

Fcγ Receptors on Mast Cells: Activatory and Inhibitory Regulation of Mediator Release

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Key Words

Mast cells · IgG · IgE · FcγRI · FcγRII · FcγRIII · FcεRI

Abstract

Mast cell activation and subsequent release of proinflammatory mediators are primarily a consequence of aggregation of the high affinity receptors for IgE (FcεRI) on the mast cell surface following antigen-dependent ligation of FcεRI-bound IgE. However, data obtained from rodent and human mast cells have revealed that IgG receptors (FcγR) can both promote and inhibit mast cell activation. These responses appear to be species and/or mast cell phenotype dependent. In CD34⁺-derived human mast cells exposed to interferon-γ, FcγRI is upregulated, FcγRII is expressed but not upregulated, and FcγRIII is not expressed. In contrast, in mouse mast cells, FcγRII and FcγRIII receptors are expressed, whereas FcγRI is not. Aggregation of FcγRI on human mast cells promotes mediator release in a manner generally similar to that observed following FcεRI aggregation. Aggregation of FcγRIIb in mouse mast cells fails to influence cellular processes; however, when coligated with FcεRI, signaling events thus activated downregulate antigen-dependent mediator release. These divergent responses are a consequence of different motifs con-

tained within the cytosolic tails of the signaling subunits of these receptors and the specific signaling molecules recruited by these receptors following ligation. The studies described imply that data obtained in rodent models regarding the influence of FcγRs on mast cells may not be directly translatable to the human. The exploitation of FcγRs for a potential therapy for the treatment of allergic disorders is discussed in this context.

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Introduction

Antigen-mediated mast cell activation plays an integral role in the initiation of cellular responses associated with atopic asthma and other allergic reactions such as urticaria, rhinitis and conjunctivitis. These reactions are traditionally considered to be dependent on the triggering of mast cells following antigen-induced aggregation of IgE-bound high affinity receptors for IgE (FcεRI) on the cell surface. The consequential activation of complex intracellular signaling cascades culminates in immediate degranulation and arachidonic acid metabolite generation, and the delayed production of cytokines and chemokines. There is emerging evidence that, in addition to the FcεRI, IgG receptors (FcγR) also regulate mast cell activa-

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tion. However, in contrast to the case of FcεRI, FcγR, under the appropriate conditions, may either induce or inhibit mast cell activation. These responses are dependent on the particular receptor(s) engaged and the subsequent intracellular signaling pathways activated. In this review, we will describe these FcγR-dependent mediated events and discuss how these pathways may regulate mast cell mediator release.

Mast Cells: Characterization and Model Systems

Mast cells are derived from CD13+, CD34+, CD117+ bone marrow progenitor cells which migrate to the blood and, ultimately, to the tissues where they complete their differentiation and maturation under the influence of cytokines contained within the microenvironment [1]. Murine and human mast cells can both be classified into two general phenotypes on the basis of preformed mediators contained in their intracytoplasmic granules and their tissue distribution. Murine mast cells have been designated as serosal and mucosal phenotypes on the basis of granule staining characteristics attributed to preformed intragranular proteoglycans, heparin or chondroitin sulfate, respectively, and on tissue distribution as reviewed elsewhere [2]. Human mast cells are also classified based on the granule components: MC_{TC} cells express both tryptase and chymase, whereas MC_T cells express only tryptase, as determined by immunohistochemistry. As in the mouse, both MC_T and MC_{TC} are differentially localized [2]. MC_T are primarily found in the gastrointestinal mucosa and alveolar walls, whereas MC_{TC} are preferentially localized in the skin and submucosa. These phenotypes display differential sensitivities to stimuli such as complement components and substance P [2].

Both human and murine mast cells can be developed in culture from hematopoietic precursors (fig. 1). Classically, mouse bone marrow-derived mast cells (BMMCs) have been obtained by culturing bone marrow cells in media containing IL-3 [3], fibroblast supernatant [4], or stem cell factor (SCF) [5]. However, it appears that in vivo the principal cytokine necessary for mast cell development is STF, since mice deficient for SCF or Kit, the receptor for SCF, are mast cell deficient [reviewed in 6]. Mouse BMMCs cultured in IL-3-containing media display more of the mucosal phenotype, whereas cells cultured in media containing SCF display more of the serosal phenotype [1]. Human mast cells can be derived from peripheral blood [7], fetal liver [8], cord blood [9] or bone

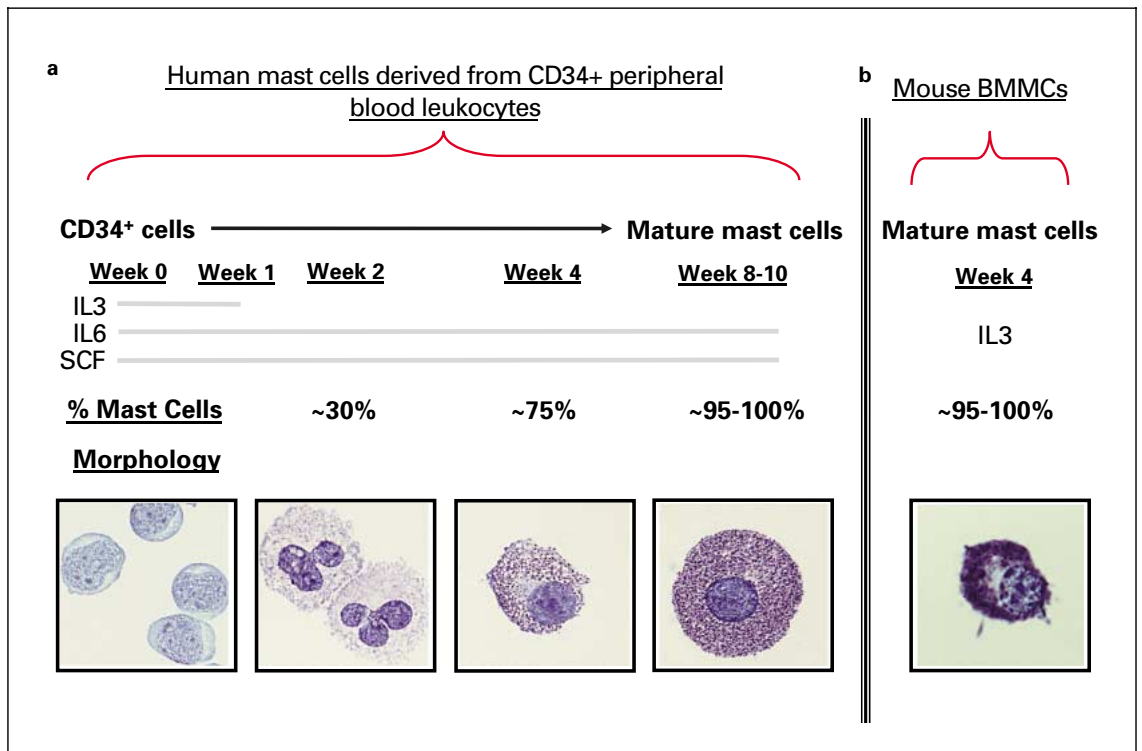
marrow [10] CD34+ precursors. In contrast to the mouse, SCF is the only cytokine capable of supporting significant human mast cell development in vitro [11]. However, IL-3, IL-6 [12] or PGE₂ [13] can act in synergy with SCF to increase the mast cell number in the cultures. Based on these observations, a technique was developed in our laboratory for obtaining mature human mast cells from CD34+ peripheral blood cells, obtained after leukaphoresis, by culturing these cells in serum-free medium containing SCF and IL-6 [12]. A pure population of human mast cells is obtained under these conditions after 8 weeks of culture (fig. 1). The populations of human mast cells cultured from progenitor cells display more of the MC_{TC} serosal-associated phenotype as assessed by flow cytometry [12].

Antigen-Mediated Mast Cell Activation via the FcεRI

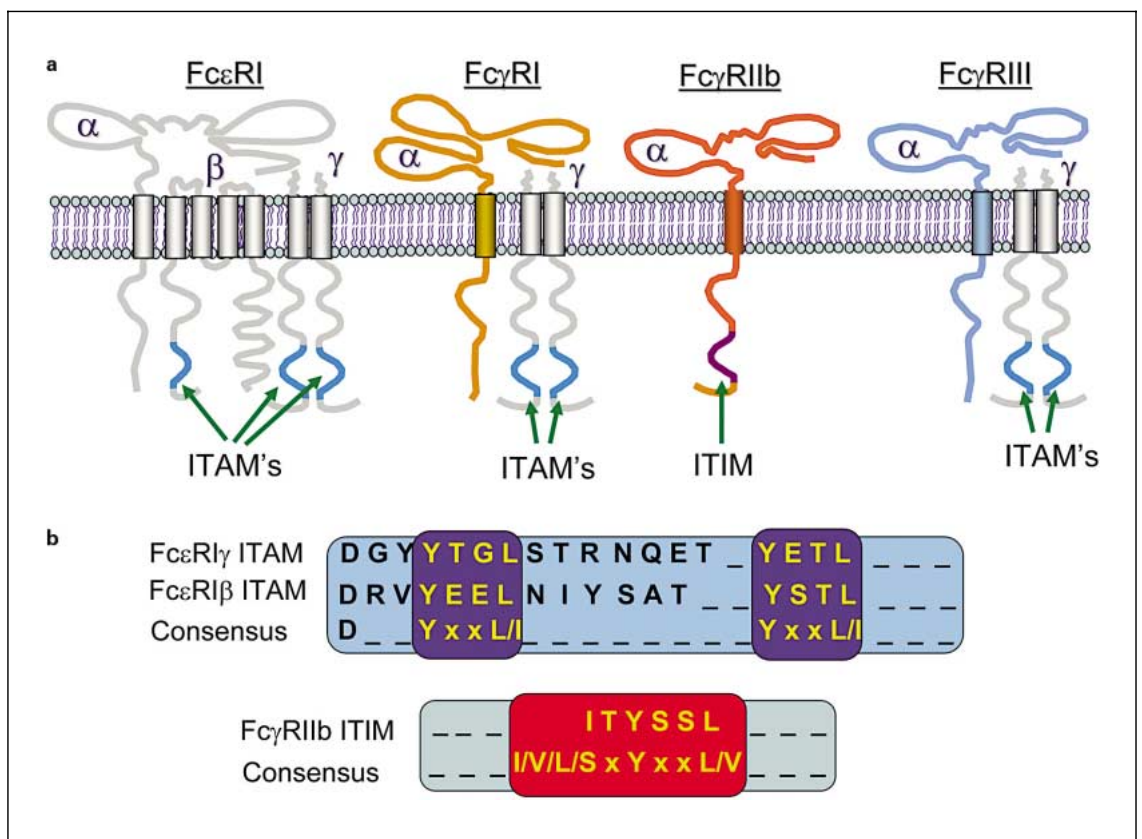
To understand how Fcγ receptors influence mast cell activation, it is necessary to first understand the mechanisms linking aggregated FcεRI to mast cell mediator generation and release. In general, antigen-mediated activation of mast cells is dependent on the Th₂-driven production of antigen-specific IgE molecules, subsequent binding of these molecules to the FcεRI expressed on the mast cell surface, and aggregation of the FcεRI, following binding of antigen to the antigen recognition sites on the receptor-bound IgE [14]. The FcεRI expressed in mast cells is comprised of the IgE-binding α subunit, a β subunit and two γ subunits which form a covalently linked homodimer [15] (fig. 2a). The γ chains are indispensable for mediating downstream signaling. Data, however, suggest that the β chain amplifies signals initiated by the γ subunits [16, 17]. In addition, in the mouse, but not human, the FcεRIβ chain is required for the expression of the FcεRI α and γ chains to the cell surface [18].

Fig. 1. Human mast cells (a) and mouse BMMCs in culture (b). a The conditions of purity and morphology during the development of mature human mast cells from CD34+ pluripotent peripheral blood cells utilizing the technique described by Kirshenbaum et al. [12] are shown. b The conditions of purity and morphology of mouse BMMCs after 4 weeks in culture are shown.

Fig. 2. a Subunits of the FcεRI and Fcγ receptors expressed in human and/or murine mast cells. b The sequences of the ITAMs and ITIM contained within the signaling subunits of these receptors are shown. The darker blue represents the consensus tyrosine containing motifs which are targets for *src* family tyrosine kinases.



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Transmission of the signals from the FcεRIγ is dependent on specific sequences contained within the cytosolic domain of the γ subunit [19]. Such a sequence is termed immunoreceptor tyrosine-based activation motif (ITAM) (fig. 2b) [20]. This motif is also found in the FcεRIβ chain [19] and other receptor subunits such as the Igα and Igβ subunits of the B cell receptor and the ζ chain of the T cell receptor [21]. The tyrosine residues contained within these motifs are targets for *src* family member tyrosine kinases which become activated following receptor ligation [19]. In the case of the FcεRI, *lyn* appears to be the major *src* kinase responsible for this action [22]. Phosphorylated ITAMs provide a docking site for *src* homology (SH)₂ domain-containing proteins and, by this means, the tyrosine kinase *Syk* is also recruited to the signaling complex where it too is tyrosine phosphorylated, thereby becoming activated [23].

Much of the machinery required for the initial signaling processes resides in specialized regions within the membrane called lipid rafts, also termed glycolipid-enriched membrane fractions [24]. These regions are enriched in certain lipids and cholesterol [25] and signaling proteins become resident in the rafts generally following palmitoylation of consensus motifs in the cytosolic juxtamembrane region [26]. The major substrates for tyrosine kinases within these regions are the transmembrane adaptor molecules LAT [27] and NTAL [28] which, when phosphorylated, provide docking sites for associating cytosolic adaptor molecules such as Grb₂, SLP76 and Vav [28–30], which in turn recruit the major signaling enzymes PLCγ₁ and PI-3 kinase. These molecules, subsequently, result in enhanced calcium flux and protein kinase C activation, essential signals for degranulation, and activation of the Ras-Raf-MAPK cascade, which is essential for arachidonic acid metabolite release and cytokine generation [31].

The distinct nature of the production and release of the three classes of mediators requires this divergency at the more receptor-distal events in the signaling cascade. Recent evidence, however, has suggested that there is also receptor proximal divergency in the signaling cascades. In this respect, it has been proposed that there is a complementary pathway to that regulated by *lyn*, which is regulated by the *src* kinase *fyn* [32]. Furthermore, data from *lyn* and *fyn* knockout mice suggest that, whereas the *lyn*-regulated pathway controls PLCγ₁ activation [32, 33], the *fyn*-dependent pathway may primarily control PI-3 kinase activation [32]. Supportive evidence for this concept of complementary pathways for FcεRI-mediated mast cell activation has come from our studies demonstrating

that antigen-dependent degranulation in human mast cells is regulated by an early PI-3 kinase-independent, PLCγ₁-dependent pathway, and a latent PI-3 kinase-dependent pathway [34]. Although it had been suggested that the PI-3 kinase may influence mast cell activation by recruiting the *src*-related kinase Btk to the membrane as a consequence of production of phosphoinositide phosphates which binds the pleckstrin homology domain contained within Btk [35], our data suggest that PI-3 kinase may regulate mast cell activation at the level of calcium influx [34].

FcγR and Mast Cell Activation

In contrast to the FcεRI, Fcγ receptors both positively and negatively regulate mast cell activation. FcγRI [36] and FcγRIII [37], expressed on mast cells under specific circumstances, when aggregated, induce degranulation. FcγRIIb, when expressed on mast cells and coligated with the FcεRI [38] or Kit [39], inhibits mast cell degranulation induced by FcεRI aggregation or mast cell proliferation induced by Kit activation, respectively. These contrasting responses are dictated by specific motifs contained within the cytosolic domains of the signaling subunits of the specific receptors termed immunoreceptor tyrosine-based inhibitory motifs (ITIMs) [20, 40] and the properties of the signaling molecules subsequently recruited into the receptor-signaling complex following *src* kinase activation.

ITAM Containing FcγR: FcγRI and FcγRIII

FcγRI

The FcγRI is comprised of a single IgG-binding chain (FcγRIα) and an FcRγ homodimer, which is also expressed as part of the FcεRI (fig. 2a). Three genes for FcγRI, FcγRIA, FcγRIB, and FcγRIC, have been identified as encoding at least six transcripts: FcγRIa1, FcγRIa2, FcγRIb1, FcγRIb2, FcγRIb3, and FcγRIc [41, 42]. The full-length FcγRI contains three Ig-like domains on the extracellular portion of its α chain. FcγRIa1 is the only full length protein, as it uniquely contains the third extracellular domain (EC3) as well as the transmembrane and cytoplasmic domains [41, 42]. Thus, FcγRIa1 is the only FcγRI gene product that binds IgG with high affinity (K_a: 10⁻⁸ to 10⁻⁹ M) [43]. FcRγ chains are required for surface expression of FcγRI on monocytes, macrophages, neutrophils, dendritic cells and eosinophils [43], and for

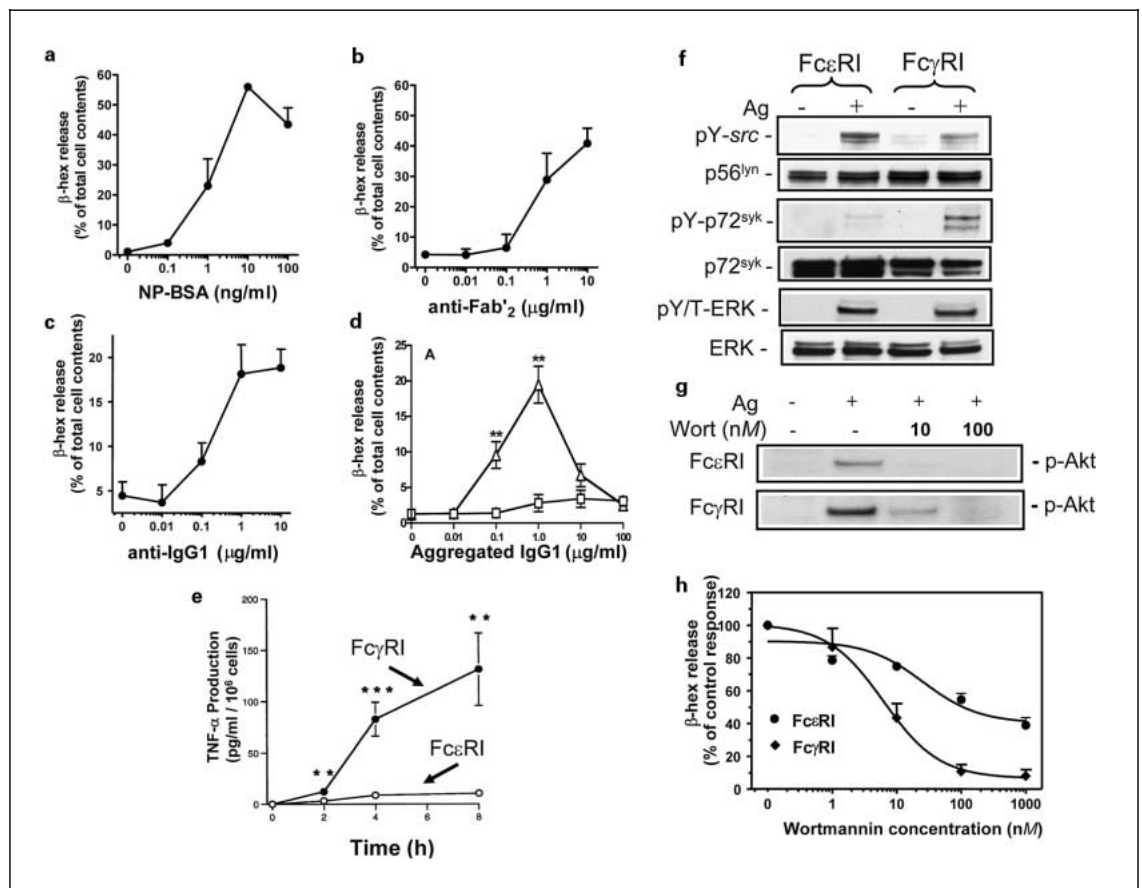


Fig. 3. Comparative activation of human mast cells via the FcεRI and FcγRI. Degranulation following FcεRI aggregation (**a**) and following FcγRI aggregation (**b–d**), as monitored by β-hexosaminidase release. **e** TNF-α production following FcεRI and FcγRI aggregation. **f** Phosphorylation of *src* kinases, *Syk* (p72^{Syk}) and ERK following FcεRI and FcγRI activation. **g** Activation of PI-3 kinase following FcεRI and FcγRI aggregation as monitored by phosphorylation of AKT, and its inhibition by wortmannin. **h** Relative ability of the PI-3 kinase inhibitor wortmannin to block degranulation in response to FcεRI and FcγRI aggregation. In these experiments, human mast cells were exposed to IFN-γ (15 ng/ml) for 48 h to induce FcγRI expression. **a, e–h** FcεRI were aggregated by sensitizing the cells overnight with anti-NP-IgE (1 μg/ml), then triggering with NP-BSA

(100 ng/ml). **b, e–h** FcγRI were aggregated by incubating the cells with F(ab')₂ fragments of anti-FcγRI (1 μg/ml), then with the indicated concentrations of goat F(ab')₂ fragments of anti-mouse F(ab')₂ fragments of IgG. For 30 min (**a, b, h**), for the indicated times (**e**), and for 5 min (**f, g**). **c, d** FcγRI were aggregated for 30 min following incubation of the cells with IgG1 (100 ng/ml) for 16 h, then the addition of the indicated concentrations of anti-IgG1 as in **c** or the addition of the indicated concentrations of aggregation IgG1 as in **d**. **a–c, f–h** From Okayama et al. [51]. **d** From Woolhiser et al. [48]. **e** From Okayama et al. [46]. They are reproduced with the permission of the publishers of these articles in **d**, ** is significantly different from controls ($p < 0.01$), and in **e** ** $p < 0.01$ and *** $p < 0.001$.

signaling mediated by FcγRI [43]. The expression of FcγRI has been shown to be influenced by immune cytokines. For example, monocytes, macrophages and neutrophils constitutively express FcγRI, whereas surface expression of FcγRI on neutrophils is strongly increased by interferon-γ (IFN-γ) [44].

Human and rodent mast cells do not constitutively express the FcγRI. However, pretreatment of human mast cells derived from CD34+ peripheral blood cells for

48 h with human recombinant IFN-γ induces a high level of expression of FcγRI at the cell surface [36]. It was observed that this response was specific for FcγRI as an increase in FcεRI, FcγRII, and FcγRIII was not observed under identical conditions [45]. The FcγRI expressed on human mast cells were demonstrated to be functional by the release of histamine and cytokines following receptor aggregation [36] (fig. 3). These data [36, 46], which were obtained by incubating the cells with a Fab'2 anti-CD64

antibody, then an anti-Fab'₂ antibody (fig. 3b), were confirmed by sensitizing human mast cells with human IgG isotype antibodies, then cross-linking with the respective anti-isotype antibodies (fig. 3c) [47]. These studies also revealed that FcγRI-mediated HuMC degranulation is optimal after IgG1 sensitization compared to IgG2, IgG3 and IgG4 [47]. More recently, it has been demonstrated that aggregated IgG [48], particularly of the IgG1 isotype, induces degranulation (fig. 3d), PGD₂ and LTC₄ production, and an increase in IL-3, IL-13, GM-CSF and TNF-α mRNA in human mast cells. When examined, these responses were potentiated by the addition of C3a. These studies provide support for the concept that, when FcγRI are expressed on human mast cells under the influence of Th₁ cytokines, immune complexes may lead to mast cell activation. Furthermore, as FcγRI aggregation in human mast cells results in TNF-α release (fig. 3e) [46], and as TNF-α has been shown to play a role in inflammation associated with rheumatoid arthritis or Crohn's disease, the above observation would suggest that, in a Th₁ environment, mast cells could be involved in autoimmune disease induction. In this respect, it is interesting to note that mast cells are more abundant in IFN-γ-rich psoriatic skin, when compared to normal skin [49] and that these mast cells express FcγRI [50].

As the FcγRI possesses the same FcRγ signaling homodimer as the FcεRI, it would be expected that the mediators released in response to FcεRI and FcγRI aggregation and the signaling pathways initiated by these receptors would be identical. When examined in human mast cells, in general this was observed to be the case although there were several notable exceptions [46, 51]. For example, although degranulation, arachidonic acid metabolite release, and the generation of a number of cytokines and chemokines in response to FcγRI aggregation were observed to be largely similar in magnitude and kinetics to that observed following FcεRI aggregation (fig. 3a, b), the mRNA and/or protein levels of other cytokines including TNF-α, IL-1β, IL-5, IL-6, IL-13, and IL-1R antagonist were markedly higher in response to FcγRI aggregation than in response to FcεRI aggregation [46] (fig. 3e). This would imply that, although the FcεRI and FcγRI may utilize common signaling pathways, there may also be differential regulation of pathways leading to the activation of specific transcription factors which lead to the expression of the individual cytokines.

Indeed, when signaling events initiated following FcγRI and FcεRI aggregation in human mast cells were examined, both similarities and differences in the pathways activated and utilized by these receptors were de-

tected [51]. In this respect, we observed similar phosphorylation of *src* kinases, *Syk*, and the MAP kinases p38, ERK1/2, and JNK in response to both stimuli, although the magnitude of *Syk* phosphorylation was higher in response to FcγRI aggregation than with FcεRI aggregation (fig. 3f) [51]. Furthermore, inhibitors of *Syk* and *src* kinases, and MAP kinases, respectively, blocked degranulation and cytokine production in response to both receptors [51]. In contrast to these results, we observed that there was differential protein tyrosine phosphorylation of a number of proteins following FcγRI aggregation compared to after FcεRI aggregation. However, the identity of these proteins was not determined [51]. In addition, degranulation in response to FcγRI aggregation was approximately 10 times more sensitive to the *src* kinase inhibitor PP2 than was degranulation in response to FcεRI [51]. Finally, studies with the PI-3 kinase inhibitor, wortmannin (fig. 3g, h), suggested the absolute requirement of the PI-3 kinase signaling pathway for FcγRI-mediated degranulation whereas as described above, FcεRI utilizes both a PI-3 kinase-dependent and a PI-3 kinase-independent pathway for degranulation [51, 52].

The similarity in the signaling pathways mediated by the FcγRI and FcεRI may be explained by the utilization of the FcRγ homodimer by both receptors for signaling [53] (fig. 4). The reasons for the differences in signaling between these receptors are currently unclear. However, there are several potential reasons for these differences: (1) the β chain which is part of the FcεRI receptor and which may contribute to its signaling does not appear to be associated with the FcγRI [54]; (2) the FcεRI and the FcγRI may utilize different *src* kinases, which may have different substrates [51]; (3) different utilization of the transmembrane adaptor molecules LAT and NTAL for signaling following differential phosphorylation or (4) distinct contributions of the FcεRIα and FcγRIα subunits to the initiation of signaling [55]. Thus several questions remain unanswered regarding the precise mechanism(s) by which the FcγRI induces mediator release from mast cells.

FcγRIII

As with the FcγRI, the FcγRIII is comprised of an IgG-binding α subunit and a homodimer of the signaling FcRγ chains (fig. 3a). Hence, these receptors, when expressed on mast cells, possess the potential to induce degranulation in a manner similar to that observed following FcεRI and FcγRI aggregation. In contrast to the FcγRIα, the FcγRIIIα binds IgG with relatively low affinity ($K_a < 10^{-7} M$) [54]. Human mast cells and IL-3-cultured

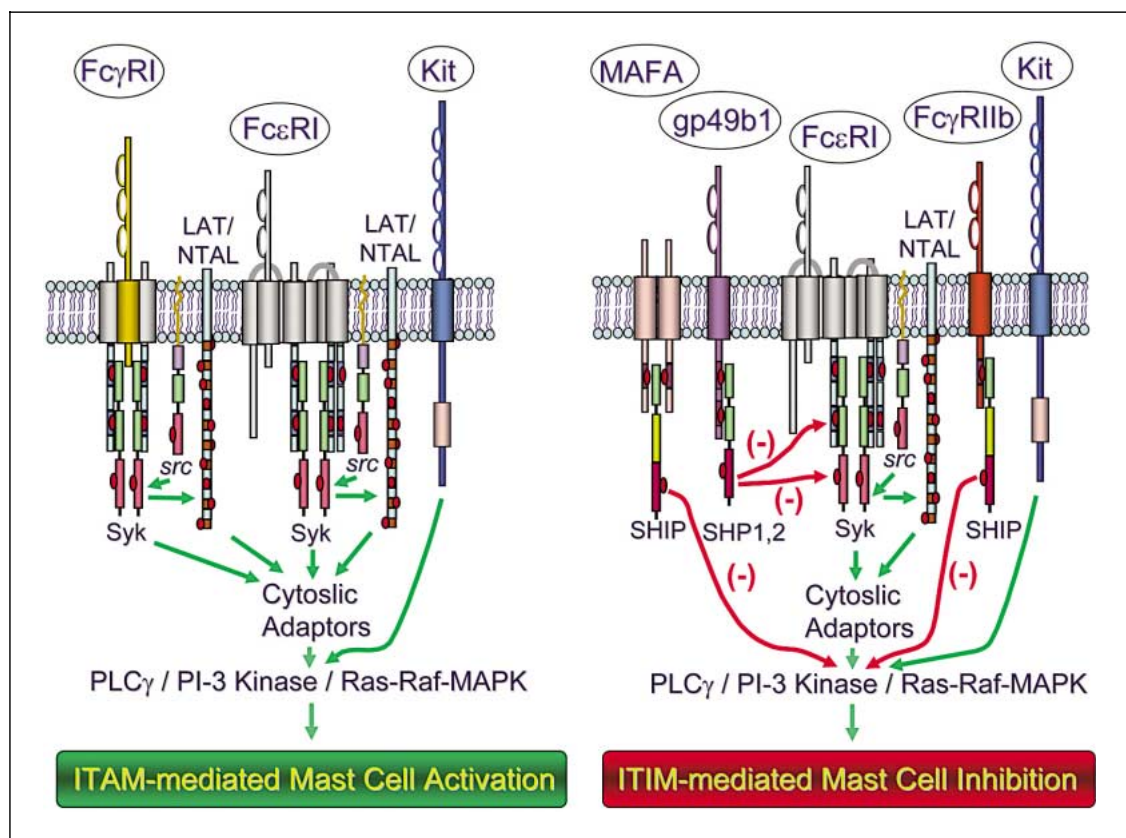


Fig. 4. Activator and inhibitor pathways regulated by Fcγ receptors. The green arrows indicate activatory pathways and the red arrows represent inhibitory pathways. *src* represents members of the SRC family of tyrosine kinases which include *lyn* and *fyn*. The small red circles represent phosphate groups.

mouse BMMCs do not constitutively express FcγRIII. However, when mouse IL-3-derived BMMCs are cultured with 3T3 fibroblasts which supply SCF, surface expression of FcγRIII is upregulated [56]. Thus, it appears that BMMCs with the mucosal phenotype do not express FcγRIII, whereas BMMCs with the serosal phenotype do. Cross-linking of FcγRIII expressed on BMMCs results in degranulation and generation of various lipid mediators [37]. Furthermore, FcγRIII aggregation has been reported to lead to the adherence of BMMCs to fibronectin [57]. Secretory responses mediated by the FcγRIII appear to be phenotype dependent as illustrated by the fact that mature serosal mast cells, which express FcγRIII but not FcγRI, degranulate when exposed to IgG complexes [58]. In contrast, the mucosal IL-3-cultured BMMCs, which express neither receptor, appear to internalize aggregated IgG without causing the release of substantial amounts of histamine [59].

ITIM-Containing FcγR: FcγRII

Unlike the FcεRI, FcγRI, and FcγRIII, the FcγRII is a single-chain receptor which does not associate with the signaling FcRγ homodimer but rather contains a specific binding site for signaling molecules in its intracellular domain in addition to the IgG binding site in its extracellular domain (fig. 2a). FcγRII binds IgG with relatively low affinity ($K_a \sim 10^{-6} M$) compared to the FcγRI [60]. FcγRII are expressed in mouse BMMCs, human mast cells [36], and human basophils [61]. There are three genes for FcγRII: FcγRIIA, FcγRIIB and FcγRIIC; however, only the FcγRIIB gene encodes for full length protein. Two isoforms of FcγRIIB are present in murine mast cells: FcγRIIB1 and FcγRIIB2. FcγRIIB1 presents an insertion of 47 amino acids in its intracytoplasmic portion compared to FcγRIIB2 [62].

Data regarding the role of FcγRII in mast cell function have been obtained from studies conducted in rodent

models. These studies demonstrated that coligation of the FcγRII with the FcεRI resulted in the downregulation of the secretory response initiated by FcεRI aggregation as reviewed by Daeron and Vivier [63]. Furthermore, FcεRI-dependent degranulation, IL-4 production, intracellular Ca²⁺ flux and histamine release were reported to be inhibited in human basophils following coligation with FcγRIIb with the FcεRI [61]. In addition to its effects on FcεRI-mediated mast cell activation, coligation of FcγRIIb with Kit results in the inhibition of Kit-dependent mouse mast cell proliferation [39]. It has been reported that, whereas only FcγRIIb2 can mediate endocytosis and phagocytosis when aggregated by specific ligands [64], both FcγRIIb1 and FcγRIIb2 can downregulate mast cell activation [38, 65].

As FcγRIII and FcγRIIb both bind IgG with low affinity, at least in the mouse, in vivo there may be a fine balance between IgG-mediated activation of mast cells and IgG-mediated inhibition of mast cell function. This balance can be influenced in vivo. For example, IL-4 upregulates in vitro FcγRIII expression on BMMCs via a STAT6-regulated pathway without affecting FcγRIIb. As a result, IgG induces, rather than inhibits, degranulation in mast cells exposed to IL-4 when compared to non-treated cells [66]. Recently, the relative roles of these two receptors in vivo, through a model of experimental allergic encephalomyelitis (EAE) disease, have been reported. This condition, which is an animal model for multiple sclerosis, is induced in susceptible rodent strains by injecting myelin components, including myelin oligodendrocyte glycoprotein (MOG) [67]. The number and/or distribution of mast cells subsequently correlate with the development of EAE. Mice lacking mast cells (W/W^v mice) develop EAE later and less severely than do control mice in response to an injection of MOG. The addition of immature mast cells to these animals then restores typical EAE susceptibility [68]. When the mast cell-deficient mice are reconstituted with FcγRIII^{-/-} mast cells, they present a reduction of severity of EAE compared to wild-type mast cell-reconstituted mice. Inversely, mice injected with FcγRIIb^{-/-} mast cells show an increase in the severity of EAE severity [69].

FcγRIIb is only one of several receptors known to be expressed in mast cells and/or basophils which downregulate FcεRI-mediated mast cell mediator release, the others being gp49b1 [70] and mast cell function-associated antigen (MAFA) [71]. In contrast to the FcRγ chains which contain ITAMs, these receptors recruit signaling molecules via a specific sequence termed ITIM (fig. 2b). When the tyrosines within these sequences are phosphorylated

by the action of *src* kinases, tyrosine or inositol phosphatases are recruited via their SH2 domains [72] (fig. 4). The major phosphatases recruited into the phosphorylated ITIMs are the SH2 domain-containing phosphatase (SHP) and SH2 domain-containing inositol phosphatase (SHIP) [54]. The ITIM contained within the FcγRIIb and MAFA cytosolic tails recruit(s) SHIP1 and/or SHIP2 [71, 73]. In contrast, the ITIM contained within the cytosolic tail of the gp49b1 receptor recruits SHP1 and SHP2 [74]. The major substrates for SHIP1 and SHIP2 are inositol phosphates [75], which are formed as a result of PI-3 kinase activation. These inositol phosphates provide a binding site for pleckstrin homology domain-containing molecules at the cell membrane [75]. Thus, coligation of FcγRII with activating receptors such as the FcεRI and/or FcγRI abrogates PI-3 kinase-mediated signaling pathways, initiated by the latter receptors (fig. 4). Supportive evidence for a role for SHIP in the FcγRII-mediated inhibition of FcεRI has come from mouse BMMCs, deficient in SHIP1, where inhibition of IgE-mediated responses was abrogated [73]. SHP1 and SHP2 are tyrosine phosphatases which can dephosphorylate the ITAMs of the β and γ chains of the FcεRI receptors and potentially other regulatory tyrosine residues on signaling proteins [76]. As a result of activation of SHP1 and or SHP2, therefore, specific tyrosine kinase-dependent signaling pathways are downregulated (fig. 4). In addition to the association with gp49b1, SHP1 and SHP2 may also associate with the β chain of the FcεRI [77]. SHIP, in addition to association with FcγRIIb1, can associate with the FcεRIγ chain [78]. Thus, these phosphatases may play a role in the endogenous negative regulation of FcεRI function.

The question of FcγRIIb expression on human mast cells and the particular phosphatases utilized for its function are potentially important considerations for exploiting the inhibitory properties of this receptor for novel therapies for the treatment of allergic disorders. As human mast cells do appear to express message for FcγRIIb [36], it is possible that agents targeted towards this receptor would show some efficacy in mast cell-driven reactions associated with these disorders, although it may be difficult to maximally inhibit FcεRI-mediated response. As discussed, the ITIM of activated FcγRIIb appears to primarily recruit the inositol phosphatase SHIP, thereby effectively terminating PI-3 kinase-dependent signaling pathways. The demonstration that there is both a PI-3 kinase-independent and a PI-3 kinase-dependent component to FcεRI aggregation [34] would imply that at least part of the response would be refractory to any inhibition imposed by coligation with FcγRIIb1. In contrast, FcγRI-

mediated degranulation is more dependent on PI-3 kinase than the FcεRI-mediated responses. Thus, coligation of FcγRIIb with FcγRI may more effectively block responses initiated by FcγRI aggregation than by FcεRI aggregation. Furthermore, inhibitory receptors which recruit the tyrosine phosphatases SHP1 and SHP2 rather than SHIP1 or SHIP2 may offer greater potential for targeting by pharmacological agents in the treatment of mast cell-dependent reactions.

Despite these arguments, it has been documented that coligation of FcγRII with FcεRI effectively blocks FcεRI-mediated degranulation in human basophils [61]. In these studies, it was found that although human basophils expressed SHP1, SHP2 and SHIP1, only SHP1 was recruited from the cytosol to the plasma membrane after FcεRI and FcγRIIb coaggregation. Although the authors did not directly demonstrate that the FcγRIIb-mediated FcεRI inhibition was due to SHP1 activity, *in vitro* experiments using ITIM peptides for FcγRIIb implied that only SHP1 could bind to ITIM motifs on FcγRIIb [61]. This contrasts with previous reports suggesting that SHIP but not SHP1 and SHP2 bind to the FcγRIIb ITIM [79]. A possible explanation could be that at low levels of FcεRI and FcγRI aggregation, ITIM motifs on FcγRIIb are insufficiently phosphorylated to recruit the two SH2 domain of SHP1 [79]. Recently, an Fcγ-Fcε fusion protein, composed of a γHinge-CHγ2-CHγ3-15 amino acids-

CHε2-CHε3-CHε4, which recognized both FcγRIIb and FcεRI on the surface of human basophil surface [80] has been described. This molecule was reported to block syk phosphorylation and histamine release from FcεRI-activated human basophils. In addition, in transgenic mice expressing human FcεRI, mast cell-mediated skin responses were reduced in animals injected with the fusion protein [80].

Conclusions

The data discussed in this review illustrate the fact that in addition to the FcεRI, Fcγ receptors, under the appropriate circumstances, can also profoundly influence mast cell activation. In this respect, aggregation of FcγRI expressed on human mast cells following IFN-γ exposure promotes mast cell inflammatory mediator release. In rodent mast cells, however, coligation of FcγRIIb with FcεRI results in downregulation of FcεRI-mediated inflammatory mediator release. Whether these differences are species related or phenotype related at this point is unclear. However, the differences in Fcγ receptor expression and signaling pathways regulated by these receptors in human mast cells compared to rodent mast cells are important considerations in the design of potential therapeutic agents based on interactions with FcγR.

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