REVIEW

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Negative signals from FcεRI engagement attenuate mast cell functions

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Abstract

Mast cells have long been recognized as the critical tissue-based effector cells in IgE-mediated allergic diseases. Ligation of the high-affinity receptor for IgE (FcεRI), constitutively expressed on mast cells, promotes cell activation and immediate release and production of pro-inflammatory mediators. Besides these positive signals, FcεRI aggregation has recently been understood to generate negative intracellular signals capable of limiting mast cell functional responses. This review is aimed at providing a summary of the mechanisms through which FcεRI engagement can generate negative signals and regulate mast-cell function. Similar mechanisms are employed by other receptors expressed by immune cells, such as T cell and B cell receptors, pointing to a general concept in negative immunoreceptor signaling.

Key words: mast cell, FcεRI, signal transduction, negative regulation.

Abbrevations: BMMC – bone marrow-derived mast cell, CIN85 – Cbl-interacting protein of 85 kDa, ERK – extracellular signal-regulated kinase, FcεRI – high-affinity receptor for IgE, ITAM – immunoreceptor tyrosine-based activation motif, ITIM – immunoreceptor tyrosine-based inhibitory motif, LAB – linker for activation of B cell, LAT – linker for activation of T cell, MAPK – mitogen-activated protein kinase, PI3K – phosphatidylinositol 3-kinase, PLC – phospholipase C, PTK – protein tyrosine kinase, PTEN – phosphatase and tensin homologue deleted on chromosome 10, RTK – tyrosine kinase receptor, SHIP – SH2 domain-containing inositol-polyphosphate 5-phosphatase, SHP – SH2 domain-containing protein tyrosine phosphatase, SLP-76 – SH2-containing leukocyte protein of 76 kDa.

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INTRODUCTION

Mast cells are key components in the initiation of allergic diseases and have essential roles in host defense against certain types of parasitic infections through the binding of antigen-specific immunoglobulin E (IgE) [13, 38]. The manifestation of mastcell-driven allergic reactions are mainly a consequence of allergen interaction with IgE bound to its high-affinity receptor, FcεRI, expressed on the mast cell surface [39, 43]. There is increasing evidence, however, that receptors for other ligands, such as pathogen-associated molecular patterns, complement components, cytokines, and stem cell factor, can markedly influence mast cell activation and participate in a wide variety of physiological and pathological processes [13, 38]. Thus, mast cells can contribute to the initiation and regulation of both innate and acquired immune responses.

Studies of mast cell activation and signal transduction, however, have mainly been driven by the acknowledged central role of FcεRI engagement in allergic inflammatory response. FcεRI is a member of the multisubunit immunoreceptor family that lacks intrinsic enzymatic activity but transduces intracellular signals through association with cytoplasmic protein tyrosine kinases (PTKs) [43, 60]. Regulation of the signals generated as a consequence of antigen interaction with specific IgE bound to FcεRI is a highly ordered process that causes a shift in the resting state equilibrium of phosphorylation and dephosphorylation that serves to maintain homeostasis. The outcome of this activated state is the release of preformed mediators and the *de novo* synthesis of eicosanoids, cytokines, and chemokines that results in both immediate and late-phase hypersensitivity reactions [3, 15, 43, 60].

Appropriate activation of mast cells upon FcεRI engagement is mediated by a number of factors, including the strength of the stimuli. Mast cell degranulation upon triggering with IgE and increasing concentrations of multivalent antigen follows a bell-shaped dose-

-response curve. The reduced response at supraoptimal antigen doses depends on excess of FcεRI cross-linking and increased receptor immobilization due to cytoskeletal interactions [36, 46, 56]. More recently, evidence has been provided that FcεRI engagement with supraoptimal doses of antigen can favor the action of negative regulators of signal propagation [14, 16].

Inhibitory stimuli can also be generated by ligation or co-ligation of inhibitory receptors with FcεRI [2, 4, 27]. Such inhibitory receptors are constitutively or inducibly expressed on the membrane of mast cells and belong to different families that share the ability to recruit and activate protein and lipid phosphatases, i.e. SH2 domain-containing protein tyrosine phosphatase (SHP) and SH2 domain-containing inositol-polyphosphate 5-phosphatase (SHIP).

During the past few years, the existence of a negative feedback loop initiated upon FcεRI engagement has also been envisaged. In fact, FcεRI signaling has been understood to consist of a mixture of positive and negative signals whose integration determines the rate and the extent of functional mast cell responses. Interestingly, signaling molecules, including PTKs, and adaptors initially thought to contribute only activating signals can also generate negative signals [55]. The concomitant discovery of increasing numbers of new inhibitory adaptors and their molecular interactions has revealed an intricate balancing system that contributes significantly to regulate the allergic responses.

Several recent and commendable reviews provide details on IgE-mediated intracellular signals and the outcome of mast cell activation [3, 15, 43, 60]. This review is intended to summarize the current understanding of the negative regulation of signals generated in response to FcεRI engagement in mast cells. A brief discussion of negative signaling induced upon coligation of FcεRI and inhibitory receptors is also included.

FcεRI-MEDIATED SIGNAL TRANSDUCTION

In both humans and rodents, FcεRI is expressed on the cell surface of mast cells (and basophils) as a heterotetramer composed of an α subunit, a four transmembrane-spanning β subunit, and two disulfide-linked γ subunits [39, 43]. The IgE-binding α-chain contains two extracellular Ig-like loops, a transmembrane region with 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-chain with the signaling-competent γ subunit. In addition, the β subunit contains a cytoplasmic tail capable of transducing intracellular signals that amplify the γ-initiated signaling events [35]. The β and γ subunits each contain a conserved immunoreceptor tyrosine-based activation motif (ITAM) within their cytoplasmic tails that is rapidly phosphorylated on tyrosines after FcεRI aggregation. Tyrosine phosphorylation of the β and γ ITAMs is mediated by the Src family PTK Lyn, which is constitutively associated with the β subunit and activated upon antigen-mediated FcεRI aggregation. This phosphorylation event leads to the recruitment and activation of the cytoplasmic PTK Syk, which binds to the tyrosine phosphorylated γ ITAM through its tandem SH2 domains [43, 60]. The activation of Syk is essential for all known FcεRI-mediated responses, including the secretion of allergic mediators and the induction of gene transcription. This was demonstrated by using Syk-deficient mast cells, which fail to degranulate, synthesize leukotrienes, and secrete cytokines after FcεRI stimulation [6].

These early phosphorylation events lead to the recruitment of molecules, e.g. linker for activation of T cells (LAT), SH2-containing leukocyte protein of 76 kDa (SLP-76), and the linker for activation of B cells (LAB, also called NTAL), [60], and to the activation of enzymes such as phospholipase Cγ (PLCγ), which regulates intracellular calcium release and protein kinase C activation by hydrolyzing the membrane phosphatidyl inositol 4,5-bisphosphate [PtdIns(4,5)-P_2] to form soluble inositol 1,4,5-trisphosphate and membrane-bound diacylglycerol (Fig. 1A).

Recruitment of adaptor proteins, such as SLP-76, Shc, and Grb2, and exchange factors, such as Sos and Vav, culminates in the activation of the small GTPases, Ras, Rac, and Rho. These GTPases regulate the activation of mitogen-activated protein kinase (MAPK) family members, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase, and p38, which control the activity of numerous transcription factors important for the synthesis and secretion of lipid mediators and cytokines [3, 15, 60].

Another PTK of the Src family, Fyn, has recently shown to associate with FcεRI and to play a complementary role in mast cells by activating the phosphatidylinositol 3-kinase (PI3K) [51]. Upon FcεRI engagement, Fyn phosphorylates the protein Gab2 that links FcεRI to the PI3K activation by binding to the p85 regulatory subunit. Once activated, PI3K catalyzes the formation of PtdIns(3,4,5)P_3, leading to the recruitment and activation of Bruton's tyrosine kinase and PLCγ1 (Fig. 1B). Thus, consistent with the requirement for more than one Src PTK in the early step of T and B cell activation, FcεRI also requires two Src family PTKs, Lyn anf Fyn, for triggering mast cell activation.

Recent evidence suggests that in addition to generating positive signals, the FcεRI β and γ subunits can also function to regulate mast cell activation and effector functions negatively [12, 29, 30, 41, 52, 67].

ITAM OF β AND γ CHAINS: A DUAL-FUNCTION MOTIF

An early description of inhibition by the ITAM-containing FcεRI β and γ subunits was reported by Torigoe et al. [67] in a study examining activation of a rat mucosal-type mast cell line, RBL-2H3. The cells were sensi-

tized with IgE against two antigenically distinct haptens that bind to their respective IgE with different affinities and then stimulated with the two antigens used alone or in combination. Phosphorylation of the main downstream signaling molecules, Syk and ERK, was decreased when the cells were exposed to the low-affinity antigen (used at a concentration 40 times higher than that of the high-affinity hapten) and, to a greater extent, in response to a mixture of the low- and high-affinity antigens. The authors hypothesized that the inhibitory effects were due to sequestration of Lyn by the high number of IgE receptors cross-linked with the low-affinity ligand. However, it is equally plausible that, under these conditions, phosphatase recruitment to the ITAMs can cause reduced activation of downstream signaling components. To this regard, more recent studies showed that SHIP, SHP-1, and SHP-2 bind to the phosphorylated FcεRI β subunit and become tyrosine phosphorylated upon receptor engagement in RBL-2H3 cells [29, 30].

An additional study [12] revealed a direct contribution of the FcεRI β subunit to negative signaling via a noncanonical tyrosine located between the two YxxL motifs of its ITAM. Mutation of this residue caused a marked increase in bone marrow-derived mast cell (BMMC) cytokine production associated with increased ERK, p38 MAPK, and NF-κB activation and decreased tyrosine phosphorylation of SHIP. All together these results suggest that the inhibition observed under certain conditions of FcεRI aggregation might be the result of phosphatase recruitment and activation.

In addition to the FcεRI β subunit, recent findings also extend a negative regulatory role to the FcεRI γ--chain. The γ-chain dimer is part of other FcR complexes, including the FcαRI expressed on blood myeloid cells [41]. Engagement of FcαRI by monomeric ligands was found to inhibit IgE-induced degranulation of RBL--2H3 transfectants expressing FcαRI, or FcαRI-FcRγ chimeras, while substained receptor clustering resulted to cell activation [52]. Such dual function was not observed in the context of FcεRI, which fails to be inhibitory when targeted monovalently. The inhibitory response observed in the context of FcαRI required an intact γ ITAM, which recruits the tyrosine phosphatase SHP-1 after weak phosphorylation.

All together, these findings indicate that the strength of the stimulus and/or the receptor context in which β and γ ITAMs are engaged might determine whether they function as positive or negative regulators of mast cell function.

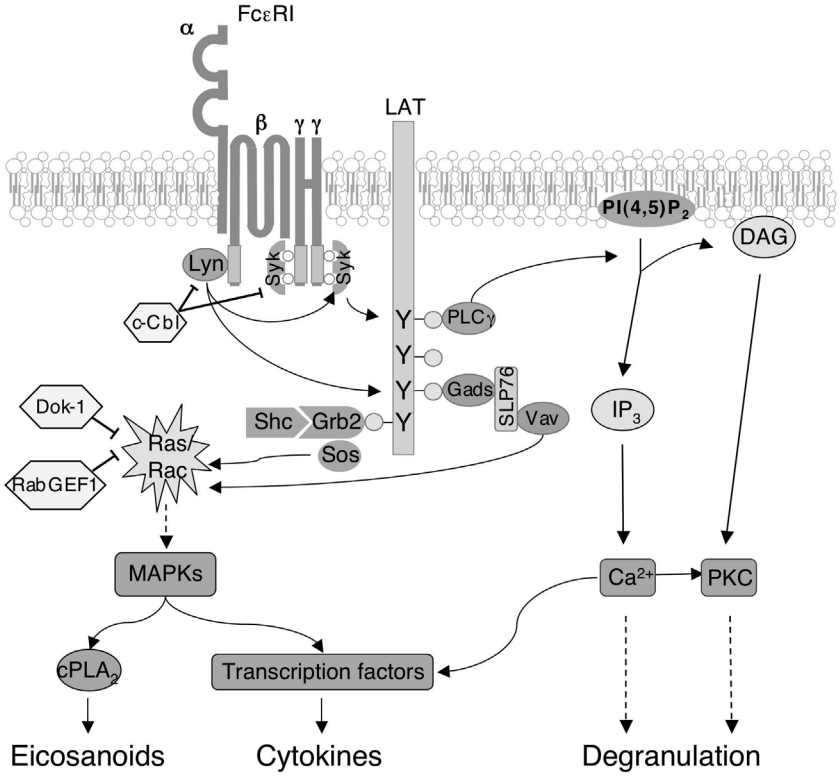

Fig. 1A. Simplified scheme of FcεRI-induced signaling events in mast cells. Upon FcεRI engagement, Lyn-mediated tyrosine phosphorylation of β and γ ITAMs leads to the recruitment and activation of Syk. Both Lyn and Syk PTKs phosphorylate the transmembrane adaptor molecules LAT and LAB, which are resident in raft microdomains. These proteins function as scaffolds and organize other adaptors, thus propagating the intracellular signal. **A** – LAT phosphorylation results in the recruitment and activation of PLCγ, which regulates Ca^{2+} mobilization and protein kinase C (PKC) activity, leading to mast cell degranulation. LAT-dependent recruitment of cytoplasmic adaptors and exchange factors culminates in the activation of the small GTPases Ras and Rac which, through activation of the MAPK cascades, control the synthesis and secretion of cytokines and eicosanoids. IP3 – 1,4,5 trisphosphate, DAG – diacylglycerol.

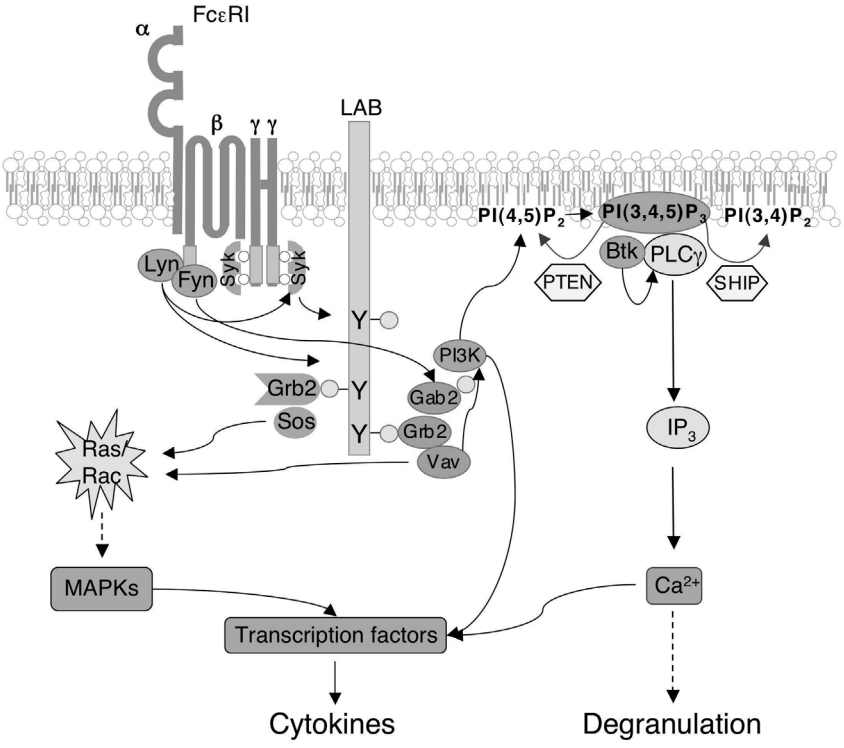

Fig. 1B. LAB phosphorylation results in the recruitment of adaptors such as Gab2 that are phosphorylated by Fyn and control PI3K activation, resulting in a complementary pathway leading to degranulation and cytokine production. As indicated in the text, FcεRI engagement activates a number of proteins that regulate these positive signalling pathway negatively (indicated by - - -). The negative regulators include c-Cbl, which promotes the ubiquitination of FcεRI, Lyn, and Syk; Dok-1 and RabGEF1, which regulates Ras/Raf/ERK pathway negatively; the lipid phosphatase PTEN, which dephosphorylates PtdIns(3,4,5)P_3 at position 3'; and the lipid phosphatase SHIP, which dephosphorylates PtdIns(3,4,5)P_3 at position 5'. Btk – Bruton's tyrosine kinase.

NEGATIVE REGULATION BY PTKs

In addition to the positive role of Lyn in initiating mast cell signaling, negative regulatory functions of Lyn have been well documented: BMMCs from Lyn-deficient mice showed increased degranulation and cytokine gene expression [28, 44], enhanced Fyn PTK activity and reduced SHIP activity [20, 44, 51], and prolonged activation of MAPKs, such as ERK-1, ERK-2, and p38 [51].

A more recent study showed that Lyn might assume a positive or negative role in mast cell activation depending on the stimulus strength [71]. Lyn functioned as a positive regulator of survival, degranulation, and cytokine production when BMMCs were stimulated with low-intensity stimuli such as monomeric IgE, IgE plus anti-IgE antibody, or IgE plus low doses of multivalent antigen (10 ng/ml), whereas BMMCs were negatively regulated by Lyn upon strong stimulation with IgE plus high doses of antigen (100 ng/ml).

The negative regulatory role of Lyn is exerted specifically through the FcεRI β subunit, which is associated with Lyn and is mainly recruited to lipid rafts upon stimulation with a high dose of antigen [71]. These observations suggest that Lyn-mediated negative regulation by strong stimulation is initiated in lipid rafts. In this regard, more recent studies demonstrated that defects in cholesterol synthesis resulted in exaggerated activation of mast cells [31].

Collectively, these findings show that Lyn PTK plays a broad negative regulatory role *in vivo* that is important for the control of mast cell responsiveness.

In contrast to the apparent negative role of Lyn, Fyn knock-out (KO) and Lyn/Fyn double KO mice were resistant to anaphylactic challenge, and BMMCs derived from these mice were markedly defective in degranulation, indicating a positive role for Fyn in promoting mast cell degranulation *in vivo* [44, 51]. However, exceptions to the role of Fyn as a positive regulator have been observed. Fyn deficiency did not affect ERK1/2 phosphorylation and increased IL-13 secretion [17], suggesting that Fyn exerts a negative regulatory role for selected cytokines. Additionally, gene expression profiling studies [17] have demonstrated increased FcεRI-dependent expression of some granule-localized enzymes (protease 4, granzyme B, and cathepsin D) involved in the inflammatory response in *fyn*$^{-/-}$ BMMCs, suggesting the existence of other possible candidate genes whose expressions are negatively regulated by Fyn.

NEGATIVE REGULATION BY LIPID PHOSPHATASES

A key step in mast cell activation is mediated through the enzymatic activity of PI3K, whose product,

PtdIns(3,4,5)P_3, binds to the pleckstrin homology domain of various signaling proteins, allowing their activation [51].

The lipid phosphatases SHIP and the phosphatase and tensin homologue deleted on chromosome 10 (PTEN) dephosphorylate PtdIns(3,4,5)P_3 at positions 5' and 3', respectively, thus affecting signal propagation [7].

SHIP deficient BMMCs exhibited enhanced responsiveness to FcεRI stimulation in terms of degranulation and proinflammatory cytokine production compared with wild-type BMMCs [24, 25]. These results support a role for SHIP in limiting murine mast cell activation. Relevant to this, Vonakis et al. [69] demonstrated that "hyper-releasable" basophils derived from highly allergic donors express lower levels of SHIP than the basophils from healthy donors, suggesting a role for SHIP as negative regulator of human basophil degranulation as well. More recently, SHIP-deficient mice were shown to develop a spontaneous allergic inflammatory response in the lung [45], suggesting that under physiological conditions, SHIP can play a role in maintaining lung homeostasis.

PTEN is recognized as a tumor suppressor and a key regulator of cell growth and apoptosis [5]. The loss of PTEN in human mast cells has revealed an increase in PtdIns(3,4,5)P_3 levels, even in the absence of FcεRI engagement [11]. Furthermore, the enzymatic activity of c-Jun and p38 MAPKs increased in PTEN deficient cells and was accompanied by a constitutive secretion of cytokines, supporting a role for PTEN in the control of mast cell homeostasis.

NEGATIVE REGULATION BY MOLECULAR ADAPTORS

Multiple adaptor proteins have been identified in mast cells as substrates of Lyn, Fyn, and Syk PTKs. These proteins function in the organization of the supramolecular complexes required for signaling and can directly influence signal propagation and, in some cases, mast cell responses [55].

LAT, LAB, and LAX

Among the various adaptors expressed in mast cells, LAT and LAB have been proposed to have both positive and negative regulatory functions on mast cells. The LAT intracytoplasmic domain contains multiple tyrosines which, when phosphorylated, provide docking sites for SH2 domain-containing cytosolic enzymes and adaptors. On the basis of mutational analysis, one tyrosine (136) was shown to recruit PLCγ and the three more distal tyrosines (175, 195, and 235) to recruit several adaptor molecules, thus contributing to signal propagation [58] (Fig. 1A).

LAT can regulate mast cell activation by generating not only positive, but also negative signals, depending on the tissue origin of the mast cell and on the strength of the stimuli. The authors examined IgE- and/or IgG--induced biochemical events and the secretory response of BMMCs derived from LAT knock-in mice in which either the PLCγ-binding tyrosine or the three adaptor--binding tyrosines were mutated. They found that LAT tyrosines differentially contribute to exocytosis and cytokine secretion: the PLCγ-binding tyrosine had a negative effect on mediator release when adaptor-binding tyrosines were mutated and, conversely, adaptor-binding tyrosines had a negative effect when the PLCγ-binding tyrosine was mutated. Furthermore, they showed that LAT differentially regulates biological responses of mucosal- and serosal-type mast cells [37].

Although LAB/NTAL is structurally similar to LAT, it lacks the binding motif for PLCγ [22]. BMMCs derived from LAB KO mice exhibited enhanced antigen-mediated calcium mobilization, ERK activation, degranulation, and cytokine production [66, 68, 74], indicating that LAB negatively regulates mast cell function. The earliest event affected was enhanced tyrosine phosphorylation of LAT accompanied by enhanced tyrosine phosphorylation and enzymatic activity of PLCγ [68]. To date there are no reports of a direct association of phosphatases with LAB which could explain its inhibitory regulation. However, one proposed scenario is that LAB could compete with LAT for lipid raft occupancy, thus possibly downregulating LAT function [74]. Interestingly, BMMCs that lack both LAT and LAB proteins have a more severe block in FcεRI-mediated signaling than *lat*$^{-/-}$ mast cells, indicating that LAB also plays a positive role [74].

LAX is another adaptor molecule recently identified in both lymphocytes and mast cells [73, 75]. In contrast to LAT and LAB, LAX is not localized to lipid rafts [73]. Upon FcεRI engagement, LAX is phosphorylated and interacts with Grb2 and the p85 subunit of PI3K [75]. *Lax*$^{-/-}$ BMMCs were hyperresponsive to stimulation via the FcεRI, as evidenced by enhanced PI3K activation and degranulation [75]. This hyperresponsiveness was a consequence of LAB down-modulation; however, how LAX regulates LAB expression remains to be determined. These results suggest a negative regulatory role of LAX in FcεRI-mediated signaling and mast cell functions.

Dok and RabGEF1

The adaptor protein downstream of tyrosine kinase (Dok)-1 is constitutively associated with FcεRI in the rat mast cell line RBL-2H3 and becomes tyrosine phosphorylated upon receptor engagement [1]. Once phosphorylated, Dok-1 binds to the exchange factor RasGAP and functions to regulate the Ras/Raf/ERK pathway and TNF-α production negatively [1].

Using functional genomics approaches, Tam et al. [63] recently identified a novel exchange factor able to regulate the Ras/Raf/ERK pathway negatively, namely RabGEF1. BMMCs derived from RabGEF1 KO mice exhibited enhanced degranulation and increased release

of lipid mediators and cytokines upon FcεRI stimulation. Interestingly, an additional study by the same group [26] revealed a delayed antigen-induced FcεRI internalization in RabGEF1-deficient BMMCs, supporting a role for the exchange factor in controlling receptor endocytosis.

The Cbl proteins

In the last few years a new class of molecular adaptors, namely Cbl family proteins, has emerged as negative regulators of immunoreceptor-mediated signals [9, 57, 64]. Cbl proteins are structurally characterized by the presence of multiple protein-protein interaction domains: an N-terminal tyrosine kinase-binding domain that includes a modified SH2 domain, a RING finger domain, a proline-rich domain, and a C-terminal ubiquitin-associated domain. Three mammalian Cbl proteins are currently identified: c-Cbl, Cbl-b, and Cbl-c (also known as Cbl-3). The function of the Cbl proteins as regulators of signal transduction pathways is largely based on their ubiquitin ligase activity. Ubiquitination is a post-translational reversible modification whereby ubiquitin, a small and highly conserved peptide, is bound to acceptor proteins that become targeted for degradation by the 26S proteasome [21, 34]. In recent years it has become evident that ubiquitination plays also an essential role in proteasome-independent functions. Polyubiquitination, a modification in which a ubiquitin chain is bound to the substrate, has been mainly associated with proteasomal degradation [10, 65]; monoubiquitination, however, can act as a signal for receptor internalization and sorting into vesicles of the secretory/endocytic pathway [23, 42].

Ubiquitination occurs via the sequential action of different enzymes, namely E1, E2, and E3 [21, 34, 70]. The E3s are ubiquitin protein ligases and provide specificity to the system: they are responsible for substrate recognition and for promoting Ub ligation to the target protein. A recently identified class of E3 ligases, including the Cbl family proteins, is characterized by the presence of a RING Finger domain that serves as a binding site for specific E2 Ub carrier enzymes [70].

Due to the presence of different protein-protein interaction domains, Cbl proteins recognize their substrates, represented by cytoplasmic signaling proteins or membrane receptors, both in a phosphorylation-dependent and -independent manner. c-Cbl and Cbl-b are both expressed in BMMCs and in the rat mast cell line RBL-2H3, become tyrosine phosphorylated upon FcεRI engagement, and translocate into lipid rafts [33, 47, 54, 72]. The first evidence for a negative role played by c-Cbl in mast cells came from experiments in which c-Cbl overexpression inhibited Syk kinase activity and receptor-mediated serotonin release in RBL-2H3 cells without affecting Lyn activity and receptor phosphorylation [48]. The molecular mechanism underlying this inhibition was elucidated by recent studies demonstrating the involvement of c-Cbl in the ubiquitination of FcεRI receptor subunits and Syk in RBL-2H3 cells [33, 50].

An earlier study from our group [49] demonstrated that the FcεRI β and γ subunits were subjected to ubiquitination upon stimulation of RBL-2H3 cells with IgE and multivalent antigen. Subcellular fractionation and confocal microscopy experiments subsequently demonstrated that c-Cbl colocalizes with FcεRI β and γ subunits into lipid rafts after receptor engagement, suggesting the involvement of Cbl in receptor ubiquitination [33].

Our group identified c-Cbl as the main E3 ligase responsible for the antigen-induced receptor ubiquitination in RBL-2H3 cells [50]. Overexpression of wild-type Cbl, but not a mutant form deleted in the RING finger domain, increased antigen-induced FcεRI β and γ ubiquitination, providing evidence for a direct role of Cbl as a ubiquitin ligase. In the same study we also demonstrated that c-Cbl is responsible for Syk ubiquitination and that Syk activity controls its own ubiquitination as well as that of the FcεRI β and γ subunits. Most of the ubiquitinated forms were also phosphorylated, suggesting that ubiquitination preferentially affects engaged receptors and the activated forms of Syk [50]. Syk and c-Cbl were previously reported to be constitutively associated in RBL-2H3 cells [47]. Thus it is conceivable that Syk, acting as an adaptor, brings Cbl in proximity of the engaged receptor, thus allowing the ligase to transfer ubiquitin to the FcεRI β and γ subunits. Our results also suggest that Syk/Cbl interaction is important because it allows the enzymes to become reciprocal substrates: Syk phosphorylates and activates Cbl which, in turn, ubiquitinates activated Syk and phosphorylated receptor subunits (Fig. 2). Furthermore, proteasome inhibition induced a persistence of tyrosine phosphorylated β chain and activated forms of Syk, supporting the involvement of Cbl-dependent ubiquitination in the down-regulation of engaged receptors and the active pool of Syk.

In addition to Syk, recent reports have demonstrated that both Lyn and Fyn are also ubiquitinated by Cbl proteins after FcεRI aggregation in RBL-2H3 cells [32]. Only the active forms of Lyn associate with c-Cbl and Cbl-b and are specifically targeted for ubiquitination, further supporting our assumption that ubiquitination preferentially affects activated forms of the kinases responsible for signal propagation. The same study also provided evidence for the requirement of lipid rafts in Cbl-mediated ubiquitination events. Lyn ubiquitination was enhanced after overexpression of a mutant form of c-Cbl that constitutively localizes in these membrane domains [32]. Moreover, overexpression of a mutant form of Cbl-b that constitutively localizes to lipid rafts reduced antigen-mediated degranulation and cytokine production [54]. At the molecular level, the membrane-targeted overexpression of Cbl-b inhibited receptor phosphorylation and kinase activity and dramatically downregulated the protein amount of Gab2 by promoting its ubiquitination.

These results demonstrate that Cbl-b can negatively regulate both the Lyn-Syk-LAT- and the Fyn-Gab2-mediated complementary signaling pathways in FcεRI-

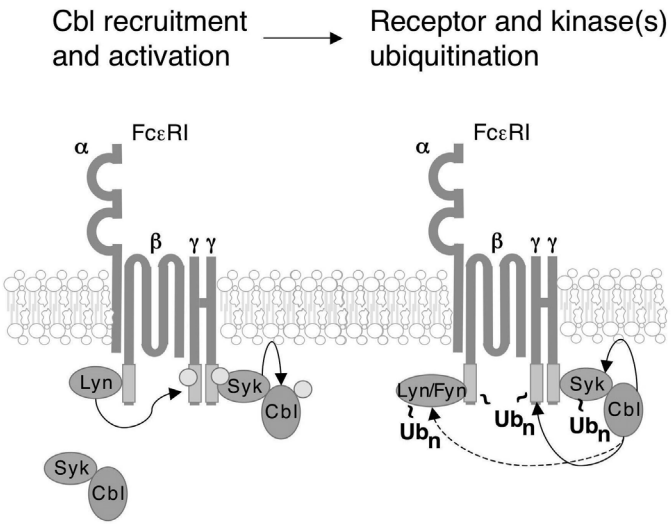

Fig. 2. Model of FcεRI and kinase ubiquitination by Syk and Cbl. Upon Lyn-mediated receptor phosphorylation, a preformed complex Syk/Cbl binds to phosphorylated γ ITAMs. Syk phosphorylates and activates Cbl that in turn ubiquitinates activated Syk, Src PTKs, and tyrosine phosphorylated receptor subunits.

-mediated mast cell activation. Thus, overexpression studies in RBL-2H3 cells have shown that c-Cbl and Cbl-b play similar negative regulatory roles in FcεRI--mediated signaling.

Gene targeting has been useful in analyzing the redundant or distinct roles of the two Cbl proteins.

The analysis of BMMCs derived from Cbl-deficient mice has revealed that c-Cbl and Cbl-b have divergent effects on FcεRI signal transduction, supporting a more prominent role for Cbl-b as a negative regulator of FcεRI-mediated signaling. In particular, the absence of Cbl-b increased receptor-mediated phosphorylation, Ca^{2+} mobilization, histamine release, and the induction of pro-inflammatory cytokines [19, 72]. Of note, Gustin et al. [19] clearly demonstrated the presence of a greater proportion of Cbl-b versus c-Cbl protein in BMMCs. This finding suggests that the differences between the BMMCs from the two Cbl KO mice can simply be due to the higher expression level of Cbl-b over c-Cbl rather than to functional differences.

CIN85

Recent evidence indicates that Cbl can promote tyrosine kinase receptor (RTK) down-modulation via a pathway that is functionally separable from its ubiquitin ligase activity and is dependent on Cbl interaction with a multidomain protein, namely CIN85 (Cbl-interacting protein of 85 kDa) [53, 61]. CIN85 is a member of a newly discovered subfamily of broadly expressed adaptor proteins that share the presence of several domains, including three SH3 domains, and a central proline-rich region [8]. The presence of different domains allowed CIN85 to form several protein-protein interactions and assemble multimeric complexes with proteins involved in signal transduction, endocytic trafficking, and cytoskeleton rearrangement.

CIN85 binding to Cbl is mediated by its SH3 domains and is enhanced by ligand-induced tyrosine phosphorylation of Cbl, while the proline-rich region of CIN85 constitutively interacts with endophilins, which are regulatory components of clathrin-coated pits. Phosphorylated Cbl mediates the association of the CIN85/endophilin complex to activated RTKs, whereas endophilin in concert with clathrin adaptors promotes clathrin-mediated receptor internalization [53, 61]. The disruption of the trimolecular complex Cbl/CIN85/endophilin by overexpression of dominant interfering forms of CIN85 inhibited receptor internalization [61]. Cbl-b shares with c-Cbl the ability to induce RTK internalization through the interaction with CIN85 [62].

We recently investigated whether c-Cbl could control the internalization of ITAM-containing receptors through a similar mechanism. CIN85 is expressed on RBL-2H3 cells and interacts with c-Cbl upon FcεRI engagement [40]. We generated transfectants stably overexpressing CIN85 using the RBL-2H3 cell line and demonstrated that overexpression of CIN85 accelerates the redistribution of engaged receptor complexes, their sorting in early endosomes, and their delivery to a lysosomal compartment for degradation [40]. RBL transfectants were also impaired in their ability to degranulate following antigen stimulation, suggesting that the accelerated internalization of activated receptors by perturbing the propagation of FcεRI signaling may contribute to limit the functional response. In conclusion, our data strongly favor a model in which after receptor engagement the Cbl/CIN85 complex is recruited to the cell membrane where it can drive internalization of engaged FcεRI complexes and their sorting into endocytic compartments, a process required for receptor degradation (Fig. 3).

We cannot declare that receptor endocytosis is the only mechanism by which CIN85 may control cell function. Indeed, more recent evidence suggest that the activity of other substrates involved in the signal propagation by RTKs could possibly be regulated by CIN85 [18, 59], and additional work will be devoted in order to their identification.

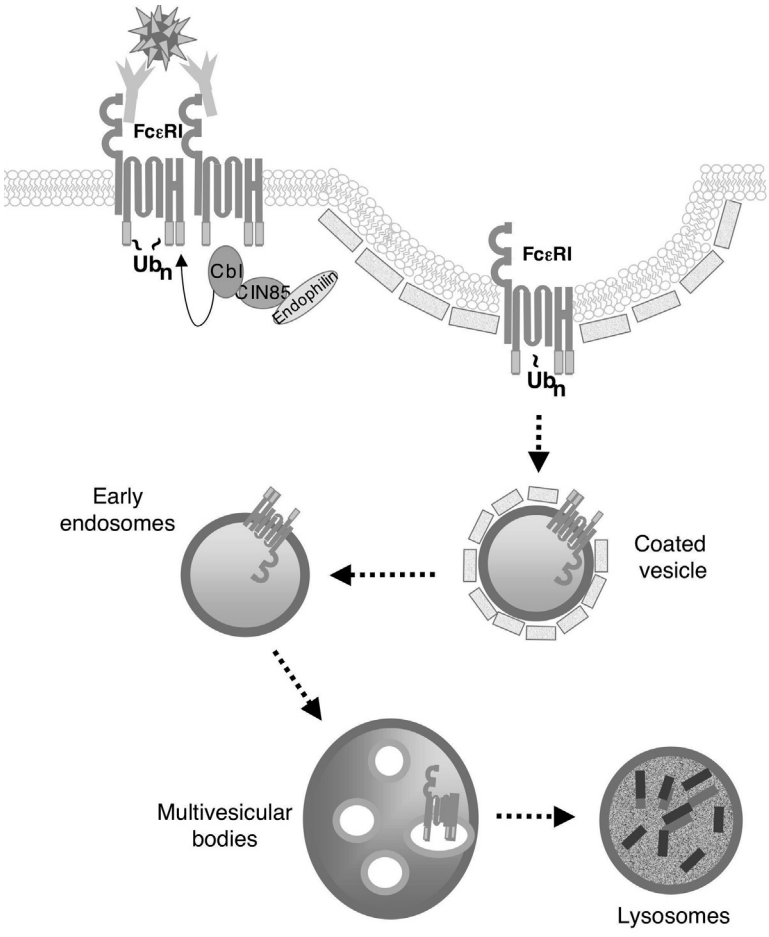

Fig 3. Schematic model of ligand-induced FcεRI down-regulation by Cbl and CIN85. Upon FcεRI engagement, Cbl promotes receptor ubiquitination and the recruitment of CIN85/endophilin in the proximity of engaged receptor complexes, whereby endophilins drive their clathrin-mediated endocytosis. Internalized receptor complexes are then sorted along the endocytic pathway and targeted to the multivesicular bodies and lysosomes for degradation.

NEGATIVE REGULATION BY INHIBITORY RECEPTORS

Over the past several years it has become apparent that mast cells express a substantial number of surface receptors that counteract FcεRI-mediated activation responses. These receptors traverse the plasma membrane once, and their N-terminal extracellular domains consist of either C-type lectin domains (e.g. mast cell-function-associated antigen, now called killer cell lectin-like receptor G1) [2] or Ig-like domains (e.g. FcγRIIB, gp49B1, paired Ig-like receptor B and signal regulatory protein α) [4, 27]. The common feature of these inhibitory receptors is the presence in their cytoplasmic tail of immunoreceptor tyrosine-based inhibitory motifs (ITIMs) that, when phosphorylated, recruit negative signaling molecules such as SHIP and SHP. Thus, mechanistically, ITIM-bearing receptors suppress FcεRI-mediated signaling by promoting dephosphorylation events. Recently, mice deficient in certain members of the ITIM receptor family have been generated, and studies with this animals have demonstrated a role for these receptors in downregulating immediate hypersensitivity reactions [27].

Thus, integration of signals from FcεRI and inhibitory receptors ensures a balanced mast cell response, and an increase in inhibitory signals might in part prevent healthy, nonatopic individuals from manifesting allergic diseases. Shifting the balance by enhancing the impact of inhibitory signals in atopic individuals is therefore a promising therapeutic option.

CONCLUDING REMARKS

In this review we described several mechanisms participating in the negative regulation of FcεRI signaling. The complex and highly regulated apparatus leading to mast cell activation is, in fact, counterbalanced by an equally sophisticated series of inhibitory mechanisms. These inhibitory mechanisms involve both ITIM and ITAM motifs, PTKs, protein and lipid phosphatases, adaptors, and ubiquitin ligases. This diverse array of molecules provides an intrinsic regulatory network in

which molecules act coordinately to achieve the desired response and limit the possible injurious effect of a persistent or excessive response. Elucidating the molecular mechanisms responsible for attenuating mast cell function may provide new insights into how mast cells may be manipulated to achieve therapeutic ends in the treatment of atopic diseases.

REFERENCES

1. Abramson J., Rozenblum G. and Pecht I. (2003): Dok protein family members are involved in signaling mediated by the type 1 Fcepsilon receptor. Eur. J. Immunol., **33**, 85–91.
2. Abramson J., Xu R. and Pecht I. (2002): An unusual inhibitory receptor-the mast cell function-associated antigen (MAFA). Mol. Immunol., **38**, 1307–1313.
3. Blank U. and Rivera J. (2004): The ins and outs of IgE--dependent mast-cell exocytosis. Trends Immunol., **25**, 266–273.
4. Bruhns P., Fremont S. and Daeron M. (2005): Regulation of allergy by Fc receptors. Curr. Opin. Immunol., **17**, 662–669.
5. Cantley L. C. and Neel B. G. (1999): New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. Proc. Natl. Acad. Sci. USA, **96**, 4240–4245.
6. Costello P. S., Turner M., Walters A. E., Cunningham C. N., Bauer P. H., Downward J. and Tybulewicz V. L. (1996): Critical role for the tyrosine kinase Syk in signaling through the high affinity IgE receptor of mast cells. Oncogene, **13**, 2595–2605.
7. Deane J. A. and Fruman D.A. (2004): Phosphoinositide 3--kinase: diverse roles in immune cell activation. Annu. Rev. Immunol., **22**, 563–598.
8. Dikic I. (2002): CIN85/CMS family of adaptor molecules. FEBS Lett., **529**, 110–115.
9. Duan L., Reddi A. L., Ghosh A., Dimri M. and Band H. (2004): The Cbl family and other ubiquitin ligases: destructive forces in control of antigen receptor signaling. Immunity, **21**, 7–17.
10. Elsasser S. and Finley D. (2005). Delivery of ubiquitinated substrates to protein-unfolding machines. Nat. Cell Biol., **7**, 742–710.
11. Furumoto Y., Brooks S., Olivera A., Takagi Y., Miyagishi M., Taira K., Casellas R., Beaven M. A., Gilfillan A. M. and Rivera J. (2006): Cutting Edge: Lentiviral short hairpin RNA silencing of PTEN in human mast cells reveals constitutive signals that promote cytokine secretion and cell survival. J. Immunol., **176**, 5167–5171.
12. Furumoto Y., Nunomura S., Terada T., Rivera J. and Ra C. (2004): The FcepsilonRIbeta immunoreceptor tyrosine--based activation motif exerts inhibitory control on MAPK and IkappaB kinase phosphorylation and mast cell cytokine production. J. Biol. Chem., **279**, 49177–49187.
13. Galli S. J., Kalesnikoff J., Grimbaldeston M. A., Piliponsky A. M., Williams C. M. and Tsai M. (2005): Mast cells as "tunable" effector and immunoregulatory cells: recent advances. Annu. Rev. Immunol., **23**, 749–786.
14. Gibbs B. F., Rathling A., Zillikens D., Huber M. and Haas H. (2006): Initial Fc epsilon RI-mediated signal strength plays a key role in regulating basophil signaling and deactivation. J. Allergy Clin. Immunol., **118**, 1060–1067.
15. Gilfillan A. M. and Tkaczyk C. (2006): Integrated pathways for mast-cell activation. Nat. Rev. Immunol., **6**, 218–230.
16. Gimborn K., Lessmann E., Kuppig S., Krystal G. and Huber M. (2005): SHIP down-regulates FcepsilonR1--induced degranulation at supraoptimal IgE or antigen levels. J. Immunol., **174**, 507–516.
17. Gomez G., Gonzalez-Espinosa C., Odom S., Baez G., Cid M. E., Ryan J. J. and Rivera J. (2005): Impaired FcepsilonRI-dependent gene expression and defective eicosanoid and cytokine production as a consequence of Fyn deficiency in mast cells. J. Immunol., **175**, 7602–7610.
18. Gout I., Middleton G., Adu J., Ninkina N. N., Drobot L. B., Filonenko V., Matsuka G., Davies A. M., Waterfield M. and Buchman V. L. (2000): Negative regulation of PI 3--kinase by Ruk, a novel adaptor protein. EMBO J., **19**, 4015–4025.
19. Gustin S. E., Thien C. B. and Langdon W. Y. (2006): Cbl-b is a negative regulator of inflammatory cytokines produced by IgE-activated mast cells. J. Immunol., **177**, 5980–5989.
20. Hernandez-Hansen V., Smith A. J., Surviladze Z., Chigaev A., Mazel T., Kalesnikoff J., Lowell C. A., Krystal G., Sklar L. A., Wilson B. S. and Oliver J. M. (2004): Dysregulated FcepsilonRI signaling and altered Fyn and SHIP activities in Lyn-deficient mast cells. J. Immunol., **173**, 100–112.
21. Hershko A. and Ciechanover A. (1998): The ubiquitin system. Annu. Rev. Biochem., **67**, 425–479.
22. Herzog S. and Jumaa H. (2007): The N terminus of the non-T cell activation linker (NTAL) confers inhibitory effects on pre-B cell differentiation. J. Immunol., **178**, 2336–2343.
23. Hicke L. (2001): Protein regulation by monoubiquitin. Nat. Rev. Mol. Cell Biol., **2**, 195–201.
24. Huber M., Helgason C. D., Damen J. E., Liu L., Humphries R. K. and Krystal G. (1998): The src homology 2-containing inositol phosphatase (SHIP) is the gatekeeper of mast cell degranulation. Proc. Natl. Acad. Sci. USA, **95**, 11330–13335.
25. Kalesnikoff J., Baur N., Leitges M., Hughes M. R., Damen J. E., Huber M. and Krystal G. (2002): SHIP negatively regulates IgE + antigen-induced IL-6 production in mast cells by inhibiting NF-kappa B activity. J. Immunol., **168**, 4737–4746.
26. Kalesnikoff J., Rios E. J., Chen C. C., Barbieri M. A., Tsai M., Tam S. Y. and Galli S. J. (2007): Roles of RabGEF1/Rabex-5 domains in regulating FcepsilonRI surface expression and FcepsilonRI-dependent responses in mast cells. Blood (in press).
27. Katz H. R. (2002): Inhibitory receptors and allergy. Curr. Opin. Immunol., **14**, 698–704.
28. Kawakami Y., Kitaura J., Satterthwaite A. B., Kato R. M., Asai K., Hartman S. E., Maeda-Yamamoto M., Lowell C. A., Rawlings D. J., Witte O. N. and Kawakami T. (2000): Redundant and opposing functions of two tyrosine kinases, Btk and Lyn, in mast cell activation. J. Immunol., **165**, 1210–1219.
29. Kimura T., Sakamoto H., Appella E. and Siraganian R. P. (1997): The negative signaling molecule SH2 domain-containing inositol-polyphosphate 5-phosphatase (SHIP) binds to the tyrosine-phosphorylated beta subunit of the high affinity IgE receptor. J. Biol.Chem., **272**, 13991–13996.
30. Kimura T., Zhang J., Sagawa K., Sakaguchi K., Appella E. and Siraganian R. P. (1997): Syk-independent tyrosine

phosphorylation and association of the protein tyrosine phosphatases SHP-1 and SHP-2 with the high affinity IgE receptor. J. Immunol., **159**, 4426–4434.
31. Kovarova M., Wassif C. A., Odom S., Liao K., Porter F. D. and Rivera J. (2006): Cholesterol deficiency in a mouse model of Smith-Lemli-Opitz syndrome reveals increased mast cell responsiveness. J. Exp. Med., **203**, 1161–1171.
32. Kyo S., Sada K., Qu X., Maeno K., Miah S. M., Kawauchi--Kamata K. and Yamamura H. (2003): Negative regulation of Lyn protein-tyrosine kinase by c-Cbl ubiquitin-protein ligase in Fc epsilon RI-mediated mast cell activation. Genes Cells, **8**, 825–836.
33. Lafont F. and Simons K. (2001): Raft-partitioning of the ubiquitin ligases Cbl and Nedd4 upon IgE-triggered cell signaling. Proc. Natl. Acad. Sci. USA, **98**, 3180–3184.
34. Laney J. D. and Hochstrasser M. (1999): Substrate targeting in the ubiquitin system. Cell, **97**, 427–430.
35. Lin S., Cicala C., Scharenberg A. M. and Kinet J.-P. (1996): The Fc(epsilon)RIbeta subunit functions as an amplifier of Fc(epsilon)RIgamma-mediated cell activation signals. Cell, **85**, 985–995.
36. Magro A. M. and Alexander A. (1974): Histamine release: *in vitro* studies of the inhibitory region of the dose--response curve. J. Immunol., **112**, 1762–1765.
37. Malbec O., Malissen M., Isnardi I., Lesourne R., Mura A. M., Fridman W. H., Malissen B. and Daeron M. (2004): Linker for activation of T cells integrates positive and negative signaling in mast cells. J. Immunol., **173**, 5086–5094.
38. Marshall J. S. (2004): Mast-cell responses to pathogens. Nat. Rev. Immunol., **4**, 787–799.
39. Metzger H. (1992): The receptor with high affinity for IgE. Immunol. Rev., **125**, 37–48.
40. Molfetta R., Belleudi F., Peruzzi G., Morrone S., Leone L., Dikic I., Piccoli M., Frati L., Torrisi M. R., Santoni A. and Paolini R. (2005): CIN85 regulates the ligand-dependent endocytosis of the IgE receptor: a new molecular mechanism to dampen mast cell function. J. Immunol., **175**, 4208–4216.
41. Monteiro R. C., Hostoffer R. W., Cooper M. D., Bonner J. R., Gartland G. L. and Kubagawa H. (1993): Definition of immunoglobulin A receptors on eosinophils and their enhanced expression in allergic individuals. J. Clin. Invest., **92**, 1681–1685.
42. Mukhopadhyay D. and Riezman H. (2007): Proteasome-independent functions of ubiquitn in endocytosis and signaling. Science, **315**, 201–205.
43. Nadler M. J., Matthews S. A., Turner H. and Kinet J.-P. (2000): Signal transduction by the high-affinity immunoglobulin E receptor Fcepsilon RI: coupling form to function. Adv. Immunol., **76**, 325–355.
44. Odom S., Gomez G., Kovarova M., Furumoto Y., Ryan J. J., Wright H. V., Gonzalez-Espinosa C., Hibbs M. L., Harder K. W. and Rivera J. (2004): Negative regulation of immunoglobulin E-dependent allergic responses by Lyn kinase. J. Exp. Med., **199**, 1491–1502.
45. Oh S. Y., Zheng T., Bailey M. L., Barber D. L., Schroeder J. T., Kim Y. K. and Zhu Z. (2007): Src homology 2 domain-containing inositol 5-phosphatase 1 deficiency leads to a spontaneous allergic inflammation in the murine lung. J. Allergy Clin. Immunol.**, 119**, 123–131.
46. Ortega E., Schweitzer-Stenner R. and Pecht I. (1988): Possible orientational constraints determine secretory signals induced by aggregation of IgE receptors on mast cells. EMBO J., **7**, 4101–4109.
47. Ota Y., Beitz L. O., Scharenberg A. M., Donovan J. A., Kinet J.-P. and Samelson L. E. (1996): Characterization of Cbl tyrosine phosphorylation and a Cbl-Syk complex in RBL-2H3 cells. J. Exp. Med., **184**, 1713–1723.
48. Ota Y. and Samelson L E. (1997): The product of the proto-oncogene c-cbl: a negative regulator of the Syk tyrosine kinase. Science, **276**, 418–420.
49. Paolini R. and Kinet J.-P. (1993): Cell surface control of the multiubiquitination and deubiquitination of high-affinity immunoglobulin E receptors. EMBO J., **12**, 779–786.
50. Paolini R., Molfetta R., Beitz L. O., Zhang J., Scharenberg A. M., Piccoli M., Frati L., Siraganian R. and Santoni A. (2002): Activation of Syk tyrosine kinase is required for c-Cbl-mediated ubiquitination of Fcepsilon RI and Syk in RBL cells. J. Biol. Chem., **277**, 36940–36947.
51. Parravicini V., Gadina M., Kovarova M., Odom S., Gonzalez-Espinosa C., Furumoto Y., Saitoh S., Samelson L. E., O'Shea J. J. and Rivera J. (2002): Fyn kinase initiates complementary signals required for IgE-dependent mast cell degranulation. Nat. Immunol., **3**, 741–748.
52. Pasquier B., Launay P., Kanamaru Y., Moura I.C., Pfirsch S., Ruffie C., Henin D., Benhamou M., Pretolani M., Blank U. and Monteiro R. C. (2005): Identification of FcalphaRI as an inhibitory receptor that controls inflammation: dual role of FcRgamma ITAM. Immunity, **22**, 31–42.
53. Petrelli A., Gilestro G. F., Lanzardo S., Comoglio P. M., Migone N. and Giordano S. (2002): The endophilin--CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met. Nature, **416**, 187–190.
54. Qu X., Sada K., Kyo S., Maeno K., Miah S. M. and Yamamura H. (2004): Negative regulation of FcepsilonRI--mediated mast cell activation by a ubiquitin-protein ligase Cbl-b. Blood, **103**, 1779–1786.
55. Rivera J. and Gilfillan A. M. (2006): Molecular regulation of mast cell activation. J. Allergy Clin. Immunol., **117**, 1214–1225.
56. Robertson D., Holowka D. and Baird B. (1986): Cross--linking of immunoglobulin E-receptor complexes induces their interaction with the cytoskeleton of rat basophilic leukemia cells. J. Immunol., **136**, 4565–4572.
57. Ryan P. E., Davies G. C., Nau M. M. and Lipkowitz S. (2006): Regulating the regulator: negative regulation of Cbl ubiquitin ligases. Trends Biochem. Sci., **31**, 79–88.
58. Saitoh S., Odom S., Gomez G., Sommers C. L., Young H. A., Rivera J. and Samelson L. E. (2003): The four distal tyrosines are required for LAT-dependent signaling in FcepsilonRI-mediated mast cell activation. J. Exp. Med., **198**, 831–843.
59. Schmidt M. H., Dikic I. and Bogler O. (2005): Src phosphorylation of Alix/AIP1 modulates its interaction with binding partners and antagonizes its activities. J. Biol. Chem., **280**, 3414–3425.
60. Siraganian R. P. (2003): Mast cell signal transduction from the high-affinity IgE receptor. Curr. Opin. Immunol., **15**, 639–646.
61. Soubeyran P., Kowanetz K., Szymkiewicz I., Langdon W. Y. and Dikic I. (2002): Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. Nature, **416**, 183–187.
62. Szymkiewicz I., Kowanetz K., Soubeyran P., Dinarina A., Lipkowitz S. and Dikic I. (2002): CIN85 participates in Cbl-b-mediated down-regulation of receptor tyrosine kinases. J. Biol. Chem., **277**, 39666–39672.
63. Tam S. Y., Tsai M., Snouwaert J. N., Kalesnikoff J.,

Scherrer D., Nakae S., Chatterjea D., Bouley D. M. and Galli S. J. (2004): RabGEF1 is a negative regulator of mast cell activation and skin inflammation. Nat. Immunol., **5**, 844–852.
64. Thien C. B. and Langdon W. Y. (2005): c-Cbl and Cbl-b ubiquitin ligases: substrate diversity and the negative regulation of signaling responses. Biochem. J., **391**, 153–166.
65. Thrower J. S., Hoffman L., Rechsteiner M. and Pickart C. M. (2000): Recognition of the polyubiquitin proteolytic signal. EMBO J., **19**, 94–102.
66. Tkaczyk C., Horejsi V., Iwaki S., Draber P., Samelson L. E., Satterthwaite A. B., Nahm D. H., Metcalfe D. D. and Gilfillan A. M. (2004): NTAL phosphorylation is a pivotal link between the signaling cascades leading to human mast cell degranulation following Kit activation and FcepsilonRI aggregation. Blood, **104**, 207–214.
67. Torigoe C., Inman J. K. and Metzger H. (1998): An unusual mechanism for ligand antagonism. Science, **281**, 568–572.
68. Volna P., Lebduska P., Draberova L., Simova S., Heneberg P., Boubelik M., Bugajev V., Malissen B., Wilson B. S., Horejsi V., Malissen M. and Draber P. (2004): Negative regulation of mast cell signaling and function by the adaptor LAB/NTAL. J. Exp. Med., **200**, 1001–1013.
69. Vonakis B. M., Gibbons S. Jr., Sora R., Langdon J. M. and MacDonald S. M. (2001): Src homology 2 domain-containing inositol 5' phosphatase is negatively associated with histamine release to human recombinant histamine-releasing factor in human basophils. J. Allergy Clin. Immunol., **108**, 822–831.
70. Weissman A. M. (2001): Themes and variations on ubiquitylation. Nat. Rev. Mol. Cell. Biol., **2**, 169–178.
71. Xiao W., Nishimoto H., Hong H., Kitaura J., Nunomura S., Maeda-Yamamoto M., Kawakami Y., Lowell C. A., Ra C. and Kawakami T. (2005): Positive and negative regulation of mast cell activation by Lyn via the FcepsilonRI. J. Immunol., **175**, 6885–6892.
72. Zhang J., Chiang Y. J., Hodes R. J. and Siraganian R. P. (2004): Inactivation of c-Cbl or Cbl-b differentially affects signaling from the high affinity IgE receptor. J. Immunol., **173**, 1811–1818.
73. Zhu M., Janssen E., Leung K. and Zhang W. (2002): Molecular cloning of a novel gene encoding a membrane--associated adaptor protein (LAX) in lymphocyte signaling. J. Biol. Chem., **277**, 46151–46158.
74. Zhu M., Liu Y., Koonpaew S., Granillo O. and Zhang W. (2004): Positive and negative regulation of FcepsilonRI--mediated signaling by the adaptor protein LAB/NTAL. J. Exp. Med., **200**, 991–1000.
75. Zhu M., Rhee I., Liu Y. and Zhang W. (2006): Negative regulation of Fc epsilonRI-mediated signaling and mast cell function by the adaptor protein LAX. J. Biol. Chem., **281**, 18408–18413.