Targeting of platelet integrin $\alpha_{\text{IIb}}\beta_3$ determines systemic reaction and bleeding in murine thrombocytopenia regulated by activating and inhibitory Fc$\gamma$R

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Abstract

Previous work on cellular destruction induced by several clinically relevant anti-platelet IgG antibodies suggested antigen-specific mechanisms in the development of immune thrombocytopenia in mice. mAb directed against mouse platelet GPIb$\alpha$ and integrin $\alpha_{\text{IIb}}\beta_3$ were highly pathogenic, and mediated their effects via different Fc-dependent ($\alpha_{\text{IIb}}\beta_3$) and Fc-independent (GPIb$\alpha$) pathways, indicating that clearance of IgG-bound platelets is only one event in the pathogenesis of murine thrombocytopenia. Here, we demonstrate that in addition to thrombocytopenia, targeting of platelet integrin $\alpha_{\text{IIb}}\beta_3$ results in acute systemic reaction and bleeding that is regulated by activating IgG Fc receptors (Fc$\gamma$R) and the inhibitory Fc$\gamma$RII. As shown by electron microscopy, anti-$\alpha_{\text{IIb}}\beta_3$ IgG mediated initial loss of $\alpha_{\text{IIb}}\beta_3$ integrin from platelet surfaces followed by rapid accumulation of $\alpha_{\text{IIb}}\beta_3$ antibody-containing immune complex (IC)-like structures in spleen and liver in vivo. In Fc$\gamma$R chain deficiency, mice resisted bleeding, but not platelet destruction, while genetic ablation of Fc$\gamma$RII resulted in uncontrolled systemic reaction and severe hemorrhage leading to enhanced mortality. Together, these results provide evidence that IC formation and engagement of Fc$\gamma$R on effector cells determines the $\alpha_{\text{IIb}}\beta_3$-specific part of the platelet pathology of the systemic reaction and bleeding in murine thrombocytopenia.

Introduction

Autoantibody-mediated cellular destruction contributes to the pathogenesis of several autoimmune diseases, and is thought to be specifically causal in the development of cytopenias such as autoimmune hemolytic anemia (AIHA) and immune thrombocytopenic purpura (ITP). In the vast majority of patients with severe ITP, pathogenic IgG autoantibodies directed against the platelet surface receptors $\alpha_{\text{IIb}}\beta_3$ integrin (GPIIbIIIa, fibrinogen receptor) and GPIIb-V-IX (von Willebrand factor receptor) are found (1–3). Immune clearance of the resulting autoantibody-opsonized platelets leading to thrombocytopenia is considered as the major cause of bleeding complications in patients suffering from severe ITP (4,5). It is currently accepted that platelet destruction is mediated mainly by cells of the reticuloendothelial system and is Fc dependent (5). Initial studies in rodents suggest a prominent role of activating IgG Fc receptors (Fc$\gamma$R) in this process (6,7). In humanized mouse models of Fc$\gamma$R, activation of the human platelet Fc receptor, hFc$\gamma$RIIA (CD32), has been reported to result in thrombosis and shock (8).

There are three classes of Fc$\gamma$R on leukocytes: the high-affinity receptor, Fc$\gamma$RI, and the two low-affinity receptors, Fc$\gamma$RII and Fc$\gamma$RIII (9,10). Both Fc$\gamma$RI and Fc$\gamma$RIII are...
multimeric receptors in association with the same FcR chain required for assembly and signaling (11). Animal studies using FcRγ or FcRRIII mutant mice revealed that activating FcRγ, especially FcRRIII, are involved in the initiation of immune complex (IC)-triggered inflammation and autoimmune disease (12–15). Most of the FcRRII- and FcRγ-triggered responses are balanced by the inhibitory FcRRII when co-expressed on the same effector cell (16–19). Moreover, the inhibitory FcRRII is modulated by IVIG in the treatment of thrombocytopenia in mice (20,21).

Recent studies using newly generated panels of mAb directed against various mouse platelet antigens, including αIIbβ3, GPIbα, GPIb-IX or GPV, have shown that differences in the antigenic specificity of anti-platelet IgG antibodies can determine their pathogenic activities (22,23). Most of these mAbs are able to mimic the effect of cytotoxic autoantibody by determining their pathogenic activities (22,23). In this study, we examined the role of the various FcR to the pathogenic effects of anti-mouse platelet αIIbβ3 mAb in normal mice as compared to IgG FcR knockout mice.

Methods

Mice

The generation of FcRγ−/−, FcRRII−/− and FcRRII+/+ mice derived from ES cells of 129 origin and backcrossed for more than eight generations with C57BL/6 mice has been described previously (11,15,16). C57BL/6 mice were obtained from Charles River (Sulzfeld, Germany). All these mice were used for experiments in accordance with the regulations of the local authorities.

Antibodies

FITC-conjugated polyclonal rabbit antibodies to human fibrinogen and rabbit anti-rat Ig were purchased from Dako (Glostrup, Denmark). Anti-mouse platelet IgG mAbs of defined specificities were established by standard techniques as described previously (23,24). Anti-mouse integrin αIIbβ3: JON1 (rat IgG2b) and JON3 (rat IgG1); anti-mouse GPIbα: p0p4 (rat IgG2b) and p0p5 (rat IgG1). F(ab)2 fragments were generated as described (23). Antibodies were conjugated with 5-nm colloidal gold by standard methods.

Experimental thrombocytopenia, systemic reaction and bleeding

Ether anesthetized mice received either a single dose of 3 μg/g of purified anti-mouse platelet IgG antibodies in 200 μl sterile PBS i.v. or, alternatively, 7 × 0.3 μg/g antibody i.p. were assayed at the indicated times for thrombocytopenia (as assessed by counting platelet numbers), for systemic reaction (as assessed by body temperature measurements with a rectal probe) and for bleeding (as assessed by levels of hematocrit (Ht)]. For determination of Ht, blood samples were collected into heparinized microhematocrit capillary tubes, centrifuged for 3 min at 12,000 r.p.m. in a microfuge and the percentage of packed erythrocytes was determined.

Platelet preparation and counting

Mice were bled under ether anesthesia from the retroorbital plexus. Blood was collected in a tube containing 10% (v/v) 7.5 U/ml heparin and platelet-rich plasma was obtained by centrifugation at 300 g for 10 min at room temperature. For determination of platelet counts, blood (20 μl) was obtained using siliconized microcapillaries and immediately diluted 1:100 in Unopette kits (Becton Dickinson, Heidelberg, Germany). The diluted blood sample was allowed to settle for 20 min in an improved Neubauer hemocytometer (Superior, Bad Mergentheim, Germany) and platelets were counted under a phase contrast microscope at ×400 magnification.

Flow cytometry

Heparinized whole blood was diluted 1:30 with modified Tyrode’s-HEPES buffer (134 mM NaCl, 0.34 mM Na2HPO4, 2.9 mM KCl, 12 mM NaHCO3, 20 mM HEPES, 5 mM glucose and 1 mM MgCl2, pH 6.6) and left for 30 min at 37°C prior to stimulation. For determination of surface αIIbβ3 levels, diluted whole blood was incubated with the fluorophore-conjugated mAb JON8 which binds to an epitope on the receptor different from that recognized by anti-αIIbβ3 JON1, JON3 mAb used in experimental thrombocytopenia in vivo.

Electron microscopy

Tissue from spleen, liver and lung were harvested from antibody-treated male mice following cervical dislocation. Following the extraction of the organ tissue, they were immersion-fixed with 0.1 M cacodylate buffered 2% glutaraldehyde/2% paraformaldehyde for 4 h. Post-fixation was performed in 2% osmium tetroxide buffered at pH 7.3 with 0.1 M sodium cacodylate at 4°C over 2 h. Specimens were rinsed in cacodylate buffer 3 times, in 1% uranyl acetate and in 70% ethanol for 3 h. Dehydration was performed in a series of graded ethanol and specimens were then embedded in araldite. Semi-thin sections of plastic-embedded specimens were cut with a glass knife on a Reichert ultramicrotome and stained with methylene blue. Ultrathin sections (30–60 nm) were obtained with the help of a diamond knife on the microtome described above, placed on copper grids and examined with a Zeiss EM902A electron microscope (Zeiss, Oberkochen, Germany).

Results

Anti-αIIbβ3 IgG-induced systemic reaction and bleeding, but not thrombocytopenia, is mediated by activating FcRγ

We previously generated panels of novel mAb recognizing various mouse platelet antigens and showed that antibodies against integrin αIIbβ3 and GPIbα are by far the most pathogenic, resulting both in surface loss of antigen and clearance of platelets in mice (23). However, we also found that the pathogenicity of anti-αIIbβ3 JON mAb is different to that induced by anti-GPIbα p0p mAb by triggering a further
acute systemic reaction and bleeding. As summarized in Fig. 1, a bolus injection of 3 μg/g of purified JON1 or JON3 (anti-αIIbβ3 rat mAb) induced hypothermia and significant decreases in Ht levels in C57BL/6 mice, whereas no such effects were observed with any other tested anti-platelet antibodies, including anti-GPIbα (Fig. 1B and C) (23). Moreover, we noted Fc-dependent (αIIbβ3) and Fc-independent (GPIbα) events causing profound thrombocytopenia in anti-platelet IgG-treated mice (Fig. 1A) (23).

As the pathogenic effects of anti-αIIbβ3, but not anti-GPIbα, mAb are Fc dependent, we determined the role of activating FcγR in platelet integrin αIIbβ3-specific pathology using FcγRIIa−/− and FcγRIII−/− mice—the latter defective in activating FcγRI and FcγRII (11). Anti-αIIbβ3 JON antibodies induced a profound thrombocytopenia in C57BL/6 control animals, or FcγRIIa−/− and FcγRIII−/− mice, as documented by decreased platelet counts after i.v. injection (Fig. 2A). However, while the cytotoxic effects of the two different antibodies, JON1 (rat IgG2b) and JON3 (rat IgG1), were virtually identical in C57BL/6 wild-type mice, JON3 was less effective in both FcγRIIa−/− and FcγRIII−/− mice, suggesting isotype-specific differences in the pathogenicity of the two antibodies. This difference became more obvious when the systemic reaction and bleeding responses to these antibodies were analyzed in the different mouse strains. As shown in Fig. 2(B), C57BL/6 mice developed severe hypothermia within minutes upon i.v. injection of either JON1 or JON3. In contrast, in FcγRIIa−/− mice, only JON1, but not JON3, induced hypothermia, whereas FcγRIII−/− mice were completely protected from JON1 and JON3-induced hypothermia. A similar picture emerged when Ht were monitored in those animals. While both JON1 and JON3 induced a marked decrease in Ht in wild-type mice, only JON1, but not JON3, had an effect in FcγRIIa−/− mice (Fig. 2C). In FcγRIII−/− mice, neither JON1 nor JON3 had a significant effect on Ht. These findings suggest that anti-αIIbβ3 antibodies elicit distinct pathogenic mechanisms for triggering thrombocytopenia or systemic reaction and bleeding, and only the latter depends on activating FcγR. While rat IgG1 strictly requires FcγRIlll for the induction of these reactions, rat IgG2b appears to act through both, FcγRI and FcγRIlll.

We have previously shown that repeated injections of low amounts of anti-αIIbβ3 antibodies still induce a marked drop in platelet counts and bleeding, but no longer hypothermia (23). To investigate whether anti-αIIbβ3 mAb-induced thrombocytopenia is the cause of bleeding complications, C57BL/6 and FcγRIII−/− mice were treated with 7 × 0.3 μg/g JON1 within 6 h. As shown in Fig. 3(A), such treatment resulted in severe thrombocytopenia in C57BL/6 mice and to a slightly lesser extent in FcγRIII−/− mice. However, while most of the control mice developed significant intestinal and s.c. bleeding, no such effects were observed in FcγRIII−/− mice despite their decreased platelet counts (see also Fig. 7C). The blood loss in C57BL/6 controls, but not in FcγRIII−/− mice, was confirmed when Ht was monitored in separate groups (Fig. 3B). Since the cytotoxic effects of anti-αIIbβ3 mAb JON1 were slightly milder in the absence of the FcγR chain, it could not fully be excluded that the normal levels of Ht and the lack of bleeding in FcγRIII−/− mice were due to the hemostatic function of the few remaining platelets. To test this possibility, FcγRIII−/− mice first received anti-αIIbβ3 mAb and after 6 h the remaining platelets were further depleted by injection of an anti-GPIbα mAb. As expected, this treatment resulted in a complete loss of platelets for ~4 days in FcγRIII−/− mice (Fig. 3C), but again the Ht levels remained virtually unchanged (Fig. 3D).

Anti-αIIbβ3 IgG-induced loss of platelet integrin αIIbβ3 and subsequent IC formation in FcγRIII−/− mice

As recently suggested, the systemic reaction induced by anti-αIIbβ3 antibodies may be based on the formation of IC composed of αIIbβ3 and the antibody. This hypothesis was based on the observation that platelets in anti-αIIbβ3-treated mice lost the integrin from the surface (23). To examine a possible involvement of FcγR in this antibody-induced loss of αIIbβ3 from the platelet surface, FcγRII−/− and C57BL/6 control mice received 7 × 0.3 μg/g anti-αIIbβ3 JON1 antibody and the circulating platelets were analyzed for the expression of αIIbβ3 integrin at different time points after injection. In both mouse strains platelets had lost ~90% of their surface αIIbβ3 (Fig. 4A)
as well as surface-bound IgG (Fig. 4B) as soon as 6 h after antibody injection and the circulating platelet population remained \( \alpha_{\text{IIb}3} \)-negative in all mice for at least 4 days.

To study the process of \( \alpha_{\text{IIb}3} \) loss in vivo, wild-type and FcR\( g \)±/± mice received 3 mg/g gold (5 nm)-conjugated JON1 or control antibody i.v. and the organs were examined by electron microscopy at different time points (Fig. 5). In wild-type mice, injection of gold-conjugated JON1 induced the formation of gold particle-containing clusters of electron dense material in the close vicinity of platelets as detected 20 min after injection. These clusters were mainly found in spleen (Fig. 5A), liver and lungs, and showed a network-like formation of the electron-dense material. At 24 h after injection of the antibody, the gold particle-containing clusters were still found in these organs. At this time point, however, the density of the cluster network appeared higher when compared to the earlier time point (Fig. 5B). Interestingly, the gold particle-containing clusters were equally observed in FcR\( g \)±/± mice, confirming that the formation of these IC-like structures occurred independently of Fc\( g \)RI and Fc\( g \)RIII (not shown). In addition to the gold particle-containing clusters in the vicinity of platelets, gold particles were also found within macrophages of wild-type, but not FcR\( g \)-deficient, mice 60 min after injection, suggesting that these immune cells had phagocy-

tosed parts of the formed IC by Fc\( g \)-dependent mechanisms (not shown). Clustering of the gold-conjugated control antibody could not be observed in the organs of wild-type or FcR\( g \) chain-deficient mice. These results suggest that anti-\( \alpha_{\text{IIb}3} \) antibodies can induce the formation of large IC in mice and indicate that these IC are recognized by Fc\( g \)-bearing cells.

**Anti-\( \alpha_{\text{IIb}3} \) IgG-induced acute systemic reaction and bleeding results in enhanced mortality in FcR\( g \)I±/± mice**

It is known that FcR\( g \)III-dependent activation responses induced by the Fc\( g \)R chain are counter-regulated by the inhibitory receptor Fc\( g \)RII when co-expressed on the same effector cells. This balance has been established in various murine disease models, and is highlighted by the increased susceptibility of FcR\( g \)II±/± mice to the pathogenic effects of antibodies in models of systemic anaphylaxis (25) and IC-triggered inflammation in several organs (17–19). To test the hypothesis that anti-\( \alpha_{\text{IIb}3} \) antibodies trigger an IC-like response, their pathogenic effects were examined in FcR\( g \)II±/± mice. A bolus injection of 3 mg/g of anti-\( \alpha_{\text{IIb}3} \) mAb led to similar thrombocytopenia, but significantly more severe hypothermia and increased mortality, in FcR\( g \)II±/± mice as compared to control mice (Fig. 6). This finding demonstrates that the FcR\( g \)-triggered systemic reaction of the anti-\( \alpha_{\text{IIb}3} \)
In this study, we have examined the specific contribution of FcγR and αIβ3 in platelet pathology. 345

dependent mechanisms in the immune clearance of platelets (6,7). More recently, however, it has been shown that the antigenic specificity of anti-platelet antibodies when directed against different mouse platelet antigens, including GP Ibα and integrin αIβ3, is of critical importance, mediating their pathogenic effects through Fc-dependent (αIβ3) or Fc-independent (GP Ibα) mechanisms (23). Surprisingly, we found that anti-αIβ3 IgG-induced thrombocytopenia, which is Fc dependent (Fig. 1) (23), is not abolished in mice lacking the activating FcγRIII or the common FcγR chain (Fig. 2), indicating a contribution of additional effectors like complement in this Fc-mediated process. Preliminary experiments in C3 mutant mice show that anti-αIβ3 IgG1 (JON3)- and IgG2b (JON1)-induced thrombocytopenia occurs independently of complement C3 activation (B. Nieswandt and J. E. Gessner, unpublished), and this may suggest redundant roles of FcγR and complement in anti-αIβ3 IgG Fc-induced platelet destruction. Studies in mice lacking both activating FcγR and complement will be required to test whether complement provides an alternative pathway, as recently suggested in an antibody-dependent model of autoimmune vitiligo (29).

While anti-platelet antibodies directed against GP Ibα (Fig. 1) or GP Ib-IX, GPV, CD9 and linear epitopes on integrin β3 (23) have mild to strong and irreversible effects on platelet counts, none of them induce significant bleeding in mice. This indicates that the clearance of circulating platelets alone may not be sufficient to account for bleeding complications. Importantly, only antibodies directed against conformational epitopes on the dominant platelet integrin, αIβ3, can induce responses leading to systemic reaction and marked blood loss in mice (Fig. 1) (22,23). FcγR chain deficiency now reveals a role of activating FcγR in the anti-αIβ3 IgG Fc-induced processes of hypothermia and severe bleeding (Figs 2 and 3). Moreover, we observe IgG isotype dependency in this model using rat IgG1 and IgG2b subclasses in mice lacking FcγRIII as compared to FcγR-deficient mice. Consistent with previous findings on mouse IgG isotypes (15,27), FcγRIII mutant mice display IgG subclass-specific protection when induced by anti-αIβ3 JON mAb of IgG1, but not IgG2b, isotypes (Fig. 2). This suggests that FcγRIII, which is the principle activatory Fc receptor for mouse IgG1, is also specific for rat IgG1 with no role of FcγRI (30). In contrast, rat IgG2b-induced effects are prevented in FcγRI, but not FcγRIII, mutant mice, thus showing that individual FcγR interact differently with rat IgG isotypes in mediating platelet pathology (Fig. 2). Together, these findings suggest that anti-αIβ3 antibodies may trigger activating FcγR on immune effector cells in a way no other anti-platelet antibodies do by inducing the formation of antigen–antibody structures particularly efficient to be recognized by FcγR with relative affinities for FcγRIII: rat IgG1 > rat IgG2b and FcγRI: rat IgG2b > rat IgG1). However, it is important to stress that although we have previously shown similar pathogenic effects for a series of different anti-αIβ3 antibodies (23), these results cannot be directly extrapolated to all antibodies to this receptor complex.

As part of the normal cellular function, platelets can release microparticles after activation by diverse stimuli and this phenomenon has now been recognized for most eukaryotic cells (31,32). HIV-1-related immune thrombocytopenia has been reported to be associated with circulating IC that consist
of platelet membrane fragments and anti-GPIIbα IgG antibodies (33). Our finding that both FcRγ-chain-negative and -positive platelets have lost αIIbβ3 integrin (GPIIbIIa) from their surfaces suggests FcRγ-independent IC formation (Fig. 4). Electron microscopic examinations using gold-conjugated anti-αIIbβ3 antibodies demonstrate the formation of IC-like structures in the vicinity of platelets, mainly in spleen, liver and lung (Fig. 5). IC-like structures were equally found in wild-type and FcRγ chain mutant mice, suggesting that activating FcγR act downstream of this process. This hypothesis is supported by the finding that gold clusters were present in macrophages of wild-type, but not FcRγ mutant mice, which strongly

![Fig. 4. Loss of surface αIIbβ3-antibody complexes in FcRγ−/− mice. C57BL/6 and FcRγ−/− mice received 7 × 0.3 μg/g anti-αIIbβ3 JON1mAb (rat IgG2b) within 6 h, each i.p. Flow cytometric detection of surface levels of αIIbβ3 (A) and surface-bound JON1 (B) on platelets from control (open circles) or FcRγ−/− (filled circles) at the indicated times after the first injection. Results are expressed as percent mean log fluorescence as compared to control platelets incubated with saturating amounts of JON1 in vitro for 30 min at 37°C for groups of six mice. Surface levels of αIIbβ3 were determined with JON8±FITC. JON1 and JON8 recognize different epitopes on αIIbβ3, and do not interfere with the binding of the other in vitro.](https://academic.oup.com/intimm/article-abstract/15/3/341/678352)

![Fig. 5. Electron microscopy of anti-αIIbβ3 IgG-induced IC formation in vivo. Representative ultrastructural images of spleen from C57BL/6 wild-type mice treated with gold-conjugated JON1 for 20 min (A) or 24 h (B). (A and B) In addition to a platelet (p), clusters (asterisks) of electron-dense material without membrane covering are shown. (Inserts) At higher magnification homogenously distributed small gold particles (5 nm) are found between the network-like distributed electron-dense material. The bar represents 250 nm (insert: 50 nm).](https://academic.oup.com/intimm/article-abstract/15/3/341/678352)

![Fig. 6. Anti-αIIbβ3 IgG-induced systemic reaction is enhanced in FcγRII−/− mice. C57BL/6 controls (open circles) or FcγRII−/− (filled circles) mice received 3 μg/g anti-αIIbβ3 JON1 mAb in 200 μl sterile PBS i.v. (A) The mean values of the body temperatures of nine mice per group at the indicated times after injection are given ± SD. Death of a mouse is indicated by a cross. (B) Platelet counts of wild-type (wt) and FcγRII−/− mice were determined at t = 1 h, using an improved Neubauer hemocytometer. Results are shown as the mean platelet count ± SD for groups of nine mice. Wild-type and FcγRII−/− mice not receiving anti-αIIbβ3 JON1 mAb all have comparable platelet counts (control).](https://academic.oup.com/intimm/article-abstract/15/3/341/678352)
Fig. 7. Increased anti-αIIbβ3 IgG-induced bleeding and mortality in FcγRII−/− mice. C57BL/6 controls (open circles) or FcγRII−/− (filled circles) mice received 7 × 0.3 μg/g anti-αIIbβ3 JON1 mAb, each i.p. Platelet counts (A) and Ht (B) were determined at the indicated times. Results are shown as the mean platelet count or Ht ± SD for groups of 10–12 mice. Death of a mouse is indicated by a cross. (C) Increased anti-αIIbβ3 IgG-induced bleeding and resulting organ failure in FcγRII−/− mice. Representative pictures from wild-type, FcγRI−/− and FcγRII−/− mice on day 3 after repeated injections of low amounts of JON1 mAb. Note the lack of bleeding in the FcγRI−/− mouse, whereas the blood loss in FcγRII−/− mice was significantly more severe than in the wild-type (WT) control, resulting in a pale appearance of the major organs, particularly liver and kidney. Such mice died from the marked blood loss and subsequent multi-organ failure.
indicates that the complexes are recognized by FcγR expressed on these cells. It is well known that IC can activate immune cells, including macrophages and mast cells, by FcγR-dependent mechanisms, thereby eliciting an acute inflammatory response (34,35). Furthermore, IC-dependent activation responses mediated by FcγRIII and the FcγR chain are regulated by the inhibitory FcγRIII (17–19). A similar coupling is also found here in anti-αδβ3 IgG-induced hyperthermia and bleeding. The genetic deletion of FcγRII results in enhanced sensitivity leading to uncontrolled acute systemic reaction and bleeding associated with profound mortality (Figs 6 and 7). These findings demonstrate the balance between FcγRII inhibition and FcγR-triggered activation on effector cells as the major immunoregulatory event in the αδβ3-specific pathology in mice.

Taken together, the results presented here suggest the existence of distinct pathogenic mechanisms that may determine the severity of hemorrhage in thrombocytopenia in mice. At least two different events are required for bleeding, one of which involves αδβ3 integrin and activating FcγR. IgG opsonization of platelets and their subsequent destruction represents the initial step in thrombocytopenia (20). However, platelet clearance appears not strictly dependent on activating FcγR and does not necessarily lead to bleeding complications in mice. Platelet destruction results in severe bleeding when integrin αδβ3 is the target of the pathogenic antibody. Targeting of αδβ3 by anti-αδβ3 IgG antibodies induces the formation of IC efficient to trigger systemic reaction through engagement of activating FcγR on effector cells. The combination of thrombocytopenia and FcγR-dependent downstream events is critical for fatal hemorrhage in mice. In accordance, a more severe systemic reaction, hemorrhage and profound mortality occurs as a consequence of changes in the balance of activating FcγR and the inhibitory FcγRII.

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Abbreviations

AIHA autoimmune hemolytic anemia
FcγR Fc receptor for IgG
FcγRII γ chain of activating FcγRII and FcγRIII receptors
Ht hematocrit
IC immune complex
ITP immune thrombocytopenic purpura

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