

Expression of a Functional High-Affinity IgG Receptor, FcγRI, on Human Mast Cells: Up-Regulation by IFN-γ¹

Yoshimichi Okayama,² Arnold S. Kirshenbaum, and Dean D. Metcalfe

Biologically relevant activation of human mast cells through Fc receptors is believed to occur primarily through the high-affinity IgE receptor FcεRI. However, the demonstration in animal models that allergic reactions do not necessarily require Ag-specific IgE, nor the presence of a functional IgE receptor, and the clinical occurrence of some allergic reactions in situations where Ag-specific IgE appears to be lacking, led us to examine the hypothesis that human mast cells might express the high-affinity IgG receptor FcγRI and in turn be activated through aggregation of this receptor. We thus first determined by RT-PCR that resting human mast cells exhibit minimal message for FcγRI. We next found that IFN-γ up-regulated the expression of FcγRI. This was confirmed by flow cytometry, where FcγRI expression on human mast cells was increased from ~2 to 44% by IFN-γ exposure. FcεRI, FcγRII, and FcγRIII expression was not affected. Scatchard plots were consistent with these data where the average binding sites for monomeric IgG1 ($K_a = 4\text{--}5 \times 10^8 \text{ M}^{-1}$) increased from ~2,400 to 12,100–17,300 per cell. Aggregation of FcγRI on human mast cells, and only after IFN-γ exposure, led to significant degranulation as evidenced by histamine release ($24.5 \pm 4.4\%$); and up-regulation of mRNA expression for specific cytokines including TNF-α, GM-CSF, IL-3 and IL-13. These findings thus suggest another mechanism by which human mast cells may be recruited into the inflammatory processes associated with some immunologic and infectious diseases. *The Journal of Immunology*, 2000, 164: 4332–4339.

The high-affinity Ig receptors FcεRI and FcγRI have tissue-specific distributions related to function (1–3). FcεRI binds monomeric IgE with a K_a of 10^{10} M^{-1} , is expressed with α-, β-, and γ-chains on mast cells and basophils (1, 3, 4) and is believed in humans to essentially be responsible for allergen-dependent allergic responses; thus, the focus on the identification of Ag-specific IgE in the evaluation of the patient with an allergic reaction (4). FcγRI, which binds monomeric IgG with a K_a of $10^8\text{--}10^9 \text{ M}^{-1}$, is reported to be expressed by monocytes, macrophages, and IFN-γ-stimulated neutrophils, eosinophils, and glomerular mesangial cells (1–3, 5). This receptor is believed to mediate phagocytosis, Ab-dependent cellular cytotoxicity, superoxide production, Ag presentation, and cytokine release (1–3, 5).

Because of the increasing body of evidence in animal models that mast cells may be recruited into allergic reactions by non-IgE-dependent mechanisms (4), and that mast cells participate in host defense mechanisms against bacteria (6–9), we hypothesized that human mast cells might also express FcγRI, perhaps influenced by specific factors produced in the microenvironment. Human mast cells were thus cultured from CD34⁺ peripheral blood-derived precursors (10) in the presence or absence of various growth factors and cytokines and examined for the expression of FcγRI. As will be shown, resting human mast cells express low levels of FcγRI and this expression is significantly up-regulated by IFN-γ.

Furthermore, aggregation of FcγRI on IFN-γ-treated mast cells leads to degranulation and enhanced cytokine expression.

Materials and Methods

CD34⁺ cell culture

Human peripheral blood CD34⁺ progenitor cells were obtained and processed, following informed consent, as described elsewhere (10), and placed in serum-free media (StemPro-34 SFM; Life Technologies, Grand Island, NY) supplemented with 2 mM L-glutamine, 50 μg/ml streptomycin, 100 IU/ml penicillin, 100 ng/ml recombinant human (rh)³ stem cell factor (SCF), 100 ng/ml rhIL-6, and 30 ng/ml rhIL-3 (first week only and to expand progenitor cells; PeproTech, Rocky Hill, NJ) (10, 11). Half of the culture media was replaced every 7 days. Mast cell purities were assessed by metachromatic staining of cytopreparations with acidic toluidine blue (pH 1.0). More than 95% of the cells were identified as mast cells 8–10 wk after the initiation of the culture (10, 11). To remove contaminating monocytes/macrophages, cultured cells were incubated in a culture dish (35 × 10 mm) for 2 h and nonadherent cells were harvested. The final purity of mast cells was >99%.

Isolation of RNA and RT-PCR

Total cellular RNA was isolated from mast cells and the human monocyte-like, histiocytic lymphoma cell line U-937 (American Type Culture Collection, Manassas, VA) with RNeasy mini kits (Qiagen, Valencia, CA) according to the manufacturer's specifications. The purity of RNA was assessed on the basis of the A_{260}/A_{280} ratio, and the integrity of RNA was verified by agarose gel electrophoresis. The yield of RNA per 10^6 mast cells was 5.2 (4.0–7.5) μg (median with range, $n = 11$). An equal amount of RNA (250 ng for FcγRI experiments, 100 ng for cytokine experiments) was used for RT-PCR analysis. PCR was performed in a thermocycler as follows: 94°C, 5 min; followed by 30 or 35 amplification cycles (94°C, 1 min; 56°C for FcγRI and adenine phosphoribosyl-transferase (APRT), or the optimal annealing temperature for cytokines as described elsewhere (12, 13), 2 min; 72°C, 3 min). The sequence of the primers for FcγRI are 5' primer, 5'-GAC AGA TTT CAC TGC TCC-3' and 3' primer, 5'-CTT TAA GAG TTA CAT ACC AT-3'. The primers of APRT, IL-2, IL-3, IL-4, IL-13, TNF-α, and IFN-γ were as described previously (12, 13). Final extension was 72°C for 10 min. Equal amounts of PCR-amplified products were visualized by ethidium bromide. In addition to Fcγ receptor-specific

Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

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² Address correspondence and reprint requests to Dr. Yoshimichi Okayama, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 10, Room 11C206, 10 Center Drive MSC 1881, Bethesda, MD 20892-1881. E-mail address: yokayama@niaid.nih.gov

³ Abbreviations used in this paper: rh, recombinant human; APRT, adenine phosphoribosyl-transferase; mBMMC, mouse bone marrow culture-derived mast cell; MEFL, molecules of equivalent fluorescein; NGF, nerve growth factor; SCF, stem cell factor.

primers, mRNA for APRT was detected as a positive control. PCR without cDNA was performed to exclude contamination. Comparative densitometric analysis was performed by running all samples within the same RT-PCR and developing the samples on the same gel. Since quantification of Fc γ RI or each cytokine involved measuring the relative expression of Fc γ RI or each cytokine with respect to APRT, the RT-PCR technique was optimized for reproducibility and accuracy. Equal amounts of cDNA were amplified for APRT and Fc γ RI or each cytokine using 25, 30, 35, and 40 cycles. Measurement of the signals by densitometric analysis with the aid of GelExpert (Nucleotech, San Carlos, CA) revealed that amplification of APRT, Fc γ RI, and each cytokine was proportional to the amount of cDNA used for amplification. Furthermore, dose-dependent amplification was not observed as the number of amplifying cycles was increased beyond 30 for APRT and 35 for Fc γ RI and each cytokine. Therefore, we employed 30 amplifying cycles for APRT and Fc γ RI and 35 amplifying cycles for cytokines.

Competitive RT-PCR using DNA competitor

To further compare the relative levels of Fc γ RI mRNA expression over time during incubation of mast cells with IFN- γ , a competitor control DNA containing 5' and 3' primer sequences of Fc γ RI was used as a standard in a competitive RT-PCR. DNA competitors were constructed using the competitive DNA construction kit (RR017; Fisher Scientific, Pittsburgh, PA) according to the manufacturer's protocol. The sequences of the Fc γ RI primers which are EC3 specific are 5' primer, 5'-GCT CCA GTG CTG AAT GCA TC-3' and 3' primer, 5'-ACT CAG GGC TGC GCT TAA GG-3' (14). The sequences of primer for the DNA competitor are 5' primer, 5'-ATT TAG GTG ACA CTA TAG AAT ACG CTC CAG TGC TGA ATG CAT CGT ACG GTC ATC ATC TGA CAC-3' and 3' primer, 5'-ACT CAG GGC TGC GCT TAA GGC GCC ATC ATC CTG GGA AGA CTC C-3'. The amplified product of the competitor was distinguished from that of the target by size. For determination of Fc γ RI mRNA levels, 10^5 – 10^9 copies of the competitor were added to PCR amplification reactions containing a constant amount of the experimental cDNA sample. Competitive PCR was performed in a thermocycler as follows: 94°C, 5 min, followed by 30 amplification cycles (94°C, 30 s; 60°C 30 s; and 72°C, 30 s). PCR-amplified products were visualized on a 2% agarose gel by ethidium bromide. Measurement of the signals by densitometric analysis with the aid of GelExpert revealed that the log of the ratios of target (Fc γ RI) to competitor PCR products at all time points, when plotted against the concentration of competitor input, yielded a linear relationship (data not shown). The amounts of target RNA were calculated to equal the copy number of the competitor when the ratio of the target to competitor PCR products equaled one (15).

Abs and flow cytometric analysis

The following mAbs were purchased: mouse anti-human Fc γ RI (clone 10.1, subclass IgG1) and mouse anti-human Fc γ RIII (clone 3G8, subclass IgG1; Caltag Laboratories, Burlingame, CA); F(ab') $_2$ fragments of mouse anti-human Fc γ RI (clone 22, subclass IgG1) and F(ab') $_2$ fragments of mouse anti-human Fc γ RII (clone IV.3, subclass IgG2b; Medarex, Annandale, NJ); mouse anti-human Fc γ RI (clone 32.2, subclass IgG1; Accurate Chemical & Scientific, Westbury, NY); mouse anti-human CD117 (subclass IgG1; Coulter-Immunotech, Miami, FL); mouse anti-human tryptase (subclass IgG1; Promega, Madison, WI); and mouse anti-human chymase (subclass IgG1; Chemicon International, Temecula, CA). FACS analysis was performed as described previously (11). In some experiments, mast cells were preincubated with 1 μ g/ml of human myeloma IgE (Calbiochem, San Diego, CA) for 12 h. Mast cells were resuspended in a mixture of PE- or PE cyanine 5-conjugated *c-kit* (CD117), biotin-conjugated goat anti-human IgE ϵ chain (BioSource International, Camarillo, CA) and FITC-conjugated mouse anti-human Fc γ receptor mAb for 30 min at 4°C. Cells were next washed and incubated with streptavidin-allophycocyanin (PharMingen, San Diego, CA) for 20 min at 4°C.

For extracellular/intracellular double staining to determine the mast cell phenotype, i.e., MC $_{TC}$ (tryptase/chymase-containing mast cells) and MC $_T$ (tryptase-containing mast cells) (16) and Fc γ RI expression on mast cell surfaces, cells were first incubated with FITC-conjugated human IgE (17) at 37°C for 1 h. Cells were then stained with PE-conjugated mouse anti-human Fc γ RI (clone 32.2) and PE cyanine 5-conjugated *c-kit* for 30 min at 4°C. After washing in PBS, cells were fixed in PBS plus 0.5% paraformaldehyde for 15 min at room temperature, followed by two washing steps in PBS. Cells were then permeabilized in 0.5% saponin for 10 min at room temperature and washed in PBS. Intracellular staining with biotinylated anti-human tryptase or anti-human chymase diluted in PBS/0.1% BSA and 1% milk plus 0.5% saponin was performed for 30 min at 4°C. Cells were then washed twice in PBS/0.1% BSA and 1% milk and developed for 20

min at 4°C by the addition of streptavidin-allophycocyanin diluted in PBS/0.1% BSA and 1% milk plus 0.5% saponin.

Cell analysis was performed using a FACScalibur (Becton Dickinson, San Jose, CA) and CellQuest software (Becton Dickinson). The median values of fluorescence intensity of mast cells were converted to the numbers of molecules of equivalent fluorescein (MEFL) using Sphero rainbow calibration particles (PharMingen) on each day an experiment was performed, as per the specifications of the manufacturer. The results were expressed as MEFL or percent positive cells. Eighty-five to 90% of cultured mast cells were routinely of the MC $_{TC}$ (17). A similar analysis was also performed on IFN- γ -treated mast cells.

Cytokine stimulation

Mast cells (10–16 wk old) were cultured in serum-free media containing rhIL-6 and rhSCF, with or without rhIL-4 (50 ng/ml), rhIL-5 (50 ng/ml), rhIL-10 (50 ng/ml), rhGM-CSF (5 ng/ml) (PeproTech), rh β -nerve growth factor (NGF) (50 ng/ml) (R&D Systems, Minneapolis, MN), or rhIFN- γ 1b (15 ng/ml) (Genentech, San Francisco, CA). After 48 h, the medium was discarded, and the mast cells were stained and analyzed by flow cytometry. The viability of mast cells in serum-free medium containing rhSCF and rhIL-6 without or with IL-4, IL-5, IL-10, GM-CSF, β -NGF, and IFN- γ for 48 h, as determined by trypan blue exclusion, was 87.2 ± 2.9 , 94.1 ± 0.9 , 96.2 ± 1.7 , 89.2 ± 3.3 , 93.4 ± 2.6 , 91.9 ± 2.9 , and $91.3 \pm 3.2\%$ (mean $\pm$ SEM, $n = 4$), respectively.

Mast cell surface iodination and immunoprecipitation

Mast cells (10^7 cells) were preincubated with IFN- γ for 48 h and labeled at room temperature with 0.5 mCi/ml of Na ^{125} I (NEN Life Science Products, Boston, MA) by the lactoperoxidase method. Cells were immediately lysed in cold lysing buffer containing 1% Triton X-100, 1% BSA, 1 mM PMSF, 1 mM iodoacetamide; 10 μ g/ml each of aprotinin, leupeptin, pepstatin, E-64, and bestatin; 20 mM EDTA; and 0.14 M NaCl in 0.01 M Tris-Cl (pH 7.4) without Ca $^{2+}$ and Mg $^{2+}$. Anti-Fc γ RI mAb 10.1 (5 μ g) immobilized on 50 μ l of protein G agarose beads (Pierce, Rockford, IL) was added to the precleared lysate, and the tubes were incubated for 12 h. After washing the agarose beads with lysing buffer, the beads were overlaid with SDS protein gel loading solution containing 5% 2-ME. The proteins were run on 4–12% Tris-glycine gels. As a positive control, immunoprecipitation of Fc γ RI proteins was performed using U-937 cells.

Iodination of human IgG1 and binding assays

Human IgG1 (Chemicon International) was iodinated using Iodo-Beads (Pierce) with Na ^{125} I (NEN Life Science Products; sp. act., 1095 Ci/mmol). For binding assays, $1-5 \times 10^6$ mast cells/ml were incubated in 1% BSA in RPMI 1640 with radioligand and varying concentrations of unlabeled ligands at 4°C for 45 min. Cell-bound ligand was quantified by centrifuging the cell suspension through 10% sucrose in PBS in duplicate and counting the radioactivity of the cell pellets by gamma scintillometry. The binding data were curve fit with the computer program Ligand (Biosoft, St. Louis, MO) to determine the affinity, number of sites, and nonspecific binding.

Cell activation

For high-affinity IgG receptor-dependent activation, mast cells were preincubated with or without IFN- γ for 48 h. The expression of Fc γ RI on IFN- γ -treated cells was then confirmed by FACS analysis. The cells were next washed and resuspended with culture medium in 96-well culture plates. Cells (5×10^2 cells for histamine assay or 2×10^5 cells for RT-PCR/200 μ l/well) were incubated with 1 μ g/ml of F(ab') $_2$ fragments of anti-Fc γ RI mAb 22 or mouse F(ab') $_2$ fragments of IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) for 30 min at 37°C. Fc γ RI was cross-linked by incubation of mast cells with goat F(ab') $_2$ fragments of anti-mouse F(ab') $_2$ fragment of IgG (10 μ g/ml; Jackson ImmunoResearch Laboratories) for 30 min for histamine assay and for 2 h for cytokine mRNA analysis. As a positive control, cells were incubated with human IgE (1 μ g/ml) for 30 min and activated with sheep anti-human IgE (10 μ g/ml; Serotec, Oxford, U.K.) for an additional 30 min for histamine assay and for 2 h for cytokine mRNA analysis. The reaction was stopped by centrifugation at 4°C, culture supernatants were collected, and histamine in the supernatants was measured using an enzyme immunoassay kit (Immuno-technique, Marseilles, France). The cell pellets were used for total RNA isolation.

Statistical analysis

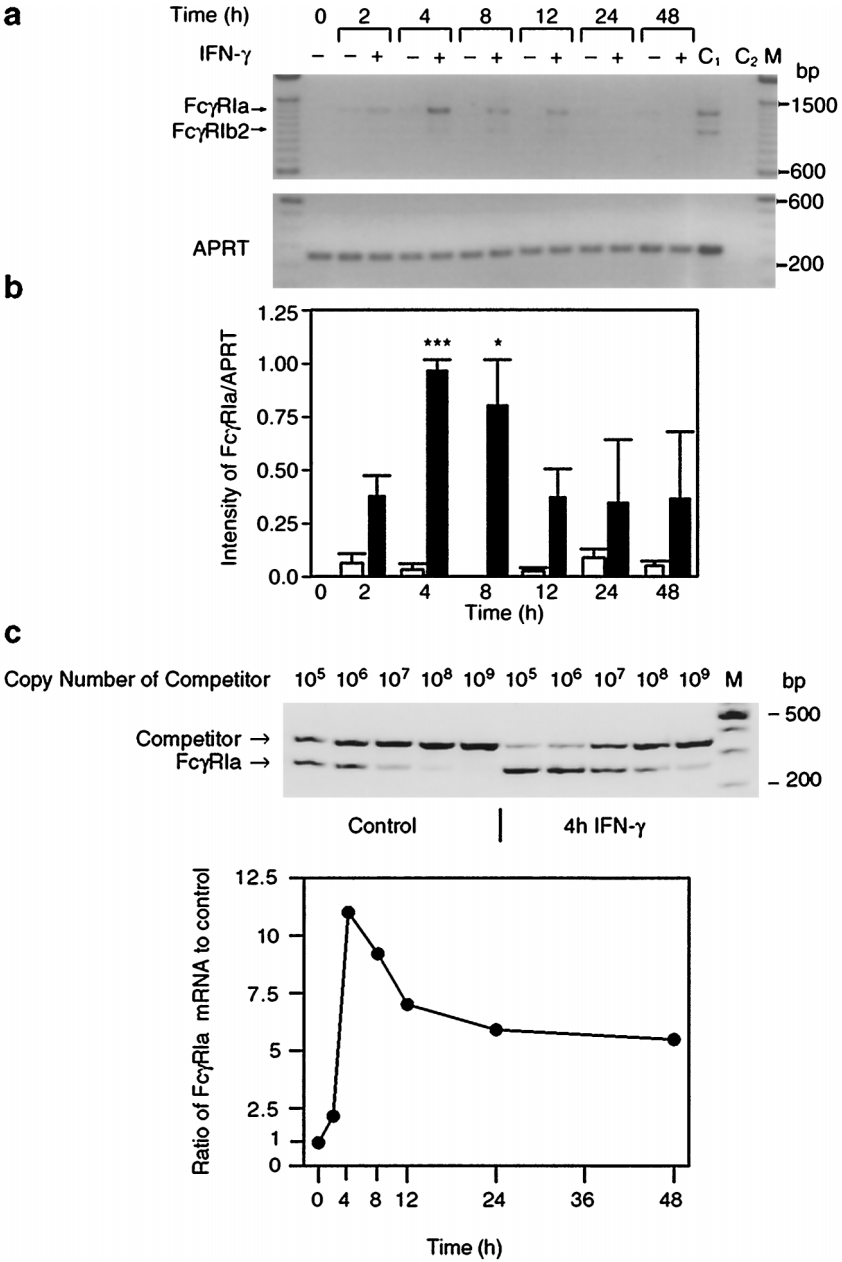
Statistical significance of differences was performed using the two-tailed unpaired Student's *t* test. Differences were considered to be significant when the *p* was < 0.05 . Data are expressed as means $\pm$ SEM.

FIGURE 1. Time course of FcγRI mRNA expression by IFN-γ-treated human mast cells. *a*, Mast cells were cultured with or without IFN-γ. Intracellular mRNA for FcγRI and APRT was amplified by RT-PCR, subjected to electrophoresis, and visualized by ethidium bromide. Total cellular RNA was extracted from U-937 cells as a positive control (C1). In the absence of cDNA, no PCR product was visualized (negative control, C2). IFN-γ-activated mast cells expressed mRNA leading to the 1291/1294 bp FcγRI PCR product, which may include FcγRIa1 (1291 bp), b1 (1294 bp), and c (1293 bp). The PCR product at ~1009 bp may contain a2 (1015 bp) and b2 (1009 bp) forms encoding for the *FcγRIIA2* and *FcγRIB2*, respectively. *b*, Semiquantification of FcγRIa was performed by measuring the band density of the relative expression of FcγRIa with respect to APRT. Mast cells were incubated without (open bars) or with (filled bars) IFN-γ. Results are presented as the means ± SEM (*n* = 3). *, *p* < 0.05, **, *p* < 0.001, when the band density of the relative expression of FcγRI with respect to APRT of mast cells incubated with IFN-γ was compared with cells incubated in the absence of IFN-γ. At 0 and 8 h, minimal signal was detected for FcγRIa. When error bars are not shown, they are too small to be diagrammed. *c*, *Upper panel*: Increase of FcγRIa mRNA expression after 4 h of stimulation by IFN-γ compared with untreated cells (control). The level of FcγRIa was measured by a competitive RT-PCR. One hundred nanograms of total RNA was used for cDNA synthesis and the synthesized cDNA was amplified by PCR in the presence of five dilutions of competitor (10⁵–10⁹ copies) using primers homologous to EC3, subjected to electrophoresis, and visualized by ethidium bromide. *Lower panel*, Time course of FcγRIa mRNA expression after IFN-γ treatment. The levels of target RNA were calculated to equal the copy number of the competitor when the ratio of the target to competitor PCR products equaled one (see *Materials and Methods*). The ratio of FcγRIa mRNA to control is the ratio of the level of target RNA from IFN-γ-treated cells to that from untreated cells.

Results

Detection of FcγRI mRNA in human mast cells: effect of IFN-γ

It is known that three highly homologous genes (A, B, and C) and at least six transcripts: two from gene A (a1 and a2), three from gene B (b1, b2 and b3), and one from gene C (c), code for a family of FcγRI proteins (14, 18, 19). Thus, using primers that detect multiple isoforms (14, 20), we performed RT-PCR using mRNA extracted from resting human mast cells and mast cells treated with IL-4, IL-5, IL-10, GM-CSF, IFN-γ, and NGF. Few, if any, products were visible after 30 cycles of amplification at 0 h or after mast cells were exposed to IL-4, IL-5, IL-10, GM-CSF, and NGF (data not shown). However, FcγRI isoforms were clearly detected by 2 h and were maximal at 4 h when human mast cells were treated with IFN-γ (Fig. 1, *a* and *b*). The PCR product detected at ~1300 bp may include FcγRIa1 (1291 bp) and b1 (1294 bp) and c (1293 bp). The PCR product at ~1000 bp may contain a2 (1015 bp) and b2 (1009 bp) forms encoding for the *FcγRIIA2* and *FcγRIB2*.



To specifically demonstrate the up-regulation of FcγRIa1 and in a more quantitative manner, we employed the 5' and 3' primer sequences for EC3 of FcγRI. As seen in Fig. 1c, we confirmed the up-regulation of FcγRIa1 mRNA expression by IFN-γ in human mast cells using RT-PCR with a DNA competitor containing the primer sequences of EC3. The *upper panel* of Fig. 1c demonstrates an increase in FcγRIa mRNA expression after 4 h of IFN-γ stimulation compared with control. FcγRIa mRNA expression is maximal between 4 and 8 h, at which time the increase was ~10-fold (*lower panel*, Fig. 1c). Thus, human mast cells exhibit FcγRI mRNA, and the level of this mRNA is up-regulated by IFN-γ among those growth factors and cytokines examined.

Analysis of FcγRI expression by human mast cells

To verify that the high-affinity IgG receptor FcγRIa, the product of *FcγRIIA*, is expressed on the human mast cell and to determine the effects of IFN-γ on this expression, we employed flow cytometry using anti-FcγRI mAb specific for EC3 (clone 10.1). In addition to

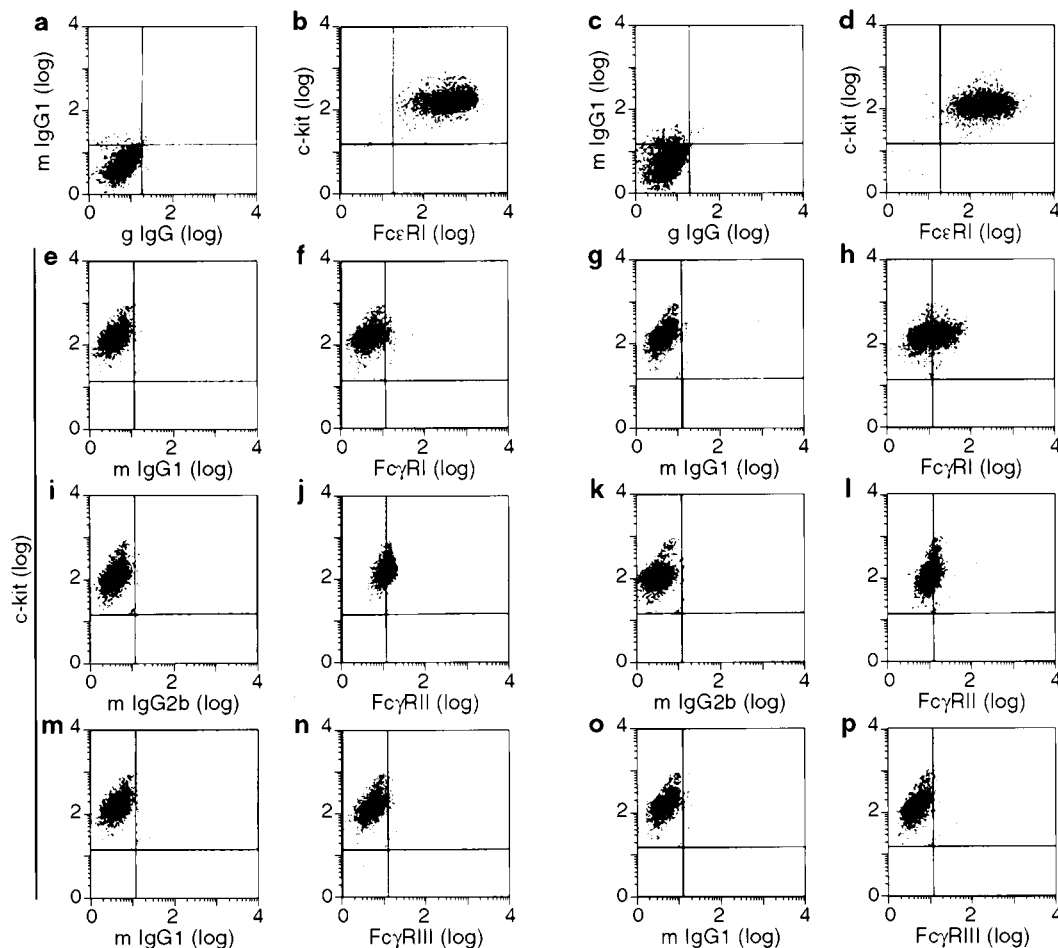


FIGURE 2. Expression of FcεRI and Fcγ receptors by human mast cells in the absence or presence of IFN-γ. Mast cells were cultured in serum-free medium without (left two panels) or with (right two panels) IFN-γ for 48 h. FcεRI was detected on *c-kit*⁺ mast cells using anti-*c-kit* mAb and anti-IgE Ab (b and d) compared with the isotype controls to anti-*c-kit* mAb and anti-IgE Ab (a and c). Fcγ receptors were detected in *c-kit*⁺ IgE⁺-gated cells using either FITC-conjugated FcγRI (f and h) or FcγRIII (n and p) and the isotype controls (e and g, or m and o (e and m are the same, g and o are the same)) and FcγRII (j and l), or the isotype control (i and k), and analyzed by flow cytometry.

analyzing the surface expression of FcγRI, we also determined the surface expression of FcγRII and FcγRIII, known to be expressed on mouse mast cells (21, 22), as well as FcεRI. As may be seen in Fig. 2, >95% of human mast cells expressed FcεRI as expected, and this expression was not affected by IFN-γ. In agreement with the up-regulation of FcγRI mRNA by IFN-γ, the number of mast cell expressing FcγRIa increased from $2.2 \pm 0.7\%$ to $43.4 \pm 8.8\%$ ($n = 4$ donors). In contrast, the expression of FcγRII was $44.7 \pm 6.3\%$ at baseline and was unchanged by IFN-γ ($45.5 \pm 5.7\%$). FcγRIII expression was minimal ($0.5 \pm 0.4\%$ at baseline) and this low level of expression was unchanged by IFN-γ ($0.2 \pm 0.6\%$). We confirmed the expression of FcγRIa using a second anti-FcγRI mAb (clone 22) which recognizes a second epitope on EC3 (23–25). The comparison of the intensity of fluorescence between these two mAbs showed no significant difference ($n = 3$; data not shown). We next determined that preincubation of mast cells with IgE did not alter the expression of Fcγ receptors on mast cells ($n = 4$; data not shown). Finally, we characterized mast cells for the presence of chymase and tryptase by flow cytometry. IFN-γ-treated FcγRI⁺ and FcγRI[−] mast cells had approximately similar phenotypes (96–97% MC_{TC} and 78–85% MC_{TC}). Thus, human mast cells express the high-affinity IgG receptor on the cell surface and the number of mast cell expressing FcγRI is increased 20-fold after incubation with IFN-γ.

As with RT-PCR, we also examined whether other proinflammatory cytokines could influence the expression of Fcγ receptors on cultured human mast cells. Mast cells were thus incubated with either IL-4, IL-5, IL-10, GM-CSF, or NGF for 48 h before flow cytometric analysis. These cytokines did not significantly affect Fcγ receptor expression ($n = 4$; data not shown).

We next analyzed the kinetics of the intensity of surface expression of FcγRI over 48 h (Fig. 3a). The intensity of surface expression of FcγRI was maximal at 24 h and appeared to plateau through 48 h, at which time 43% of the mast cells expressed FcγRI (see also Fig. 1h). Thus, FcγRI on the human mast cell surface appeared to be up-regulated over the first several hours of exposure to IFN-γ and to remain expressed over at least 48 h.

The presence of FcγRI on the mast cell surface was further confirmed by immunoprecipitation (Fig. 3b). As expected, the human mast cell FcγRI had a molecular mass of ~72 kDa. This is the same molecular mass as the FcγRI expressed by human monocytes (23).

Determination of IgG1 binding sites and affinity on human mast cells

FcγRI was the only receptor up-regulated with surface of IFN-γ-treated human mast cells as assessed by flow cytometry. To further confirm the up-regulation of this receptor and to determine receptor number, we performed binding assays with ¹²⁵I-labeled human

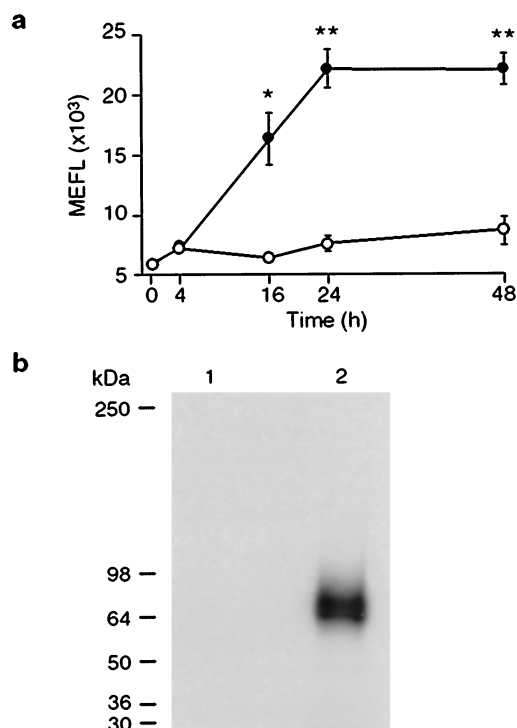


FIGURE 3. Fc γ RI surface expression by IFN- γ -treated human mast cells. *a*, Membrane expression of Fc γ RI on mast cells cultured without (open circles) or with (filled circles) IFN- γ . Mast cells were identified as c-kit⁺IgE⁺ cells. Data are expressed as MEFL (mean $\pm$ SEM; $n = 3$). *, $p < 0.05$, **, $p < 0.01$, when MEFL of cells incubated with IFN- γ was compared with the results without IFN- γ . When error bars are not shown, they are too small to be diagrammed. *b*, Immunoprecipitation of Fc γ RI protein from membranes of mast cells preincubated with IFN- γ for 48 h. Cell surfaces were labeled with ¹²⁵I, the cells were lysed, and Fc γ RI was precipitated with anti-Fc γ RI mAb 10.1 (lane 2) or with mouse IgG1 used as a negative control (lane 1). The proteins were run on a 4–12% Tris-glycine gel.

IgG1 using human mast cells, an aliquot of which had been pre-treated with IFN- γ for 48 h. Scatchard analysis revealed that resting mast cells bound IgG1 with a K_a of $4.2 \times 10^8 \text{ M}^{-1}$ with a calculated 2420 binding sites/cell. IFN- γ -treated mast cells bound IgG1 with a K_a of $4.9 \times 10^8 \text{ M}^{-1}$ with a calculated 12,110 binding sites/cell (Fig. 4). Unlabeled IgG1 fully competed with ¹²⁵I-labeled IgG1 for Fc γ RI with IC_{50} of $3 \times 10^{-8} \text{ M}$. To confirm these observations, a second mast cell culture from a separate donor was also examined. Data obtained were similar, where IFN- γ -treated mast cells bound IgG1 with a K_a of $4.4 \times 10^8 \text{ M}^{-1}$ with a calculated 17,250 binding sites/cell.

Fc γ RI aggregation is followed by histamine release and up-regulation of cytokine mRNA expression

To examine whether the aggregation of Fc γ RI on the human mast cell surface is capable of mast cell activation, we exposed cultured mast cells to a mAb against Fc γ RIa and followed histamine release and cytokine mRNA induction. To circumvent participation of Fc γ RII (26), which is constitutively expressed on mast cells (Fig. 2, *j* and *l*), F(ab')₂ fragments of mAbs were employed. After induction of Fc γ RI expression by IFN- γ , cells were activated with F(ab')₂ fragments of anti-Fc γ RI mAb 22 cross-linked with goat F(ab')₂ fragments of anti-mouse F(ab')₂ fragments of IgG. No histamine release from human mast cells was observed unless they had first been exposed to IFN- γ (Fig. 5). This aggregation of Fc γ RI on IFN- γ -treated mast cell surfaces led to significant his-

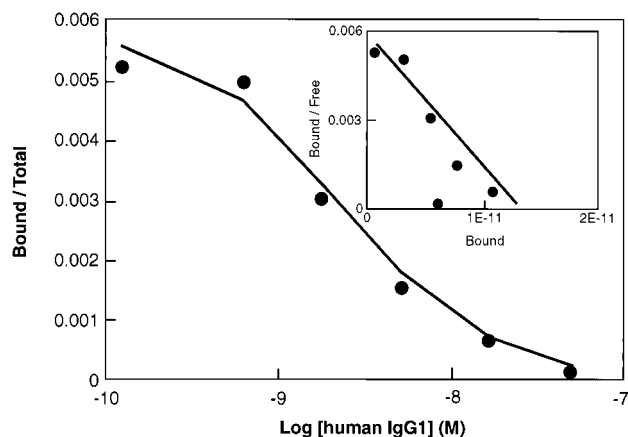


FIGURE 4. Analysis of ¹²⁵I-labeled human IgG1 binding to human mast cells. A representative ligand displacement curve is shown, and Scatchard plot transformation of the binding data is presented in the inset. Human mast cells were preincubated with IFN- γ for 48 h. Human mast cells (1×10^5 cells/tube) were incubated with ¹²⁵I-labeled human IgG1 (1095 Ci/mmol) in the presence or absence of increasing concentrations of unlabeled ligand for 45 min at 4°C.

tamine release ($24.5 \pm 4.4\%$ vs $4.0 \pm 0.9\%$). For comparison, Fc ϵ RI was also aggregated on human mast cells with or without pretreatment with IFN- γ . In both cell populations, Fc ϵ RI-mediated histamine release was similar ($40.5 \pm 6.7\%$ vs $43.0 \pm 11.0\%$). Thus, it appears that human mast cells must first be exposed to IFN- γ before Fc γ RI receptors are sufficient in number to provoke degranulation when aggregated. This effect appears to be specific for Fc γ RI in that IFN- γ did not alter histamine release due to Fc ϵ RI aggregation.

The profile of cytokine mRNA expression by mast cells induced by the aggregation of Fc ϵ RI or Fc γ RI was next examined. As expected, the aggregation of the high-affinity IgE receptor Fc ϵ RI led to induction or increased accumulation of IL-3, IL-4, IL-13, GM-CSF, and TNF- α transcripts in IFN- γ -treated mast cells (Fig.

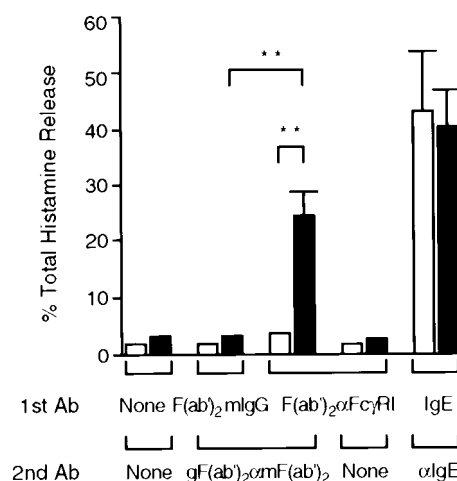
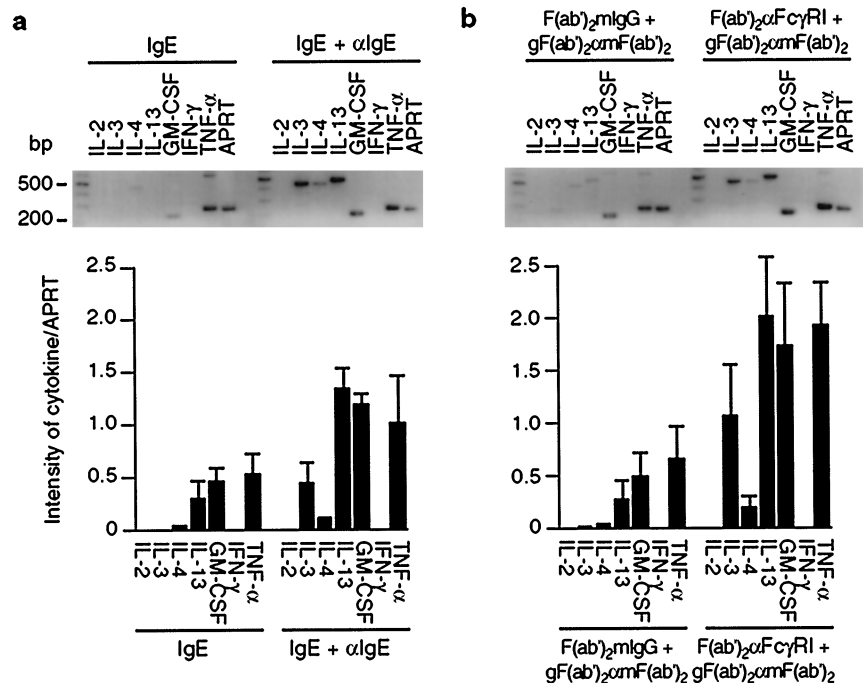


FIGURE 5. Histamine release from human mast cells following aggregation of Fc γ RI or Fc ϵ RI. Mast cells were incubated for 48 h without (open bars) or with (filled bars) IFN- γ . Mast cells were then placed in 96-well plates and incubated with IgE or a mAb (1st Ab) for 30 min at 37°C, followed by addition of anti-human IgE or goat F(ab')₂ fragments of anti-mouse F(ab')₂ fragments of IgG (2nd Ab) for 30 min at 37°C. Plates were then centrifuged, and the released histamine was measured. Results are presented as the means $\pm$ SEM ($n = 6$); **, $p < 0.01$. When error bars are not shown, they are too small to be diagrammed.

FIGURE 6. Cytokine mRNA expression in human mast cells following aggregation of Fc ϵ RI or Fc γ RI. Mast cells were preincubated for 48 h with IFN- γ , followed by exposure to human IgE for 30 min at 37°C, followed by the addition of anti-human IgE for 2 h at 37°C (a), or exposure to F(ab')₂ fragments of anti-Fc γ RI mAb 22, or F(ab')₂ fragments of mouse IgG for 30 min at 37°C, followed by the addition of goat F(ab')₂ fragments of anti-mouse F(ab')₂ fragments of IgG for 2 h at 37°C (b). Total RNA was then isolated and intracellular mRNA for each cytokine or APRT was amplified by RT-PCR, subjected to electrophoresis, and visualized by ethidium bromide. The expected product sizes of cDNAs of IL-2, IL-3, IL-4, IL-13, GM-CSF, IFN- γ , TNF- α , and APRT are 255, 455, 449, 500, 215, 270, 254, and 246 bp, respectively (*upper panel*). Semi-quantification of cytokine expression was performed by measuring the band density of the relative expression of each cytokine with respect to APRT (*lower panel*). Results are presented as the means $\pm$ SEM ($n = 3$). When error bars are not shown, they are too small to be diagrammed.



6a), but neither IL-2 or IFN- γ mRNA. The aggregation of Fc γ RI by anti-Fc γ RI (mAb 22) induced a similar cytokine mRNA profile to that observed after aggregation of Fc ϵ RI in IFN- γ -treated mast cells. Thus, activation of human mast cells via aggregation of Fc γ RI is capable of inducing up-regulation of mRNAs for cytokines, including TNF- α , GM-CSF, IL-3, and IL-13.

Discussion

In this paper, we demonstrate that human mast cells express the high-affinity IgG receptor Fc γ RI and that this receptor is up-regulated by IFN- γ (Figs. 1, 2, and 3a). We further demonstrate that receptor aggregation is followed by mast cell degranulation (Fig. 5) and up-regulation of mRNAs for a number of cytokines (Fig. 6). This demonstration of a functional high-affinity IgG receptor on mast cells may help explain *in vivo* observations which document the participation of the mast cells in host defense mechanisms against bacteria (9) and suggests that aggregation of the high-affinity receptors for IgG should be considered as a possible mechanism by which mast cells could be recruited to contribute to inflammation associated with viral diseases which induce IFN- γ production (27).

Although we believe this to be the first definitive report that mast cells may express Fc γ RI, there have been extensive studies on the expression of Fc γ RII and Fc γ RIII. IL-3-dependent, mouse bone marrow culture-derived mast cells (mBMMCs) express the low-affinity IgG receptors Fc γ RIIb1 and Fc γ RIIb2, and mouse serosal mast cells additionally express Fc γ RIII (21, 28). Mature serosal mast cells degranulate when exposed to IgG complexes (29), whereas mBMMCs appear to internalize aggregated IgG without causing the release of substantial amounts of histamine (28). In later studies, it was reported that Fc γ RIIb inhibits Fc γ RI-mediated degranulation of mBMMCs and rat basophilic leukemia cells (30, 31) because of the ability of Fc γ RIIb to recruit Src homology 2 domain-bearing inositol 5-phosphatase (32). Mouse mast cells alter the surface expression of certain IgG receptors (33). Thus, mBMMCs up-regulate surface expression of Fc γ RIII when cultured with 3T3 fibroblasts (33), which may be functionally important because cocultured mBMMCs degranulate and generate various lipid me-

diators when their Fc γ RIII receptors are cross-linked (29). Fc γ RIII signaling may further lead to the adherence of mBMMCs to fibronectin (34). Mast cells have also been shown in a mouse model to play a role in immune complex-induced injury, related to the expression of IgG receptors (35–37). The relationship of Fc γ RI expression reported in this paper to the expression of Fc γ RII and Fc γ RIII by human mast cells (Fig. 2) remains to be determined.

Three genes for Fc γ RI, i.e., *Fc γ RIA*, *Fc γ RIB*, and *Fc γ RIC*, have been identified as encoding at least six transcripts: Fc γ Rla1, Fc γ Rla2, Fc γ Rlb1, Fc γ Rlb2, Fc γ Rlb3, and Fc γ Rlc (14, 18, 19). The Fc γ RIA gene product Fc γ Rla1 uniquely contains the EC3 as well as transmembrane and cytoplasmic domains (18, 19). The presence of these domains, as well as the presence of EC2, allows this gene product to bind monomeric IgG with high affinity and to initiate IgG-mediated cellular responses (25). Two transcripts (b1 and c) have stop codons in the third extracellular domain (EC3) and are believed to only code for soluble secreted proteins (18). Three transcripts are alternatively spliced isoforms, one (a2) from gene A and two (b2 and b3) from gene B (19). Fc γ Rlb2 lacks EC3 (18) and is not expressed on the cell surface (38). Fc γ Rlb3 lacks the signal sequences (S)2, EC1, and EC3 (19). Fc γ Rla2 lacks the S2 and EC1 (19). The protein product and function of Fc γ Rla2 and Fc γ Rlb3 are unknown (19). As can be seen in Fig. 1, Fc γ RI mRNA expression is induced in human mast cells exposed to IFN- γ . This expression is maximal at 4 h in a kinetic pattern similar to that observed in neutrophils and monocytes (39). However, it should be noted that the RT-PCR techniques employed do not distinguish among Fc γ RIa, Fc γ Rlb1, and Fc γ Rlc. Thus, by RT-PCR, the *Fc γ RIA* gene product cannot be confirmed. However, all known Abs to Fc γ RI identify an epitope on EC3, which is found only on Fc γ RIa (1, 23–25). Employing such Abs, we could confirm that IFN- γ treatment of human mast cells resulted in the up-regulation of Fc γ RIa on the mast cell surface (Fig. 2h). Note that we also detected Fc γ Rlb2 mRNA (Fig. 1a). However, Fc γ Rlb2 is not believed to be present on the surface membrane (38), does not bind either monomeric or complexed IgG, nor is it recognized by Abs to the high-affinity IgG receptor (38). Thus, as analyzed by

RT-PCR and flow cytometry, human mast cells express the high-affinity IgG receptor, the gene product of *Fc γ RIA*, on the cell surface within hours after exposure to IFN- γ .

After up-regulation of mRNA for Fc γ RI by IFN- γ , the resulting cell surface expression of the protein was evident by 16 h and maximal at 24 h (Fig. 3a). Scatchard plots using ^{125} I-labeled human IgG1 (Fig. 4) revealed a K_a of $4\text{--}5 \times 10^8 \text{ M}^{-1}$, consistent with the expression by human mast cells of a class of Fc receptors binding IgG1 with a high affinity. Furthermore, the average number of IgG1 binding sites on mast cells after incubation with IFN- γ was increased 5-fold, as has been observed with human monocytes (40). The immunochemical assessment of the size of this cell surface receptor determined it was identical in size to the human Fc γ RI on human monocytes (23) (Fig. 3b). This protein was recognized by two different anti-Fc γ RI mAbs which recognize the EC3. The mAb 10.1 blocks IgG binding (23) while mAb 22 recognizes an epitope which is distinct from the binding site for the Fc portion of human IgG (24).

IFN- γ treatment of human mast cells significantly up-regulates surface Fc γ RI (Figs. 2 and 3a). IFN- γ had little effect on the surface expression of Fc ϵ RI or on the surface expression of Fc γ RII and Fc γ RIII, as also has been reported in human monocytes (41). Although IL-10 has been shown to up-regulate the expression of Fc γ RI in human monocytes (42), IL-10 failed to induce the surface expression of Fc γ RI in mast cells, as has been reported in neutrophils (43). Also, neither IL-4, IL-5, GM-CSF, nor NGF up-regulated Fc γ RI (1, 2, 41–47). Thus, IFN- γ might be expected to up-regulate IgG-dependent responses in mast cells in pathologic situations associated with IFN- γ production at the tissue level. Such IFN- γ production has been reported in association with viral infection (27). Studies are thus underway to determine whether the surface expression of Fc γ RI on mast cells as assessed by histochemistry is up-regulated in such diseased tissues.

The aggregation of Fc γ RI was examined by first exposing IFN- γ -treated human mast cells to F(ab') $_2$ fragments of an Ab to Fc γ RI. These cells were then exposed to goat F(ab') $_2$ fragments of an Ab directed to mouse F(ab') $_2$ fragments of IgG. This strategy avoids the possibility that intact Abs might directly bind to other surface receptors, specifically Fc γ RII or Fc γ RIII. Fc γ RI aggregated by this strategy induced histamine release only from IFN- γ -treated mast cells (Fig. 5), which averaged $24.5 \pm 4.4\%$. For comparison, mast cell degranulation was also induced through aggregation of Fc ϵ RI. Fc ϵ RI aggregation, which also led to histamine release, was not effected by IFN- γ pretreatment and approximated 35–40%.

Triggering of mast cells by aggregation of Fc γ RI with F(ab') $_2$ fragments of anti-Fc γ RI mAb also led to induction of specific cytokine mRNA expression (Fig. 6). Aggregation of Fc γ RI on human monocytes induces IL-6 and TNF- α production (48, 49). Activation of human mast cells through Fc γ RI appeared to up-regulate mRNAs for IL-3, IL-13, GM-CSF, and TNF- α (Fig. 6b). Overall, the pattern of cytokine mRNA produced following Fc ϵ RI or Fc γ RI aggregation appeared to be similar (compare Fig. 6, a to b). These data are in agreement with the observation that biological responses triggered by FcR with immunoreceptor tyrosine-based activation motifs seem to depend more on the cell type than on the receptor (3).

We have thus, for the first time, definitely shown that human mast cells or, for that matter, mast cells from any species, may be activated through Fc γ RI. The expression of the functional Fc γ RI required several hours of exposure to IFN- γ . This exposure, however, does not affect mast cell activation through Fc ϵ RI. Thus, IFN- γ production associated with specific disease states, including

bacterial or viral infections (27, 50), provides a novel means by which the mast cell may be recruited into both immunologic and infectious diseases and may help explain the etiology of cellular reactions to Ag where Ag-specific IgE cannot be identified.

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