Supporting Information

Khodoun et al. 10.1073/pnas.1105695108

SI Materials and Methods

Reagents. Hybridomas were obtained from the following sources: rat IgG2b anti-mouse FcγRII/RIII mAb (2.4G2) (1), mouse IgE anti-TNP (IGEL 2a), and mouse IgG1 anti-TNP (1B7.11, used as an isotype control) from the ATCC; mouse anti-mouse FcγRIIb (Ly17.2) (2) from Ulrich Hammerling; rat IgG2b anti-NP (J1.2, used as an isotype control) from John Abrams; and rat IgG2a anti-mouse IgE mAb (EM-95) (3) from Zelig Eshhar. Hybridomas were grown as ascites in Pristane-primed athymic nude mice and purified by ammonium sulfate precipitation, followed by DE-52 cation exchange chromatography. PE-anti-mouse FcγRIII mAb was purchased from R&D Systems; APC-anti-mouse IL-3R, APC-anti-F4/80, APC-anti-mouse c-kit, biotin-anti-mouse c-kit, APC-anti-human FcγRII, PE-anti-human FcγRIII, FITC-anti-human CD15, and PerCP/Cy5.5-anti-human CD163 mAbs were purchased from eBioscience; FITC-anti-B220, FITC-anti-mouse CD19, PE-anti-mouse IL-4Rα, PerCP-anti-mouse CD8, FITC-anti-mouse Ly6G, APC-anti-DX5 (CD49b), FITC-anti-mouse IgE, PerCP-anti-mouse CD11b, PerCP-Cy5.5-anti-CD11c, FITC- and APC-anti-mouse CD4, PE-anti-DX5, streptavidin-PE, streptavidin-PerCP, and corresponding isotype controls were purchased from BD Biosciences. Abs for measurement of in vivo IL-4 secretion (biotin-BVD4-1D11 and BVD6-24G2.3) were obtained from BD. Platelet activating factor was purchased from BIOMOL (now Enzo Life Sciences International). Histamine was purchased from Sigma-Aldrich. Water-soluble peanut extract (4) and TNP-OVA (5) were prepared as previously described. IgG immune complexes (ICs) were prepared by mixing affinity-purified azide-free anti-human IgG and human IgG (Bethyl Laboratories) at a 1:1 ratio and incubating the mixture at room temperature for 30 min.

1. Unkeless JC (1979) Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. *J Exp Med* 150:580–596.
2. Gessner JE, Heiken H, Tamm A, Schmidt RE (1998) The IgG Fc receptor family. *Ann Hematol* 76:231–248.
3. Baniyash M, Eshhar Z (1984) Inhibition of IgE binding to mast cells and basophils by monoclonal antibodies to murine IgE. *Eur J Immunol* 14:799–807.
4. Khodoun M, et al. (2009) Peanuts can contribute to anaphylactic shock by activating complement. *J Allergy Clin Immunol* 123:342–351.
5. Strait RT, Morris SC, Finkelman FD (2006) IgG blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and FcγRIIb crosslinking. *J Clin Invest* 118:833–841.

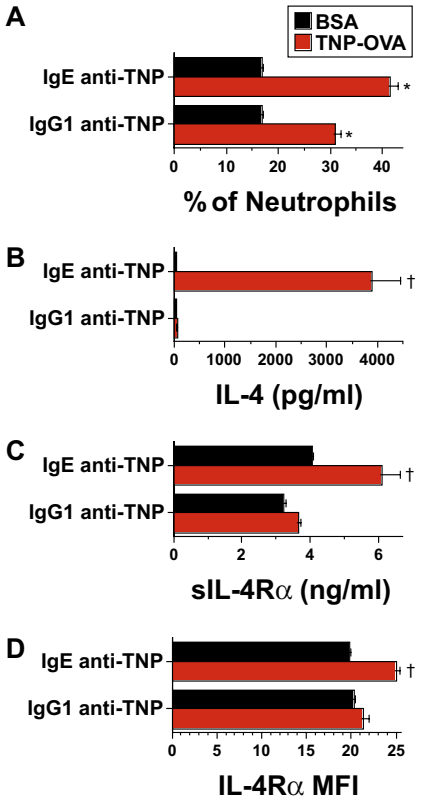

Fig. S1. Markers selective for IgE-mediated anaphylaxis. BALB/c mice were passively immunized i.v. with 10 μg of IgEαTNP mAb or 100 μg of IgG1αTNP mAb and challenged the next day with 40 μg of trinitrophenyl-ovalbumin (TNP-OVA) or BSA. Mice were injected at the time of challenge with 10 μg of biotin-BVD4-1D11 (anti-IL-4) mAb for in vivo cytokine capture assay. Mice were bled 4 h after challenge, and the percentage of neutrophils in peripheral blood (*A*), IL-4 secretion (*B*), serum levels of sIL-4Rα (*C*), and $CD4^+$ T-cell IL-4Rα expression (*D*) were determined.

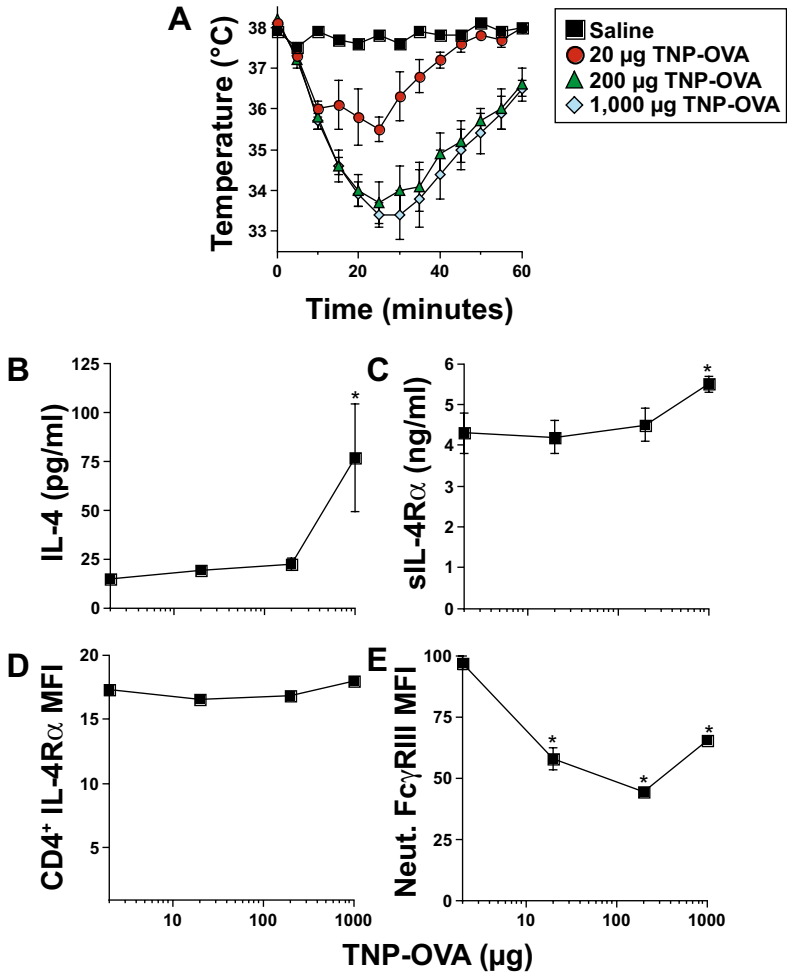

Fig. S2. Dose-dependence of IgG-mediated anaphylaxis. BALB/c mice were passively immunized with 100 μg of IgG1 anti-TNP mAb and challenged i.v. with 20, 200, or 1,000 μg of TNP-OVA. Mice were injected at the time of challenge with 10 μg of biotin-BVD4-1D11 (anti-IL-4) mAb for in vivo cytokine capture assay. (*A*) Rectal temperatures were determined during the 60 min after challenge. Mice were bled 4 h after challenge, and IL-4 secretion (*B*), serum sIL-4Rα levels (*C*), $CD4^+$ T-cell IL-4Rα expression (*D*), and neutrophil FcγRIII expression (*E*) were determined.

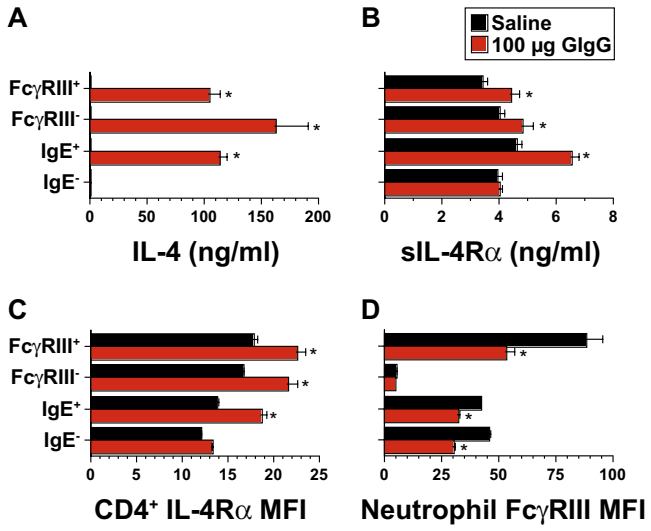

Fig. S3. IgE- and FcγRIII-dependence of markers for the classical and alternative anaphylaxis pathways. C57BL/6 wild-type ($Fc\gamma RIII^+$) and FcγRIII-deficient ($Fc\gamma RIII^-$) and FVB/N wild-type (IgE^+) and IgE-deficient (IgE^-) mice were actively immunized with 0.2 mL of goat anti-mouse IgD antiserum and challenged 14 d later with 100 μg of goat IgG (GIgG). Mice were injected at the time of challenge with 10 μg of biotin-BVD4-1D11 (anti-IL-4) mAb for in vivo cytokine capture assay. Mice were bled 4 h after challenge, and IL-4 secretion (*A*), serum sIL-4Rα levels (*B*), $CD4^+$ T-cell IL-4Rα expression (*C*), and neutrophil FcγRIII expression (*D*) were determined.

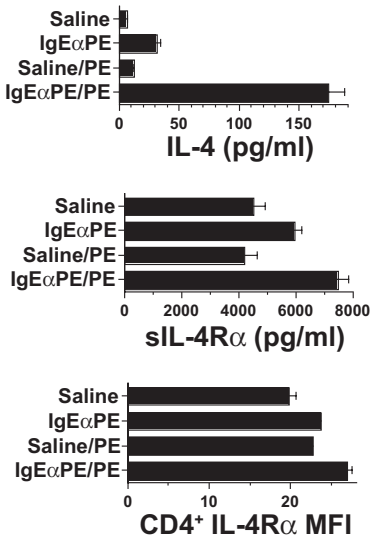

Fig. S4. Antigen activation of human IgE-primed cells in mice that express human FcεRIα induces increases in IL-4 secretion, serum sIL-4Rα concentration, and $CD4^+$ T-cell IL-4Rα expression. BALB/c mice in which the mouse IL-4Rα gene is replaced by the homologous human gene were injected with saline or IgG-depleted serum from a peanut-allergic individual, then challenged 4 d later with saline or PE. IL-4 secretion, serum sIL-4Rα concentration, and $CD4^+$ T-cell IL-4Rα expression were measured 4 h later. *Significant increase compared with all other groups.

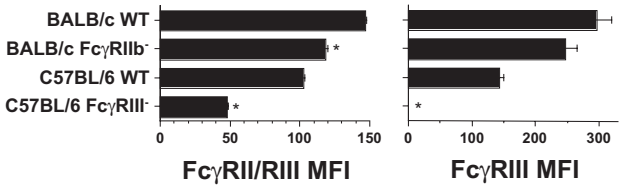

Fig. S5. Demonstration of specificity of anti-FcγRIII mAb. Peripheral blood neutrophils from BALB/c wild-type (WT) and FcγRIIb-deficient ($FcγRIIb^-$) and C57BL/6 wild-type and FcγRIII-deficient ($FcγRIII^-$) mice were stained with fluorochrome-labeled anti-FcγRII/RIII mAb (2.4G2, *Left*) or anti-FcγRIII mAb (*Right*) and analyzed for the mean intensity of surface fluorescence (MFI) by flow cytometry. Intensity of staining with a fluorochrome-labeled isotype control mAb was subtracted to generate the values shown.

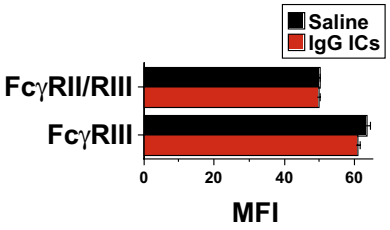

Fig. S6. IgG/antigen ICs do not directly block anti-FcγR mAb binding to neutrophils. Three million peripheral blood nucleated cells from BALB/c mice were cultured on ice in the presence of 0.2% sodium azide with ICs produced by mixing 10 μg of IgG1 anti-TNP mAb with 3 μg of TNP-OVA, then washed, stained with fluorochrome-labeled anti-FcγRII/RIII or anti-FcγRIII mAb, and analyzed for surface fluorescence by flow cytometry.

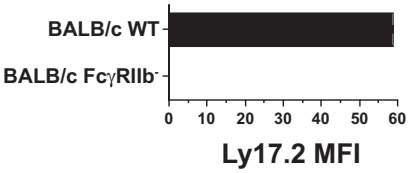

Fig. S7. Demonstration of specificity of anti-FcγRIIb mAb. Peripheral blood neutrophils from BALB/c wild-type (WT) and FcγRIIb-deficient (FcγRIIb) mice were stained with fluorochrome-labeled anti-Ly17.2 mAb and analyzed for the mean intensity of surface fluorescence (MFI) by flow cytometry. Intensity of staining with a fluorochrome-labeled isotype control mAb was subtracted to generate the values shown.