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Adaptive and Innate Immune Reactions Regulating Mast Cell Activation: from Receptor-mediated Signaling to Responses

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Under physiologic and experimental conditions, mast cells can be activated to release a wide variety of inflammatory mediators [1]. As described in other articles in this issue, these mediators profoundly affect such physiologic processes as airway responsiveness, contractility and diameter, mucous secretion, and mucosal membrane integrity. In terms of the immunologic processes that lead to mast cell activation, the traditional view is that of an adaptive immune response involving antigen-dependent aggregation of the high-affinity IgE receptors (Fcε receptor I [FcεRI]) expressed on mast cells [2]. More recently, however, it has been demonstrated that a specific family of receptors called Toll-like receptors (TLRs), which are expressed on a number of cell types, including mast cells, can relay signals for cell activation by recognizing specific sequences found in pathogenic agents such as bacteria and viruses, called pathogen-activating molecular patterns (PAMPs) [3]. This constitutes an innate immune regulation of mast cell activation.

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As discussed in a recent review article [4], it is likely that pathogen- and antigen-dependent mast cell activation in vivo is modified by other factors contained in the surrounding milieu. By differentially or synergistically regulating receptor-mediated signaling events, these various stimuli can result in a significantly different suite of mediators being released [4]. Thus, how mast cells are activated in vivo may have a significant impact on the physiologic manifestations of this reaction. In the following sections, we describe how adaptive and innate immune responses influence mast cell activation, how these responses may be influenced by other receptors expressed on mast cells, and the signaling pathways involved in these reactions.

Mast cells and adaptive immune responses

Antigen-dependent activation of mast cells via the FcεRI (Fig. 1) is initiated by the generation of antigen-specific IgE molecules by B lymphocytes following antigen presentation and release of interleukin (IL)-4 from T helper (Th) 2 cells [5]. At least under experimental conditions, it has been demonstrated that the Th1 cytokine, interferon (IFN)-γ upregulates the high-affinity IgG receptor (Fcγ receptor I [FcγRI]) on human mast cells [6] and that these receptors can be activated via aggregated IgG_1 to induce the release of a similar suite of mediators as that observed after FcεRI aggregation (see Fig. 1) [7]. To what extent this occurs in normal or diseased physiologic settings remains to be determined. The FcεRI and FcγRI belong to the immunoglobulin receptor superfamily. These receptors are composed of an immunoglobulin-binding α subunit and signaling subunits, which, in the case of the FcεRI and FcγRI, are the γ chain homodimers [8]. The FcεRI (but not the FcγRI) also possesses a signaling β subunit, which appears to play a role in amplifying FcεRI-dependent signaling pathways initiated by the γ chains [9].

The most immediate secretory event occurring upon antigen-mediated mast cell activation is degranulation. By this mechanism, presynthesized mediators, such as histamine, proteases, carboxypeptidases, and β-hexosaminidase, are rapidly released into the extracellular spaces and surrounding tissues, where they exert their effects by binding to specific receptors or by enzymatic processes, respectively. Almost concurrent events with degranulation are the generation and release of eicosanoids, such as prostaglandin (PG) D_2 and leukotrienes (LTs) B_4 and C_4, which are generated from arachidonic acid liberated from membrane phospholipids. A further lipid-derived mediator, sphingosine-1-phosphate (S1P), is generated and released from activated mast cells [10,11]. Secreted S1P can subsequently act in an autocrine or paracrine manner to induce mast cell chemotaxis and activation depending on the particular S1P receptors occupied [12]. These early secretory responses are ultimately followed by the induction of gene expression and, within 4 to 8 hours, generation and release of cytokines and chemokines. In addition to these antigen-mediated IgE-dependent responses, it has been

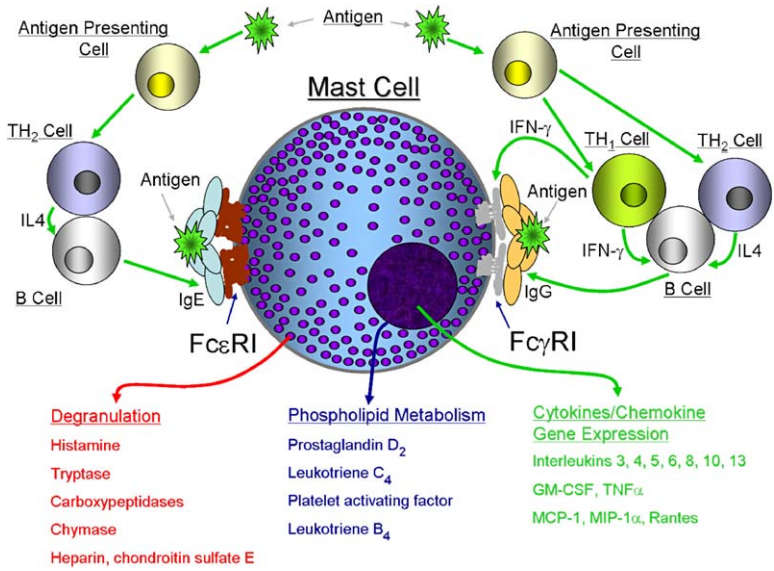

Fig. 1. Adaptive immune responses regulating mast cell activation. Following IgE and IgG production by B lymphocytes, these molecules bind to FcεRI and FcγRI, respectively. In contrast to the FcεRI, the FcγRI requires IFNγ for surface expression on mast cells. Subsequent exposure of the receptor-bound antibodies to specific antigen results in mast cell activation, leading to the release of the three groups of inflammatory mediators outlined. GM-CSF, granulocyte-macrophage colony-stimulating factor; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RANTES, regulated upon activation, normal T cell expressed and secreted; Th, T helper.

suggested that monomeric IgE can by itself induce signals to promote mast cell survival and enhance chemokine production [2,13]. These signals are not sufficient to induce degranulation, however.

The low-affinity receptors for IgG, FcγRIIb and FcγRIII, are also expressed on mast cells [6,14]. By possessing the same signaling γ subunits as the FcεRI and FcγRI, the FcγRIII activates rodent mast cells in a similar manner to these aforementioned receptors [15]. In contrast, the FcγRIIb is one of a number of receptors, including gp49B1 [16], mast cell function–associated antigen (MAFA) [17], myeloid-associated immunoglobulin-like receptor (MAIR) 1 and 2 [18], paired immunoglobulin-like receptor B (PIRB) [19], CD200R [20], and CD300a [21], expressed on mast cells, which, when ligated or coligated with the FcεRI, are inhibitory for antigen-dependent mast cell activation. Whether these receptors similarly influence human mast cell biology in vivo is currently unresolved. An Fcγ-Fcε fusion protein that coligates the FcεRI and FcγRIIb has been documented to block antigen-dependent mast cell and basophil activation in vivo effectively as well as mast cell–mediated skin reactions in transgenic mice expressing the human FcεRI, however [22]. This provides promise of a therapeutic approach for the treatment of allergic reactions based on these inhibitory receptors.

Innate responses in mast cells

In addition to the adaptive immune reactions described previously, it is now recognized that mast cells play an important role in innate immune defense against pathogens. This is attributable to their ability to recognize and engulf bacteria and to release a variety of inflammatory mediators, including IL-4, IL-6, IL-10, tumor necrosis factor-α (TNFα), chemokines, and leukotrienes, after recognition of PAMPs found in these and other pathogenic agents. These PAMPs are recognized by a specific group of receptors expressed on mast cells called Toll-like receptors (TLRS) [3].

Ten members of the TLR family have been described in humans (1–10) and 12 in mice (1–9, 11–13) [23]. Of these, the mRNAs for TLR2, TLR3, TLR4, TLR7, and TLR9 have been reported to be present in murine mast cells; for TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, and TLR7, the mRNAs have been reported to be present in mature human mast cells [24–27]. TLRs belong primarily to the class of type 1 membrane proteins [28]; however, TLR3, TLR7, TLR8, and TLR9 are intracellular and largely localized to the endoplasmic reticulum [23]. The cytoplasmic tail, which confers the signaling properties to the membrane-associated TLRs, has a high similarity to that of the IL-1 receptor family and has therefore been called the Toll/IL-1 receptor (TIR) domain [29].

Ligands for TLR2 (lipoteichoic acid, lipopeptides, and peptidoglycan [PGN] from gram-positive bacteria), TLR3 (double-stranded RNA), and TLR4 (lipopolysaccharide [LPS] from gram-negative bacteria [30]) have been documented to influence mast cell activation (Fig. 2). There are conflicting data, however, regarding the ability of TLR ligands to induce degranulation. For example, although there are reports of the TLR2 ligand PGN inducing degranulation of human mast cells [27], other groups have been unable to find evidence of degranulation in response to a variety of TLR ligands [24,25]. Similarly, TLR2 but not TLR4 stimulation of mouse bone marrow–derived mast cells (BMMCs) has been reported to mediate degranulation [31]. A contrasting report, however, observed no degranulation of mouse BMMCs with PGN or LPS [32]. Finally, although Pam_3Cys [synthetic tripalmitoyl lipopeptide Pam_3 Cys-Serine (Ser) $(Lys)_4$], which heterodimerizes TLR2/1, was reported to mediate degranulation in human mast cells [25], although this ligand was unable to induce degranulation in BMMCs [33].

Although there are fewer reports of the effects of TLR ligands on eicosanoid production, there is some evidence that heterodimerization of TLR2/TLR6 but not TLR2/1 induces LTC_4 release from human mast cells in a dose-dependent manner similar to the IgE/antigen response [25]. There is substantial evidence, however, that TLR ligands induce cytokine generation in mast cells. In human mast cells, for example, PGN-mediated TLR2 activation induces the generation of TNFα, IL-1β, IL-5, IL-10, IL-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (see Fig. 2) [25,27]. Furthermore, human mast cells challenged with the TLR3 ligand

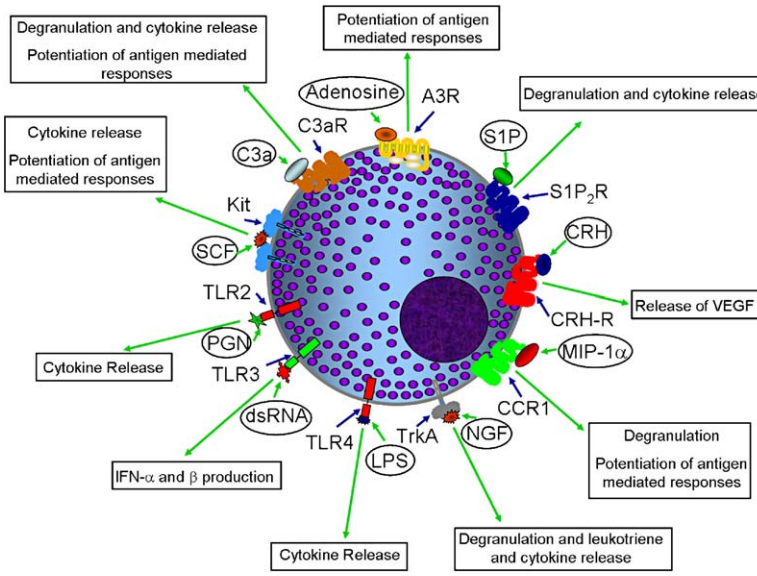

Fig. 2. The influence of TLRs and other receptors (*blue arrows*) on mast cell activation. The ligands for these receptors are indicated by the ovals and small green arrows, and their responses are indicated by the large green arrows. C3a, complement component 3a; CCR1, CC chemokine receptor 1; CRH, corticotrophin-releasing hormone; dsRNA, double-stranded RNA; MIP-1α, macrophage inflammatory protein-1α; NGF, nerve growth factor; R, receptor; SCF, stem cell factor; TrkA, tropomyosin receptor kinase A; VEGH, vascular endothelial growth hormone.

polyinosine-polycytidylic acid (polyI:C) induced the production of IFNα and IFNβ but not TNFα, IL-1β, IL-5, or GM-CSF (see Fig. 2) [24]. These responses were, however, only partially reversed in TLR3$^{-/-}$ mouse BMMCs compared with wild-type responses, suggesting that the response to polyI:C was also mediated by a TLR3-independent mechanism [24]. In IL-4–primed human mast cells, TLR activation mediated by LPS resulted in TNFα as well as IL-5, IL-10, and IL-13 production [27]. Similarly, in mouse BMMCs, PGN enhanced TNFα, IL-4, IL-5, IL-6, and IL-13 but not IL-1β production [31] and LPS has been reported to enhance TNFα, IL-1β, IL-6, IL-13 [34], IL-5, and IL-10 [35] production.

Complementary receptors

As described in a contemporary review [4], there is an increasing volume of evidence to suggest that the events described previously may also be substantially influenced by other receptors expressed on mast cells. Indeed, a recent study has demonstrated that the signals induced by the TLR2/1 ligand Pam_3 Cys synergizes with those induced by the FcεRI to dramatically enhance cytokine but not eicosanoid production or degranulation

dramatically [33]. The microenvironment in which mast cells are activated in vivo also is likely to contain multiple factors that have been demonstrated in vivo to modify antigen-mediated mast cell activation or to activate mast cells by themselves [4].

Receptors for the complement components C3a and C5a are expressed on human mast cells [36]. The C3a receptor is one of a number of G protein–coupled receptors (GPCRs) on mast cells that influence their activation (see Fig. 2). Studies using the LAD2 human mast cell line have demonstrated that C3a induces degranulation and generation of the chemokines monocyte chemoattractant protein (MCP)-1/CC chemokine ligand 2 (CCL2) and regulated upon activation, normal T cell expressed and secreted (RANTES)/CCL5, whereas C5a induced MCP-1/CCL2 production in the absence of degranulation [37]. Furthermore, C3a potentiates FcγRI-mediated degranulation from CD34+ peripheral blood leukocyte–derived human mast cells [38]. Because FcεRI and FcγRI share common signaling pathways [39], it is likely that the C3a-induced signals would also interact with those induced by the FcεRI to potentiate mediator release from mast cells.

In a similar manner, other GPCRs, including the A3 adenosine receptor [40], the $S1P_2$ receptor [10,11,41,42], the macrophage inflammatory protein (MIP)-1α chemokine receptor [43–45], corticotrophin-releasing factor receptors (see Fig. 2) [46], and the β_2-adrenergic receptor [47–49], can modify basal or antigen-dependent mast cell mediator release. The responses mediated by these receptors differ, however. In this respect, whereas the $S1P_2$ receptor [41] and MIP-1α [45] can directly induce degranulation and, in the case of MIP-1α, can further potentiate antigen responses [50], the activated A3 adenosine receptor cannot induce mast cell activation but can potentiate antigen-mediated responses [40]. In contrast corticotropin-releasing hormone (CRH) receptor activation does not induce degranulation or cytokine production but does induce the production of vascular endothelial growth hormone (VEGH) [46], and β_2 receptors inhibit antigen-mediated mast cell activation [48]. These differences may be due, in part, to different G proteins coupled to these receptors or to differences in the downstream signaling pathways. The physiologic relevance of these responses for mast cell function is unclear; however, at least for the MIP-1α receptor, studies conducted in MIP-1α knockout mice have suggested that this cytokine may be an important coactivator of mast cells in vivo [50].

Finally, the stem cell factor (SCF) receptor Kit, which is essential for mast cell growth, differentiation, and survival [51], has been demonstrated to dramatically potentiate antigen-mediated mast cell degranulation and cytokine production in vitro [52–57]. Although as yet not directly demonstrated, the absolute requirement that human mast cells have for SCF for survival suggests that such interactions take place in a physiologic setting, where SCF is likely to be present in the immediate mast cell microenvironment [4].

Early signaling events

Although, as described previously, multiple receptors expressed on mast cells may induce or modify antigen-mediated mast cell activation, much of the research on the signaling mechanisms mediating these responses has historically focused on delineating the pathways initiated upon antigen-dependent FcεRI aggregation. Recently, however, there has been increased interest on determining how other receptors, such as GPCRs, Kit, and TLRs, influence mast cell activation. In the following sections, we discuss what is currently known about the general principles of these mechanisms.

Activation of tyrosine kinases and dissociation of G protein subunits

For the FcεRI, and possibly other receptors that activate mast cells, the initial signal transduction events appear to occur in discrete but heterogeneous regions within the plasma membrane that are rich in glycosylphosphatidylinositol (GPI)-anchored proteins, glycolipids, and cholesterol, which are termed *lipid rafts* [58,59]. Upon aggregation, FcεRIs translocate to these microdomains, where they then engage critical signaling molecules [59,60]. The biochemical reactions thus initiated require activation of tyrosine kinases and subsequent receptor phosphorylation (Fig. 3) [61–63]. Because the FcεRI does not possess inherent tyrosine kinase activity, it requires recruitment of cytosolic tyrosine kinases, namely, the Src family kinase member Lyn and the ZAP-70–related tyrosine kinase Syk, to elicit downstream events [64,65].

Lyn induces phosphorylation of critical tyrosine residues in the C termini of the β and γ chains of FcεRI, which are contained within specific motifs called immunoreceptor tyrosine-based activation motifs (ITAMs) [66,67]. Following phosphorylation, the β and γ ITAMs, respectively, bind Lyn and Syk. The activity of Syk is subsequently increased following its autophosphorylation and its phosphorylation by Lyn, leading to the phosphorylation cascade that is central to downstream signaling [64,65]. Tyrosine-phosphorylated residues provide docking sites for recognition motifs called Src homology 2 (SH2) domains, which are found in a variety of signaling molecules [68], although other phosphotyrosine binding motifs have been described [68]. Thus, receptor aggregation, and subsequent tyrosine phosphorylation, permits critical signaling molecules to become associated with the receptor-signaling complex (see Fig. 3) [69]. Recently, it has become apparent that another Src kinase, Fyn, may regulate a complementary pathway for mast cell activation (see Fig. 3) [70]. Although the downstream players in this pathway have been identified, it is still unclear exactly how Fyn is regulated by these initial events and how Fyn interacts with the receptor signaling complex. The role of Fyn is discussed later in this article.

Kit is a single chain receptor that has inherent tyrosine kinase activity, however, Src kinases such as Lyn may also be required by Kit for specific

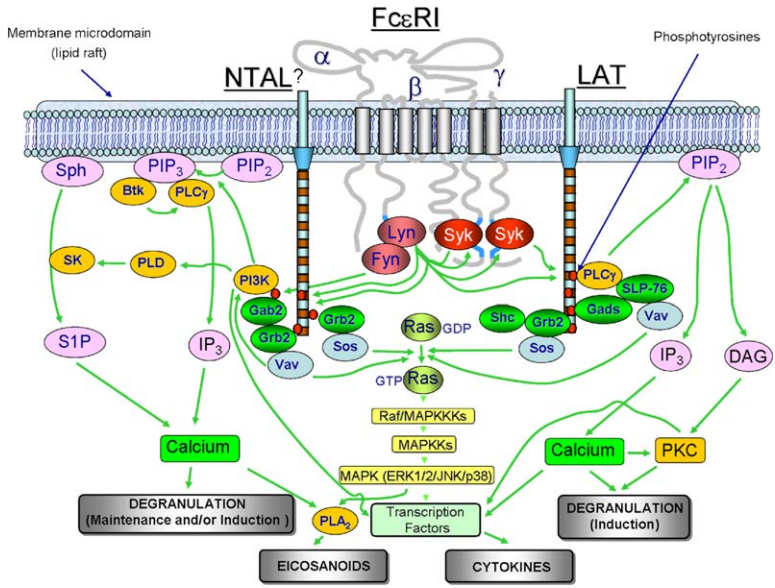

Fig. 3. The signal transduction cascade initiated by FcεRI aggregation. Following antigen-induced receptor aggregation, the FcεRIβ and γ chains are phosphorylated by the activation of Lyn. The subsequent recruitment of Lyn and Syk results in the phosphorylation of the transmembrane adaptor molecules LAT and NTAL. These provide multiple docking sites for cytosolic adaptor molecules, such as Grb2 (to LAT and NTAL) and Gads (to LAT), and the signaling enzyme PLCγ (directly to LAT and indirectly to LAT via SLP76 and Gads). The PLCγ-mediated hydrolysis of PIP_2 produces IP_3 and DAG, which, respectively, liberate intracellular calcium and activate PKC, leading to degranulation and activation of transcription factors for cytokine production. An alternative pathway mediated by Fyn leads to PI3K activation via Gab2 phosphorylation. This signal further promotes the calcium signal, leading to degranulation and activation of transcription factors, which are also dependent on the MAPK cascade. Other pathways outlined in this figure are described in the text.

responses, such as chemotaxis [71]. Initiation of signaling by Kit requires SCF-induced receptor dimerization, consequential activation of its inherent kinase activity, and autophosphorylation of specific tyrosine residues in its cytosolic tail [71]. The tyrosine residues phosphorylated in Kit are not contained within ITAMs. Rather, these residues are contained within sequences that, once phosphorylated, become direct binding sites for the SH2 domains of cytosolic adaptor molecules, such as SH2-domain-containing transforming protein C (SHC), and signaling enzymes, such as phosphoinositide 3-kinase (PI3K) and phospholipase $C\gamma_1$ ($PLC\gamma_1$) [71].

As discussed earlier, a number of receptors expressed on mast cells possess inhibitory function when ligated or coligated with the FcεRI. The FcγRI, gp49B1, and other receptors of this class are single chain receptors that possess inhibitory motifs (ITIMs) in their cytosolic tails [68]. Following phosphorylation, these receptors recruit the phosphatases SH2-domain-

containing transforming protein tyrosine phosphatase (SHP)1, SHP2, and SH2-domain-containing inositol-5-phosphatase (SHIP), thereby terminating signaling following dephosphorylation of critical signaling molecules [72]. The readers are referred to a recent review article for a more in-depth discussion of this topic [73].

The C3a, adenosine, S1P, and other GPCRs are single chain receptors that have seven hydrophobic transmembrane spans. These receptors do not require tyrosine phosphorylation for relaying signals; instead, they relay their signals through guanosine triphosphate (GTP)–binding proteins (G proteins) [74]. These signaling elements are composed of an α subunit and a $\beta\gamma$ complex. Under resting conditions, guanosine diphosphate (GDP) is bound to the α subunit, thus maintaining the complex in an inactive configuration [74]. The conformational changes in the receptor that binding of the ligand induces result in GDP being replaced by GTP, with consequential dissociation of the $\beta\gamma$ complex from the α subunit. The α and $\beta\gamma$ subunits regulate the downstream signaling process, and multiple isoforms of α subunits provide further flexibility in the effector function of these molecules [74].

Phosphorylation and recruitment of adaptor molecules

The terminal events that regulate receptor-induced mediator release are dependent on recruitment of critical signaling molecules into the signaling complex within the lipid rafts [75]. These events are coordinated by a diverse group of proteins called adaptor molecules [4,68,69]. In the resting state, adaptor molecules are located within the membrane (transmembrane adaptor molecules) and within the cytosol (cytosolic adaptor molecules) [68,69,76]. Adaptor molecules provide docking sites for critical signaling proteins, and such interactions can be constitutive or inducible, depending on the binding motifs employed [68,76].

Three major transmembrane adaptor molecules have been described to function in mast cells; LAT (linker for activation of T cells), NTAL (non-T-cell activation linker), and Cbp/PAG (Csk-binding protein/phosphoprotein associated with glycosphingolipid-enriched microdomains [GEMs]). These molecules are predominantly resident within the lipid rafts, where following phosphorylation by Lyn and/or Syk, they provide multiple docking sites for associating molecules [69]. LAT positively regulates the signaling pathways required for FcεRI-dependent mediator release [53,77], Cbp/PAG negatively regulates release [76], and NTAL has been suggested to regulate degranulation positively in a complementary manner with LAT [53,78] and to act in a negative manner to regulate release [78,79]. NTAL is also phosphorylated by Kit, and it has been proposed that NTAL plays a role in the Kit-mediated augmentation of FcεRI-dependent degranulation [53]. Whether these transmembrane adaptor molecules are utilized by other receptors regulating mast cell function is currently unknown.

The ability of LAT to regulate downstream signaling leading to degranulation is a direct consequence of the recruitment of the cytosolic adaptor molecules, Grb2 and Gads, and the signaling enzyme PLCγ_1, which binds to LAT directly and indirectly via Gads-linked SH2-domain-containing leukocyte protein of 76kDa (SLP76) (see Fig. 3) [69,76]. The GTP exchangers Vav, and potentially Sos, also bind to LAT indirectly via SLP76/Gads and Grb2, respectively. Vav has been demonstrated to regulate pathways leading to cytokine production and arachidonic acid metabolite production [80–82]. These latter events are dependent on the ability of Sos, and potentially Vav, to regulate the Ras/Raf/mitogen-activated protein (MAP) kinase (MAPK) pathway by activating the small GTP-binding protein, Ras [80,82]. As with LAT, phosphorylated NTAL binds Grb2 [83–85], which may explain how NTAL may positively regulate FcεRI- and Kit-mediated mast cell responses. It has yet to be determined exactly how NTAL may negatively regulate FcεRI-mediated mast cell activation, although it has been hypothesized that phosphorylated NTAL may compete with LAT for the same pool of critical signaling molecules [79]. The ability of Cbp/PAG to inhibit mast cell activation, however, is related to its ability to recruit the tyrosine kinase Csk into the signaling complex. Csk, in turn, phosphorylates an inhibitory tyrosine contained in Lyn, thereby terminating its activity [86].

Downstream signaling events

The final stages of receptor-mediated signaling that lead to degranulation, lipid-metabolite production, and cytokine generation are regulated by four major processes: lipid metabolism and phosphorylation, calcium mobilization, protein phosphorylation, and activation of transcription factors (see Fig. 3).

FcεRI aggregation induces the activation of the phospholipases PLA_2, PLC, and PLD (see Fig. 3). PLA_2 hydrolyses phospholipids to yield free arachidonic acid, thus providing substrate for the production of leukotrienes via 5-lipoxygenase and prostaglandins via cyclooxygenase [87]. The molecules produced by the action of PLC and PLD play roles in downstream signaling events. PLC hydrolyses phosphoinositide 4,5-bisphosphate (PIP_2) to yield inositol trisphosphate (IP_3) and diacylglycerol (DAG) [88,89], and PLD hydrolyses primarily phosphatidylcholine to yield phosphatidic acid and free choline [90,91]. The phosphatidic acid produced by this mechanism can be subsequently metabolized to form DAG by the action of phosphatidic acid phosphohydrolase [90–92].

Multiple isoforms of PLC are expressed in mast cells, and these are differentially used by the specific classes of receptors that mediate mast cell activation. The PLCγ_1 and PLCγ_2 isoforms are regulated by tyrosine phosphorylation, and are thus linked to FcεRI and Kit signaling pathways (see Fig. 3; Fig. 4) [52,93], and PLCβ is regulated by G protein α and βγ

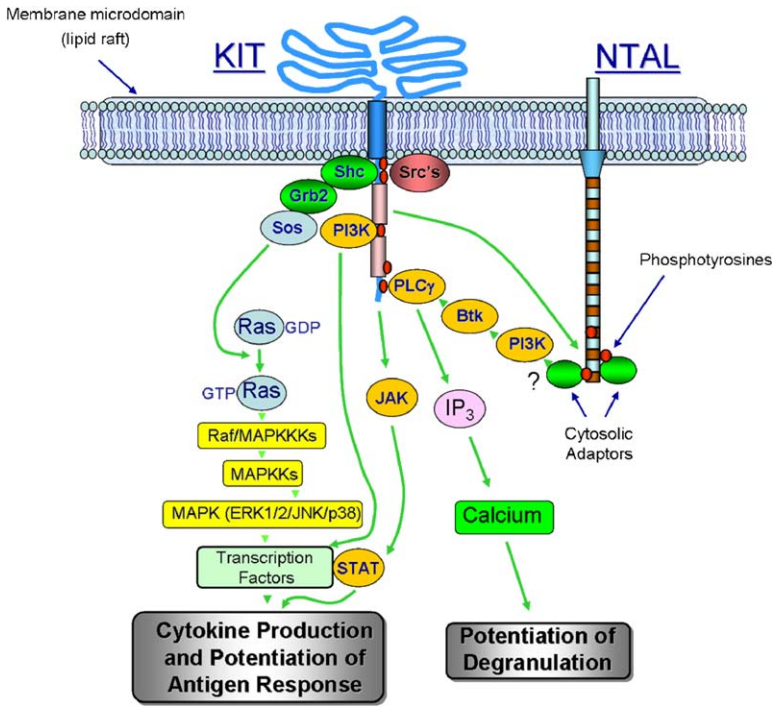

Fig. 4. The signaling pathways regulated by Kit that may lead to the potentiation of antigen-mediated degranulation and cytokine production. SCF-induced dimerization of Kit results in autophosphorylation, recruitment of signaling molecules, and activation of PLCγ and PI3K. These signals on their own are not sufficient to induce degranulation, however, likely due to the lack of detectable LAT phosphorylation and protein kinase C (PKC) activation. The ability of Kit to enhance FcεRI-mediated degranulation may be linked to a mechanism involving phosphorylation of NTAL, activation of Btk, and subsequent enhancement of calcium mobilization. The ability of Kit to induce cytokine induction likely is mediated by the Ras-Raf-MAPK pathway, PI3K-dependent pathways, and/or Janus kinases (JAKs) and signal transducer and activator of transcription (STATs).

subunits, and is thus involved in GPCR-regulated signaling pathways (Fig. 5) [94]. Regardless of the isoform involved, the net result of PLC activation, is the IP_3-mediated mobilization of intracellular calcium from the endoplasmic reticulum and the DAG-dependent activation of protein kinase C (PKC), both of which are obligatory for degranulation to proceed [89,95]. Although the role of PLD in mast cell activation is less clear, it has also been proposed to be involved in PKC activation by the indirect production of DAG [90] as well as in activation of sphingosine kinase [42]. Sphingosine kinase phosphorylates sphingosine at the 1′ position to yield S1P [10,12]. Although this molecule can act in an autocrine function on cell surface receptors [41,42], it also may act as an intracellular mediator for the liberation of intracellular calcium in a manner similar to IP_3 [96,97].

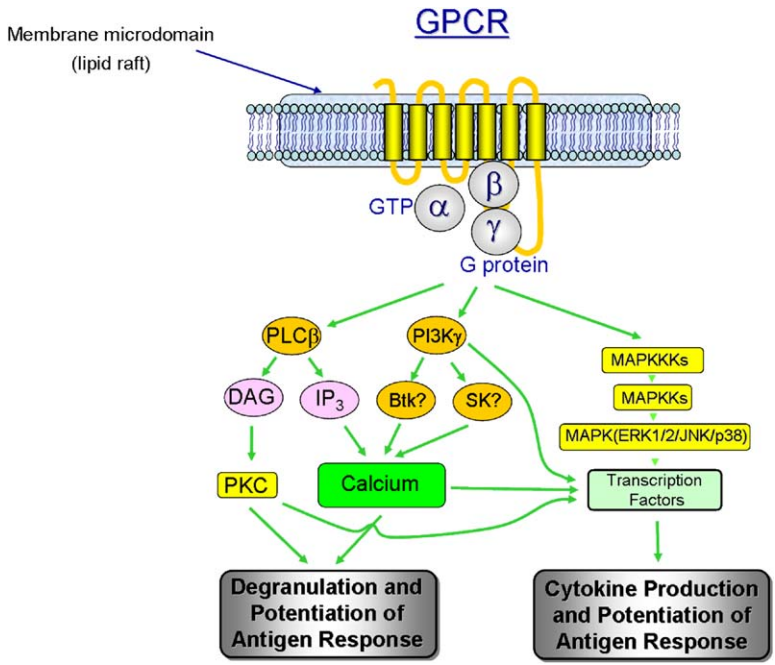

Fig. 5. The signaling pathways regulated by GPCRs that may lead to the potentiation of antigen-mediated degranulation and cytokine production. Binding of the appropriate ligand to GPCRs, such as C3a, S1P, and adenosine, induces a conformational change in the receptor that results in GTP displacing GTP on the α subunit of G proteins, allowing the α subunit to dissociate from the βγ subunits. These subunits subsequently activate PLCβ and PI3Kγ, which contribute to the regulation of downstream signaling as outlined for PLCγ and PI3Kδ in Fig. 3, thus leading to degranulation and cytokine and/or chemokine generation.

Together with PLC, the lipid kinase PI3K appears to be one of the major players in the intermediary signaling events that follow the formation of the adaptor molecule scaffolding complex within the lipid rafts (see Fig. 3). PI3K catalyzes the phosphorylation of membrane-associated phosphoinositides at the 3′ position [98]. The different PI3K catalytic subunit isoforms expressed in mast cells are activated by alternative mechanisms, depending on the nature of the receptor eliciting their activation. The major p110 isoform required for Kit- and FcεRI-mediated signaling is p110δ [99], whereas, it is the p110γ isoform that appears to be responsible for transducing the signals associated with GPCRs [40]. The membrane-associated 3′ phosphorylated phosphoinositides formed by the actions of PI3K p110δ or PI3K p110γ provide docking sites for pleckstrin homology (PH) domains, which are contained within a number of important signaling proteins, including PLCγ, Btk, and AKT [68].

In activated mast cells, the activities of PLCγ and PI3K are regulated independently but function in a complementary manner to control receptor-

distal signaling events (see Fig. 3) [93]. The principal pathway mediating FcεRI-dependent PLCγ_1 activation is that regulated by binding to the LAT-Gads-SLP76 complex [100], whereas PI3K activation appears to be mediated by the Fyn-Gab2 pathway (see Fig. 3) [70,101]. Although both of these pathways are activated concurrently upon FcεRI aggregation, the onset of PI3K activation is delayed compared with that for PLCγ activation. In contrast to FcεRI and Kit, GPCRs mediate activation of PLCβ and PI3K P110γ via G protein α and βγ subunits, and thus apparently do not require adaptor molecules for these reactions (see Fig. 5) [74].

The roles of PLCγ and PI3K in mast cell activation have been largely delineated using selective inhibitors of these enzymes and by the use of knockout and transgenic mice [4,93,99,102,103]. These studies have provided evidence that the initial calcium signal required for degranulation is regulated by PLCγ independently of PI3K. PI3K is required, however, for the subsequent maintenance of this signal for optimal degranulation to occur [93,99]. The PI3K signaling pathways that help to regulate the calcium signal required for FcεRI-mediated degranulation and which contribute to the potentiation of this pathway by Kit have still not been elucidated, but they may involve a positive feedback loop regulated by Btk for the maintenance of the normally transient activation of PLCγ_1 [57]. The calcium signal produced by PLCγ and PI3K is essential for multiple processes within the signaling cascade, including activation of PLD and PLA_2, activation of the calcium-dependent isoforms of PKC, and regulation of the transcription factor nuclear factor of activated T cells (NFAT) via the calcium-binding protein calmodulin [57,104–107].

Downstream protein phosphorylation and activation of transcription factors

In contrast to the early stages of mast cell activation, which are primarily regulated by tyrosine kinases, the receptor-distal events are largely regulated by serine, threonine, and/or mixed acting kinases belonging to the PKC and MAPK families (see Fig. 3). Selective inhibitors of PKC or degradation of specific forms of PKC by long-term exposure to phorbol ester [108] result in the inhibition of FcεRI-mediated degranulation, cytokine generation, and early, but not late, release of arachidonic acid in mast cells [109–112], thus implying a critical role for PKC in these responses. Mast cells express PKCα, PKCβ, PCKγ, PKCδ, PKCε, and PKCζ isoforms, and it is the β and δ isoforms that seem to positively regulate mast cell degranulation [89,113,114]. This may be attributable to the PKC-dependent phosphorylation of specific fusion proteins that regulate the exocytotic process [113] or to the ability of PKC to regulate other signaling molecules (eg, PLD) [91,107]. The PKCβ and ε isoforms have been demonstrated to regulate the generation of the transcription factors Fos and Jun [114], which may account for the ability of PKC to regulate cytokine production. In contrast to

this observation, the α and ε isoforms seem to be inhibitory for mast cell degranulation [108]; however, the mechanism of this inhibition is unclear.

By regulating the phosphorylation of specific transcription factors, the MAPKs ERK1/2, ERK5, p38, and JNK are important for the production of cytokines and chemokines in activated mast cells [39,115–117]. Furthermore, ERK1/2 regulates the activation of PLA_2, and thus the production of eicosanoids following FcεRI aggregation in mast cells [117]. The activation of ERK1/2 is regulated by a series of phosphorylation events initiated by the Sos- or Vav-dependent activation of the small GTP binding-protein Ras and activation of the MAPK kinase kinase Raf [80,118,119]. The pathways leading to the activation of the MAPK cascade regulating JNK and p38 in mast cells are less clear, but they may involve PKC- and/or PI3K-regulated mechanisms [52,115].

In addition to MAPKs, PKC, PI3K, and the elevated calcium signal all contribute to transcriptional regulation by the activation of specific transcription factors following FcεRI aggregation (see Fig. 3) [33,52,120–123]. The factors involved in these interactions, including nuclear factor (NF)-κB, NFAT, activated T-cell factor (ATF)-2, and the activator protein (AP) 1 components Fos and Jun, bind to the multiple recognition sites for these proteins in the promoter regions of the genes for cytokines, including IL-4, TNFα, and IL-13, thus regulating the expression of these and other cytokines and chemokines [123–128]. Enhancement of transcription by these factors is controlled by several processes. C-fos and c-jun, for example, are early response genes, whose products, Fos and Jun, are rapidly synthesized in a PKC-dependent manner [114] and then subsequently phosphorylated in a MAPK- and PI3K-dependent manner [52,129] after FcεRI aggregation. The activation of NFAT and NF-κB may also be regulated by MAPK and PI3K [84,130]; however, the activity of NFAT is also regulated in a calcium-dependent manner via the calcium-binding protein calcineurin [131,132]. It is likely that similar transcription regulation occurs after GPCR and Kit activation in mast cells.

Toll-like receptor signaling

Unlike the signaling pathways described previously, there are only limited studies describing the signaling pathways used by TLRs for the generation and release of inflammatory mediators in mast cells. The mechanistic principles of these pathways obtained from studies conducted in other cell types likely apply to those operating in mast cells, however (Fig. 6). The ability of membrane-associated TLRs to regulate cellular responses is dependent on the TIR domains contained within the cytosolic tails of these receptors [133]. Receptor occupancy results in the association of the adaptor molecule, myeloid differentiation factor 88 (MyD88) with the TLRs, although TLR2 and TLR4 signaling also involves the adaptor MyD88

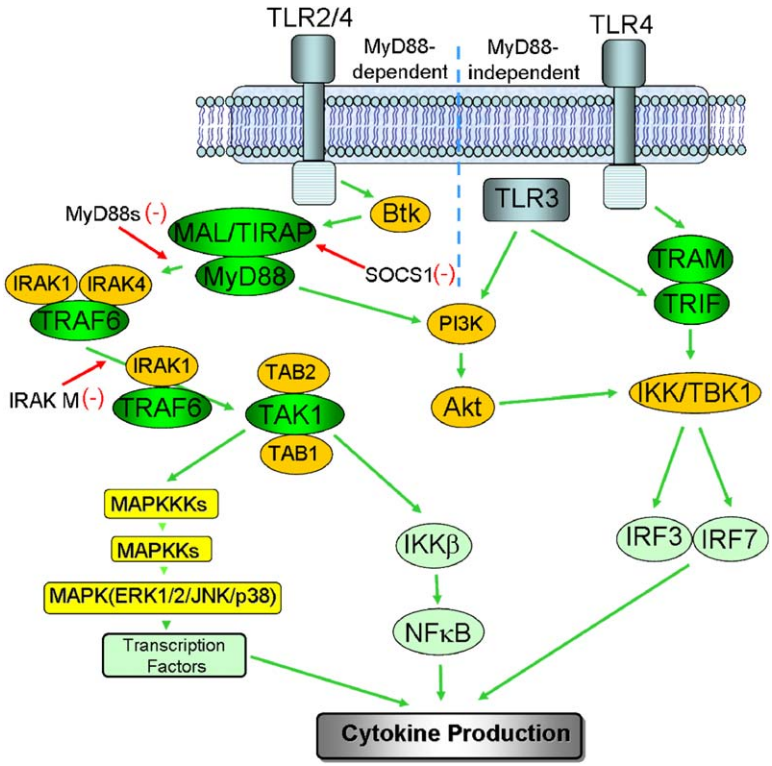

Fig. 6. The signaling pathway initiated by TLRs that may lead to the activation of mast cells for the generation of cytokines. Binding of ligand leads to a myeloid differentiation factor 88 (MyD88)-dependent or MyD88-independent signaling pathway. TLR2 and TLR4 use MyD88, but the signaling from these receptors also involves another adaptor molecule, MyD88 adapter-like (MAL). MyD88 recruits IL-1R-associated protein kinases (IRAKs), which ultimately leads to I-κB kinase kinase (IKK)-β and MAPK activation, resulting in cytokine production. The MyD88-independent pathway involves adaptor molecules, such as TIR domain–containing adaptor-inducing IFNβ protein (TRIF) and TRIF-related adaptor molecule (TRAM). TLR3 only involves TRIF, whereas TLR4, which also signals in a MyD88-independent manner, uses TRIF and TRAM. The signaling leads to activation of the transcription factors interferon regulatory factor (IRF)-3 and IRF-7, which results in cytokine production. Negative regulation of the TLR response (*red arrows*) has been shown to be mediated by several molecules, including IRAK-M, MyD88s, and suppressor of cytokine signaling (SOCS)1.

adapter-like (MAL)/Toll/IL-1R domain-containing adaptor protein (TIRAP), which is phosphorylated by Btk [134]. Binding to IL-1R–associated protein kinases (IRAKs) and tumor necrosis factor receptor–associated factor 6 (TRAF6) [135] results in the recruitment of this latter complex to the receptor. IRAK1 and TRAF6 subsequently dissociate from the receptor complex to form a new complex, together with transforming growth factor-β–activated kinase (TAK-1) and TAK-1–binding proteins (TABs) [135]. TRAF6 activates TAK-1, which, together with TABs, activates the

IκB kinase (IKK) complex, leading to nuclear translocation of NF-κB and transcription of cytokine genes [135]. This pathway is used by TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, and TLR9 [29].

In addition, MyD88-independent pathways have been shown to operate in TLR signaling (see Fig. 6). These pathways use other adaptor molecules, including TIR domain–containing adaptor-inducing IFNβ protein (TRIF) and TRIF-related adaptor molecule (TRAM) [133]. In MyD88-deficient cells, TLR3 and TLR4 are capable of activating the transcription factor interferon regulatory factor-3 (IRF-3) through TBK1 and IKKi/IKKε, which leads to production of IFNβ, even though production of inflammatory cytokines is abrogated. It has been shown that in this MyD88-independent pathway, TRIF is essential for TLR3 and TLR4 signaling, whereas TRAM is only involved in TLR4 signaling. Both pathways lead to activation of IRF-3/IRF-7 and production of IFNβ [29]. In the TLR3 pathway, it has been described that TLR3 interacts with PI3K and activates AKT, which leads to further phosphorylation, and thereby maximum activation of IRF-3 and IRF-7 [136].

Regardless of the adaptor molecules employed, MyD88-dependent and -independent pathways lead to similar downstream signaling cascades (activation of PI3K, MAPKs, or transcription factors). Thus, the major difference between these cascades and those initiated by the FcεRI is the apparent inability of TLRs to activate PLC, PKC, or induce calcium mobilization. PI3K interacts directly with the TLR receptor through an YxxM-binding motif), which only is found in TLR1, TLR2, and TLR6 but not in TLR3, TLR4, or TLR5. Regarding MyD88, it has been suggested that it also contains a sequence that could act as a binding site for PI3K. This interaction has been demonstrated to be inducible after LPS stimulation of mouse macrophages [137]. Furthermore, it has been described that TLR interacts with PI3K to activate AKT. This results in the phosphorylation, and thus optimal activation, of IRF-3 and IRF-7 [136]. As with FcεRI, GPCRs, and Kit, specific signaling molecules are known to regulate the TLR response negatively. Among those are IRAK-M, MyD88s, and suppressor of cytokine signaling (SOCS)1, which have mainly been studied in monocytes and macrophages (see Fig. 6) [29]. IRAK-M prevents the dissociation of the IRAK1-IRAK4 complex from MyD88, thereby preventing further downstream signaling [29]. MyD88s is an alternative spliced form of MyD88, which does not bind IRAK4, and thus does not induce IRAK phosphorylation [29]. SOCS1 has recently been demonstrated to interact with MAL/TIRAP, resulting in polyubiquitination and degradation [138].

Whether these positive and negative regulatory pathways exist in mast cells has not yet been adequately investigated. What has been demonstrated, however, is that LPS-induced cytokine production is associated with activation of NF-κB activation in human mast cells [139] and that polyI:C-induced IFNα and IFNβ are associated with NF-κB, p38, c-Jun, and MAPKs [24] in the same model. The activation of NF-κB by LPS appears

to be mediated by I-κB-α but not by JNK [34], despite the fact that MAPKs, including ERK1/2, JNK, and p38, are activated by LPS stimulation of BMMCs [35]. Finally, in the innate response against bacteria, it has been reported that JAK (Janus kinase)-3 and the JAK/signal transducer and activator of transcription (STAT) pathway may play an important role, because $JAK^{-/-}$ mice have reduced TNFα release in response to this kind of challenge [140]. Bacterial phagocytosis was similar in $JAK^{-/-}$ and $JAK^{+/+}$, however, which indicates that mediator release and phagocytosis are controlled by two different pathways [140].

Summary

In this article, we have described studies that have demonstrated that mast cells can be activated as a consequence of adaptive and innate immune reactions and that these responses can be modified by ligands for other receptors expressed on the surface of mast cells. These various stimuli differentially activate multiple signaling pathways within the mast cells required for the generation and/or release of inflammatory mediators. Thus, the composition of the suite of mediators released and the physiologic ramifications of these responses are dependent on the stimuli and the microenvironment in which the mast cells are activated. Knowledge of the different signaling molecules used by cell surface receptors may allow selective pharmacologic targeting such that inhibiting the adverse effects of mast cell activation can be achieved without influencing the beneficial effects of mast cell activation. The exact interconnections between the signaling pathways initiated by the surface receptors described in this article remain to be completely worked out; thus, this remains a topic for future investigation.

Acknowledgments

Because of space restrictions, the authors could not cite all the literature pertinent to the topic of this review. This does not imply that these studies are of lesser merit.

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