

Mast cell activation: A complex interplay of positive and negative signaling pathways

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Abstract

Mast cells regulate the immunological responses causing allergy, autoimmunity, and contribute to the tumor microenvironment through generation and secretion of a broad array of preformed-granule stored and de novo synthesized bioactive compounds. The release and production of mast cell mediators is the result of a coordinated signaling machinery, followed by the FcεRI and FcγR antigen ligation. In this review we present the latest understanding of FcεRI and FcγR signaling, required for the canonical mast cell activation during allergic responses and anaphylaxis. We then describe the cooperation between the signaling of FcR and other recently characterized membrane-bound receptors (i.e. IL-33R and TSLPR) and their role in the chronic settings, where the mast cell activation is crucial for the development and the sustainment of chronic diseases, such as asthma or airway inflammation. Finally we report how the FcR activation could be used as a therapeutic approach to treat allergic and atopic diseases by mast cell inactivation. Understanding the magnitude and the complexity of mast cell signaling is necessary to identify the mechanisms underlying the potential effector and regulatory roles of mast cells in the biology and pathology of those disease settings in which mast cells are activated.

Introduction

Mast cells originate in the bone marrow from a lineage-specific multipotent hematopoietic progenitor, circulate as CD34⁺ precursors until they migrate to tissues and mature into effector cells in the proximity of organs and blood vessels. Mast cells respond to antigenic stimulation through the cross-linking of immunoglobulin E (IgE)-bound to high affinity receptors for IgE (FcεRI) and the activation of the FcγR after IgG binding (reviewed in [1, 2]). Upon activation, mast cells release either preformed, granule-stored mediators, such as histamine and proteases, or newly generated mediators, such as eicosanoids, cytokines, and chemokines [3]. Although the basic molecular mechanisms of Ig:Fc activation have been extensively studied in the past 15 years [4-6] new molecules regulating the FcR-signaling cascades have recently been characterized, suggesting not only a still vivid interest in the field of mast cell activation and signaling, but also in identifying putative new therapeutic targets for intervention in mast cell-dependent disorders.

This review will focus on three aspects of mast cell activation/function. First, we give an essential but complete overview of the FcR-mediated activation in mast cells and report on recent advances in IgE- and IgG-mediated signaling. For clarity, we will dissect and analyze the signals derived from each pathway, with particular attention to the newly identified positive or negative molecular players describing - when possible - their implication towards degranulation and cytokine production. Second, we highlight the growing interest around the biology and signaling of IL-33 and TSLP receptors in the mast cell field, focusing on their impact towards IgE- and IgG-mediated signals, and their importance in mast cell-dependent pathology. Third, we discuss the emerging concept of mast cell desensitization and anergy of FcR-mediated activation, which reviews the tuning of mast cell activation in atopic diseases.

Signaling in FcεRI-induced activation

The best studied mechanism by which mast cells become activated is through the cross-linking of the FcεRI [4]. The FcεRI is constitutively expressed on mast cells as a tetrameric receptor composed of the IgE-binding α chain, the membrane-tetraspanning β chain, and the disulfide-linked homodimer of the γ chains [4]. The level of expression of the FcεRI on the mast cell surface can be influenced by several factors, such as IgE availability or IgE binding [5]. Antigen (Ag) ligation of IgE-bound FcεRI initiates phosphorylation cascades that cause profound morphological and transcriptional modifications. The exocytosis of pre-stored compounds, the modification of the membrane shape and density (ruffling), the activation of gene transcription and the new synthesis of proteins and lipid mediators are all events observed upon Ag triggering. As FcεRI lacks of intrinsic tyrosine kinase activity, its activation requires the downstream phosphorylation of several Src kinases. To date, pathways of four Src kinases (Lyn, Syk, Fyn and Hck) have mainly been studied in mast cell. It is important to clarify that, despite use of distinct adapters/kinases/phosphatases to transduce signals downstream, the activity of the Src kinases is interconnected and FcεRI ligation causes the simultaneous activation of all the Src kinases and gives rise to a complex signalosome, which regulates mast cell exocytosis, vesicular trafficking and gene transcription (Figure 1 A-C and Table 1).

Lyn-Syk

FcεRI associates with the Lyn tyrosine kinase, whose activity is crucial for transphosphorylation of the tyrosine residues in its immunoreceptor tyrosine-based activation motifs (ITAMs) on β and γ chains of FcεRI. Lyn also activates Syk tyrosine kinase and several adaptor proteins ([5]). Syk in turn phosphorylates many signaling proteins required

for the assembly of membrane localized signaling networks. Among these proteins, the linkers for activation of T cells 1 (LAT1) and LAT2 play essential roles as scaffolds in organizing, coordinating and propagating the generated signals (as a consequence of the phosphorylation of several serines, threonines and tyrosines) [7]. The subsequent recruitment of PLC- γ is promoted by the formation of a complex that includes the scaffold proteins SLP-76 (Src homology 2 domain-containing leukocyte protein of 76 kDa) and LAT (linker for activation of T cells) itself, and the guanosine triphosphate (GTP) exchangers Sos and Vav1 [7]. Accordingly, LAT-deficient and SLP-76-deficient mast cells showed impaired response to the Ag, resulting in a reduced capacity to degranulate and to generate cytokines [8].

PLC- γ hydrolyzes phosphatidylinositol-4,5-bisphosphate to form soluble inositol-1,4,5-trisphosphate (IP₃) and membrane-bound diacylglycerol (DAG). IP₃ and DAG are second messengers which induce a series of complex modifications in the mast cell physiology. The binding of IP₃ to its receptor causes the “first wave” of calcium (Ca²⁺) mobilization, which is the transient release of Ca²⁺ from endoplasmic reticulum stores; this in turn induces a prolonged “second wave” of Ca²⁺ through store-operated calcium entry (SOCE, through the association of the proteins STIM-1 in the ER and Orai-1 on the plasma membrane, [9]) (Figure 1A). The massive Ca²⁺ entry is necessary for the activation of the NF- κ B and NFAT transcription factors, both crucial for the transcription of many cytokine genes, including IL-6, TNF and IL-13 [9]. Similar to IP₃, DAG is also required for normal mast cell responses. Both phospholipase D1/2 (PLD1/2), and DAG kinase ζ (DGK ζ) play a critical role in the metabolism of DAG following Fc ϵ RI stimulation and degranulation, resulting in the formation of phosphatidic acid (PA), which is necessary for mast cell degranulation [10-12]. New regulators of the Lyn pathway have recently been described and their role and effect on degranulation and cytokine production been reported. Ainsua-Enrich et al. demonstrated that

the SH3-binding protein 2 (3BP2) directly interacts with Lyn, Syk and PLC- γ , and is required for optimal Ag responses such as degranulation, and IL-8 and GM-CSF secretion, in human mast cells [13].

Lipin-1 is another example of newly identified player in the Lyn cascade. Lipin-1 hydrolyzes PA to produce DAG. Shin and colleagues demonstrate that lipin-1 deficiency increases Fc ϵ RI-mediated degranulation in mast cells in vitro and in vivo, but not late mast cell responses such as the production of IL-6 and TNF- α . Moreover, lipin-1 directly modulates exocytosis of granules through the activation of PKC-SNAP-23 signaling [14].

DJ-1 is a regulator of signals derived from oxidative stress, whose effect in mast cells and Lyn-Syk pathway has been recently reported by Kim et al. [15].

Oxidative stress and reactive oxygen species (ROS) are best known to be involved in phagocytosis and bacterial clearance. However, it is known that low levels of ROS serve as second messengers in mast cell signaling, leading to degranulation, eicosanoid production, and cytokine secretion [16]. In this view, DJ-1 seems to mediate such “ROS-dependent signaling” in mast cells. DJ-1 deficiency decreases Syk phosphorylation but enhances LAT phosphorylation, resulting in increased PLC- γ and MAPK activation. Differential effects of increased reactive oxygen species (ROS) on SH2 tyrosine phosphatase (SHP)-2 and SHP-1 activities, explain these paradoxical observations. Increased ROS enhance SHP-2 activity, resulting in dephosphorylation and inactivation of Syk, while increased ROS simultaneously inhibit SHP-1 activity, resulting in increased LAT phosphorylation and activation. These signaling effects correlate with increased mast cell degranulation and cytokine production in vitro, and increased severity of cutaneous anaphylaxis in vivo [15].

While the precise series of events describing the dynamics and the chronological events upon Ag triggering have been described in detail [5], the link between mast cell activation and de novo gene transcription machinery is poorly understood.

Zeb2 is a member of the Smad-interacting transcription factor family and necessary for the development of the vertebrate embryos [17]. Zeb2 has recently been characterized in mast cells as a positive regulator of both FcεRI-induced degranulation (via Syk-LAT activation) and TNF-α, IL-13 and CCL-4 production through the activation NF-κB and NFAT [18]. Recent evidence describes a role of STAT-3 following Lyn activation. STAT-3 signaling has been reported in other immune cells, mainly T and B cells, where it is important for the formation of germinal centers and for the development of high-affinity antibody, including IgE. A recent study with patients with hyper IgE syndrome bearing mutations in the STAT-3 locus suggests a previously unrecognized role for STAT-3 in mast cell degranulation. The authors clarify this finding in vitro and in *Stat-3*-deficient mice as a consequence of Lyn/Syk/PLC-γ activation, although the chronological events necessary for STAT-3 activation remain still unclear [19]. Figure 1A summarizes the content of this section and shows the newly identified proteins involved in the Lyn-dependent signaling.

Fyn

In addition to Lyn and Syk, FcεRI triggering also activates the Src kinase Fyn. Fyn is a positive regulator of mast cell degranulation and its activation is complementary to the Lyn cascade. Fyn phosphorylation activates PI3K through a multi-protein complex composed of Fyn and the adaptor protein Gab2 which is activated by Fyn itself ([5] and Table 1). PI3K is crucial for the propagation of the Fyn cascade, since it induces the activation of Akt, the production of PIP₃ (by phosphorylation of PIP₂), and thereby provides tethering to the Tec

kinase Btk. Fyn appears to be important in the calcium influx signaling deriving from the activation of non-selective Ca^{2+} channels such as TRPC-1 [20], which contributes to SOCE. Fyn signaling may be finely modulated by the action of membrane phosphatases, such as the SHPs, which have been the object of investigation in a series of recent papers [21-23]. Loss of function of SHP-1 leads to hyperresponsive mast cell phenotype [21]. The association of 3BP2 itself (3BP2 is active in the Lyn pathway as well), to SHP-1 regulates SHP-1-mediated production of $\text{TNF-}\alpha$ without effects on mast cell degranulation [22]. Moreover, SHP-2 enhances degranulation, calcium mobilization, and the synthesis of $\text{IL-1}\beta$, IL-10 , and monocyte chemoattractant protein 1 (MCP-1) transcripts [23].

The roles of newly characterized Fyn-dependent downstream molecules have been recently investigated [24]. Shin et al. described a previously unknown mechanism of mTOR regulation also in mast cell biology [24-26]. mTOR is an evolutionarily conserved serine/threonine kinase activated by PI3K/Akt signaling. mTOR forms two functionally and structurally distinct complexes, mTORC1 and mTORC2 which can be both activated after Ag triggering in mast cells, and are responsible of mast cell homeostasis, survival, chemotaxis and cytokine production following antigenic ligation [27]. mTORCs are negatively regulated by the tuberous sclerosis complex (TSC). Loss of either TSC1 or TSC2 leads to the constitutively active status of the mTORC1 pathway [24]. The authors show fine modulation of mTOR and regulation of the tuberous sclerosis complex 1 (TSC1). TSC1 deficiency resulted in increased cytokine production and impaired degranulation (via $\text{PKC-}\delta$ activation) [24].

The entire apparatus necessary for mast cell degranulation, as well as granule trafficking, involves the rearrangement of cytoskeletal actin and myosin microfilaments, in a way that largely requires the activation of Fyn [5]. In these regards, p21 activated kinases (Pak) have

been recently characterized to transduce the Fyn-dependent signaling to the cytoskeleton. Pak1 is necessary for F-actin arrangement [25], while Pak2 deficiency increases RhoA-GTPase signaling activity to downstream effectors, including myosin light chain and MAPK activity, resulting in degranulation and cytokine exocytosis [26]. Figure 1B summarizes the content of this section and shows the newly identified proteins involved in the Fyn-dependent signaling.

Hck and other Src kinases

Besides the better studied Lyn-Syk-Fyn pathways, mast cells express other members of the Src kinase family, Fgr and Hck, but studies exploring their activation in mast cells are still quite limited. Fgr associates with FcεRI, phosphorylates Syk and acts as a positive regulator of Ag-induced degranulation and cytokine production [28]. Moreover, it has recently been demonstrated that activation of FcεRI by low affinity-Ags requires the activation of Fgr and signals through LAT1 and LAT2 adapter proteins, specifically activating LAT2 and dampening LAT1 phosphorylation and Ca²⁺ signaling [29]. Hck is a positive regulator of Lyn activity. Hck-deficient bone marrow-derived mast cells display reduced degranulation and impaired phosphorylation of several adapters and molecules necessary for the FcεRI cascade. The relationship between Hck and Fyn remains unknown [30]. More recently, a mechanistic study indicated that protein tyrosine phosphatase (PTP)-α, which is able to activate Lyn and Fyn and suppress Hck, is a regulator of Ag-induced degranulation [31]. Finally, one study published during the 1990s identified the activation of the Src kinase Yes (better analyzed in the context of malignancies than mast cells) upon FcεRI engagement in mast cells [32]. Figure 1C summarizes the content of the non-Lyn and non-Fyn-dependent signaling section.

Kit enhances FcεRI-mediated activation

KIT (CD117) is a tyrosine protein kinase present on mast cell membrane and becomes activated after binding to its ligand stem cell factor (SCF). As shown in mast cell deficient KIT-mutant W/W^v , W/W^{sh} and SCF-mutant Sl/Sl^d mice, signaling through KIT is crucial for correct development, growth, differentiation, survival and homing of mast cells [33]. Consistently, in in vitro studies with both human and mouse mast cells, KIT activation is not able by itself to induce mast cell degranulation, but can enhance mast cell degranulation and cytokine production in combination with antigenic stimulation [34, 35]. In contrast to FcεRI, KIT is a single chain receptor with intrinsic tyrosine kinase activity, although it shares downstream signaling proteins in common with the FcεRI-dependent cascade. SCF/FcεRI combined signaling induces hyperphosphorylation of PI3K and MAPK, PLC- γ and enhances Ca^{2+} influx [35], but has marginal effects towards LAT activation [36].

Signaling in FcγRs-induced activation

Mast cells express several receptors for IgG and their expression differs between humans and mice. Human mast cells constitutively express (h)FcγRIIA on their surface which mediates positive signaling upon receptor activation [37]. Under certain conditions, such as stimulation with IFN- γ , human mast cells can also express (h)FcγRI which promotes degranulation and mRNA expression for specific cytokines including TNF- α , GM-CSF, IL-3 and IL-13 [38]. Mouse mast cells express FcγRIIB (inhibitory) and FcγRIII (activating).

The γ chain homodimer present in FcγRI and FcγRIII is identical to the one expressed in FcεRI [39] and, as it could be expected, common γ chain activation and propagation of the FcγRI/FcγRIII signal involves some components that also play a role in the FcεRI cascade. In fact, FcγR engagement results in the recruitment and phosphorylation of the adapters LAT,

NTAL and PI3K [39]. In mice, Fc γ RIII seems to have crucial *in vivo* functions, since passive cutaneous anaphylaxis (PSA) and Arthus reactions do not occur in Fc γ RIII-deficient mice [40]. More recently, Arias et al. have clearly shown the combined contribution of IgE- and IgG1-mediated signaling to peanut induced anaphylaxis (which is reduced in the absence of Fc γ RIII) [41]. Lyn but not Fyn kinase is required for IgG mediated anaphylaxis in mice, despite the activation of both Fyn and Lyn kinases (and downstream activation of Akt and MAPK) as recently reported. Indeed Fc γ R-mediated activation was enhanced in Lyn-deficient mast cells, but decreased in Fyn-deficient mast cells. More importantly, Lyn-deficient mice were subjected to stronger PSA, whereas no major changes were observed for Fyn-deficient mice [42].

Fc γ RIIB is a single-chain receptor which does not have the ability to induce mast cell activation *per se*, but it can influence the activation of Fc ϵ RI when co-ligated to Fc ϵ RI itself, by the recruitment of the phosphatase SHIP to the membrane and its association to Fc ϵ RI [39]. Recently Fc γ RIIB has also been identified as a regulator of mast cell viability through the aggregation of Fc γ receptors by IgG immune complexes [43]. The authors reported that the aggregation of either Fc γ RIIB or Fc γ RIII by IgG immune complexes induced apoptosis of mouse bone marrow-derived mast cells.

IL-33R signaling and modulation of FcR activation

Lately, studies focusing on mast cells and the biology of IL-33 have been the object of growing attention and investigation, driven by an understanding of IL-33's unique immunomodulatory properties. IL-33 acts as an alarmin (released by necrotic tissues), but it can also have a role in coordinating/enhancing the adaptive immune response during infections, asthma, cardiovascular diseases and more generally in the context of tissue

inflammation, with paracrine and autocrine effects on IL-33R⁺ cells. The receptor for IL-33 (IL-33R, formerly known as ST2) is a member of the IL-1R family, and was first discovered on the surface of T helper (Th) cells, concretely Th2 [44]. Interestingly, mast cells can produce IL-33 in response to IgE/Ag stimulation [45, 46] and may themselves be targets of IL-33, as a consequence of the high levels of IL-33R expressed on the mast cell membrane. Indeed, the authors reported both IL-33 intracellular detection and mRNA increase following IgE/Ag stimulation of BMMCs. Failure in detection of IL-33 protein in the supernatant of stimulated BMMCs might be ascribed to the relatively immature phenotype of BMMCs as a model chosen for in vitro experiments, or to the presence in the supernatant of Ag-stimulated BMMCs of mast cell released proteases, such as MCPT4, which cleaves IL-33 [47].

Proximal events for the amplification and propagation of IL-33R signaling require the phosphorylation of IL-1R-associated kinase 1 (IRAK1) and IRAK4 which induce the activation of NF- κ B and MAPK [48]. In mast cells, activation of IL-33R, either alone or simultaneously with Fc ϵ RI, leads to, or enhances, the production of TNF- α , IL-6, IL-13, MCP-1, MCP-3, and MIP-1 α via the Src and MAPK cascade [49]. This activation mirrors enhanced in vivo mast cell response in a mast cell-dependent model of psoriasis [50] and airway inflammation [51]. Indeed, IL-33 is highly expressed in asthmatics [52] and in the bronchoalveolar fluid of asthmatic mice [53], and causes eosinophilic inflammation and airway hyperresponsiveness dependent on IL-33R expression on mast cells [44] and on the IL-33R-dependent mast cell production of IL-13 [54]. Despite an apparent pro-inflammatory signal derived from the engagement of IL-33R in mast cells, IL-33/IL-33R's physiology and functions might be more complex, since other authors have shown reduced mast cell activation after longer exposure to IL-33 [55]. Indeed, "chronic" exposure of human and mouse mast cells to IL-33 induces a hypo-responsive phenotype in mast cells, as a

consequence of the downregulation of the expression of PLC- γ 1 and Hck, raising the intriguing possibility that IL-33 might actually have a protective rather than a causative role in chronic inflammation settings, such as asthma [55].

Interestingly, a recent paper showed that IL-33-“primed” mast cells can be activated by IgG immune complexes via Fc γ RIII in a murine model of arthritis [56] suggesting that IL-33R may co-signal with Fc γ Rs and not only with Fc ϵ RI *in vivo*. The IL-33/IL-33R axis may thus represent a novel target for therapeutics involving mast cell activation across a wide range of diseases, diseases for which only symptomatic management is available today.

The TSLP/TSLPR axis

Thymic stromal lymphopoietin (TSLP) is an IL-7-like pleiotropic cytokine involved in the onset or propagation of allergic and non-allergic derived pathologies that affect several organs, such as skin, airways and gut [57]. TSLP is produced in the epidermis, airway and skin epithelia and sub-mucosa, playing a crucial role in the sensitization process and exacerbation of allergic diseases [57]. Similar to IL-33/IL-33R, mast cells can either produce TSLP through IgE/Ag-mediated signaling (via caspase-1/NF- κ B activation in the HMC-1 mast cell line, [58]), or be the target of TSLP activation through membrane-bound TSLP receptor (TSLPR), which associates with IL-7Ra to form a fully functional receptor with high affinity to TSLP compared to TSLPR alone [59]. Mature mast cells release Th2-cytokines in response to TSLP and IL-1, suggesting a mechanism that may skew asthmatic patients to a Th2-driven response [60]. Recent work on airway smooth muscle cells (ASMCs) revealed that these cells express Fc ϵ RI [61] and are able to induce TSLP production through the activation of Syk kinase [57], as occurs in mast cells following Ag triggering.

A TSLPR blocking antibody was shown to significantly reduce eosinophilic airway inflammation and Th2 differentiation in a mouse asthma model and to reduce airway inflammation and IL-13 production in the broncho-alveolar fluid in monkeys [62]. Although the authors did not show a direct effect of the blocking antibody on mast cell activation, they identified a promising target for the treatment of allergic lung inflammation. The pathways involved in the activation of TSLPR in mast cells are almost completely unknown and further studies are necessary to clarify the molecular signals following TSLP stimulation and their relationship with the IgE- and non-IgE-activation pathways in order to design a more efficient strategy for therapy in inflammatory/allergic settings.

Mast cell desensitization limits FcεRI activation

Mast cell desensitization is a process in which the mast cells are rendered hypo-responsive to Ag challenge. This can be obtained either by exposure to low Ag doses or by exposure to incremental doses of Ag [63]. Mast cell desensitization is used clinically to achieve temporary tolerance in order to deliver fully therapeutic doses of drugs for patients who develop an allergic response to them, and in clinical trials to prevent anaphylaxis to foods. Several recent studies have investigated the feasibility and techniques for mast cell desensitization [64-67].

In vitro findings in bone marrow-derived mast cells have reported desensitization in mast cells through the incubation with increasing concentrations of specific Ag in the presence of physiologically relevant extracellular calcium levels (necessary for degranulation [64]). Increasing doses of Ag at fixed time intervals induced an Ag-specific and prolonged mast cell hypo-responsiveness to triggering doses of Ag. Although the mechanisms involved are not detailed, this protocol induced early and late stage hypo-responsiveness, with almost

complete inhibition of the release of both pre-stored β -hexosaminidase and newly synthesized TNF- α and IL-6, through the reduced activation of p38 and STAT-6 and impaired internalization of the Fc ϵ RI/IgE complex [64]. In a recent work, Oka and co-workers [65] developed a similar protocol to induce desensitization of peritoneal mast cells. In this study, desensitization was achieved by internalization of the IgE complex during rapid desensitization [65], suggesting that this process might be ascribed either to the phosphorylation of the receptor or to the enhancement of endocytic signaling, rather than to the modification of the Src activation cascade itself. However, the differences in mast cell desensitization might also reflect the different phenotypic plasticity of the mast cell population analyzed in these two studies, as well as the different IgE clones used in each experiment. The reduction of the number of Fc ϵ RI molecules available for Ag triggering on the mast cell membrane is consistent with other recent findings reported by Gasparrini et al [66]. This study showed a Syk-dependent modulation of Fc ϵ RI expression and availability on the mast cell membrane, through the regulation of the activation of the early endosome-associated kinase Hrs, which orchestrates the endocytosis of activated Fc ϵ RI [66] (Figure 1D).

Other studies about mast cell desensitization have analyzed the use of either anti-IgE or anti-Fc ϵ RI antibodies in vivo as inducers of the desensitization [67]. In addition to this, it is important to note that current protocols of allergen-specific immunotherapy induce allergen-specific IgG, which correlate to clinical improvement and protection against the allergen [68]. Recently a paper by Uermösi et al., showed that IgG antibodies are actively involved in down-regulation of Fc ϵ RI-bound IgE without inducing mast cell degranulation [69]. Experiments in mice have shown that IgG cross-linking of the Fc γ RIIB results in IgE internalization and removal of long-lived IgE Abs on mast cells. A likely mechanism

responsible for IgE desensitization requires the Fc γ RIIB-mediated activation of the inositol phosphatase SHIP-1, which prevents Fc ϵ RI-dependent signaling and degranulation [70] (Figure 1D). Although the information summarized in Figure 1D are still quite fragmented, SHIP-1 appears crucial in the desensitization process (beyond its involvement in Fc γ RIIB-mediated signaling), since its ability to associate to the phosphorylated C-terminus of Syk [71].

These new intriguing therapeutic possibilities potentially overcome the limitations of Ag-induced desensitization (i.e. the risk related to being exposed to the Ag itself) and could be used for patients who are allergic to multiple Ags. In fact, it is correct to speculate that Ag sensitization in its current form can be risky since the presence of serum antibodies - including IgG - which can bind inoculated Ags, may render the Ag sensitization itself ineffective and dangerous for the patient, since the second dose of allergen may elicit a severe systemic reaction by inducing mast cell degranulation.

Concluding remarks

Mast cells are well-recognized initiators of acute allergic reactions associated with urticaria, rhinitis, atopy, and anaphylaxis. In these settings, mast cells become activated by Ag ligation of IgE-bound Fc ϵ RI or of IgG bound Fc γ Rs on their membrane. The activation of these receptors leads to a complex cascade of signals that culminates into mast cell degranulation of pre-stored mediators and de novo synthesized lipid mediators, pro-inflammatory cytokines and chemokines. Increased knowledge of the signal pathways and the molecular interplay between activatory and inhibitory molecules in mast cells might lead to develop more accurate therapeutic strategies (i.e. mast cell desensitization) against mast cell-dependent disorders in the near future.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

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Figure 1. Schematic and simplified signaling in FcεRI-induced activation in mast cells. (A-C) Schematic representation of early events following IgE-bound FcεRI crosslinking by Ag, with subsequent activation of the Src kinases (A) Lyn/Syk, (B) Fyn and (C) Hck, Fgr and Yes. Arrows indicate positive activation signals, red bars negative inhibitory signals. (D) Schematic representation of molecular events necessary for mast cell desensitization. Arrows with a cross indicate the positive signaling pathways affected by desensitization. DAG, diacyl-glycerol; ER, endoplasmic reticulum; IP₃, inositol triphosphate; ITAM, immunoreceptor tyrosine-based activation motif; MAPK, mitogen-activated kinase; PAK, p21 activated kinase; PIP₂, phosphatidylinositol 4,5-bisphosphate; PIP₃, phosphatidylinositol (3,4,5)-trisphosphate; PKC, protein kinase C; PLC-γ, phospholipase C gamma; PTP, protein tyrosine phosphatase; SHIP, Src homology 2-containing inositol phosphatase; SHP, Src-homology-2-domain-containing protein tyrosine phosphatase; SOS, son of sevenless.

Figure 1

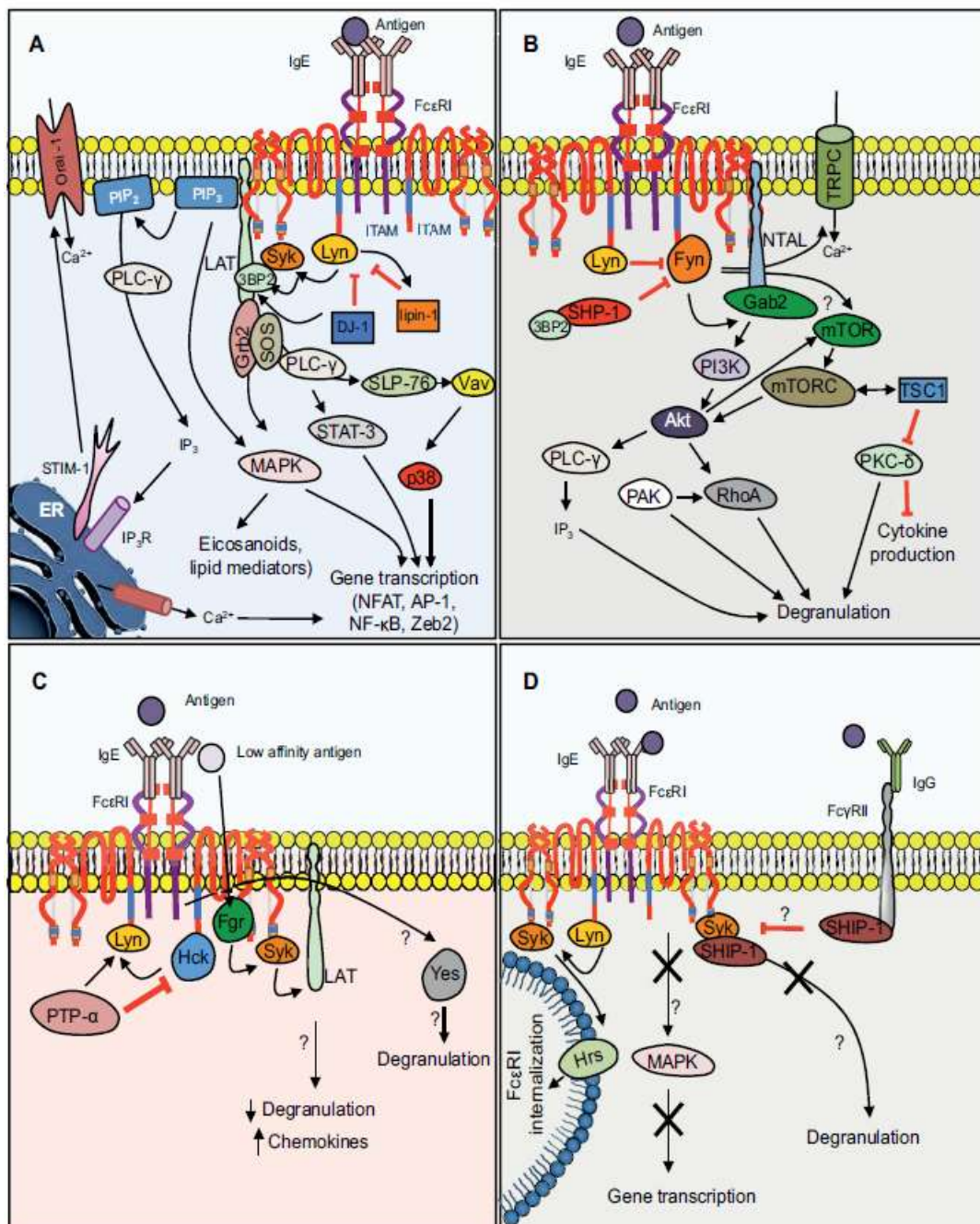


Table 1. List of major signaling proteins involved in FcR upstream activation cascade, description of function and phenotypic characterization of the protein-deficient mast cell/mouse. Primary reference papers to the protein-deficient mast cell/mouse phenotypes are provided in parentheses. *with regards to mast cell-dependent *in vivo* activation and anaphylaxis.

Protein	MC function	Protein MC-deficient and protein-deficient mouse phenotype*
Lyn	Src-protein tyrosine kinase (PTK)	Enhanced MC degranulation, enhanced Fyn activity; negative regulation of IgE production and anaphylaxis (Penhallow <i>et al.</i> , 1995).
Syk	PTK	Complete loss of degranulation and release of cytokines; suppressed allergic response (Shiue <i>et al.</i> , 1995).
LAT	Adapter	Impaired calcium influx and degranulation; suppressed allergic response and anaphylaxis (Saitoh <i>et al.</i> , 2000)
SLP-76	Adapter	Impaired histamine and IL-6 release; resistance to anaphylaxis (Pivniouk <i>et al.</i> , 1999).
Sos	Adapter/guanine nucleotide exchange factor	Impaired MAPK activation and cytokine production. Sos-deficient animals are not viable (Yamasaki <i>et al.</i> , 2007).
Vav	Adapter/guanine nucleotide exchange factor	Impaired calcium response, cytokine production/release and degranulation; suppressed anaphylaxis (Manetz <i>et al.</i> , 2001).
Grb2	Adapter	Loss of degranulation; impaired MAPK activation (Jabril-Cuenod <i>et al.</i> , 1996).
Fyn	Src-PTK (complementary to Lyn)	Defective Syk activation, impaired degranulation and resistance to anaphylaxis (Parravicini <i>et al.</i> , 2002).
Gab2	Adapter	Impaired activation of MAPK, proliferation and degranulation; markedly impaired allergic and skin reactions (Gu <i>et al.</i> , 2001).
Btk	PTK	Partial reduction in the capacity to degranulate in response to Ag, c-Kit dependent degranulation abrogated (Kawakami <i>et al.</i> , 1994).
NTAL	Adapter	Impaired degranulation due to incorrect microtubule nucleation (Brdicka <i>et al.</i> , 2002).
SHP-2	Phosphatase	Positive effector of proliferation via c-kit

		reduced IgE/Ag dependent TNF- α production (Kimura <i>et al.</i> , 1997).
PTP- α	Phosphatase	Reduced Lyn/Fyn activation, Hck activation, hyper-degranulation and anaphylaxis (Samayawardhena and Pallen, 2010).
Hck	Src-PTK (complementary to Lyn and Fyn)	Reduced degranulation and cytokine production due to impaired Gab2 activation and microtubule formation. Reduced anaphylaxis (Hong <i>et al.</i> , 2007).