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Adapters in the organization of mast cell signaling

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Summary

Mast cells are pivotal in innate immunity and play an important role in amplifying adaptive immunity. Nonetheless, they have long been known to be central to the initiation of allergic disorders. This results from the dysregulation of the immune response whereby normally innocuous substances are recognized as non-self, resulting in the production of IgE antibodies to these ‘allergens’. Preformed and newly synthesized inflammatory (allergic) mediators are released from the mast cell following allergen-mediated aggregation of allergen-specific IgE bound to the high-affinity receptors for IgE (FcεRI). Thus, the process by which the mast cell is able to interpret the engagement of FcεRI into the molecular events necessary for release of their allergic mediators is of considerable therapeutic interest. Unraveling these molecular events has led to the discovery of a functional class of proteins that are essential in organizing activated signaling molecules and in coordinating and compartmentalizing their activity. These so-called ‘adapters’ bind multiple signaling proteins and localize them to specific cellular compartments, such as the plasma membrane. This organization is essential for normal mast cell responses. Here, we summarize the role of adapter proteins in mast cells focusing on the most recent advances toward understanding how these molecules work upon FcεRI engagement.

Introduction

Mast cells (MCs) play an important role in the initiation and regulation of immune responses. They are not only essential in the clearance of parasitic infections but also the key to an effective immune response against bacterial infections and virus attacks (1–3). MCs are tissue-resident cells found throughout the body, where they reside in vascularized tissues and the serosal cavity (4). They are most abundant in the tissues exposed to the external environment like the skin, gastrointestinal, and respiratory tract, and together with dendritic cells are among the first cells to encounter invading pathogens (1–3). In addition to their role in host defense, analysis of mouse models and the use of MC-deficient mice (*W/W^v* or *Wsh/Wsh*) have shown that MCs are not only involved in directing and/or mediating a protective immune response but can also serve a role in tolerance (5–7).

Although the beneficial role of MCs in immunity has been the focus of much of the recent research, these cells contribute to several pathophysiological states. MCs are best known as the primary effector cells in type I hypersensitivity reactions, such as allergic rhinitis, allergic asthma, and anaphylaxis (8–11). In addition, mouse models have implicated MCs in autoimmune diseases such as rheumatoid arthritis and experimental autoimmune encephalomyelitis (12,13). While activation of MCs via their high-affinity receptors for immunoglobulin (Ig) E (FcεRI) is central in allergic disease, it is clear that a wide range of stimuli, including Fcγ receptors, Toll-like receptors, complement receptors, and a variety of G-protein coupled receptors (GPCRs), are able to cause MC activation and are likely to elicit the involvement of MCs in various pathophysiological states (10,14).

Receptor engagement initiates cellular signaling and responses in a manner that is specific to the particular receptor. Thus, a given cell has the necessary intrinsic molecular framework to respond to a specific stimulus in a mode that is selective and that results only in the desired

response. This implies that organization and compartmentalization of molecular signals is necessary as in this way the cell can selectively activate or organize the events leading to the desired response. Adapter proteins provide this molecular framework. They function to organize and coordinate the activity of signaling proteins. Herein, we focus on how adapters organize the molecular signals downstream of FcεRI stimulation. This review is not all inclusive; instead, we describe those adapters that are most well studied in MCs. Where pertinent, however, the role of an adapter in another cell type, or in response to another stimulus, is discussed.

The mast cell and its high-affinity IgE receptor (FcεRI)

FcεRI is a member of the Fc receptor family, which is characterized by the binding the crystallizable fragment (Fc portion) of immunoglobulins (Igs) (15) (Fig. 1). Family members bind a variety of Igs (16), but on MCs, Fc receptors bind IgE or IgG (Fcγ receptors). The Fcγ receptors found on MCs are of three types: the FcγRI binds IgG2a with high affinity whereas the FcγRIIB and FcγRIII are low-affinity receptors, which prefer to bind IgG1 (16,17). Unlike FcγRI and FcγRIII, FcγRIIB is an inhibitory receptor whose colligation with the FcεRI abrogates the latter's ability to activate MCs (17,18). Here, we briefly review the properties of FcεRI in binding IgE antibodies and the consequences of the encounter of IgE-occupied FcεRI with antigen.

FcεRI binds monovalent IgE antibodies secreted by plasma cells (Fig. 1). IgE antibodies are bound with high affinity ($K_A \geq 10^{10} \text{M}^{-1}$), but binding is reversible (19,20). Nonetheless, in a normal *in vivo* setting, the FcεRI on MCs is extensively occupied with IgE, as the amount of circulating IgE favors the binding rather than the dissociation of IgE from FcεRI. In host defense or in a pathophysiological setting, this equips the MCs with receptors that are ready for an encounter with the antigen. For many years, it was viewed that the binding of monovalent IgE had no significant consequence with regards to MC function. However, in the recent past, we have begun to appreciate that the binding of IgE itself, even in the absence of a known antigen, may induce cytokine production and have a role in MC survival and adhesion (21–24). However, it seems that aggregation of FcεRI is still required (22), implying that some IgE may cross react with undefined antigens (25). Regardless, the most potent MC responses are seen when antigen-specific IgE bound to FcεRI encounters the specific antigen. This results in the release of a variety of allergic mediators that are stored in intracellular granules and also initiates *de novo* synthesis and secretion of inflammatory lipid mediators, such as leukotriene C₄ and prostaglandin D₂, and a diverse spectrum of cytokines and chemokines (26,27) (Fig. 1).

The FcεRI is a member of the multichain immune recognition receptor (MIRR) superfamily (28). In MCs, FcεRI is a tetrameric complex consisting of the IgE binding α-chain, a signal amplifying membrane-tetraspanning β-chain, and a γ-chain homodimer (Fig. 1). The β- and γ-chains encode an immunoreceptor tyrosine-based activation motif (ITAM), which is characteristic of MIRR family members and endows them with the ability to transduce signals. This ability is a consequence of the phosphorylation of canonical tyrosine residues found in the ITAMs that form novel docking sites for other signaling proteins (discussed further in the next section). In humans, the FcεRI can also be found as an αγ₂-heterotrimer in some cells, such as Langerhans cells (29). However, in human MCs, only the tetrameric form of this receptor is expressed (30). In the mouse, only the tetrameric form of FcεRI has been found, and in the absence of the β-chain, there is no cell surface expression of this receptor (30). The ITAM sequences of the β-chain and the γ-chain are functionally distinct (30–32). The β-chain functions to amplify FcεRI signaling, whereas the γ-chain is able to initiate weak signals from the FcεRI even in the absence of the β-chain (31–33). This division of function may underlie the distinct role of the trimeric receptor as an antigen-

presenting receptor versus that of the tetrameric receptor (30), which elicits strong cellular responses. A characteristic of MIRRs, which includes the B-cell receptor (BCR) and the T-cell receptor (TCR), is the lack of intrinsic kinase activity. These receptors must associate with tyrosine kinases to elicit ITAM phosphorylation. Src family protein tyrosine kinases (Src PTKs) are the key initiators of this event, and multiple Src kinases can play this role depending on cell type or stimulus (34). For FcεRI, two Src kinases (Fyn and Lyn) are known to be proximal to this receptors function (35) (Fig. 1). However, as discussed in the following section, these Src PTKs have distinct roles in FcεRI signaling.

Early signaling events proximal to FcεRI

The Src PTK Lyn is responsible for the phosphorylation of the canonical ITAM tyrosines of both the β and γ chains (Fig. 1). Small amounts of Lyn have been found to constitutively associate with the FcεRI β-chain (36,37) and are able to transphosphorylate a neighboring receptors β- and γ-chains (38). There is also considerable evidence that this initial event is further fostered by recruitment of additional Lyn molecules (36) to the receptor and that the surrounding lipid environment is an important contributor in FcεRI phosphorylation (39). Like other Src PTKs, Lyn is anchored via its palmitoyl and myristoyl moieties in the plasma membrane, and its activity closely correlates with its localization within cholesterol–sphingolipid-enriched plasma membrane domains (commonly referred to as lipid rafts). FcεRI stimulation also causes an increased presence of these receptors in lipid raft domains (39–41). Lyn purified from lipid rafts was found to be more active than Lyn purified from the rest of the plasma membrane (42), thus exposing lipid raft-localized FcεRI to increased Lyn activity. Furthermore, the localization of Lyn in lipid rafts was demonstrated as necessary for sustained FcεRI phosphorylation and for maintenance of an active Lyn kinase (42–44).

There is increasing evidence, however, that in resting cells a co-localization of Lyn with FcεRI takes place in small highly dynamic membrane domains (45,46). These small highly dynamic domains may well explain the observed but limited constitutive association of FcεRI and Lyn (36–38), which may be crucial for the initial phosphorylation. Sustaining FcεRI phosphorylation may require increased FcεRI and Lyn interactions, and recent studies demonstrated that antigen aggregation of FcεRI induced membrane changes that foster the generation of larger lipid rafts (47,48). Thus, this may be a mechanism for increased recruitment of FcεRI into lipid rafts, where it becomes exposed to additional Lyn molecules that are concentrated within these domains and are highly active (42,49). This would likely provide the necessary amplification of signaling and sustained phosphorylation of FcεRI. This scenario for FcεRI phosphorylation is in agreement with studies showing that redistribution of these receptors is absolutely dependent on receptor aggregation by antigen but is independent of Lyn activity (39,50). The requirement for localization of the FcεRI in lipid rafts, for efficient and sustained receptor phosphorylation, is likely due to the protective phosphatase-depleted environment that prevents dephosphorylation of FcεRI and exposes this receptor to highly active Lyn (42) rather than as a requirement for the initiation of FcεRI phosphorylation (43,44). While still hypothetical, this view is supported by data showing that hapten-mediated disruption of FcεRI aggregates causes a rapid loss of receptor from lipid rafts and dephosphorylation of the receptor (51–53), whereas the partitioning of Lyn in lipid raft domains is not dramatically altered by such treatment.

The phosphorylation of the γ-chain ITAMs by Lyn creates binding sites for the tandem Src homology 2 (SH2) domains of the tyrosine kinase Syk (spleen tyrosine kinase) (54) (Fig. 1), which shares homology with ζ-associated protein of 70 kDa (ZAP-70) expressed in T cells. Binding to the phosphorylated FcεRIγ allows Syk to adopt an active conformation, leading to its phosphorylation by Lyn and subsequently transphosphorylation of other Syk molecules, which amplifies Syk activation and allows downstream phosphorylation of its targets (55).

Among these targets are adapter molecules like the linker for activation of T cells (LAT1) (56) and possibly Gab2 (57) (discussed in more detail in a following section). Syk is critically important in amplification of signaling, and inhibition of its activity or loss of its expression in MCs results in a marked and generalized inhibition of MC responses. Thus, this kinase has engendered considerable interest as a therapeutic target in allergic and inflammatory diseases (58).

Although multiple Src PTKs are expressed in MCs (26,59), besides Lyn, only Fyn kinase has been shown to have a receptor proximal role in FcεRI-mediated activation of MCs (35,60) (Fig. 1). Fyn is not involved in the phosphorylation of the receptor itself but rather is responsible for the phosphorylation of several downstream targets that are essential components in FcεRI-mediated MC activation. Fyn can be co-immunoprecipitated with the β-chain of the FcεRI (35) and appears to require the aggregation of this receptor to become active. Unlike Lyn, which can be found primarily in lipid rafts, only a small fraction of Fyn seems to reside in these domains (44). This difference in distribution of Fyn and Lyn is consistent with the view that Fyn- and Lyn-dependent signals function to a large extent independent of but complementary to each other (35). In addition, there is evidence demonstrating that the Fyn-dependent signals can be activated selectively depending on the strength or type of stimulus (61,62).

Fyn signals initiated upon FcεRI stimulation are important in the activation of the phosphatidylinositol-3 kinase (PI3K) (35), a kinase that is essential for the production of the lipid second messenger phosphatidylinositol 3,4,5-trisphosphate (PIP₃) (Fig. 1). This seems to be mediated via Fyn (and possibly Syk) dependent phosphorylation of the adapter protein Grb2-associated binding protein 2 (Gab2), which is known to be required for PI3K activity (35,57,63) (discussed further in a following section). Several signaling proteins like phospholipase C-γ (PLC-γ), Bruton's tyrosine kinase (Btk), and protein kinase B (PKB/Akt) need to be recruited to the plasma membrane by binding to PIP₃ (Fig. 1) to be activated (64). Thereby, Fyn is an important positive regulator of FcεRI signaling and MC responses (60). In addition, recent data from our laboratory suggests that Fyn is directly involved in the regulation of the extracellular Ca²⁺ influx (R. Suzuki and J. Rivera, unpublished data). Regardless, the paradigm for an independent and complementary signaling pathway is not that straightforward. For example, it is clear that Lyn controls the activity of Fyn kinase (65,66). Loss of Lyn expression in MCs enhances Fyn activity through an apparent loss of negative regulation by the adapter C-terminal src kinase (Csk)-binding protein (Cbp) and the association of the negative regulatory kinase Csk (67) (Fig. 2). Moreover, recent studies have demonstrated that another Src PTK, Hck, controls Lyn activity and cell responses in a manner that is independent of Fyn (68). This complexity suggests that the view of Fyn and Lyn as initiators of independent but complementary pathways is oversimplified, and we are yet to define how these signals are coordinated and where they overlap.

Why do we need adapters in MC signaling?

Central for signal amplification and coordination of the activation process is the phosphorylation by the aforementioned kinases of adapter proteins (Fig. 3). In general, although not exclusively, these are proteins without intrinsic enzymatic function that serve as platforms for the coordination of signaling events (69). Adapters have multiple functions: they are responsible for determining the localization of signaling proteins in the cell, they coordinate the necessary but diverse signals required for cell activation, and they bring together the required enzymes and substrates that drive the activation process. Adapters can be transmembrane proteins, reside in the cytoplasm under resting conditions, and be recruited to the membrane upon activation, or be localized by specific interactions in intracellular compartments such as the endoplasmic reticulum (ER), cytosol, etc. A common

feature of adapter proteins, regardless of their cellular localization, is their modular structure (Fig. 3). In general, they contain one or more types of domains that allow their interaction with several other proteins, thus being able to assemble a macromolecular structure. However, there are some specific features that can distinguish a membrane adapter from a cytosolic one (Fig. 3). Membrane adapters act like anchored scaffolds organizing larger macromolecular complexes, relying mainly on their ability to form novel docking sites through the phosphorylation of multiple tyrosine residues (Fig. 3). In contrast, cytosolic adapters have a more varied array of motifs (such as SH2, SH3 domains, and phosphotyrosines) that allow them to act both in a non-phosphotyrosine or phosphotyrosine-dependent manner bringing proteins to the sites where these adapters reside or are recruited. SH2 domains [and several other domains like phosphotyrosine-binding (PTB) domain (70)] interact with phosphorylated tyrosines. SH3 domains [and others like EVH1 (71)] bind to proline-rich sequences (72). Adapters may also have motifs that are responsible for protein-lipid interaction like the pleckstrin homology (PH) domains (73,74). All of these domains are used to coordinately regulate the assembly and function of signals generated from such macromolecular complexes (69,75).

In MCs, Lyn and Syk activation result in phosphorylation of the transmembrane adapter protein LAT1 (Fig. 1), which is localized in lipid rafts (76). The phosphorylation of LAT1 together with the generation of PIP_3 in the lipid raft environment recruits proteins such as PLC- γ 1 and PLC- γ 2 (56) and Btk (77,78), a kinase that phosphorylates PLC- γ and enhances its activity (79,80) (Figs 1 and 4). Activated PLC- γ cleaves phosphatidylinositol-4,5-bisphosphate [$\text{PI}(4,5)\text{P}_2$], which is also found to increase in the lipid raft environment, to yield two important second messengers, inositol-1,4,5-trisphosphate (IP_3), and 1,2-diacylglycerol (DAG) (Fig. 1). IP_3 binds to its receptors in the membrane of the ER, stimulating depletion of Ca^{2+} from the organelle. The depletion of Ca^{2+} from the ER activates STIM1 and eventually leads to the opening of the Ca^{2+} release-activated Ca^{2+} channels at the plasma membrane resulting in a strong influx of Ca^{2+} into the cytoplasm (81,82). The Ca^{2+} influx is an essential trigger for fusion of the MC granules to the membrane and the breakdown of the actin cytoskeleton and activation of several Ca^{2+} -dependent signaling molecules (for example protein kinase C- β) that participate in MC degranulation (Fig. 1). In addition, transcription factors such as the nuclear factor of activated T cells (NFAT) also require Ca^{2+} influx for their activation and regulation of gene expression (26,27). It should be noted, however, that at the same time LAT1 is also complexed with other proteins that mediate the multiple tasks required for cellular responses (Figs 1 and 4). Guanine nucleotide exchange factors (GEFs), such as Vav and son-of-sevenless (SOS), are also found to associate with LAT1. These are required for activation of small GTPases of the Rho and Ras families and thus modulate cytoskeletal rearrangements, vesicle movement, and the activation of mitogen-activated protein kinases (MAPKs) extracellular signal-regulated kinase (Erk), c-Jun N-terminal kinase (JNK), and p38 (83–85). Thus, not only does the LAT1 assembled complex contribute to MC degranulation, but it is also involved in generating and regulating signals into the nucleus (27,86,87).

The assembly of macromolecular signaling complexes through adapter proteins must be tightly controlled. One might imagine the troublesome consequences of assembling the constituent components of an active macromolecular complex when the MC encounters a weak antigen, as this might lead to disease. A kinetic proofreading paradigm has been proposed, and several studies provide strong evidence that such control mechanisms exist in MCs (52,88–90). Kinetic proofreading stipulates that each molecular signal needs to be sustained for sufficient time to allow the subsequent molecular event to occur, thus leading to a productive MC response. It argues that a weak versus a strong stimulus might be distinguished on the basis of the molecular signals engendered or on the basis of what molecular assemblies are formed. As one might imagine, the extent of IgE-occupied $\text{Fc}\epsilon\text{RI}$

aggregation by an antigen would be a likely determinant in both the assembly of a macromolecular signaling complex and the outcome of the cellular response (61). It has been shown that high concentrations of a low affinity antigen can elicit FcεRI phosphorylation (52). However, under these conditions, degranulation is absent, suggesting that only a subset of the molecular events initiated by high-affinity antigen is elicited. Our own studies provide evidence of this, as weak stimulation or low receptor occupancy was found to preferentially, although not exclusively, activate Fyn-dependent signals in MCs (61). Moreover, non-toxic stress conditions induced by hydrogen peroxide treatment of MCs also led to selective activation of Fyn-dependent signals relative to those initiated by Lyn kinase (62). How is the cell able to distinguish the extent of FcεRI aggregation and interpret this into selective signals? We have no clear cut answer, and this is an area of intensive research focus. Nonetheless, there may be some potential clues in the findings that the FcεRI is found in plasma membrane regions that are distinct from those in which the adapter LAT1 is found (45). Additionally, even the closely related adapters LAT1 and LAT2 [formerly called non-T-cell activation linker (NTAL) or linker for activation of B cells (LAB)] appear to be differentially localized in the plasma membrane (91,92). Yet after FcεRI stimulation, these molecules are active, suggesting that colocalization or communication of these molecules occurs. This could require certain morphological changes that may be engendered by the extent of FcεRI aggregation as has been observed in several studies (47,48,93). In fact, one of these studies suggested that the actin cytoskeleton is also a likely participant by rearranging actin filament bundles in a manner that sequesters the movement of FcεRI upon its aggregation, enhancing its interaction with other receptors and likely other proteins included in these regions (93). However, it is still unclear as to whether receptor mobility or immobility is a requirement for macromolecular complex formation and signal transduction.

Below we review what is known about the function of the individual adapters known to be expressed in MCs and utilized by FcεRI. We divide the adapters into two classes, membrane versus cytosolic, in hopes that this distinction provides a clearer focus on the distinct properties of each.

Membrane adapters

The assembly of macromolecular signaling complexes in the plasma membrane as well as in organelle membranes requires the tethering of an adapter to these membranes. This can be accomplished by various means such as lipid modification (myristoylation and palmitoylation), which targets the adapter to membranes, by the presence of a hydrophobic transmembrane domain in the adapter protein, or by protein–protein interactions that tether the adapter proximal to the membrane (such as interaction with actin that localizes an adapter submembraneous). In MCs, the best studied adapters are transmembrane adapters, and we focus on these in this section. An important classification criterion for transmembrane adapters is whether the adapter is located exclusively in lipid rafts (via lipid modification), whether the adapter is recruited to these microdomains upon cell activation, or whether it is entirely excluded from these membrane regions. Below we provide examples of transmembrane adapters in MCs that fulfill the first and last classification criteria.

LAT1

One of the most studied and relevant adapters in immune cells is LAT1 (Figs 1 and 4). This adapter was first described in T cells as a 36–38 kDa transmembrane protein with a short three-residue N-terminal extracellular domain, a unique hydrophobic portion constituting the single membrane-spanning region, and a long cytoplasmic tail at the C-terminus with 10 tyrosine residues (nine of them are conserved in mice) that after phosphorylation may constitute SH2 domain-binding sites. Analysis of LAT1 sequence and the initial *in vitro*

studies characterizing its function demonstrated its role in binding Grb2, PI3K, and PLC- γ 1, and showed the importance of these molecular associations in T-cell activation (94). A mutant of the Jurkat T-cell line, J.CaM2, lacks LAT1 expression and was shown to be defective in Ca^{2+} mobilization and T-cell activation after TCR engagement, demonstrating the importance of this adapter in coupling early events of TCR engagement with downstream Ca^{2+} signals (95). Initial signaling events after TCR activation, such as TCR ζ -chain and ZAP-70 phosphorylation, are not affected by the absence of LAT1, but PLC- γ 1, Vav, and SH2 domain-containing protein of 76 kDa (SLP-76) phosphorylation were severely diminished placing LAT1 downstream of early TCR proximal events (94,95). The critical role of LAT1 in immunity was demonstrated by genetic disruption in mice, which caused an arrest in thymocyte development into all T-cell subsets, rendering these animals deficient in mature $\alpha\beta$ T cells in lymph nodes and spleen as well as in $\gamma\delta$ T cells (96).

LAT1 undergoes a lipid modification that determines its cellular localization. Two cysteine residues located in the LAT1 juxtamembrane region (C26 and C29 in the human sequence), become palmitoylated and are responsible for targeting of this protein to lipid rafts. Mutants of these residues fail to become dually acylated and thus cannot localize in lipid raft domains (76). However, *in vitro* studies with acylated and non-acylated LAT1 peptides demonstrated that acylation alone is necessary but not sufficient for the lipid raft localization of LAT1 and that other regions of the protein contributed to its targeting to lipid rafts (97). Early studies also showed that in transfected Jurkat cells, localization to lipid rafts was required for effective phosphorylation of LAT1 on its multiple tyrosine residues. Moreover, LAT1 cysteine mutants were phosphorylated less efficiently after TCR activation (76). However, more recent experiments with transgenic mice expressing a chimeric protein of LAT1 with the transmembrane domain of the non-raft adapter LAX showed that LAT1 phosphorylation was normal and it effectively mediated T-cell activation and T-cell development, suggesting that targeting of LAT1 to lipid rafts was not essential for its function (98). It is not clear why the results of the aforementioned studies differ with regards to the lipid raft requirement for LAT1 phosphorylation. However, given that LAX can be phosphorylated outside of lipid rafts (99,100), the chimeric LAX-LAT1 protein may become a target for a kinase that is associated with or localized in regions where LAX is found.

LAT1 is expressed in MCs and becomes strongly tyrosine phosphorylated after Fc ϵ RI engagement (56) (Fig. 1). LAT1 tyrosine phosphorylation is maximal within 2 min after antigen stimulation, and because its activity depends on phosphorylation, this observation implies its participation in Fc ϵ RI-mediated signals. The distribution of LAT1 was studied by high-resolution immunoelectron microscopy on native membrane sheets of non-activated RBL-2H3 cells, where it was found in small membrane clusters that were distinct from those containing Fc ϵ RI (45). The Fc ϵ RI clusters were found to contain other signaling mediators such as Lyn, Syk kinase, and PLC- γ , and the cytoplasmic adapters Grb2 and Gab2, and as suggested (45) might be thought of as the primary signaling domain. After activation, LAT1-containing clusters or secondary signaling domains were found to enlarge and intersect (although not mix) with Fc ϵ RI clusters (45). These findings provided evidence for the distinct compartmentalization of Fc ϵ RI and LAT1 (Fig. 1).

The importance of LAT1 in MC responses was demonstrated by experiments in *lat*^{-/-} mice. Unlike for T-cell development, LAT1 deficiency does not alter MC development and maturation both *in vivo* and *in vitro* (56). Despite normal MC numbers, *lat*^{-/-} mice are resistant to IgE-mediated passive systemic anaphylaxis. When analyzed *in vitro*, LAT1-deficient MCs showed a profound defect in degranulation after antigen engagement of IgE-occupied Fc ϵ RI as well as a failure to mount a normal Ca^{2+} response. As observed in LAT-deficient T cells following TCR stimulation, early signaling events in MCs were still intact. Fc ϵ RI β - and γ -chain phosphorylation as well as Syk phosphorylation were unaltered in

LAT-null MCs (Fig. 1). In contrast, PLC- γ 1, PLC- γ 2, and SLP-76 phosphorylation were markedly reduced when compared to wildtype MCs (Figs 1 and 4). As mentioned above, these proteins associate with LAT1 and thus become membrane localized. However, given that Fc ϵ RI and LAT1 may be localized in different membrane domains, it remains unclear how Fc ϵ RI engagement is translated to the phosphorylation of LAT1. While biochemical studies argue for a mixing of Fc ϵ RI with LAT1 upon the latter's engagement and show that Syk is important for LAT1 phosphorylation, the high-resolution topographical studies (45,91) argue that distinct compartmentalization exists, as no mixing of the respective primary (Fc ϵ RI-containing) or secondary (LAT1-containing) domain components was observed. Thus, the steps in engaging the activation of the secondary domain components are unclear but seemingly require the proximity of distinct lipid raft domains.

The four membrane distal tyrosines of LAT1 are crucial for its phosphorylation and function in MCs (Fig. 4). Mutation of these tyrosine residues largely ablates LAT1 phosphorylation and markedly decreases its function (101). Experiments in which LAT1-null MCs were reconstituted by retroviral infection with LAT1, carrying tyrosine to phenylalanine mutations of the four membrane distal cytoplasmic tyrosines, provided considerable insight on the role of these tyrosines in LAT1 function. In the mouse, Y136 which is found in the context of YLVV motif, constitutes the major docking site for PLC- γ 1. Mutation of this tyrosine to phenylalanine caused marked defect in MC degranulation as well as defective Ca²⁺ mobilization (101). However, individual mutations of Y175 (YVNV), Y195 (YVNV), and Y235 (YENL) also had some effect on the aforementioned response, albeit to a lesser extent. These three tyrosine residues have the consensus binding site YxN for the adapters Grb2 (or another family member, Gads), and mutational analysis revealed that all three sites can bind these adapters (101) (Fig. 4). In addition, Grb2 binding to LAT1 is also necessary for optimal phosphorylation of PLC- γ 1 and PLC- γ 2. This is likely due to multiple interactions of PLC- γ with other proteins found to complex with LAT1, which may serve to stabilize the interaction of PLC- γ 1/2 with LAT1 and the recruitment of Btk, a kinase necessary for phosphorylation and function of PLC- γ , into the complex (Fig. 4). In addition, there is evidence for the cooperative recruitment of PLC- γ to LAT1 both directly and indirectly via SLP-76 (101). Thus, the apparent redundant interactions and the intracomplex molecular interactions among constituent proteins provide a robust signaling complex whose level of response may be determined by the type and amount of proteins assembled in the complex. Evidence for this view is provided by analysis of Ca²⁺ mobilization, whereby mutation of each individual Grb2-binding motif had a partial effect on Ca²⁺ mobilization, whereas mutation of all four distal tyrosine decreases the Ca²⁺ response to that seen in *lat*^{-/-} MCs (101). Nonetheless, it should be noted that while Ca²⁺ signals are considerably reduced they are not totally abolished in *lat*^{-/-} MCs (56). The adapter Gads can also bind to LAT1 and appears to be favored with regards to coupling of SLP-76 with LAT1 (Fig. 4). In T cells, the Gads docking sites are Y175 and Y195 (102). These sites are likely to bind Gads in MCs as well, as this protein is expressed in these cells. Elimination of Gads binding by mutagenesis causes a marked decrease in SLP-76 phosphorylation following Fc ϵ RI stimulation (101). As previously mentioned, LAT1 binds to multiple signaling proteins including GEFs such as Vav1 and SOS, which regulate the activity of small GTPases such as Rho and Ras (Fig. 4). Thus, it is not surprising that Fc ϵ RI-dependent activation of MAPKs, which are regulated by these small GTPases, is also impaired by the absence of LAT1 in MCs (56,101). Activation of ERKs, p38MAPK, and JNK were all reduced in *lat*^{-/-} MCs. A concomitant reduction in various cytokines [interleukin (IL)-3, IL-4, IL-6, and tumor necrosis factor (TNF- α)] produced after Fc ϵ RI stimulation was also observed (56). Reconstitution of LAT1-null MCs with LAT1 mutants (tyrosine to phenylalanine) showed that all four distal tyrosines contributed to optimal activation of MAPKs in MCs (101). The results once again suggest cooperativity of the proteins that associate with LAT1 in the regulation of MAPK activation. The findings clearly demonstrate that the LAT1 complex

plays an important and rather global role in positively regulating MC signaling and responses.

There is increasing evidence, however, for additional complexity in the role of LAT1 in MC activation and responses. Recently, a negative regulatory role for this adapter was described in MCs as well as in T cells. In T cells, LAT1 organizes an inhibitory macromolecular complex that includes the adapter Dok-2 and the SH2 domain-containing inositol phosphatase (SHIP), which serves to dampen the extent of early activation upon TCR engagement (103). It is unclear whether this LAT1 inhibitory complex is independent of the LAT1 stimulatory complex seen in T cells or whether this association occurs in a dynamic manner, following dissociation of stimulatory proteins in the LAT1-scaffolded complex. However, based on the kinetics of the association of Dok-2 and SHIP with LAT1, an evolving view is that LAT1 complexes may be heterogeneous, some possessing stimulatory and others inhibitory signaling proteins. In MCs, LAT1 can also exert an inhibitory role under certain circumstances. Analysis of MCs carrying LAT1 with mutations in individual tyrosine residues also showed the recruitment of SH2 domain-containing inositol phosphatase-1 (SHIP-1) into the FcεRI-mediated signaling complex (104). Binding of SHIP-1 to LAT1 seemed to require tyrosine residues Y136 and Y235. Interestingly, SHIP-1 is recruited in a cooperative manner in which binding of Grb2 to tyrosine Y195 potentiates the binding of SHIP-1 to tyrosine Y235. SHIP-1-dependent negative regulatory pathways are known to affect MC proliferation (105). However, in the case of LAT1-dependent negative regulation, survival is compromised but proliferation is normal. Thus, this observation suggests that the compartmentalized effect of SHIP-1 may differ from the more global effect of SHIP-1 deficiency. In addition, another study showed a differential LAT1-dependent regulation of MC responses (like degranulation) in two models of MCs, namely peritoneal derived MCs, which are a more mature MC population reminiscent of serosal MCs, and bone marrow-derived MCs that are a model of mucosal MCs (106). The difference observed in the role of LAT1 for each cell type might be partly explained by differing amounts of other signaling molecules expressed in the given cell type. These findings show the importance of balance, in terms of the levels of expression of signaling molecules in a cell, toward integration of cellular responses.

LAT2

LAT2, formerly named NTAL or LAB, was first identified and purified from HL-60 and THP-1 cell lines as a non-T-cell molecule related to LAT1 (107,108) that is expressed by B cells, natural killer cells, and myeloid cells including MCs. Originally described as absent in naive T cells, it is now known that LAT2 is upregulated after T-cell activation via the TCR (109). LAT2 is a 30-kDa molecule in humans and a 25-kDa molecule in mice. Like LAT1, LAT2 is a single-spanning transmembrane protein with a long cytoplasmic tail bearing 10 tyrosine residues susceptible of phosphorylation (Figs 3 and 4). An important difference between LAT2 and LAT1 is that the former is lacking the PLC-γ1-binding motif (110). However, LAT2 can still recruit PLC-γ1 in an indirect manner after phosphorylation of the Grb2-binding sites Y193 and Y233 (111). Similar to LAT1, LAT2 also has a palmitoylation site (CXXC) adjacent to the transmembrane domain that localizes it to lipid rafts (Fig. 4). Despite their structural similarities, it has been shown by immunolabeling of isolated plasma membrane sheets followed by high-resolution electron microscopy that LAT1 and LAT2 occupy distinct domains in the plasma membrane of RBL-2H3 and bone marrow-derived MCs in non-activated and antigen-stimulated cells (91). However, whether these molecules can under different stimuli or circumstances colocalize and compete for signaling molecules is still an open question.

The role of LAT2 in MCs is still unclear. Results obtained from MCs from LAT2-null mice suggested a negative regulatory role for this adapter, as these cells were hyperreactive in

their degranulation response as well as in cytokine production after IgE antigen stimulation. An enhanced degranulation phenotype was supported by higher PLC- γ 1 and PLC- γ 2 phosphorylation, leading to increased IP₃ and DAG production (112,113). However, *in vivo* data showed that the response of LAT2 mice to a passive systemic anaphylactic challenge is equivalent to WT mice (113). Moreover, RNA silencing of LAT2 in human MCs rendered these cells hyporesponsive after Fc ϵ RI engagement (114). Thus, based on the collective results, it is unclear whether LAT2 plays a positive or negative regulatory role in MC responses elicited via Fc ϵ RI. Nonetheless, LAT1/LAT2 double-deficient MCs showed a defect in degranulation after Fc ϵ RI stimulation, reminiscent of the phenotype observed in LAT1-null MCs, favoring the view that both of these molecules may be needed to drive the degranulation response (113). Whether LAT2 plays a positive or negative role in the MCs' response may well depend upon the context in which it is functional. A clue in support of this view is provided by the finding that LAT2's presence in lipid raft domains is increased in LAT1-null MCs and vice versa, indicating that these molecules may be able to substitute for each other in these domains (112,113,115). Moreover, they may compete for some of the same molecular constituents (27); thus in some situations, LAT2 may have dominance by effectively outcompeting LAT1 for the same signaling molecules, whereas other stimuli may lead to LAT1 dominance.

Given the interdependence and apparent dual roles, it is likely that LAT2 and LAT1 coordinately function in the regulation of MC responses and survival (27,115) (Figs 1 and 4). It may not be possible to clarify the distinct roles of these adapters by studying them separately or individually. Instead, a systematic study of individual tyrosine mutants of both molecules expressed simultaneously may shed some light on the key roles for each molecule.

Cbp/PAG

Another transmembrane adapter found in MCs is Cbp/PAG (Cbp/phosphoprotein associated with glycosphingolipid-enriched microdomains) (Fig. 2). It was first purified as a 80–90 kDa protein interacting with the negative regulatory kinase called Csk (116,117). Cbp is a transmembrane protein with structural similarity to LAT1 and LAT2 (Fig. 3). This adapter is also myristoylated and palmitoylated due to a dicysteine motif (CXXC) located in the cytoplasmic region immediately after its transmembrane domain. This targets Cbp to lipid raft domains of the plasma membrane where it is exclusively localized. Phosphorylation of Cbp (specifically at Y317) causes the recruitment and binding of Csk via its SH2 domain (116). Csk relocation to the membrane allows this kinase to phosphorylate an inhibitory tyrosine residue located at the C-terminus of the catalytic domain of Src family kinases (i.e. Y508 in Lyn), causing an intramolecular interaction between the phosphorylated tyrosine residue and the SH2 domain of that molecule (67) (Fig. 2). This causes a conformational change that sequesters the catalytic domain (and ATP-binding site) of Src family PTKs effectively repressing kinase activity. Unlike adapters, such as LAT1 and LAT2, which are expressed mainly in hematopoietic cells, Cbp is ubiquitously expressed (116,117).

Initial studies on the role of Cbp in MCs showed that this adapter was exclusively localized in lipid rafts, and its overexpression caused a marked inhibition of Fc ϵ RI-mediated degranulation and Ca²⁺ flux (118). This finding suggests that Cbp is important for controlling MC responsiveness through targeting of Src PTK activity. In mouse MCs, Cbp is phosphorylated by Lyn kinase (Fig. 2). Phosphorylation of Cbp and Csk binding is seen in resting cells, but both are dramatically enhanced after Fc ϵ RI stimulation (65,118). This observation suggests that Cbp-dependent recruitment of Csk to the plasma membrane is important for controlling the activation of MCs. Strikingly, Fyn phosphorylation and activity was increased in Lyn-null MCs, which are defective in Cbp phosphorylation (65). Moreover, these cells showed a hyperresponsive phenotype, where both degranulation and cytokine

production were enhanced. Additional studies revealed that the hyperresponsive degranulation phenotype was influenced by the genetic background of the mice from which the MCs were derived and was associated with the amount of Fyn kinase expressed in the cells from given genetic background (an increased ratio of Fyn:Lyn was associated with increased degranulation) (119). Cbp phosphorylation was not reduced in Fyn-null MCs, confirming the unique role of Lyn in phosphorylation of this adapter. Other studies have demonstrated the hypophosphorylation of Cbp in mice with low levels of Lyn activity (ASK mice) and have shown that these mice are anaphylaxis-prone (120). Importantly, Fyn kinase was hyperactive in MCs from these mice (120), consistent with the observations in Lyn-null MCs (65). Additional models have been reported that reflect the key role for Cbp–Csk regulation of MC responses. Inactivation of the 3 β -hydroxysterol 7-reductase gene results in altered lipid rafts due to the loss of cholesterol synthesis, an inborn error of metabolism seen in patients with Smith–Lemli–Opitz syndrome (121). MCs from mice carrying a deletion of the 3 β -hydroxysterol 7-reductase gene showed a reduced Cbp phosphorylation and increased Fc ϵ RI induced degranulation and cytokine production (44). This hyperresponsive phenotype is most likely explained by increased Fyn activity in these cells (44). Thus, it appears that Cbp plays a key role in controlling Fyn activity (Fig. 2) and that dysregulation of the Cbp–Csk interaction by hypo-phosphorylation of Cbp is likely to lead to an increased MC-mediated inflammatory response.

LAX

LAX is another transmembrane adapter initially cloned from T cells (99), but it is also expressed in B cells (100) and in MCs (122). LAX does not have significant sequence homology with LAT1. However, both molecules display a similar structural organization including the presence of 10 tyrosine residues at the COOH-terminus that can be phosphorylated (Fig. 3). Unlike LAT1, LAT2, or Cbp/PAG, the LAX protein does not contain a dicysteine motif (Fig. 3) and is excluded from lipid rafts in Jurkat T cells (99). In TCR-mediated signaling, LAX has been described to have a negative regulatory role in regulating IL-2 production and proliferation after CD3/CD28 stimulation (123,124).

Similar to its role in T cells, LAX exerts a negative regulatory role on Fc ϵ RI-mediated MC signals (122). LAX becomes phosphorylated after Fc ϵ RI-stimulation of MCs and phosphorylated LAX interacts with the p85 regulatory subunit of PI3K, as well as with the adapters Grb2 and Gads. LAX-null MCs showed a higher degranulation response after Fc ϵ RI stimulation when compared to wildtype cells. Surprisingly, however, the Ca²⁺ mobilization of LAX-null MCs was not considerably different from that of wildtype cells. LAX deficiency, however, led to increased phosphorylation of p38 MAPK and Akt. This was accompanied by a modest increase in the production of IL-3, IL-6, and TNF- α . LAT2 protein expression (and mRNA) was downregulated in LAX-null MCs following Fc ϵ RI stimulation (122). This observation suggests that the phosphorylation of LAX promotes signals that stabilize LAT2 expression. Why LAX signals may be required for LAT2 expression following Fc ϵ RI stimulation is unclear. However, one might speculate that this regulation could be relevant to the aforementioned balance of positive and negative roles for LAT1 and LAT2 and might suggest that different stimuli may determine the dominant expression of LAT1 or LAT2 through a LAX-mediated regulatory pathway.

Compatible with the enhanced phosphorylation of Akt in LAX-null cells, LAX deficiency in MCs led to an enhanced growth relative to wildtype cells. This result was due to increased cell survival that was also evident after withdrawal of the growth factor IL-3 (122). Interestingly, this enhanced survival phenotype was not seen *in vivo* where the numbers of MCs in tissues appeared to be similar between LAX-null and wildtype mice. Moreover, the hyperresponsive degranulation seen *in vitro* was not observed *in vivo* upon a systemic anaphylactic challenge of *lax*^{−/−} mice. Thus, it appears that other factors may modulate the

LAX-null phenotype *in vivo*. Alternatively, the studies on *in vitro* differentiated MCs are not likely to fully reflect the *in vivo* phenotype of *lax*^{-/-} MCs.

Cytosolic adapter proteins

The absence of a putative hydrophobic transmembrane domain marks this category of adapters for residence in the cytosol (Fig. 3). However, cytosolic adapters are not simply free floating proteins in the cytosol, as they can be found associated with the cytoskeleton, membranes, and organelles (125,126). Importantly, cytosolic adapter proteins can be recruited to form macromolecular signaling complexes upon cell stimulation, where they participate in organizing and coordinating signals. Cytosolic adapters can interact with more than one protein, thus membrane adapters, such as LAT1 and LAT2, recruit these cytosolic adapters to promote interactions with various signaling proteins, which also stabilizes the signaling complex. Cytosolic adapters also play key roles by promoting the recruitment of effector or enzymatic proteins into a signaling complex, thus serving to activate and coordinate their activities. The importance of such adapters in cell signaling is revealed by the fact that genetic deletion of some of them results in embryonic lethality or marked defects in development and function (125).

SLP-76 and Gads

One of the main components of LAT1-scaffolded signaling complexes formed after FcεRI stimulation of MCs is the adapter protein SLP-76 (Figs 1 and 4). SLP-76 is a member of a small family of adapter proteins most abundantly expressed in hematopoietic cells (127). Although they display only minor sequence homology, these adapters possess the same overall structure (Fig. 3). They have an N-terminal acidic region, contain several tyrosine phosphorylation sites, a central proline-rich SH3-binding region, and a C-terminal SH2-domain. Other members of this family are the cytokine-dependent hematopoietic linker (Clnk) and SLP-65 (which is not expressed in MCs) (127). SLP-76 is not involved in MC differentiation *in vivo* or *in vitro*. SLP-76-null MCs showed defective degranulation and cytokine secretion after FcεRI stimulation (128). Thus, as might be expected, the *in vivo* response of MCs was also defective, as SLP-76-null mice showed little response to an anaphylactic challenge with barely detectable increases in circulating histamine levels (128).

SLP-76 is strongly tyrosine-phosphorylated upon FcεRI stimulation, in a Syk-dependent manner (129) (Fig. 1). The predominant phosphorylated tyrosine residues are Y113, Y128, and Y145 (Fig. 3), which provide binding sites for Vav1, Nck, and Btk, promoting their recruitment to LAT1 (130). SLP-76 recruitment of the GEF Vav1 to the LAT1-scaffolded complex is not only important for its function, as an activator for the Rho family of small G-proteins that regulate cytoskeletal changes and MAPK activation, but also for stabilizing the recruitment and activation of PLC-γ and the regulation of Ca²⁺ responses (127,131). In addition, the recruitment and activation of Btk in a LAT1/SLP-76-dependent manner also contributes to PLC-γ activation in MCs (132). To date, we know little about the function of the SLP-76-recruited adapter protein Nck in MCs, but it may well have a similar function as in T cells. In T cells, Nck couples to p21-activated kinase (PAK1) and Wiskott-Aldrich syndrome protein (WASP), proteins that are involved in the regulation of the actin cytoskeleton and participate in regulating the activity of JNK and cytokine production (133,134). In MCs, both PAK1 and WASP have been shown to play an important role in cell responses upon activation of FcεRI (133,134). PAK1-null MCs were defective in degranulation and this phenotype could be linked to reduced Ca²⁺ responses and defective depolymerization of F-actin (134). WASP-null MCs also showed a marked defect in degranulation and cytokine production that was linked to the loss of PLC-γ phosphorylation and defective calcium responses (133). Not surprisingly, SLP-76-null MCs also showed a marked defect in calcium responses, while signaling proximal to the FcεRI was unaffected.

The overall protein tyrosine phosphorylation and Syk phosphorylation was normal following FcεRI stimulation. In contrast, PLC-γ1 phosphorylation was reduced and PLC-γ2 phosphorylation was almost entirely abolished (128,130).

A detailed analysis of the different protein interaction sites in SLP-76 revealed that the acidic N-terminus, with its tyrosine phosphorylation sites, and the proline-rich regions (Fig. 3) were required for Ca²⁺ responses and degranulation but were only partly involved in cytokine secretion. Mutagenesis of the Btk-binding site showed a marked effect on degranulation, whereas cytokine production was more severely affected by mutation of the Vav1 binding site. Correspondingly and as previously described (84), the Vav1-binding mutant also had the most drastic effect in JNK activation (130). In contrast, there were only minor effects on p38 MAPK and ERK activation in SLP-76-null MCs and its role seems to be in sustaining their activation (130). Somewhat unexpectedly, Vav1 tyrosine phosphorylation was not affected in SLP-76-null MCs (128), suggesting that its phosphorylation may be independent of an association with SLP-76 and possibly with LAT1. The SH2 domain of SLP-76 had no major role in MC degranulation but was necessary for cytokine production and JNK activation (130). However, how the SH2 domain of SLP-76 contributes to JNK activation is not known. The SH2 domain of SLP-76 is known to bind the adapter protein SLP-76-associated protein of 130 kDa (SLAP-130) (135,136) (Fig. 3). This adapter regulates the actin cytoskeleton and thus in this manner it may be able to influence JNK activation. SLAP-130 is discussed in greater detail in a following section.

SLP-76 does not directly couple to LAT1, but rather, it is recruited to the LAT1 signaling complex via another adapter protein, Gads (Figs 1 and 4). Like Grb2 (which is discussed later), Gads is small adapter proteins that consists of two SH3 domains and a central SH2 domain. Gads is found to constitutively associate with proline-rich region localized in the middle part of SLP-76. Recruitment of Gads (via its SH2 domain) to the LAT1 protein complex after FcεRI stimulation is absolutely essential for SLP-76 function. This localizes SLP-76 to lipid rafts where it can modulate calcium responses (130,137,138). In MCs, disruption of the Gads-SLP-76 interactions caused a marked impairment in degranulation and cytokine production (138). In T cells, it was shown that targeting of a SLP-76 mutant (unable to bind Gads) to lipid rafts rescued the defect observed by interfering with SLP-76 binding to Gads (139). Thus, it appears that the key role for Gads is to target SLP-76 to lipid rafts by interacting with LAT1 (Fig. 4). As might be expected, the genetic deletion of Gads in MCs greatly diminished SLP-76 binding to LAT1 and almost completely abolished cytokine secretion (140). However, Ca²⁺ influx and degranulation were less affected in Gads-null MCs than in SLP-76-deficient MCs, suggesting a Gads-independent interaction of SLP-76 and LAT1 that is sufficient to induce some Ca²⁺ responses (140).

Clnk

Another SLP-76 family member expressed in MCs is Clnk (also termed MIST). Clnk shares the overall structure of SLP-76 (Fig. 3); however, this adapter is missing the interaction sites for Vav and Nck (141,142). Clnk becomes rapidly tyrosine phosphorylated after FcεRI stimulation of MCs, and like SLP-76, it associates with LAT1, Grb2, and PLC-γ and probably indirectly with Vav1 (142). Clnk also interacts with SLAP-130 and also forms a complex with Src kinase-associated phosphoprotein of 55 kDa (SKAP-55) in FcεRI-activated MCs (143). In contrast to SLP-76, the interaction between Clnk and SLAP-130 is constitutive. Depending on the level of expression of the Src PTKs Lyn and Fyn, in different types of MCs, Clnk can associate with Fyn or Lyn following FcεRI stimulation. In the presence of both Src PTKs, the SH2-domain of Fyn seems to have a higher affinity for tyrosine phosphorylated Clnk (143). In contrast to many other adapter proteins expressed in MCs (including SLP-76), tyrosine phosphorylation of Clnk seems to be directly mediated by Src PTKs rather than by Syk (142). Overexpression of a mutant form of Clnk, in which all

tyrosines were mutated to phenylalanine, showed a significant suppression of Ca^{2+} responses and MC degranulation (142). However, contrary to all expectations, Clnk deficiency did not alter MC signaling or response when cells were stimulated via Fc ϵ RI *in vitro* (144). This outcome might be explained, in part, by possible redundancy with SLP-76, as it has been suggested that Clnk may be able to partially compensate for SLP-76 function, as its residual activation was observed in SLP-76-null MCs (130). Alternatively, as unlike SLP-76 Clnk expression is cytokine inducible (141), its role may not be easily evident *in vitro* as it may require the appropriate milieu for functional competence. Thus, an appropriate immune challenge in an *in vivo* setting may be required to reveal the role of Clnk in immunity and in MC function.

SLAP-130 (FynB/FyB/ADAP)

One of the most prominent SLP-76-interacting proteins in MCs is the adapter protein SLAP-130 (129,145). SLAP-130 is a large adapter with a wide range of different protein interaction motifs and domains, such as two SH3 domains and several SH2 domain-binding sites (Fig. 3). After Fc ϵ RI stimulation, SLAP-130 was found to be strongly tyrosine-phosphorylated and translocated to F-actin-rich membrane ruffles (127,146). SLP-76 binds SLAP-130 via the former's SH2 domain (Fig. 4), and deletion or mutation of the SH2 domain of SLP-76 inhibits Fc ϵ RI-mediated MC activation (130). As the SH2 domain of SLP-76 is required for its interaction with Gads and LAT1, its interaction with SLAP-130 would imply that several pools of SLP-76 are required for MC function. Studies in the MC line, RBL-2H3, showed that overexpression of SLAP-130 increased adhesion to fibronectin and was able to enhance MC degranulation (146,147). It has been suggested that SLAP-130 could serve as a platform for the integration of Syk-and Fyn-dependent signaling, as SLAP-130 (which is apparently phosphorylated by Fyn in T cells) interacts with SLP-76, which is phosphorylated in a Syk-dependent manner (35,148). In MCs, SLAP-130 was shown to interact with Fyn (145). However, in contrast to the overexpression experiments in RBL-2H3 cells, SLAP-130 deficiency did not alter Fc ϵ RI-dependent MC degranulation nor affect adhesion to fibronectin *in vitro*. In B or T cells, SLAP-130 was shown to connect these immune receptors to integrin signaling (127,149). At this point, one cannot exclude that SLAP-130 may be important for connecting Fc ϵ RI signaling to integrin signaling in an *in vivo* setting; however, one can conclude that MC degranulation responses are unaffected as the anaphylaxis was unaltered in SLAP-130-null mice (127). The apparent discrepancy of a strong phosphorylation of SLAP-130 after Fc ϵ RI stimulation but no functional alterations in SLAP-130-null MCs remains to be resolved. Redundancy may be a factor, but the known SLAP-130 homolog PRAM-1 (PML-RAR α target gene encoding an adapter molecule-1) has not been shown to be expressed in MCs (150).

In T cells, SLAP-130 constitutively interacts with the adapter protein SKAP-55, and SKAP-55 has also been shown to link TCR signaling to LFA-1 clustering and the regulation of integrin-mediated adhesion (151,152). In MCs, SLAP-130 interactions with SKAP-55 and Clnk have also been described (143), but whether this interaction links Fc ϵ RI to integrins is unknown. MCs also express the SKAP-homolog (SKAP-HOM/SKAP-55R) (153, E. Lessmann, unpublished data). Like SLAP-130-null MCs, SKAP-HOM-null MCs showed normal Fc ϵ RI responses when stimulated *in vitro* (E. Lessmann and M. Togni, unpublished data). In B cells, SKAP-HOM deletion resulted in lower proliferation and reduced adhesion to fibronectin and ICAM following BCR stimulation (154). However, a role for SKAP-HOM in the activation of integrins in MCs has not been demonstrated.

3BP2

Another LAT1-interacting adapter protein is the c-Abl SH3 domain binding protein-2 (3BP2), which for MCs has been studied in the RBL-2H3 cell line. These studies and the

analysis of 3BP2-deficient B cells implicate this adapter as having a potentially important role in regulating MC function. 3BP2 consists of a PH domain, several proline-rich regions, several tyrosine phosphorylation sites, and a C-terminal SH2 domain (155) (Fig. 3). Studies on 3BP2-deficient primary B cells showed that 3BP2 served as a scaffold to mediate PLC- γ 2 phosphorylation by tyrosine kinases. In contrast, 3BP2 seems not to be involved in signaling from the TCR (156).

In RBL-2H3 cells, Fc ϵ RI stimulation led to rapid tyrosine phosphorylation of 3BP2 in a Syk-dependent manner (157,158). The tyrosine phosphorylation of 3BP2 was shown to create novel binding sites for Lyn and caused increased Lyn phosphorylation. While the functional outcome of this increased Lyn phosphorylation is unclear, it could provide a feedback loop that serves to enhance Lyn activity (158). This could promote both positive and negative consequences in MC responses, given the dual role of Lyn kinase (27,34,86). Overexpression of the SH2 domain of 3BP2 inhibited PLC- γ 1 and PLC- γ 2 phosphorylation, which translated to reduced Ca²⁺ responses and degranulation (159). This result was not due to the previously proposed function of 3BP2 in regulating Syk activity (160), as the overall tyrosine phosphorylation of Syk was unaltered. Rather, 3BP2's binding to LAT1 seemed to be necessary for downstream activation of PLC- γ (157), although how 3BP2 influences PLC- γ activation is unclear. It is apparent that we still have much to learn about the role of 3BP2 in the LAT1-scaffolded complex and in MC function.

Gab2

Although it has been long recognized that PI3K signaling is important for the regulation of Fc ϵ RI-mediated activation of MCs (161–163), the molecular link between Fc ϵ RI and activation of PI3K remained a mystery for many years. This long-standing unknown was partly unraveled by the discovery of the adapter protein Gab2 (164) and the demonstration of its function in MCs (63) (Fig. 1). This 97 kDa adapter protein belongs to the family of DOS/Gab adapters and has an N-terminal PH domain, two proline-rich regions, and several tyrosine phosphorylation sites (Fig. 3). This structure endows Gab2 with the ability to bind multiple proteins, thereby coordinating important signaling events downstream of the Fc ϵ RI and c-Kit (57,63). A detailed analysis of Gab2 signaling and function revealed that it acts in a Fyn-dependent manner but complementary to the already well-characterized Lyn-LAT1-dependent signaling pathway (35,63) (Fig. 1). Upon Fc ϵ RI stimulation, Gab2 was found to translocate to the plasma membrane, where it becomes heavily tyrosine phosphorylated (63,165). In agreement with its role in Fc ϵ RI signaling, Gab2 was shown to localize in plasma membrane domains that contain the Fc ϵ RI (45). Association of another family member Gab1 with LAT2 was demonstrated in a B-cell line (107); thus as Gab2 is dominantly expressed in MCs, it is likely that it interacts with LAT2 via its multiple Grb2-binding sites (111) (Fig. 1). However, analysis of different Gab2 mutants suggested that Gab2 may also be recruited to the Fc ϵ RI β -chain by a mechanism similar to its recruitment to the 'common β -chain' utilized by the cytokine receptors for IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF). As for the common β -chain, Gab2 was proposed to be recruited to the receptor via its interaction with the adapter proteins Shc and Grb2 (57).

How Gab2 is phosphorylated in MCs is not clearly understood. Two potential modes of phosphorylation have been described that are not mutually exclusive. Analysis of Gab2 in Fyn-null MCs showed a defect in Gab2 phosphorylation (Fig. 1). Furthermore, Fyn (and not Lyn) was found to bind to Gab2 upon Fc ϵ RI stimulation (35). This was consistent with later reports, where Gab2 phosphorylation had been described to be normal or enhanced in Lyn-null MCs (44,65,66). In contrast, Gab2 was found to bind Lyn in RBL-2H3 cells (165), and later analysis of Lyn- and Fyn-null MCs reported that Gab2 tyrosine phosphorylation was strictly dependent on Lyn and Syk kinase and only partly dependent on Fyn kinase (57). The

reason for this apparent discrepancy is not clear. However, the recently described differences in the phenotype of Lyn-null MCs (119,166), when derived from mice of different genetic backgrounds, has shown that the relative expression levels of Fyn and Lyn can determine their function in the MC. This observation suggests that under certain circumstances Lyn and Fyn can exchange roles, and thus Fyn may also be involved in the activation of Syk. Indeed, Yu *et al.* (57) observed reduced Syk phosphorylation in response to FcεRI activation in Fyn-null MCs, whereas this had not been observed in the initial analysis of Fyn-null MCs (35). Thus, the extent of Fyn, Lyn, or Syk's contribution to Gab2 phosphorylation may well depend on the genetic background or the levels to which these proteins are expressed.

Gab2-null MCs showed impaired degranulation upon FcεRI stimulation and were also defective in cytokine secretion. Accordingly, Gab2-null mice showed diminished responses in cutaneous and systemic anaphylaxis. Nonetheless, phosphorylation of the FcεRI and activation of tyrosine kinases, like Lyn and Syk, appeared to be normal (63). LAT1-dependent signals were also unaffected (63). In contrast, Gab2-deficiency caused a defect in PI3K activation in MCs (Fig. 1). Following FcεRI stimulation, the p85 regulatory subunit of PI3K was shown to bind to Gab2, a step associated with the activation of PI3K and the production of PIP₃ (Figs 3 and 4). Thus, in MCs, the absence of Gab2 caused a marked diminution in PIP₃ levels (63). As many signaling proteins are dependent on PIP₃ for their recruitment to the plasma membrane and for increased activity, the consequences of a marked loss of PIP₃ are likely to be amplified and lead to the impairment of various MC responses. Therefore, it is not surprising that Gab2-null MCs were defective in degranulation and cytokine production (63). Interestingly, the PH domain of Gab2 appears to have a preferential affinity for PIP₃ and PI(3,4)P₂, thereby providing an amplification mechanism by which Gab2 membrane binding and protein interactions are stabilized (57).

Unexpectedly, overexpression of Gab2 in the RBL-2H3 cell line was shown to strongly suppress FcεRI-mediated signaling (165). Similar to Gab2-null MCs, degranulation and cytokine production were significantly impaired. This suppression effect was PI3K independent, as the activity of this lipid kinase appeared to be normal (165). A possible explanation for this observation may be that Gab2 competes for binding of Lyn and PLC-γ (as observed in this study) (165), dampening the Lyn-LAT1 pathway (Fig. 1) required for degranulation and cytokine production. An alternate explanation may be that overexpression of Gab2, which also binds the tyrosine phosphatase SHP-2 (63), could increase the presence of this phosphatase on the membrane and impair FcεRI-induced signals.

Besides its function in PI3K activation, Gab2 is also directly involved in the regulation of the vesicle transport during the degranulation process. Nishida *et al.* (167) showed that Fyn-mediated Gab2-dependent signaling events led to the activation of RhoA and induced the formation of microtubule networks necessary for the movement of granules to the plasma membrane. Thereby, Gab2 appears to function in the degranulation process downstream of its role in PI3K activation and early signaling events. Recently, it has been reported that Gab2-dependent signals can be manipulated independently of other signals in MCs. Kraft *et al.* (168) showed that antibodies directed against the tetraspanin CD63 suppressed FcεRI-mediated degranulation. Preincubation of MCs with anti-CD63 antibodies was shown to specifically downregulate Gab2-dependent signaling, whereas Lyn-LAT1-dependent signaling was still intact (168). Similarly, Sinomenine, an alkaloid extracted from a Chinese medical plant used for treatment of autoimmune diseases, selectively inhibits Gab2 phosphorylation and Gab2-dependent signaling after FcεRI activation in RBL-2H3 cells, in the absence of effects on other signaling pathways (169). This result is consistent with the previously reported selective induction of Fyn-mediated signaling and Gab2 phosphorylation upon an MC's encounter with a weak stimulus (61,62).

Grb2/Shc/IRS

Grb2 is an adapter protein of 25 kDa molecular mass that is ubiquitously expressed in mammalian cells. It serves as a linker downstream of several tyrosine kinase receptors as well as immune receptors. However, in contrast to tyrosine kinase receptors like the epidermal growth factor receptor, Grb2 does not directly bind to the phosphorylated ITAMs of immune receptors, but rather, it is recruited to the activated immune receptor complexes via its association with the adapter protein Shc (170–172) (Figs 1 and 4).

As for its related family member Gads, Grb2 consists of an N- and C-terminal SH3-domain with a central SH2-domain (Fig. 3). Grb2 constitutively interacts with the guanine-exchange factor SOS (Fig. 1). Recruitment of Grb2 to activated receptors and membrane-localized adapters brings SOS into close proximity to its substrate and leads to Ras activation and subsequently ERK activation (173). Although alternative pathways for Ras activation in MCs have been described (174), the activation via Grb2 and SOS seems to be an important pathway in FcεRI-mediated Erk activation. There are only a few studies on the role of Grb2 in MCs; however, several binding partners of Grb2 have been identified after FcεRI activation. Beside Shc and SOS (175), Grb2 has been described to interact with LAT1, LAT2, SLP-76, and Gab2 (102,111,129). However, its contribution and function in such partnerships can only be inferred from mutational analysis of putative Grb2-binding sites on its interacting partners, like LAT1 (101) (Fig. 4).

Like Grb2, Shc is ubiquitously expressed in mammalian cells. Nonetheless, this adapter protein serves an important role downstream of immune receptors. Two isoforms of Shc are expressed in MCs, 46- and 52-kDa proteins. Both isoforms have two domains that recognize phosphotyrosines, an N-terminal PTB domain and a C-terminal SH2 domain (Fig. 3). Shc can also be tyrosine phosphorylated at three sites in a central collagen-binding domain (CH1), creating novel docking sites for SH2-domains. In addition, Shc has two proline-rich regions mediating interactions with other proteins (176).

While Shc and SOS have been described to associate with LAT1 or LAT2 via Grb2 (Fig. 1), Shc also associates with the tyrosine-phosphorylated ITAMs of the FcεRI and becomes tyrosine phosphorylated in a Syk-dependent manner. It has been shown to preferentially interact with the FcεRI β-chain (170–172). Phosphorylation of Shc leads to the recruitment of Grb2 and SOS and thereby induces Ras activation (176). It has been suggested that immune receptors use Shc to amplify the activation of Ras, as Shc contains two Grb2-binding sites, leading to increased targeting of SOS to the plasma membrane where Ras is found (176). However, this may not be the only mode of FcεRI induction of Ras and ERK activity, as under conditions where Shc is not efficiently phosphorylated, the activation of ERK appears to be relatively normal (174). Besides its possible function in the activation and amplification of signaling from the FcεRI, Shc also plays an important role in the negative regulation of FcεRI activation. This adapter is a prominent binding partner for the SHIP-1. In fact, SHIP-1 was first described as a 145-kDa tyrosine-phosphorylated protein that associated with Shc after cytokine stimulation of MCs, and this complex was shortly thereafter described in FcεRI-activated MCs (174,177). Interestingly, Shc interacts with SHIP-1 but not SHIP-2, which might explain the difference in function described for the two different SHIP homologs in MCs (178). SHIP-1 is an important negative regulator of FcεRI signaling in MCs, as this lipid phosphatase regulates PIP₃ levels and thereby controls the levels of this second messenger after FcεRI activation (174). The Shc/Grb2 complex also seems to be important for the recruitment of Gab2 to the β-chain of the FcεRI (57). Thus, it appears that Shc may serve to couple the PI3K activity associated with Gab2 to the negative regulatory partner SHIP-1. Another point of negative regulation that may involve SHIP-1 is that it may compete with Grb2 binding to Shc that is associated with the FcεRI, thereby interfering with Ras recruitment and activation (179). SHIP-1 interaction with Shc seems to

be important for Shc tyrosine phosphorylation, as it is compromised in SHIP-1-deficient MCs (174). The mechanism for this is unknown, but it suggests that SHIP-1 binding may reveal tyrosine residues on Shc that are otherwise masked.

The adapter protein insulin-receptor substrate-2 (IRS-2) is a ubiquitously expressed adapter that has an important function in proliferation and survival. Although IRS-1 and IRS-2 were originally described to be absent in MCs (180), unpublished work has found that IRS-2 is expressed in MCs and becomes tyrosine phosphorylated in response to FcεRI stimulation (E. Lessmann and M. Huber, unpublished data). IRS-2 was found to interact with the SH2 domain of SHIP-1, indicating a potential role in the regulation of MC responses. However, its role and function in FcεRI signaling is not known. Nonetheless, given its role in cell proliferation and survival as well as the recent recognition of a role for IRS molecules in actin rearrangement (181), IRS-2 may be important in FcεRI signaling and MC responses.

Dok

The adapter protein family Dok (downstream of tyrosine kinase) has been shown to interact exclusively with negative regulators of FcεRI signaling in MCs. Dok family proteins have an N-terminal PH domain, a PTB domain, numerous tyrosine residues that can be phosphorylated, and several PXXP motifs that bind SH3 domains (Fig. 3). These adapter proteins have been shown to interact with an array of different signaling proteins (182,183). Of the different isoforms described, Dok-1, Dok-2, and Dok-3 are expressed in MCs (183,184). FcεRI stimulation leads to the tyrosine phosphorylation of Dok-1 and Dok-2 but not Dok-3. Nevertheless, Dok-3 associates with tyrosine-phosphorylated proteins upon FcεRI stimulation, implicating a yet undefined function for this adapter protein downstream of the receptor (183).

Dok-1, also called p62^{dok}, is the most well-studied isoform and has been shown to play an important role downstream of negative regulatory receptors that encode immune receptor tyrosine-based inhibitory motifs (ITIMs) (Fig. 5). Dok-1 forms a constitutive complex with the Ras GTP-binding protein-activating protein (RasGAP) and associates with SHIP-1 upon activation of ITIM-bearing receptors. This protein complex is directly recruited to the tyrosine phosphorylated ITIMs via the interaction of the SH2 domain of SHIP-1 (185) (Fig. 5). Thus, this negative regulatory complex of SHIP-1/RasGAP/Dok-1 downregulates PIP₃ levels via the action of SHIP-1 and inhibits Ras activation via RasGAP. This complex of Dok-1, RasGAP, and SHIP-1, which was first described after co-aggregation of the BCR with FcγRIIB (185), was also described in MCs after FcεRI and FcγRIIB co-aggregation (182,186). This negative regulatory complex is also utilized downstream of other negative regulatory MC receptors like MAFA (187). Dok-1 has also been involved downstream of activating receptors, like FcεRI, by associating with and negatively regulating signals without the involvement of inhibitory receptors (182,183). How Dok-1 interacts with FcεRI is not clearly understood. However, Dok-1 was shown to bind to phosphorylated receptors directly via its PTB domain (188), and in RBL-2H3 cells, Dok-1 was found to associate with the FcεRI β-chain (183). Interestingly, mutation of the FcεRI β-chain ITAM's non-canonical tyrosine residue (that lies in the middle of the ITAM) was shown to cause increased cytokine production in MCs, suggesting that this tyrosine residue might interact with a negative regulator (32). Nevertheless, Dok-1 deficiency did not affect MC activation, suggesting a possible redundant function for the different isoforms expressed in these cells (182). Dok family proteins are associated solely with negative regulation in MCs, although these adapters may also be involved in promoting activation (183,189,190).

Discussion and perspectives

Adapter proteins (Fig. 3) are essential in the spatiotemporal organization of cell signaling. They serve to coordinate, amplify, and regulate the molecules involved in signaling, thus creating a highly organized network of events that are key in promoting and regulating cell activation and effector responses. As described herein for FcεRI-mediated MC activation, the initial events that promote signaling are organized around the receptor itself (i.e. receptor phosphorylation), but the formation of novel docking sites for signaling proteins on the receptor itself is not sufficient for signal amplification and downstream events (52,89,191). Adapter proteins, like LAT1, LAT2, Grb2, and Gab2 appear to be essential for this signal propagation (27,69,75,87,115) (Fig. 1). In the case of the FcεRI, transmembrane adapters like LAT1, LAT2, LAX, and Cpb/PAG function to assemble macromolecular complexes at the plasma membrane (Figs 2 and 4), and this coordination of signals at the plasma membrane is essential for promoting and controlling FcεRI-mediated MC responses. Many of these events take place in the lipid raft environment, as molecules such as LAT1 and LAT2 and Cbp are found in these domains. Moreover, the FcεRI is also found in these cholesterol-rich lipid raft domains following its engagement. At first glance, it might seem that these adapter-containing macromolecular complexes can be found in close proximity of the receptor-associated tyrosine kinases, thus linking receptor events to signal amplification that drives the cellular response. However, multiple studies (44,45,91,92,192) provide evidence for the heterogeneity of the lipid raft domains. In fact, as mentioned above LAT1 and LAT2 appear to reside in lipid raft domains that are distinct from those in which the FcεRI resides (45,91,92). Moreover, high-resolution electron microscopic studies suggest that domains containing LAT1 do not mix with the domains containing FcεRI but instead seem to localize close to or intersect with the domains containing this receptor (45). This is in sharp contrast to the recruitment of cytosolic adapters like Gab2, which are found in the lipid raft domains containing FcεRI (45). Thus, how FcεRI is able to communicate to the LAT1- and LAT2-assembled signaling complexes remains an unresolved question. Interestingly, FcεRI engagement by a weak versus strong stimulus seems to be distinguished by whether LAT1 is not phosphorylated or is phosphorylated, respectively (61, R. Suzuki and J. Rivera, unpublished data). Exploration of these requirements may shed new light on the issue of how these molecules communicate. In the absence of transmembrane adapter phosphorylation, some cytosolic adapters like Gab2 can be phosphorylated (61). These 'activated' adapters should be able to bind other signaling proteins, thereby localizing them into proximity to their substrates, and in this manner promote selective signals and MC responses (61).

While some general principles (and many adapter molecules) may be shared by different receptor systems in different immune cells, there are observed differences that do not allow one to directly extrapolate the function of a given adapter from one cell to another. For example, SLP-76 is required for T-cell development but is dispensable for the development of MCs (193). Moreover, LAT2 is constitutively expressed in MCs and is strongly phosphorylated by c-KIT and FcεRI stimulation (194), and its absence leads to enhanced or reduced MC responses depending on the experimental approach used to downregulate it (27,115). In contrast, in T cells, this adapter is expressed only in activated cells, and its absence leads to enhanced IL-2 production and autoimmunity (109). This paradigm of differential use of signaling molecules extends beyond adapter molecules and can take place within the same cell type, depending on the expression levels of a signaling protein. Thus, in MCs the ratio of the two Src kinases Fyn and Lyn (which differs with the genetic background of the mouse from which the cells are derived) may lead to different nuances in the use of an adapter like Clnk and thus differences in MC responses (119,143,166). Another interesting aspect of differential use of adapter molecules is seen in signal integration. This is of special importance because cells *in vivo* usually are exposed to more than one stimulus

at a time. For example, in tissues MCs are exposed to their growth factor, SCF, and this exposure is likely to influence FcεRI-mediated responses, as has been shown to occur *in vitro* (114,195). Not only does SCF enhance the FcεRI-mediated responses, but it also enables MC activation under conditions where no FcεRI response is usually observed (114,195,196). This signal integration between c-KIT and FcεRI has been ascribed to the role of the adapter LAT2 in MCs. Thus, adapter proteins can serve as platforms for the integration of signaling and for the regulation of signal magnitude (114,115,194).

Understanding adapter molecule function in the context of FcεRI-stimulation (as well as for other immune receptors) requires strategies that focus on the study of the dynamic interactions of these molecules. High-resolution imaging of living cells and other biophysical methods are now shedding some light into the dynamics of the FcεRI in the plasma membrane and how this might relate to signaling (47–50,93). While we are still limited in our knowledge of the potential interacting partners for many adapters expressed in MCs, determining the spatiotemporal events underlying adapter function is likely to be important in defining how FcεRI proximal signaling events are propagated and in determining the importance of a particular adapter molecule to specific MC responses. Ultimately, an increased understanding of the role of individual adapter molecules in MC responses may well provide new paradigms for modulating MC function in health and disease.

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Figure 1. Schematic representation of the FcεRI-stimulated assembly of macromolecular signaling complexes in mast cells (MCs)

FcεRI aggregation by cell surface-bound IgE antibody interaction with a multivalent antigen leads to the phosphorylation of the receptors β- and γ-chain immunoreceptor tyrosine-based activation motifs (ITAMs) by Lyn kinase. Fyn and Syk kinases are then recruited to the receptor and initiate complementary signals that cooperate in MC responses. Fyn and possibly Syk phosphorylate adapters like Gab2 that are essential for PI3K activation. Syk also phosphorylates the adapters LAT1 and LAT2 in several tyrosine residues. LAT1 organizes a complex that binds phospholipase C-γ (PLC-γ), SLP-76, and son-of-sevenless (SOS) via adapters such as Gads, Grb2, and Shc. Activation of PLC-γ in this complex causes the Ca^{2+} hydrolysis of PI(4,5)P₂ into IP₃ and DAG, increasing intracellular Ca concentrations and activating PKC. In addition to LAT1, the adapter LAT2 is also phosphorylated after FcεRI aggregation and organizes a signaling complex by recruiting Grb2 to its several YXN motifs. Grb2 then participates in activating the PI3K pathway through its association with the adapter Gab2. Like LAT1, LAT2 is able to recruit the guanine nucleotide exchange factors Vav1 and SOS, which in turn activate MAPKs such as ERK. LAT2 may also bind PLC-γ indirectly by its association with Grb2. LAT1 is well documented to be involved in Ca²⁺ mobilization, which is crucial for MC degranulation, NFAT activation, and cytokine production. PKC activation is also essential for MC degranulation, activation of NF-κB, and cytokine production.

Figure 2. Negative regulation of Fyn kinase by Lyn-phosphorylated Cbp/PAG

The adapter Cbp is a transmembrane protein that is targeted to lipid rafts due to its palmitoylation. In mast cells (MCs), Cbp is moderately phosphorylated in resting cells by Lyn kinase and shows a moderate association with the negative regulatory kinase Csk, which phosphorylates Src PTKS and inactivates them. FcεRI stimulation greatly enhances the phosphorylation of Cbp (on Y317) in a Lyn-dependent manner. This allows additional recruitment of Csk, which phosphorylates Fyn at its C-terminal tyrosine (Y525) causing an intra-molecular folding (via interaction with its own SH2 domain) that inactivates it. This negative regulation of Fyn (and possibly of other Src PTKS) controls the responsiveness of the MC. In the absence of Lyn, MCs are hyperresponsive due to loss of this negative regulatory mechanism.

Figure 3. Adapter proteins in mast cells (MCs)

Schematic model of the adapter proteins expressed in MCs, structural motifs that allow protein–protein or protein lipid–interactions, and molecular mass. Binding partners confirmed in MCs are indicated.

Figure 4. Model of FcεRI-induced LAT1 and LAT2 multiprotein complexes in mast cells (MCs) FcεRI stimulation causes LAT1 tyrosine phosphorylation, which allows association with Gads, Grb2, SLP-76, Btk, Vav1, SLP-130, and phospholipase C-γ (PLC-γ). Structural motifs for interactions are shown. The small adapters Grb2 and Gads bring molecules like son-of-sevenless (SOS) and SLP-76 into the complex. SLP-76 can recruit two additional adapters, SLP-130 and most probably Nck. Other signaling molecules, like Vav1 and Btk, can bind to tyrosine-phosphorylated SLP-76 via their SH2 domains. Like LAT1, LAT2 also becomes tyrosine phosphorylated upon FcεRI stimulation and is also able to recruit Grb2 and SOS. In contrast to LAT1, the adapter LAT2 associates with Vav1 primarily via binding to Grb2. In addition, through a second Grb2-interaction site, the adapter Gab2 may be indirectly recruited to the LAT2 multiprotein complex. Grb2 mediates recruitment of Gab2 via its SH2 domain binding to LAT2 and its SH3 domain binding to the proline-rich region of Gab2. Tyrosine phosphorylation of Gab2 results in the recruitment of the regulatory PI3K subunit p85 via its SH2 domain subsequently leading to PI3K activation.

Figure 5. Negative regulation of FcεRI signaling by the SHIP-1/Dok-1/RasGAP complex
 Upon co-aggregation of the FcεRI with the FcγRIIB by a multivalent antigen, SHIP-1 is recruited to the tyrosine-phosphorylated ITIM (immune receptor tyrosine-based inhibitory motif) of the FcγRIIB (via its SH2-domain) in a Lyn kinase-dependent manner. Tyrosine phosphorylated SHIP-1 becomes associated with the adapters Shc and Grb2. Grb2 binds to another C-terminal phosphorylated tyrosine in the FcγRIIB, which stabilizes the complex (197). In addition, Dok-1, which is constitutively associated with RasGAP, is also recruited to tyrosine phosphorylated SHIP-1. One known effect of recruitment of SHIP-1 to the plasma membrane is the downregulation of PIP3 levels, which leads to decreased phospholipase C-γ (PLC-γ) activity, IP₃ production, and impaired Ca²⁺ fluxes, thus inhibiting mast cell degranulation. Recruitment of RasGAP to Dok-1 inhibits son-of-sevenless (SOS) and thereby Ras activation, leading to decreased cytokine and eicosanoid production. Similar to the FcγRIIB, the tyrosine-phosphorylated ITAM (immunoreceptor tyrosine-based activation motif) sequence of the β-chain of the FcεRI has been shown to recruit SHIP-1 and Grb2, and an association of Dok-1, but not RasGAP, with the FcεRI has been demonstrated.