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Fcγ Receptors Inhibit Mouse and Human Basophil Activation

Lydie Cassard,††1 Friederike Jönsson,*††1 Ségolène Arnaud,*†† and Marc Daéron*††

Besides high-affinity IgE receptors (FceRI), 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 (FcγRIIA) 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 FceRI 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 (FceRI) that are associated with the two ITAM-containing subunits, FcRγ and FcRβ. They are activated upon FceRI 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 FcγRI 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 FceRI, and on mast cells (10), as IgE-induced passive systemic anaphylaxis does (11, 12). Also, IgG1-induced passive systemic anaphylaxis, which depends on FcγRIIA (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 ITAM-containing inhibitory receptors (21). Human basophils also express minute amounts of the GPI-anchored low-affinity IgG receptor FcγRIIB (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 FcγRI (27). Whether mouse basophils express one, the other, or both FcγRI 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 FceRI 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.
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 FceRI-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 FceRI by immune complexes in basophils, as follows: 1) negative signals may be dominantly generated by FcγR and antagonize positive signals generated by FceRI; 2) positive signals may be dominantly generated by FcγR and synergize with positive signals generated by FceRI; and 3) positive and negative signals generated by FcγR may neutralize each other and have no effect on FceRI 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

KRN9° 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 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 × 10^6 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 its isotype controls from Miltenyi Biotec; Alexa647-conjugated anti-human IgE, human IgG, ε PE-conjugated anti-CD203c was from Beckman Coulter; allophycocyanin-labeled Abs and reagents were purchased from Charles River, and FcγRIIIA (anti-mFcγRIIIA IV.3) from BioLegend; and human recombinant cytokines from ImmunoTools. Anti-FcyRI mAb (9E9) was provided by J. Ravetch (Rockefeller University, New York, NY). Anti-fcγRIIIB (2B6N297Q) (33), Alexa488-2B6N297Q, and isocontrol type (Alexa488-CH4420) were provided by R. Hayman.

Abs and reagents

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-FcγRIIIB 2B6N297Q or 25 μg/ml IV.3 F(ab')2 anti-FcγRIIIB was added 30 min before stimulation with MAR.

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-FcγRIIIB 2B6N297Q or 25 μg/ml IV.3 F(ab')2 anti-FcγRIIIB was added 30 min before stimulation with MAR.

Activation via IgG receptors. PBMC were incubated with 5 ng/ml hIgE or without, and 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 hIgE 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 (Fab')2, 5 ng/ml hIgE or without.

Activation with specific Ag. PBMC were incubated with supernatant of mAb 2682-1 (200 ng/ml mIgE) overnight, washed, and incubated for 30 min at 37°C with medium, TNF-α, alone, or preformed immune complexes made of mouse polyclonal IgG anti-gpi and TNF-α. To assess human basophil activation by flow cytometry, cells were stained with PE-conjugated anti-CD203c and allophycocyanin-conjugated anti-FcγRIIa.

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 × 10^6 cells/ml with 1 ng/ml IL-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 mFcyRIIB-specific K9.361/Ly17.3 (35) F(ab')2 were added 10 min before stimulation.

Activation via IgG receptors. Mice were injected i.v. with 50 μg rIgE IR162 or mIgE 2682-1 24 h before BM was harvested. BM-derived basophils were also activated 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, TNF-gpI and TNP-gpI anti-IgG immune complexes.

Immunofluorescence

Extracellular. Human or mouse cells were washed in cold PBS containing 0.5% BSA and 0.09% NaN3 (PBS-BSA), incubated with labeled Ab 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 PermFix and PermWash (BD Biosciences). Cells were then labeled with anti-IL-4 PE, washed, and analyzed on a MACSQuant (Miltenyi Biotec).
Activation via IgG receptors. BM-derived basophils or BM cells were incubated with 10 μg/ml mAb anti-FcγRIIA 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+/FcεRI+ 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+IgE+ 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 FcγRI with comparable expression levels (Fig. 1G). They were stained by the FcγRIIB-specific mAb Ly17.1/2 (35) and by the mFcγRIIA-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 FcγRIIA-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 FcγR-associated FcγRIIA in mice, but the single-chain FcγRIIA in humans. Inhibitory receptors are FcγRIIB in humans and mice. Whether FcγRIIB 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 FcγRIIA and FcγRIIB. As expected, NK cells expressed FcγRIIB but not FcγRIIA, whereas B cells expressed FcγRIIB but not FcγRIIA. T cells expressed neither FcγRIIA nor FcγRIIB (Fig. 2C). Murine basophils expressed at least as much FcγRIIA 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.
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 FcγRIIB with F(ab')2 fragments of FcγRII-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 sample preparations (Supplemental Fig. 3B). Human basophils were therefore activated upon FcγRIIA aggregation, but not upon FcγRIIB 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 FcγRIIA−/− mice, with anti-FcγRIIA 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. 4D) 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 mBM, unless cells were preincubated with anti-mFcγRIIB F(ab')2 fragments. Immune complexes also induced CD200R1 upregulation in mBMC from FcγRIIB−/−, but not from FcγRIIIA−/− mice (Fig. 4C). Similar results were observed with polyclonal (gpi–anti-gpi) and with monoclonal (ova–anti-ova) complexes. Likewise, no IL-4 secretion was induced by immune complexes in wt BM (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), but not 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...
FcgRI-bound IgE via their Fab portions, but also to FcyR via their Fc portion. F(ab\')2 could therefore aggregate FcRI, whereas IgG could coaggregate FcRI with FcyR 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 FcRI only is occupied by hlgE and, as expected (40), rlgE dose dependently bound to basophils upon incubation with PBMC (Supplemental Fig. 2C). Human basophils sensitized with rlgE 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 rlgE was induced by IgG MAR than by F(ab\')2 MAR (Fig. 5B).

We next investigated whether FcyRIIB 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 rlgE sensitized human basophils were incubated with the anti-hFcRIIB mAb K9.361 before stimulation (Fig. 5C). This mAb bears a N297Q mutation that removes a glycosylation site that abrogates FcRII-dependent inhibition is therefore dominant over 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 mlgE anti-DNP, human basophils were dose dependently activated by a trinitrophenylated protein (TNP-gpi), as assessed by CD203c upregulation. Indeed, hFcRI can bind mlgE as well as rlgE (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 rlgE 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 mBM, whereas cells were preincubated with the anti-mFcyRIIB 2B6N297Q, before stimulation (Fig. 5G). This mAb bears a N297Q mutation that removes a glycosylation site that abrogates the ability of its Fc portion to bind to FcyR (33). IgG MAR-induced CD200R1 upregulation was enhanced neither when rlgE sensitized human basophils were incubated with the anti-hFcyRIIB 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 FcyR (33). IgG MAR-induced CD200R1 upregulation was enhanced neither when rlgE sensitized human basophils were incubated with the anti-hFcyRIIB 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 FcyR (33). IgG MAR-induced CD200R1 upregulation was enhanced neither when rlgE sensitized human basophils were incubated with the anti-hFcyRIIB 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 FcyR (33). IgG MAR-induced CD200R1 upregulation was enhanced neither when rlgE sensitized human basophils were incubated with the anti-hFcyRIIB 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 FcyR (33). IgG MAR-induced CD200R1 upregulation was enhanced neither when rlgE sensitized human basophils were incubated with the anti-hFcyRIIB 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 FcyR (33). IgG MAR-induced CD200R1 upregulation was enhanced neither when rlgE sensitized human basophils were incubated with the anti-hFcyRIIB 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 FcyR (33). IgG MAR-induced CD200R1 upregulation was enhanced neither when rlgE sensitized human basophils were incubated with the anti-hFcyRIIB 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 FcyR (33). IgG MAR-induced CD200R1 upregulation was enhanced neither when rlgE sensitized human basophils were incubated with the anti-hFcyRIIB 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 FcyR (33). IgG MAR-induced CD200R1 upregulation was enhanced neither when rlgE sensitized human basophils were incubated with the anti-hFcyRIIB 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 FcyR (33).
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 monoclonal IgG anti-gpi Abs + gpi. Histograms show CD200R1 staining on gated BM basophils (CD49+/IgE+) after a 1-h incubation with anti-FcγRIIIA (A). IL-4 concentration in the supernatants of mBM (B) and mMBMC (C) was measured by ELISA after a 24-h incubation with anti-FcγRIIIA or without. (D–F) Wt mBM and mMBMC, preincubated with anti-mFcγRIIB K9.361 F(ab′)2, or without, FcγRIIIA−/− mBM and mMBMC, or FcγRIIB−/− mBM and mMBMC 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 mMBMC (F) by ELISA after a 24-h incubation with medium, Ag, Abs, or immune complexes. (G) wt, FcγRIIIA−/−, or FcγRIIB−/− mMBMC were incubated with medium, anti-FcγRIIa, 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.

**FIGURE 4.** Mouse basophils can be activated by anti-FcγRIIIA mAb, but not by IgG immune complexes. (A–C) mBM cells or mMBMC from wt, FcγRIIIA−/−, and FcγRIIB−/− mice were incubated with 10 μg/ml anti-FcγRIIIA (275003) 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 mMBMC (C) was measured by ELISA after a 24-h incubation with anti-FcγRIIIA or without. (D–F) Wt mBM and mMBMC, preincubated with anti-mFcγRIIB K9.361 F(ab′)2, or without, FcγRIIIA−/− mBM and mMBMC, or FcγRIIB−/− mBM and mMBMC 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 mMBMC (F) by ELISA after a 24-h incubation with medium, Ag, Abs, or immune complexes. (G) wt, FcγRIIIA−/−, or FcγRIIB−/− mMBMC were incubated with medium, anti-FcγRIIa, 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.

**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 63.2 ± 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
with RAHE, 13 showed inhibition. FcyRIIB-dependent negative regulation is therefore dominant over FcyR-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 FcyRII expression on human monocytes (44), we examined FcyRII 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 FcyRIIB and FcyRIIB expression (Fig. 8A, 8B). FcyRIIB upregulation was, however, of a higher magnitude than FcyRIIA upregulation. We therefore examined the effect of IL-3 on FcyRIIB-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 FcyRIIA, FcyRIIB (19, 20), and FcyRIIB (22). FcyR were also observed on mouse basophils (25), but not identified. What FcyR 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 FcyRIIA on human basophils and FcyRIIB on mouse basophils. Responses of human and mouse basophils, triggered by FcyRIIA and by FcyRIIB, respectively, differed quantitatively, FcyRIIA triggered robust secretory responses of a comparable magnitude as responses triggered by FceRI, in mouse basophils, whereas FcyRIIA triggered weak responses, of a much lower magnitude than responses triggered by FceRI, in human basophils, even after IL-3 priming. These functional differences may be
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 FcγRIIA as other FcγRIIA-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 asso-
ciate 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, respect-
ively, did not trigger identical responses (46). As previously observed (22), we failed to activate human basophils by engaging FcγRIIB.

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 un-
detected 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 is 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.
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. Därön, 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 FcγRIIB2/2 basophils, compared with wt

**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.

**FIGURE 8.** IL-3 increases FcyRII 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.
basophils. These IgG Abs could indeed engage not only FcεRIIA 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 mFcεRIIA 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 FcεRIIIA. 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 phenomenon of murine cells that coexpress FcγRIIB and FcεRIIIA. Our results differ from those of Karasuyama and colleagues (15), who reported that IgG1 immune complexes induced CD49b+ 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 FcγRII-specific mAbs. When coengaged with FcεRI, FcγRIIA, and FcγRIIB, FcγRIIB therefore generate potent negative signals that control human basophil activation, and FcγRIIB-dependent negative regulation induced by IgG MAR requires neither FcγRIIA nor FcγRIIB. When assessing basophil responses with intact anti-IgE Abs, as it is commonly done in clinical practice, one therefore assesses the resultant of FcεR1-dependent activation and FcγRIIB-dependent inhibition, rather than FcεR1-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 FcεRII-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 FcεRII-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 FcγRIIB in our experimental setting. Also, bispecific fusion proteins capable of binding both to FcγRIIB and to FcγRI 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
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


**Figure S1.** Expression of FcγRIIIA and FcγRIIB on murine BMCB and IL-4 production by basophils in BMCB cultures and bm. (A) BMCB from wt, FcγRIIIA−/− and FcγRIIB−/− mice were identified as CD49b+/kit- cells. Dot plots show the staining of BMCB with anti-FcγRIIB (Ly17.1 and Ly17.2) and anti-FcγRIIIA (275003). (B,C) BM cells derived in presence of IL-3 for 7 days were sensitized with rIgE (B) or not (C), washed and stimulated with medium, F(ab')2 MAR (B) or anti-FcγRIIIA (C) in presence of brefeldin A for 5 hours. Basophils (DX5+/c-kit-) but not mast cells (DX5-/c-kit+) or other bone marrow-derived cells (DX5-/c-kit-) produce IL-4 as detected by intracellular FACS. (D) Bone marrow cells from C57BL/6 mice injected with rat IgE i.v. and treated 24 hours earlier with 100 μg Ba103 mAb or not treated, were analyzed by flow cytometry with anti-DX5 and anti-IgE antibodies. Aliquots of these cells were challenged with medium, F(ab')2 MAR or an equimolar concentration of IgG MAR. IL-4 secreted in cell supernatants was measured by ELISA.
**Figure S2.** Human basophils are activated by F(ab')$_2$ anti-IgE. (A) Human PBMC were labeled with anti-CD203c-PE, anti-FcεRIα-APC and anti-hIgE-FITC. (B) Human PBMC were stimulated in duplicate with indicated concentrations of F(ab')$_2$ RAHE. Cells were labeled with anti-CD203c-PE and anti-FcεRI-APC. Graphs show basophil activation as assessed by the up-regulation of CD203c MFI on FcεRI$^+$ cells as a function of F(ab')$_2$ RAHE 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 10 normal donors. (C) Human PBMC were incubated overnight at 37°C with the indicated concentrations of rIgE, washed and labeled with anti-CD203c-PE, anti-FcεRIα-APC and F(ab')$_2$ MAR-FITC. The dot plot (left) shows the binding of 10 μg/ml rIgE on CD203c$^+$ basophils gated on FcεRIα$^+$ cells. The graph (right) shows the binding of rIgE, as determined by the Mean Fluorescence Intensity (MFI) of FITC labeling, as a function of rIgE concentrations. (D) Human PBMC were incubated overnight at 37°C with 3 μg/ml rIgE, washed and stimulated in duplicate with indicated concentrations of F(ab')$_2$ MAR. Basophil activation was measured as in Suppl. Figure 2B. (E,F) Human PBMC were incubated without (E) or with (F) 3 μg/ml rIgE overnight at 37°C, washed and stimulated in duplicate with increasing concentrations of F(ab')$_2$ RAHE or MAR. Results are mean ± SD of histamine concentration measured in supernatants by ELISA. (G) Human PBMC were incubated with (black bars) or without (white bars) hIL-3 overnight at 37°C, washed and 5 x 10$^6$ cells were incubated in duplicate with medium alone or 10$^{-7}$ M of F(ab')$_2$ RAHE with hIL-3 or without for 24 hours at 37°C. Results are mean ± SD of IL-4 concentration measured in supernatants by ELISA.
Figure S3. Human basophils are neither activated by IgG immune complexes nor by FcγRIIIB aggregation. (A) Human PBMC and purified neutrophils from the same donor were incubated in the presence of immune complexes formed with mouse polyclonal anti-gpi antibodies and gpi or with heat-aggregated human IgG. The left graph shows basophil activation as assessed by an up-regulation of CD203c on FcεRIα+ cells. ΔMFI was calculated by subtracting the CD203c MFI of unstimulated cells from the CD203c MFI of stimulated cells. The inset shows CD203c up-regulation induced by F(ab')2 RAHE in cells from the same donor. The right graph shows neutrophil activation as assessed by a down-regulation of CD62L. (B) FcγRIIIB engagement does not activate human basophils. Human PBMC were incubated with various concentrations of F(ab')2 anti-FcγRIII mAb (3G8), washed and challenged with F(ab')2 fragments of Goat anti-Mouse Ig or without. Aliquots of cells that were not incubated with 3G8 were challenged with F(ab')2 RAHE. CD203c up-regulation and CD11b up-regulation were monitored by flow cytometry on basophils and on NK cells, respectively. (C) FcγRIIIB do not contribute to negative regulation when co-engaged with FcεRI, FcγRIIA and FcγRIIB by IgG MAR. Human PBMC were incubated overnight at 37°C with 3 µg/ml rIgE, washed and incubated with medium, 2B6N297Q anti-FcγRIIB or 3G8 F(ab')2 anti-FcγRIII 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 ΔMFI.
Figure S4. (A) IgG anti-gpi antibodies do not prevent TNP-gpi from binding to basophil-bound IgE anti-DNP. mBMCB from FcγRI-/-FcγRIIB-/-FcγRIIIA-/- mice that express no IgG receptors were sensitized with mIgE anti-DNP 2682-I, washed, and incubated with immune complexes made with the indicated concentrations FITC-conjugated TNP-gpi and the indicated concentrations of IgG anti-gpi antibodies used in Fig. 6DE. Cell-bound fluorescence was monitored by flow cytometry. (B) Intracellular signaling triggered by aggregating FcγRI or by co-aggregating FcγRI with FcγR in mBMCB. Kit-depleted mBMCB from C57Bl/6 wt, FcγRIIB-/- or FcγRIIIA-/- mice were sensitized with rIgE and challenged with medium (unst.) F(ab')2 MAR of IgG MAR. Cells were lysed, electrophoresed and Western blotted with indicated antibodies.