanisms underlying the effect of IVIG in the treatment of ITP. First, Samuelsson et al. reported that the protective effect of IVIG in an anti-platelet antibody–mediated murine model of ITP was dependent on the presence of FcγRIIB and that IVIG administration increased the expression of this inhibitory receptor by splenic macrophages (3). These findings have since been confirmed and dramatically extended. An elegant series of preclinical studies by Lazarus's group (20) examined the downstream signaling pathways of FcγRs, and the results indicated that the therapeutic effect of IVIG in antibody-mediated murine ITP resulted from its interaction with DCs, in that DCs pre-incubated with IVIG in vitro could recapitulate the therapeutic effect of IVIG. These IVIG-primed leukocytes only took effect when the recipient mouse expressed FcγRIIB, although FcγRIIB was not required on the "initiator" DCs, indicating that FcγRIIB was not the direct target of IVIG but a critical downstream mediator (20). Furthermore, the ability of DCs to ameliorate ITP was maintained in immunodeficient mice lacking T and B cells, suggesting that DCs do not merely act via modulation of antibody production by B cells or of the T cell compartment, but directly interact with phagocytes of the innate immune system to prevent destruction of opsonized platelets. The findings of Samuelsson et al. (3) and the subsequent studies demonstrated the clinical importance of this hitherto unrecognized central role for FcγRIIB and set the stage for the current study by Asahi and colleagues of the mechanism of the effect of *H. pylori* to exacerbate and/or perpetuate ITP (5).

H. pylori–associated thrombocytopenia in ITP

It is generally accepted that the presence of *H. pylori* infection may contribute to the

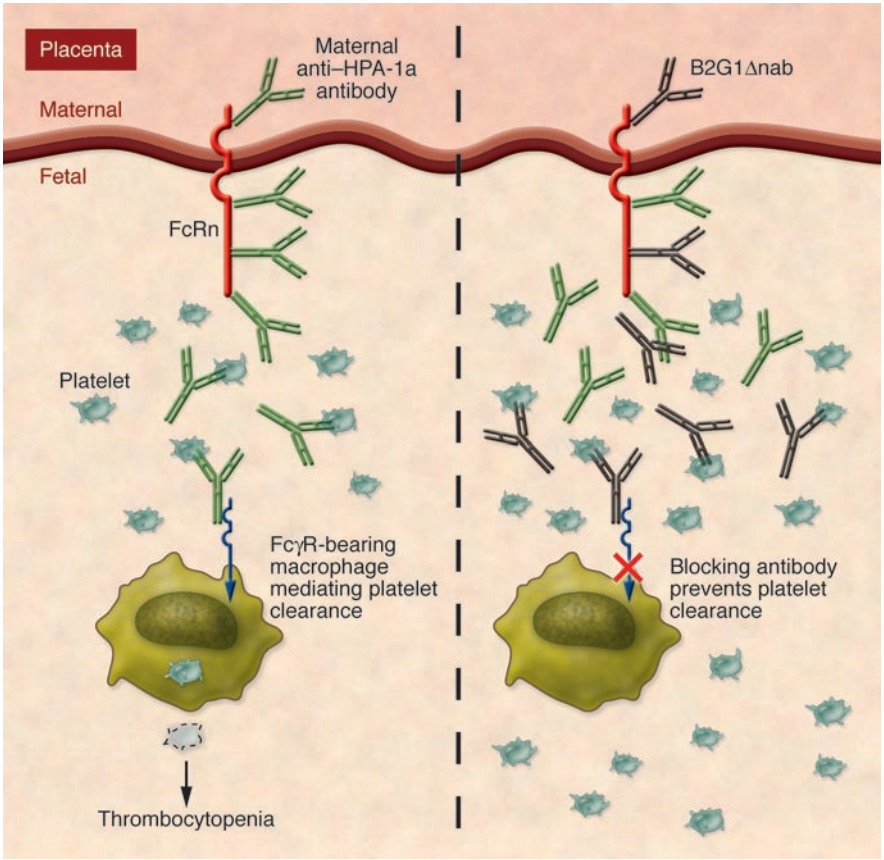

Figure 2

Interfering with FcγR-mediated platelet phagocytosis in FMAIT. In FMAIT, maternal anti-platelet antibodies are transferred across the placenta by the neonatal Fc receptor (FcRn) and mediate clearance of fetal platelets by FcγR-bearing phagocytes (macrophages) in the reticuloendothelial system. In the model proposed by Ghevaert et al. in their current study in this issue of the *JCI* (6), administration of their newly designed antibody B2G1Δnab would saturate available HPA-1a–binding sites on platelets but not activate FcγR signaling, thereby preventing platelet destruction characteristic of FMAIT.

pathogenesis and persistence of immune thrombocytopenia in patients with ITP and that eradication of the organism can result in an increase in platelet count in a substantial fraction of infected patients. Despite this clinical wisdom, the underlying mechanism of the effect of *H. pylori* to cause thrombocytopenia has remained unclear. Suggested hypotheses have included molecular mimicry of *H. pylori* antigens by platelet/megakaryocyte glycoproteins and infection-related perturbation of the immunoregulatory system, thereby promoting the production of autoreactive antibodies (21). The platform of immunological assays employed by Asahi et al. in their current study (5) suggests that *H. pylori* causes or exacerbates ITP by downregulating FcγRIIB and that eradicating *H. pylori* restores the balance by shifting toward the inhibitory FcγR phenotype with less active opsonophagocytosis, thereby ameliorating the immune-mediated platelet destruction.

The very high degree of homology in the extracellular domains of FcγRIIA and FcγRIIB has been an almost insurmountable obstacle to previous studies of these receptor subtypes (22). In their current study, Asahi et al. (5) employed cell permeabilization in order to use intracellular antibodies specific to the C-terminal portion of FcγRIIB for flow cytometric assays, in combination with mRNA analysis to determine the FcγRIIA/FcγRIIB balance. Future investigation utilizing the very recently developed specific discriminatory antibodies may allow finer delineation of receptor expression and an expanded understanding of the factors that alter the expression of FcγRIIB. Support that the changes in the FcγRIIA/FcγRIIB balance are clinically relevant comes from the temporal correlation of the changes in FcR subtype expression with changes in both phagocytosis assays and the increase in platelet numbers in responders. While this report does not explain why only a portion of *H. pylori*–infected ITP patients will benefit from *H. pylori* eradication in terms of their ITP, it provides sound evidence that an imbalance in FcγR signaling may be the most important clinical mechanism underlying *H. pylori*–associated ITP. We believe this to be the first time that an infection has been shown to alter the natural balance of activating and inhibitory FcRs, with the eradication of the infection being the means to restore the FcR balance.

Fc-FcR interactions in neonatal alloimmune thrombocytopenia

The report by Ghevaert et al. (6) in this issue of the Journal exploits the FcγR pathway by a totally different approach from that of Asahi et al. (5), this time with the aim of treating FMAIT. FMAIT results from transplacental transfer of maternal antibodies that develop in response to alloimmunization against paternal human platelet antigens (HPAs) expressed on fetal platelets. The HPA-1a antigen is responsible for the great majority of cases of severe FMAIT in individuals of mixed European descent (23). FMAIT is uncommon (affecting approximately 1 in 1,000 births), but it is the most important cause of severe fetal/neonatal thrombocytopenia and is associated with substantial morbidity and mortality due to intracranial hemorrhage, the risk of which is higher if a previous sibling was similarly affected (24). Currently, there is no routine screening for this condition during pregnancy, and antenatal management of siblings of fetuses affected by FMAIT relies on administration of large quantities of IVIG to the mother (1–2 g/kg/wk) with varying degrees of invasive intrauterine monitoring and occasional intrauterine infusions of HPA-compatible platelets.

In contrast to the studies by Asahi et al. (5) on infection-related thrombocytopenia and mechanism of treatment effect, the approach of Ghevaert et al. (6) involves manipulation of the Fc portion of IgG in order to change its interaction with FcRs. Why develop such a complicated treatment for FMAIT? FMAIT presents a more complicated immunopathology than ITP by virtue of involving not only the immunobiology of the mother, fetus, and placenta

but also the pregnancy-associated changes to the maternal immune system. Therefore, the challenge faced by Ghevaert et al. was to design an antibody that would bind with high affinity to HPA-1a on platelets, not initiate FcγR-mediated immune clearance of these platelets, and yet have an intact Fc fragment able to interact with FcRn and thereby be transferred via the placenta to the fetus (Figure 2). Previous preclinical studies by this group support the achievement of these aims.

Ghevaert et al. (6) engineered a specific, non-FcγR–activating antibody construct (termed B2G1Δnab) that saturates available antigenic sites and blocks binding of the pathological maternal antibodies to fetal platelets. The need for preserved interaction with FcRn prevented the use of either single-chain antibodies or of deglycosylating the Fc fragment of the antibody, and of other modifications such as the crude digestions of IgG described above, which would prevent activating interaction with FcγRs. Therefore, the construct was engineered using IgG subclass 2 and 4 residues substituted into an IgG1 backbone (25). IgG2 and IgG4 are known not to activate complement and have a 20- to 100-fold lower affinity for FcγR than IgG1 and IgG3. Using a dually perfused isolated human placental model, they confirmed that transplacental transfer via FcRn was intact (26).

In 19 of 20 maternal sera tested in vitro, up to 95% inhibition of anti–HPA-1a–binding to platelets was achieved using the B2G1Δnab antibody (6). Murine studies confirmed that this recombinant antibody abrogated FcγR-mediated antibody-coated platelet clearance, encouraging further exploration of the feasibility of this approach in patients.

While teasing out the exact mechanism of IVIG therapy in FMAIT has proved difficult, a leading hypothesis is that high-dose weekly infusions of IVIG administered to the mother may block FcRn-mediated transplacental transport of the anti–HPA-1a antibody. This treatment is relatively effective (but very expensive, especially if initiated at 12 weeks of gestation); involves the infusion of large quantities of salt, protein, and water; and is a human blood product. In contrast, there is the enticing possibility that infusion of this synthetic monoclonal antibody B2G1Δnab (6) would only need to be given to the mother, albeit weekly, and therefore, direct intrauterine delivery could potentially be avoided. Extensive in vivo studies of this potential approach will be required to confirm its feasibility.

Designing more specific FcR-targeted therapies: the challenge

The race to design effective biological therapies for use in autoimmune thrombocytopenias that have a similar or improved efficacy over IVIG without having the disadvantages of involving human blood products has been in process, although without dramatic success thus far. Two monoclonal antibodies to FcγRIII (3G8 and GMA161) have been used in clinical trials for the treatment of ITP with only moderate efficacy (11, 27). A Syk kinase inhibitor that targets signaling pathways downstream of FcγRs, including FcγRIIA and FcγRIIB, has been shown to achieve a platelet response that could be maintained with continued administration in a majority of patients, albeit with some gastrointestinal toxicity (28).

Why have therapies targeted to the Fc-FcR system not been more successful thus far? As the complexities of the system continue to be revealed, perhaps it is becoming clear that the real challenge is to recapitulate the "social networking" or "class action" of native, intact immunoglobulin. As the current reports by Asahi et al. (5) and Ghevaert et al. (6) illustrate, manipulating FcR-mediated phagocytosis in immune thrombocytopenias remains a highly attractive target for the design of immunomodulatory therapies. In addition, disturbances to the relative expression of the inhibitory receptor FcγRIIB may, as is the case for ITP patients simultaneously infected with *H. pylori*, underlie other causes of thrombocytopenia in ITP.

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Fifty years later: the disk goes to the prom

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Although age-related macular degeneration is the most prevalent macular disease in the world, numerous discoveries regarding the molecular bases of vision have been made through genetic association studies of rare inherited maculopathies. In this issue of the *JCI*, Yang et al. present a functional genetics study that identifies a role for prominin 1 (PROM1), best known as a stem cell and/or progenitor cell marker, in the biogenesis of retinal photoreceptor disk arrays (see the related article beginning on page 2908). This study supports an established model in which disk morphogenesis occurs through membrane evagination and extends other recent studies assigning PROM1 important functions outside of the stem cell niche.

Essentials of photoreceptor organization

More than 50 years ago, the first ultrastructural evidence of photoreceptor disk organization was published by noted electron microscopist Fritiof Sjöstrand (1). Subsequent studies provided more detailed characterizations of the evolutionarily conserved arrangement of rod and cone photoreceptors into inner and outer segments within Bilateria (2). It is in this outer segment region that thousands of rhodopsin-containing bilayered disks form an array of photovoltaic cells that transmit visual stimuli to the neural retinal components. Without the organized development and maintenance of these precious subcellular elements, the eye cannot fulfill its raison d'être.

Many congenital and acquired diseases that result in vision loss are caused by photoreceptor degeneration. The most widely studied of these pathologies is age-related macular degeneration (3), an epidemic in the developed world affecting approximately 30–50 million people, rivaling the prevalence of cancer (4). However, the study of other, more rare hereditary macular diseases has also yielded fundamental knowledge that has greatly advanced our understanding of the molecular bases of vision. Historically, many of these major studies were published in 2 phases: the genetic association data was followed by insights into the functional implications of an identified polymorphism obtained via the use of transgenically engineered mice. In this issue of the *JCI*, Yang et al. give us the best of both worlds by presenting a combined functional genetics investigation of the critical nature of prominin 1 (*PROM1*; also known as *CD133* and *AC133*) expression during photoreceptor disk morphogenesis that provides essential insight into the molecular programming of disk formation and the ever-expanding roles for PROM1 (5).

Discovery of PROM1

PROM1 is still best known for its original use as a human stem cell–specific marker (6), yet its known biological functions continue to reach far beyond this role. The protein is constructed of 5 transmembrane domains, 2 large extracellular loops containing 8 N-linked glycosylation sites, and a cytoplasmic tail. Variable glycosylation of these extracellular loops may account for the monoclonal antibody specificity for certain tissue types and circulating stem cells. Contemporaneous with the characterization of AC133 for hematopoietic cell lineage analysis, another group reported the discovery of a mouse protein, termed PROM1, found to be expressed on specific embryonic and adult epithelia and localized to plasma membrane protrusions (7). Although it was quickly realized in an exchange of public letters by the 2 laboratories that the human stem cell marker was the likely homolog of mouse PROM1, with more than 60% sequence overlap, an entire body of literature emerged in which the antigen was used to identify specific cell populations. In a recent *JCI* article, previously unchallenged claims that PROM1 was a marker of tumor-initiating metastatic colon cancer cells were rebutted in a study that demonstrated the initiation of colon cancer tumors in xenografts by PROM1-negative cells (8). Thus, it appears that PROM1 is not as lineage specific or functionally determined as it once was purported to be.

PROM1 mutations are associated with hereditary macular degeneration

There is mounting evidence that PROM1 is critical to the organization of photoreceptor disks. In 2000, a group that included members from the team that initially described mouse PROM1 found a genetic association between a human PROM1 frameshift mutation and a form of autosomal-recessive retinal degeneration in a small Indian pedigree (9). This polymorphism resulted in premature termination of the protein, which prohibited it from

Nonstandard abbreviations used: PROM1, prominin 1.

Conflict of interest: The authors have declared that no conflict of interest exists.

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