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Fcγ Receptors Inhibit Mouse and Human Basophil Activation

Lydie Cassard,*,†,[1] Friederike Jönsson,*,†,[1] Ségolène Arnaud,*,† and Marc Daëron*,†

Besides high-affinity IgE receptors (FcεRI), human basophils express activating (FcγRIIA) and inhibitory (FcγRIIB) low-affinity IgG receptors. IgG receptors (FcγR) were also found on mouse basophils, but not identified. We investigated in this study FcγR and the biological consequences of their engagement in basophils of the two species. We found the following: 1) that mouse basophils also express activating (FcγRIIIA) and inhibitory (FcγRIIB) low-affinity FcγR; 2) that activating FcγR can activate both human and mouse basophils, albeit with different efficacies; 3) that negative signals triggered by inhibitory FcγR are dominant over positive signals triggered by activating FcγR, thus preventing both human and mouse basophils from being activated by IgG immune complexes; 4) that the coengagement of FcεRI with inhibitory and activating FcγR results in a FcγRIIB-dependent inhibition of IgE-induced responses of both human and mouse basophils; 5) that FcγRIIB has a similar dominant inhibitory effect in basophils from virtually all normal donors; and 6) that IL-3 upregulates the expression of both activating and inhibitory FcγR on human basophils from normal donors, but further enhances FcγRIIB-dependent inhibition. FcγR therefore function as a regulatory module, made of two subunits with antagonistic properties, that prevents IgG-induced and controls IgE-induced basophil activation in both mice and humans. ***The Journal of Immunology*****, 2012, 189: 2995–3006.**

Human basophils have long been thought to play a major role in allergy. They express high-affinity IgE receptors (FcεRI) that are associated with the two ITAM-containing subunits, FcRγ and FcRβ. They are activated upon FcεRI aggregation, when specific Ag binds to receptor-bound IgE Abs (1). Activated basophils release vasoactive granular mediators and secrete cytokines, especially of the Th2 type, that promote allergic inflammation. Supporting their contribution in allergy, basophils were found at sites of allergic inflammation, where they displayed an activated phenotype in asthma (2) or atopic dermatitis (3) patients.

Contrasting with the in vivo roles of human basophils, which can be primarily documented with correlations, the in vivo roles of mouse basophils can be investigated experimentally in wild-type (wt) mice, the basophils of which were depleted by appropriate Abs, or in genetically-modified mice that lack basophils. Thus, IgE-induced chronic inflammation was abrogated in the absence of basophils (4, 5). Mouse basophils express FcεRI that are assumed to trigger similar signals as human basophil FcεRI. When activated, they secrete large amounts of IL-4, IL-6, and IL-13 that contribute to Th2 polarization (6–9) at the initiation of adaptive immune responses, and to the effector phase of inflammation.

We recently found that active systemic anaphylaxis primarily depends on IgG Abs, on IgG receptors (FcγR), and on neutrophils, rather than on IgE Abs, on FcεRI, and on mast cells (10), as IgE-induced passive systemic anaphylaxis does (11, 12). Also, IgG1-induced passive systemic anaphylaxis, which depends on FcγRIIIA (13), was observed in mast cell-deficient mice (14), but it was abrogated by basophil depletion (15). It was, however, not abrogated in basophil-deficient mice (5). Whether IgG and FcγR contribute to anaphylaxis in humans or to allergic inflammation is not known. Like mouse (16) and human (17) mast cells, mouse and human basophils express not only IgE receptors, but also IgG receptors.

IgG were long ago reported to bind to human basophils (18) on which two low-affinity IgG receptors were subsequently identified: FcγRIIA and FcγRIIB (19, 20). FcγRIIA are single-chain ITAM-containing activating receptors, whereas FcγRIIB are single-chain ITIM-containing inhibitory receptors (21). Human basophils also express minute amounts of the GPI-anchored low-affinity IgG receptor FcγRIIIB (22), whose biological properties are unclear. They express neither FcγRIIIA nor high-affinity IgG receptors (FcγRI) and, unlike in human mast cells (23), FcγRI were not inducible by IFN-γ in human basophils (24). Mouse basophils were stained by 2.4G2 (25), a mAb that recognizes the two murine low-affinity IgG receptors: FcγRIIB, which have the same ITIM as in humans (26), and FcγRIIIA, which associate with the same ITAM-containing subunits as FcεRI (27). Whether mouse basophils express one, the other, or both FcγR is not known.

Anti-allergen IgG Abs were described in nonallergic donors (28), but no convincing evidence that IgG can activate human basophils has been published. The possibility that FcγR can negatively regulate human basophil activation was suggested by experiments showing that bifunctional molecules, which coligated FcεRI with FcγRII, inhibited IgE-induced basophil activation (29, 30). On the contrary, IgG1 immune complexes induced $CD49^+$ cells, among which are mouse basophils, to secrete platelet-activating factor in vitro, and they triggered basophil-dependent passive systemic anaphylaxis in vivo (15). Whether FcγRIIB can negatively regulate mouse basophil activation was not investigated.

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Abbreviations used in this article: BM, bone marrow; DAM, donkey anti-mouse IgG; gpi, glucose-phosphate isomerase; hFc, human Fc; hIL-3, human IL-3; MAR, mouse anti-rat IgG; mBM, mouse BM; mBMB, mBM basophil; mBMCB, BM-derived cultured basophil; mFc, mouse Fc; mIgE, mouse IgE; mIL-3, mouse IL-3; RAHE, rabbit anti-human IgE; rIgE, rat IgE; TNP, trinitrophenyl; wt, wild-type.

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We examined which FcγR are expressed on normal human and murine basophils, and investigated their biological properties when engaged by IgG Abs. We found that human and mouse basophils coexpress activating and inhibitory low-affinity FcγR, and that inhibitory FcγR exert a dominant effect over activating FcγR, thus preventing basophils from being activated by IgG immune complexes. We next examined the effect of FcγR on FcεRI-dependent basophil activation. One can indeed envision IgG receptors to have three effects on IgE-induced responses, when activating and inhibitory IgG receptors are coengaged with FcεRI by immune complexes in basophils, as follows: 1) negative signals may be dominantly generated by FcγR and antagonize positive signals generated by FcεRI; 2) positive signals may be dominantly generated by FcγR and synergize with positive signals generated by FcεRI; and 3) positive and negative signals generated by FcγR may neutralize each other and have no effect on FcεRI signals. We found that inhibitory FcγR also exert a dominant effect over activating FcγR when all receptors are coengaged by immune complexes and dampened IgE-induced responses.

Materials and Methods

Mice

KRN^{tg} mice (31) were provided by D. Mathis, C. Benoist (Harvard Medical School, Boston, MA), and Institut de Génétique et de Biologie Moléculaire et Cellulaire (Strasbourg, France). The wt C57BL/6J mice were purchased from Charles River, and $FcγRIIIA^{-/-}$ and $FcγRIIB^{-/-}$ C57BL/6J mice were from The Jackson Laboratory. Mice were 6–8 wk old. All mouse protocols were approved by Animal Care and Use Committees (Paris, Ile de France, France).

Mouse bone marrow-derived basophils

Mouse bone marrow (mBM) cells were seeded at 5×10^5 cells/ml and cultured for 8 d in Opti-MEM supplemented with 10% FCS, 100 IU/ml penicillin, 100 μg/ml streptomycin, 50 mM 2-ME (Complete Opti-MEM), and 1 ng/ml mouse IL-3 (mIL-3). After 24 h of culture, adherent cells were discarded. Cells were split once during this period. On day 7, 3 μg/ml IR162 was added to cultures. The next day, basophil-rich cultures were further enriched by depleting Kit^+ cells using the CD117-Microbead kit and an AutoMACS (Miltenyi Biotec), according to the manufacturer's instructions. The purity of basophils thus obtained in these bone marrow-derived cultured basophils (mBMCB) was generally 90%.

Human basophils

Whole blood from normal donors collected in EDTA was obtained from the Etablissement Français du Sang (Paris, France) in accordance with a convention between Institut Pasteur and Etablissement Français du Sang. This study was approved by the Comité de Protection des Personnes and the Ministère de l'Education Nationale de la Recherche et de Technologie (Déclaration Collective 2008-68). All donors provided written informed consent for the collection of samples and subsequent analysis. PBMC were isolated by Ficoll-Hypaque density gradient centrifugation. PBMC from a total of 52 normal blood donors were examined.

Abs and reagents

PE-conjugated anti-CD203c was from Beckman Coulter; allophycocyanin-conjugated anti-human FcεRIα (anti-hFcεRIα) and mouse CD115-PE from eBioscience; allophycocyanin-conjugated anti-hCD64 (anti-FcγRI), anti-mouse FcγRIIIA (anti-mFcγRIIIA) (275003), and anti-mFcγRI (290322) from R&D Systems; FITC-conjugated anti-FcγRIIA IV.3 from Stem Cells Technologies; FITC-conjugated anti-human IgE, human IgG, mouse monoclonal anti-OVA IgG (OVA-14), OVA grade V, DNP-has, and glucose-phosphate isomerase (gpi) from Sigma-Aldrich; anti-mouse B220-PE, CD3ε-allophycocyanin, CD11b-FITC, CD117-allophycocyanin, DX5-PE, FcγRIIB/IIIA (2.4G2)-PE, Gr1-allophycocyanin, IgE-FITC, NK1.1-PE-Cy7, SiglecF-PE, IL-4 PE, PE-conjugated anti-human CD14, CD19, CD3, CD56, CDw125, and corresponding isotype controls from BD Biosciences; mouse CD45-VioBlue, allophycocyanin-conjugated anti-CD16, and its isotype controls from Miltenyi Biotec; Alexa647-conjugated anti-CD200R1 (MCA2281) from Serotec; anti–mFcγRIIB-FITC (Ly17.1/2) from Caltag; FITC-F(ab′)2 mouse anti-rat IgG (FITC-MAR), FITC-F(ab′)2 donkey anti-mouse IgG (FITC-DAM), IgG and F(ab′)2 fragments of mouse anti-rat IgG (MAR), F(ab′)2 fragments of DAM and of donkey anti-human IgG from Jackson ImmunoResearch Laboratories; rabbit anti-human IgE (RAHE) from DakoCytomation; rat IgE (rIgE; IR162) from IMEX (Brussels, Belgium); mouse IgE (mIgE) anti-DNP mAb 2682-I (32) used as culture supernatants or as purified Abs and mIL-3 from BioLegend; and human recombinant cytokines from ImmunoTools. Anti-FcγRIV mAb (9E9) was provided by J. Ravetch (Rockefeller University, New York, NY). Anti-hFcγRIIB (2B6N297Q) (33), Alexa488-2B6N297Q, and isotype control (Alexa488-CH4420) were provided by MacroGenics. IgG from anti–gpi-rich K/B×N serum (34), anti-mFcγRIIB (K9.361) (35), and anti-FcγRIIA (IV.3) (36) were purified by affinity chromatography on protein G-Sepharose. F(ab′)2 fragments from K9.361, IV.3, and RAHE were prepared by pepsin digestion. gpi (Sigma-Aldrich) was trinitrophenylated with trinitrobenzene sulfonic acid (Sigma-Aldrich). Trinitrophenyl $(TNP)_{11}$ gpi thus obtained was recognized by mIgE anti-DNP mAb 2682-I, as assessed by ELISA (data not shown).

Immunofluorescence

Extracellular. Human or mouse cells were washed in cold PBS containing 0.5% BSA and 0.09% NaN_3 (PBS-BSA), incubated with labeled Abs for 20 min at 4°C, and washed. Fluorescence was analyzed by flow cytometry using a FACSCalibur (BD Biosciences) or a MACSQuant Analyzer (Miltenyi Biotec). Postacquisition analysis was done using the software FlowJo (Tree Star).

Intracellular. Kit nondepleted mBMCB were sensitized or not with 2 μg/ml on day 6 of culture. Cells were stimulated the next day in the presence of 10 μg/ml brefeldin A (Sigma-Aldrich) for 5 h. Cells were washed and stained with anti-DX5 and anti-kit prior to fixation and permeabilization with Perm/Fix and Perm/Wash (BD Biosciences). Cells were then labeled with anti–IL-4 PE, washed, and analyzed on a MACSQuant (Miltenyi Biotec).

Human basophil activation

PBMC were cultured at 1×10^6 cells/ml in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 1% sodium pyruvate, 1% nonessential amino acids, 1% HEPES buffer, and 1% penicillin-streptomycin (complete RPMI 1640) with 3 μg/ml rIgE or without overnight at 37°C. In some experiments, 5 ng/ml human IL-3 (hIL-3) was added.

Activation via IgE receptors. PBMC from 24 among the 52 donors were sensitized with rIgE and challenged both with RAHE and with MAR for 30 min at 37°C; PBMC from 12 donors tested were sensitized with rIgE and challenged with MAR only; cells from 16 donors were challenged with RAHE only. Forty-seven donors were included in the study. Five donors were excluded because their basophils did not respond to challenge to $F(ab')_2$ fragments of anti-IgE Abs. In some experiments, 10 μg/ml anti-FcγRIIB 2B6N297Q or 25 μg/ml IV.3 $F(ab')_2$ anti-FcγRIIA was added 30 min before stimulation with MAR.

Activation via IgG receptors. PBMC were incubated with 5 ng/ml hIL-3 or without, and with IV.3 $F(ab')_2$, washed, and stimulated with DAM $F(ab')_2$ for 30 min at 37°C. In some cases, PBMC were incubated for 30 min at 37°C with heat-aggregated human IgG and with 5 ng/ml hIL-3 or without. In other experiments, PBMC were incubated for 30 min at 37°C with preformed immune complexes made of human IgG and donkey anti-human IgG $F(ab')_2$, with 5 ng/ml hIL-3 or without.

Activation with specific Ag. PBMC were incubated with supernatant of mAb 2682-I (200 ng/ml mIgE) overnight, washed, and incubated for 30 min at 37°C with medium, TNP-gpi alone, or preformed immune complexes made of mouse polyclonal IgG anti-gpi and TNP-gpi. To assess human basophil activation by flow cytometry, cells were stained with PE-conjugated anti-CD203c and allophycocyanin-conjugated anti-FcεRIα.

Mouse basophil activation

Mouse basophils were stimulated in complete Opti-MEM. In the case of bone marrow (BM)-derived basophils, cells were stimulated at 5×10^5 cells/ml with 1 ng/ml mIL-3. BM cells were stimulated at 5×10^6 cells/ml without IL-3. Cell activation was monitored by assessing CD200R1 up-regulation on basophils by flow cytometry after 1 h and by measuring IL-4 in culture supernatants after 24 h. For FcγRIIB-blocking experiments, 10 μg/ml mFcγRIIB-specific K9.361/Ly17.2 (35) $F(ab')_2$ were added 10 min before stimulation.

Activation via IgE receptors. Mice were injected i.v. with 50 μg rIgE IR162 or mIgE 2882-I 24 h before BM was harvested. BM-derived basophils were sensitized overnight with 3 μg/ml rIgE IR162 before purification. Cells were washed twice and incubated with the indicated concentrations of $F(ab')_2$ MAR, with equimolar concentrations of IgG MAR, TNP-gpi or TNP-gpi–anti-gpi IgG immune complexes.

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Activation via IgG receptors. BM-derived basophils or BM cells were incubated with 10 μg/ml mAb anti-FcγRIIIA or with preformed immune complexes made with 100 μg/ml mAb OVA-14 and 100 μg/ml OVA or with 100 μg/ml polyclonal IgG anti-gpi and 30 μg/ml gpi. To assess mouse basophil activation by flow cytometry, erythrocytes from total blood or BM samples were lysed, and cells were incubated with a mixture of anti-IgE/DX5/CD200R1 for 30 min at 0˚C.

Histamine and cytokine measurements

Histamine was measured by ELISA, according to the manufacturer's instructions (Neogen). Mouse and human IL-4 were measured by ELISA, according to the manufacturer's instructions (R&D Systems).

Statistical analysis

Data were analyzed using the Student *t* test. The *p* values ≤ 0.05 were considered significant.

Results

Human and murine basophils coexpress activating and inhibitory IgG receptors

In accordance with previous works, human basophils, identified in PBMC from normal donors as $CD203c^+/Fc\varepsilon RI^+$ cells (37) (Fig. 1A), expressed FcγRII, but not FcγRI (Fig. 1B). They contained FcγRIIA (data not shown)- and FcγRIIB-specific transcripts (Fig. 1C), and they were stained by $F(ab')_2$ fragments of the FcγRIIA-specific mAb IV.3 (36) and by the hFcγRIIB-specific mAb 2B6N297Q (33) (Fig. 1B).

Murine basophils were identified as $CD49b^+/mIgE^+$ cells in peripheral blood (mouse blood basophils [mBB]) (Fig. 1D) and in BM (mBM basophils [mBMB]) (Fig. 1E). mBMCB were generated by culturing mouse BM cells with mIL-3. Following enrichment by negative selection, $CD49b^+/Kit^-$ basophils accounted for 83% cells (Fig. 1F). Murine basophils from all three sources expressed FcεRI and the same pattern of FcγR with comparable expression levels (Fig. 1G). They were stained by the FcγRIIB-specific mAb Ly17.1/2 (35) and by the mFcγRIIIA-specific mAb 275003, but not by the FcγRI-specific mAb 290322 or the FcγRIV-specific mAb 9E9. mBMCB from wt mice were stained by both Ly17.1/2 and 275003, mBMCB from FcγRIIB-deficient mice by 275003 only, and mBMCB from FcγRIIIA-deficient mice by Ly17.1/2 only (Supplemental Fig. 1A).

Human and mouse basophils therefore express low-affinity, but not high-affinity IgG receptors. Basophils from both species coexpress activating and inhibitory IgG receptors. Activating receptors are the FcRγ-associated FcγRIIIA in mice, but the single-chain FcγRIIA in humans. Inhibitory receptors are FcγRIIB in humans and mice. Whether FcγRIIIB found in minute amounts on human basophils are activating or inhibitory is unknown.

Basophils express as much or more inhibitory IgG receptors than other leukocytes

Like basophils, human monocytes coexpressed FcγRIIA and FcγRIIB. Neutrophils and eosinophils expressed FcγRIIA, but not FcγRIIB, whereas B cells expressed FcγRIIB but not FcγRIIA. T cells and NK cells expressed neither FcγRIIA nor FcγRIIB (Fig. 2A). Noticeably, human basophils expressed much less FcγRIIA than monocytes, neutrophils, and eosinophils, but they expressed much more FcγRIIB than monocytes and even B cells. The same expression pattern was found in basophils from all normal donors tested ($n = 13$) (Fig. 2B).

Like basophils, mouse monocytes, eosinophils, and possibly neutrophils coexpressed FcγRIIIA and FcγRIIB. As expected, NK cells expressed FcγRIIIA but not FcγRIIB, whereas B cells expressed FcγRIIB but not FcγRIIIA. T cells expressed neither FcγRIIIA nor FcγRIIB (Fig. 2C). Murine basophils expressed at least as much FcγRIIIA as monocytes, neutrophils, and eosinophils, and similar amounts of FcγRIIB as monocytes, but more FcγRIIB than neutrophils, eosinophils, and B cells.

Murine basophils therefore express at least as much FcγRIIB, and human basophils much more than other blood cells.

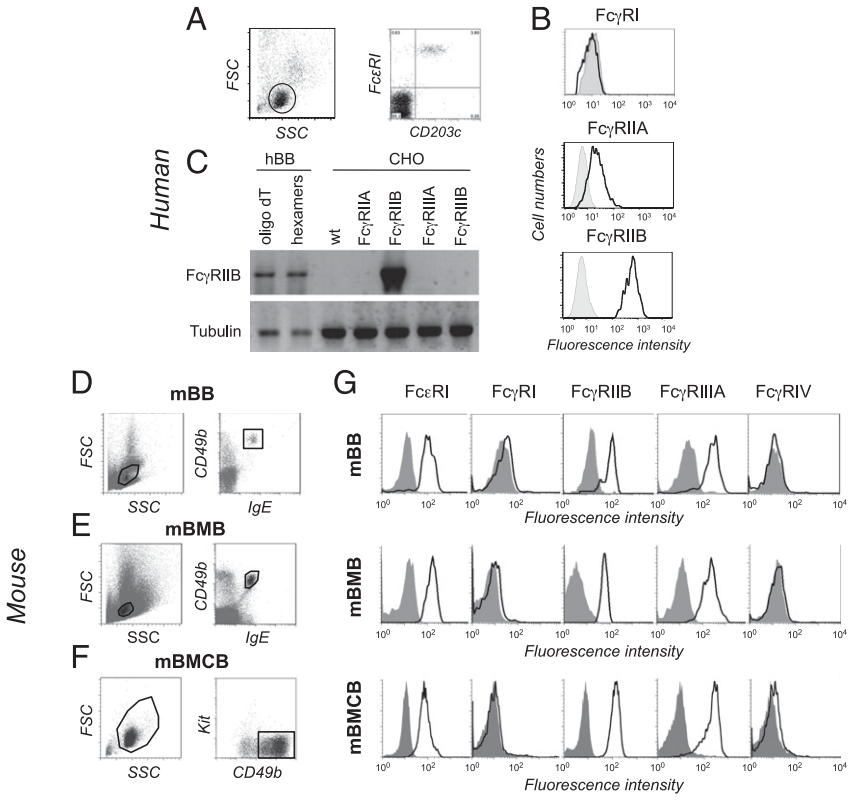

FIGURE 1. Human and mouse basophils coexpress activating and inhibitory FcγR. (**A**) Human blood basophils were identified as $CD203c^+/Fc\varepsilon RI\alpha^+$ cells in PBMC. (**B**) Histograms show the binding of anti-FcγRI, anti-FcγRIIA (FITC-IV.3), and anti-FcγRIIB (Alexa488-2B6N297Q) (solid lines) or their isotype controls (shaded histograms) on $CD203c^+$, $Fc\varepsilon RI\alpha^+$-gated cells. (**C**) cDNA from purified human basophils, Chinese hamster ovary wt, and Chinese hamster ovary transfectants was prepared using deoxythymine oligonucleotides or hexamer primers. FcγRIIB and tubulin transcripts were detected by RT-PCR using corresponding primers. (**D**) Mouse blood basophils (mBB) and (**E**) mBMB were identified as $CD49b^+/IgE^+$ cells; (**F**) mBMCB were identified as $CD49b^+/kit^-$ cells. (**G**) Histograms show overlays of anti-FcεRIα (MAR-1), anti-FcγRI (290322), anti-FcγRIIB (clone Ly17.2), anti-FcγRIIIA (275003), or anti-FcγRIV (9E9) (solid lines) and their isotype controls staining (shaded histograms) on basophils from the three sources. (A)–(G) are representative of at least two independent experiments.

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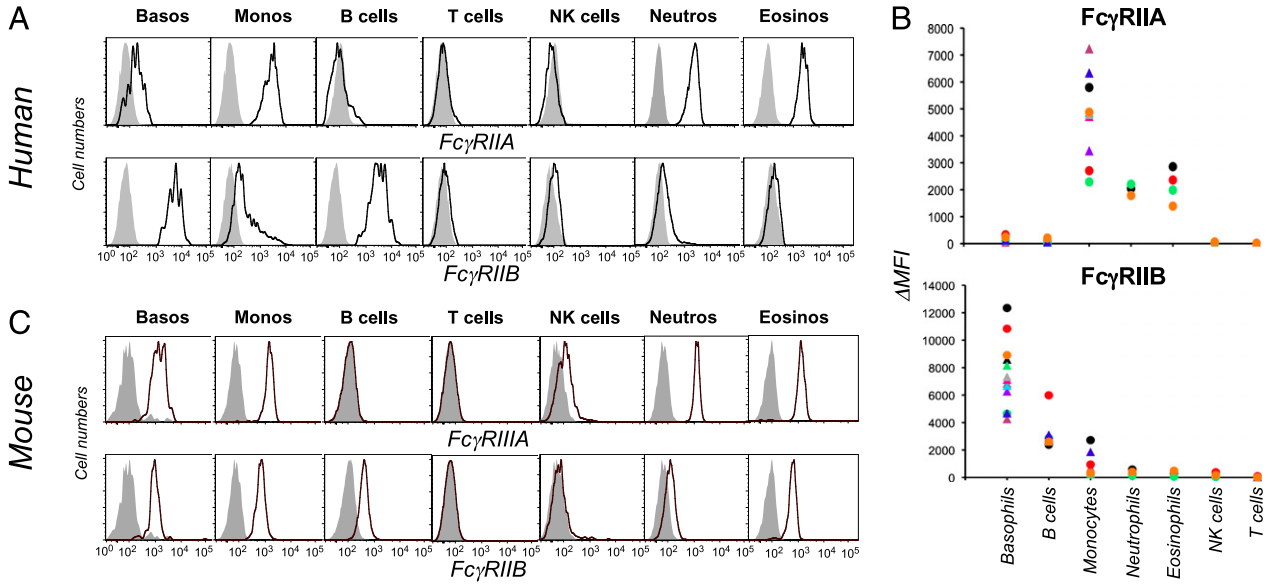

FIGURE 2. Human and mouse basophils express high levels of inhibitory FcγR. (**A**) Histograms show the binding of anti-FcγRIIA (FITC-IV.3) and anti-FcγRIIB (Alexa488-2B6N297Q) (solid lines) or their isotype controls (shaded histograms) on $CD203c^+$ (basophils)-, $CD14^+$ (monocytes)-, $CD19^+$ (B cells)-, $CD3^+$ (T cells)-, $CD56^+$ (NK cells)-, $CD16^{high}$ (neutrophils)-, or $CDw125^+$ (eosinophils)-gated human blood cells. (**B**) Human blood cells (circles) or PBMC (triangles) were incubated with anti-FcγR Abs. Graphs show Δ mean fluorescence intensity (MFI) of FcγRIIA or FcγRIIB on blood cells ($n = 4$) or PBMCs ($n = 9$) from normal donors. ΔMFI was calculated by subtracting isotype control MFI from anti-FcγRIIA or anti-FcγRIIB MFI. Each color represents an individual donor. (**C**) Mouse blood cells were incubated with anti-FcγR Abs. Histograms show the binding of anti-FcγRIIIA (275003) and anti-FcγRIIB (Ly17.1/2) (solid lines) or their isotype controls (shaded histograms) on $CD45^{low}/DX5^+/IgE^+$-(basophils), $CD11b^+/CD115^+$-(monocytes), $B220^+$-(B cells), $CD3^+$-(T cells), $DX5^+/NK1.1^+$-(NK cells), $CD11b^+/Gr1^{high}$-(neutrophils), or $Gr1^{int}/SiglecF^+$ (eosinophils)-gated cells. (A)–(C) are representative of at least two independent experiments.

Human and murine basophils do not respond to IgG immune complexes

All blood basophils from normal donors carried human IgE (hIgE) (Supplemental Fig. 2A). As a consequence, $F(ab')_2$ fragments of rabbit anti-hIgE Abs [$F(ab')_2$ RAHE], which can aggregate FcεRI-bound hIgE, dose dependently activated human basophils, as assessed by CD203c upregulation (38) (Supplemental Fig. 2B). Human basophils were also activated, although to a much lower degree, when sensitized with anti-FcγRIIA $F(ab')_2$ fragments and challenged with $F(ab')_2$ fragments of DAM Abs. Activation was enhanced by priming cells with hIL-3 (Fig. 3A). Human basophils were, however, not detectably activated by human IgG, whether heat aggregated (Fig. 3B) or in complexes, even when cells were primed with IL-3 (Fig. 3C). The same human IgG aggregates activated human neutrophils (Supplemental Fig. 3A). As in our previous works (22), we failed to activate human basophils by aggregating FcγRIIIB with $F(ab')_2$ fragments of FcγRIII-specific mAb 3G8, whether aggregated with $F(ab')_2$ goat anti-mouse or not (Supplemental Fig. 3B), even if cells were primed with IL-3 (data not shown). NK cells were activated, however, in the same blood samples (Supplemental Fig. 3B). Human basophils were therefore activated upon FcγRIIA aggregation, but not upon FcγRIIIB aggregation or upon aggregation of total FcγR, suggesting that weak activation signals generated by FcγRIIA may be inhibited by inhibitory receptors when all basophil FcγR are coengaged by IgG immune complexes.

mBMB could be activated by incubating BM cells from wt or $FcγRIIB^{-/-}$, but not from $FcγRIIIA^{-/-}$ mice, with anti-FcγRIIIA mAb, as assessed by CD200R1 upregulation (Fig. 4A). CD200R1 upregulation indeed correlates with mouse basophil activation (39). Anti-FcγRIIIA Abs also induced BM cells from wt, but not from $FcγRIIIA^{-/-}$ mice to secrete IL-4 (Fig. 4B). Under these conditions, IL-4 could originate from FcγRIIIA-expressing BM cells other than basophils. Purified wt, but not $FcγRIIIA^{-/-}$ mBMCB, however, secreted higher amounts of IL-4 when challenged with anti-FcγRIIIA mAb (Fig. 4C). Noticeably, anti-FcγRIIIA–induced IL-4 secretion was markedly enhanced in $FcγRIIB^{-/-}$ mBM (Fig. 4B) and even more (up to 100-fold) in $FcγRIIB^{-/-}$ mBMCB (Fig. 4C), and IL-4 was detected intracellularly in basophils, but not in other BM-derived cells (Fig. 4G and Supplemental Fig. 1C). This suggests that FcγRIIB could dampen FcγRIIIA-dependent basophil activation. Indeed, when binding to FcγRIIIA via their Fab portions, intact IgG Abs could also engage FcγRIIB via their Fc portion. Supporting this interpretation, IgG immune complexes induced no CD200R1 upregulation in wt mBMB, unless cells were preincubated with anti-mFcγRIIB $F(ab')_2$ fragments. Immune complexes also induced CD200R1 upregulation in mBMB from $FcγRIIB^{-/-}$, but not from $FcγRIIIA^{-/-}$ mice (Fig. 4D). Similar results were observed with polyclonal (gpi–anti-gpi) and with monoclonal (OVA [ova]–anti-ova) complexes. Likewise, no IL-4 secretion was induced by immune complexes in wt mBM (Fig. 4E) or mBMCB (Fig. 4F) unless cells were preincubated with anti-mFcγRIIB $F(ab')_2$ fragments. Even higher amounts of IL-4 were secreted in response to the same complexes by $FcγRIIB^{-/-}$ mBM (Fig. 4E) or mBMCB (Fig. 4F), but $FcγRIIIA^{-/-}$ basophils secreted no IL-4 (Fig. 4E, 4F). Likewise, IgG immune complexes induced detectable amounts of intracellular IL-4 in $FcγRIIB^{-/-}$, but not in wt or in $FcγRIIIA^{-/-}$ mBMCB (Fig. 4G). FcγRIIB-dependent inhibition is therefore dominant over FcγRIIIA-dependent activation in mouse basophils.

FcγR modulate IgE-induced responses in human and murine basophils

We next investigated whether FcγR might regulate IgE-induced human and murine basophil activation. To investigate this possibility, we used $F(ab')_2$ fragments and intact IgG against IgE. Unlike $F(ab')_2$ fragments, IgG anti-Ig can indeed bind not only to

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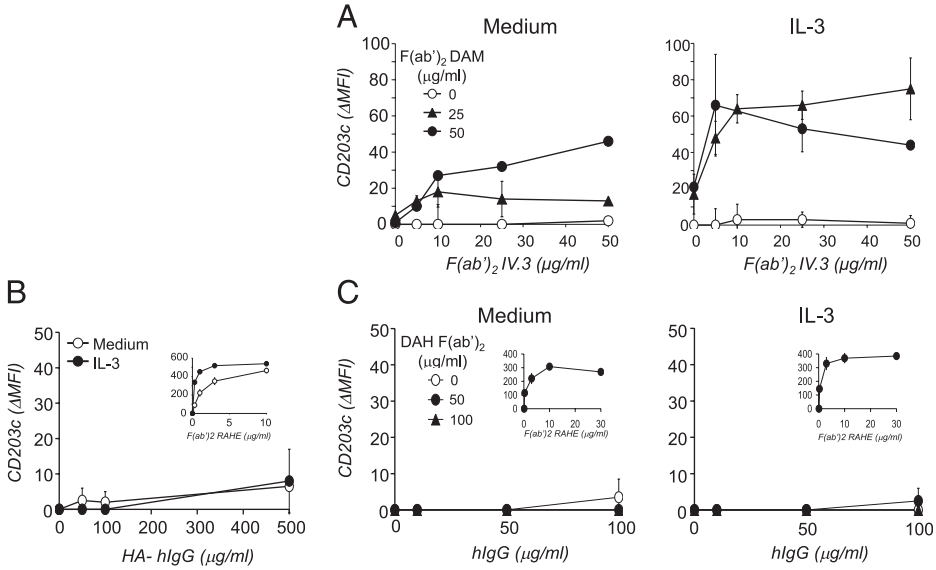

FIGURE 3. Human basophils can be activated by anti-FcγRIIA F(ab′)$_2$, but not by IgG complexes. (**A**) Human PBMC were primed by an overnight incubation with hIL-3 or without. They were incubated in duplicate with the indicated concentrations of F(ab′)$_2$ anti-FcγRIIA IV.3, washed, and challenged or not with DAM F(ab′)$_2$. Graphs show basophil activation induced by FcγRIIA aggregation measured by the upregulation of CD203c mean fluorescence intensity (MFI) on FcεRI$^+$ cells as a function of IV.3 F(ab′)$_2$ concentrations. Results are mean ± SD of CD203c ΔMFI. ΔMFI was calculated by subtracting the CD203c MFI of unstimulated cells from the CD203c MFI of stimulated cells. Results are representative of PBMC from three normal donors. (**B**) Human PBMC were primed by an overnight incubation with hIL-3 or without, and stimulated in duplicate with the indicated concentrations of heat-aggregated human IgG (HA-hIgG). Graphs show CD203c upregulation, as measured in (A). The *inset* shows CD203c upregulation induced by F(ab′)$_2$ RAHE in cells from the same donor, primed with hIL-3 or without. (**C**) Human PBMCs were primed by an overnight incubation with hIL-3 or without, and stimulated in duplicate with indicated concentrations of human IgG donkey anti-human IgG F(ab′)$_2$ complexes. Graphs show CD203c upregulation as measured in (A). *Insets* show CD203c upregulation induced by F(ab′)$_2$ RAHE in cells from the same donor. Results are representative of PBMC from three independent normal donors.

FcεRI-bound IgE via their Fab portions, but also to FcγR via their Fc portion. F(ab′)$_2$ could therefore aggregate FcεRI, whereas IgG could coaggregate FcεRI with FcγR on basophils.

In human basophils, significantly lower activation was induced by IgG RAHE than by equimolar concentrations of F(ab′)$_2$ RAHE (Fig. 5A). A fraction of basophil FcεRI only is occupied by hIgE and, as expected (40), rIgE dose dependently bound to basophils upon incubation with PBMC (Supplemental Fig. 2C). Human basophils sensitized with rIgE were dose dependently activated not only by F(ab′)$_2$ RAHE (data not shown), but also by F(ab′)$_2$ fragments of MAR (Supplemental Fig. 2D). As with RAHE, a significantly lower activation of basophils sensitized with rIgE was induced by IgG MAR than by F(ab′)$_2$ MAR (Fig. 5B).

We next investigated whether FcγRIIB could account for the differential ability of IgG Abs and of F(ab′)$_2$ fragments to activate human basophils. IgG MAR-induced, but not MAR F(ab′)$_2$-induced CD203c upregulation was indeed enhanced when rIgE-sensitized human basophils were incubated with the anti-hFcγRIIB 2B6N297Q, before stimulation (Fig. 5C). This mAb bears a N297Q mutation that removes a glycosylation site that abrogates the ability of its Fc portion to bind to FcγR (33). IgG MAR-induced CD203c upregulation was enhanced neither by incubating cells with anti-FcγRIIA F(ab′)$_2$ fragments (Fig. 5C) nor by incubating cells with anti-FcγRIII F(ab′)$_2$ fragments (Supplemental Fig. 3C). FcγRIIB, but not FcγRIIA or FcγRIIIB, therefore accounts for FcεRI-dependent human basophil activation when IgE and IgG receptors are coengaged on human basophils by IgG MAR.

The aggregation of FcεRI by F(ab′)$_2$ anti-IgE induced not only a CD203c upregulation, but also the release of histamine (Supplemental Fig. 2E, 2F) by human basophils and, if cells were primed with IL-3, the secretion of IL-4 (Supplemental Fig. 2G). IgG anti-IgE, whether RAHE or MAR, induced significantly lower histamine release (Fig. 5D) or IL-4 secretion (Fig. 5E) than equimolar concentrations of F(ab′)$_2$ fragments of the same specificity.

To mimic more closely physiological conditions, we set up another experimental system based on the use of a soluble Ag and specific IgE and IgG Abs directed against distinct moieties of this Ag. When passively sensitized with mIgE anti-DNP, human basophils were dose dependently activated by a trinitrophenylated protein (TNP-gpi), as assessed by CD203c upregulation. Indeed, hFcεRI can bind mIgE as well as rIgE (40), and anti-DNP Abs (41, 42), including mAb 2682-I (43), are well known to cross-react with TNP. Ag-induced IgE-dependent basophil activation was decreased when TNP-gpi was in complex with polyclonal mouse IgG anti-gpi. Inhibition increased with the concentration of IgG Abs used to form immune complexes (Fig. 5F).

Similar results were obtained with BM cells from mice injected with rIgE i.v. 24 h earlier and challenged with F(ab′)$_2$ MAR or IgG MAR. F(ab′)$_2$ MAR induced CD200R1 upregulation of a comparable magnitude in wt mBMB, whether cells were preincubated or not with F(ab′)$_2$ fragments of the anti-mFcγRIIB mAb K9.361 in FcγRIIB$^{-/-}$ mBMB, and in FcγRIIIA$^{-/-}$ mBMB. IgG MAR induced CD200R1 upregulation in anti-mFcγRIIB F(ab′)$_2$ fragment-treated wt and FcγRIIB$^{-/-}$ basophils, but not in untreated wt or FcγRIIIA$^{-/-}$ basophils (Fig. 6A).

Likewise, both mBM cells sensitized in vivo with rIgE (Fig. 6B) and mBMCB sensitized in vitro with rIgE (Fig. 6C) dose dependently secreted IL-4 upon challenge with F(ab′)$_2$ MAR, whether wt, FcγRIIB$^{-/-}$, or FcγRIIIA$^{-/-}$. IL-4 originated from basophils both in mBM as shown by basophil depletion (Supplemental Fig. 1D) and in mBMCB as shown by intracellular staining (Supplemental Fig. 1B). IgG MAR induced IL-4 secretion by FcγRIIB$^{-/-}$ cells, but not, or very little, by wt or FcγRIIIA$^{-/-}$ cells. IgG MAR, however, induced IL-4 secretion by wt mBM and, to a lower extent, by mBMCB if cells were preincubated with anti-mFcγRIIB F(ab′)$_2$ fragments. FcγRIIB-dependent inhibition is therefore dominant over

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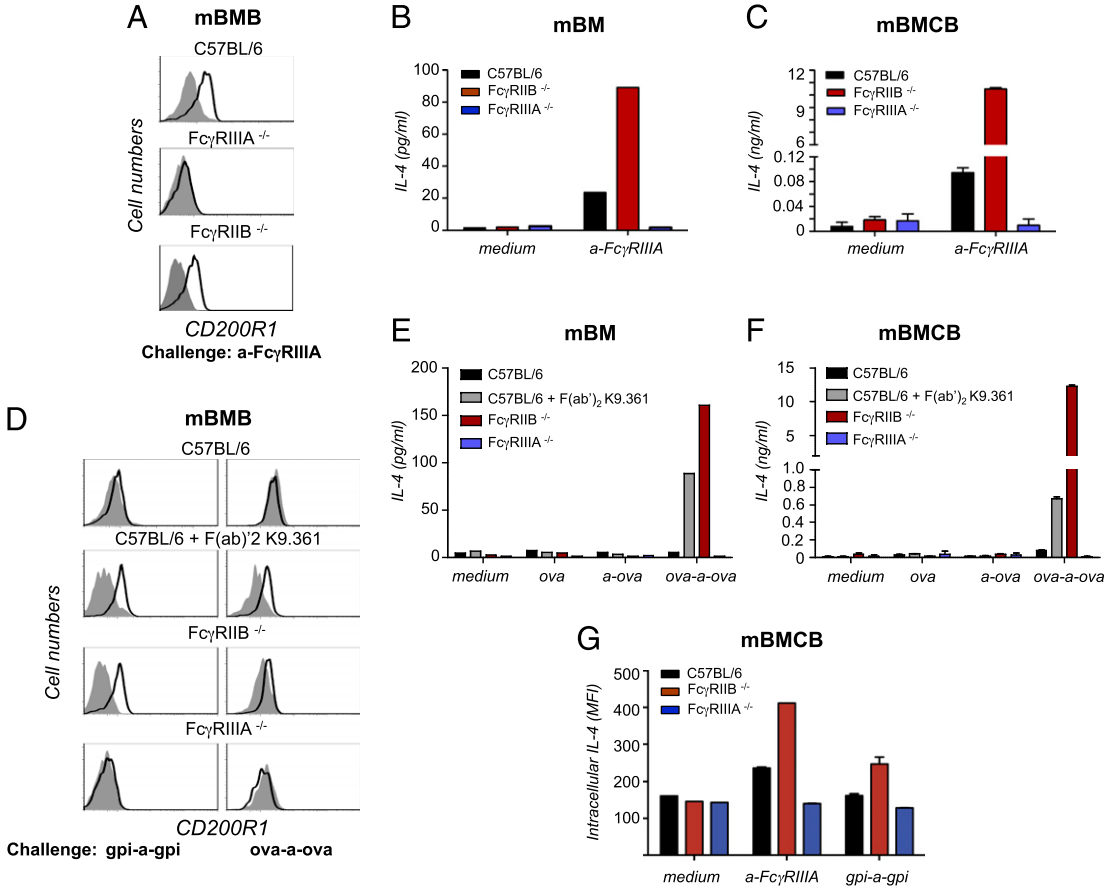

FIGURE 4. Mouse basophils can be activated by anti-FcγRIIIA mAb, but not by IgG immune complexes. (**A**–**C**) mBM cells or mBMCB from wt, FcγRIIIA$^{-/-}$, and FcγRIIB$^{-/-}$ mice were incubated with 10 μg/ml anti-FcγRIIIA (275003). Histograms show CD200R1 staining on gated BM basophils (CD49^{+}/IgE^{+}) after a 1-h incubation with (solid lines) or without (shaded histograms) anti-FcγRIIIA (A). IL-4 concentration in the supernatants of mBM (B) and mBMCB (C) was measured by ELISA after a 24-h incubation with anti-FcγRIIIA or without. (**D**–**F**) Wt mBM and mBMCB, preincubated with anti-mFcγRIIB K9.361 F(ab′)$_2$ or without, FcγRIIIA$^{-/-}$ mBM and mBMCB, or FcγRIIB$^{-/-}$ mBM and mBMCB were incubated with immune complexes formed with monoclonal IgG1 anti-OVA + OVA or polyclonal anti-gpi Abs + gpi. Histograms show CD200R1 staining on gated BM basophils (CD49^{+}/IgE^{+}) after a 1-h incubation with indicated immune complexes (solid lines) or without (shaded histograms). IL-4 concentration was measured in the supernatants of mBM (E) and mBMCB (F) by ELISA after a 24-h incubation with medium, Ag, Abs, or immune complexes. (**G**) wt, FcγRIIIA$^{-/-}$, or FcγRIIB$^{-/-}$ mBMCB were incubated with medium, anti-FcγRIIIA, or immune complexes formed with polyclonal IgG anti-gpi + gpi. IL-4 in DX5^{+}/kit^{-} cells (basophils) was detected by intracellular FACS and represented as mean fluorescence intensity (MFI). (C), (F), and (G) are represented as mean ± SEM. (A)–(G) are representative of at least two independent experiments.

FcγRIIIA, and dampens FcεRI-dependent activation when the three receptors are coengaged on murine basophils.

Finally, like CD203c upregulation in human basophils, CD200R1 upregulation induced by TNP-gpi in mBMB from mice injected with mIgE anti-DNP 24 h earlier was decreased when TNP-gpi was in complex with polyclonal mouse IgG anti-gpi. Inhibition increased with the concentration of IgG Abs used to form immune complexes (Fig. 6D). Inhibition was not due to steric hindrance as FITC-labeled TNP-gpi bound similarly to FcεRI-bound mIgE anti-DNP, whether in complex with IgG anti-gpi or not, on basophils lacking FcγR (Supplemental Fig. 4A). IgG anti-gpi did not prevent Ag-induced FcεRI engagement either, as these receptors were similarly downregulated upon challenge with TNP-gpi, whether in complex with IgG anti-gpi Abs or not (Fig. 6E).

Altogether, these results indicate that, when basophil FcγR are coengaged with FcεRI, negative signals generated by FcγRIIB are dominant over positive signals generated by FcγRIIA in human basophils or by FcγRIIIA in mouse basophils, and dampen FcεRI-dependent cell activation. Similar effects were observed when FcγR were coengaged with FcεRI by anti-IgE Abs or by IgG immune complexes in basophils from both species.

FcγRIIB inhibit IgE-induced human basophil activation in most normal donors

To determine whether the above results are a rule or an exception in humans, FcγRIIB-dependent negative regulation was investigated in basophils from a panel of normal blood bank donors. Cells were sensitized or not with rIgE. Nonsensitized cells were challenged with either F(ab′)$_2$ RAHE or IgG RAHE, whereas sensitized cells were challenged with either F(ab′)$_2$ MAR or IgG MAR. At an equimolar concentration (1.5×10^{-7} M), intact IgG induced a lower CD203c upregulation than F(ab′)$_2$ fragments in basophils from all individual donors tested, whether induced via hIgE or rIgE. The percentage of inhibition was 43.2 ± 5.3% and 65.2 ± 4.7% with RAHE and MAR, respectively (Fig. 7A). Similar results were obtained when comparing CD203c upregulation induced by a wide range of F(ab′)$_2$ and IgG concentrations in the same donors, whether RAHE or MAR (Fig. 7B). Forty-seven donors were included in these experiments. Among the 19 donors whose basophils were challenged with both RAHE and MAR, 16 showed inhibition in both systems. All the 13 donors whose basophils were challenged only with MAR showed inhibition. Among the 15 donors whose basophils were challenged

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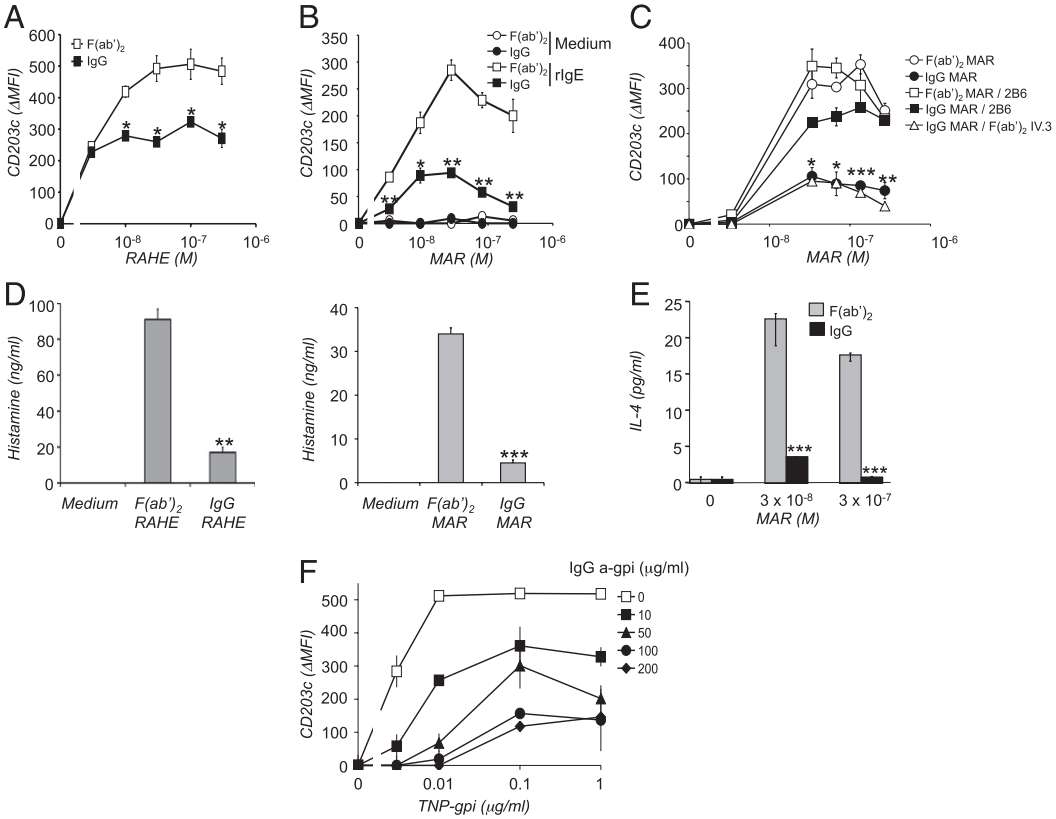

FIGURE 5. FcγRIIB negatively regulate IgE-induced human basophil activation. (**A** and **B**) Human PBMC were incubated overnight at 37°C with 3 μg/ml rIgE or without, washed, and stimulated in duplicate with equimolar concentrations of F(ab′)$_2$ RAHE or IgG RAHE (A) or of F(ab′)$_2$ MAR or IgG MAR (B). Basophil activation was measured as in Fig. 3A. (**C**) Human PBMC were incubated overnight at 37°C with 3 μg/ml rIgE, washed, and incubated with or without 10 μg/ml 2B6N297Q anti-FcγRIIB or 25 μg/ml IV.3 F(ab′)$_2$ anti-FcγRIIA for 30 min at 37°C before being stimulated in duplicate with equimolar concentrations of F(ab′)$_2$ MAR or IgG MAR. Basophil activation was measured as in Fig. 3A. Results are mean ± SD of CD203c Δ mean fluorescence intensity (MFI). (**D**) Human PBMC were incubated without (*left*) or with (*right*) 3 μg/ml rIgE overnight at 37°C, washed, and stimulated in duplicate with 3×10^{-7} M F(ab′)$_2$ or IgG RAHE (*left*) or MAR (*right*). Results are mean ± SD of histamine concentration measured in supernatants by ELISA. (**E**) Human PBMC were incubated with hIL-3 overnight at 37°C and washed, and 5×10^6 cells were incubated in duplicate with medium alone or indicated concentrations of MAR in the presence of hIL-3 for 24 h at 37°C. Results are mean ± SD of IL-4 concentration measured in supernatants by ELISA. (**F**) Human PBMC were incubated with supernatant of mIgE 2682-I overnight at 37°C, washed, and stimulated in duplicates with immune complexes formed with indicated concentrations of polyclonal anti-gpi Abs + indicated concentrations of TNP-gpi. Basophil activation was measured as in Fig. 3A. (A)–(F) are representative of at least two independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

with RAHE, 13 showed inhibition. FcγRIIB-dependent negative regulation is therefore dominant over FcγR-dependent positive regulation in human basophils from the vast majority (89.4%) of normal donors.

IL-3 modulates the expression and function of IgG receptors on human basophils

As cytokines were reported to modulate FcγRII expression on human monocytes (44), we examined FcγRII expression in human basophils exposed to cytokines overnight. Whereas IL-4, IL-13, IL-10, or IFN-γ had no detectable effect (Fig. 8A), IL-3 dose dependently upregulated FcγRIIA and FcγRIIB expression (Fig. 8A, 8B). FcγRIIB upregulation was, however, of a higher magnitude than FcγRIIA upregulation. We therefore examined the effect of IL-3 on FcγRIIB-dependent inhibition of IgE-induced basophil activation.

As expected, CD203c upregulation induced by F(ab′)$_2$ RAHE was dose dependently enhanced following an overnight incubation of basophils with increasing concentrations of IL-3 (Fig. 8C). The same effect was observed, and even more pronounced, when basophils were sensitized with rIgE and challenged with F(ab′)$_2$ MAR (Fig. 8D). In marked contrast, CD203c upregulation induced by IgG RAHE (Fig. 8C) or IgG MAR (Fig. 8D) was not enhanced by IL-3. IL-3 therefore increases not only IgE-dependent activation, but also IgG-dependent inhibition of basophil activation.

Discussion

IgG receptors were described long ago on human basophils (18) and identified as FcγRIIA, FcγRIIB (19, 20), and FcγRIIIB (22). FcγR were also observed on mouse basophils (25), but not identified. What FcγR are doing on basophils is either not or poorly known in both species. We show in this study that, besides activating high-affinity IgE receptors, human and murine basophils express both activating and inhibitory low-affinity IgG receptors that, when coengaged, inhibit both IgG- and IgE-induced activation signals.

Human and mouse basophils express activating IgG receptors. These are FcγRIIA on human basophils and FcγRIIIA on mouse basophils. Responses of human and mouse basophils, triggered by FcγRIIA and by FcγRIIIA, respectively, differed quantitatively. FcγRIIIA triggered robust secretory responses of a comparable magnitude as responses triggered by FcεRI, in mouse basophils, whereas FcγRIIA triggered weak responses, of a much lower magnitude than responses triggered by FcεRI, in human basophils, even after IL-3 priming. These functional differences may be

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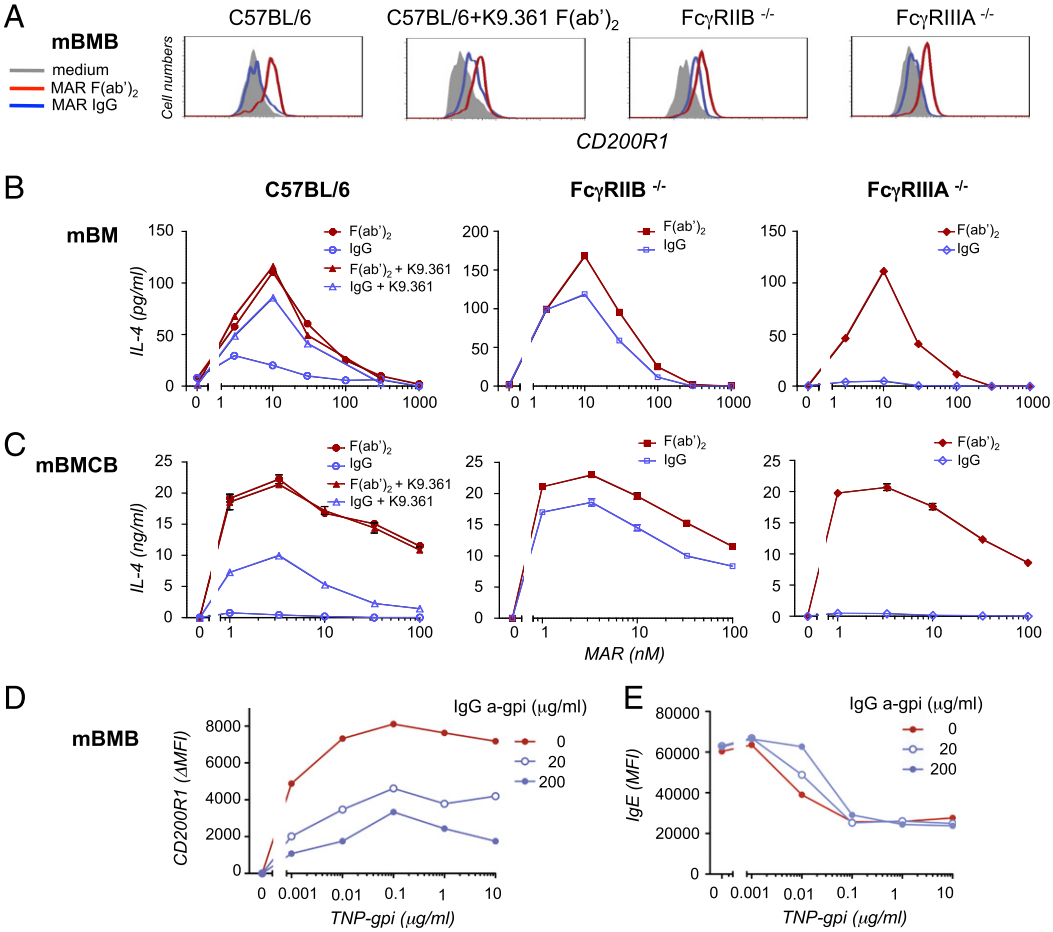

FIGURE 6. FcγRIIB negatively regulate IgE-induced mouse basophil activation. Wt mBM and mBMCB, preincubated with anti-mFcγRIIB K9.361 F(ab′)$_2$ or without, FcγRIIIA$^{-/-}$ mBM and mBMCB were sensitized with rIgE and challenged with F(ab′)$_2$ MAR or IgG MAR. (**A**) Histograms show CD200R1 staining on gated BM basophils (CD49$^+$/IgE$^+$) after a 1-h incubation with medium (shaded histogram), 100 nm F(ab′)$_2$ MAR (red line), or 100 nm IgG MAR (blue line). (**B** and **C**) IL-4 concentration in the supernatants of mBM (B) and mBMCB (C) cells from wt, FcγRIIB$^{-/-}$, and FcγRIIIA$^{-/-}$ mice was measured by ELISA after a 24-h incubation with indicated concentrations of F(ab′)$_2$ MAR (red) or IgG MAR (blue) in triplicate. (**D** and **E**) CD200R1 and IgE expression on mBMB sensitized with mIgE anti-DNP 2682-I and stimulated with TNP-gpi or TNP-gpi–anti-gpi immune complexes. Δ Mean fluorescence intensity (MFI) was calculated by subtracting the CD200R1 MFI of nonstimulated cells from that of stimulated cells. All graphs shown are representative of at least two independent experiments.

explained by differences in the expression levels of FcγR. Human basophils indeed express minute amounts of FcγRIIA, compared with other FcγRIIA-positive human blood cells, whereas mouse basophils express at least as much FcγRIIIA as other FcγRIIIA-positive murine blood cells. Functional differences might also be explained by structural differences. FcγRIIA are single-chain receptors that contain one ITAM only, whereas mFcγRIIIA associate with the FcR common subunits FcRγ and, in mast cells and basophils, with FcRβ (27) that contain two and one ITAM, respectively. FcγRIIA, however, are constitutively expressed as homodimers (45). In addition, the FcγRIIA and mFcγRIIIA ITAM are different. When expressed in murine B cells and aggregated by the same extracellular ligands, chimeric molecules containing the intracytoplasmic domains of FcγRIIA or of FcRγ, respectively, did not trigger identical responses (46). As previously observed (22), we failed to activate human basophils by engaging FcγRIIIB.

Human and mouse basophils express inhibitory IgG receptors; these are FcγRIIB in both species. hFcγRIIB and mFcγRIIB are very similar. They are single-chain low-affinity receptors that contain the same ITIM, which endows them with the same inhibitory properties (26). These depend on the recruitment of the same phosphatase (47) that accounts for inhibition. Human and mouse FcγRIIB, however, differ in their relative expression on hematopoietic cells. Human basophils express much more FcγRIIB than other blood leukocytes, including B cells. This finding was surprising as B lymphocytes are usually considered as the prototype of FcγRIIB-expressing cells in humans. Murine basophils express higher or similar amounts of FcγRIIB as monocytes, but higher amounts than neutrophils, eosinophils, and B cells. In both humans and mice, basophils are therefore among the blood cells that express the highest amounts of FcγRIIB. Such a high FcγRIIB expression on human basophils is in sharp contrast with the undetected FcγRIIB expression on human skin mast cells (17). As a consequence, FcγRIIB/FcγRIIA ratio is markedly different on these two classical effector cells of allergy in humans. One can therefore expect allergic reactions to be differentially controlled by IgG immune complexes, depending on the relative contributions of mast cells and basophils to these reactions.

Although both express activating receptors for IgG, neither human nor mouse basophils could be detectably activated by IgG immune complexes. The nonresponse of human basophils to IgG in vitro has been reported in several works (18, 48). Unlike basophils, human monocytes were readily activated by IgG aggregates (49). Nonresponse to IgG is therefore not a general phenotype of human cells that coexpress FcγRIIB and FcγRIIA.

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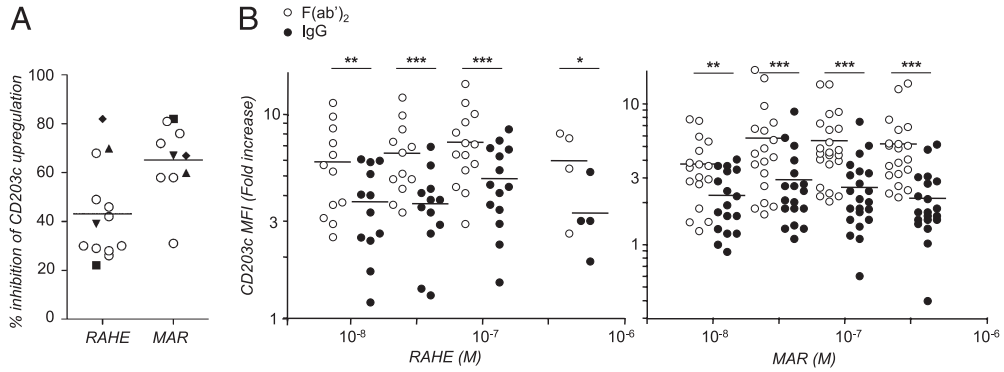

FIGURE 7. FcγRIIB-dependent negative regulation is dominant over FcγRIIA-dependent human basophil activation in most normal donors. (**A**) Human PBMC were incubated overnight at 37°C with 3 μg/ml rIgE or without, washed, and stimulated with 1.5×10^{-7} M F(ab′)$_2$ RAHE or IgG RAHE ($n = 13$ donors) or 1.5×10^{-7} M F(ab′)$_2$ MAR or IgG MAR ($n = 9$ donors). Basophil activation was measured as in Fig. 3A. Results are the percentage of inhibition of CD203c upregulation calculated as follows: (1 − [(CD203c mean fluorescence intensity of cells with IgG RAHE − CD203c MFI of unstimulated cells)/(CD203c mean fluorescence intensity of cells with F(ab′)$_2$ RAHE − CD203c mean fluorescence intensity of unstimulated cells)]) × 100. Closed symbols represent cells from individual donors that were tested with the two systems within the same experiment. (**B**) Human PBMC were incubated overnight at 37°C with 3 μg/ml rIgE or without, washed, and stimulated with increasing concentrations of F(ab′)$_2$ RAHE or IgG RAHE ($n = 20$ donors) or F(ab′)$_2$ MAR or IgG MAR ($n = 29$ donors). Basophil activation was measured as in Fig. 3A. Results are the fold increase of CD203c MFI calculated by dividing CD203c MFI of cells challenged with F(ab′)$_2$ or IgG by CD203c MFI of cells challenged with medium alone. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Whether the nonresponse of human basophils to IgG is due to FcγRIIB-dependent inhibition is uncertain. That the affinity of FcγRIIA is 50-fold higher than that of hFcγRIIB for human IgG1 (D. Mancardi, F. Jönsson, P. Bruhns, and M. Daëron, unpublished data) (50) does not support this possibility. Perhaps more importantly, we failed to observe a detectable response of human basophils treated with a blocking mAb anti-hFcγRIIB in response to IgG (data not shown). The magnitude of positive signals generated by FcγRIIA may therefore be insufficient not only to trigger basophil activation, but also to launch inhibition by FcγRIIB. Inhibition indeed requires that the FcγRIIB ITIM is phosphorylated by a kinase activated by ITAM-containing receptors (51) when the magnitude of activation signals is high enough.

The nonresponse of mouse basophils to IgG immune complexes was not due to a lack of activation by FcγRIIIA, but to an inhibition by FcγRIIB. Basophils from FcγRIIB-deficient mice and basophils from wt mice treated with anti-mFcγRIIB mAb were indeed activated by IgG immune complexes. The potent inhibitory effect of FcγRIIB is exemplified by the 100-fold higher responses to anti-FcγRIIIA mAb of FcγRIIB$^{-/-}$ basophils, compared with wt

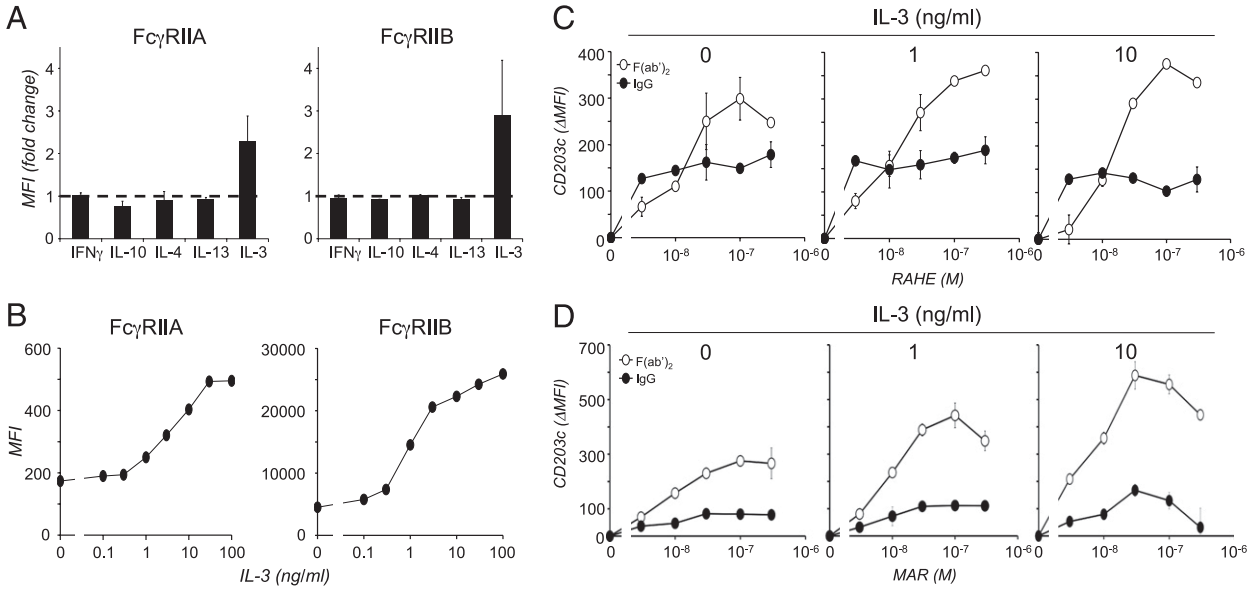

FIGURE 8. IL-3 increases FcγRII expression and IgG-induced inhibition of IgE-dependent basophil activation. (**A**) Human PBMC were incubated overnight at 37°C with 100 ng/ml IFN-γ, IL-4, or IL-13 or 10 ng/ml IL-10 or IL-3 or without, washed, and incubated with anti-CD203c PE and anti-FcγRIIA (FITC-IV.3) or anti-FcγRIIB (Alexa488-2B6N297Q). Graphs show the fold change of FcγRII mean fluorescence intensity (MFI) induced by cytokines calculated by dividing FcγRII MFI of cells incubated with cytokine by FcγRII MFI of cells incubated with medium alone. Results are mean ± SD of MFI fold change from three donors. (**B**) Human PBMC were incubated overnight at 37°C with the indicated concentrations of IL-3 or without, washed, and stained for FcγRIIA or FcγRIIB. Graphs show FcγRIIA and FcγRIIB MFI on CD203c$^+$ gated cells. (**C** and **D**) Human PBMC were incubated overnight at 37°C with the indicated concentrations of hIL-3 and with 3 μg/ml rIgE (D) or without (C), washed, and stimulated in duplicate with increased concentrations of F(ab′)$_2$ RAHE or IgG RAHE (C) or of F(ab′)$_2$ MAR or IgG MAR (D). Basophil activation was measured as in Fig. 3A. Results are mean ± SD of CD203c ΔMFI. Results are representative of PBMC from three independent normal donors.

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basophils. These IgG Abs could indeed engage not only FcγRIIIA by their Fab portions, but also FcγRIIB by their Fc portion. Possibly explaining such a strong, dominant-negative effect, the affinity of mFcγRIIB is 10-fold higher than that of mFcγRIIIA for IgG1 (50, 52). Mouse basophils therefore resemble bone marrow-derived mast cells that are similarly nonresponsive to IgG immune complexes, although they coexpress FcγRIIB and FcγRIIIA. They, however, differ from peritoneal mast cells, which express the same IgG receptors, but respond vigorously to IgG immune complexes (53). Nonresponse to IgG is therefore not a general phenotype of murine cells that coexpress FcγRIIB and FcγRIIIA. Our results differ from those of Karasuyama and colleagues (15), who reported that IgG1 immune complexes induced $CD49^+$ spleen cells to secrete platelet-activating factor in vitro. CD49b is, however, expressed not only by basophils, but also by NK cells that express FcγRIIIA, but not FcγRIIB. Finally, mouse basophils may be a better functional model of human basophils than anticipated, as cells from both species are similarly responsive to IgE Abs and, although for different reasons, similarly nonresponsive to IgG Abs.

When coengaged with FcεRI, FcγR dampen IgE-induced basophil activation. This was suggested in mouse basophils that were activated less efficiently by intact IgG Abs anti-IgE than by $F(ab')_2$ fragments. A similar experimental system, using intact IgG or $F(ab')_2$ fragments of anti-Ig Abs, has been extensively used to study FcγRIIB-dependent negative regulation of B cell activation (54). It was demonstrated using mFcγRIIB-specific blocking mAbs on basophils from wt mice and confirmed using basophils from FcγRIIB-deficient mice. Partial effect of blocking Abs can be easily explained by competition. The remaining mild inhibition seen in FcγRIIB-deficient basophils is more difficult to explain. As expected, Akt and Erk1/2 phosphorylation were reduced in wt basophils challenged with IgG MAR, compared with basophils challenged with $F(ab')_2$ MAR, but not in FcγRIIB-deficient basophils (Supplemental Fig. 4B). Likewise, human basophils were activated less efficiently by intact IgG Abs anti-IgE than by $F(ab')_2$ fragments. Importantly, the efficacy of intact Abs to activate basophils was enhanced by a hFcγRIIB-specific mAb, the Fc portion of which had been genetically engineered to prevent it from binding to FcγR. It was not enhanced by $F(ab')_2$ fragments of FcγRIIA- or FcγRIII-specific mAbs. When coengaged with FcεRI, FcγRIIA, and FcγRIIIB, FcγRIIB therefore generate potent negative signals that control human basophil activation, and FcγRIIB-dependent negative regulation induced by IgG MAR requires neither FcγRIIA nor FcγRIIIB. When assessing basophil responses with intact anti-IgE Abs, as it is commonly done in clinical practice, one therefore assesses the resultant of FcεRI-dependent activation and FcγRIIB-dependent inhibition, rather than FcεRI-dependent activation as it is usually thought. Supporting our results obtained by comparing the effects of intact IgG versus $F(ab')_2$ fragments of anti-IgE Abs, IgG anti-gpi Abs inhibited IgE anti-DNP–induced human and mouse basophil activation triggered upon challenge with TNP-gpi. Contrary to inhibition induced by IgG anti-IgE Abs, inhibition induced by IgG immune complexes might have been due to steric hindrance, as it was reported in experimental anaphylaxis (55). Steric hindrance could be excluded, however, as complexation with IgG Abs prevented Ag neither from binding to FcεRI-bound IgE nor from engaging and downregulating FcεRI.

That FcγRIIB can negatively regulate human basophil activation was previously suggested. Rabbit IgG anti-pollen (Lol p1) was shown to inhibit Lol p1-induced histamine release by basophils without having the same epitopic specificity as IgE Abs (56). We reported that the coaggregation of receptor-bound mouse anti-FcγRII mAb and FcεRI-bound mIgE by anti-mouse $F(ab')_2$ fragments could inhibit human basophil activation (26). We later described an anti-FcγRII/anti-IgE bispecific molecule that could inhibit histamine release by human basophils passively sensitized with IgE and challenged with specific Ag (29). IgG Abs are thought to contribute to specific immunotherapy, during which high titers of IgG anti-allergen Abs, especially IgG4 (57), are induced. IgG Abs may inhibit basophil activation not only by competing with IgE for allergen, but also by engaging FcγRIIB when under the form of immune complexes. Immune complexes made of allergen and autologous Abs improved allergy to grass pollen (58). Supporting this in vivo observation, IgG anti-allergen Abs were reported to inhibit allergen-induced basophil activation (20, 59). With this system, Cady et al. (20) found that FcγRIIA was required for inhibition. We could not confirm a contribution of FcγRIIA in our experimental setting. Also, bispecific fusion proteins capable of binding both to FcγRIIB and to FcεRI could inhibit allergen-induced histamine release by basophils from normal (60) and allergic donors (30, 61). Whether inhibition observed in these studies was due to FcγRIIB engagement is, however, unclear.

Using the $F(ab')_2$ versus IgG assay described in this study, we found that, altogether, FcγRIIA and FcγRIIB could dampen IgE-induced basophil activation in 89.4% normal donors (42 of 47). Whether the five donors whose basophil activation was not lower in response to IgG Abs than to $F(ab')_2$ fragments of the same specificity were healthy donors as assumed is not known. One expects a significant percentage of individuals to be allergic among blood donors. Our results may explain in part why healthy donors, who are in contact with allergens and whose serum contains anti-allergen IgG Abs (28, 62), are not allergic. Noticeably, IL-3 enhanced FcγRIIB, and, to a lower extent, FcγRIIA expression. Although it enhanced IgE-induced responses, IL-3 further enhanced IgG-induced inhibition. FcγRIIB-dependent inhibition of basophil activation may therefore remain dominant in the context of an allergen-driven Th2 response in normal individuals. Whether the same applies to basophils from allergic donors is not known.

Finally, the expression of activating IgG receptors that cannot activate human basophils is puzzling. One can notice the contrast between the modest expression of FcγRIIA that poorly activates human basophils and the high expression of FcγRIIB that potently inhibits cell activation. Negative regulation seems oversized for controlling weak IgG-induced positive signals. Under these conditions, human basophils are highly unlikely to be activated by IgG immune complexes, even though (or possibly, as) they are bathed by several mg/ml IgG in blood. Noticeably, however, the expression of an activating IgG receptor that, because its signals are strongly inhibited by FcγRIIB, fails to activate mouse basophils was conserved during evolution through the substitution for another activating IgG receptor that does not activate human basophils either, because it has a low level of expression. One therefore wonders about the biological significance of FcγRIIA on human basophils. Pairs of molecules with antagonistic properties, such as ITAM-containing and ITIM-containing receptors, are often found in mammalian cells (63). The delicate balance of positive and negative signals generated by these receptors is a mean to finely tune biological responses. One can envision the FcγRIIA–FcγRIIB pair on human basophils as a regulatory module formed upon coengagement by IgG immune complexes, which generates a mixture of antagonistic signals, the proportions of which depend on the relative expression of each receptor.

In conclusion, we show in this work that, in both mice and humans, normal basophils express a pair of IgG receptors with

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antagonistic properties. As they are coengaged by IgG Abs, these receptors function in concert as a regulatory module that prevents basophils from being activated by IgG Abs and that controls IgE-induced basophil activation. Whether this regulatory system is impaired in allergic patients is an attractive possibility that needs to be investigated.

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Disclosures

The authors have no financial conflicts of interest.

References

1. Turner, H., and J. P. Kinet. 1999. Signalling through the high-affinity IgE receptor Fc epsilonRI. *Nature* 402: B24–B30.
2. Lichtenstein, L. M., and B. S. Bochner. 1991. The role of basophils in asthma. *Ann. N. Y. Acad. Sci.* 629: 48–61.
3. Atkins, P. C., L. B. Schwartz, N. F. Adkinson, C. von Allmen, M. Valenzano, and B. Zweiman. 1990. In vivo antigen-induced cutaneous mediator release: simultaneous comparisons of histamine, tryptase, and prostaglandin D2 release and the effect of oral corticosteroid administration. *J. Allergy Clin. Immunol.* 86: 360–370.
4. Obata, K., K. Mukai, Y. Tsujimura, K. Ishiwata, Y. Kawano, Y. Minegishi, N. Watanabe, and H. Karasuyama. 2007. Basophils are essential initiators of a novel type of chronic allergic inflammation. *Blood* 110: 913–920.
5. Ohnmacht, C., C. Schwartz, M. Panzer, I. Schiedewitz, R. Naumann, and D. Voehringer. 2010. Basophils orchestrate chronic allergic dermatitis and protective immunity against helminths. *Immunity* 33: 364–374.
6. Denzel, A., U. A. Maus, M. Rodriguez Gomez, C. Moll, M. Niedermeier, C. Winter, R. Maus, S. Hollingshead, D. E. Briles, L. A. Kunz-Schughart, et al. 2008. Basophils enhance immunological memory responses. *Nat. Immunol.* 9: 733–742.
7. Karasuyama, H., K. Mukai, K. Obata, Y. Tsujimura, and T. Wada. 2011. Nonredundant roles of basophils in immunity. *Annu. Rev. Immunol.*
8. Seder, R. A., W. E. Paul, A. M. Dvorak, S. J. Sharkis, A. Kagey-Sobotka, Y. Niv, F. D. Finkelman, S. A. Barbieri, S. J. Galli, and M. Plaut. 1991. Mouse splenic and bone marrow cell populations that express high-affinity Fc epsilon receptors and produce interleukin 4 are highly enriched in basophils. *Proc. Natl. Acad. Sci. USA* 88: 2835–2839.
9. Yoshimoto, T., K. Yasuda, H. Tanaka, M. Nakahira, Y. Imai, Y. Fujimori, and K. Nakanishi. 2009. Basophils contribute to T(H)2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4+ T cells. *Nat. Immunol.* 10: 706–712.
10. Jönsson, F., D. A. Mancardi, Y. Kita, H. Karasuyama, B. Iannascoli, N. Van Rooijen, T. Shimizu, M. Daëron, and P. Bruhns. 2011. Mouse and human neutrophils induce anaphylaxis. *J. Clin. Invest.* 121: 1484–1496.
11. Dombrowicz, D., V. Flamand, K. K. Brigman, B. H. Koller, and J. P. Kinet. 1993. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor alpha chain gene. *Cell* 75: 969–976.
12. Martin, T. R., S. J. Galli, I. M. Katona, and J. M. Drazen. 1989. Role of mast cells in anaphylaxis: evidence for the importance of mast cells in the cardiopulmonary alterations and death induced by anti-IgE in mice. *J. Clin. Invest.* 83: 1375–1383.
13. Hazenbos, W. L., J. E. Gessner, F. M. Hofhuis, H. Kuipers, D. Meyer, I. A. Heijnen, R. E. Schmidt, M. Sandor, P. J. Capel, M. Daëron, et al. 1996. Impaired IgG-dependent anaphylaxis and Arthus reaction in Fc gamma RIII (CD16) deficient mice. *Immunity* 5: 181–188.
14. Miyajima, I., D. Dombrowicz, T. R. Martin, J. V. Ravetch, J. P. Kinet, and S. J. Galli. 1997. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaRIII: assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J. Clin. Invest.* 99: 901–914.
15. Tsujimura, Y., K. Obata, K. Mukai, H. Shindou, M. Yoshida, H. Nishikado, Y. Kawano, Y. Minegishi, T. Shimizu, and H. Karasuyama. 2008. Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. *Immunity* 28: 581–589.
16. Benhamou, M., C. Bonnerot, W. H. Fridman, and M. Daëron. 1990. Molecular heterogeneity of murine mast cell Fc gamma receptors. *J. Immunol.* 144: 3071–3077.
17. Zhao, W., C. L. Kepley, P. A. Morel, L. M. Okumoto, Y. Fukuoka, and L. B. Schwartz. 2006. Fc gamma RIIa, not Fc gamma RIIb, is constitutively and functionally expressed on skin-derived human mast cells. *J. Immunol.* 177: 694–701.
18. Ishizaka, T., A. R. Sterk, and K. Ishizaka. 1979. Demonstration of Fcgamma receptors on human basophil granulocytes. *J. Immunol.* 123: 578–583.
19. Kepley, C. L., J. C. Cambier, P. A. Morel, D. Lujan, E. Ortega, B. S. Wilson, and J. M. Oliver. 2000. Negative regulation of FcepsilonRI signaling by FcgammaRII costimulation in human blood basophils. *J. Allergy Clin. Immunol.* 106: 337–348.
20. Cady, C. T., M. S. Powell, R. J. Harbeck, P. C. Giclas, J. R. Murphy, R. K. Katial, R. W. Weber, P. M. Hogarth, S. Johnson, E. Bonvini, et al. 2010. IgG antibodies produced during subcutaneous allergen immunotherapy mediate inhibition of basophil activation via a mechanism involving both FcgammaRIIA and FcgammaRIIB. *Immunol. Lett.* 130: 57–65.
21. Daëron, M. 1997. Fc receptor biology. *Annu. Rev. Immunol.* 15: 203–234.
22. Meknache, N., F. Jönsson, J. Laurent, M. T. Guinnepain, and M. Daëron. 2009. Human basophils express the glycosylphosphatidylinositol-anchored low-affinity IgG receptor FcgammaRIIIB (CD16B). *J. Immunol.* 182: 2542–2550.
23. Okayama, Y., A. S. Kirshenbaum, and D. D. Metcalfe. 2000. Expression of a functional high-affinity IgG receptor, Fc gamma RI, on human mast cells: up-regulation by IFN-gamma. *J. Immunol.* 164: 4332–4339.
24. Anselmino, L. M., B. Perussia, and L. L. Thomas. 1989. Human basophils selectively express the Fc gamma RII (CDw32) subtype of IgG receptor. *J. Allergy Clin. Immunol.* 84: 907–914.
25. Mack, M., M. A. Schneider, C. Moll, J. Cihak, H. Brühl, J. W. Ellwart, M. P. Hogarth, M. Stangassinger, and D. Schlöndorff. 2005. Identification of antigen-capturing cells as basophils. *J. Immunol.* 174: 735–741.
26. Daëron, M., S. Latour, O. Malbec, E. Espinosa, P. Pina, S. Pasmans, and W. H. Fridman. 1995. The same tyrosine-based inhibition motif, in the intracytoplasmic domain of Fc gamma RIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. *Immunity* 3: 635–646.
27. Kurosaki, T., I. Gander, U. Wirthmueller, and J. V. Ravetch. 1992. The beta subunit of the Fc epsilon RI is associated with the Fc gamma RIII on mast cells. *J. Exp. Med.* 175: 447–451.
28. Ciprandi, G., M. De Amici, M. Tosca, and G. Marseglia. 2010. Allergen-specific Ig classes in non-allergic individuals. *J. Biol. Regul. Homeost. Agents* 24: 335–340.
29. Tam, S. W., S. Demissie, D. Thomas, and M. Daëron. 2004. A bispecific antibody against human IgE and human FcgammaRII that inhibits antigen-induced histamine release by human mast cells and basophils. *Allergy* 59: 772–780.
30. Zhu, D., C. L. Kepley, K. Zhang, T. Terada, T. Yamada, and A. Saxon. 2005. A chimeric human-cat fusion protein blocks cat-induced allergy. *Nat. Med.* 11: 446–449.
31. Ji, H., K. Ohmura, U. Mahmood, D. M. Lee, F. M. Hofhuis, S. A. Boackle, K. Takahashi, V. M. Holers, M. Walport, C. Gerard, et al. 2002. Arthritis critically dependent on innate immune system players. *Immunity* 16: 157–168.
32. Liu, F. T., J. W. Bohn, E. L. Ferry, H. Yamamoto, C. A. Molinaro, L. A. Sherman, N. R. Klinman, and D. H. Katz. 1980. Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation, and characterization. *J. Immunol.* 124: 2728–2737.
33. Veri, M. C., S. Gorlatov, H. Li, S. Burke, S. Johnson, J. Stavenhagen, K. E. Stein, E. Bonvini, and S. Koenig. 2007. Monoclonal antibodies capable of discriminating the human inhibitory Fcgamma-receptor IIB (CD32B) from the activating Fcgamma-receptor IIA (CD32A): biochemical, biological and functional characterization. *Immunology* 121: 392–404.
34. Monach, P. A., D. Mathis, and C. Benoist. 2008. The K/B×N arthritis model. *Curr. Protoc. Immunol.* Chapter 15: Unit 15.22.
35. Holmes, K. L., R. G. Palfree, U. Hammerling, and H. C. Morse, III. 1985. Alleles of the Ly-17 alloantigen define polymorphisms of the murine IgG Fc receptor. *Proc. Natl. Acad. Sci. USA* 82: 7706–7710.
36. Looney, R. J., G. N. Abraham, and C. L. Anderson. 1986. Human monocytes and U937 cells bear two distinct Fc receptors for IgG. *J. Immunol.* 136: 1641–1647.
37. Bühring, H. J., P. J. Simmons, M. Pudney, R. Müller, D. Jarrossay, A. van Agthoven, M. Willheim, W. Brugger, P. Valent, and L. Kanz. 1999. The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multipotent and unipotent progenitors. *Blood* 94: 2343–2356.
38. Bühring, H. J., A. Streble, and P. Valent. 2004. The basophil-specific ectoenzyme E-NPP3 (CD203c) as a marker for cell activation and allergy diagnosis. *Int. Arch. Allergy Immunol.* 133: 317–329.
39. Torrero, M. N., D. Larson, M. P. Hübner, and E. Mitre. 2009. CD200R surface expression as a marker of murine basophil activation. *Clin. Exp. Allergy* 39: 361–369.
40. Conrad, D. H., J. R. Wingard, and T. Ishizaka. 1983. The interaction of human and rodent IgE with the human basophil IgE receptor. *J. Immunol.* 130: 327–333.
41. Little, J. R., W. Border, and R. Freidin. 1969. The binding reactions of antibodies specific for the 2,6-dinitrophenyl group. *J. Immunol.* 103: 809–817.
42. Little, J. R., and H. N. Eisen. 1969. Specificity of the immune response to the 2,4-dinitrophenyl and 2,4,6-trinitrophenyl groups: ligand binding and fluorescence properties of cross-reacting antibodies. *J. Exp. Med.* 129: 247–265.
43. Zhang, L., and M. A. McCloskey. 1995. Immunoglobulin E receptor-activated calcium conductance in rat mast cells. *J. Physiol.* 483: 59–66.
44. Pricop, L., P. Redecha, J. L. Teillaud, J. Frey, W. H. Fridman, C. Sautès-Fridman, and J. E. Salmon. 2001. Differential modulation of stimulatory and inhibitory Fc gamma receptors on human monocytes by Th1 and Th2 cytokines. *J. Immunol.* 166: 531–537.
45. Powell, M. S., N. C. Barnes, T. M. Bradford, I. F. Musgrave, B. D. Wines, J. C. Cambier, and P. M. Hogarth. 2006. Alteration of the Fc gamma RIIa dimer interface affects receptor signaling but not ligand binding. *J. Immunol.* 176: 7489–7494.
46. Van den Herik-Oudijk, I. E., M. W. Ter Bekke, M. J. Tempelman, P. J. Capel, and J. G. Van de Winkel. 1995. Functional differences between two Fc receptor ITAM signaling motifs. *Blood* 86: 3302–3307.

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47. Isnardi, I., P. Bruhns, G. Bismuth, W. H. Fridman, and M. Daëron. 2006. The SH2 domain-containing inositol 5-phosphatase SHIP1 is recruited to the intracytoplasmic domain of human FcgammaRIIB and is mandatory for negative regulation of B cell activation. *Immunol. Lett.* 104: 156–165.
48. Ishizaka, K., and T. Ishizaka. 1970. Biological function of gamma E antibodies and mechanisms of reaginic hypersensitivity. *Clin. Exp. Immunol.* 6: 25–42.
49. Jönsson, F., D. A. Mancardi, W. Zhao, Y. Kita, B. Iannascoli, H. Khun, N. van Rooijen, T. Shimizu, L. B. Schwartz, M. Daëron, and P. Bruhns. 2012. Human FcγRIIA induces anaphylactic and allergic reactions. *Blood* 119: 2533–2544.
50. Bruhns, P., B. Iannascoli, P. England, D. A. Mancardi, N. Fernandez, S. Jorieux, and M. Daëron. 2009. Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. *Blood* 113: 3716–3725.
51. Malbec, O., D. C. Fong, M. Turner, V. L. Tybulewicz, J. C. Cambier, W. H. Fridman, and M. Daëron. 1998. Fc epsilon receptor I-associated lyn-dependent phosphorylation of Fc gamma receptor IIB during negative regulation of mast cell activation. *J. Immunol.* 160: 1647–1658.
52. Nimmerjahn, F., and J. V. Ravetch. 2005. Divergent immunoglobulin G subclass activity through selective Fc receptor binding. *Science* 310: 1510–1512.
53. Malbec, O., K. Roget, C. Schiffer, B. Iannascoli, A. R. Dumas, M. Arock, and M. Daëron. 2007. Peritoneal cell-derived mast cells: an in vitro model of mature serosal-type mouse mast cells. *J. Immunol.* 178: 6465–6475.
54. Phillips, N. E., and D. C. Parker. 1983. Fc-dependent inhibition of mouse B cell activation by whole anti-mu antibodies. *J. Immunol.* 130: 602–606.
55. Strait, R. T., S. C. Morris, and F. D. Finkelman. 2006. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc gamma RIIb cross-linking. *J. Clin. Invest.* 116: 833–841.
56. van Ree, R., and R. C. Aalberse. 1995. Rabbit IgG directed to a synthetic C-terminal peptide of the major grass pollen allergen Lol p I inhibits human basophil histamine release induced by natural Lol p I. *Int. Arch. Allergy Immunol.* 106: 250–257.
57. Aalberse, R. C., S. O. Stapel, J. Schuurman, and T. Rispens. 2009. Immunoglobulin G4: an odd antibody. *Clin. Exp. Allergy* 39: 469–477.
58. Machiels, J. J., M. A. Somville, M. G. Jacquemin, and J. M. Saint-Remy. 1991. Allergen-antibody complexes can efficiently prevent seasonal rhinitis and asthma in grass pollen hypersensitive patients: allergen-antibody complex immunotherapy. *Allergy* 46: 335–348.
59. Uermösi, C., R. R. Beerli, M. Bauer, V. Manolova, K. Dietmeier, R. B. Buser, T. M. Kündig, P. Saudan, and M. F. Bachmann. 2010. Mechanisms of allergen-specific desensitization. *J. Allergy Clin. Immunol.* 126: 375–383.
60. Zhu, D., C. L. Kepley, M. Zhang, K. Zhang, and A. Saxon. 2002. A novel human immunoglobulin Fc gamma Fc epsilon bifunctional fusion protein inhibits Fc epsilon RI-mediated degranulation. *Nat. Med.* 8: 518–521.
61. Zhang, K., C. L. Kepley, T. Terada, D. Zhu, H. Perez, and A. Saxon. 2004. Inhibition of allergen-specific IgE reactivity by a human Ig Fcgamma-Fcepsilon bifunctional fusion protein. *J. Allergy Clin. Immunol.* 114: 321–327.
62. Saint-Remy, J. M., P. M. Lebrun, S. J. Lebecque, and P. L. Masson. 1988. Human immune response to allergens of house dust mite, *Dermatophagoides pteronyssinus*: isotypic analysis of antibodies in atopic and non-atopic subjects. *Allergy* 43: 338–347.
63. Daëron, M., S. Jaeger, L. Du Pasquier, and E. Vivier. 2008. Immunoreceptor tyrosine-based inhibition motifs: a quest in the past and future. *Immunol. Rev.* 224: 11–43.

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