



Activation of human mast cells through the high affinity IgG receptor

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Abstract

Mast cells are known to participate in the induction of inflammation through interaction of antigen with specific IgE bound to the high affinity receptor for IgE (FcεRI). Human mast cells, derived from CD34⁺ hematopoietic precursors, not only express FcεRI but also express high affinity receptors for IgG (FcγRI), the latter only after IFN-γ exposure. Human mast cells that express FcγRI are activated following FcγRI aggregation, either using antibodies directed to the receptor or through IgG bound to the receptor. This activation results in degranulation, with the release of granule-associated mediators, and the generation of metabolites of arachidonic acid and secretion of chemokines and cytokines. These observations provide evidence that human mast cells may also be recruited into inflammation through IgG-dependent mechanisms.

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1. Introduction

Mast cells are generally thought of in the context of immediate hypersensitivity type I reactions, where activation follows IgE-dependent FcεRI aggregation (Metcalfe et al., 1997). Activated mast cells release preformed mediators such as proteases and histamine (Segal et al., 1977), generate arachidonic acid derivatives (Nakamura et al., 1991), and both release and synthesize certain cytokines and chemokines (Gordon and Galli, 1990). There is also data that demonstrate that mast cells may be activated by non-IgE-dependent mechanisms. This is illustrated in a murine model where IgE-deficient mice still present some manifestations of anaphylaxis (Oettgen et al., 1994).

One alternative to IgE-dependent activation may involve IgG receptors. Murine mast cells are known to express low affinity receptors for IgG, including FcγRIIb1, FcγRIIb2 (Benhamou et al., 1990) and FcγRIII (Katz et al., 1990). Among these, FcγRIII has been shown to induce mast cell degranulation (Katz and Lobell, 1995) and promote adhesion after IgG-mediated aggregation (Dastyk et al., 1997).

More recently, it has been reported that human mast cells express FcγRI (CD64) on their surface following IFN-γ

treatment (Okayama et al., 2000). Aggregation of these FcγRI on the human mast cell surface induces granule exocytosis, generation of mediators derived from arachidonic acid, and the up-regulation of mRNA for a number of cytokines and chemokines. While this mediator profile resembles that observed in human mast cells after IgE-dependent degranulation, there are differences in the profile of cytokine production.

2. Structure of FcγRI

FcγRI is the only FcγR which binds IgG with high affinity (K_a : 10^{-8} to 10^{-9} M). It is expressed at the cell surface as a $\alpha\gamma_2$ complex (Van de Winkel and Anderson, 1991). The full length FcγRI presents three extracellular Ig-like domains on the extracellular portion of its α chain. Fcγ chains are required for surface expression of FcγRI on monocytes, macrophages, neutrophils, dendritic cells and eosinophils (Heijnen and Van de Winkel, 1997) and for signaling mediated by FcγRI (Ernst et al., 1992). FcγRI expression has been shown to be influenced by immune cytokines. For example, monocytes, macrophages and neutrophils constitutively express FcγRI. However, surface expression of FcγRI on neutrophils is strongly increased by IFN-γ (Pan et al., 1990). FcγRI is coded by three genes A, B, C. Alternative splicing induces six different isoforms

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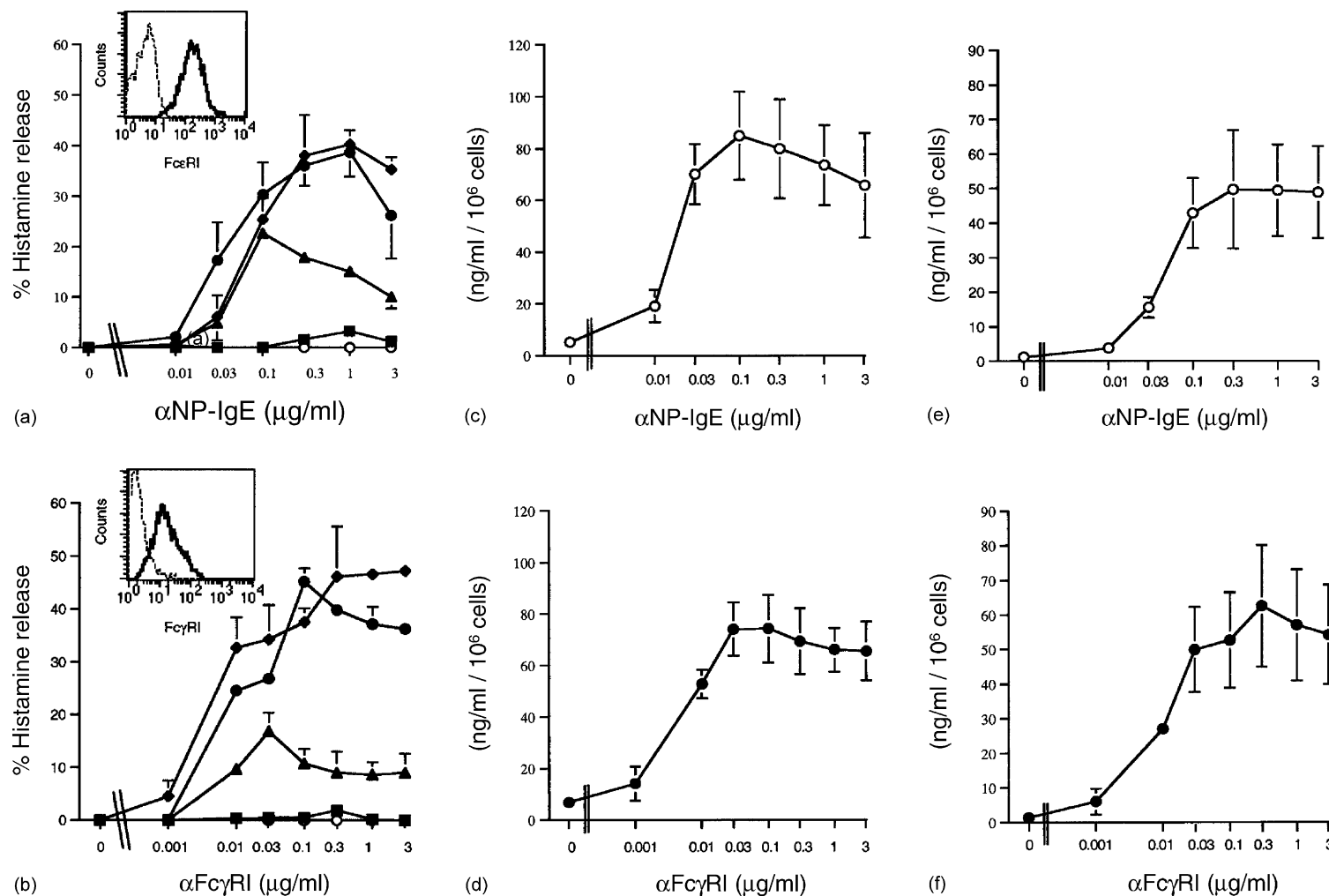


Fig. 1. Histamine, PDG₂ and LTC₄ release after Fc γ RI and Fc ϵ RI aggregation on IFN- γ -treated human mast cells. IFN- γ -treated human mast cells were incubated with anti-NP-IgE (a, c, e) or F(ab')₂ of mouse anti-human Fc γ RI mAb (b, d, f). Cells were then stimulated with NP-BSA (a) at 0 (○), 0.1 (■), 1 (▲), 10 (●), or 100 ng/ml (◆), or goat F(ab')₂ of anti-mouse F(ab')₂ of IgG (b) at 0 (○), 0.1 (■), 1 (▲), 10 (●), or 30 μ g/ml (◆) for 30 min at 37 °C, and histamine release determined. For PDG₂ (c, e) and LTC₄ (d, f) assay, cells were stimulated by addition of either NP-BSA (○), or goat F(ab')₂ of anti-mouse F(ab')₂ of IgG (●). Fc ϵ RI (b) was detected using anti-IgE Ab compared to the isotype control to anti-IgE (dotted line). Reproduced from Okayama et al. (2001a) by copyright permission of The American Association of Immunologists.

a₁, a₂, b₁, b₂, b₃ and c. However, FcγRIa₁ is the only full length gene product (Ernst et al., 1998).

3. Expression of FcγRI on IFN-γ-treated human mast cells

Human mast cells, derived from CD34⁺ progenitors in peripheral blood, were obtained following culture of progenitor cells for one week with recombinant human (rh) IL-3, rh IL-6 and stem cell factor (SCF), then for 6–8 weeks with rh IL-6 and SCF (Kirshenbaum et al., 1999). These culture conditions led to a cell population of 97% human mast cells as assessed by toluidine blue staining. These mast cell cultures were then enriched to greater than 99.9% mast cells by removing monocytes by adherence. To examine possible expression of FcγRI on human mast cells, mRNA of cultured cells was extracted and analyzed for the expression of different FcγRI transcripts (Okayama et al., 2000). To examine whether FcγRI expression was up-regulated by cytokines, human mast cells were pretreated with IL-4, IL-5, IL-10, granulocyte-macrophage colony stimulating factor (GM-CSF), IFN-γ or nerve growth factor (NGF). RT-PCR studies demonstrated that resting human mast cells cultured only with SCF and IL-6 did not express mRNA for FcγRI isoforms. However, following culture with IFN-γ, mRNA for the FcγRIa₁ isoform was detected after 2 h, maximizing at 4 h (Okayama et al., 2000). Subsequent flow cytometry experiments, using specific antibodies, demonstrated that FcγRIa₁, the product of FcγRIa, is expressed on human mast cells after several hours of exposure to IFN-γ (Fig. 1). IFN-γ treatment did not, however, affect the expression of FcεRI on human mast cells.

Expression of low affinity receptors for IgG was also examined on CD34⁺ progenitor cell-derived human mast cells. Human mast cells expressed mRNAs for FcγRIIb₁, FcγRIIb₂ and FcγRIII, however significant surface expression was only observed for FcγRII. The mRNA expression for these receptors was unaffected by IFN-γ treatment. Moreover, aggregation of FcγRII, using available monoclonal antibodies, did not induce mast cell degranulation (Okayama et al., 2001b).

4. FcγRI aggregation induces degranulation and arachidonic acid metabolite secretion by IFN-γ-treated human mast cells: comparison to FcεRI activation

To examine whether FcγRI aggregation influenced human mast cell activation, IFN-γ-treated human mast cells, expressing FcγRI, were incubated with mouse F(ab')₂ anti-FcγRI, to avoid non-specific binding to FcγRII. Goat anti-mouse IgG F(ab')₂ fragments were then added to aggregate FcγRI (Okayama et al., 2000). As illustrated in Fig. 1, histamine release was observed after FcγRI aggregation on human mast cells. The level of histamine release

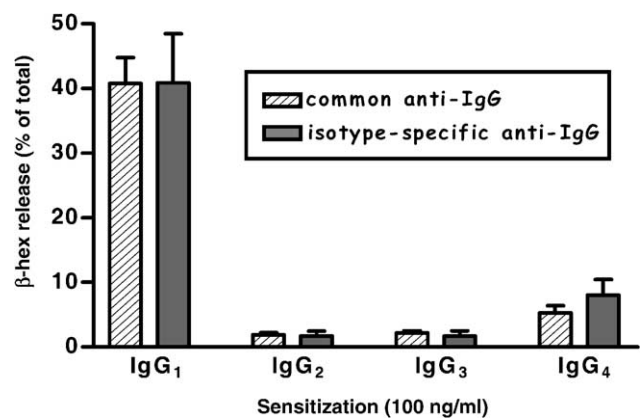


Fig. 2. β-Hexosaminidase release after IFN-γ-treated-human mast cell sensitization with different IgG isotypes. Human mast cells were cultured with IFN-γ and then sensitized with purified human myeloma monomeric IgG₁, IgG₂, IgG₃ or IgG₄. Surface bound IgG was cross-linked using either isotype specific anti-IgG (hatched bars) or a common anti-IgG (solid bars) recognizing all four isotypes. β-hexosaminidase release was determined after stimulation for 30 min at 37 °C. Reproduced from Woolhiser et al. (2001) by copyright permission of Wiley-VCH editors.

was similar to that observed following FcεRI activation. IFN-γ pretreatment did not alter FcεRI-mediated mast cell degranulation. Similarly, FcγRI or FcεRI aggregation on IFN-γ-treated-human mast cells generated the arachidonic acid derivatives, PGD₂ and LTC₄ (Okayama et al., 2001a). These observations were confirmed in freshly purified normal human lung mast cells which were cultured with IFN-γ, which led to the expression of FcγRI. Aggregation of FcγRI did induce more TNF-α release compared to FcεRI-mediated activation (Okayama et al., 2001a).

It has also now been observed that IFN-γ-treated human mast cells degranulate after sensitization with human IgG immunoglobulins followed by the addition of antibodies specific to each IgG isotype (Fig. 2). Sensitization with IgG₁ was most effective at promoting granule exocytosis, and mediator release and cytokine generation. Minimal degranulation was observed when mast cells were incubated with IgG₂, IgG₃ and IgG₄ followed by the appropriate antibody to aggregate the IgG bound receptors (Woolhiser et al., 2001).

5. Comparison of cytokine mRNA induction and release after FcγRI or FcεRI aggregation

Mast cell degranulation following FcεRI aggregation is known to lead to the synthesis and release of both chemokines and cytokines (Gordon and Galli, 1990). This led us to focus on cytokine mRNA regulation in FcγRI or FcεRI activated-IFN-γ-treated human mast cells. Initial RNase protection assay experiments revealed mRNAs for TNF-α, GM-CSF, IL-1β, IL-5, IL-6, IL-1Ra and IL-13 were up-regulated after FcγRI aggregation when compared to FcεRI activation (Okayama et al., 2001a,b). The results

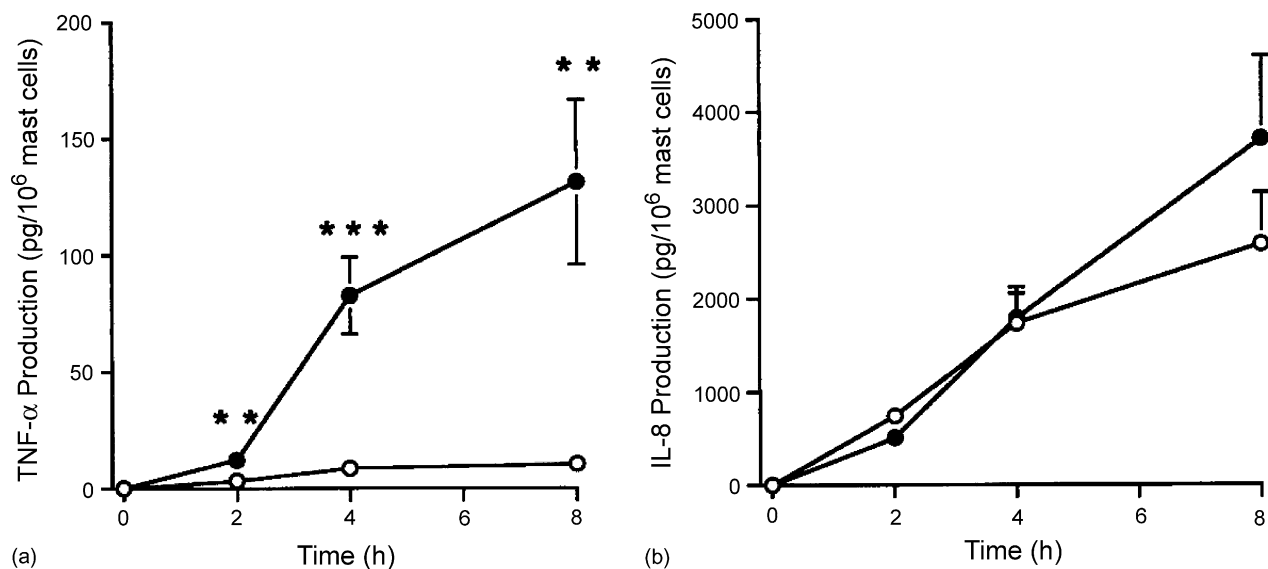


Fig. 3. Kinetics of TNF- α and IL-8 secretion after Fc γ RI or Fc ϵ RI aggregation on human mast cells. Mast cells were cultured with IFN- γ , and incubated with 1 μ g/ml of anti-NP-IgE for 16 h (○) or 1 μ g/ml goat F(ab')₂ of anti-mouse (●). At the indicated time points, Fc ϵ RI and Fc γ RI were cross-linked by addition of NP-BSA (○) or of goat F(ab')₂ of IgG (●). TNF- α and IL-8 were measured by ELISA. Reproduced from Okayama et al. (2001a) by copyright permission of The American Association of Immunologists.

from RNase protection assays were in part confirmed by ELISA experiments demonstrating that TNF- α protein was also secreted to a greater degree after Fc γ RI activation, than when mast cells were activated employing anti-CD64

F(ab')₂ fragments (Okayama et al., 2001a) (Fig. 3) or IgG₁ antibody (Woolhiser et al., 2001). The authors also examined the secretion of IL-8. There was no difference in IL-8 release after Fc ϵ RI or Fc γ RI aggregation of IFN- γ -treated

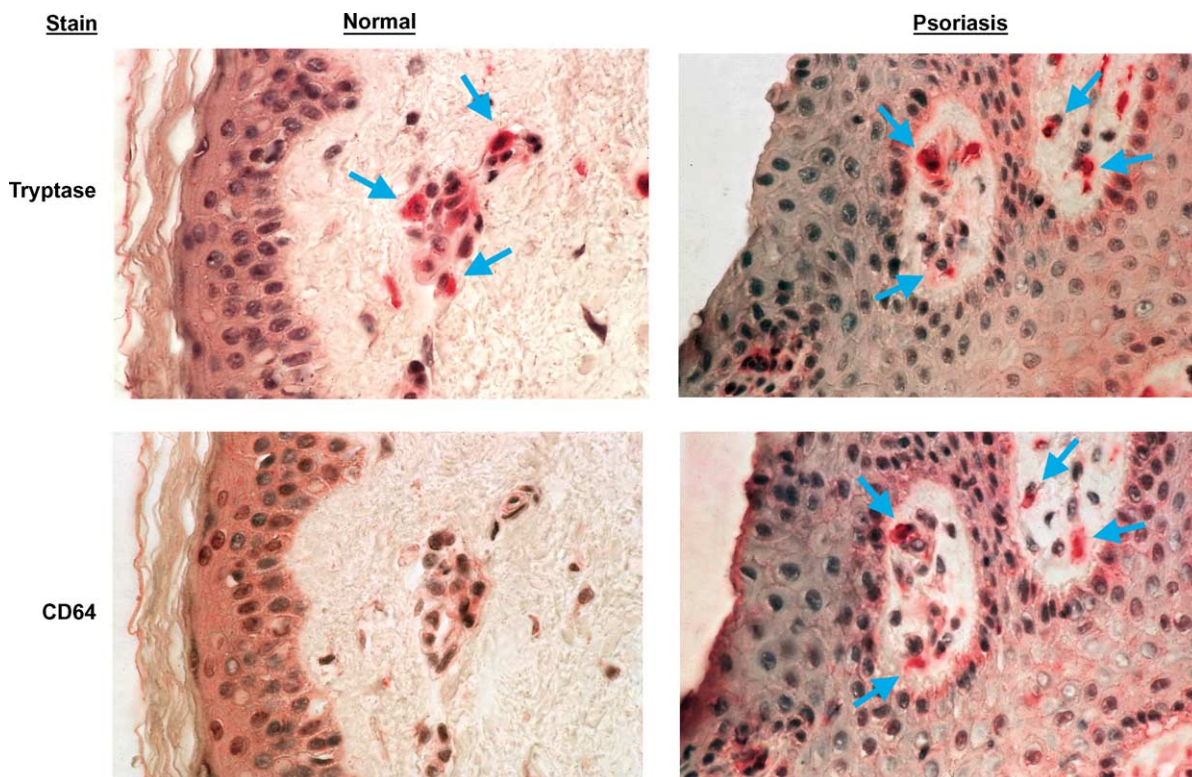


Fig. 4. Detection of Fc γ RI in psoriatic tissue. Skin biopsies were taken from psoriatic and normal skin, then fixed prior to staining with selective antibodies. Mast cells were identified by staining for tryptase. A sequential section was subsequently stained with an anti-Fc γ RI (anti-CD64) antibody.

mast cells (Okayama et al., 2001a) (Fig. 3). These results suggest that a Th₁ environment could recruit mast cells into a Th₁ type inflammation. This appears true in vivo, through expression of FcγRI, as human mast cells in IFN-γ-rich psoriatic skin (Barker, 1991) express FcγRI. This is not observed in normal skin (Fig. 4).

6. FcγRI-mediated signaling in human mast cells

FcεRI and FcγRI aggregation on IFN-γ-treated human mast cells induced degranulation to a similar degree, but TNF-α release differed. These results led us to examine the signaling pathways in human mast cell following activation through these receptor. Preliminary data indicates that *src* kinases and p72^{syk} are both phosphorylated after either FcγRI or FcεRI receptor aggregation, although p72^{syk} appears activated to a greater degree after FcγRI aggregation. IFN-γ-treated mast cells incubated with specific inhibitors revealed that *src* kinase proteins and p72^{syk} were both required for FcγRI or FcεRI-mediated degranulation in IFN-γ-treated human mast cells. However, it appears that the PI-3 kinase pathway may only be required for degranulation induced by FcγRI aggregation. It has also been reported that cytokine secretion, after FcεRI aggregation, is controlled by the MAP kinase pathway in rodent mast cells (Zhang et al., 1997). Activation of MAP kinases such as ERK1/2, p38 and JNK appeared to be similar after FcεRI or FcγRI-mediated human mast cell degranulation.

7. Conclusions

Following IFN-γ-treatment, human mast cells express FcγRI. Aggregation of these receptors results in degranulation. Human mast cells then generate arachidonic acid metabolites, and synthesize and release both chemokines and cytokines. The signaling pathways activated after FcγRI aggregation generally resemble those in other cell types that may be activated via FcγRI. The signaling pathways initiated in human mast cells following FcεRI aggregation also are similar to those initiated in human mast cells following FcγRI aggregation, although there do appear to be quantitative differences. In addition, some inhibitors of signaling proteins alter FcγRI, compared to FcεRI signaling, to a greater degree. These data suggest that human mast cells may be recruited into sites of inflammation through IgG-dependent mechanisms. The biologic consequences would be similar to those observed following IgE-dependent activation. Finally, studies with inhibitors of signaling molecules indicate the potential exists to identify pharmacologic agents that preferentially inhibit one pathway compared to the other.

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