Published in final edited form as:

J Allergy Clin Immunol. 2009 October ; 124(4): 639–648. doi:10.1016/j.jaci.2009.08.035.

Mechanisms of mast cell signaling in anaphylaxis

Dean D. Metcalfe, MD, Richard D. Peavy, PhD, and Alasdair M. Gilfillan, PhD

From the Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health., Bethesda, Md

Abstract

The recent development of a consensus definition and proposed diagnostic criteria for anaphylaxis offers promise for research efforts and a better understanding of the epidemiology and pathogenesis of this enigmatic and life-threatening disease. This review examines basic principles and recent research advances in the mechanisms of mast cell signaling believed to underlie anaphylaxis. The unfolding complexity of mast cell signaling suggests that the system is sensitive to regulation by any of several individual signaling pathways and intermediates and that complementary pathways regulate mast cell activation by amplified signals. The signaling events underlying anaphylactic reactions have largely been identified through experiments in genetically modified mice and supported by biochemical studies of mast cells derived from these mice. These studies have revealed that signaling pathways exist to both upregulate and downregulate mast cell responses. In this review we will thus describe the key molecular players in these pathways in the context of anaphylaxis.

Keywords

Anaphylaxis; mast cells; FcεRI; signal transduction

“Anaphylaxis is a serious allergic reaction that is rapid in onset and may cause death.”¹ This simple definition for anaphylaxis was developed through consensus by representatives of several organizations from the United States and other countries at 2 symposia sponsored by the National Institute of Allergy and Infectious Diseases and the Food Allergy and Anaphylaxis Network in 2004 and 2005 (summaries and a discussion of the symposia are available in 2 reports in the *Journal of Allergy and Clinical Immunology*).^{1,2} The goal of the symposia was to address the century-old problems of the lack of a universally accepted definition of anaphylaxis and consensus on its diagnostic criteria. This definition was developed to encourage progress in epidemiologic, clinical, and laboratory research on anaphylaxis, as well as therapeutic development and practice. In addition to defining anaphylaxis, symposia participants developed a set of 3 clinical criteria for diagnosis, presentation of any of which would indicate an anaphylactic reaction.¹ The symposia participants also reviewed established methods of treatment for anaphylaxis and identified important goals for research, such as gaining a better understanding of pathophysiologic responses and the mechanisms of mast cell activation involved in anaphylaxis.¹ At a symposium in 2006, researchers from the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergology and Clinical Immunology met to review issues on risk assessment for anaphylaxis and identified a research agenda that included an investigation of mast cell signaling pathways.³ The present review examines recent progress in research into the molecular mechanisms of mast cell signaling in anaphylaxis.

The absence of consistent criteria for diagnosis has made it difficult to gather and analyze the data required to estimate the incidence and prevalence of anaphylaxis.⁴ In addition, differences in study methodologies, sample size, population representation, and environmental/allergen exposure, and incomplete and inconsistent data collection have made it a challenge to compare results from different studies.⁵ The American College of Allergy, Asthma and Immunology Epidemiology of Anaphylaxis Working Group met in 2004 to review the literature and to estimate the occurrence of anaphylaxis. Based on a qualitative review, the group estimated the frequency of anaphylaxis to be 50 to 2,000 episodes per 100,000 persons or a lifetime prevalence of approximately 0.05% to 2.0%; they acknowledged that because of underdiagnosis and underreporting, this estimate did not represent the true prevalence.⁶ The consensus definition and diagnostic criteria for anaphylaxis proposed by the National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network symposia representatives might be used to standardize the classification of anaphylaxis and improve future epidemiologic studies.

MAST CELL MEDIATORS AND MECHANISMS OF RELEASE

Anaphylaxis occurs rapidly and systemically, affecting 1 or more organ systems, generally where mast cells reside in relative abundance.^{2,7} Underlying the pathophysiology of anaphylaxis is exposure to allergen or other factors that activate mast cells or basophils, prompting degranulation and immediate (5–30 minutes) release of preformed mediators (histamine, tryptase, carboxypeptidase A, and proteoglycans), synthesis of arachidonic acid metabolites (prostaglandins, leukotrienes), and *platelet-activating factor* (PAF), and delayed-phase (2–6 hours) generation of cytokines (*TNF- α*) and chemokines resulting from increased gene expression. These mediators are thought to be responsible for the signs and symptoms that can be variably present and that relate to the respiratory tract (laryngeal edema and bronchospasm), cardiovascular system (hypotension, syncope, and arrhythmias), epidermis and dermis (urticaria and angioedema), and gastrointestinal tract (nausea, vomiting, and cramping). Histamine stimulates vasodilation, vascular permeability, heart rate, cardiac contraction, and glandular secretion. Prostaglandin D₂ acts as a bronchoconstrictor, pulmonary and coronary vasoconstrictor, and peripheral vasodilator. Leukotrienes and PAF increase bronchoconstriction and vascular permeability. *TNF- α* is reportedly released as a preformed mediator that activates neutrophils, recruits other effector cells, and increases chemokine synthesis; it also acts as a late-phase mediator with other cytokines and chemokines, leading, in some cases, to a biphasic or protracted response.⁸

Anaphylaxis can occur after exposure to certain foods (eg, peanuts and tree nuts), drugs (eg, antibiotics, vaccines, and anesthetics), insect venoms (eg, hymenoptera), latex, and immunotherapy injections or without an identifiable cause (idiopathic anaphylaxis).^{2,9–11} Anaphylaxis is generally thought to be mediated by IgE, although there are reports of IgE-independent, IgG-dependent, or nonimmunologic mechanisms.¹² The alternative pathways described arise from complement generation of anaphylatoxin, neuropeptide release, immune complex formation, cytotoxicity, or T-cell activation.³ The mechanisms underlying IgG-dependent anaphylaxis in human subjects are not well understood.¹³ Two mechanisms of anaphylaxis have been demonstrated in murine models: an IgE-dependent pathway that results in release of histamine and PAF and an IgG-dependent pathway that results in release of PAF, rather than histamine, as its mediator.¹²

Recent studies have revealed the complexity of mast cell signaling and the sensitivity of this system to regulation by individual pathways and intermediates. Murine knockout and knockdown studies have demonstrated the effect of single components resulting in either resistance or sensitivity to anaphylaxis.¹² The expression of the D816V activating mutation in the *tyrosine kinase* KIT in human mast cells results in mast cell proliferation and mastocytosis.

¹⁴ Studies of mast cell proliferation and the prevalence of episodes of hypotension in patients with mastocytosis led to the recognition of association between systemic mastocytosis and anaphylaxis; the D816V KIT mutation has been identified in mast cells from patients with mastocytosis and recurrent unexplained anaphylaxis.^{15–18} **Omalizumab**, which inhibits the binding of IgE to its high-affinity receptor, FcεRI, was effective for treating anaphylaxis in 2 patients with systemic mastocytosis.¹⁹ The association of the activating mutation in KIT with mastocytosis and anaphylaxis indicates that there might be other mutations in mast cell signaling components that contribute to the hypersensitive phenotype and that cause a predisposition to allergic diseases, such as anaphylaxis.²⁰ These recent research reports, as well as others, have highlighted signaling pathways and components involved in mast cell activation and degranulation that affect the regulation or threshold of activation that might serve as key events leading to mediator release and the pathophysiology of anaphylaxis.

MAST CELL SIGNALING

The signaling cascades that regulate mast cell activation have been extensively investigated during the past few years and are described in depth in recent reviews.^{21–23} In this review the basic principles involved in the regulation of mast cell activation are described relative to the anaphylactic response and focus on the contributions of degranulation and immediately released mediators. The relevance of these studies to our understanding of the role of basophils in anaphylaxis is not clear. The models that are most widely used to study anaphylactic responses are passive cutaneous anaphylaxis (PCA) and passive systemic anaphylaxis (PSA), which can be examined in genetically modified murine strains. PCA is induced in mice by means of injection of antigen-specific IgE, and then vascular extravasation is monitored at the sites of antigen injection, usually the back or ear dermis, through the release of Evans blue dye into these sites. PSA is induced by sensitization, followed by systemic injection of antigen. Responses can be monitored by measuring the levels of circulating histamine. Measurement of core temperature has also been used to monitor mast cell-mediated physiology *in vivo*. Although these models do not provide a complete picture of the spectrum of responses associated with human anaphylaxis, they have provided much information about the signaling molecules that regulate mast cell activation in a physiologic setting. These studies have also supported more comprehensive *in vitro* signaling studies conducted in bone marrow-derived mast cells (BMMCs) of knockout and transgenic mice.

A point of note, however, is that such defined anaphylactic reactions observed in mice are strain dependent. The C57BL/6 and 129/Sv strains show skewing of their immune response to T_H1 and T_H2, respectively. As a result, 129/Sv mice have higher levels of circulating IgE, increased expression of FcεRI, and a greater degree of PSA reactions compared with those seen in C57BL/6 mice. Mast cells derived from the bone marrow of 129/Sv mice also show greater degranulation on FcεRI aggregation.²⁴ However, the phenotypes observed in specific knockout mice can be dramatically different based on the genetic background of the mice, requiring that experiments performed in different murine strains be compared with caution, a lesson for human studies on anaphylaxis.

Early signaling events: FcεRI-Lyn-Syk signaling

Antigen-specific IgE binds with high affinity to FcεRI, and in the presence of specific antigen, these complexes aggregate.²³ However, other receptors expressed on mast cells, such as KIT and various **G protein-coupled receptors** (GPCRs), can modify this response when they are activated.^{21,25} The FcεRI consists of an IgE-binding α subunit and signal-transducing β and homodimeric γ subunits (Fig 1).²³ The γ homodimer is indispensable for the generation of the signals required for mast cell activation.²⁶ However, the β chain appears to serve primarily as a modulator of the signals regulated by the γ chains.²³ The ability of the γ chain to initiate downstream signaling events and the β chain to modulate these responses depend on

immunoreceptor tyrosine-based activation motifs (ITAMs), which are in the cytosolic domains of the FcεRI β and γ chains.²³

After aggregation, FcεRI coalesces with glycolipid-enriched membrane domains (lipid rafts), allowing the tyrosines within the ITAM sequences (YxxL) to become phosphorylated by the Src kinase Lyn,²⁷ which is preferentially activated within these domains.²⁸ Although this would suggest that Lyn is essential for the ability of mast cells to promote an anaphylactic response, studies conducted in *Lyn*^{-/-} mice and mast cells derived from these animals have produced conflicting data. Although it was initially reported that PCA reactions did not occur in *Lyn*^{-/-} mice,²⁹ later studies reported enhanced PSA reactions in this model.³⁰ Furthermore, although consistently increased FcεRI-mediated cytokine production has been observed in BMMCs of *Lyn*^{-/-} mice, normal,³¹ partially reduced,³² and enhanced³⁰ FcεRI-mediated degranulation has been reported in this model.

These apparent disparities have now been determined to be primarily linked to the different genetic backgrounds used to generate the *Lyn*^{-/-} BMMCs. *Lyn*^{-/-} BMMCs derived from the C57Bl/6 strain have a hyporesponsive degranulation phenotype when challenged with antigen, whereas *Lyn*^{-/-} BMMCs derived from the 129/Sv strain have a hyperresponsive degranulation phenotype.²⁴ The hyperresponsive phenotype was recapitulated in human mast cells in which Lyn levels were reduced after lentiviral transduction of Lyn-targeted shRNA.²⁴ In addition to helping to clarify the role for Lyn in the anaphylactic response, these studies illustrate the potential pitfalls of relying on only 1 murine strain to study the role of specific signaling molecules in anaphylactic reactions.

The phosphorylation of the γ chains and subsequent recruitment and activation of the Zeta-chain-associated protein kinase 70 (ZAP70)-related tyrosine kinase Syk are certainly crucial for mast cell activation,³³ and indeed, PSA responses are ablated in FcεRI γ chain knockout mice.³⁴ Nevertheless, findings from studies of *Lyn*^{-/-} BMMCs indicate that despite the reported role of Lyn in phosphorylating the FcεRI β and γ chains, other unidentified tyrosine kinases might substitute for Lyn in these early events.

Transmembrane adaptor molecules

After these early receptor-proximal events, downstream signaling processes are coordinated through the assembly of macromolecular complexes through both inducible and constitutive protein-protein interactions.³⁵ The activation of Syk (possibly in conjunction with other tyrosine kinases, such as Lyn, Fyn, and **Bruton's tyrosine kinase** [Btk]) promotes inducible protein-protein interactions by phosphorylating tyrosine-containing motifs that recognize specific binding domains, such as the **Src homology 2** (SH2) and PTP domains on other proteins.³⁵ Thus formed, these signaling complexes are tethered, either directly or indirectly through cytosolic adaptor molecules, to the plasma membrane by means of interaction with transmembrane adaptor molecules, such as linker for activation of T cells (**LAT**) and LAT2 (non T cell activation linker [NTAL]/linker for activation of B cells [LAB]) after their phosphorylation (Fig 1).³⁵ By this means, key intermediary signaling enzymes required for the release of the inflammatory mediators that contribute to the anaphylaxis response are recruited into the receptor-signaling complex. The central role of LAT in this process has been illustrated by the absence of PSA in *LAT*^{-/-} mice and the reduced degranulation and cytokine production in mast cells derived from these animals.³⁶

The role of LAT2, however, is more enigmatic. *LAT2*^{-/-} knockout mice have an increased PSA reaction that is associated with enhanced mast cell mediator release.^{37,38} However *LAT*^{-/-}/*LAT2*^{-/-} (double-knockout) BMMCs have a greater defect in degranulation than that observed in the *LAT*^{-/-} BMMCs,³⁸ indicating that anaphylaxis might also be reduced in these animals. In human mast cells, gene knockdown studies have provided evidence that antigen-

mediated degranulation and the ability of stem cell factor to enhance this response depends on LAT2 phosphorylation.³⁹ The above data and those of other studies have led to the conclusion that, whereas LAT might control a principal pathway for the regulation of mast cell activation and hence anaphylactic reactions, LAT2 might serve to modify this response by either upregulating or downregulating the ongoing LAT-regulated signaling cascade.²¹ The exact circumstances under which either modification occurs is unclear.

LAT-Gads-SLP76, phospholipase C γ , and calcium

The major signaling enzyme recruited to phosphorylated LAT⁴⁰ and potentially LAT2³⁹ is phospholipase C (PLC) γ (Fig 1). There are 2 isoforms of PLC γ : PLC γ_1 and PLC γ_2 , which are both expressed by mast cells.⁴¹ Although PLC γ activation is essential for initiating the calcium signal required for antigen-mediated mast cell activation, because of the embryonic lethality associated with the PLC $\gamma_1^{-/-}$ mice, the relative contributions of the 2 isoforms to the anaphylactic response is unknown. In human mast cells activated with antigen, however, the activation of PLC γ_1 is greater than that of PLC γ_2 ,⁴¹ indicating that PLC γ_1 might have a greater role in mediating human anaphylaxis. PLC γ binds to phosphorylated LAT directly, through interaction of its SH2 domain with phospho-Y132 (in the human LAT sequence).⁴⁰ This interaction is stabilized through a second indirect interaction of PLC γ with LAT through the cytosolic adaptor molecules Gads and SLP76.⁴² Accordingly, PSA is substantially reduced in *Gads*^{-/-43} and *SLP76*^{-/-44} mice. The antigen-induced, PLC γ -mediated calcium signal that regulates degranulation and cytokine production is attenuated in BMMCs of these mice.

PLC γ hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP₂) to yield diacylglycerol and inositol trisphosphate. Diacyl-glycerol regulates the activation of protein kinase C. Although various protein kinase C isoforms undoubtedly play major roles in the regulation of mast cell activation,⁴⁵ there is little information about their role in mediating anaphylaxis in mice and human subjects. It is the inositol trisphosphate liberated by the action of PLC γ_1 that initiates the calcium signal by binding to receptors on the endoplasmic reticulum, thereby inducing the liberation of calcium from the endoplasmic reticulum into the cytosol.⁴⁶ This emptying of the intracellular calcium stores leads to an influx of extracellular calcium through store-operated calcium channels, resulting in a prolongation of the calcium signal.⁴⁷ The molecular sensor for the store emptying on the endoplasmic reticulum membrane has recently been identified as Stromal interaction molecule 1 (STIM1),^{48,49} and the equivalent plasma-membrane calcium channel has been identified as ORAI1.^{50,51} On depletion of calcium stores within the endoplasmic reticulum, STIM1 oligomerizes, the endoplasmic reticulum localizes to the plasma membrane, and ORAI1 forms functional calcium channels through interaction with STIM1 and oligomerization. Hence mice deficient in either STIM1⁵² or ORAI1⁵³ display significantly impaired PCA reactions; BMMCs from these animals have a reduced capacity to degranulate or generate cytokines after exposure to antigen. A group of transmembrane proteins termed transient receptor potential canonical channels (TRPCs) also might contribute to the influx of calcium. In the RBL2H3 rat mast cell line, TRPC5 acts in conjunction with STIM1 and ORAI1 to regulate antigen-mediated calcium flux and degranulation.⁵⁴ The function of mast cells in mice that lack specific TRPC channels has yet to be described, and therefore it is currently unclear whether these channels influence anaphylactic reactions *in vivo*.

The calcium signal is terminated after depolarization of the membrane through the calcium-activated, nonselective cation channel transient receptor cation channel, subfamily M (TRPM4), which downregulates calcium influx.⁵⁵ Consequently, mice deficient in TRPM4 have exaggerated PCA reactions, and BMMCs of these mice show enhanced degranulation and cytokine production.⁵⁵ After TRPM4-dependent termination of calcium influx, excess cytosolic calcium is restored to resting levels by the shunting of excess calcium ions into the

endoplasmic reticulum, mitochondria, and extracellular stores by Ca^{2+} -AT-Pase pumps (for a review of calcium regulation, see Ma and Beaven⁵⁶).

Fyn–GAB2–phosphoinositide 3-kinase

In addition to $\text{PLC}\gamma$, the other major signaling enzyme that is essential for intermediary signaling in mast cells is phosphoinositide 3-kinase (PI3K; Fig 1). PI3K regulates multiple processes within mast cells that are required not only for degranulation and cytokine production but also for mast cell proliferation, differentiation, and survival.⁵⁷ PI3K is a family of enzymes that catalyze the conversion of PIP_2 to phosphatidylinositol 3,4,5-trisphosphate, thereby providing docking sites for pleckstrin homology domain-containing proteins.⁵⁷ Several classes of PI3K exist. However, mast cell activation is primarily regulated by class 1A and class 1B PI3Ks.⁵⁷ Class 1A PI3Ks are linked to receptors, such as $\text{Fc}\epsilon\text{RI}$, that signal through tyrosine kinases, whereas class 1B PI3Ks are linked to G protein-coupled receptors (GPCRs). Null transgenic mice, knockout mice, or both have been generated for the class 1A PI3K $\text{p110}\delta$ and the class 1B PI3K $\text{p110}\gamma$. Studies conducted in BMMCs of transgenic mice expressing inactive $\text{p110}\delta$ revealed that this isoform has a major role in stem cell factor-mediated mast cell responses, such as proliferation, chemotaxis, and enhancement of antigen-induced mediator release,⁵⁸ as well as the ability of antigen to promote degranulation and cytokine production.⁵⁸ Similarly, studies conducted in BMMCs of $\text{p110}\gamma$ -deficient mice demonstrated a role for this isoform in the potentiation of antigen-mediated degranulation by GPCR agonists, such as adenosine.⁵⁹ Surprisingly, the antigen-mediated degranulation response was also attenuated in these cells,^{59,60} indicating that, at least in culture, part of the antigen-mediated degranulation response is secondary to the release of GPCR agonists, which then act on a cell-surface GPCR to enhance degranulation.

The relative *in vivo* roles of the $\text{p110}\delta$ and $\text{p110}\gamma$ isoforms in anaphylaxis have been controversial. A study published in 2002 reported that antigen-mediated PSA was virtually absent in $\text{p110}\gamma$ -deficient mice.⁵⁹ A subsequent *in vivo* study, however, indicated that it was the $\text{p110}\delta$ isoform that was responsible for the anaphylactic response.⁵⁸ A study published in 2008 compared the effects of blocking $\text{p110}\delta$ and $\text{p110}\gamma$ by administering selective inhibitors of each isoform to wild-type mice. This study supported a role for $\text{PI3K}\delta$, but not $\text{PI3K}\gamma$, in anaphylaxis.⁶⁰ The reasons for the disparity between the various reports remain unclear, and thus we await further experimentation to clarify this issue. However, it is clear that PI3K-regulated pathways are essential for antigen-mediated mast cell activation.⁵⁷

PI3K must be recruited to the membrane-associated receptor-signaling complex to function in mast cell activation. This localization is regulated by the inducible interaction of the p85 or p55 adaptor subunits of PI3K with the cytosolic adaptor molecule GAB2 after its phosphorylation by the Src family tyrosine kinase Fyn. Antigen-mediated PI3K activation in mast cells also appears to be positively regulated by Ras guanyl nucleotide-releasing protein (RasGRP1)⁶¹ and negatively regulated by regulator of G protein signaling (RGS13).⁶² Hence anaphylactic reactions are attenuated in $\text{GAB2}^{-/-}$,⁶³ $\text{Fyn}^{-/-}$,⁶⁴ and $\text{RasGRP1}^{-/-}$ ⁶¹ mice and enhanced in $\text{RGS13}^{-/-}$ mice.⁶² Similarly, the ability of antigen to induce degranulation, cytokine production, or both is substantially reduced in BMMCs developed from $\text{GAB2}^{-/-}$, $\text{Fyn}^{-/-}$, and $\text{RasGRP1}^{-/-}$ mice and enhanced in BMMCs developed from $\text{RGS13}^{-/-}$ mice. The actions of PI3K are reversed by the inositol phosphatases PTEN and SH2-containing inositol phosphatase 1 (SHIP1), which dephosphorylate PIP_2 at the D3 and D5 positions, respectively.⁵⁷ Activation of SHIP1 through coligation of $\text{Fc}\gamma\text{RIIb}$ with $\text{Fc}\epsilon\text{RI}$ with fusion proteins effectively blocks anaphylactic reactions in mice.⁶⁵ The role of PTEN in the anaphylactic response has not been assessed *in vivo*.

Studies on the relative roles of LAT, LAT2, GADS, GAB2, PI3K, $\text{PLC}\gamma$, and Fyn in mast cells have led to the concept of complementary pathways regulating mast cell activation.^{21,64}

Therefore it is assumed that these complementary pathways would operate for the regulation of anaphylactic reactions *in vivo*. It is proposed that at early stages of mast cell activation, the LAT-GADS-SLP76-PLC γ_1 pathway plays a predominant role after the activation of Syk, whereas the pathways that lead to the activation of PI3K might serve to prolong and amplify ongoing PLC γ -dependent responses.²¹ It is not clear how the LAT-GADS-SLP76-PLC γ_1 pathway affects the activation of PI3K. However, based on the observation that the reduced PSA in *Vav1*^{-/-} mice is associated with a decrease in PLC γ phosphorylation and PI3K activation,⁶⁶ Vav1 might have a role in this interaction. On the other hand, PI3K appears to modulate the activation of PLC γ through the Tec kinase Btk. Btk contains a pleckstrin homology domain, and thus is recruited to the plasma membrane and activated in a PI3K-dependent manner after Fc ϵ RI aggregation through its binding to membrane-associated PIP₂.⁴⁵ Defects in the Btk gene are associated with X-linked immunodeficiency in mice and X-linked agammaglobulinemia in human subjects. Mice with X-linked immunodeficiency have a defective PCA reaction, and mast cells from these mice, *Btk*^{-/-} mice, or both have partially reduced degranulation and cytokine responses.⁶⁷ These defects can be attributed to a similar decrease in the PLC γ_1 -mediated calcium response observed in these cells.

Sphingosine 1-phosphate

The lipid metabolite *sphingosine-1-phosphate* (S1P) has recently been shown to have a role in anaphylactic reactions in mice.⁶⁸ Formation of S1P from sphingosine is catalyzed by sphingosine kinase (SphK; Fig 1), which exists in 2 forms: SphK1 and SphK2. Both forms have been reported to be expressed in human and murine mast cells,^{68,69} although 1 study failed to find expression of SphK2 in human BMMCs.⁷⁰ Studies conducted in SphK1- and SphK2-knockout mice demonstrated that each kinase contributes to anaphylactic reactions by inducing the generation of S1P.⁶⁸ However, whereas SphK2 appears to function within mast cells to regulate the calcium flux necessary for activation of protein kinase C and anaphylaxis, SphK1 appears to contribute to anaphylaxis by generating circulating S1P, which acts on surface receptors.⁷¹ Mast cells express at least 2 receptors for S1P: S1P₁ and S1P₂. After binding of S1P, these receptors regulate mast cell chemotaxis and degranulation, respectively.⁷¹ In contrast, intracellular pools of S1P have been proposed to contribute to the calcium signal required for mast cell activation, although the mechanism responsible is largely unclear.

CONCLUSION

Advances in mast cell signaling research have improved our understanding of the pathophysiology of anaphylaxis. The identification of signaling pathways and components that amplify signals or alter the threshold of activation of mast cells, leading to degranulation and mediator release, has the promise of identifying novel approaches for prevention and treatment of anaphylaxis. The discovery of polymorphisms and mutations in components that regulate mast cell signaling might lead to ways to identify subjects who are most susceptible to life-threatening episodes of anaphylaxis caused by mast cell activation.

INFORMATION FOR CATEGORY 1 CME CREDIT

Credit can now be obtained, free for a limited time, by reading the review articles in this issue. Please note the following instructions.

Method of Physician Participation in Learning Process

The core material for these activities can be read in this issue of the Journal or online at the JACI Web site: www.jacionline.org. The accompanying tests may only be submitted online at www.jacionline.org. Fax or other copies will not be accepted.

Date of Original Release

October 2009. Credit may be obtained for these courses until September 30, 2011.

Copyright Statement

Copyright © 2009–2011. All rights reserved.

Overall Purpose/Goal

To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

Target Audience

Physicians and researchers within the field of allergic disease.

Accreditation/Provider Statements and Credit Designation

The American Academy of Allergy, Asthma & Immunology (AAAAI) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians. The AAAAI designates these educational activities for a maximum of 1 *AMA PRA Category 1 Credit*TM. Physicians should only claim credit commensurate with the extent of their participation in the activity.

List of Design Committee Members

Authors: Dean D. Metcalfe, MD, Richard D. Peavy, PhD, and Alasdair M. Gilfillan, PhD

Activity Objectives

1. To understand the predominant mast cell mediators underlying the pathophysiology of anaphylaxis.
2. To understand mechanisms of signaling after antigen-mediated mast cell activation.
3. To understand abnormalities in mast cell signaling that alter the threshold for activation and amplify signaling strength in mast cells.

Recognition of Commercial Support

This CME activity is supported by an educational grant from Merck & Co., Inc.

Disclosure of Significant Relationships with Relevant Commercial Companies/Organizations

The authors have declared that they have no conflict of interest.

Acknowledgments

Supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Glossary

Abbreviations used

BMMC	Bone marrow–derived mast cell
PAF	Platelet-activating factor
PCA	Passive cutaneous anaphylaxis
PIP₂	Phosphatidylinositol 4,5-bis-phosphate

PI3K	Phosphoinositide 3-kinase
PLC	Phospholipase C
PSA	Passive systemic anaphylaxis
RasGRP	Ras guanyl nucleotide–releasing protein
RGS13	Regulator of G protein signaling
SHIP1	SH2-containing inositol phosphatase 1
SphK	Sphingosine kinase
TRPC	Transient receptor potential canonical channel

GLOSSARY

BRUTON'S TYROSINE KINASE (Btk), first described for its importance in B-cell development, is also important for antigen-mediated and stem cell factor–enhanced mast cell activation. In the absence of Btk, antigen-mediated and stem cell factor–enhanced degranulation, cytokine production, PLC γ activation, and nuclear factor κ B phosphorylation are all impaired.

IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIFS Immunoreceptor tyrosine-based inhibitory motif (ITIM) are activating and inhibiting motifs that interact with cellular kinases and phosphatases to regulate cell signaling after their phosphorylation by specific tyrosine kinases. Examples of proteins containing ITAMs are the β and γ chains of the Fc ϵ RI, and an example of a protein containing an ITIM is Fc γ RIIb.

G PROTEIN-COUPLED RECEPTORS G protein-coupled receptors present in eukaryotic cells with functions that require binding to guanosine triphosphate, G protein–coupled receptors (GPCRs) have 3 subunits, α , β , and γ , which dissociate into α and $\beta\gamma$ subunits after receptor ligation. These dissociated subunits then induce downstream signaling events, including PLC β and PI3K activation and cyclic AMP production. Examples of GPCRs are the cysteinyl leukotriene and leukotriene B₄ receptors.

LAT A transmembrane adaptor molecule that is a substrate for tyrosine kinases activated on T-cell receptor engagement in T cells and Fc ϵ RI aggregation in mast cells. LAT is expressed on natural killer cells, T cells, platelets, and mast cells. Phosphorylated LAT recruits critical signaling molecules, such as PLC γ , into the receptor-signaling complex. Although LAT-deficient mice have normal numbers of mast cells, these mast cells display decreased antigen-mediated degranulation associated with a marked reduction in PLC γ activation and calcium mobilization. The reduced mitogen-activated protein kinase activation observed on antigen stimulation in LAT-deficient mast cells might account for the reduced capacity to generate cytokines in these cells.

OMALIZUMAB Omalizumab is a humanized, monoclonal anti-IgE antibody used for the treatment of severe allergic asthma. Omalizumab has also been used for the treatment of eosinophilic gastrointestinal diseases.

PLATELET-ACTIVATING FACTOR (PAF) released from mast cells and basophils has a number of pathologic outcomes, including bronchoconstriction and vascular

permeability. Patients with severe anaphylaxis might have higher levels of serum PAF, perhaps because of decreased PAF acetylhydrolase activity.

SPHINGOSINE-1-PHOSPHATE S1P is important in lymphocyte trafficking because modulation of S1P function with chemical blockers decreases CD4⁺ T-cell and mast cell infiltration into the large intestine in a murine model of intestinal food allergy. S1P production is enhanced in mast cells after FcεRI aggregation and, intracellularly, might help in the regulation of the calcium signal required for degranulation. Extracellular S1P has been documented to act as a mast cell chemotactic agent and to trigger mast cell degranulation.

Src HOMOLOGOUS 2 DOMAIN (SH2) domains are stretches of approximately 100 amino acids that allow signaling proteins containing these sequences to bind to specific tyrosine-containing motifs (eg, ITAMs) after the phosphorylation of the tyrosines contained within these motifs by tyrosine kinases. This contributes to the formation of a macromolecular signaling complex after receptor aggregation. SH2 domains are contained in multiple signaling molecules, including the tyrosine kinases Btk, Lyn, and Syk; PLCγ; and the p85 adaptor subunit of PI3 K.

TNF-α TNF-α is also known as “cachetin,” is produced primarily by monocytes/macrophages, and has effects similar to those of IL-1. Mast cell–derived TNF-α has been implicated in priming effects on sensory neurons, vascular permeability, and neutrophil recruitment. TNF blockers include etanercept, infliximab, and adalimumab, which are used in rheumatoid arthritis, psoriasis/psoriatic arthritis, and Crohn disease.

TYROSINE KINASE The most common receptor-signaling mechanisms involves receptor tyrosine kinases (RTKs). Two major forms of tyrosine kinases play critical roles in immunologic processes: growth factor receptors with inherent tyrosine kinase activity, such as KIT, and cytosolic tyrosine kinases, such as Lyn, Syk, and Btk, which are critical for FcεRI-mediated signaling in mast cells. The tyrosine kinase inhibitor imatinib can be used to treat the subtype of hypereosinophilic syndrome caused by the fusion of platelet-derived growth factor receptor α and FIP1L1, which results in a constitutively active RTK.

The Editors wish to acknowledge Seema Aceves, MD, PhD, for preparing this glossary.

REFERENCES

1. Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF, Bock SA, Branum AB, et al. Second symposium on the definition and management of anaphylaxis: summary report Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *J Allergy Clin Immunol* 2006;117:391–397. [PubMed: 16461139]
2. Sampson HA, Munoz-Furlong A, Bock SA, Schmitt C, Bass R, Chowdhury BA, et al. Symposium on the definition and management of anaphylaxis: summary report. *J Allergy Clin Immunol* 2005;115:584–591. [PubMed: 15753908]
3. Simons FE, Frew AJ, Ansotegui IJ, Bochner BS, Golden DB, Finkelman FD, et al. Risk assessment in anaphylaxis: current and future approaches. *J Allergy Clin Immunol* 2007;120:S2–S24. [PubMed: 17602945]
4. Lieberman P. Epidemiology of anaphylaxis. *Curr Opin Allergy Clin Immunol* 2008;8:316–320. [PubMed: 18596588]
5. Lieberman P, Camargo CA Jr, Bohlke K, Jick H, Miller RL, Sheikh A, et al. Epidemiology of anaphylaxis: findings of the American College of Allergy, Asthma and Immunology Epidemiology of Anaphylaxis Working Group. *Ann Allergy Asthma Immunol* 2006;97:596–602. [PubMed: 17165265]

6. Clark S, Camargo CA Jr. Epidemiology of anaphylaxis. *Immunol Allergy Clin North Am* 2007;27:145–163. [PubMed: 17493495]
7. Simons FE. 9. Anaphylaxis. *J Allergy Clin Immunol* 2008;121:S402–S407. [PubMed: 18241691]
8. Ogawa Y, Grant JA. Mediators of anaphylaxis. *Immunol Allergy Clin North Am* 2007;27:249–260. [PubMed: 17493501]
9. Kemp SF, Lockey RF. Anaphylaxis: a review of causes and mechanisms. *J Allergy Clin Immunol* 2002;110:341–348. [PubMed: 12209078]
10. Lenchner K, Grammer LC. A current review of idiopathic anaphylaxis. *Curr Opin Allergy Clin Immunol* 2003;3:305–311. [PubMed: 12865776]
11. Ring J, Darsow U. Idiopathic anaphylaxis. *Curr Allergy Asthma Rep* 2002;2:40–45. [PubMed: 11895624]
12. Finkelman FD. Anaphylaxis: lessons from mouse models. *J Allergy Clin Immunol* 2007;120:506–515. [PubMed: 17765751]
13. Nieuwenhuizen N, Herbert DR, Lopata AL, Brombacher F. CD4 + T cell-specific deletion of IL-4 receptor alpha prevents ovalbumin-induced anaphylaxis by an IFN- γ -dependent mechanism. *J Immunol* 2007;179:2758–2765. [PubMed: 17709489]
14. Nagata H, Worobec AS, Oh CK, Chowdhury BA, Tannenbaum S, Suzuki Y, et al. Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associate hematologic disorder. *Proc Natl Acad Sci USA* 1995;92:10560–10564. [PubMed: 7479840]
15. González de Olano D, de la Hoz Caballer B, Núñez López R, Sánchez Muñoz L, Cuevas Augustín M, Diéguez MC, et al. Prevalence of allergy and anaphylactic symptoms in 210 adult and pediatric patients with mastocytosis in Spain: a study of the Spanish network on mastocytosis (REMA). *Clin Exp Allergy* 2007;37:1547–1555. [PubMed: 17883734]
16. Brockow K, Jofer C, Ring J. Anaphylaxis in patients with mastocytosis—a prospective study on prevalence, severity and trigger factors in 121 patients [abstract]. *J Allergy Clin Immunol* 2006;117:S307.
17. Akin C, Scott LM, Kocabas CN, Kushnir-Sukhov N, Brittain E, Noel P, et al. Demonstration of an aberrant mast-cell population with clonal markers in a subset of patients with “idiopathic” anaphylaxis. *Blood* 2007;110:2331–2333. [PubMed: 17638853]
18. Akin C, Metcalfe DD. Occult bone marrow mastocytosis presenting as recurrent systemic anaphylaxis in patients with systemic mastocytosis. *J Allergy Clin Immunol* 2003;111:S206.
19. Carter MC, Robyn JA, Bressler PB, Walker JC, Shapiro GG, Metcalfe DD. Omalizumab for the treatment of unprovoked anaphylaxis in patients with systemic mastocytosis. *J Allergy Clin Immunol* 2007;119:1550–1551. [PubMed: 17481708]
20. Brown JM, Wilson TM, Metcalfe DD. The mast cell and allergic disease: role in pathogenesis and implications for therapy. *Clin Exp Allergy* 2008;38:4–18. [PubMed: 18031566]
21. Gilfillan AM, Tkaczyk C. Integrated signalling pathways for mast-cell activation. *Nat Rev Immunol* 2006;6:218–230. [PubMed: 16470226]
22. Rivera J, Gilfillan AM. Molecular regulation of mast cell activation. *J Allergy Clin Immunol* 2006;117:1214–1225. [PubMed: 16750977]
23. Kraft S, Kinet JP. New developments in Fc ϵ RI regulation, function and inhibition. *Nat Rev Immunol* 2007;7:365–378. [PubMed: 17438574]
24. Yamashita Y, Charles N, Furumoto Y, Odom S, Yamashita T, Gilfillan AM, et al. Cutting edge: genetic variation influences Fc ϵ RI-induced mast cell activation and allergic responses. *J Immunol* 2007;179:740–743. [PubMed: 17617561]
25. Kuehn HS, Gilfillan AM. G protein-coupled receptors and the modification of Fc ϵ RI-mediated mast cell activation. *Immunol Lett* 2007;113:59–69. [PubMed: 17919738]
26. Repetto B, Bandara G, Kado-Fong H, Larigan JD, Wiggan GA, Pocius D, et al. Functional contributions of the Fc ϵ RI α and Fc ϵ RI γ subunit domains in Fc ϵ RI-mediated signaling in mast cells. *J Immunol* 1996;156:4876–4883. [PubMed: 8648137]
27. Sheets ED, Holowka D, Baird B. Critical role for cholesterol in Lyn-mediated tyrosine phosphorylation of Fc ϵ RI and their association with detergent-resistant membranes. *J Cell Biol* 1999;145:877–887. [PubMed: 10330413]

28. Young RM, Holowka D, Baird B. A lipid raft environment enhances Lyn kinase activity by protecting the active site tyrosine from dephosphorylation. *J Biol Chem* 2003;278:20746–20752. [PubMed: 12670955]
29. Hibbs ML, Tarlinton DM, Armes J, Grail D, Hodgson G, Maglitta R, et al. Multiple defects in the immune system of Lyn-deficient mice, culminating in autoimmune disease. *Cell* 1995;83:301–311. [PubMed: 7585947]
30. Odom S, Gomez G, Kovarova M, Furumoto Y, Ryan JJ, Wright HV, et al. Negative regulation of immunoglobulin E-dependent allergic responses by Lyn kinase. *J Exp Med* 2004;199:1491–1502. [PubMed: 15173205]
31. Nishizumi H, Yamamoto T. Impaired tyrosine phosphorylation and Ca²⁺ mobilization, but not degranulation, in lyn-deficient bone marrow-derived mast cells. *J Immunol* 1997;158:2350–2355. [PubMed: 9036984]
32. Kawakami Y, Kitaura J, Satterthwaite AB, Kato RM, Asai K, Hartman SE, et al. Redundant and opposing functions of two tyrosine kinases, Btk and Lyn, in mast cell activation. *J Immunol* 2000;165:1210–1219. [PubMed: 10903718]
33. Costello PS, Turner M, Walters AE, Cunningham CN, Bauer PH, Downward J, et al. Critical role for the tyrosine kinase Syk in signalling through the high affinity IgE receptor of mast cells. *Oncogene* 1996;13:2595–2605. [PubMed: 9000133]
34. Miyajima I, Dombrowicz D, Martin TR, Ravetch JV, Kinet JP, Galli SJ. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and FcγIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J Clin Invest* 1997;99:901–914. [PubMed: 9062348]
35. Tkaczyk C, Iwaki S, Metcalfe DD, Gilfillan AM. Roles of adaptor molecules in mast cell activation. *Chem Immunol Allergy* 2005;87:43–58. [PubMed: 16107762]
36. Saitoh S, Arudchandran R, Manetz TS, Zhang W, Sommers CL, Love PE, et al. LAT is essential for FcεRI-mediated mast cell activation. *Immunity* 2000;12:525–535. [PubMed: 10843385]
37. Zhu M, Liu Y, Koonpaew S, Granillo O, Zhang W. Positive and negative regulation of FcεRI-mediated signaling by the adaptor protein LAB/NTAL. *J Exp Med* 2004;200:991–1000. [PubMed: 15477350]
38. Volna P, Lebduska P, Draberova L, Simova S, Heneberg P, Boubelik M, et al. Negative regulation of mast cell signaling and function by the adaptor LAB/NTAL. *J Exp Med* 2004;200:1001–1013. [PubMed: 15477348]
39. Iwaki S, Spicka J, Tkaczyk C, Jensen BM, Furumoto Y, Charles N, et al. Kit- and FcεRI-induced differential phosphorylation of the transmembrane adaptor molecule NTAL/LAB/LAT2 allows flexibility in its scaffolding function in mast cells. *Cell Signal* 2008;20:195–205. [PubMed: 17993265]
40. Saitoh S, Odom S, Gomez G, Sommers CL, Young HA, Rivera J, et al. The four distal tyrosines are required for LAT-dependent signaling in FcεRI-mediated mast cell activation. *J Exp Med* 2003;198:831–843. [PubMed: 12953098]
41. Tkaczyk C, Beaven MA, Brachman SM, Metcalfe DD, Gilfillan AM. The phospholipase Cγ1-dependent pathway of FcεRI-mediated mast cell activation is regulated independently of phosphatidylinositol 3-kinase. *J Biol Chem* 2003;278:48474–48484. [PubMed: 13129935]
42. Silverman MA, Shoag J, Wu J, Koretzky GA. Disruption of SLP-76 interaction with Gads inhibits dynamic clustering of SLP-76 and FcεRI signaling in mast cells. *Mol Cell Biol* 2006;26:1826–1838. [PubMed: 16479002]
43. Yamasaki S, Takase-Utsugi M, Ishikawa E, Sakuma M, Nishida K, Saito T, et al. Selective impairment of FcεRI-mediated allergic reaction in Gads-deficient mice. *Int Immunol* 2008;20:1289–1297. [PubMed: 18664516]
44. Pivniouk VI, Martin TR, Lu-Kuo JM, Katz HR, Oettgen HC, Geha RS. SLP-76 deficiency impairs signaling via the high-affinity IgE receptor in mast cells. *J Clin Invest* 1999;103:1737–1743. [PubMed: 10377180]
45. Gilfillan AM, Rivera J. The tyrosine kinase network regulating mast cell activation. *Immunol Rev* 2009;228:149–169. [PubMed: 19290926]

46. Furuichi T, Yoshikawa S, Miyawaki A, Wada K, Maeda N, Mikoshiba K. Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P400. *Nature* 1989;342:32–38. [PubMed: 2554142]
47. Hoth M, Penner R. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* 1992;355:353–356. [PubMed: 1309940]
48. Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr, et al. STIM is a Ca^{2+} sensor essential for Ca^{2+} -store-depletion-triggered Ca^{2+} influx. *Curr Biol* 2005;15:1235–1241. [PubMed: 16005298]
49. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, et al. STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *J Cell Biol* 2005;169:435–445. [PubMed: 15866891]
50. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 2006;441:179–185. [PubMed: 16582901]
51. Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, et al. CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. *Science* 2006;312:1220–1223. [PubMed: 16645049]
52. Baba Y, Nishida K, Fujii Y, Hirano T, Hikida M, Kurosaki T. Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses. *Nat Immunol* 2008;9:81–88. [PubMed: 18059272]
53. Vig M, DeHaven WI, Bird GS, Billingsley JM, Wang H, Rao PE, et al. Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. *Nat Immunol* 2008;9:89–96. [PubMed: 18059270]
54. Ma HT, Peng Z, Hiragun T, Iwaki S, Gilfillan AM, Beaven MA. Canonical transient receptor potential 5 channel in conjunction with Orai1 and STIM1 allows Sr^{2+} entry, optimal influx of Ca^{2+} , and degranulation in a rat mast cell line. *J Immunol* 2008;180:2233–2239. [PubMed: 18250430]
55. Vennekens R, Olausson J, Meissner M, Bloch W, Mathar I, Philipp SE, et al. Increased IgE-dependent mast cell activation and anaphylactic responses in mice lacking the calcium-activated nonselective cation channel TRPM4. *Nat Immunol* 2007;8:312–320. [PubMed: 17293867]
56. Ma HT, Beaven MA. Regulation of Ca^{2+} signaling with particular focus on mast cells. *Crit Rev Immunol* 2009;29:155–186. [PubMed: 19496745]
57. Kim MS, Radinger M, Gilfillan AM. The multiple roles of phosphoinositide 3-kinase in mast cell biology. *Trends Immunol* 2008;29:493–501. [PubMed: 18775670]
58. Ali K, Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C, et al. Essential role for the p110 δ phosphoinositide 3-kinase in the allergic response. *Nature* 2004;431:1007–1011. [PubMed: 15496927]
59. Laffargue M, Calvez R, Finan P, Trifilieff A, Barbier M, Altruda F, et al. Phosphoinositide 3-kinase γ is an essential amplifier of mast cell function. *Immunity* 2002;16:441–451. [PubMed: 11911828]
60. Ali K, Camps M, Pearce WP, Ji H, Ruckle T, Kuehn N, et al. Isoform-specific functions of phosphoinositide 3-kinases: p110 δ but not p110 γ promotes optimal allergic responses *in vivo*. *J Immunol* 2008;180:2538–2544. [PubMed: 18250464]
61. Liu Y, Zhu M, Nishida K, Hirano T, Zhang W. An essential role for RasGRP1 in mast cell function and IgE-mediated allergic response. *J Exp Med* 2007;204:93–103. [PubMed: 17190838]
62. Bansal G, Xie Z, Rao S, Nocka KH, Druey KM. Suppression of immunoglobulin E-mediated allergic responses by regulator of G protein signaling 13. *Nat Immunol* 2008;9:73–80. [PubMed: 18026105]
63. Gu H, Saito K, Klamann LD, Shen J, Fleming T, Wang Y, et al. Essential role for Gab2 in the allergic response. *Nature* 2001;412:186–190. [PubMed: 11449275]
64. Parravicini V, Gadina M, Kovarova M, Odom S, Gonzalez-Espinosa C, Furumoto Y, et al. Fyn kinase initiates complementary signals required for IgE-dependent mast cell degranulation. *Nat Immunol* 2002;3:741–748. [PubMed: 12089510]
65. Mertsching E, Bafetti L, Hess H, Perper S, Giza K, Allen LC, et al. A mouse Fc γ -Fc ϵ protein that inhibits mast cells through activation of Fc γ RIIB, SH2 domain-containing inositol phosphatase 1, and SH2 domain-containing protein tyrosine phosphatases. *J Allergy Clin Immunol* 2008;121:441–447. [PubMed: 17949802]

66. Manetz TS, Gonzalez-Espinosa C, Arudchandran R, Xirasagar S, Tybulewicz V, Rivera J. Vav1 regulates phospholipase C γ activation and calcium responses in mast cells. *Mol Cell Biol* 2001;21:3763–3774. [PubMed: 11340169]
67. Hata D, Kawakami Y, Inagaki N, Lantz CS, Kitamura T, Khan WN, et al. Involvement of Bruton's tyrosine kinase in Fc ϵ RI-dependent mast cell degranulation and cytokine production. *J Exp Med* 1998;187:1235–1247. [PubMed: 9547335]
68. Olivera A, Mizugishi K, Tikhonova A, Ciaccia L, Odom S, Proia RL, et al. The sphingosine kinase-sphingosine-1-phosphate axis is a determinant of mast cell function and anaphylaxis. *Immunity* 2007;26:287–297. [PubMed: 17346996]
69. Olivera A, Urtz N, Mizugishi K, Yamashita Y, Gilfillan AM, Furumoto Y, et al. IgE-dependent activation of sphingosine kinases 1 and 2 and secretion of sphingosine 1-phosphate requires Fyn kinase and contributes to mast cell responses. *J Biol Chem* 2006;281:2515–2525. [PubMed: 16316995]
70. Melendez AJ, Khaw AK. Dichotomy of Ca²⁺ signals triggered by different phospholipids pathways in antigen stimulation of human mast cells. *J Biol Chem* 2002;277:17255–17262. [PubMed: 11856736]
71. Jolly PS, Bektas M, Olivera A, Gonzalez-Espinosa C, Proia RL, Rivera J, et al. Trans-activation of sphingosine-1-phosphate receptors by Fc ϵ RI triggering is required for normal mast cell degranulation and chemotaxis. *J Exp Med* 2004;199:959–970. [PubMed: 15067032]

FIG 1.

Signaling pathways that lead from activated KIT and aggregated FcεRI to mast cell responses. Aggregation of IgE-occupied FcεRI induces activation of the Src family tyrosine kinase Lyn, whereas stem cell factor-induced dimerization of KIT induces activation of its intrinsic kinase. Phosphorylation (indicated by *red circles*) of tyrosine residues in the intracellular domains of each of these receptors recruits SH2 domain-containing signaling molecules. In the case of FcεRI, Syk is recruited through FcεRI by ITAMs contained in γ chain cytoplasmic domains. Resulting activation of Syk leads to phosphorylation of LAT and LAT2. These proteins then serve as scaffolds for multimolecular signaling complexes for the binding of cytosolic adapter molecules, such as Gads, Grb2, SLP76, and SHC; guanosine triphosphate exchangers, including Sos and Vav1; and the signaling enzymes PLCγ₁ and PLCγ₂. PLCγ catalyzes the hydrolysis of PIP₂ to yield diacylglycerol (DAG) and inositol trisphosphate (IP₃), which, respectively, result in the activation of protein kinase C (PKC) and the liberation of intracellular calcium. These signals lead to mast cell degranulation and eicosanoid generation and contribute to activation of transcription factors required for cytokine and chemokine production. In parallel, PI3K is activated after binding to Gab2 on phosphorylation of this cytosolic adapter molecule by Fyn, Syk, or both; phosphorylation of the p85 adapter subunit of PI3K; and activation of the catalytic subunit by small guanosine triphosphate (GTP)-binding proteins. In the case of KIT, the p85 subunit directly binds to the phosphorylated molecule. The subsequent

formation of membrane-associated phosphatidylinositol 3,4,5-trisphosphate (*PIP*₃) results in the recruitment of pleckstrin homology (*PH*) domain-containing signaling molecules, such as Btk and PLD. PI3K-regulated pathways serve to enhance/maintain LAT/PLC γ ₁-regulated degranulation. KIT- and Fc ϵ RI-mediated activation of the Ras–Raf–mitogen-activated protein kinase (*MAPK*) pathway after Sos- and Vav-regulated guanosine diphosphate (*GDP*)–GTP exchange of Ras contributes to these processes. MAPK–extracellular signal-regulated kinase (*ERK*) 1/2 signaling also regulates phospholipase A₂ (*PLA*₂) activation, which leads to the generation of eicosanoids. LAT2 can both upregulate and downregulate antigen-mediated responses and appears to be required for KIT to enhance Fc ϵ RI-dependent degranulation. In addition to its role in mast cell mediator release, KIT signaling regulates mast cell proliferation, differentiation, survival, migration, and adhesion. *JAK*, Janus kinase; *STAT*, signal transducer and activator of transcription. For other abbreviations, please refer to text. This figure is based on one published in the article by Gilfillan and Rivera.⁴⁵