

A Current Understanding of FcεRI-dependent Mast Cell Activation

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Mast cell activation via the high-affinity immunoglobulin (Ig) E receptor FcεRI is a topic of extensive investigation with therapeutic potential in allergic disease. The protein tyrosine kinases Fyn, Lyn, and Syk are intimately linked with the early events initiated by allergen-mediated aggregation of IgE-occupied FcεRI. Fyn and Lyn initiate signaling events that are organized by adaptor molecules, which compartmentalize and coordinate the activity of activated protein and lipid kinases and phospholipases to generate lipid products essential for signal amplification and mast cell function. Fyn and Lyn counter-regulate phosphatidylinositol 3-OH kinase (PI3K), controlling the produced amount of phosphatidylinositol (3,4,5)-trisphosphate (PIP₃), a key regulator of mast cell degranulation. Fyn and Lyn also activate sphingosine kinases (SphK), which generate sphingosine-1-phosphate (S1P), thus contributing to mast cell chemotaxis and degranulation. Here, we summarize the current knowledge and future challenges and directions.

Introduction

Mast cells are a key cellular component of innate immunity [1]. These tissue resident cells provide host defense against pathogens via various receptors that activate the mast cells and cause recruitment of other immune cells leading to infection resolution. Mast cell activation results in the secretion of several immune response modulators, among which the mast cell can produce a variety of factors (eg, interleukin [IL]-4, IL-6, and IL-13) that promote and enhance B-cell immunoglobulin (Ig) production and class switching to IgG and IgE, thus amplifying acquired immunity. Dysregulation of this

latter process, which can result in production of IgE antibodies to normally innocuous substances, makes the mast cell a central player in allergic disease as the effector cell that binds allergen-specific IgE (via its high-affinity receptor FcεRI) and responds to allergen challenge [2]. This unconventional role has garnered much interest since the discovery of IgE almost 40 years ago [3]. Recent effort has focused on identifying the molecules needed to elicit mast cell effector functions (eg, degranulation) with the hope that these molecules might be suitable targets for therapeutic intervention in allergic diseases [4].

In the past 5 years, our knowledge has expanded considerably regarding the molecular mechanisms that lead to mast cell activation upon engagement of FcεRI [5,6]. The evolving view is one of increasing molecular complexity as new molecules are identified and their role in FcεRI signal transduction is investigated. New insights are emerging from this increasing complexity. For example, we now know that regulation of mast cell response is determined by molecules with preferential positive or negative regulatory roles and by molecules that can play both roles [5]. One key point in this process is the regulation of phosphatidylinositol 3-OH kinase (PI3K) activity, which is primarily initiated by Fyn kinase activity and negatively regulated by Lyn kinase [5]. Moreover, the requirement for coactivation of other cell surface receptors for FcεRI-mediated mast cell activation has also been revealed [6]. New findings demonstrate a key role for sphingosine-1-phosphate (S1P) and S1P receptors in determining mast cell responsiveness [6]. These emerging areas promote conceptual changes in the paradigm of how mast cells are activated in response to allergens.

The High-affinity Receptor for IgE (FcεRI)

FcεRI is primarily expressed on mast cells and basophils and is a tetrameric receptor [7]. The α chain binds IgE, whereas the membrane tetraspanning β chain amplifies receptor signaling, and the disulfide-linked homodimer of γ chain provides the receptor its signaling competence [7]. Signaling competence and signaling amplification are imparted by immunoreceptor tyrosine-based activation motifs (ITAMs) encoded in the γ and β chains,

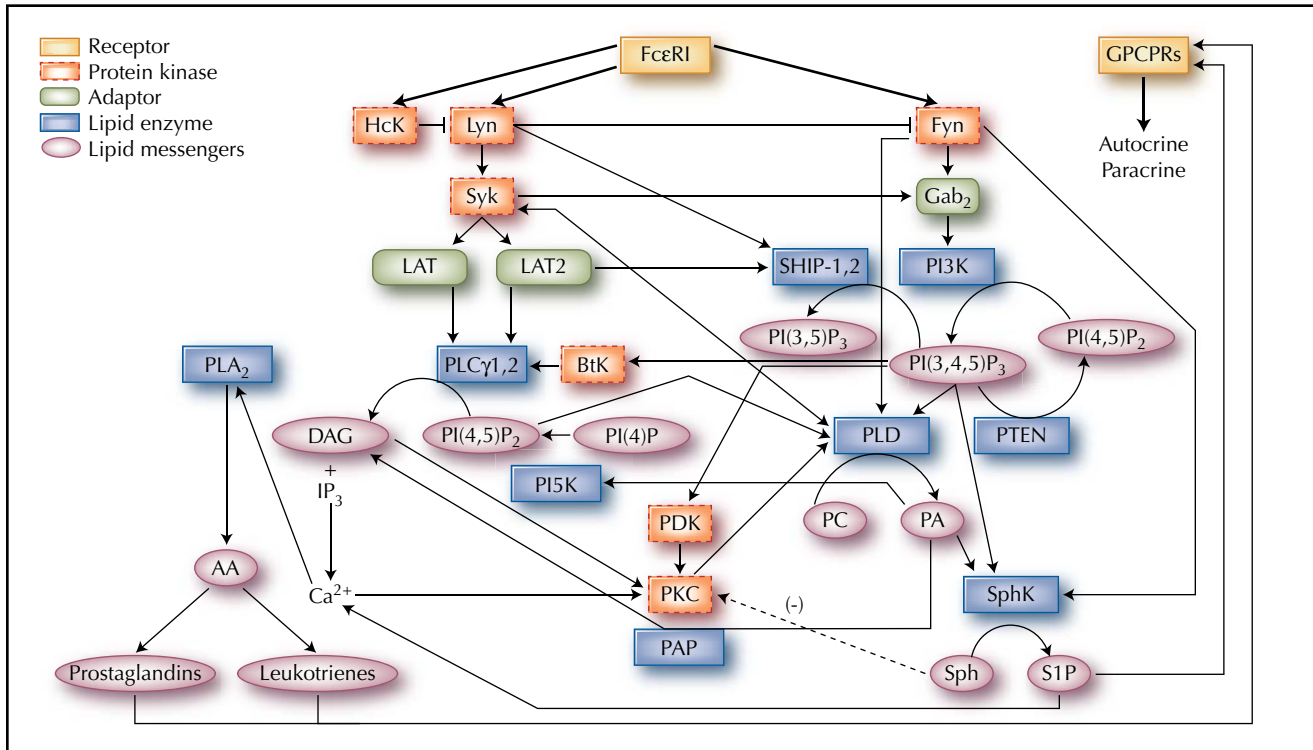


Figure 1. The dominant pathways, interactions, and regulation in mast cell activation. After engagement of high-affinity immunoglobulin E receptor (FcεRI), protein tyrosine kinases (eg, Lyn, Fyn, Hck, and Syk) contribute at multiple levels by phosphorylating tyrosine residues on various protein substrates, modifying their function. Their substrates include receptors, kinases, lipases, phosphatases, and adaptor proteins. Once phosphorylated, adaptor proteins coordinate signaling by bringing proteins and lipids together in the proximity of the membrane. Lipids are important in targeting, activating, and regulating signaling protein function, which is key for the full response of the mast cell. These pathways provide the dynamic signals that adjust spatiotemporally to fine-tune mast cell responses. Some lipids can also be exported from mast cells (or are derived from other cells) and bind G-protein–coupled receptors, initiating autocrine and paracrine loops of signaling that are important for mast cell function. DAG—diacylglycerol; IP₃—inositol trisphosphate; LAT—linker for activation of T cells; PA—phosphatidic acid; PIP₃—phosphatidylinositol (3,4,5)-trisphosphate; PKC—protein kinase C; PL—phospholipase; S1P—sphingosine-1-phosphate; Sph—sphingosine; SphK—sphingosine kinases.

respectively. Phosphorylation of the tyrosine residues in the ITAMs by Lyn kinase is the key event that initiates receptor signaling (Fig. 1). This modification creates novel binding sites on the receptor that recognize specific proteins (detailed below) that function in signal transduction and amplification. Phosphorylation of the receptor requires liquid-ordered phase domains in the plasma membrane, which are enriched in cholesterol, sphingolipids, and other saturated phospholipids (lipid rafts) [8]. Current data support a role for lipid rafts in maintaining the phosphorylated state of the FcεRI rather than as essential for initiating its phosphorylation [5,9,10]. This is likely a consequence of the high concentration of Lyn kinase in these domains [11], which are also largely devoid of phosphatase activity [12]. Arguably, this view would suggest that these domains might be important determinants of signal propagation in response to FcεRI engagement. As discussed in the following sections, many of the key molecules that regulate mast cell activation are found within, or are recruited to, these domains following FcεRI stimulation.

Protein Tyrosine Kinases in Mast Cell Activation

Early signaling events are intimately linked to the activity of protein tyrosine kinases. Two Src-family protein tyrosine kinases, Fyn and Lyn, are proximal in initiating FcεRI signaling. As indicated above, phosphorylation of the FcεRI ITAM occurs through transphosphorylation by Lyn kinase [13], which interacts with the β chain of the receptor and is the first identified component of FcεRI-mediated mast cell activation. However, although it causes a marked reduction in phosphorylation of FcεRI, loss of Lyn kinase does not necessarily abrogate the degranulation or cytokine response of mast cells [14]. This finding was puzzling because one might have predicted that the absence of this first step in mast cell activation would abrogate the cell's responses, though some residual phosphorylation of FcεRI was noted. However, in another study, it became evident that mast cell activation required Fyn, a second Src-family kinase [15], whose activity was negatively regulated by Lyn [14] (Fig. 1). Thus, in the absence of Lyn, Fyn activity is dramatically increased, promoting mast cell effector responses when minimal

FcεRI phosphorylation is observed. Active Fyn did not phosphorylate FcεRI but instead caused increased production of phosphatidylinositol (3,4,5)-trisphosphate (PIP₃), through regulation of PI3K [16] (Fig. 1), which enhances activity of signaling proteins that can promote mast cell degranulation and cytokine production.

However, contradictory evidence demonstrated that the loss of Lyn kinase resulted in decreased mast cell degranulation rather than the increased responses mentioned previously [17,18]. Recently, investigators explored this apparent contradiction by testing the major variable in the conducted studies—the genetic background of the mice or mast cells used. Mast cell responses in the genetic background of C57/BL6 or 129/Sv differed considerably. The genetic loss of Lyn in mast cells from C57/BL6 mice caused a decrease in mast cell degranulation, whereas the genetic loss of Lyn in mast cells from 129/Sv mice caused an enhanced degranulation response [19]. This work revealed that 129/Sv mast cells had twice the amount of Fyn kinase compared with mast cells from C57/BL6 mice. Moreover, the defective degranulation of C57/BL6 Lyn-deficient mast cells could be reversed by ectopic expression of Fyn kinase. The findings demonstrated the importance of genetics in mast cell responsiveness and showed that Fyn kinase is a contributory determinant. However, they also presented the dilemma of the appropriate genetic background for mast cell response studies in rodent models. To address this issue, the expression of Fyn and Lyn was individually reduced in CD34⁺-derived peripheral human mast cells using a silencing RNA approach. Loss of Fyn caused reduced degranulation of human mast cells, whereas loss of Lyn enhanced degranulation of these cells. This suggests that the responses observed in the 129/Sv mice and mast cells were more similar to that seen in human cells, and begs the question of the appropriate genetic background for study of allergic disease in mouse models.

Once phosphorylated, the (phospho)-ITAMs bind a variety of proteins with both positive and negative regulatory control on signal amplification [5]. A key positive amplifier is the tyrosine kinase Syk (Fig. 1). This kinase binds to the phospho-ITAMs of the FcεRI γ chain via its dual tandem SH2 domains and becomes active. Its binding and its ability to activate other Syk molecules through transphosphorylation are essential for signal amplification [20]. Syk phosphorylates a large number of substrates, and in doing so further amplifies signaling (Fig. 1). In particular, its role in the phosphorylation of adaptor proteins (discussed in the following section) is important because adaptors serve the key role of organizing signaling modules that coordinate and amplify signaling events. Syk-deficient mast cells fail to degranulate or produce cytokines, yet activity of Lyn and FcεRI phosphorylation is largely unaffected. Thus, intervening in Syk activation has been intensely pursued as a therapeutic strategy in allergic diseases [4]. Lyn kinase is required for complete Syk activation. However, in Lyn-deficient mast cells Syk can still be activated, albeit at markedly reduced

levels relative to wild-type cells, likely due to the minimal FcεRI phosphorylation seen in these cells. As mentioned above, enhanced Fyn activation may be important in causing the enhanced degranulation response of Lyn-deficient mast cells, thus overcoming the defect in Syk activation. Additionally, a connection between Syk and Fyn kinase may exist in mast cells (Fig. 1). Several studies in other immune cells have demonstrated the interaction of these two kinases. To date, the evidence of a role for Fyn in the activation of Syk in mast cells is limited to the description of impaired and transient activation of Syk in Fyn-deficient mast cells [21]. Thus, the possibility exists that both Lyn and Fyn may influence Syk activity. This would define a point of cross-talk for these Src-family kinases that would likely be a key in regulating cellular responses. However, mast cells express a variety of Src-family kinases (eg, Lyn, Fyn, Fgr, Hck, Src, Yes), many of which could contribute to Syk activation and may play a role in mast cell activation [7].

Adaptor Proteins, Signal Amplification, and Regulation

After FcεRI stimulation, the protein tyrosine kinases Fyn, Lyn, and Syk phosphorylate key scaffolding (transmembrane) adaptor molecules that organize and coordinate activities of signaling molecules, thus amplifying the signaling intensity required to mediate cell effector responses [5,7]. Their phosphorylation allows recruitment of signaling proteins that contain domains (eg, SH2 or PDB domains) that recognize phosphotyrosines. Because scaffolding adaptor molecules contain multiple tyrosines that can be phosphorylated, they recruit diverse proteins such as protein and lipid kinases, phospholipases, and other adaptor molecules. The latter, in turn, mediate the formation of large membrane-localized macromolecular signaling complexes by recruitment of additional proteins to this complex.

Two principal scaffolding adaptors have been identified to function in mast cells as a consequence of FcεRI stimulation. The transmembrane adaptor molecule linker for activation of T cells (LAT) is a lipid raft resident protein that is phosphorylated by Syk in multiple tyrosine residues, becoming a scaffold for organization of signaling proteins [22]. Another adaptor, recently renamed LAT2, is also a lipid raft resident and is also rapidly phosphorylated after engagement of FcεRI [23]. Both molecules are concentrated in lipid rafts where they bind multiple signaling proteins including phospholipases, lipid phosphatases, and PI3K, targeting them to membrane regions where their lipid substrates are concentrated (Fig. 1). Evidence of both a positive and negative regulatory role for these molecules on mast cell activation has been proposed [24]. LAT deficiency or mutation of key tyrosine residues on LAT caused defective calcium responses and impaired degranulation and cytokine production [24]. LAT has also been demonstrated to have an inhibitory role depending on the tissue origin of the mast cell and on the stimulus [25]. Downregulation of LAT2 by small interfering

(si) RNA resulted in a reduction of mast cell degranulation [26]. In contrast, LAT2 deficiency caused increased mast cell degranulation [27,28]. Thus, it is unclear if LAT2 has a dual role or if the study strategies might underlie the observed differences. Regardless, once phosphorylated, LAT and LAT2 appear to integrate both positive and negative signals. This is likely to be mediated by their interaction with other adaptor molecules (eg, Grb2, Gads, and SLP-76) and effector molecules (eg, Itk, PLCγ, Vav1, Sos, and PI3K). Also, the recently identified interactions of LAT with negative regulatory molecules (eg, SHP-2, SHIP, and Dok) [29] provide the molecular basis for a negative role of this adaptor (and possibly of LAT2) in various cell types.

Multiple adaptor proteins are activated and recruited into signaling complexes upon FcεRI stimulation. Many of these are cytoplasmic adaptors, including Gab2, Shc, Grb2, MIST1/Clnk, and ADAP, whose function has been well detailed in previous reviews [5,2,30] and is not covered here. However, Gab2 (Fig. 1) plays an important role in activation of PI3K in mast cells [31], and Fyn appears to be important for its phosphorylation. Loss of Fyn expression caused a marked reduction in phosphorylation of Gab2 and in PI3K activity [15]. Phosphorylated Gab2 binds the p85 regulatory subunit of PI3K class IA, resulting in its targeting to the membrane. The generation of local PIP₃ by PI3K may further strengthen the interaction of PI3K with the membrane by enhancing the Gab2 PH domain interactions with membranes [21]. However, as mentioned earlier, this work suggests that Gab2 phosphorylation is Syk dependent, but that Fyn might regulate Syk activation. To add further complexity, Hong et al. [32•] recently reported a reduction in Gab2 phosphorylation in Hck-deficient mast cells, suggesting the contributions of various Src kinases in FcεRI-mediated Gab2 phosphorylation and PI3K activation. Furthermore, another recent study indicated that PI3K activation also requires the contribution of RasGRP1/N-Ras through its potential effect on p110 catalytic subunit activity [33]. Although the findings to date suggest involvement of a type IA class PI3K (Fig. 1), it is not clear what regulatory and catalytic subunits of PI3K participate in FcεRI signaling. Loss of p110δ function, similar to Fyn and Gab2 deficiency, caused defective mast cell degranulation, cytokine production, and anaphylaxis [34], which supports the involvement of this class 1A PI3K catalytic subunit. However, mast cells lacking the most abundant of the regulatory type IA class p85α subunit of PI3K had normal responses to FcεRI stimulation, and mice lacking this regulatory subunit exhibited normal passive systemic anaphylaxis [35]. Regardless, the importance of PI3K activity in driving mast cell responses is evident from studies using inhibitory drugs or genetic alteration of its expression.

Other Proteins Involved in Generating Lipid Second Messengers

Signaling by lipid kinases/lipases/phosphatases is not dissociated from signaling by protein kinases/phospha-

tases (Fig. 1). Phosphorylation can regulate the activity and location of lipid enzymes, and reciprocally, lipid metabolites affect both the regulation and location of protein kinases/phosphatases and other lipid enzymes. Lipid mediators such as diacylglycerol (DAG), phosphatidic acid (PA), PIP₃, and S1P have been demonstrated to be key players mediating mast cell responses [36]. The production of these and other lipid mediators depends on proteins such as phospholipases C (PLC) and D (PLD), and PI3Ks (described earlier) and sphingosine kinases (SphK) (Fig. 1). Importantly, phosphatidylinositol (4,5)-bisphosphate (PIP₂) is a substrate of both PLC and PI3K. In mast cells, PLCγ activation generates inositol trisphosphate (IP₃) and DAG, causing the release of calcium from intracellular stores and activating the serine/threonine protein kinase C (PKC), respectively, while PI3K will generate PIP₃ (Fig. 1). Because both enzymes appear to be essential for mast cell responses, the availability of their common substrate PIP₂ and their activities and location in mast cells are tightly controlled through scaffolding adaptor protein association. Another phospholipase (PLD) generates PA from phosphatidylcholine (Fig. 1). PA can be an additional source for DAG other than the one generated by PLCγ, and thus, PLD also affects PKC activity. Additionally, SphKs convert the sphingomyelin metabolite sphingosine (Sph) into S1P. Sphingomyelin is highly concentrated in lipid rafts; thus, its metabolites can be found in these domains. S1P is a potent and pleiotropic signaling molecule that has been involved in multiple biologic and pathophysiologic processes [37]. Because the roles of PIP₂, PIP₃, DAG, and PA were recently reviewed elsewhere [36], here we focus on S1P's effects on mast cell activation and responsiveness.

Regulation of SphK Activation and S1P as an Intrinsic and Extrinsic Regulator of Mast Cell Activation

Sph and S1P are bioactive lipids involved in a variety of cell processes, including the regulation of mast cell function [37,38] (Fig. 1). The ratio of Sph, a negative regulator, to S1P, a positive regulator, has been implicated as a rheostat that controls FcεRI-dependent mast cell responsiveness [38]. The amount of S1P inside cells is very low but rapidly increases in response to certain SphK-activating stimuli. Two isoforms of SphK (SphK1 and SphK2) phosphorylate Sph into S1P. SphKs are rapidly activated and membrane translocated after FcεRI stimulation. The translocation of SphKs in mast cells, unlike in other cells, does not require PKC activation or calcium mobilization (Olivera and Rivera, unpublished observations) but requires both Fyn and Lyn kinases. Translocation may be mediated by the direct interaction of SphKs with Fyn and Lyn [39,40•]. Fyn activity is also required for activation of SphKs by FcεRI, and Lyn activity is also important for the rapid rise of SphK activity [39,40•]. Activation of

both SphK1 and SphK2 isoforms requires Fyn; however, whereas SphK1 activation is mediated through the Gab2/PI3K/PLD pathway, SphK2 activation is independent of Gab2/PI3K/PLD signals [40•].

Our recent data using liver-derived mast cells from SphK1- and SphK2-deficient mice demonstrated a major role for SphK2 but not SphK1 in mast cell degranulation and cytokine production [41••]. This is in contrast to the demonstrated importance of SphK1 in rat basophilic leukemia and human mast cells [42,43]. This apparent discrepancy has not yet been resolved; however, we also found that SphK2 has a role in S1P production in bone marrow-derived mast cells. In liver-derived mast cells, the absence of SphK2 inhibited S1P production in mast cells and impaired two important signaling events: calcium responses and PKC activation. SphK2-deficient mast cells were impaired in calcium uptake, while calcium release from intracellular stores as well as PLC γ phosphorylation and IP $_3$ production were unaffected in these cells. Given that calcium is also important for activation of PKC β , the calcium-regulated isoform found to contribute to mast cell degranulation [44], the effect on mast cell degranulation is not unexpected. However, how SphK2 regulates calcium influx remains to be explored.

In response to Fc ϵ RI crosslinking, S1P is rapidly generated inside cells and is also exported outside cells later (Fig. 1). S1P is a ligand for a family of G-protein coupled receptors, termed S1P $_{1-5}$ [37]. These receptors differ by their coupling to the heterotrimeric G proteins α_i , α_q , and $\alpha_{12/13}$, and thus elicit varied responses depending on the combination of receptors expressed and engaged in a particular cell type [45]. Mast cells express S1P $_1$ (an α_i -coupled receptor) and S1P $_2$ (mostly coupled to α_q and $\alpha_{12/13}$), and Fc ϵ RI-induced S1P formation results in the transactivation of these receptors [42]. Knockdown of S1P $_1$ by antisense oligonucleotides showed the requirement of this receptor for chemotaxis of mast cells toward low concentrations of antigen, whereas genetic deletion of S1P $_2$ demonstrated its importance for degranulation [42]. The specific signals by which S1P $_1$ and S1P $_2$ affect migration and degranulation, respectively, in mast cells are not yet defined. However, the mode of action of S1P in mast cell function is not solely restricted to transactivation of its receptors. The defects in calcium responses and degranulation by genetic deletion of SphK2 were also not fully compensated by adding exogenous S1P to these cells, demonstrating the importance of an intracellular role for S1P.

Exogenous S1P may also play an important role in vivo. S1P has broad functions in endothelial, epithelial, smooth muscle, lymphocytes, and other cell types. It has long been appreciated that mast cells constitute a heterogeneous population of cells whose phenotypes vary from tissue to tissue. These variations are the result of a number of factors [1]. Recently we found that S1P levels in the blood can alter mast cell responsiveness to an Fc ϵ RI stimulus in vivo [41••]. This effect of S1P seems to be distinct

from the role of S1P as an intrinsic modulator of mast cell signaling (discussed earlier), and from its autocrine function on mast cells. Our findings also demonstrated that mast cells do not contribute to the normal homeostasis of circulating S1P. Instead, red blood cells [46••], and possibly endothelial cells [47], appear to be important regulators of S1P in blood, and this regulation depends mainly on SphK1 [41••,46••]. Mice with a genetic deletion of SphK1 and with reduced levels of circulating S1P were resistant to anaphylaxis even though mast cells from these mice functioned normally in vitro. Interestingly, mice with a deletion in the SphK2 gene showed enhanced levels of blood S1P relative to wild-type mice [41••,48] and responded normally to an anaphylactic challenge [41••]. This was unexpected, because mast cells from these mice were defective in S1P production and degranulation in vitro. However, deletion of one allele for SphK1 in the SphK2-deficient mice decreased the blood S1P levels to normal and conferred resistance to an anaphylactic challenge, even though levels of S1P in their mast cells were unchanged compared to those from the SphK2-deficient mice. These findings point to a key extrinsic role for S1P in regulating mast cell responsiveness (Fig. 1), and suggest that high enough levels of S1P in the blood could overcome the intrinsic defects of SphK2-null mast cells. This finding also reveals the complexity of regulation and function of S1P, demonstrating that its presence in different compartments and its varying modes of action may be key in determining mast cell responsiveness.

Conclusions

Although recent advances provide new insights into the mechanism underlying Fc ϵ RI-mediated activation of mast cells, much is left to be uncovered. The activation requirements for Fyn (and other Src-family kinases like Hck) upon Fc ϵ RI stimulation are unknown. In addition, the relationship of Fyn/Hck and other Src-family kinases to Syk activation needs further exploration. Preliminary evidence also suggests that the negative regulatory role of Lyn on mast cell effector responses may occur in a Fc ϵ RI-dependent and Fc ϵ RI-independent manner [49], and these steps or events are not completely deciphered. Moreover, delineating the isoforms of PI3K that are activated upon Fc ϵ RI engagement is important because isoform-specific inhibitors are now being developed. How S1P regulates mast cell calcium influx is also of considerable interest, and whether these events occur in lipid rafts should also be explored. Recent advances also point to the importance of S1P and its receptors in regulating mast cell responsiveness in vivo. This prompts further exploration of how Fyn might activate SphKs and toward understanding how the circulating levels of S1P (extrinsic component) can regulate mast cell function. Given the rapid advance in knowledge in the past 5 years, it is reasonable to conclude that the coming years will reveal new areas of investigation with therapeutic promise in allergic diseases.

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Disclosure

No potential conflict of interest relevant to this article was reported.

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