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Signal transduction and chemotaxis in mast cells

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Abstract

Mast cells play crucial roles in both innate and adaptive arms of the immune system. Along with basophils, mast cells are essential effector cells for allergic inflammation that causes asthma, allergic rhinitis, food allergy and atopic dermatitis. Mast cells are usually increased in inflammatory sites of allergy and, upon activation, release various chemical, lipid, peptide and protein mediators of allergic reactions. Since antigen/immunoglobulin E (IgE)-mediated activation of these cells is a central event to trigger allergic reactions, innumerable studies have been conducted on how these cells are activated through cross-linking of the high-affinity IgE receptor (FcεRI). Development of mature mast cells from their progenitor cells is under the influence of several growth factors, of which the stem cell factor (SCF) seems to be the most important. Therefore, how SCF induces mast cell development and activation via its receptor, KIT, has been studied extensively, including a cross-talk between KIT and FcεRI signaling pathways. Although our understanding of the signaling mechanisms of the FcεRI and KIT pathways is far from complete, pharmaceutical applications of the knowledge about these pathways are underway. This review will focus on recent progresses in FcεRI and KIT signaling and chemotaxis.

Keywords

Mast cell; IgE receptor; KIT receptor; Signal transduction; Chemotaxis; Plasma membrane

1. Introduction

Mast cells are terminally differentiated cells of the hematopoietic origin that are involved in both innate and adaptive immunity (Bischoff, 2007; Kalesnikoff and Galli, 2008; Abraham

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and St John, 2010). Mast cells originate from myeloid precursors that are released from bone marrow into blood circulation. Once they acquire proper signals through their chemoattractant receptors, they migrate to the target tissues that are strategically located at the host-environment interface (Okayama and Kawakami, 2006; Halova et al., 2012). Numbers of tissue mast cells are tightly regulated not only by migration, but also by proliferation and survival, as mast cells are long-lived cells capable of surviving for months. Under pathological conditions, tissue mast cell homeostasis could be disturbed and the number and distribution of mast cells quickly changed (Okayama and Kawakami, 2006). Mast cell chemoattractants include antigens recognized by immunoglubulins E (IgE), stem cell factor (SCF), different chemokines, cytokines, and leukotrienes. Many of them are also produced by mast cells to attract various cell types of the immune system as well as other mast cells and their precursors to modulate their amount by autocrine and/or paracrine mechanisms (Halova et al., 2012).

Mature mast cells express on their plasma membrane numerous receptors which, after binding of the corresponding ligands, can induce cell activation leading to the release of various inflammatory mediators. The most prominent is the high-affinity receptor for IgE (FcεRI), which has been implicated in an array of acute as well as chronic reactions including allergic rhinitis, asthma, anaphylaxis and atopic dermatitis. Antigen/IgE-mediated activation of mast cells is a multistep process, eventually leading to degranulation of preformed granules containing histamine, heparin, various proteases, tumor necrosis factor (TNF)-α, and other inflammatory mediators and *de novo* synthesis of cytokines, chemokines, eicosanoids, and other immune mediators. FcεRI-mediated activation events are modulated by engagement of other surface receptors such as KIT, adenosine receptors, prostaglandin (PG) receptors and many others. These receptors play multiple roles in differentiation, proliferation, chemotaxis and in setting a threshold for mast cell triggering (Gilfillan and Tkaczyk, 2006).

In industrial countries, mast cell-associated diseases are a serious problem, solution of which requires new strategies for development of new therapeutics. Detailed understanding of mast cell signaling events at the molecular levels could contribute to such developments. In this review we summarize recent findings on the early stages of antigen- and SCF-induced mast cell activation as well as mast cell chemotaxis.

2. Signal transduction

Mast cells express on their plasma membrane numerous receptors that are involved in cell migration and activation. The most extensively studied are FcεRI and KIT.

2.1. FcεRI signaling

2.1.1. FcεRI—FcεRI belongs to the multichain immune receptor family that includes the T and B cell receptors and other Fc receptors. In mast cells and basophils the receptor is expressed as a tetrameric structure composed of one IgE-binding α subunit, one membrane-tetraspanning β subunit and a dimer of disulphide-linked γ subunits (Blank et al., 1989). In other cells such as monocytes, Langerhans cells and dendritic cells, FcεRI is also found in a trimeric form lacking the β subunit (Kinet, 1999). The chain is responsible for binding the Fc

part of IgE. The β chain stabilizes the receptor complex (Donnadieu et al., 2000) and amplifies spleen tyrosine kinase (SYK) phosphorylation resulting in higher magnitude of calcium mobilization while the γ chain dimer functions as an autonomous activation module (Lin et al., 1996). Each β and γ chain possesses one immunoreceptor tyrosine-based activation motif (ITAM) located in their cytoplasmic tails which are responsible for signal transduction and after phosphorylation serve as docking sites for molecules containing one or two Src homology (SH)2 domains (Cambier, 1995; Kinet, 1999). The β and γ chains are shared with other Fc receptors.

2.1.2. Protein kinases and phosphatases—Transduction of the signal from FcεRI is mediated and regulated via several kinases and phosphatases (Figure 1). The Src family protein tyrosine kinases (SFKs) have a well-defined structure containing five functional domains: a variable N-terminal domain, an SH2 domain, an SH3 domain, a kinase domain and a C-terminal regulatory tail (Okada, 2012). LYN, FYN, HCK and FGR are the SFKs that have been shown to be involved in early stages of the FcεRI signaling. LYN is the most abundant SFK expressed in mast cells and its activity is essential for initial tyrosine phosphorylation of the ITAMs of the FcεRI β and γ chains. LYN plays both positive and negative regulatory roles in mast cell signaling but exact molecular mechanisms of its action still remain controversial. Discordant results were obtained from studies using LYN knockout mice (Table 1). All experiments concluded that in the absence of LYN Ca^{2+} mobilization is decreased (Nishizumi and Yamamoto, 1997; Kawakami et al., 2000; Parravicini et al., 2002; Hernandez-Hansen et al., 2004). However, some studies showed increased degranulation in bone marrow-derived cultured mast cells (BMMCs) from LYN knockout mice (Parravicini et al., 2002; Hernandez-Hansen et al., 2004; Odom et al., 2004), whereas in others absence of LYN had no effect on degranulation (Nishizumi and Yamamoto, 1997; Kawakami et al., 2000). An early study described opposite roles of LYN after activation of mast cells with high or low intensity; low-intensity stimulation suppressed LYN kinase activity and its association with FcεRI receptor, whereas high-intensity stimulation had an opposite effect (Xiao et al., 2005). Also studies on passive cutaneous anaphylaxis (PCA) and/or passive systemic anaphylaxis (PSA) gave different results. A first study showed an absence of PCA in LYN knockout mice (Hibbs et al., 1995). Later it was described that PSA in LYN knockout mice depends on age of the mice; in young mice (4 weeks old) it was increased, but in older mice (more than 7 weeks old) it was decreased (Odom et al., 2004). A follow-up study showed that the genetic background of mice affects the results. When BMMCs from Lyn knockout mice were compared to those from wild-type mice, antigen-induced degranulation was either decreased when derived from C57BL/6 mice, or increased when derived from 129/Sv mice (Yamashita et al., 2007). The authors suggested that different expression of FYN kinase in different mouse strains could be responsible for the observed differences.

It has been found that LYN-deficient mast cells exhibit enhanced FYN-dependent signals and degranulation, but reduced calcium responses (Parravicini et al., 2002). In contrast, FYN deficiency resulted in impaired degranulation, whereas calcium response was normal. Both FYN and LYN were found to be associated with the β chain of FcεRI, but unlike LYN, FYN did not participate in the phosphorylation of FcεRI (Gomez et al., 2005a). Instead, FYN was

found to play a role in regulating the activity of phosphatidylinositol 3-kinases (PI3K) (Parravicini et al., 2002).

Less is known about the role of FGR and HCK in mast cell function and activation. HCK deficiency resulted in enhanced LYN activity and FcεRI phosphorylation but decreased phosphorylation of SYK and degranulation without effect on calcium response (Hong et al., 2007). FGR was found to associate with FcεRI and positively regulate mast cell signaling via promoting phosphorylation of SYK and its substrates (Lee et al., 2011). Studies on FGR and HCK suggest that these two kinases also contribute to signaling and SFKs exhibit a hierarchical relationship, i.e., HCK inhibits LYN and LYN inhibits FYN.

SYK is a member of another non-receptor tyrosine kinase family, composed of N-terminal two tandem SH2 domains, a kinase domain, a short C-terminal tail, and two interdomains. SYK-deficient mice are lethal and mast cells derived from fetal liver of SYK knockout mice are deficient in degranulation and cytokine release (Costello et al., 1996). In line with this, SYK-deficient RBL-2H3 rat mast cell lines lack the ability to degranulate and produce cytokines (Zhang et al., 1996). Furthermore, *in vivo* inducible SYK knockout mice exhibited impaired PCA, as expected (Wex et al., 2011). SYK is associated with the γ chain of the FcεRI. Binding of SYK to phosphorylated γ chain ITAM through the SYK SH2 domains induces a conformational change in the kinase, leading to its increased enzymatic activity (Zhang et al., 2000; Siraganian et al., 2010).

Tec family kinases represent another class of non-receptor protein tyrosine kinases that are implicated in FcεRI-mediated activation. The Tec kinases are located in cytosol and contain an N-terminal pleckstrin homology (PH) domain, a Tec homology domain, an SH2 domain, an SH3 domain, and a C-terminal catalytic domain (Gilfillan and Rivera, 2009). Three members of this family, Bruton's tyrosine kinase (BTK), interleukin (IL)-2-inducible T-cell kinase (ITK) and tyrosine kinase expressed in hepatocellular carcinoma (TEC) are expressed in mast cells and are activated upon antigen-induced activation (Kawakami et al., 1994; Kawakami et al., 1995). Regulatory steps in Tec kinases activation include recruitment to the membrane through their PH domain, phosphorylation by SFKs and subsequent autophosphorylation. A single point mutation in the PH domain of *Btk* gene leading to X-linked immunodeficiency (*xid*) confirms the importance of BTK membrane localization for its proper function (Rawlings et al., 1993). Studies with *xid* and BTK knockout mice showed the importance of BTK for PCA. Furthermore, BMMCs obtained from these mice exhibited mild impairment in degranulation, and more severe defects in the production of various cytokines (Hata et al., 1998). Absence of ITK led to impaired PCA as well as decreased degranulation of airway mast cells *in vivo* (Forssell et al., 2005). Another study showed that degranulation of ITK knockout BMMCs is unaffected, but their cytokine production is increased (Iyer and August, 2008). Further analyses of ITK/BTK double knockout mast cells suggested that although they may share substrates, ITK has both positive and negative regulatory roles, while BTK is primarily a positive regulator of FcεRI signaling (Iyer et al., 2011). In contrast to ITK and BTK, TEC knockout mice displayed normal histamine levels in an anaphylactic model and also degranulation was not affected, but TEC was found to play an important role in generation of leukotrienes and cytokines (Schmidt et al., 2009).

The tyrosine kinase network is regulated by dephosphorylation events that are mediated by protein tyrosine phosphatases, which selectively catalyze the removal of a phosphate group from the tyrosine residue. In mast cells, the cytoplasmic SH2-domain-containing protein tyrosine phosphatase (SHP)-1 and SHP-2 are implicated in the control of cellular proliferation, survival and signaling (Heneberg and Dráber, 2002). They contain two adjacent N-terminal SH2 domains, and SHP-2 also possesses a proline-rich sequence at the C-terminus that can interact with SH3 domains of other proteins. After FcεRI engagement, these phosphatases negatively regulate mast cell activation via the immunoreceptor tyrosine-based inhibitory motif (ITIM) as documented by experiments with genetically modified mice.

2.1.3. Lipid kinases and phosphatases—PI3Ks are a family of lipid kinases that are involved in diverse biological functions. PI3Ks phosphorylate phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) to phosphatidylinositol-3,4,5-trisphosphate ($PI(3,4,5)P_3$). Mast cells possess class IA PI3Ks, consisting of a catalytic subunit (p110α, β or δ) and a regulatory subunit (p85α, p85β or p50α). The p85 subunits carry two SH2 domains, which allow the p85/p110 complex to be recruited to phosphotyrosine residues. Class IA PI3Ks are utilized after activation of FcεRI and KIT receptors, whereas class IB PI3K, consisting of a catalytic subunit p110γ and a regulatory subunit p84 or p101, is activated through G protein-coupled receptors (GPCRs) (Rommel et al., 2007; Kim et al., 2008). Whereas mice lacking p110α or p110β are embryonic lethal, p110δ-null mice are viable, with specific defects in B and T cells (Okkenhaug and Vanhaesebroeck, 2003). BMMCs isolated from mice deficient in p85 subunit exhibited no defects in antigen-induced signaling (Fukao et al., 2002; Tkaczyk et al., 2003), suggesting that class IA PI3Ks are not important for FcεRI signaling. However, subsequent studies with mice expressing either wild-type p110δ or $p110\delta^{D910A}$, a loss-of-function allele of p110δ, as well as a chemical inhibition of p110δ, showed indispensability of these PI3Ks for mast cell activation (Ali et al., 2004). Thus, in the absence of p85, p110 may be capable of cooperating with the remaining regulatory subunits. However, a recent study called into question the role of p110δ in mast cell activation (see below).

Two phosphatases oppose activity of PI3Ks. SH2-containing inositol phosphatase (SHIP)1 removes a phosphate group from $PI(3,4,5)P_3$ at the 5′ position (Rauh et al., 2003). Absence of SHIP1 leads to hyperresponsiveness of mast cells to antigen stimulation under in vivo or in vitro conditions (Huber et al., 1998; Haddon et al., 2009). Phosphatase and tensin homologue deleted on chromosome ten (PTEN) dephosphorylates $PI(3,4,5)P_3$ at the 3′ position. Deficiency in PTEN had a very similar effect as the absence of SHIP1 (Furumoto et al., 2011).

2.1.4. Adaptor proteins—Adaptor proteins play important roles as scaffolds enabling the assembly of large spacio-temporarily controlled signaling complexes that contribute to degranulation, cytokine production, chemotaxis and other physiological events. Adaptors are proteins without intrinsic enzymatic function composed of multiple protein-protein or protein-lipid interacting domains. Membrane-bound adaptors are anchored scaffolds that provide docking sites mainly through phosphorylation of tyrosine residues. Cytosolic

adaptors usually possess more motifs that allow them to bind other molecules (Alvarez-Errico et al., 2009; Draber et al., 2012).

The transmembrane adaptor protein linker for activation of T-cells (LAT), also known as LAT1, is the first adaptor described in T cells as playing an important role after T-cell receptor engagement (June et al., 1990). In mast cells, LAT is phosphorylated by SYK and associates with numerous cytoplasmic proteins, including growth factor receptor-bound protein 2 (GRB2), phospholipase (PLC)γ1, guanine nucleotide exchange factor VAV, SH2-domain-containing leukocyte protein of 76 kDa (SLP-76), Casitas B-lineage lymphoma (CBL) and GRB2-related adaptor downstream of SHC (GADS). LAT deficiency causes reduced phosphorylation of PLCγ1 and SLP-76, decreased mitogen-activated protein kinase (MAPK) activity, Ca^{2+} signaling and degranulation (Saitoh et al., 2000). Non-T-cell activation linker (NTAL), also termed as LAB or LAT2, is an adaptor structurally related to LAT, but it lacks a motif for PLCγ1 binding (Brdička et al., 2002). It is phosphorylated by SYK and LYN (Iwaki et al., 2008). There are conflicting results about NTAL function in mast cells. NTAL-deficient BMMCs exhibited enhanced antigen-induced degranulation, calcium mobilization, and phosphorylation of LAT, PLCγ1, and extracellular signal-regulated protein kinase (ERK) (Volná et al., 2004; Zhu et al., 2004). This was in contrast with the results obtained with human mast cells and RBL cells in which LAT knockdown (KD) by RNA silencing techniques resulted in reduced degranulation (Tkaczyk et al., 2004; Draberova et al., 2007). A recent study showed that mouse BMMCs with NTAL KD have similar properties as BMMCs from NTAL knockout mice (Polakovicova et al., 2014). These data indicate that NTAL in mouse BMMCs is a negative regulator of FcεRI signaling, independently of possible compensatory developmental alterations in NTAL-deficient mice.

Phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), also called C-terminal SRC kinase (CSK)-binding protein (CBP), is phosphorylated by LYN and FYN but not SYK. It can bind the negative regulatory kinase CSK (Brdicka et al., 2000; Kawabuchi et al., 2000). Experiments with RBL cells showed that PAG is a negative regulator of FcεRI signaling (Ohtake et al., 2002). Interestingly, BMMCs from PAG-deficient mice exhibited impaired degranulation, calcium mobilization and tyrosine phosphorylation of FcεRI subunits and PLCγ, suggesting that PAG could also have positive regulatory roles (Draberova et al., 2014).

Cytosolic adaptor proteins lack the hydrophobic transmembrane domain that would anchor them to the plasma membrane; instead they possess various motifs which allow them to associate with plasma membrane molecules, cytoskeleton and/or other organelles (Alvarez-Errico et al., 2009). One of the cytosolic adaptors being recruited to tyrosine phosphorylated LAT is SLP-76 (Saitoh et al., 2000). Mast cells lacking SLP-76 show impaired FcεRI signaling, including decreased degranulation, PLCγ and PI3K activity (Pivniouk et al., 1999). Upon FcεRI triggering, SLP-76 becomes tyrosine-phosphorylated by SYK and provide binding sites for VAV, non-catalytic region of tyrosine kinase (NCK), and BTK (Kettner et al., 2003). SLP-76 is recruited to LAT by another cytosolic adaptor, GADS, which constitutively associates with proline-rich regions of SLP-76. This interaction is important for proper FcεRI-mediated degranulation (Silverman et al., 2006). The absence of GADS causes disruption of association of SLP-76 to LAT, and impaired degranulation and

calcium mobilization; however this effect is not as strong as SLP-76 deletion (Yamasaki et al., 2008).

GRB2-associated-binding protein 2 (GAB2) is a cytosolic adaptor important in PI3K signaling (Gu et al., 1998). An initial study suggested that GAB2 is phosphorylated by FYN and is involved in downstream signaling events from FcεRI and KIT, but a later study showed that the kinase activity of SYK is reduced in FYN-deficient mast cells and SYK phosphorylates GAB2 (Yu et al., 2006). The FYN signaling is complementary to the LYN-LAT pathway (Gu et al., 2001; Parravicini et al., 2002). GAB2-deficient mast cells show impaired degranulation, cytokine production, and PI3K activation (Gu et al., 2001).

Related to GAB2 is GRB2, which interacts with the guanine exchange factor SOS and thus activates the RAS-ERK cascade (Lowenstein et al., 1992). Besides SOS, GRB2 was reported to interact with LAT, NTAL, SLP-76 and GAB2 (Alvarez-Errico et al., 2009).

Adaptors are involved not only in promoting signaling but also act in a negative regulatory manner. For instance, downstream of tyrosine kinase 1 (DOK1) is constitutively associated with RAS GTPase-activating protein (RAS-GAP). After SHIP recruitment to the ITIM in a LYN-dependent manner, DOK1 associates with SHIP and the negative regulatory complex SHIP/RAS-GAP/DOK1 down-regulates PI(3,4,5)P_3 levels and inhibits RAS activation via Ras-GAP (Kepley et al., 2004; Tamir et al., 2000). Nevertheless, DOK1-deficient mast cells show no defect in activation (Ott et al., 2002).

2.1.5. FcεRI signaling pathways—The binding of multivalent antigen to IgE-FcεRI complexes on the plasma membrane causes aggregation of the complexes and triggers a series of activation events starting with enhanced phosphorylation of the FcεRI β and γ chains by LYN kinase. The exact molecular mechanism behind this event is not completely understood, but it is expected that there are changes in access of LYN to tyrosine residues in the ITAM motifs of β and γ chains and/or decrease dephosphorylation of the target phosphotyrosines (Bugajev et al., 2010). Phosphorylation of FcεRI β and γ chains facilitates recruitment of LYN and SYK, respectively, and their activation. Activated SYK in FcεRI signalosome is instrumental in downstream propagation of the signal through phosphorylation of many substrates, including adaptor proteins LAT, NTAL, and SLP-76. Phosphorylated LAT serves as an anchor for PLCγ, which after phosphorylation promotes calcium signaling.

Another pathway of FcεRI signaling is initiated by FYN. After FcεRI aggregation, FYN phosphorylates the adaptor GAB2 and AKT. Phosphorylated GAB2 serves as a docking site for additional FYN and promotes degranulation by its subsequent association with PI3K. PI3K then phosphorylates PI(4,5)P_2 to PI(3,4,5)P_3. FYN is required for normal mast cell degranulation and maintenance and/or amplification of Ca^{2+} signal (Parravicini et al., 2002).

The transmembrane adaptor LAT, phosphorylated by SYK recruits cytosolic adaptors, such as GADS, GRB2 and SLP-76, guanine nucleotide exchangers VAV and SOS, and signaling enzymes PLCγ1 and PLCγ2. Assembly of this complex and recruitment of PI3K enables PLCγ phosphorylation by SYK and BTK. BTK recruitment to the plasma membrane is

regulated via its PH domain-mediated binding to $PI(3,4,5)P_3$, generated by activity of PI3K. Activated PLCγ catalyzes the hydrolysis of membrane bound lipid, $PI(4,5)P_2$, to generate two important second messengers, 1,2-diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). DAG activates conventional protein kinase C (PKC) isoforms that together with the free Ca^{2+} released via the action of IP_3 initiates the degranulation event and its maximum is reached by sustained high levels of $PI(3,4,5)P_3$ and Ca^2 (Kraft and Kinet, 2007).

2.1.6. Calcium mobilization—IP_3 generated by activity of PLCγ diffuses through the cytosol and induces cytosolic calcium mobilization via binding to IP_3 receptors located in the membrane of the endoplasmic reticulum (ER). IP_3 receptors are Ca^{2+} channels allowing the release of Ca^{2+} from the ER stores to the cytoplasm. The depletion of Ca^{2+} from intracellular stores leads to opening of plasma membrane store-operated Ca^{2+} release-activated Ca^{2+} (CRAC) channels that allow a strong Ca^{2+} influx into the cytoplasm. The stromal interaction molecule 1 (STIM1) located in the membrane of ER is a Ca^{2+} sensor detecting the ER Ca^{2+} concentration (Roos et al., 2005; Liou et al., 2005; Draber and Draberova, 2005). STIM1-deficient mast cells had impaired FcεRI-mediated Ca^{2+} influx, degranulation, cytokine production, and activation of the transcription factors NF-κB and nuclear factor for T cell activation (NFAT) (Baba et al., 2008). Depletion of Ca^{2+} from ER induces clustering of STIM1 in the ER membrane which then moves to the close proximity of the plasma membrane, and STIM1 directly interacts with the CRAC channel subunit ORAI1. This enables entering of Ca^{2+} into the cells, leading to numerous changes, including reorganization of microtubules in activated mast cells (Hajkova et al., 2011). Mast cell degranulation is critically dependent on increased intracellular calcium and activation of PKC (Ozawa et al., 1993). The Ca^{2+} influx triggers the fusion of mast cell granules to the membrane and is essential for actin cytoskeleton reorganization.

Calcium mobilization has an impact on activation of many signal-transducing proteins including VAV, GRB2, SOS, Rho GTPases and MAPKs. A key role in Ca^{2+} mobilization is played by LAT which after phosphorylation allows anchoring of PLCγ1, GRB2, and GADS via their SH2 domains. Guanine nucleotide exchange factors (GEFs) VAV and SOS associate with these complexes and activate the RAS and Rho family GTPases and thus modulating cytoskeletal organization and vesicle movement as well as initiating MAPKs activation. Activated RAS positively regulates the RAF-dependent pathway and activates the ERK, JNK, and p38 MAPK pathways, leading to activation of FOS and JUN, NFAT, NF-κB, ATF2, and ELK1 (Gilfillan and Rivera, 2009; Gilfillan and Beaven, 2011).

An increase in free intracellular calcium concentration activates a protein phosphatase calcineurin by binding a regulatory subunit and activating calmodulin binding. Calcineurin dephosphorylates NFAT and the activated NFAT then translocates to the nucleus, where it regulates the transcription of several cytokine genes (Rivera and Gilfillan, 2006).

Another second messenger for mast cell activation and calcium mobilization is sphingosine 1-phosphate (S1P). LYN and FYN kinases activate sphingosine kinases SPHK1 and SPHK2, which induce conversion of sphingosine into S1P. S1P works as a ligand for a subset of G protein-coupled S1P receptors, which are known to regulate a variety of cellular responses

including cytoskeletal reorganization, formation of adherent junctions, proliferation, angiogenesis, and cell movement (Spiegel and Milstien, 2003). Mast cells activated via the FcεRI secrete S1P through transporter ABCC1 (a member of the ATP-binding cassette transporter family) and the extracellular S1P binds to $S1P_1$ and $S1P_2$ receptors (Prieschl et al., 1999; Jolly et al., 2004). Mast cells isolated from mice lacking SPHK1 didńt show any marked alteration of degranulation and calcium response while SPHK2-deficient cells were found to have impaired degranulation, cytokine secretion, and defective calcium influx (Olivera et al., 2007). In contrast to murine mast cells, S1P induced degranulation of human mast cells and SPHK1, but not SPHK2, has been shown to play a critical role in this process (Oskeritzian et al., 2008). Direct interaction of SPHK1 with LYN, but not with SYK, causes SPHK1 recruitment to membrane rafts and to FcεRI (Oskeritzian et al., 2008; Urtz et al., 2004). The sphingosine kinase activity induced by FcεRI crosslinking plays a role in maintaining the balance between sphingosine and S1P. While high levels of sphingosine are associated with apoptosis, high levels of S1P with cell proliferation (Hait et al., 2006).

2.1.7. Regulation of the FcεRI signaling—A recent study showed that FcεRI is capable of reacting differently to high- and low-affinity stimuli (Suzuki et al., 2014). The low-affinity stimulation led to reduced degranulation and leukotriene B4 and cytokine production, but enhanced chemokine production. The observed differences were not due to variable receptor phosphorylation but to differences of the size, mobility, and distribution of the receptor clusters. In low-affinity stimulation bigger and less mobile receptor clusters were formed. While in high-affinity stimulation LAT, PLCγ1 and PLCγ2 were strongly phosphorylated, in low-affinity stimulation the signal was shifted to NTAL that exhibited higher phosphorylation and more extensive colocalization with the FcεRI. Also the association of FGR with the receptor was increased.

Mast cells also express inhibitory cell-surface receptors to control the effector functions. These receptors contain ITIMs, which are used to suppress the activation by promoting the dephosphorylation carried out by the phosphatases. Lipid phosphatase SHIP binds to the low-affinity receptor for IgG, FcγRIIB, and to β and γ subunits of the FcεRI and inhibits the degranulation. Through interactions of its SH2 domain with ITIMs, SHIP is recruited to the plasma membrane where it degrades $PI(3,4,5)P_3$ to $PI(4,5)P_2$, subsequently leading to reduced BTK activation and PLCγ-mediated Ca^{2+} mobilization. PTEN opposes PI3K activity. PTEN catalyzes the hydrolysis of $PI(3,4,5)P_3$ to $PI(4,5)P_2$, thus functioning as a negative regulator of FcεRI-induced calcium flux, degranulation and cytokine production (Furumoto et al., 2006).

2.2. KIT signaling

2.2.1 KIT receptor—An important receptor localized on the plasma membrane of mast cells is KIT (CD117), the receptor for SCF. KIT activation is crucial for growth, survival, differentiation and homing of mast cells into target tissues. SCF, existing in two isoforms, soluble and membrane bound, that arise by alternative splicing of one RNA, is produced by different cell types including fibroblasts and endothelial cells. SCF, similarly to IgE-antigen complexes, can be an activator/co-activator of mast cells (Gilfillan and Tkaczyk, 2006). When activated by antigen, SCF markedly increases mast cell degranulation and potentiates

and prolongs calcium signals. It also influences mast cell chemotaxis and adhesion (Vosseller et al., 1997). KIT is a single chain receptor with protein-tyrosine kinase activity. Its extracellular part comprises of five immunoglobulin-like domains, the first three of which bind SCF and the fourth is important for the receptor dimerization. The intracellular part has a split catalytic domain. SCF binding to KIT leads to dimerization and auto/transphosphorylation at tyrosine residues.

2.2.2. KIT signaling pathways—Signaling pathways elicited by KIT share several similar features with FcεRI-mediated events such as involvement of SFKs, PLCγ1, PI3K, calcium mobilization and MAPK-cascade activation (Figure 2). However, activated KIT does not recruit or activate SYK or phosphorylate LAT. The ability of KIT to potentiate FcεRI-dependent degranulation seems to be due to the involvement of NTAL and BTK and their ability to regulate PLCγ1-dependent calcium mobilization and PKC activation (Gilfillan and Beaven, 2011). KIT can phosphorylate tyrosine residues in NTAL, which are different from those phosphorylated by SYK in FcεRI-activated cells (Iwaki et al., 2008).

After SCF-induced KIT dimerization and activation of its intrinsic kinase activity, phosphorylated tyrosine residues recruit SH2-domain containing proteins, including cytosolic adaptors SHC and GRB2, SFKs (LYN and FYN), PLCγ, and PI3K (Linnekin et al., 1997; Roskoski, Jr., 2005). This creates a signaling complex that further propagates the downstream signaling leading to activation of other signaling molecules. Among them are components of the JAK-STAT and RAS-RAF-MAPK pathways, which lead to mast cell growth, differentiation, survival, adhesion and chemotaxis. KIT receptor can directly bind the p85α subunit of PI3K and in this way contributes to subsequent generation of membrane-associated $PI(3,4,5)P_3$ (Gilfillan and Rivera, 2009).

2.3. Other surface receptors

Besides FcεRI and KIT, mast cells express other surface receptors that enable them to perform various functions including recognition of microbes, signaling to other immune cells, interactions with neurons and positive or negative modulation of other surface receptors. Several recent reviews have dealt with such receptors in mast cells (Pundir and Kulka, 2010; Sandig and Bulfone-Paus, 2012; Gilfillan and Beaven, 2011).

Several surface receptors are important for recognition and/or defense against pathogens. Toll-like receptors (TLRs) enable mast cells to respond to products of Gram-negative and Gram-positive bacteria. The tissue origin of mast cells and/or culture conditions can influence the expression of different TLRs (Marshall and Jawdat, 2004). TLR2 and TLR4, the most well-studied TLRs in mast cells, act synergistically with antigen to enhance cytokine production (Gilfillan and Beaven, 2011). Closely related is ST2 (also known as IL1RL1), which binds IL-33 and forms a complex with IL-1 receptor accessory protein (IL1RAP). IL-33 can activate NF-κB and MAP kinase signaling pathways, drives maturation of human mast cells and directly stimulates them to produce several proinflammatory cytokines and chemokines (Allakhverdi et al., 2007). IL-33 has a pro-inflammatory role in rheumatoid arthritis and promotes anaphylaxis by mast cell activation. Furthermore, increased levels of IL-33 were reported in asthmatic patients, and in skin cells

of patients with atopic dermatitis (Liew et al., 2010). Nucleotide-binding oligomerization domain-like receptors (NLRs) such as NOD1-5 and NLRP1-14 are involved in intracellular recognition of bacterial infection. NLRs are expressed in the cytosol of epithelial cells and antigen presenting cells, and some of them are also in mast cells (Haidl et al., 2011). Antimicrobial peptides are ancient and essential elements expressed by different immune cells to execute innate immune response against pathogens. Antimicrobial peptides can induce mast cell degranulation independently of antigen stimulation. Many of these peptides work through GPCRs but their identity and mechanisms of action are poorly understood (Pundir and Kulka, 2010).

Mast cells not only produce a variety of cytokines but also express various receptors for cytokines such as IL-3R, IL-4R, IL-5R, IL-10R, and TGFβR1. Binding of IL-3 and IL-4 to their receptors causes increased FcεRI-dependent histamine release (Gebhardt et al., 2002; Bischoff et al., 1999), while IL-5 binding to IL-5R has no effect on degranulation. IL-10 has an anti-inflammatory effect on mast cells by inhibiting their release of TNF-α, IL-8 and histamine (Royer et al., 2001). It also causes suppression of FcεRI expression (Kennedy et al., 2008). Mast cell-derived TGF-β also acts as a negative regulator, working in an autocrine manner on mast cells. TGF-β inhibits the release of histamine and TNF-α from mast cells (Bissonnette et al., 1997) and the surface expression of FcεRI (Gomez et al., 2005b).

FcεRI-mediated mast cell activation can be modified by GPCRs, which are the most common targets of anti-allergy therapy. GPCRs with 7 membrane-spanning regions associate with heterotrimeric G proteins, composed of α,β, and γ subunits. In the resting state, the α subunit of a G protein is bound to GDP. After ligand binding to the receptor, the bound GDP is exchanged for GTP resulting in temporary dissociation of this subunit from the β and γ subunits and allowing the free α subunits to mediate downstream signaling (Kuehn and Gilfillan, 2007). Mast cells express many classes of GPCRs including the receptors for the complement components and chemokines, S1P, PGE_2, adenosine, neuropeptide and antimicrobial peptides.

Mast cells express complement receptors for C3a and C5a, which can act as mast cell chemoattractants (Nilsson et al., 1996). C3a (anaphylatoxin peptide) can modulate mast cell degranulation and the release of chemokines (Woolhiser et al., 2004). The chemokine receptor CCR3 seems to play an important role in allergic diseases. This receptor binds a variety of chemokines including CCL2/MCP-1, CCL5/RANTES and CCL11/eotaxin-1, which either induce mast cell chemotaxis or degranulation. Also expressed are CCR1, CCR5, and CXCR4 (Kuehn and Gilfillan, 2007; Marshall and Jawdat, 2004).

As described above, S1P can act as a second messenger for intracellular calcium mobilization and regulate mast cell activation and chemotaxis by binding to one of the two S1P receptors, $S1P_1R$ and $S1P_2R$, expressed on mast cells (Choi et al., 1996; Jolly et al., 2004). These two receptors have distinct roles; $S1P_1R$ induces mast cell chemotaxis while $S1P_2R$ modifies degranulation and chemokine and cytokine release (Jolly et al., 2004).

PGE_2 is an important modulator of inflammatory responses that is involved in several inflammatory diseases including rheumatoid arthritis. It is produced by various cell types

and multiple GPCRs for PGE_2 have been described. Mast cells express two of them, EP2 and EP3 receptors. The EP2 receptor is linked with down-regulation of antigen-mediated degranulation and cytokine production in mast cells. In contrast, the EP3 receptor enhances FcεRI-mediated degranulation and production of cytokines (Feng et al., 2006).

Adenosine has been shown to mediate pathogenic mechanisms of asthma and rheumatoid arthritis. The adenosine A_{2A} receptor is expressed on most inflammatory cells implicated in asthma including mast cells. This receptor down-regulates mast cell activation, while the A_{2B} receptor up-regulates degranulation and cytokine secretion. Finally, the A_3 receptor seems to be uninvolved in the regulation of degranulation and is mainly expressed by lung mast cells in mice (Brown et al., 2008).

During an inflammatory response mast cells may respond to stimuli such as neuropeptides in an FcεRI-independent manner. Since mast cells are often located in close proximity to neurons and blood vessels, they may respond to neuropeptides produced by nearby neurons. Acute psychological stress can trigger skin mast cell degranulation (Singh et al., 1999). Different types of mast cells were reported to respond to various neuropeptides such as substance P, vasoactive intestinal polypeptide (VIP), neuropeptide Y, and calcitonin gene-related peptide (CGRP). Substance P and VIP induce degranulation and cytokine and chemokine release in mast cells. These cells constitutively express the receptor for substance P, neurokinin 1 receptor (NK1R), the receptor for VIP, VIP receptor type 2 (VPAC2), and neuropeptide receptors NK2R and NK3R. FcεRI-mediated activation of human mast cells up-regulated expression of VPAC2, NK2R, and NK3R, suggesting that exposure to allergen may enhance neuronal inflammation (Kulka et al., 2008). Mouse mast cells express the neuropeptide Y receptor, Y1, and to a lesser extent Y2 and Y5. Recently, the overexpression of neuropeptide Y was shown to contribute to the development of atherosclerosis. Neuropeptide Y causes a significant increase in perivascular mast cell activation and induced pro-inflammatory mediator release from isolated mast cells (Lagraauw et al., 2014).

Antigen-induced responses can be modulated by various inhibitory receptors. They contain in their cytoplasmic domains up to four ITIMs that recruit phosphatases and other inhibitory signaling molecules. Most of the inhibitory receptors recruit the phosphatases SHP-1 and SHP-2, while FcγRIIB primarily recruits SHIP-1. FcγRIIB binds allergen-specific IgG and prevents activation of mast cells caused by allergen-specific IgE bound to FcεRI (Kraft and Kinet, 2007). Other inhibitory receptors include sialic acid-binding immunoglobulin-like lectin (Siglec) family, among them the most studied Siglec 8, mast cell function-associated antigen (MAFA), platelet endothelial cell adhesion molecule (PECAM1), Gp49B1, and many others whose properties have been reviewed elsewhere (Li and Yao, 2004; Shik and Munitz, 2010). CD200, also known as OX2, is a ligand for surface receptor CD200R that lacks ITIMs but can also suppress FcεRI signaling (Kraft and Kinet, 2007).

Apart from the inhibitory FcγRIIB, mast cells express other activating high- or low-affinity receptors for IgG. FcγRI (CD64) and FcγRIII (CD16) share the same γ subunit dimer with FcεRI, but their α chains bind IgG. Their expression may vary according to the local environment, presence of cytokines, and species origin of the cells. The types of Fcγ

receptors and differences in expression on human and mouse have been extensively reviewed (Malbec and Daeron, 2007; Jonsson and Daeron, 2012).

3. Chemotaxis

3.1. FcεRI-mediated chemotaxis

As mentioned above, binding of IgE to the α chain of FcεRI leads to sensitization of mast cells which is a prerequisite for their activation by multivalent antigen recognized by the IgE. The chain of activation events is initiated by phosphorylation of the ITAM motifs of the FcεRI β and γ subunits by LYN (and other SFKs). Inhibition of enzymatic activity of the SFKs with PP2 and SYK with piceatannol or ER-27319 diminished FcεRI-mediated migration, whereas inhibition of TEC kinase with terreic acid had no effect. Experiments with mast cells from mice deficient in LYN or FYN kinase showed that for FcεRI-mediated migration the presence of LYN is more important than FYN. Furthermore, cells deficient in SYK were unable to migrate towards (Kitaura et al., 2005). On the other hand, BTK-deficient mast cells did not migrate towards antigen, but exhibited normal migration towards SCF and PGE_2 (Kuehn et al., 2010). However, the role of SYK in mast cell physiology is more complex; recent studies with inducible SYK knockout mice showed that SYK is dispensable for mast cell chemotaxis towards SCF (Wex et al., 2011). SYK propagates the signal, among others, by phosphorylating LAT and NTAL. Different roles of these two adaptors in chemotaxis and degranulation have been demonstrated: NTAL was found to be a predominantly negative regulator of chemotaxis (Tumova et al., 2010), whereas LAT was found to be expendable in chemotaxis (Halova et al., 2013). Interestingly, NTAL/LAT doubly deficient mast cells had diminished phosphorylation of numerous substrates, calcium release and degranulation, but exhibited increased migration towards antigen when compared to wild-type cells. However, this increase was lower in magnitude when compared to NTAL knockout cells, suggesting that in the absence of NTAL, LAT could negatively regulate chemotaxis (Halova et al., 2013). These results are consistent with the results obtained with SYK-deficient cells and suggest that the differences in activation leading to the release of secretory granules and chemotaxis are larger than previously thought. The negative regulatory role of NTAL in antigen-induced chemotaxis seems to be mediated trough small GTPase RhoA and its kinase ROCK; on the other hand NTAL does not seem to be involved in chemotaxis towards SCF (Tumova et al., 2010). RhoA is known to regulate cortical filamentous (F)-actin disassembly depending on levels of free cytoplasmic calcium (Sullivan et al., 1999). The importance of SOCE influx on mast cell chemotaxis was shown in cells with decreased levels of STIM1. These cells exhibited decreased calcium influx, formation of microtubule protrusions and impaired chemotaxis (Hajkova et al., 2011). In accordance with these data are findings that knockdown of the Ca^{2+} entry channel protein ORAI1 led to inhibition of spontaneous motility as well as directional chemotaxis (Lee et al., 2012). Recent study also indicated that chemotaxis towards antigen is positively regulated by adaptor protein PAG, which, however, had no effect on KIT-mediated chemotaxis in BMMCs (Draberova et al., 2014).

The role of PI3K isoforms in mast cell migration is controversial. In initial studies with p110δ knockout mice, $p110\delta^{D910A}$ transgenic mice with inactive catalytic subunit, and

p110δ-selective inhibitor IC87114 showed importance of this subunit for SCF-mediated proliferation, adhesion and chemotaxis, as well as antigen-induced degranulation and cytokine production (Ali et al., 2004). Further *in vivo* comparative analyses showed that p110δ, but not p110γ, is important for antigen-dependent hypersensitivity responses in mice (Ali et al., 2008). Studies with IC 87114 and AS 252424, which selectively block PI3Kδ and PI3Kγ, respectively, showed PI3Kδ as the major isoform regulating the responses to antigen alone, or in combination with other stimulants and PI3Kγ contributing only in GPCR activation (Kuehn et al., 2010). The absence of PI3Kγ inhibited G protein-coupled signaling and mast cell degranulation *in vitro*, and protected mice from antigen-induced PSA by disrupting an adenosine-dependent autocrine/paracrine signaling loop (Laffargue et al., 2002). PI3Kγ is also critically involved in an autocrine/paracrine mast cell migration towards antigen (Kitaura et al., 2005; Endo et al., 2009). In contrast to the results obtained previously (Kuehn et al., 2010; Ali et al., 2008), the most recent work (Collmann et al., 2013) ruled out the role of PI3Kδ in antigen-mediated mast cell chemotaxis. Using p110γ knockout mice, $p110\gamma^{KR}$ mice (expressing catalytically inactive subunit of p110γ), p110δ knockout mice, the p110δ-specific inhibitor IC87114 and a novel p110γ-specific inhibitor NVS-PI3-4, the authors showed that PI3Kγ alone is responsible for accumulation of mast cells in IgE-challenged skin, cytokine release, and mast cell/endothelial interaction and chemotaxis. As expected, NVS-PI3-4 also blocked direct migration through GPCRs; PI3Kδ was necessary only for migration and adhesion caused by SCF. The blocking of mast cell recruitment protected mice against anaphylaxis. These *in vivo* results demonstrated the advantage of blocking chemotaxis in treatment because they required 10 times lower drug doses than those used for inhibition of degranulation. Interestingly, transient inhibition of PI3Kγ enzymatic activity was sufficient to block the recruitment of mast cell progenitors into target tissues. In addition to PI3Kγ, another GPCR signal transducer PLC-β3 plays an important role in FcεRI signaling, as PLC-β3-deficient mast cells exhibit reduced antigen-induced chemotaxis and cytokine production but normal degranulation (Xiao et al., 2011). Interestingly, PLC-β3 constitutively interacts with FcεRI, LYN and SHP-1. These observations might simply indicate that multiple signaling molecules are commonly used by GPCRs and FcεRI pathways. Alternatively, FcεRI might interact with some GPCR(s) at a receptor or G protein level as well.

As already mentioned, an amount of $PI(3,4,5)P_3$ produced by PI3K is negatively regulated by activity of two lipid phosphates, PTEN and SHIP1. It has been shown that PTEN-deficient human mast cells exhibited increased calcium levels, cytokine production, constitutive phosphorylation of AKT, p38 MAPK, and JNK as well as enhanced cell survival (Furumoto et al., 2006). Further studies showed that PTEN knockout mice exhibited mast cell hyperplasia in various organs. Selective depletion of PTEN in mast cells revealed that this phenomenon is intrinsic to the mast cell (Furumoto et al., 2011). In this study no changes in mast cell chemotaxis towards antigen and SCF in PTEN-deficient mast cell were found. However, PTEN was important for migration of macrophages (Papakonstanti et al., 2007), and one cannot exclude the possibility that the increased number of mast cells in PTEN knockout mice could be caused not only by hyper-proliferation but also by increased migration of their progenitors. Similarly, SHIP1 knockout mice also exhibited mast cell hyperplasia, increased cytokine production and anaphylactic response (Haddon et al., 2009).

A new small-molecule SHIP1 activator, AQX-1125, reduced cytokine production in splenocytes, inhibited the activation of mast cells and human leukocyte chemotaxis (Stenton et al., 2013b; Stenton et al., 2013a). However, the role of these phosphatases in mast cell chemotaxis remains to be determined.

3.2. KIT-mediated chemotaxis

SCF is a major chemotactic attractant for mast cells and their precursors (Chabot et al., 1988; Meininger et al., 1992; Nilsson et al., 1994; Nilsson et al., 1998). Once bound to KIT, SCF causes KIT tyrosine phosphorylation and formation of docking sites for SH2 domain-containing molecules, such as LYN and FYN, SFKs that are recruited to phosphorylated Y567 of the KIT. This results in activation of the kinases and further propagation of the signal. Activation of FYN leads to phosphorylation of GAB2 and subsequent activation of the small GTPase RAC that is responsible for the cytoskeletal reorganization and mast cell migration (Linnekin et al., 1997; Timokhina et al., 1998; Ueda et al., 2002; Samayawardhena et al., 2007). Proper functioning of the GAB2/SHP2/VAV/PAK/RAC/JNK signaling axis requires protein tyrosine phosphatase PTPα as determined by reduced migration towards SCF in PTPα knockout mice (Samayawardhena and Pallen, 2008). Chemotaxis towards SCF also requires another phosphatase, SHP2. BMMCs from conditional SHP2 knockout mice exhibit decreased chemotaxis. SHP2 directly activated LYN kinase by dephosphorylation of its inhibitory tyrosine. This resulted in enhanced phosphorylation of VAV1, RAC activation and F-actin polymerization. In accordance with these data, chemotaxis was also reduced in cells exposed to the SHP2 inhibitor, II-B08 (Sharma et al., 2014). An important activation step is phosphorylation of KIT Y719 that is crucial for recruitment of PI3K (Ueda et al., 2002). As already mentioned PI3K plays an important role in mast chemotaxis towards different chemoattractants.

3.3. FcεRI and KIT cross-talk in chemotaxis

SCF- and antigen/IgE-activation pathways converge into a final common pathway leading to cell migration towards a chemoattractant. Therefore, it is of importance to comprehend cooperative actions of these pathways during cell migration. This topic is controversial. An early study showed that pretreatment of mast cells with SCF causes inhibition of migration towards antigen (Sawada et al., 2005). The authors proposed that SCF at sites of inflammation could lead to inhibition of migration towards antigen. This would result in preferential accumulation and degranulation of mast cells at sites of high levels of antigen. However, further experiments showed that when the IgE-sensitized cells are exposed to antigen and SCF simultaneously the chemotactic response is higher than when the cells are exposed to the individual activators (Kuehn et al., 2010). Another study showed that the response of cells exposed simultaneously to antigen and SCF was higher than that toward antigen alone, but lower than that induced by SCF alone (Tumova et al., 2010). These discrepancies could be due to differences in experimental procedures. Clarification of this issue requires further study.

4. Pharmacological targeting of signal transduction and chemotaxis pathways

Current pharmacological approaches to inhibit activity of mast cells and basophils have been summarized in a recent review (Harvima et al., 2014). Three inhibitory levels have been identified: (1) targeting soluble mediators released from the cells, (2) interfering with the intracellular signaling pathways, and (3) modulating the action of surface activating or inhibitory receptors. In the category of intracellular signal transduction targets the promising drugs are those which inhibit key enzymes involved in signal transduction from the plasma membrane receptors to the cytoplasmic effectors. To fulfill a set of the criteria as a therapeutic, they should be specific to mast cells and their action should not cause severe effects in other cell types. For this reason, SFKs may not be suitable targets, because they are expressed in numerous other cells types. SYK has a more limited distribution and therefore is more suitable as a drug target. In fact, several SYK inhibitors have been developed and some of them have entered clinical trials. These include fostamatinib, also called R-788 (Braselmann et al., 2006; Weinblatt et al., 2013) and PRT062607 (Simmons, 2013). Fostamanitib, an oral SYK inhibitor, has been successfully used in a phase II clinical trial for treatment of patients with rheumatoid arthritis (Weinblatt et al., 2010; Weinblatt et al., 2013). When administrated to mice it caused a significant reduction of neutrophils and macrophages, whereas systemic leukocyte counts were not affected (Hilgendorf et al., 2011). BTK can be selectively inhibited by ibrutinib that, via its binding to the active site, disables its phosphorylation and thus causes inactivation. It forms a specific covalent bond with cysteine 481 in BTK and abrogates its full activation by inhibiting autophosphorylation at Tyr-223 (Pan et al., 2007; Honigberg et al., 2010). Another drug AVL-292/CC-292 was designed on the same principle and is under investigation in clinical trials to treat rheumatoid arthritis (Evans et al., 2013).

Promising as targets are also PI3Ks which are involved in various signaling pathways, although these kinases are expressed in many cell types. IC87114 and its chemical derivatives CAL-101 and CAL-263 are highly selective inhibitors of PI3Kδ and have clinical potential for treatment of allergic rhinitis (Blunt and Ward, 2012), asthma (Lee et al., 2006) and rheumatoid arthritis (Randis et al., 2008). IPI-145, a small-molecule inhibitor of PI3K-δ and PI3K-γ, showed potent activity in collagen-induced arthritis, ovalbumin-induced asthma, and systemic lupus erythematosus in rodent models. This drug blocked neutrophil migration, lymphocyte proliferation, and reduce basophil and mast cell activation (Winkler et al., 2013). IPI-145 is in clinical trials for various hematologic malignancies and inflammatory diseases, such as asthma and rheumatoid arthritis. A wide range of other PI3K inhibitors have entered clinical trials (Marone et al., 2008).

Enhancement of the activity of negative regulators of degranulation and termination of the activation events can be achieved by stimulating the phosphatase SHIP1. A recent double blind (placebo/controlled) study with AQX-1125, a novel oral SHIP1 activator, reports significantly reduced late responses to allergen challenge, with a trend to reduce airway inflammation and claims AQX-1125 as a safe and well tolerated drug (Leaker et al., 2014).

Several diseases including mastocytosis are associated with a gain of function mutation (D816V) in KIT (Ustun et al., 2011). Also an aberrant bone marrow mast-cell population carrying the clonal markers found in mastocytosis was reported in patients with recurrent anaphylaxis (Akin et al., 2007). The KIT receptor is related to platelet-derived growth factor receptor (PDGFR), and the known KIT inhibitors also inhibit PDGFR and some other molecules with tyrosine kinase activity. Inhibition of KIT activity is mainly desired in myeloid leukemia but could also be used for treatment of systemic mastocytosis. Several KIT inhibitors varying in their inhibitory specificity and sensitivity to the activating mutation are available, including dasatinib (Kneidinger et al., 2008), nilotinib (Verstovsek et al., 2006), imatinib (Vega-Ruiz et al., 2009) and masitinib (Humbert et al., 2009; Paul et al., 2010). Two KIT inhibitors, masitinib and imatinib, that are promising for treatment of mastocytosis, arthritis and allergen-induced asthma, were also found to be efficient inhibitors of mast cell migration, where masitinib was more efficient then imatinib (Dubreuil et al., 2009).

5. Future directions and concluding remarks

First of all, one should realize that our understanding of FcεRI signaling is far from complete. Although everyone would agree on the fundamental importance of tyrosine and lipid phosphorylation in FcεRI signaling pathways, multiple studies fail to agree on detailed mechanisms by which SFKs and PI3K isoforms regulate mast cell activation, as discussed above. Furthermore, more than one hundred tyrosine-phosphorylated proteins (Cao et al., 2007) await to be investigated. There are also many poorly explored areas in FcεRI signaling, including potential roles of other post-translational modifications, miRNAs and other noncoding RNAs, epigenetic regulation of signaling molecules, etc. However, as we are in the post-human/mouse genome era with an armamentarium of proteomics, next-generation sequencing, epigenetics, siRNA and CRISPR/Cas techniques, super-resolution microscopy, multi-photon microscopy, etc., our pace of discoveries in FcεRI signaling per se will be accelerated in the next few years. Second, one needs to understand more how FcεRI signaling in mast cells contributes to in vivo allergic inflammation. This issue involves not only the influence of soluble factors on FcεRI signaling, but also that of direct mast cell-other cell interactions. Studies on interactions of mast cells with T cells, eosinophils, and other immune cells have already begun to provide novel insights into the latter area (Mekori and Hershko, 2012; Gangwar and Levi-Schaffer, 2014). This type of studies will deepen our understanding of the cellular and molecular mechanisms of allergic diseases. Third, one need to pay more attention to the effect of the developmental stages of mast cells and different types of mast cells on FcεRI signaling. In this regard, Mcpt5-Cre mice, which allows to generate conditional null alleles in connective tissue-type mast cells, are very useful (Scholten et al., 2008; Reber et al., 2013; Oh et al., 2014). As described above, all these aspects of new knowledge will be subsequently followed by pharmaceutical development of anti-allergic drugs.

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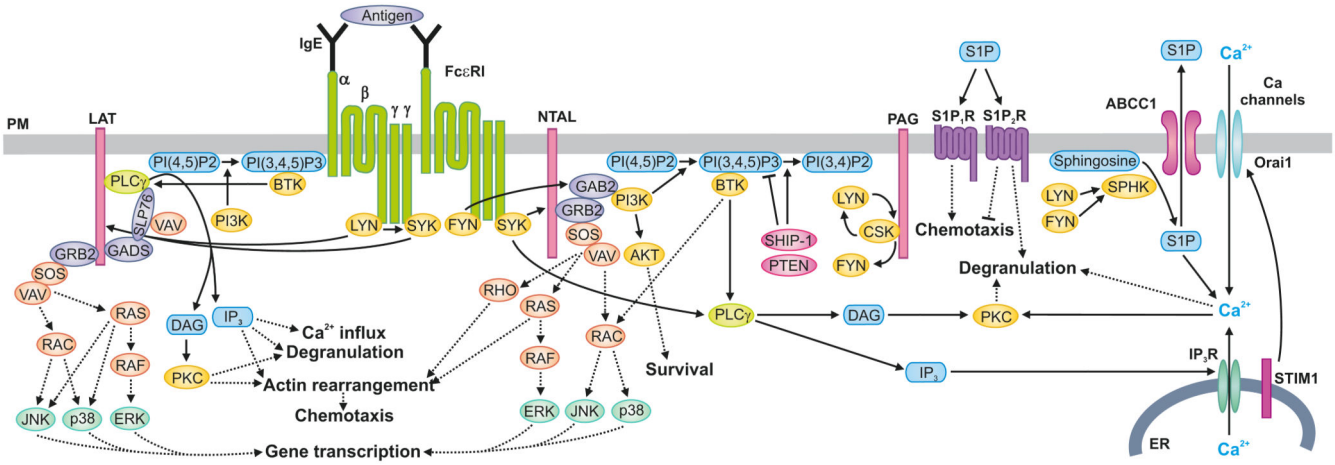

Fig. 1.

FcεRI-mediated signaling events. The first biochemically defined step in antigen-mediated aggregation of the IgE-FcεRI complexes is tyrosine phosphorylation of the FcεRI β and γ subunits by LYN kinase. This is followed by binding of SYK to FcεRI γ subunit leading to phosphorylation and activation of SYK. These kinases then phosphorylate downstream signaling targets. SYK phosphorylates transmembrane adaptor proteins NTAL and LAT and thus creates binding sites for various SH2-containing proteins like GRB2. In this way PI3K and GAB2 are brought to the plasma membrane (PM). PM-bound PI3K phosphorylates $PI(4,5)P_2$ and generates $PI(3,4,5)P_3$. Production of $PI(3,4,5)P_3$ can be negatively regulated by SHIP-1 and PTEN by conversion of $PI(3,4,5)P_3$ to $PI(3,4)P_2$ and $PI(4,5)P_2$, respectively. Several PH domain-containing proteins, including BTK and PLCγ, are recruited to the membrane-bound $PI(3,4,5)P_3$. PLCγ hydrolyzes $PI(4,5)P_2$ to generate the second messengers, diacylglycerol (DAG) and IP_3. IP_3 binds to the ER-bound IP_3 receptor (IP_3R) and triggers the release of Ca^{2+} from the ER. Depletion of Ca^{2+} from the ER leads to interaction of STIM1 with the Orai1 PM-associated protein, opening the PM-bound calcium channels and influx of extracellular Ca^{2+} into the cytoplasm. LYN and FYN kinases also activate SPHKs which induce conversion of sphingosine into S1P, which is secreted from the cell through ABCC1 (a member of the ATP-binding cassette transporter family). The extracellular S1P binds to $S1P_1R$ and $S1P_2R$, which are involved in cell migration and degranulation. Some other signaling proteins (SLP-76, VAV, GADS, SOS, RAC, RAS, RAF, JNK, p38, ERK, PKC, RHO, AKT, PAG, and CSK), which are involved in Ca^{2+} influx, degranulation, actin rearrangement, chemotaxis, and/or gene transcription, are also indicated.

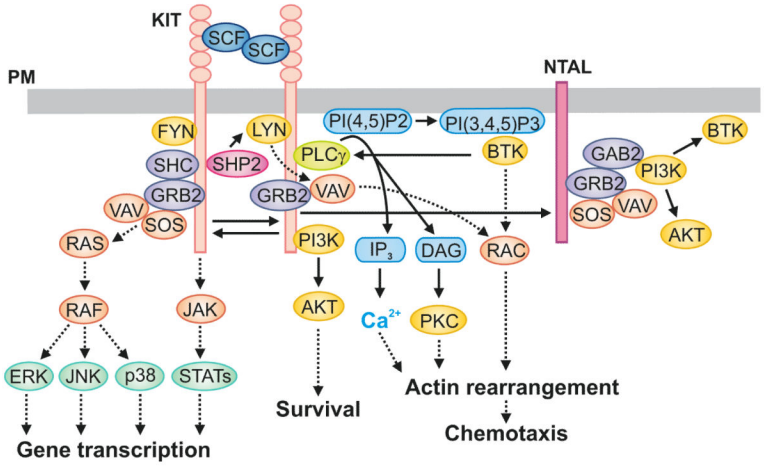

Fig. 2.

KIT-mediated signaling events. Dimerization of the plasma membrane (PM)-anchored KIT by SCF leads to the receptor auto-transphosphorylation. This is followed by recruitment of SFKs LYN and FYN, SHP2, PI3K, GRB2, SHC and other SH2 domain-containing proteins to phosphorylated KIT. Activated PI3K generates PI(3,4,5)P_3 through which PH domain-containing proteins such as BTK are recruited to the PM and further propagate the signal. An increased activity of PLCγ leads to production of DAG and IP_3, responsible for enhanced levels of free cytoplasmic Ca^{2+}. This is followed by actin rearrangements and chemotactic response. KIT also phosphorylates NTAL that can bind other adaptor proteins, including GRB2. Activated GRB2 orchestrates activation of RAS-RAF pathway by recruiting GEFs, SOS and VAV, which subsequently leads to activation of MAP kinases ERK, JNK and p38. Some other signaling proteins (JAK, STATs, AKT, PKC, and RAC), which are involved in gene transcription, survival, actin rearrangement, and/or chemotaxis, are also indicated.

Table 1

The effect of gene knockout of key signaling molecules on SCF- or antigen-mediated chemotaxis, degranulation, Ca^{2+} response, PCA, or PSA

Knockout	Chemotaxis		Degranulation	Ca^{2+} release	PCA/PSA
	SCF	Antigen	Antigen	Antigen	
Protein kinases					
LYN	↓(O'Laughlin-Bunner et al., 2001)	↓(Kitaura et al., 2005)	↑ (Parravicini et al., 2002; Hernandez-Hansen et al., 2004) No (Nishizumi and Yamamoto, 1997; Kawakami et al., 2000)	↓ (Parravicini et al., 2002); Nishizumi and Yamamoto, 1997)	↑↓ * (Odom et al., 2004) ↓ (Hibbs et al., 1995)
FYN	↓ (Samayawardhena et al., 2006; Olivera et al., 2006)	↓ (Kitaura et al., 2005; Olivera et al., 2006)	↓ (Parravicini et al., 2002)	No (Parravicini et al., 2002) ↓ (Sanchez-Miranda et al., 2010)	↓ (Odom et al., 2004)
LYN/FYN			↓ (Odom et al., 2004)		↓ (Odom et al., 2004)
LYN/BTK			↓ (Kawakami et al., 2000; Iwaki et al., 2005)	↓ (Kawakami et al., 2000; Iwaki et al., 2005)	
HCK		No (Kitaura et al., 2005)	↓ (Hong et al., 2007)	No (Hong et al., 2007)	
SYK	No (Wex et al., 2011)	↓ (Kitaura et al., 2005) No (Wex et al., 2011)	↓ (Costello et al., 1996)	↓ (Costello et al., 1996)	↓ (Wex et al., 2011)
BTK	No (Kuehn et al., 2010)	No (Kitaura et al., 2005) ↓ (Kuehn et al., 2010)	↓ (Iwaki et al., 2005; Setoguchi et al., 1998; Hata et al., 1998) No (Kawakami et al., 2000)	↓ (Setoguchi et al., 1998; Kawakami et al., 2000; Iwaki et al., 2005)	↓ (Hata et al., 1998)
ITK			↓ (Forssell et al., 2005) ↑ (Iyer et al., 2011)	No (Iyer et al., 2011)	↓ (Forssell et al., 2005)
BTK/ITK			↓ (Iyer et al., 2011)	↓ (Iyer et al., 2011)	↓ (Iyer et al., 2011)
SPHK1		↓(Dillahunt et al., 2013)	No (Olivera et al., 2007)	No (Olivera et al., 2007)	No (Dillahunt et al., 2013)
SPHK2		↓(Dillahunt et al., 2013)	↓ (Olivera et al., 2007)	↓ (Olivera et al., 2007)	↓(Dillahunt et al., 2013)
SPHK1/2			↓ (Olivera et al., 2007)	↓ (Olivera et al., 2007)	
Protein phosphatases					
SHP-1			↓ (Nakata et al., 2008) ↑ (Zhang et al., 2010)	↓ (Nakata et al., 2008)	↑ (Zhou et al., 2013)
SHP-2	↓ (Sharma et al., 2014)		No (McPherson et al., 2009)		↓ (Sharma et al., 2012)
PTPα	↓ (Samayawardhena and Pallen, 2008)		↑ (Samayawardhena and Pallen, 2010)		↑ (Samayawardhena and Pallen, 2010)
Lipid kinases					
$p110\delta^{D910A}$	↓ (Ali et al., 2004)	No (Collmann et al., 2013)	↓ (Ali et al., 2004) No (Collmann et al., 2013)		↓ (Ali et al., 2004) No (Collmann et al., 2013)
p110γ	No (Collmann et al., 2013)	↓ (Kitaura et al., 2005;	↓ (Laffargue et al., 2002; Ali et al., 2008;		↓ (Laffargue et al., 2002; Collmann et al., 2013)

Knockout	Chemotaxis		Degranulation	Ca^{2+} release	PCA/PSA
	SCF	Antigen	Antigen	Antigen	
		Collmann et al., 2013)	Collmann et al., 2013)		No (Ali et al., 2008)
p85α	↓ (Tan et al., 2003)		No (Tkaczyk et al., 2003; Fukuoka et al., 1992)	No (Tkaczyk et al., 2003)	No (Fukuoka et al., 1992)
p85β			No (Tkaczyk et al., 2003)	No (Tkaczyk et al., 2003)	
Lipid phosphatases					
SHIP1			↑ (Huber et al., 1998)	↑ (Huber et al., 1998)	↑ (Haddon et al., 2009)
PTEN	No (Furumoto et al., 2011)	No (Furumoto et al., 2011)	↑ (Furumoto et al., 2011)		↑ (Furumoto et al., 2011)
Adaptors					
LAT		No (Halova et al., 2013)	↓ (Saitoh et al., 2000)	↓(Saitoh et al., 2000)	↓ (Saitoh et al., 2000)
NTAL	No (Tumova et al., 2010)	↑(Tumova et al., 2010)	↑(Volná et al., 2004; Zhu et al., 2004)	↑ (Volná et al., 2004; Zhu et al., 2004)	↑ (Volná et al., 2004) No (Zhu et al., 2004)
LAT/NTAL		↑ (Halova et al., 2013)	↓ (Volná et al., 2004; Zhu et al., 2004)	↓ (Volná et al., 2004; Zhu et al., 2004)	
PAG	↓ (Draberova et al., 2014)	↓ (Draberova et al., 2014)	↓ (Draberova et al., 2014)	↓ (Draberova et al., 2014)	↓ (Draberova et al., 2014)
GAB2			↓ (Gu et al., 2001)	↓ (Gu et al., 2001)	↓ (Gu et al., 2001)
SLP-76			↓ (Pivniouk et al., 1999)	↓ (Pivniouk et al., 1999)	↓ (Pivniouk et al., 1999)
GADS			↓ (Yamasaki and Saito, 2008)	↓ (Yamasaki and Saito, 2008)	↓ (Yamasaki and Saito, 2008)
Others					
STIM1			↓ (Baba et al., 2008)	↓ (Baba et al., 2008)	↓ [++](Baba et al., 2008)
VAV1			↓ (Manetz et al., 2001)	↓ (Manetz et al., 2001)	↓ (Manetz et al., 2001)
DOK1			No (Ott et al., 2002)	No (Ott et al., 2002)	No (Ott et al., 2002)

↓, decreased response; ↑, increased response; No, no change in response

[*] PSA was increased in younger mice (4 weeks old) and decreased in older mice (more than 7 weeks old).

[++] STIM1 knockout mice die in utero and therefore STIM1 heterozygotic mice were used.