

Rapid desensitization of mice with anti-Fc γ RIIb/Fc γ RIII mAb safely prevents IgG-mediated anaphylaxis

Marat V. Khodoun, PhD,^{a,b,*} Zeynep Yesim Kucuk, MD,^{c,*} Richard T. Strait, MD,^{a,d,*} Durga Krishnamurthy, PhD,^{a,b,‡} Kevin Janek, BA,^e Corey D. Clay, MD, PhD,^b Suzanne C. Morris, PhD,^{a,b} and Fred D. Finkelman, MD^{b,f,g} Cincinnati, Ohio

Background: Stimulatory IgG receptors (Fc γ Rs) on bone marrow-derived cells contribute to the pathogenesis of several autoimmune and inflammatory disorders. Monoclonal antibodies that block Fc γ Rs might suppress these diseases, but they can induce anaphylaxis.

Objective: We wanted to determine whether a rapid desensitization approach can safely suppress IgG/Fc γ R-mediated anaphylaxis.

Methods: Mice were injected with serially increasing doses of 2.4G2, a rat mAb that blocks the inhibitory Fc γ R, Fc γ RIIb, and the stimulatory receptor, Fc γ RIII. Rectal temperature was used to detect the development of anaphylaxis. Passive and active IgG-mediated anaphylaxis were evaluated in mice that had been rapidly desensitized with 2.4G2 or mock-desensitized in mice in which monocyte/macrophages, basophils, or neutrophils had been depleted or desensitized and in mice in which Fc γ RI, Fc γ RIII, and/or Fc γ RIV had been deleted or blocked.

Results: Rapid desensitization with 2.4G2 prevented 2.4G2-induced shock and completely suppressed IgG-mediated anaphylaxis. Rapid desensitization of ovalbumin-sensitized mice with 2.4G2 was safer and more effective than rapid desensitization with ovalbumin. 2.4G2 treatment completely blocked Fc γ RIII and removed most Fc γ RI and Fc γ RIV from nucleated peripheral blood cells. Because IgG_{2a}-mediated anaphylaxis was partially Fc γ RI and Fc γ RIV dependent, the effects of 2.4G2 on Fc γ RI and Fc γ RIV were probably crucial for its complete inhibition of IgG_{2a}-mediated anaphylaxis. IgG_{2a}-mediated anaphylaxis was partially inhibited by depletion or desensitization of monocyte/macrophages, basophils, or neutrophils.

Conclusion: IgG-mediated anaphylaxis can be induced by ligation of Fc γ RI, Fc γ RIII, or Fc γ RIV on monocyte/macrophages, basophils, or neutrophils and can be safely suppressed by rapid desensitization with anti-Fc γ RII/RIII mAb. A similar approach may safely suppress other Fc γ R-dependent immunopathology. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Anaphylaxis, IgG, Fc γ R, mouse, macrophage, basophil, neutrophil, rapid desensitization

Antibody-dependent effector functions are mediated, to a large extent, by receptors for antibody Fc domains that are expressed by multiple bone marrow-derived cell types. In the mouse, these include 3 tyrosine kinase-associated receptors that stimulate receptor-expressing cells when they are ligated by IgG/antigen complexes (Fc γ RI, Fc γ RIII, and Fc γ RIV) and 1 tyrosine phosphatase-associated receptor (Fc γ RIIb) that transmits inhibitory signals.^{1,2} Human bone marrow-derived cells express similar receptors, although some differences exist.^{1,2} Although stimulatory Fc γ Rs can contribute to host protection against microbial pathogens,^{3,4} these receptors are also important in the pathogenesis of autoimmune and inflammatory diseases, including mouse models of rheumatoid arthritis,⁵ systemic lupus erythematosus,⁶ antibody-mediated hemolytic anemia,⁷ glomerulonephritis,^{8,9} myasthenia gravis,¹⁰ blistering skin diseases,¹¹ and IgG-mediated anaphylaxis.^{2,12} Indeed, the therapeutic efficacy of intravenous IgG in a large number of inflammatory diseases is thought to depend, in part, on its interaction with Fc γ Rs.¹³

These considerations have suggested that an agent that blocks Fc γ Rs might be a useful therapeutic for antibody-dependent, Fc γ R-dependent disorders. Consistent with this, a rat IgG_{2b} mAb, 2.4G2, that blocks IgG immune complex binding to mouse Fc γ RIIb and Fc γ RIII and may also interact with Fc γ RI and Fc γ RIV^{14,15} has been shown to suppress IgG-mediated anaphylaxis¹² and immune thrombocytopenic purpura.¹⁶ Unfortunately, the potential use of this mAb is limited by its ability to activate Fc γ R-expressing cells. The resulting activation stimulates the production of the potent vasoactive mediator, platelet-activating factor (PAF), which causes an anaphylactic response.¹²

Recently, we found that the anaphylactic response caused by a mAb specific for the high-affinity IgE receptor, Fc ϵ RI, could be prevented by administering that mAb through a protocol that resembles the rapid desensitization approach used by allergists to suppress the induction of anaphylaxis by a specific allergen.¹⁷ That is, treatment of mice with anti-Fc ϵ RI mAb, starting with a dose too small to induce detectable disease and doubling that dose every hour, allowed safe administration of a dose of that mAb that would induce anaphylaxis in

From ^athe Department of Research, Cincinnati Veterans Affairs Medical Center; ^bthe Division of Immunology, Allergy and Rheumatology, Department of Medicine, University of Cincinnati School of Medicine; the Divisions of ^cAllergy and Immunology and ^dEmergency Medicine, Cincinnati Children's Hospital Medical Center; ^ethe University of Cincinnati School of Medicine; ^fthe Department of Medicine, Cincinnati Veterans Affairs Medical Center; and ^gthe Division of Cellular and Molecular Immunology, Cincinnati Children's Hospital Medical Center.

*These authors contributed equally to this work.

‡Durga Krishnamurthy is currently affiliated with the Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria.

Supported by the US Department of Veterans Affairs Merit Award (F.D.F.).

Disclosure of potential conflict of interest: M. Khodoun, D. Krishnamurthy, and F. Finkelman have a provisional patent, and there is potential that money may be paid to them and their institution. Z. Kucuk has received a grant from the National Institutes of Health. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication February 27, 2013; revised July 29, 2013; accepted for publication September 12, 2013.

Corresponding author: Fred D. Finkelman, MD, Division of Immunology, Allergy and Rheumatology, University of Cincinnati College of Medicine, Health Professions Building, 3255 Eden Ave, Suite 350, Cincinnati, OH 45267-0563. E-mail: finkelfd@ucmail.uc.edu.

0091-6749

<http://dx.doi.org/10.1016/j.jaci.2013.09.008>

Abbreviations used

ATCC: American Type Culture Collection

EW: Egg white

HN: Hanks balanced salt solution plus 10% newborn bovine serum

HNA: HN plus 0.2% NaN₃

mAb: Monoclonal antibody

OVA: Ovalbumin

PAF: Platelet-activating factor

TNP: Trinitrophenyl

WT: Wild-type

previously untreated mice and, in fact, blocked all IgE-mediated anaphylaxis. Desensitization resulted from both the induction of mast cell anergy and, eventually, the removal of all FcεRI and IgE from mast cells.¹⁷ These observations suggested that a similar approach with anti-FcγRII/RIII mAb (2.4G2) might avoid the induction of shock but still block IgG-mediated anaphylaxis. The studies described here indicate that this is indeed the case and increase understanding of the mechanisms involved in IgG-mediated anaphylaxis by showing the importance of 3 different FcγR-expressing cell types, monocyte/macrophages, basophils, and neutrophils and each of the 3 stimulatory FcγRs in this disorder.

METHODS**Mice**

Female BALB/c and C57BL/6 mice were purchased from Taconic (Hudson, NY) and were used at 7 to 12 weeks of age. FcγRIII-deficient and FcγRI-deficient mice on a BALB/c background^{1,18} were a gift of Dr Jeffrey Ravetch (Rockefeller University, New York, NY); these mice were bred to each other to generate mice deficient in both FcγRI and FcγRIII. F2 offspring were typed by PCR to identify double-deficient offspring. FcγRIIb-deficient mice on a C57BL/6 background were purchased from the Jackson Laboratory (Bar Harbor, Me). FcεRIα-deficient mice on a BALB/c background were a gift of Jean-Pierre Kinet (Harvard University, Cambridge, Mass).¹⁹

Antibodies

Monoclonal antibodies or the hybridomas that produce them were obtained from the following sources: 2.4G2 (rat IgG_{2b} anti-mouse FcγRII/RIII mAb; American Type Culture Collection [ATCC], Rockville, Md), GK1.5²⁰ (rat IgG_{2b} anti-mouse CD4; ATCC), MAR-1 (hamster anti-mouse FcεRIα mAb; eBioscience, San Diego, Calif), 5B11 (rat IgG_{2a} anti-mouse IL-3R mAb; eBioscience), M1/70²¹ (rat IgG_{2b} anti-mouse CD11b mAb; ATCC), 1A8 (rat IgG_{2a} anti-mouse Ly6G mAb; BD Pharmingen, San Diego, Calif), DX5 (rat IgM anti-mouse CD49b; BD Pharmingen), ACK-2 (rat IgG anti-mouse c-kit mAb²²; Richard Grencis, University of Manchester, Manchester, United Kingdom), 1B7.11 (mouse IgG₁ anti-trinitrophenyl [TNP] mAb; ATCC), HY1.2 (mouse IgG_{2a} anti-TNP mAb; Shozo Izui, University of Geneva, Geneva, Switzerland), J1.2 (rat IgG_{2b} anti-4-hydroxy-3-nitrophenylacetyl, used as a control mAb; John Abrams, DNAX Research Institute, Palo Alto, Calif), x54-5/7.1 (rat IgG anti-mouse FcγRI mAb; eBioscience), K9.361²³ (anti-Ly17.2, a mouse IgG anti-mouse FcγRIIb mAb, a gift of Ulrich Hammerling, Sloan-Kettering, New York, NY), clone 275003 (rat IgG_{2a} anti-mouse FcγRIII; R&D Systems, Minneapolis, Minn), and 9F9²⁴ (hamster IgG anti-mouse FcγRIV mAb; a gift of Jeffrey Ravetch, Rockefeller University, New York, NY). mAbs produced as ascites in pristane-primed athymic nude mice were purified as described.²⁵ A goat antiserum to mouse IgD was produced as described.²⁵

Antigens, pharmaceuticals, and chemicals

Ovalbumin (OVA) and BSA were purchased from Sigma (St Louis, Mo). OVA and BSA were labeled with 2,4,6-trinitrophenyl-ε-aminocaproyl-O-succinamide (Biosearch Technologies, Novato, Calif).

Immunization of mice

BALB/c and C57BL/6 female mice 8 to 12 weeks old were immunized intraperitoneally with 50 μg of chicken egg white (EW) mixed with 1 mg of alum on days 0 and 12. Mice were challenged intravenously with 200 μg of EW on day 19.

IL-4C

A long-acting complex of IL-4 that dissociates *in vivo* to release free, biologically active IL-4 was produced by mixing recombinant mouse IL-4 (PeproTech, Rocky Hill, NJ) with anti-IL-4 mAb (BVD4-1D11) at a 2:1 molar (1:5 weight) ratio for at least 5 minutes before injection.²⁶

Detection of systemic anaphylaxis

Mice were challenged intravenously with the appropriate antigen, after which body temperature was determined every 5 to 15 minutes with a rectal probe to detect and quantify hypothermia, a sign of shock.²⁷

Preparation of peritoneal mast cells

Peritoneal lavage was collected by intraperitoneal injection of 10 mL of sterile Hanks balanced salt solution with 5% newborn bovine serum and plated on 25-cm² cell culture flasks (Corning, Corning, NY) for 2 hours at 37°C with 5% CO₂. Nonadherent cells were collected and washed with Hanks balanced salt solution plus 10% newborn bovine serum (HN).

Preparation of nucleated blood cells

Blood obtained by tail vein incision was collected in microtainers with K₂EDTA (BD Biosciences, San Jose, Calif). Red blood cells were lysed with ammonium-chloride-potassium lysis buffer; cells were washed twice with Hanks balanced salt solution and resuspended in HN plus 0.2% NaN₃ (HNA).

Immunofluorescence staining

Cells (1×10^6) in 0.1 mL of HNA were incubated with 2.4G2 for 15 minutes on ice and then stained with 1 μg each of mAbs to CD49b, IgE, and IL-3R to identify basophils; with mAbs to IgE, c-kit, and IL-3R to identify mast cells; with M1/70, 1A8 (anti-Ly6G), and F4/80 to identify neutrophils and monocytes; with 6B2 (anti-B220) to identify B cells; and with GK1.5 (anti-CD4) and 2.43 (anti-CD8) to identify these T-cell populations. Cells were washed twice, fixed with 2% paraformaldehyde, and analyzed with a FACScalibur (BD Biosciences). Basophils were also distinguished as having relatively low forward and side scatter, whereas mast cells have high forward and side scatter, and neutrophils have high side scatter and intermediate forward scatter. Dendritic cell subpopulations were identified as previously described.¹⁷

Cellular depletion

To deplete neutrophils, mice were injected intraperitoneally daily for 7 days with 20 mg of hydroxyurea. Mice were also injected intraperitoneally on days 6 and 7 with 0.1 mg of RB6-8C5 anti-Gr-1 mAb. This treatment caused a decrease in blood neutrophil count of >95%.²⁸ Basophil depletion²⁹ and monocyte/macrophage desensitization and depletion²⁸ were performed as described.

Preparation of F(ab')₂ fragment of 2.4G2

2.4G2 was dialyzed into a 20 mmol/L acetate buffer, pH 4.5, and digested for 30 hours at 37°C with immobilized pepsin (Thermo

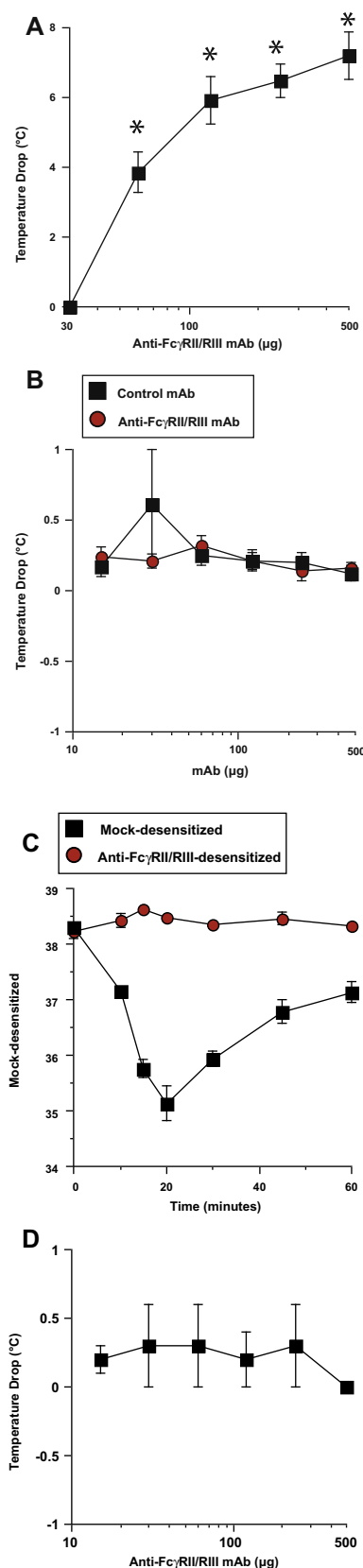


FIG 1. Anaphylaxis and rapid desensitization with anti-FcγR mAb. **A**, BALB/c mice were injected intravenously with the doses of anti-FcγRIIb/RIII mAb (2.4G2) shown. Rectal temperatures were determined. Mean maximum temperature decrease is shown. **B**, Mice were rapidly

Fisher Scientific, Waltham, Mass) while being rotated, then dialyzed against 0.15 mol/L NaCl and concentrated by pressure filtration. An aliquot was reduced with 2-mercaptoethanol and compared with reduced undigested 2.4G2 by SDS-PAGE with a 10% polyacrylamide gel (Fig 4, D).

Statistics

A 2-tailed Mann-Whitney *t* test and a Fischer exact χ^2 test were used, when appropriate, to test for statistical significance. A *P* value less than .05 was considered significant.

RESULTS

Rapid desensitization with anti-FcγRII/RIII mAb (2.4G2) avoids direct toxicity and prevents IgG-mediated anaphylaxis

To determine whether a rapid desensitization approach could prevent the development of anaphylaxis in mice injected with 2.4G2, we first determined the dose of 2.4G2 required to induce shock (hypothermia) in BALB/c mice (Fig 1, A). A significant and substantial decrease in temperature was observed in mice injected with 63 μg or more of this mAb but not in mice injected with 31 μg. In contrast, serial, hourly injections of mice with doubling doses of 2.4G2, starting with a dose of 15 μg and culminating in a dose of 500 μg, failed to induce shock (Fig 1, B). Challenge of these mice intravenously the next day with 500 μg induced shock in the mock-desensitized mice but not in the 2.4G2-desensitized mice (Fig 1, C). Rapid desensitization with 2.4G2 failed to induce hypothermia even when mice were first treated with a long-acting preparation of IL-4 (IL-4C),²⁶ to increase their sensitivity to mediators such as PAF³⁰ (Fig 1, D).

To determine whether 2.4G2 could prevent IgG-mediated anaphylaxis in a passive immunization system, we first primed immunologically naive mice with mouse IgG₁ or IgG_{2a} mAb to TNP, then rapidly desensitized them with 2.4G2 or mock-desensitized them with an isotype-matched control rat mAb and challenged them with TNP-BSA. Results (Fig 2, A) indicated that rapid desensitization with 2.4G2 completely blocked the anaphylactic responses in mice primed with either isotype of anti-TNP mAb. To determine whether 2.4G2 could safely and effectively desensitize mice that had been actively immunized with OVA, we compared the abilities of OVA and 2.4G2 to rapidly desensitize these mice and to inhibit anaphylaxis after subsequent challenge with OVA. In an initial experiment, no EW-immune mice developed temperature drops during desensitization with either EW or 2.4G2 (not shown). We then repeated this

desensitized by intraperitoneal injection hourly for 6 hours with doubling doses of anti-FcγRIIb/RIII mAb or mock-desensitized with an isotype control mAb, starting with a dose of 15 μg. Mean maximum rectal temperature decreases for the 60 minutes after each injection. **C**, Anti-FcγRII/RIII mAb-desensitized and mock-desensitized mice were challenged the next day with 500 μg of anti-FcγRII/RIII mAb. Rectal temperatures were followed for the next 60 minutes. **D**, Mice were first pretreated with IL-4C to increase their sensitivity to vasoactive mediators, then rapidly desensitized the next day with anti-FcγRII/RIII mAb as in panel B. Total of 8 mice per group, pooled from 2 experiments, for each panel, except 4 mice per group for panel C. Asterisks in panel A indicate significantly greater temperature drop than with a dose of 30 μg (*P* < .05).

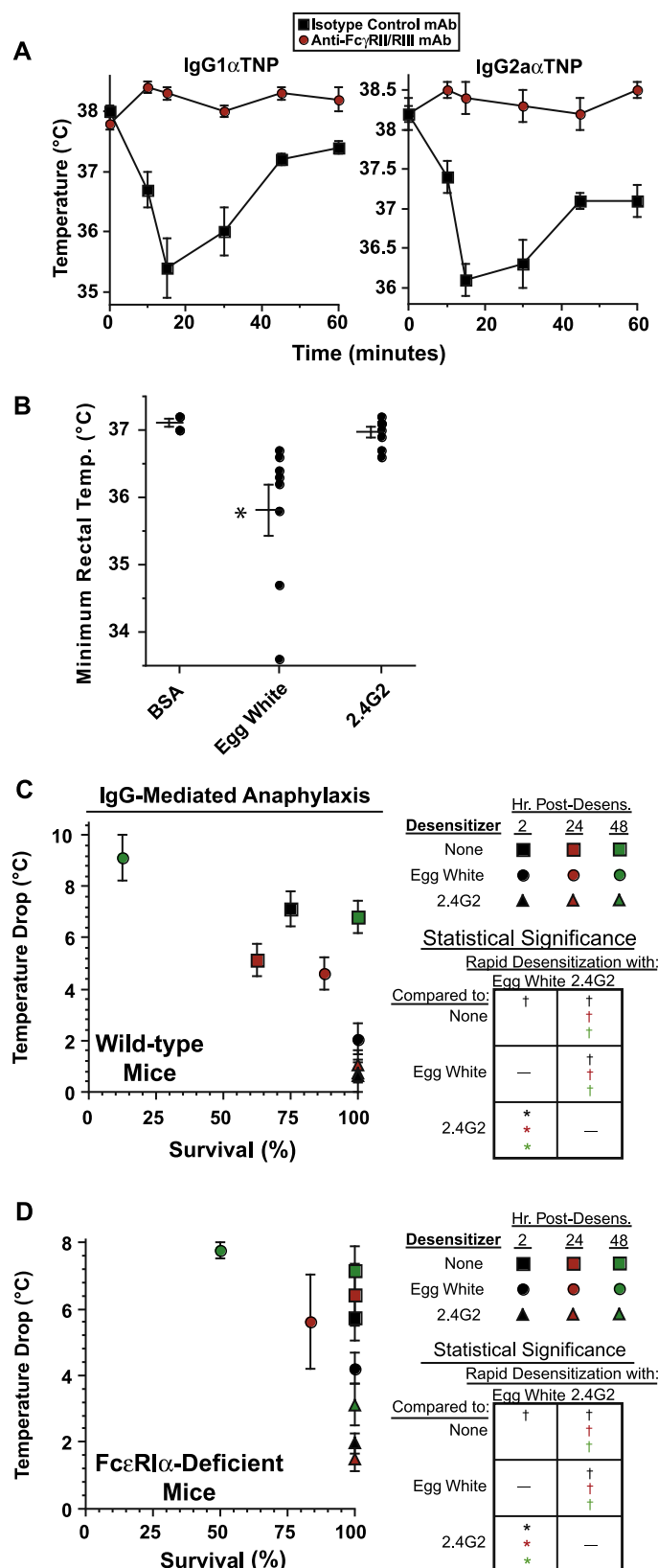


FIG 2. 2.4G2 suppression of IgG-mediated anaphylaxis in mice sensitized by active or passive immunization. **A**, BALB/c mice were pretreated with 500 μ g of IgG₁ or IgG_{2a} anti-TNP mAb, then rapidly desensitized as in Fig 1, **B**, with anti-Fc γ RII/RIII or control mAb (J1.2). Mice were challenged by intravenous injection of 100 μ g of TNP-BSA 2 hours after desensitization. Rectal temperatures were determined. Four mice per group; representative of 2 experiments performed. **B**, BALB/c mice were immunized intraperitoneally

procedure in mice that had been made more sensitive to vasoactive mediators (and perhaps better resemble persons with allergy who produce increased quantities of T_H2 cytokines) by pretreating them with IL-4C.²⁶ Rapid desensitization with 2.4G2 or mock desensitization with a control antigen (BSA) failed to cause shock in any of these mice (Fig 2, B). By contrast, rapid desensitization with EW caused shock in several of these mice (Fig 2, B). Thus, rapid desensitization with anti-FcγRII/RIII mAb appears to be safer than rapid desensitization with antigen. This may be because the dose of 2.4G2 that binds to FcγR-expressing cells is more predictable than the dose of OVA/IgG anti-OVA antibody complex that interacts with these cells.

Rapid desensitization with either EW or 2.4G2 effectively prevented anaphylaxis when EW-sensitized mice, in which IgE-mediated anaphylaxis had been blocked, were challenged with EW 2 hours after the completion of rapid desensitization (Fig 2, C). However, rapid desensitization with 2.4G2 was still completely effective 48 hours after the completion of rapid desensitization, whereas the effectiveness of rapid desensitization with EW was lost within 24 hours. In addition, rapid desensitization with EW actually increased the severity of anaphylaxis in mice challenged at 48 hours (Fig 2, C) (this may be because of increased IgG anti-OVA antibody secretion and/or basophil or T-cell secretion of IL-4 in OVA-desensitized mice). To confirm this result in another model in which anaphylaxis can be mediated by IgG, but not IgE, we immunized FcεRIα-deficient BALB/c background mice intraperitoneally with EW/alum, rapidly desensitized them with EW or 2.4G2, and challenged them intravenously with EW 2 hours, 1 day, or 2 days later. Results of this experiment again showed less severe anaphylaxis in mice desensitized with 2.4G2 than in mice desensitized with EW (Fig 2, D) (even the 2.4G2-desensitized mice developed some temperature drop in this experiment, possibly because the absence of FcεRIα is associated with increased signaling by FcγRs³¹). Taken together, our observations indicate that rapid desensitization with anti-FcγR mAb is safer and prevents IgG-mediated anaphylaxis longer than rapid desensitization with antigen.

FcγR involvement in rapid desensitization with 2.4G2

Differences of opinion exist about the FcγRs that are involved in murine IgG-mediated anaphylaxis^{2,12,32,33} and about the ability of 2.4G2 to bind to the intermediate-affinity FcγR, FcγRIV, and the high-affinity FcγR, FcγRI.^{15,34} We investigated these issues because they should affect the ability of 2.4G2 to prevent FcγR-mediated disease. To do this, we first used mice deficient in specific FcγRs and mAbs specific for particular FcγRs to determine the relative roles of the 3 activating FcγRs in IgG-mediated anaphylaxis. The severity of anaphylaxis induced by challenging IgG_{2a} anti-TNP mAb-primed mice with TNP-OVA was greatly decreased in FcγRIII-deficient mice and further decreased in mice deficient in both FcγRI and FcγRIII (Fig 3, A). A blocking anti-FcγRIV mAb did not induce anaphylaxis and did not, by itself, affect anaphylaxis severity in wild-type (WT) mice, but it further decreased the severity of anaphylaxis in both FcγRIII-deficient and FcγRI/FcγRIII dual-deficient mice (Fig 3, A). Thus, although FcγRIII is predominantly responsible for IgG_{2a}-mediated anaphylaxis, IgG immune complex interactions with FcγRI and FcγRIV also contribute. This is consistent with the ability of IgG_{2a} to interact with all 3 of these receptors.^{2,18} In contrast, IgG₁-dependent anaphylaxis depends solely on FcγRIII,^{12,35} the only stimulatory FcγR with which it interacts.^{2,18}

These observations raised questions about how 2.4G2 can completely suppress IgG_{2a}-mediated anaphylaxis when incubation of neutrophils and monocytes with 2.4G2 under noncapping (*in vitro*, 4°C) condition fails to block the binding of mAbs specific for FcγRI or FcγRIV, although it completely blocks the binding of anti-FcγRIIb and anti-FcγRIII mAbs to these cells (Fig 3, B, left panels). This issue may be explained by the ability of 2.4G2 to substantially decrease expression of FcγRI and FcγRIV when injected into mice; in fact, the extent of suppression of these receptors by 2.4G2 was similar to the extent to which 2.4G2 binding to blood mononuclear cells and neutrophils decreased in 2.4G2-treated mice (Fig 3, C). Thus, 2.4G2 directly blocks binding to FcγRIIb and FcγRIII and indirectly blocks binding to FcγRI and FcγRIV by decreasing the expression of these receptors.

with egg white/alum on days 0 and 12, then treated intraperitoneally with IL-4C on day 16, 17, or 18. The day after IL-4C treatment, mice were rapidly desensitized with BSA (negative control, 6 doubling doses, starting at 6 μg), egg white (6 doubling doses, starting at 6 μg), or 2.4G2 (6 doubling doses, starting at 15 μg). Mice were challenged intravenously with egg white on day 19. The minimum rectal temperature for each mouse during rapid desensitization is shown, along with the mean ± SEM of minimum rectal temperature for the group. Data were pooled from 2 experiments except 1 experiment for the BSA (negative control) group; 4 mice/group. Total of 8 mice/group, except 4 mice for the BSA group. *Significant lowering in rectal temperature compared with BSA group. **C**, BALB/c mice were immunized with egg white as in panel B, then injected intraperitoneally 17 days after the initial immunization with 100 μg of EM-95 to suppress IgE-mediated anaphylaxis. These mice were then rapidly desensitized with egg white or 2.4G2 or mock-rapidly desensitized and challenged intravenously with 200 μg of egg white 2 hours, 1 day, or 2 days after rapid desensitization. All mice were challenged with egg white 1 to 2 days after EM-95 injection; 2 experiments, total of 8 mice/group. Statistical significance box: †significantly lower decrease in temperature drop and/or mortality for a group above the box compared with a group to the right of the box; *significantly greater decrease in temperature drop and/or mortality for a group above the box compared with a group to the right of the box. Colors code for comparisons for groups challenged 2 hours (black), 24 hours (red), or 48 hours (green) after rapid desensitization. **D**, BALB/c FcεRIα-deficient mice were immunized with egg white as in panel B. These mice were then rapidly desensitized with egg white or 2.4G2 or mock-rapidly desensitized with BSA on day 17, 18, or 19 and challenged intravenously with 200 μg of egg white on day 19, 2 hours, 1 day, or 2 days after rapid desensitization; 3 experiments, total of 6 mice/group. Statistics as for panel C, with identical results.

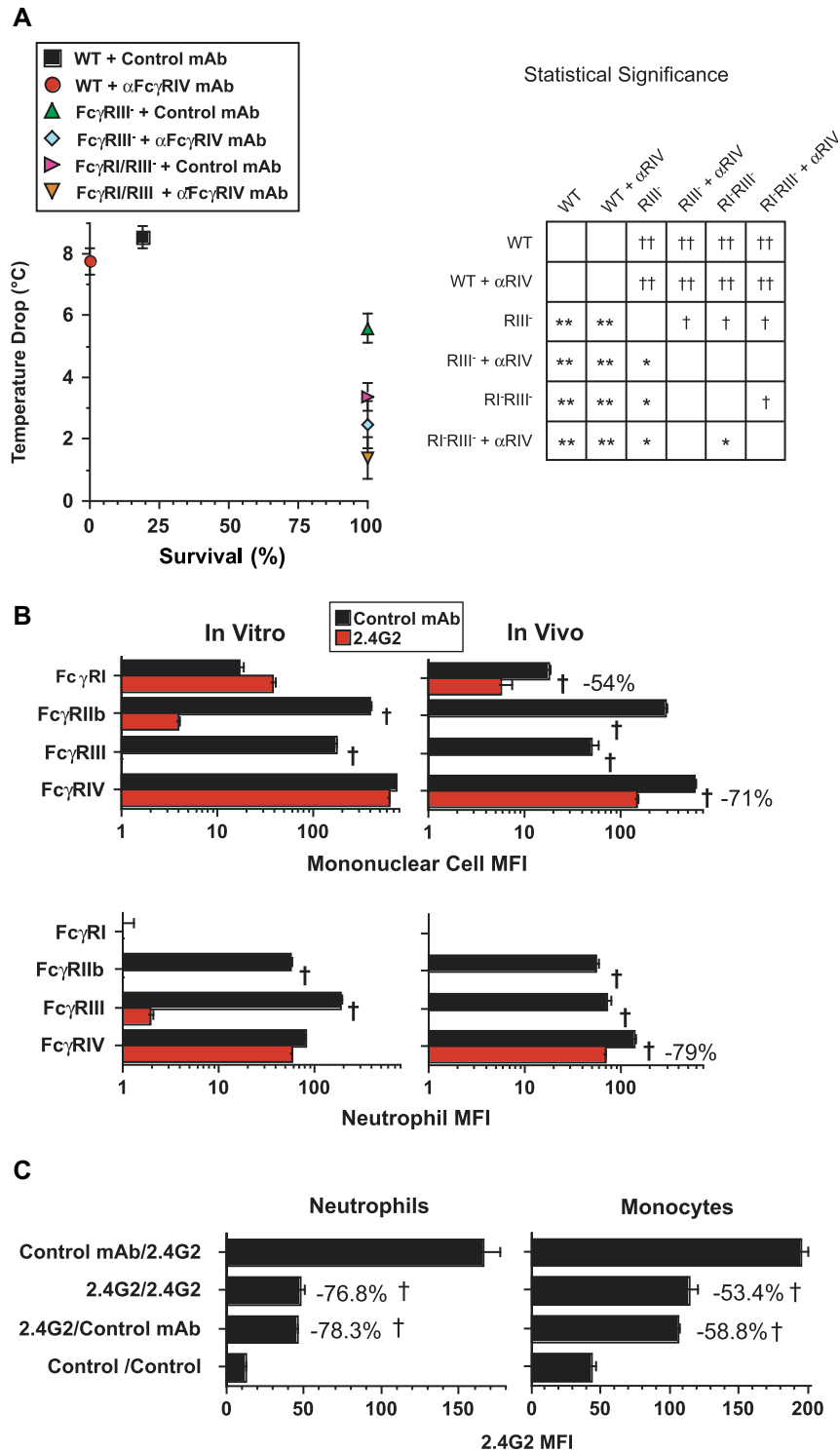


FIG 3. Effects of 2.4G2 on Fc γ R expression and contributions to anaphylaxis. **A**, WT and Fc γ RIII^{-/-}C57BL/6 mice and Fc γ RI/RIII^{-/-}B10.D2 mice were primed by intravenous injection of 500 μ g of IgG_{2a} anti-TNP mAb, then treated intraperitoneally with 200 μ g of anti-Fc γ RIV or control mAb and challenged the next day intravenously with 100 μ g of TNP-OVA. Rectal temperatures were determined for the next 60 minutes; mean maximal decrease in rectal temperature and percentage of survival for >2 hours is shown for each group of mice (pooled from 2 experiments, total of 8 mice/group). Statistical significance box compares values for temperature decrease and mortality for groups above box with each group to right of box. *Greater temperature drop; **greater temperature drop and mortality; †smaller temperature drop; ††smaller temperature drop and less mortality. **B**, *Left panels*: Blood leukocytes (10^6) were incubated for 30 minutes at 4°C in the presence of NaN₃ with 1 μ g of anti-Fc γ RI/RIII or control mAb, then stained for Fc γ RI, Fc γ RIIb, Fc γ RIII, or Fc γ RIV and analyzed by flow cytometry. *Right panels*: BALB/c mice were injected with

2.4G2 might modulate FcγRI and FcγRIV expression by interacting with these receptors through its antibody binding site or through its own Fc domain. To determine whether the second putative mechanism was possible, we evaluated the ability of mice injected with another rat IgG_{2b} mAb that binds to monocytes and neutrophils, M1/70 (anti-CD11b mAb), to decrease the expression of each of the stimulatory FcγRs (Fig 4, A). M1/70 injection decreased FcγRI, FcγRIII, and FcγRIV expression on monocytes and neutrophils to almost the same extent as expression of these receptors was reduced by 2.4G2 (44% to 73%). This suggests that 2.4G2 interferes with FcγRI and FcγRIV expression primarily by interacting with them through its own Fc domain, rather than through its antigen binding site. However, M1/70 was much less effective than 2.4G2 at blocking IgG_{2a}-mediated anaphylaxis in passively immunized mice (Fig 4, B and C), possibly because M1/70 was much less effective than 2.4G2 at blocking binding to FcγRIII (compare Fig 3, B, with Fig 4, A).

To further evaluate the requirement for an interaction between the Fc domains of 2.4G2 and stimulatory FcγRs other than FcγRIII to completely suppress IgG_{2a}-mediated anaphylaxis, we generated 2.4G2 F(ab')₂ that contained undetectable intact 2.4G2 (>0.5%; Fig 4, D). Because F(ab')₂ fragments of IgG have a much shorter *in vivo* half-life than intact IgG,³⁶ it was necessary to use much more 2.4G2 F(ab')₂ than intact 2.4G2 to saturate FcγRIIb and FcγRIII 1 hour after injection; even 2 mg of the F(ab')₂ fragment was less effective than 100 μg of the intact molecule at blocking fluorochrome-labeled 2.4G2 binding and staining with anti-FcγRIII mAb (Fig 4, E). Although the F(ab')₂ fragment significantly suppressed neutrophil FcγRIV expression at this time, suppression was modest and much less than was seen with intact 2.4G2 (Fig 4, E); this suggests that FcγRIV expression is suppressed both by FcγRIIb/FcγRIII cross-linking and by an interaction between the Fc domains of 2.4G2 and FcγRIV. We were unable to compare the effects of intact 2.4G2 versus 2.4G2 F(ab')₂ on FcγRI expression because injection of either form of this mAb temporarily depletes nearly all FcγRI-expressing cells from peripheral blood (data not shown).

IgG_{2a}-mediated anaphylaxis was completely blocked 24 hours after the completion of rapid desensitization with intact 2.4G2 (maximum dose, 500 μg) and was blocked by approximately 90% by rapid desensitization with 2.4G2 F(ab')₂ (maximum dose, 2 mg) (Fig 4, F). Although we cannot tell whether the somewhat decreased ability of the F(ab')₂ fragment to suppress anaphylaxis resulted from decreased modulation of FcγRIV (and possibly FcγRI) or from the less complete saturation of FcγRIIb and FcγRIII by the F(ab')₂ fragment (Fig 4, G), our observations indicate that IgG_{2a}-mediated anaphylaxis is strongly inhibited by the cross-linking of FcγRIIb and FcγRIII, even in the absence of direct binding to FcγRI and FcγRIV.

This raised the alternative possibility that the greater effectiveness of 2.4G2 than M1/70 at blocking IgG_{2a}-mediated anaphylaxis might be the ability of the former mAb to interact directly with the inhibitory FcγR, FcγRIIb, which might allow it to give a direct inhibitory signal to FcγRIIb-expressing cells through that receptor. This possibility is eliminated by evidence that 2.4G2 completely blocks IgG_{2a}-mediated anaphylaxis in FcγRIIb-deficient mice (Fig 5). Surprisingly, the severity of anaphylaxis in mice passively immunized with IgG_{2a} anti-TNP mAb was less in FcγRIIb-deficient mice than in WT mice on the same genetic background (Fig 5), whereas elimination of an inhibitory receptor might have been expected to increase the severity of anaphylaxis. One possible explanation for this phenomenon is the spontaneous development of autoantibodies and, thus, IgG immune complexes, in FcγRIIb-deficient mice³⁷; these putative immune complexes might partially desensitize mice to IgG-mediated anaphylaxis. Regardless of the explanation, our results show that FcγRIIb was not required for rapid desensitization with 2.4G2.

Roles of different cell types in IgG_{2a}-mediated anaphylaxis

Disagreement exists about the importance of different FcγR-expressing cell types, particularly monocyte/macrophages, basophils, and neutrophils in murine IgG-mediated anaphylaxis.^{12,32,33} We evaluated the possible roles of these 3 cell types in anaphylaxis in BALB/c and C57BL/6 mice that had been passively immunized with IgG_{2a} anti-TNP mAb by depleting neutrophils with hydroxyurea plus a low dose of anti-Ly6C/6G mAb (RB6-8C5), by depleting or desensitizing monocytes and macrophages with gadolinium, or by depleting basophils with anti-CD200R3 mAb. Results of these experiments indicated that (1) IgG_{2a}-mediated anaphylaxis was more severe in C57BL/6 mice than in BALB/c mice and (2) depletion of any of these 3 cell types significantly decreased the severity of anaphylaxis in both mouse strains (Fig 6). The importance of monocyte/macrophages in IgG_{2a}-mediated anaphylaxis is also supported by our evidence that FcγRI contributes to this disorder, inasmuch as FcγRI is expressed by monocytes and macrophages but not by either basophils or neutrophils.^{2,24}

Because 2.4G2 could have decreased the contribution of these cell types to IgG_{2a}-mediated anaphylaxis either by killing these cells or by blocking or modulating their stimulatory FcγRs, we looked at the effects of 2.4G2 on the numbers of several different cell types in different organs. Results of this study (Fig 7) show that 2.4G2 treatment had no effect on numbers of monocytes, macrophages, T cells, dendritic cells, or mast cells; may have caused a partial redistribution of basophils from blood to spleen; and somewhat decreased

500 μg of anti-FcγRII/RIII or control mAb. Blood cells obtained 24 hours later were stained for FcγRI, FcγRIIb, FcγRIII, or FcγRIV and analyzed by flow cytometry; 4 mice/group, representative of 2 experiments. **C**, BALB/c WT mice were rapidly desensitized with 500 μg of biotin-labeled 2.4G2 or control mAb. Blood leukocytes obtained the next day were incubated on ice with biotin-2.4G2 or biotin-control mAb, then stained with fluorochrome-labeled streptavidin, as well as with mAbs to identify neutrophils and monocytes; 4 mice/group, representative of 2 experiments. **B** and **C**, †Decreased receptor expression compared with control mAb-treated mice. Percentages to right of bars are mean decreases in expression. *MFI*, Mean fluorescent intensity.

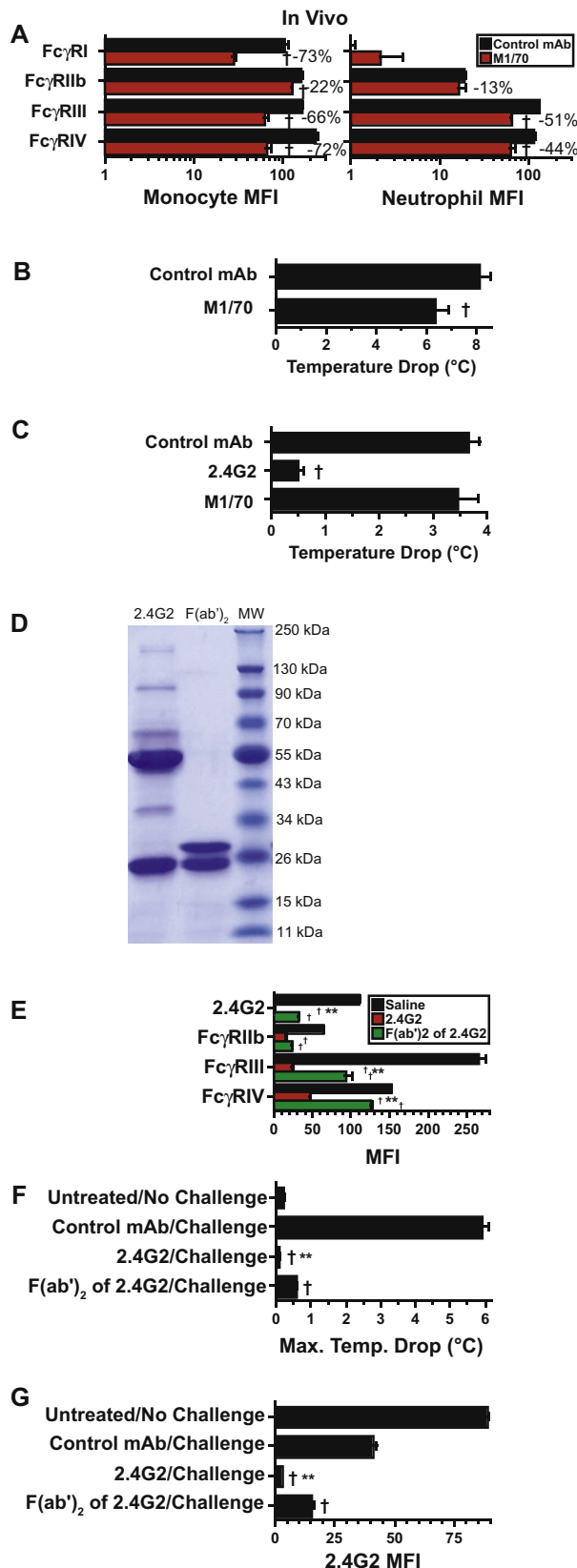


FIG 4. Effects of rat IgG_{2b} anti-mouse CD11b mAb on FcγR expression and IgG-mediated anaphylaxis. **A**, WT BALB/c mice were injected with 500 μg of anti-CD11b mAb (M1/70) or control mAb. Blood leukocytes obtained the next day were stained with mAbs to FcγRI, FcγRIIb, FcγRIII, FcγRIV, and

the number of neutrophils in blood but not in spleen. Thus, the predominant suppressive effect of 2.4G2 on IgG_{2a}-mediated anaphylaxis appears to result more from the blocking or modulation of stimulatory FcγRs than from the elimination of cells that contribute to anaphylaxis. An alternative possibility, that FcγR expression and/or severity of anaphylaxis is decreased nonspecifically by cell death, is unlikely because administration of GK1.5 (rat IgG_{2b} anti-CD4 mAb), which kills CD4⁺ T cells,²⁰ had no effect on either parameter (Fig 8).

DISCUSSION

The results of our studies indicate that rapid desensitization with an IgG mAb specific for FcγRIIb and FcγRIII *safely* allows complete inhibition of IgG-mediated anaphylaxis. Although shock is induced in mice given a single injection of 63 μg of 2.4G2, mice injected hourly with doubling doses of that mAb, starting with a dose of 15 μg, failed to anaphylax in response to 500 μg of 2.4G2, even if pretreated with a long-acting formulation of IL-4 that considerably increases sensitivity to this mAb.³⁰ Rapid desensitization with 2.4G2 prevented IgG-mediated anaphylaxis in both passively and actively immunized mice and was both safer and longer lasting in actively immunized mice than rapid desensitization with antigen. The completeness of suppression of IgG_{2a}-mediated anaphylaxis was somewhat surprising, because this isotype can mediate anaphylaxis to some extent through FcγRI and FcγRIV, as well as through FcγRIII (Fig 3, A), and 2.4G2 has never convincingly been shown to bind to FcγRI or FcγRIV.^{2,34} Our observation that 2.4G2 and another rat IgG_{2b} mAb that binds to monocyte/macrophages, M1/70 (anti-CD11b), both decrease FcγRI and FcγRIV expression by >50% when injected *in vivo* suggests that inhibition of

with fluorochrome-labeled streptavidin, as well as with mAbs to identify neutrophils and monocytes; 8 mice/group, pooled from 2 experiments. Percentages to right of bars are mean decreases in expression. **B**, WT BALB/c mice were primed by intravenous injection of 500 μg of IgG_{2a} anti-TNP mAb and treated intraperitoneally with 500 μg of M1/70 or control mAb. Mice were challenged intravenously with 100 μg of TNP-OVA 1 day after M1/70 treatment. Rectal temperatures were obtained during the subsequent 60 minutes; pooled from 3 experiments, total 12 mice/group. **C**, BALB/c mice were injected intravenously with 500 μg of IgG_{2a} anti-TNP mAb, then rapidly desensitized with 2.4G2, M1/70, or control mAb. Mice were challenged intravenously the next day with 100 μg of TNP-OVA. Rectal temperatures were obtained during the subsequent 60 minutes; 1 experiment, 4 mice/group. **D**, Intact 2.4G2 and 2.4G2 that had been digested with immobilized pepsin were reduced with 2-mercaptoethanol and analyzed by SDS-10% PAGE. **E**, BALB/c mice (3/group) were injected intravenously with saline, 100 μg of 2.4G2, or 2 mg of 2.4G2 F(ab')₂. One hour later, blood neutrophils were analyzed by flow cytometry for binding of fluorochrome-labeled 2.4G2 and mAbs specific for FcγRIIb, FcγRIII, and FcγRIV. **F**, BALB/c mice (4/group) were initially left untreated or were injected intravenously with 500 μg of IgG_{2a} anti-TNP mAb, then rapidly desensitized intraperitoneally with doubling doses of intact 2.4G2 (initial dose, 15 μg; final dose, 480 μg) or 2.4G2 F(ab')₂ (initial dose, 62.5 μg; final dose, 2 mg). One day later, mice were challenged intravenously with 100 μg of TNP-OVA. **G**, Blood neutrophils obtained 4 hours after TNP-OVA challenge were analyzed for staining with fluorochrome-labeled 2.4G2. †Significantly decreased staining or less severe anaphylaxis than saline-treated mice (panel E) or control mAb mock-desensitized mice (panels F and G). **Significantly decreased staining or less severe anaphylaxis than mice treated with 2.4G2 F(ab')₂. MFI, Mean fluorescent intensity.

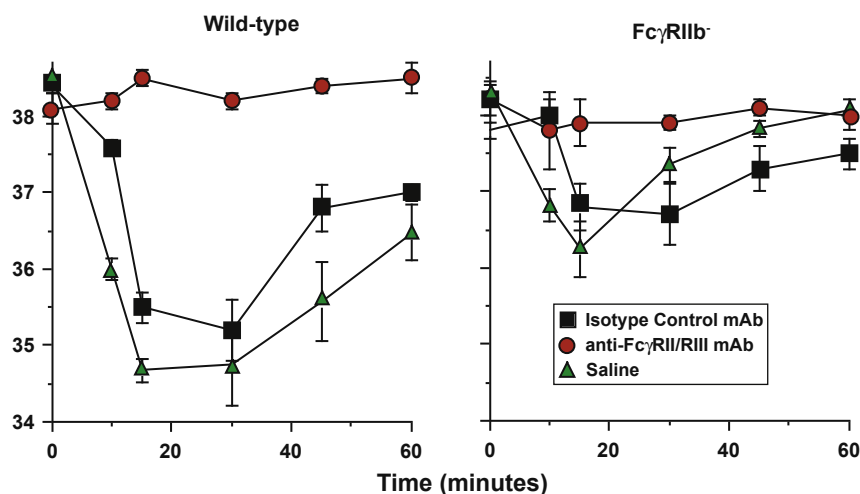


FIG 5. 2.4G2 inhibits IgG_{2a}-mediated anaphylaxis in FcγRIIb-deficient mice. WT and FcγRIIb-deficient mice (FcγRIIb^{-/-}) were pretreated with 500 μg of IgG_{2a} anti-TNP mAb, then rapidly desensitized with anti-FcγRII/RIII or control mAb or injected with saline and challenged intravenously with 100 μg of TNP-BSA. Rectal temperatures were determined. Data were pooled from 2 experiments; total 8 mice/group.

the expression of these 2 FcγRs may result mostly from the interaction of the Fc domains of these mAbs with these FcγRs after they have been focused onto cells by binding to their primary targets. This “nonspecific” FcγR modulation may need to be considered in interpreting the results of any studies in which animals are injected with an IgG antibody that binds to a cell member antigen. However, our studies with 2.4G2 F(ab')₂ indicate that the cross-linking of the 2 FcγRs bound by this mAb also somewhat suppresses FcγRIV expression.

It is unlikely that 2.4G2 inhibits IgG-mediated anaphylaxis primarily by sending an inhibitory signal to targeted cells through FcγRIIb, because rapid desensitization with 2.4G2 still inhibits IgG-mediated anaphylaxis in FcγRIIb-deficient mice. In contrast, 2.4G2 inhibition of FcγRI- and FcγRIV-mediated anaphylaxis might involve FcγRIII-mediated induction of cellular hyporesponsiveness (anergy) in addition to partial modulation of FcγRI and FcγRIV. Induction of anergy may also be involved in the rapid desensitization process itself, inasmuch as it is not clear how the rapid desensitization process allows the administration of large doses of 2.4G2 without the induction of anaphylaxis. It is possible that anaphylaxis is avoided by ligating so little FcγR on monocyte/macrophages, basophils, and neutrophils at any one time that the PAF released by these cells is never sufficient to induce shock (because PAF has an *in vivo* half-life of approximately 5 minutes,³⁸ successive waves of PAF production would not cause a cumulative increase in concentration). However, we favor the possibility that slow increases in 2.4G2 concentration also lead to monocyte/macrophage, basophil, and neutrophil anergy (partial suppression of FcγR-activated signaling pathways), because this effect was observed in our previous studies of rapid desensitization of mast cells with an anti-FcεRI mAb.¹⁷

Although we have focused on inhibition of IgG-mediated anaphylaxis, it is likely that rapid desensitization with 2.4G2 would also inhibit or ameliorate other disorders that have been shown by 2.4G2 administration studies or studies performed

with FcγR-deficient mice to be primarily mediated by these receptors. We also suspect that our observations in mice apply to humans. Even though human IgG-mediated anaphylaxis has never been proven to exist, the likelihood that this occurs in humans is supported by several descriptions of anaphylaxis in patients repeatedly infused with large quantities of a foreign protein who had high serum concentrations of IgG antibodies but no detectable IgE antibodies to the infused antigen and who failed to develop an increased serum tryptase.³⁹⁻⁴⁵ In addition, human macrophages, neutrophils, and basophils express stimulatory FcγRs^{1,18} and can be induced to secrete PAF,⁴⁶⁻⁴⁸ which has been associated with human anaphylaxis.^{49,50} Human and mouse FcγRI, FcγRIIb, and FcγRIII (FcγRIIIa and FcγRIIIb in humans) are homologous and murine FcγRIV is thought to be homologous to human FcγRIIIa.^{1,2} Humans have additional activating FcγRs (FcγRIIa and FcγRIIc) that lack mouse homologs^{1,2}; however, the ability of anti-FcγRIIb/RIII mAb (2.4G2) to block all activating signaling through murine FcγRs, including those to which it does not bind directly through its antigen binding site, suggests that an antibody to human FcγRIIIa will have a similar effect. *In vitro* studies are planned to determine whether mAbs to human FcγRIIIa and/or FcγRIIIb will modulate other FcγRs on human macrophages. In addition, we plan *in vivo* experiments with immunodeficient mice reconstituted with human myeloid cells to determine whether any anti-human FcγR mAbs can (1) induce anaphylaxis in this model, and (2) if so, whether it is possible to rapidly desensitize mice to prevent anaphylaxis mediated in this model by human IgG antibodies. If successful, clinical studies could be performed to determine whether desensitization with appropriately humanized anti-FcγR mAbs could be used to treat any of the inflammatory or autoimmune disorders, mentioned in the Introduction, in which FcγRs have a pathogenic role.

Any consideration of using rapid desensitization-facilitated FcγR blockade to suppress putative IgG-mediated anaphylaxis or FcγR-associated disorders in humans will need to take into account potential side effects that are unrelated to direct

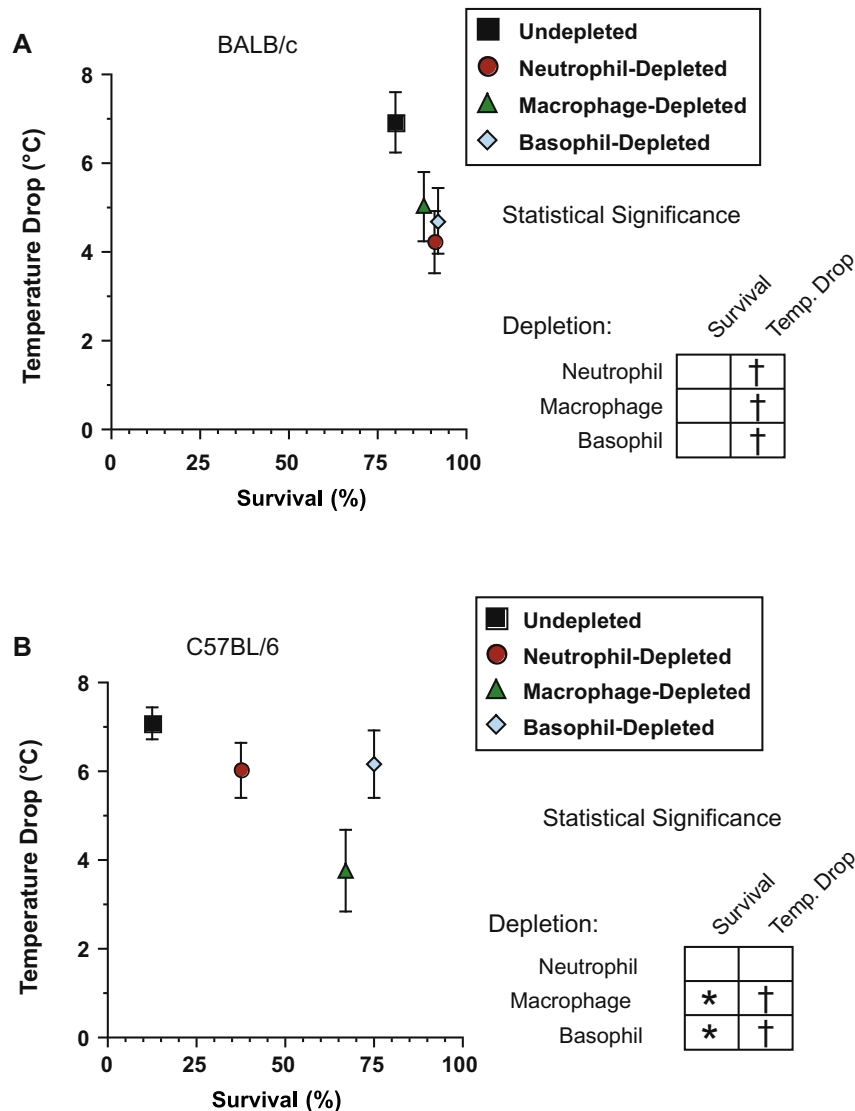


FIG 6. Contributions of myeloid cell types to IgG-mediated anaphylaxis. **A**, BALB/c mice were depleted of neutrophils by treatment with hydroxyurea and anti-Gr-1 mAb (RB6-8C5), subjected to gadolinium depletion/desensitization of monocytes and macrophages, or depleted of basophils with anti-CD200R3 mAb. Mice were then primed by intravenous injection of 500 μ g of IgG_{2a} anti-TNP mAb, followed by intravenous challenge 2 hours later with 100 μ g of TNP-OVA. Rectal temperature was determined during the next 60 minutes. Mean maximum temperature drops and survival for >2 hours for each group are shown (pooled from 2 experiments, total of 8 mice/group). Depletion of monocytes and macrophages with clodronate liposomes had a similar effect to treatment with gadolinium (not shown). Statistical significance box compares survival and temperature drop in groups that had neutrophil, macrophage, or basophil depletion with the group with no cellular depletion. *Less mortality; †lower temperature drop. **B**, An experiment similar to that shown in panel A was performed with C57BL/6 mice.

mAb-mediated toxicity. Stimulatory Fc γ R_s would be expected to promote antibody-mediated host protection against infectious agents, although the results of murine and human studies indicate that these receptors enhance protection against some bacteria, viruses, and protozoa but have no effect against others and, in some cases, actually increase invasiveness and pathogenicity.^{3,4,51-59} Blockade of the inhibitory receptor, Fc γ RIIb, might enhance autoimmunity and increase responses of inflammatory cells.^{1,37,60} although this problem may be less serious when stimulatory Fc γ R_s are blocked as well, and the

problem might be eliminated by using a reagent that selectively blocked stimulatory receptors. Further murine studies should help to determine whether it is feasible to reduce potential risks while maintaining beneficial effects and whether trials in human disorders that are currently treated with dangerous and incompletely effective medications would be worth the potential risk.

We thank Jeffrey Ravetch for his gift of a monoclonal anti-mouse Fc γ RIV mAb and Fc γ RI- and Fc γ RIII-deficient mice.

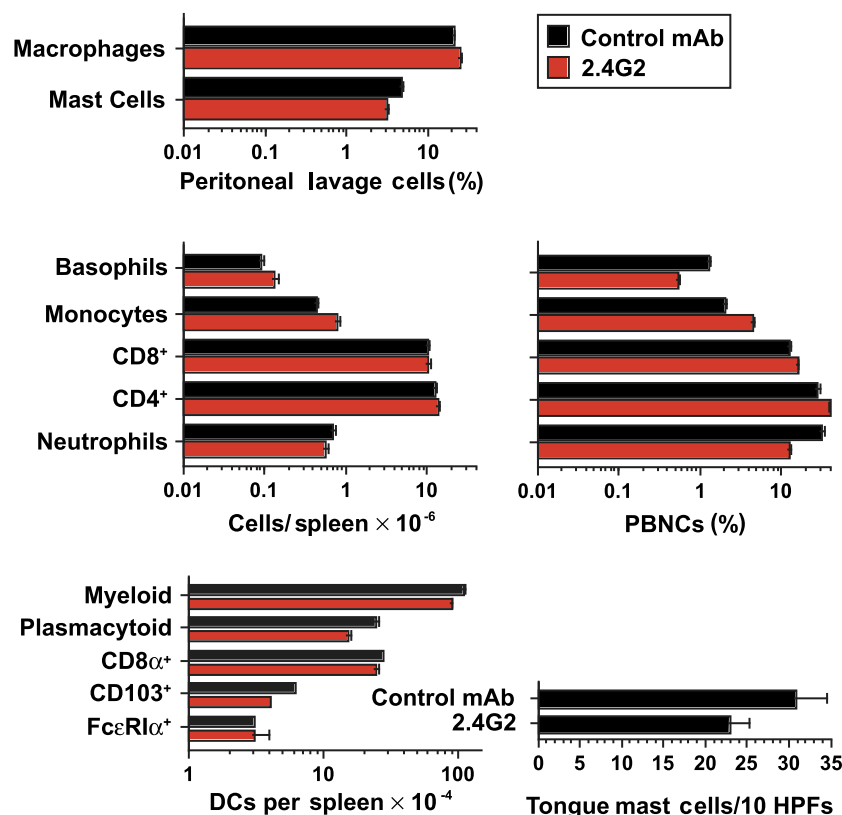


FIG 7. Effects of 2.4G2 on cell number. BALB/c mice (2 experiments, total of 8 mice/group) were rapidly desensitized with 2.4G2 or isotype control mAbs. The percentages or numbers of the cell populations shown in peritoneal lavage, spleen, and peripheral blood were determined 3 days after rapid desensitization. Cell types were identified and enumerated by flow cytometry, using the strategies described in Methods. In addition, tongue mast cell numbers per 10 high-powered fields were determined. No significant effects were found on any cell population when data are corrected for multiple comparisons. DC, Dendritic cell; HPF, high-power field; PBNC, peripheral blood nuclear cell.

Key messages

- Rapid desensitization with 2.4G2, an anti-Fc γ R2/RIII mAb, can safely inhibit IgG-mediated anaphylaxis.
- IgG_{2a}-mediated anaphylaxis is predominantly Fc γ R2/RIII dependent but partially Fc γ R1 and Fc γ R4 dependent.
- Treatment of mice with 2.4G2 blocks Fc γ R2/RIII and removes most Fc γ R1 and Fc γ R4 from monocyte/macrophages and dendritic cells and Fc γ R4 from neutrophils.
- Monocyte/macrophages, basophils, and neutrophils all contribute to IgG_{2a}-mediated anaphylaxis.

REFERENCES

1. Nimmerjahn F, Ravetch JV. Fc γ receptors as regulators of immune responses. *Nat Rev Immunol* 2008;8:34-47.
2. Bruhns P. Properties of mouse and human IgG receptors and their contribution to disease models. *Blood* 2012;119:5640-9.
3. Huber VC, Lynch JM, Bucher DJ, Le J, Metzger DW. Fc receptor-mediated phagocytosis makes a significant contribution to clearance of influenza virus infections. *J Immunol* 2001;166:7381-8.
4. Chung KM, Nybakken GE, Thompson BS, Engle MJ, Marri A, Fremont DH, et al. Antibodies against West Nile Virus nonstructural protein NS1 prevent lethal infection through Fc γ receptor-dependent and -independent mechanisms. *J Virol* 2006;80:1340-51.
5. Ioan-Facsinay A, de Kimpe SJ, Hellwig SM, van Lent PL, Hofhuis FM, van Ojik HH, et al. Fc γ R1 (CD64) contributes substantially to severity of arthritis, hypersensitivity responses, and protection from bacterial infection. *Immunity* 2002;16:391-402.
6. Bergtold A, Gavhane A, D'Agati V, Madaio M, Clynes R. FcR-bearing myeloid cells are responsible for triggering murine lupus nephritis. *J Immunol* 2006;177:7287-95.
7. Meyer D, Schiller C, Westermann J, Izui S, Hazenbos WL, Verbeek JS, et al. Fc γ R2/RIII (CD16)-deficient mice show IgG isotype-dependent protection to experimental autoimmune hemolytic anemia. *Blood* 1998;92:3997-4002.
8. Gessner JE, Heiken H, Tamm A, Schmidt RE. The IgG Fc receptor family. *Ann Hematol* 1998;76:231-48.
9. Radeke HH, Janssen-Graalfs I, Sowa EN, Chouchakova N, Skokowa J, Loscher F, et al. Opposite regulation of type II and III receptors for immunoglobulin G in mouse glomerular mesangial cells and in the induction of anti-glomerular basement membrane (GBM) nephritis. *J Biol Chem* 2002;277:27535-44.
10. Tuzun E, Saini SS, Yang H, Alagappan D, Higgs S, Christadoss P. Genetic evidence for the involvement of Fc γ R2/RIII in experimental autoimmune myasthenia gravis. *J Neuroimmunol* 2006;174:157-67.
11. Hashimoto T, Ishii N, Ohata C, Furumura M. Pathogenesis of epidermolysis bullosa acquisita, an autoimmune subepidermal bullous disease. *J Pathol* 2012;228:1-7.
12. Strait RT, Morris SC, Yang M, Qu XW, Finkelman FD. Pathways of anaphylaxis in the mouse. *J Allergy Clin Immunol* 2002;109:658-68.
13. Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV. Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. *Science* 2008;320:373-6.

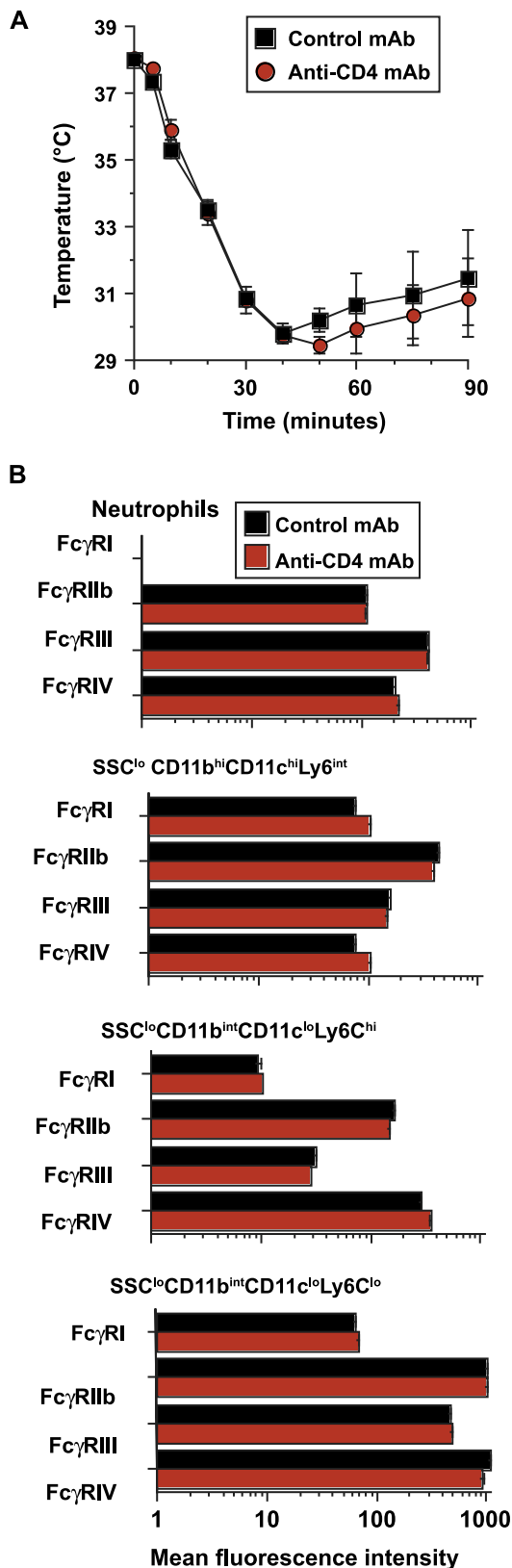


FIG 8. Neither FcγR expression nor severity of IgG_{2a}-mediated anaphylaxis is decreased in mice treated with a cytotoxic anti-CD4 mAb. BALB/c WT mice were injected intravenously with 0.5 mg of GK1.5 anti-CD4 mAb or an isotype control mAb. One day later, one cohort of mice (4/group) was injected with IgG_{2a} anti-TNP mAb and challenged intravenously 2 hours after

- Unkeless J. Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. *J Exp Med* 1979;150:580-96.
- Hirano M, Davis RS, Fine WD, Nakamura S, Shimizu K, Yagi H, et al. IgE immune complexes activate macrophages through FcγRIV binding. *Nat Immunol* 2007;8:762-71.
- Teeling JL, Jansen-Hendriks T, Kuijpers TW, de Haas M, van de Winkel JG, Hack CE, et al. Therapeutic efficacy of intravenous immunoglobulin preparations depends on the immunoglobulin G dimers: studies in experimental immune thrombocytopenia. *Blood* 2001;98:1095-9.
- Khodoun M, Kucuk ZY, Strait RT, Krishnamurthy D, Janek K, Lewkowich IP, et al. Rapid polyclonal desensitization with antibodies to IgE and FcεRIα. *J Allergy Clin Immunol* 2013;133:1555-64.
- Ravetch JV, Bolland S. IgG Fc receptors. *Annu Rev Immunol* 2001;19:275-90.
- Dombrowicz D, Flamand V, Brigman KK, Koller BH, Kinet JP. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor alpha chain gene. *Cell* 1993;75:969-76.
- Wilde DB, Marrack P, Kappler J, Fitch FW. Evidence implicating L3T4 in class II MHC antigen reactivity: monoclonal antibody GK1.5 (anti-L3T4) blocks class II MHC antigen-specific proliferation, release of lymphokines, and binding by cloned murine helper T lymphocyte lines. *J Immunol* 1983;131:2178-83.
- Springer T, Galfre G, Secher DS, Milstein C. Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. *Eur J Immunol* 1979;9:301-6.
- Okada S, Nakauchi H, Nagayoshi K, Nishikawa S, Miura Y, Suda T. Enrichment and characterization of murine hematopoietic stem cells that express c-kit molecule. *Blood* 1991;78:1706-12.
- Schiller C, Janssen-Graalfs I, Baumann U, Schwerter-Strumpf K, Izui S, Takai T, et al. Mouse FcγRII is a negative regulator of FcγRIII in IgG immune complex-triggered inflammation but not in autoantibody-induced hemolysis. *Eur J Immunol* 2000;30:481-90.
- Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV. FcγRIV: a novel FcR with distinct IgG subclass specificity. *Immunity* 2005;23:41-51.
- Finkelman FD, Kessler SW, Mushinski JF, Potter M. IgD-secreting murine plasmacytomas: identification and partial characterization of two IgD myeloma proteins. *J Immunol* 1981;126:680-7.
- Finkelman FD, Madden KB, Morris SC, Holmes JM, Boiani N, Katona IM, et al. Anti-cytokine antibodies as carrier proteins. Prolongation of in vivo effects of exogenous cytokines by injection of cytokine-anti-cytokine antibody complexes. *J Immunol* 1993;151:1235-44.
- Miyajima I, Dombrowicz D, Martin TR, Ravetch JV, Kinet JP, Galli SJ. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and FcγRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J Clin Invest* 1997;99:901-14.
- Strait RT, Hicks W, Barasa N, Mahler A, Khodoun M, Kohl J, et al. MHC class I-specific antibody binding to nonhematopoietic cells drives complement activation to induce transfusion-related acute lung injury in mice. *J Exp Med* 2011;208:2525-44.
- Khodoun M, Strait R, Orekov T, Hogan S, Karasuyama H, Herbert DR, et al. Peanuts can contribute to anaphylactic shock by activating complement. *J Allergy Clin Immunol* 2009;123:342-51.
- Strait RT, Morris SC, Smiley K, Urban JF Jr, Finkelman FD. IL-4 exacerbates anaphylaxis. *J Immunol* 2003;170:3835-42.
- Dombrowicz D, Flamand V, Miyajima I, Ravetch JV, Galli SJ, Kinet JP. Absence of FcεRI chain results in upregulation of FcγRIII-dependent mast cell degranulation and anaphylaxis. Evidence of competition between FcεRI and FcγRIII for limiting amounts of Fcεβ and γ chains. *J Clin Invest* 1997;99:915-25.
- Jonsson F, Mancardi DA, Kita Y, Karasuyama H, Iannascoli B, Van Rooijen N, et al. Mouse and human neutrophils induce anaphylaxis. *J Clin Invest* 2011;121:1484-96.
- Tsujimura Y, Obata K, Mukai K, Shindou H, Yoshida M, Nishikado H, et al. Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. *Immunity* 2008;28:581-9.
- Mancardi DA, Iannascoli B, Hoos S, England P, Daeron M, Bruhns P. FcγRIV is a mouse IgE receptor that resembles macrophage FcεRI in humans and promotes IgE-induced lung inflammation. *J Clin Invest* 2008;118:3738-50.

that with 100 μg of TNP-BSA and followed for 90 minutes for severity of anaphylaxis (A). Blood neutrophils and 3 populations of blood mononuclear myeloid cells (CD11b⁺) from a second cohort were evaluated for FcγR expression by flow cytometry (B) 1 day after GK1.5 injection. SSC, Side scatter.

35. Strait RT, Morris SC, Finkelman FD. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc γ RIIB cross-linking. *J Clin Invest* 2006;116:833-41.
36. Waldmann TA, Strober W. Metabolism of immunoglobulins. *Prog Allergy* 1969; 13:1-110.
37. Bolland S, Ravetch JV. Spontaneous autoimmune disease in Fc γ RIIB-deficient mice results from strain-specific epistasis. *Immunity* 2000;13:277-85.
38. Yoshida H, Satoh K, Koyama M, Hiramoto M, Takamatsu S. Deficiency of plasma platelet-activating factor acetylhydrolase: roles of blood cells. *Am J Hematol* 1996;53:158-64.
39. Schmidt AP, Taswell HF, Gleich GJ. Anaphylactic transfusion reactions associated with anti-IgA antibody. *N Engl J Med* 1969;280:188-93.
40. Cheifetz A, Smedley M, Martin S, Reiter M, Leone G, Mayer L, et al. The incidence and management of infusion reactions to infliximab: a large center experience. *Am J Gastroenterol* 2003;98:1315-24.
41. Miele E, Markowitz JE, Mamula P, Baldassano RN. Human antichimeric antibody in children and young adults with inflammatory bowel disease receiving infliximab. *J Pediatr Gastroenterol Nutr* 2004;38:502-8.
42. Baert F, Noman M, Vermeire S, Van Assche G, D'Haens G, Carbonez A, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med* 2003;348:601-8.
43. Hedin H, Richter W, Messmer K, Renck H, Ljungstrom KG, Laubenthal H. Incidence, pathomechanism and prevention of dextran-induced anaphylactoid/anaphylactic reactions in man. *Dev Biol Stand* 1980;48:179-89.
44. Umeda Y, Fukumoto Y, Miyauchi T, Imaizumi M, Shimabukuro K, Mori Y, et al. Anaphylactic shock related to aprotinin induced by anti-aprotinin immunoglobulin G antibody alone; report of a case. *Kyobu Geka* 2007;60: 69-71.
45. Bergamaschini L, Mannucci PM, Federici AB, Coppola R, Guzzoni S, Agostoni A. Posttransfusion anaphylactic reactions in a patient with severe von Willebrand disease: role of complement and alloantibodies to von Willebrand factor. *J Lab Clin Med* 1995;125:348-55.
46. Camussi G, Aglietta M, Coda R, Bussolino F, Piacibello W, Tetta C. Release of platelet-activating factor (PAF) and histamine, II: the cellular origin of human PAF: monocytes, polymorphonuclear neutrophils and basophils. *Immunology* 1981;42:191-9.
47. Sanchez-Crespo M, Alonso F, Egido J. Platelet-activating factor in anaphylaxis and phagocytosis, I: release from human peripheral polymorphonuclears and monocytes during the stimulation by ionophore A23187 and phagocytosis but not from degranulating basophils. *Immunology* 1980;40: 645-55.
48. Camussi G, Bussolino F, Tetta C, Piacibello W, Aglietta M. Biosynthesis and release of platelet-activating factor from human monocytes. *Int Arch Allergy Appl Immunol* 1983;70:245-51.
49. Vadas P, Gold M, Perelman B, Liss GM, Lack G, Blyth T, et al. Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. *N Engl J Med* 2008;358: 28-35.
50. Vadas P, Perelman B, Liss G. Platelet-activating factor, histamine, and tryptase levels in human anaphylaxis. *J Allergy Clin Immunol* 2012;131:144-9.
51. Araujo-Jorge T, Rivera MT, el Bouhdi A, Daeron M, Carlier Y. An Fc γ RII-, Fc γ RIII-specific monoclonal antibody (2.4G2) decreases acute *Trypanosoma cruzi* infection in mice. *Infect Immun* 1993;61:4925-8.
52. Ouma C, Davenport GC, Garcia S, Kempaiah P, Chaudhary A, Were T, et al. Functional haplotypes of Fc gamma (Fc γ) receptor (Fc γ RIIA and Fc γ RIIB) predict risk to repeated episodes of severe malarial anemia and mortality in Kenyan children. *Hum Genet* 2012;131:289-99.
53. Sole-Violan J, Garcia-Laorden MI, Marcos-Ramos JA, de Castro FR, Rajas O, Borderias L, et al. The Fc γ receptor IIA-H/H131 genotype is associated with bacteremia in pneumococcal community-acquired pneumonia. *Crit Care Med* 2011;39:1388-93.
54. Endeman H, Cornips MC, Grutters JC, van den Bosch JM, Ruven HJ, van Velzen-Blad H, et al. The Fc γ receptor IIA-R/R131 genotype is associated with severe sepsis in community-acquired pneumonia. *Clin Vaccine Immunol* 2009;16:1087-90.
55. Domingo P, Muniz-Diaz E, Baraldes MA, Arilla M, Barquet N, Pericas R, et al. Associations between Fc γ receptor IIA polymorphisms and the risk and prognosis of meningococcal disease. *Am J Med* 2002;112:19-25.
56. Yee AM, Phan HM, Zuniga R, Salmon JE, Musher DM. Association between Fc γ RIIA-R131 allotype and bacteremic pneumococcal pneumonia. *Clin Infect Dis* 2000;30:25-8.
57. Pinheiro da Silva F, Aloulou M, Skurnik D, Benhamou M, Andreumont A, Velasco IT, et al. CD16 promotes *Escherichia coli* sepsis through an Fc γ inhibitory pathway that prevents phagocytosis and facilitates inflammation. *Nat Med* 2007;13:1368-74.
58. de Haas M, Kleijer M, van Zwieten R, Roos D, von dem Borne AE. Neutrophil Fc γ RIIB deficiency, nature, and clinical consequences: a study of 21 individuals from 14 families. *Blood* 1995;86:2403-13.
59. van de Winkel JG, de Wit TP, Ernst LK, Capel PJ, Ceuppens JL. Molecular basis for a familial defect in phagocyte expression of IgG receptor I (CD64). *J Immunol* 1995;154:2896-903.
60. Ujike A, Ishikawa Y, Ono M, Yuasa T, Yoshino T, Fukumoto M, et al. Modulation of immunoglobulin (Ig)E-mediated systemic anaphylaxis by low-affinity Fc receptors for IgG. *J Exp Med* 1999;189:1573-9.