

Activating and inhibitory signaling in mast cells: New opportunities for therapeutic intervention?

Vanessa L. Ott, PhD, and John C. Cambier, PhD *Denver, Colo*

Immune responses are tightly controlled by the activities of both activating and inhibitory signals. At the cellular level, these signals are generated through engagement of membrane-associated receptors and coreceptors. The high-affinity IgE receptor FcεRI is expressed on mast cells and basophils and, on cross-linking by multivalent antigen (allergen), stimulates the release of inflammatory mediators that induce acute allergic responses. Activation signals mediated by a variety of immune receptors (eg, B-cell receptor, T-cell receptor, and FcεRI) are subject to negative regulation by a growing family of structurally and functionally related inhibitory receptors. Recent studies indicate that mast cells express multiple inhibitory receptors that may regulate FcεRI-induced mast cell activation through similar mechanisms. The ability of inhibitory receptors to attenuate IgE-mediated allergic responses implicates them as potential targets for therapeutic intervention in the treatment of atopic disease. Indeed, coaggregation of activating and inhibitory receptors has been suggested as one possible mechanism to explain the beneficial effects of specific immunotherapy in the treatment of allergy. In this review we summarize the current knowledge of inhibitory receptors expressed in mast cells and the mechanisms through which they regulate mast cell function. (*J Allergy Clin Immunol* 2000;106:429-40.)

Key words: Mast cell, FcεRI, FcγRIIB, inhibitory receptors, signal transduction

The incidence of atopic diseases, including asthma, rhinitis, and eczema, has increased dramatically in recent years in both developed and developing countries.^{1,2} Ironically, this trend is paralleled by advances in our understanding of the cellular and molecular mechanisms that control allergic responses, ranging from the initial events that stimulate the production of IgE to antigen-

Abbreviations used

BCR:	B-cell receptor
BMMC:	Bone marrow-derived mast cells
Btk:	Bruton's tyrosine kinase
ER:	Endoplasmic reticulum
IAP:	Integrin-associated protein
InS:	Inositol
IRS:	Inhibitory receptor superfamily
ITAM:	Immunoreceptor tyrosine-based activation motif
ITIM:	Immunoreceptor tyrosine-based inhibition motif
KIR:	Killer cell inhibitory receptor
MAFA:	Mast cell function-associated antigen
MIRR:	Multisubunit immune response receptor
NK:	Natural killer
PI3 kinase:	Phosphatidylinositol 3 kinase
PIR:	Paired Ig-like receptor
PtdIns:	Phosphatidylinositol
SIRP:	Signal regulatory protein
TCR:	T-cell receptor

induced activation of mast cells and other effector cells. In individuals predisposed to atopic disease, T-cell recognition of allergen presented by dendritic cells or other antigen-presenting cells drives a T_H2-type T-cell response characterized by the production of IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13.³ Of these, IL-4, IL-5, and IL-13 are critical for the development of the allergic response. Production of IL-4 (and the closely related cytokine IL-13) stimulates Ig class-switching, leading to production of IgE, which binds to its high-affinity receptor (FcεRI) on the surface of mast cells and basophils. Production of IL-5 by T_H2-type T cells contributes to the allergic response by stimulating the recruitment of eosinophils to sites of inflammation.

Mast cells have long been recognized as the critical tissue-based effector cells that mediate IgE-dependent allergic responses. Binding of multivalent antigen (allergen) to FcεRI-bound IgE on the surface of mast cells stimulates a complex series of signaling events that culminate in the release of proinflammatory mediators that contribute to both acute and late-phase allergic responses.⁴ Effector molecules released in response to FcεRI-mediated signal-

From the Department of Immunology, University of Colorado Health Sciences Center and, National Jewish Medical and Research Center, Denver. Supported by research and training grants from the US Public Health Service. J. C. C. is Ida and Cecil Green Professor of Cell Biology.

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Reprint requests: John C. Cambier, PhD, 1400 Jackson St, K1004, Denver, CO 80206.

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ing can be classified into 3 groups. FcεRI aggregation stimulates the release of preformed cytoplasmic granules containing histamine, proteoglycans, and mast cell-specific proteases and the *de novo* synthesis of lipid-derived mediators, including platelet-activating factor and the arachidonic acid metabolites PGD₂ and leukotriene C₄. Together, these mediators are responsible for the symptoms of the acute allergic response. Third, mast cells synthesize and secrete a wide range of cytokines (eg, IL-3, IL-4, IL-5, IL-6, IL-8, IL-13, IL-16, TNF-α, and GM-CSF) and chemokines (eg, monocyte chemotactic protein 1, monocyte inhibitory protein 1α/β, and RANTES) that stimulate the recruitment and activation of secondary effector cells, including eosinophils and lymphocytes, thereby contributing to the late-phase response.

Current therapeutic strategies for the treatment of atopic disease rely heavily on the use of anti-inflammatory drugs, such as corticosteroids (for asthma) and antihistamines (for allergic rhinitis and atopic dermatitis).⁵ In addition, inhibitors of cysteinyl leukotrienes have been used in the treatment of asthma with some success. Although these treatments can be highly effective at controlling disease, they are often associated with systemic side effects at higher doses and variable patient response. Therefore efforts have been undertaken to develop novel therapies that specifically target the atopic disease process. One promising approach involves the use of anti-IgE antibodies to block IgE binding to cell surface FcεRI.^{6,7} In a recent study treatment of atopic individuals with a humanized murine mAb directed against the FcεRI-binding domain of IgE (rhAb-E25) led to decreased serum IgE levels and reduced early and late-phase responses to inhaled allergen.⁸ Finally, specific immunotherapy, also known as desensitization, involves repeated injection of allergen and has been used successfully for years in the treatment of allergy; however, the immunologic mechanisms underlying this approach are not known.^{9,10}

In an effort to define novel therapeutic approaches, some laboratories, including our own, have attempted to define molecular mechanisms that function as negative regulators of mast cell function. These studies have identified several inhibitory receptors that attenuate FcεRI-induced mast cell activation. These inhibitory receptors are members of a growing family of structurally and functionally related molecules that are expressed throughout the immune system and modulate activating signals transduced by a variety of receptors. The ability of inhibitory receptors to modulate immune responses implicates them and their downstream effectors as potential targets for the therapeutic intervention in the treatment of disease. Here we review the mechanisms that underlie the opposing effects of activating and inhibitory mast cell receptors.

FCεRI-MEDIATED SIGNAL TRANSDUCTION

FcεRI is a member of the multisubunit immune response receptor (MIRR) family of cell surface receptors that lack intrinsic enzymatic activity but transduce intracellular signals through association with cytoplas-

mic tyrosine kinases.¹¹ In rodents FcεRI is expressed on mast cells and basophils as a heterotetramer consisting of a single IgE-binding α-subunit, a β-subunit, and two disulfide-linked γ-subunits (Fig 1). The α-chain contains two extracellular Ig-like loops, a single transmembrane region containing an aspartic acid residue, and a short cytoplasmic domain that lacks signal transduction motifs. The charged amino acid within the transmembrane domain mediates the association of the α-subunit with the signaling-competent γ-subunit. The γ-subunit also associates with other activating immune receptors (eg, FcγRI, FcαR, and the TCR-CD3 complex) through a similar mechanism and is required for ligand-induced signal transduction.¹²⁻¹⁵ The β-subunit consists of 4 membrane-spanning domains and a cytoplasmic tail capable of transducing intracellular signals that amplify γ-mediated signaling events.

In rodents all 3 subunits (α, β, and γ) are required for the cell surface expression of FcεRI.^{11,16} In the absence of the β and γ subunits, the ligand-binding α-subunit is retained in the endoplasmic reticulum (ER) because of ER retention signals located within its cytoplasmic domain. Assembly of the 3 subunits masks the ER retention signal and allows cell surface expression of the αβγ₂ complex. In contrast, two FcεRI isoforms (αβγ₂ and αγ₂) are expressed in humans. In addition to its expression in mast cells and basophils, human FcεRI is expressed in monocytes and macrophages, Langerhans cells, dendritic cells, and eosinophils.¹¹ Interestingly, the tetrameric complex (αβγ₂) is expressed in human mast cells and basophils, whereas αγ₂ is expressed in monocytes, Langerhans cells, and dendritic cells. However, it is possible that human mast cells and basophils express both receptor complexes. As will be discussed below, the tyrosine kinase Lyn associates with the FcεRI β-subunit and is necessary for FcγRIIB-mediated inhibitory signaling in mast cells. Therefore αγ₂ may be less susceptible to inhibition by FcγRIIB compared with αβγ₂.

The β- and γ-subunits each contain a conserved immunoreceptor tyrosine-based activation motif (ITAM) within their cytoplasmic tails that is rapidly phosphorylated on tyrosine after FcεRI aggregation.^{16,17} The ITAM consensus sequence (YxxLx₆₋₈YxxL) is present in the cytoplasmic tails of a number of MIRR family members (eg, B-cell receptor [BCR], T-cell receptor [TCR], FcεRI, FcγRIIA, FcγRI, and FcαR) and is both necessary and sufficient for receptor-induced signal transduction.¹⁸ Tyrosine phosphorylation of the β and γ ITAMs is mediated by Lyn, which is constitutively associated with the β-subunit and activated after antigen-mediated FcεRI aggregation.^{16,17} FcεRI aggregation leads to the recruitment and activation of the tyrosine kinase Syk, which binds to the tyrosine phosphorylated γ ITAMs through its tandem SH2 domains.^{16,17} Recruitment of Syk occurs upstream of several signal transduction pathways and is a critical event in FcεRI-mediated signal transduction. This is demonstrated by using Syk-deficient mast cells, which fail to degranulate, synthesize leukotrienes, and secrete cytokines after FcεRI stimulation.¹⁹

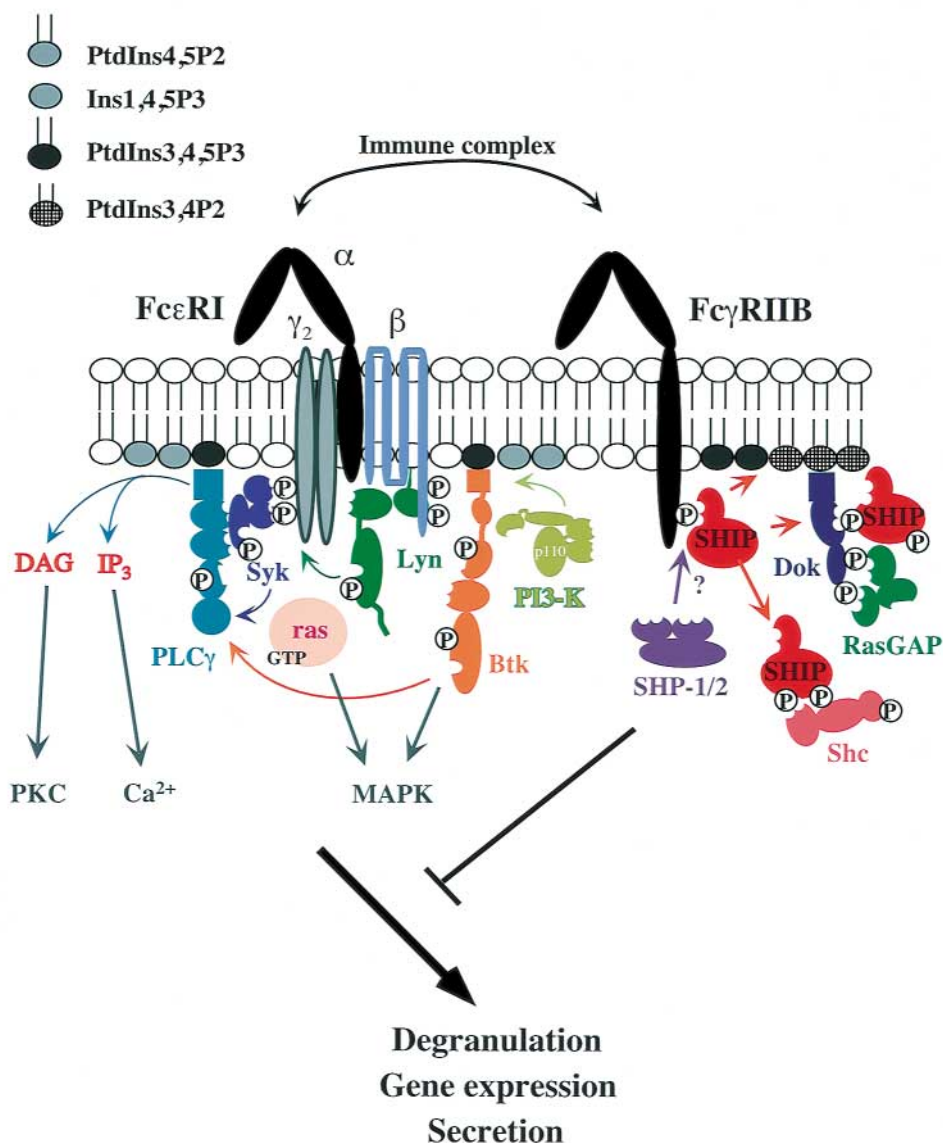


FIG 1. Inhibition of FcεRI signaling pathways by FcγRIIB. DAG, Diacylglycerol; PKC, protein kinase C; IP3, Ins (3,4,5) P₃.

A major downstream target of FcεRI-mediated signaling is phospholipase Cγ1, which catalyzes the hydrolysis of phosphatidylinositol (PtdIns)(4,5)P₂ to inositol (Ins)(1,4,5)P₃ and diacylglycerol second messengers.^{16,17} Ins(1,4,5)P₃ and diacylglycerol modulate intracellular calcium levels and activation of some protein kinase C isozymes, respectively, which are critical events for FcεRI-induced cytoskeletal organization, secretion, and gene expression. Activation of phospholipase Cγ1 requires its recruitment to the plasma membrane, where it is a substrate for tyrosine phosphorylation by Syk and Bruton's tyrosine kinase (Btk).^{17,20} In addition, Btk is required for FcεRI-induced activation of JNK/SAPK and, to a lesser extent, p38 MAP kinases.²¹ Activation of Btk requires its recruitment to the plasma membrane, where it binds through its pleckstrin homology domain to

PtdIns(3,4,5)P₃, which is synthesized from PtdIns(4,5)P₂ by phosphatidylinositol 3 (PI3) kinase.^{22,23} In B cells inhibition of PI3 kinase with a pharmacologic inhibitor results in decreased Btk phosphorylation and activity and inhibition of BCR-induced calcium mobilization and Ins(3,4,5)P₃ production.^{24,25} Similarly, in mast cells cross-linking of FcεRI activates PI3 kinase, and inhibition of PI3 kinase results in decreased Ins(3,4,5)P₃ production and FcεRI-induced histamine secretion and leukotriene production.^{26,27} These data support a model in which PI3 kinase catalyzes the formation of PtdIns(3,4,5)P₃, leading to the recruitment and activation of Btk and phospholipase Cγ1.

Antigen-mediated cross-linking of FcεRI also stimulates the recruitment and activation of p21^{ras}, which has been implicated in FcεRI-induced transcription and

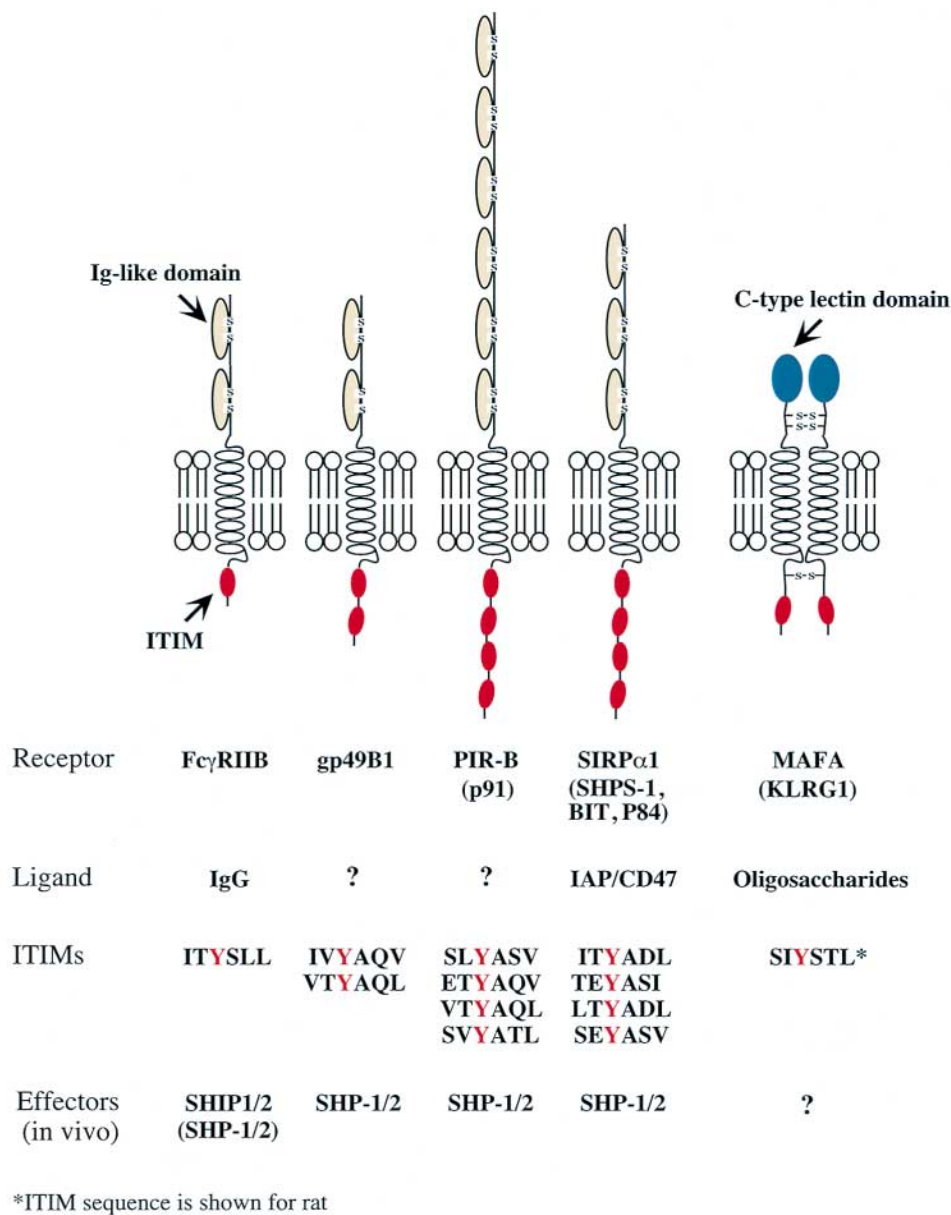


FIG 2. ITIM-containing inhibitory receptors expressed in mast cells. *SHPS-1*, src homology 2 domain-containing phosphatase substrate 1; *BIT*, brain Ig-like molecules with tyrosine-based activation motifs; *KLRG1*, killer cell lectin-like receptor G1.

secretion.^{16,17} Targets of p21^{ras} include, but are not limited to, the classical Ras/Raf-1/MEK/Erk cascade, JNK and p38 MAP kinases, PI3 kinase, and other GTPases (eg, Rac and Rho). Activation of p21^{ras} pathways is critical for the nuclear translocation of several transcription factors (eg, nuclear factor-κB, nuclear factor of activated T cells, and Elk-1) that regulate the synthesis of a variety of cytokines and chemokines.¹⁶ MAP kinases also mediate activation of phospholipase A₂, leading to the production of platelet-activating factor, PGD₂, and leukotriene C₄.

INHIBITORY RECEPTOR SUPERFAMILY

It has become increasingly clear that activation signals mediated by ITAM-containing immune receptors are negatively regulated by members of a growing family of structurally similar cell surface receptors known as the inhibitory receptor superfamily (IRS).²⁸ Members of the IRS are single-pass transmembrane receptors that contain either C-type lectin (ie, CD22, Ly49, NKG2, and mast cell function-associated antigen [MAFA]) or Ig-like (ie, FcγRIIB, killer cell inhibitory receptor [KIR], gp49B1,

Ig-like transcripts/leukocyte Ig-like receptors/monocyte/macrophage Ig-like receptors, leukocyte-associated Ig-like receptor, paired Ig-like receptor [PIR-B], and signal regulatory protein [SIRP α]) extracellular domains (Fig 2). IRS members are expressed in hematopoietic and nonhematopoietic cells and regulate a diverse array of cellular responses. For example, Fc γ RIIB is expressed in numerous cell types and inhibits cellular activation when coaggregated with the BCR, TCR, activating Fc receptors (eg, Fc γ RIII, Fc γ RIIA, and Fc ϵ RI) and cytokine receptors (eg, c-kit).²⁹ All members of the IRS contain at least one copy of a conserved sequence (I/VxYxxL), termed the *immunoreceptor tyrosine-based inhibition motif* (ITIM).³⁰ Coaggregation with activating receptors (eg, Fc ϵ RI and BCR) leads to the rapid phosphorylation of the ITIM tyrosine, providing a docking site for the SH2 domain-containing protein tyrosine phosphatases SHP-1 (65 kd) and SHP-2 (70 kd) and the Ins 5-phosphatases SHIP (145 kd) and SHIP2 (160 kd).^{30,31}

ITIM-containing inhibitory receptors differentially recruit SHP-1, SHP-2, and SHIP *in vivo*.^{32,33} For example, in recent studies comparing effector recruitment by Fc γ RIIB and the ITIM-containing KIR, Fc γ RIIB associates with SHIP, whereas KIR recruits SHP-1.³² These data suggest that different inhibitory receptors may elicit distinct biologic responses by recruiting specific effector molecules. Supporting this hypothesis, Fc γ RIIB inhibitory signaling is abrogated in SHIP-deficient B cells but unaffected in SHP-1-deficient cells. Conversely, KIR-mediated inhibitory signaling was significantly reduced in SHP-1-deficient cells but unaffected in SHIP-deficient cells. Recent studies indicate that the relative recruitment of SHIPs versus SHPs by Fc γ RIIB may be a function of coaggregate size. For example, coaggregation of Fc γ RIIB and the BCR leads to the recruitment and activation of SHIP; however, superclustering of the two receptors leads to SHP-1 activation.³⁴ In addition, results from *in vitro* studies indicate that SHIP binds to phosphorylated ITIM peptides immobilized to beads over a range of peptide densities, whereas SHP-1 binds only when the density of ITIM peptides is high (Daëron, unpublished observations). Therefore immune complexes containing different amounts of Fc γ RIIB ligand (ie, IgG) may evoke the activity of different effectors. These observations suggest an explanation for reports that Fc γ RIIB recruits both SHP-1 and SHIP during negative signaling in B cells.^{35,36} In addition, Fc γ RIIB mediates the translocation of SHP-1 from the cytosol to the plasma membrane in human basophils.³⁷

Recruitment of SHP-1, SHP-2, and SHIP is dependent on the phosphorylation of the ITIM tyrosine; however, additional residues within the ITIM contribute to effector specificity. For example, substitution of the amino acid residue in the Y-2 position in either the carboxyl-terminal (I; single letter amino acid code) or amino-terminal (V) ITIM in KIR prevents binding to SHP-1 and SHP-2, whereas the corresponding mutation in Fc γ RIIB (I $\rightarrow$ A) has no effect on SHIP binding.³⁸ The unique ability of Fc γ RIIB to bind SHIP is a function of specific amino acid residues in the Y+1 (S) and Y+2 (L) positions relative to the

ITIM tyrosine, which in KIR are occupied by alanine (A) and glutamine (Q) (Bruhns and Daëron, unpublished observations).

Fc γ RIIB

The first identified and most extensively studied member of the IRS is Fc γ RIIB, a low-affinity IgG receptor that functions in capping, endocytosis, and phagocytosis in response to binding multivalent antigen.³⁹ Fc γ RIIB is expressed throughout the hematopoietic system and exists in 3 alternatively spliced isoforms designated Fc γ RIIB1, Fc γ RIIB1', and Fc γ RIIB2, which differ only in their cytoplasmic domain sequences. All 3 isoforms contain two extracellular Ig-like loops and a single conserved ITIM within their cytoplasmic tails. Fc γ RIIB1 and Fc γ RIIB2 are expressed in both mice and humans, whereas Fc γ RIIB1' is expressed only in mice. The role of Fc γ RIIB in transducing inhibitory signals was first described in B cells. Coaggregation of Fc γ RIIB and the BCR, which occurs *in vivo* when B cells encounter antigen complexed with antigen-specific IgG, leads to inhibition of BCR-induced blastogenesis and proliferation^{40,41} and stimulates apoptosis.⁴² The importance of Fc γ RIIB inhibitory signaling in regulating immune responses is evident in Fc γ RIIB-deficient mice, which exhibit enhanced antibody production, anaphylactic responses, and autoimmunity.^{43,44}

All 3 Fc γ RIIB isoforms are expressed in murine mast cells.⁴⁵ When coaggregated with Fc ϵ RI in rat basophilic leukemia (RBL-2H3) cells, Fc γ RIIB1 and Fc γ RIIB2 inhibit Fc ϵ RI-mediated release of serotonin and TNF- α .^{46,47} In addition, Fc γ RIIA-induced mast cell degranulation is inhibited after Fc γ RIIA coaggregation with human Fc γ RIIB1 or Fc γ RIIB2. Coaggregation of Fc γ RIIB and Fc ϵ RI leads to the rapid tyrosine phosphorylation of the Fc γ RIIB ITIM tyrosine by Fc ϵ RI-associated Lyn.^{45,48} Synthetic peptides corresponding to the tyrosine phosphorylated Fc γ RIIB ITIM bind SHP-1, SHP-2, and SHIP from mast cell lysates; however, only SHIP associates with Fc γ RIIB *in vivo*. Supporting a predominant role for SHIP in Fc γ RIIB inhibitory signaling, bone marrow-derived mast cells (BMMCs) from SHP-1-deficient mice are equally susceptible to Fc γ RIIB inhibitory signaling compared with wild-type cells. Fc γ RIIB also inhibits c-kit-induced mast cell proliferation, demonstrating that Fc γ RIIB inhibitory activity is not limited to ITAM-mediated activation signals.⁴⁹ Coaggregation of Fc γ RIIB and c-kit is accompanied by the tyrosine phosphorylation of Fc γ RIIB and recruitment of SHIP but does not affect ligand-induced tyrosine phosphorylation of c-kit. Although this inhibition is of questionable physiologic significance, it raises the possibility of inhibiting mast cell production by using agents that dimerize c-kit and Fc γ RIIB.

In contrast to mast cells, Fc γ RIIB recruits both SHP-1^{34,50} and SHIP^{35,51} in B cells after coaggregation with the BCR. These data suggest a role for multiple effectors in Fc γ RIIB inhibitory signaling in B cells. Supporting this hypothesis, Fc γ RIIB1 fails to inhibit BCR-induced cell proliferation, but not calcium mobilization, in B cells

isolated from SHP-1-deficient mice.^{50,52} These data suggest that Fc γ RIIB may regulate mast cell and B-cell activation through distinct mechanisms. Recently, a second isoform of SHIP (SHIP2) has been cloned from humans, rats, and mice.⁵³ In B cells Fc γ RIIB recruits both SHIP (also known as SHIP1) and SHIP2 after coaggregation with the BCR, although the biologic significance of this observation is not known.³¹ To date, the role of SHIP2 in Fc γ RIIB inhibitory signaling in mast cells has not been investigated.

Recent studies have provided insight into the mechanisms through which SHIP mediates Fc γ RIIB inhibitory signaling in mast cells. When coaggregated with Fc ϵ RI, Fc γ RIIB inhibits Fc ϵ RI-induced calcium mobilization but does not affect Fc ϵ RI-induced tyrosine phosphorylation of the Fc ϵ RI β - and γ -subunits.^{48,54} In addition, Fc ϵ RI-induced tyrosine phosphorylation and activation of Syk are unaffected after coaggregation with Fc γ RIIB. SHIP contains an enzymatic domain that catalyzes the removal of the 5-phosphate from PtdIns(3,4,5)P₃ and Ins(1,3,4,5)P₄.⁵³ Recent evidence implicates SHIP in the direct cleavage of PtdIns(3,4,5)P₃,⁵⁵ which in mast cells would inhibit Fc ϵ RI-induced recruitment and activation of Btk and phospholipase C γ 1 and downstream signaling events required for Fc ϵ RI-mediated degranulation and gene expression (Fig 1). In B cells mutation of critical residues within the catalytic domain of SHIP blocks Fc γ RIIB-mediated inhibition of BCR signaling.³² However, results from a recent study suggest that the catalytic domain of SHIP alone is insufficient to mediate Fc γ RIIB inhibitory signaling.⁵⁶

In addition to its enzymatic activity, SHIP contains two carboxyl-terminal NPXY motifs and several proline-rich regions through which it may bind signaling molecules containing phosphotyrosine-binding and SH3 domains, respectively. In B cells coaggregation of Fc γ RIIB and the BCR induces the tyrosine phosphorylation of SHIP and subsequent binding to Shc and the RasGAP-binding protein p62^{dok} (Fig 1).^{57,58} It has been hypothesized that SHIP inhibits BCR-induced p21^{ras} activation by recruiting Shc away from the Grb2-Sos complex.⁵⁹ In another model Fc γ RIIB-BCR coaggregation stimulates the tyrosine phosphorylation of SHIP and subsequent binding to p62^{dok}.⁵⁸ Under these conditions, p62^{dok} is also phosphorylated on tyrosine and binds RasGAP, which is thought to inhibit p21^{ras} by enhancing its intrinsic GTPase activity.⁶⁰ Supporting a role for p62^{dok} in Fc γ RIIB inhibitory signaling, coaggregation of the BCR with a chimeric receptor in which the cytoplasmic domain of Fc γ RIIB is replaced with the RasGAP-binding region of p62^{dok} inhibits BCR-induced Ras/Erk activation.⁵⁸ Similarly, Fc γ RIIB fails to inhibit BCR-induced MAP kinase activation and cell proliferation in B cells from p62^{dok}-deficient mice.⁶¹ However, p62^{dok} is dispensable for Fc γ RIIB-mediated inhibition of calcium mobilization, suggesting that p62^{dok} is not the only downstream effector required for Fc γ RIIB inhibitory signaling.

To date, Fc γ RIIB is the only ITIM-containing inhibitory receptor known to recruit SHIP in vivo. However, BMMCs from SHIP-deficient mice readily degranulate

in response to IgE binding alone, whereas BMMCs from wild-type mice do not.⁶² These data suggest that SHIP mediates inhibitory signals other than those transduced by Fc γ RIIB. The mechanism or mechanisms through which SHIP mediates this effect are not known. Surprisingly, the SH2 domain of SHIP binds to the tyrosine phosphorylated ITAMs of the Fc ϵ RI β - and γ -subunits in vitro.^{63,64} Although these interactions have not been demonstrated in vivo, they suggest that SHIP may be activated directly in response to binding of IgE to prevent degranulation in the absence of antigen-induced Fc ϵ RI cross-linking. Alternatively, or in addition, SHIP may function downstream of other ITIM-containing inhibitory receptors that are constitutively activated by an unknown ligand or ligands.

gp49

gp49 was originally identified as a 49-kd cell surface glycoprotein preferentially expressed in BMMCs.⁶⁵ Screening of a mast cell cDNA library with oligonucleotides corresponding to the amino-terminal amino acid sequence identified 3 homologous cDNAs, now designated gp49A, gp49B1, and gp49B2.⁶⁶ The cDNAs for gp49A and gp49B1 predict type I integral membrane proteins containing two extracellular Ig-like loops that are 89% identical at the amino acid level. The cytoplasmic domains of gp49A and gp49B1 are 88% identical in the first 34 amino acids; however, gp49B1 contains an additional 32 amino acids at the carboxyl-terminus that contains two ITIMs. The gp49B2 cDNA predicts a protein identical to gp49B1, except it lacks the entire transmembrane domain, suggesting that gp49B2 may either be secreted or retained in the cytoplasm.

An inhibitory function for gp49B1 in mast cells was recently demonstrated. Coaggregation of gp49B1 and Fc ϵ RI in BMMCs inhibits Fc ϵ RI-induced secretion and synthesis of leukotriene C₄.⁶⁷ Tyrosine phosphorylated peptides corresponding to either ITIM sequence associate with SHP-1 and SHP-2 from RBL-2H3 cell lysates; in contrast, only the second ITIM (ETQDVITYAQLCIR) binds to SHIP.⁶⁸ This is a surprising result because the serine and leucine residues in the Y+1 and Y+2 positions in Fc γ RIIB, respectively, are critical for its binding to SHIP (see above). Association of gp49B1 and SHIP has not been demonstrated in vivo, and therefore the physiologic relevance of this interaction is unclear. Interestingly, mutation of either ITIM tyrosine results in a partial loss of gp49B1 inhibitory activity, whereas mutation of both ITIM tyrosines completely abolishes activity.⁶⁹ These data demonstrate that both ITIMs are required for full gp49B1 inhibitory function, possibly through the recruitment of multiple effector molecules. When coaggregated with Fc ϵ RI in BMMCs, gp49B1 associates transiently with SHP-1, but not SHP-2 or SHIP, suggesting a dominant role for SHP-1 in gp49B1 inhibitory signaling. However, gp49B1 still partially inhibits Fc ϵ RI-induced exocytosis in BMMCs derived from SHP-1-deficient mice, demonstrating that SHP-1 is not the exclusive mediator of gp49B1 inhibitory signaling.

In addition to mast cells, gp49B1 is expressed in natural killer (NK) cells cultured in the presence of IL-2.^{70,71} When expressed as a chimeric receptor with the extracellular domain of the ITIM-containing receptor KIR, gp49B1 inhibits NK cell-mediated killing of target cells.⁷⁰ These data suggest a role for gp49B1 in regulating both mast cell and NK cell function, possibly through a common ligand. At the present time, the ligand or ligands for gp49 family members have not been identified. Interestingly, the extracellular domains of gp49A and gp49B1 are postulated to form distinct 3-dimensional structures despite their high degree of amino acid homology.⁷² Therefore gp49A and gp49B1 may bind to different ligands, which could contribute to distinct biologic function. Alternatively, gp49A and gp49B1 could bind to the same or similar ligand but transduce distinct intracellular signals (activating vs inhibitory) by virtue of their divergent cytoplasmic domains.

PIR (also p91)

In 1997, two novel genes (*PIRA* and *PIRB*) were identified and cloned from a mouse splenic cDNA library by using a human Fc α R probe to identify novel Ig-superfamily members.^{73,74} *PIRA* and *PIRB* encode type I transmembrane proteins that contain 6 extracellular Ig-like loops but different transmembrane and cytoplasmic domains. *PIR-A* contains a short cytoplasmic domain (16 amino acids) that lacks obvious signal transduction motifs. However, when expressed as a chimeric receptor with the extracellular domain of Fc γ RIIB, the transmembrane and cytoplasmic domains of *PIR-A* stimulate calcium mobilization and degranulation in RBL-2H3 cells.^{75,76} Like other activating receptors (eg, Fc ϵ RI, Fc α R, Fc γ RI, and Fc γ RIIA), *PIR-A* associates with the ITAM-containing γ -subunit through a mechanism dependent on transmembrane charged amino acids, and this association is required for the stimulatory activity of *PIR-A* in mast cells.⁷⁶⁻⁷⁸

In contrast to *PIR-A*, *PIR-B* lacks charged amino acids within its transmembrane domain and contains a long cytoplasmic tail (178 amino acids) with 4 ITIMs.⁷⁴ When expressed as a chimeric receptor with the extracellular domain of Fc γ RIIB, *PIR-B* inhibits BCR- and Fc ϵ RI-induced B-cell and mast cell activation, respectively.^{75,79,80} Using tyrosine phosphorylated peptides corresponding to each of the 4 ITIMs, Yamashita et al⁸¹ showed that the third (ESQDVTYAQLCSR) and fourth (PEEPSVYATLAIH) ITIMs both bind SHP-1 and SHP-2. Surprisingly, the third ITIM also exhibits weak binding to SHIP. Interestingly, the sequence of the third ITIM is very similar to the SHIP-binding ITIM in gp49B1 (see above). In contrast, the first and second show little if any binding to SHP-1, SHP-2, or SHIP, again suggesting differential recruitment of specific effectors by individual ITIMs. Analogous to gp49B1, mutation of either the third or fourth ITIM in *PIR-B* either fails to inhibit or only partially inhibits *PIR-B* activity, whereas a double mutant shows a much greater response.^{79,81} However, retention of the ability of the double mutant to partially abrogate *PIR-B* signaling suggests a role for the other

ITIMs, or perhaps other transmembrane or cytoplasmic sequences, in transducing intracellular signals.

PIR-B recruits SHP-1 in RBL-2H3 cells after coaggregation with Fc ϵ RI.⁷⁹ Surprisingly, SHP-1 associates constitutively with *PIR-B* in primary macrophages.⁸² However, Ho et al⁸³ recently showed that *PIR-B* is constitutively phosphorylated and associates with SHP-1 in freshly isolated splenic B lymphocytes, macrophages, and granulocytes, but not in B-cell, macrophage, or mast cell lines. Supporting a role for SHP-1 (and SHP-2) in *PIR-B* inhibitory signaling, *PIR-B*-mediated inhibition of BCR signaling is significantly reduced in SHP-1 and SHP-2 double-deficient DT40 B cells but unaffected in SHIP-deficient cells.⁸⁰ In addition, *PIR-B* inhibitory signaling is only slightly reduced in SHP-1- or SHP-2-deficient cells, suggesting that SHP-1 and SHP-2 may have partially redundant functions.

The mechanism or mechanisms by which SHP-1, SHP-2, or both inhibit activation signals are not well understood. In B cells coaggregation of *PIR-B* and the BCR inhibits BCR-induced calcium mobilization.⁸⁰ However, unlike Fc γ RIIB, *PIR-B* inhibits membrane-proximal signaling events, including BCR-induced tyrosine phosphorylation of Ig α /Ig β , Syk, Btk, and phospholipase C γ 2.⁸⁴ Overexpression of a dominant negative SHP-1 mutant prevents *PIR-B*-mediated inhibition of tyrosine phosphorylation of Syk, Btk, and phospholipase C γ 2. These data suggest a model in which SHP-1 dephosphorylates Syk and Btk, leading to reduced kinase activity and decreased activation of phospholipase C γ 2. Supporting a role for SHP-1 in inhibiting Syk activity, B cells overexpressing a dominant negative SHP-1 mutant demonstrate enhanced BCR-induced calcium mobilization and ERK activation that are accompanied by enhanced Syk tyrosine phosphorylation and kinase activity.⁸⁵ These data suggest a critical role for SHP-1 in attenuating BCR-induced signaling, although the mechanism by which SHP-1 is activated in this system is not known.

The ligand or ligands for *PIR-A* and *PIR-B* are unidentified. *PIR* molecules are closely related to the human ILT/LIR/MIR family of inhibitory receptors, and ILT2 and ILT4 bind to classical or nonclassical MHC class I alleles.⁸⁶ Endogenous *PIRB* tyrosine phosphorylation is significantly reduced in β_2 m-deficient mice, implicating MHC class I as a potential *PIR* ligand.⁸³ However, the significance of this result is unclear because *PIR-B* tyrosine phosphorylation is unaffected in mice deficient in TAP1, which is required for stable MHC class I surface expression. Therefore it remains quite possible that *PIR-B* ligands are unrelated to MHC class I molecules.

SIRP (also src homology 2 domain-containing phosphatase substrate 1 [SHPS-1], brain Ig-like molecules with tyrosine-based activation motifs [BIT], and P84)

SIRP, also known as src homology 2 domain-containing phosphatase substrate 1, brain Ig-like molecules with tyrosine-based activation motifs, and P84, was originally

identified by virtue of its association with the protein tyrosine phosphatases SHP-1 and SHP-2 in non-hematopoietic cells.^{87,88} Subsequent cloning of SIRP cDNAs reveals a multigene family of transmembrane Ig-like molecules that can be divided into two subtypes based on the presence (SIRP α) or absence (SIRP β) of a cytoplasmic domain.⁸⁸ SIRP α 1 is the most characterized member of this family and consists of 3 extracellular Ig-like loops, a single hydrophobic transmembrane region, and a cytoplasmic domain containing at least two ITIMs.

Tyrosine phosphorylation of SIRP α 1 and subsequent association with SHP-2 has been reported in response to growth factor receptor activation (eg, insulin receptor, epidermal growth factor receptor, growth hormone, and nerve growth factor),⁸⁸⁻⁹⁰ stimulation with mitogens (eg, serum, insulin, and lysophosphatidic acid), and cell adhesion to extracellular matrix (eg, fibronectin and laminin).^{87,91} Overexpression of SIRP α 1 in NIH3T3 cells inhibits growth factor-induced DNA synthesis, MAP kinase activation, and oncogene-mediated transformation.⁸⁸ This activity requires the cytoplasmic domain of SIRP α 1 and, more specifically, tyrosine residues within the cytoplasmic domain.

In hematopoietic cells SIRP α 1 is highly expressed in macrophages and to a lesser extent in monocytes, granulocytes, and dendritic cells.⁹²⁻⁹⁴ In addition, transcripts for SIRP α 1 have been detected in human mast cell lines.⁹⁵ Although SIRP α 1 recruits primarily SHP-2 in nonhematopoietic cells, SHP-1 is the primary effector recruited in macrophages.^{82,92} The biologic significance of SHP-1 versus SHP-2 recruitment in different cell types is not understood. In contrast to SHP-1, SHP-2 has been implicated in the transduction of activating signals downstream of growth factor and cytokine receptor activation.⁹⁶ Results from genetic and biochemical studies implicate SHP-2 in the activation of the Ras-MAP kinase pathway downstream of growth factor receptor activation. In this regard SIRP α 1 is postulated to function as a positive regulator of growth factor signaling.

However, SHP-2 has also been implicated in the negative regulation of IFN-induced cytotoxic activity through inhibition of the Jak/STAT pathway.⁹⁷ In addition, SHP-2 has been implicated in cytotoxic T lymphocyte antigen-4-mediated inhibitory signaling in T cells.⁹⁸ Furthermore, a chimeric receptor containing the amino-terminal KIR ITIM recruits SHP-2 but not SHP-1 and is sufficient to inhibit BCR-induced IL-2 secretion and calcium mobilization in B cells and Fc ϵ RI-induced release of serotonin in mast cells.⁹⁹ When expressed as a chimeric receptor with the extracellular domain of Fc γ RIIB and coaggregated with Fc ϵ RI, SIRP α 1 blocks Fc ϵ RI-induced release of serotonin and TNF- α in RBL-2H3 cells.⁹⁵ In this system SIRP α 1 recruits SHP-2 and to a lesser extent SHP-1 after coaggregation.

Integrin-associated protein (IAP/CD47) has recently been identified as a ligand for SIRP α 1.^{100,101} IAP is a ubiquitously expressed cell surface glycoprotein that contains 5 membrane-spanning domains and is involved in the regulation of integrin function.^{102,103} IAP has been

implicated in the regulation of several adhesion-dependent processes. For example, IAP binds extracellular matrix molecules, including thrombospondin and vitronectin,^{104,105} and has been implicated in transendothelial and transepithelial migration of neutrophils.^{106,107} Antibodies specific for either SIRP α 1 or IAP inhibit the migration of neutrophils across endothelial monolayers, suggesting that an SIRP α 1/IAP receptor-ligand interaction is important in this process.¹⁰¹

SIRP α 1 and IAP have also been implicated in adhesion-mediated signaling in nonhematopoietic cells. SIRP α 1 and IAP colocalize in synapse-rich regions of the central nervous system, and SIRP α 1-coated substrates promote binding of cortical neurons and stimulate neurite outgrowth.^{100,108} These data support a model in which SIRP α 1 binding to IAP is involved in the formation, regulation, or both of synaptic function. Finally, IAP is also highly expressed in peripheral T lymphocytes and stimulates enhanced proliferation and IL-2 secretion in CD3-activated T cells.^{109,110} A SIRP α 1 homologue (MyD-1) was recently cloned from cattle and is expressed in monocytes and dendritic cells.⁹⁴ Interestingly, anti-MyD-1 antibodies inhibit antigen-specific proliferation of T cells induced by MyD-1⁺ monocytes, presumably by blocking MyD-1 binding to its cognate receptor on T cells. Mast cells express MHC class II molecules and, in the presence of antigen, stimulate proliferation of antigen-specific T cells through an MHC class II-dependent mechanism.^{111,112} Therefore SIRP α 1 expressed in mast cells may function as a coreceptor for antigen-dependent T-cell activation by binding to IAP.

MAFA (also KLRG1 and MAFA-L)

MAFA was originally identified as a novel membrane receptor expressed in RBL-2H3 cells that, when aggregated with specific antibodies, inhibits Fc ϵ RI-induced degranulation and secretion of de novo synthesized inflammatory mediators.^{113,114} Unlike other ITIM-containing receptors, cross-linking of MAFA alone generates inhibitory signals. However, recent studies indicate that MAFA is either localized in close proximity or directly associated with Fc ϵ RI on the cell surface.^{115,116} Cloning and expression of the full-length MAFA cDNA reveal a type II integral membrane protein bearing significant homology to members of the C-type (calcium dependent) lectin family of cell surface receptors.^{114,117}

MAFA is expressed at the plasma membrane as both a monomer and a disulfide-linked homodimer. The extracellular domain displays sequence homology with the carbohydrate-binding domain of calcium-dependent lectins and binds terminal mannose residues in a calcium-dependent manner.¹¹⁸ The cytoplasmic tail contains a single ITIM-like motif (SIYSTL); however, its orientation is reversed relative to a typical ITIM. Cross-linking of MAFA or Fc ϵ RI alone or coaggregation of both receptors stimulates phosphorylation on serine and tyrosine residues, presumably within this motif.¹¹⁷ Tyrosine phosphorylated peptides corresponding to the ITIM-like motif bind SHP-1, SHP-2, and SHIP from mast cell

lysates.¹¹⁹ In addition, the serine residue at the Y-2 position provides selectivity for peptide binding to the SH2 domains of SHP-1 and SHP-2 in vitro, demonstrating characteristic features of an ITIM. However, these associations have not been demonstrated in vivo.

Recently, the mouse homologue of rat MAFA (rMAFA) has been identified and cloned.¹²⁰⁻¹²² This molecule, known as killer cell lectin-like receptor G1 (KLRG1; formerly mouse MAFA or 2F1-Ag), displays 89% similarity at the amino acid level compared with its rat homologue and is expressed as a homodimer of glycosylated 30- to 38-kd subunits. However, unlike rMAFA, KLRG1 is highly expressed in NK cells and a small population of T cells but not detected in mouse mast cell lines, BMMCs, or peritoneal mast cells. Furthermore, a recently described human homologue of rMAFA, designated MAFA-like receptor (MAFA-L), shows 54% homology to rMAFA and is expressed in both NK cells and basophils.¹²³ Together, these data suggest a role for MAFA in other cell types, although its function in these cells has not been determined.

CD81 (also target of antiproliferative antibody 1 [TAPA-1])

CD81 is a member of the tetraspan family of cell surface molecules that includes CD9, CD53, CD63, and CD82.¹²⁴ Expression of CD81 is detected in a wide range of immune cells, including B and T lymphocytes, granulocytes, and monocytes. In B cells CD81 is localized to the CD19-CD21 complex and functions as a costimulatory molecule in BCR signaling.¹²⁵ In T cells CD81 has been implicated in both stimulatory and inhibitory signaling.^{126,127}

In an attempt to identify membrane proteins that regulate FcεRI signaling, mAbs raised against RBL-2H3 cells were screened for their ability to inhibit FcεRI-induced mast cell degranulation.¹²⁸ Two antibodies that inhibit FcεRI-induced degranulation were found to recognize CD81. Anti-CD81 antibodies inhibit IgE-dependent passive cutaneous anaphylaxis reactions, confirming an inhibitory role for CD81 in normal mast cells in vivo. However, unlike typical inhibitory receptors, CD81 lacks an ITIM in its cytoplasmic domain and fails to inhibit FcεRI-induced tyrosine phosphorylation, calcium mobilization, and leukotriene C₄ production. This effect is specific for FcεRI-induced signaling pathways because CD81 fails to inhibit degranulation induced by phorbol ester or calcium ionophore. Together, these data indicate that CD81 inhibits mast cell degranulation but not other FcεRI-induced responses through a mechanism distinct from ITIM phosphorylation and phosphatase recruitment.

CONCLUDING REMARKS

Mast cells express multiple ITIM-containing inhibitory receptors that have the potential to regulate antigen-mediated FcεRI signal transduction and cell activation. The biologic significance of multiple inhibitory receptors is not well understood; however, identification of the li-

gands for many of these receptors should provide insight into their role in the regulation of mast cell function. Of the inhibitory receptors discussed above, only FcγRIIB is known to coaggregate with FcεRI under physiologic conditions (ie, in the presence of allergen complexed with IgG) and may therefore be relevant to FcεRI signaling in vivo. Coaggregation of FcγRIIB and FcεRI suggests a possible mechanism to explain the beneficial effects of specific immunotherapy in the treatment of allergy. As increasing doses of allergen are administered to allergic patients, an allergen-specific IgG response develops, leading to the formation of allergen-IgG complexes that are postulated to inhibit IgE-induced mast cell activation by coaggregating FcεRI and FcγRIIB.^{9,129} This mechanism of inhibition may have important implications in the treatment of atopic disease. For example, administering of allergen-IgG complexes to patients with allergic asthma significantly attenuated allergic symptoms.¹³⁰ Although the biologic function of many inhibitory receptors expressed in mast cells is not known, therapeutic strategies designed to coaggregate inhibitory receptors with FcεRI may provide novel approaches for the treatment of atopic disease.

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