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The multiple roles of phosphoinositide 3-kinase in mast cell biology

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Abstract

Mast cells play a central role in the initiation of inflammatory responses associated with asthma and other allergic disorders. Receptor-mediated mast cell growth, differentiation, homing to their target tissues, survival, and activation, are all controlled, to varying degrees, by phosphoinositide-3-kinase (PI3K)-driven pathways. It is not fully understood how such diverse responses can be differentially regulated by PI3K. However, recent studies have provided greater insight into the mechanisms which control, and those which are controlled by, different PI3K subunit isoforms in mast cells. In this review, we discuss how PI3K influences the mast cell processes described above. Furthermore, we describe how different mast cell receptors utilize alternative isoforms of PI3K for these functions, and discuss potential downstream targets of these isoforms.

Keywords

Mast cells; PI3K; FcεRI; Kit; p110δ; p110γ

Regulation of mast cell processes by Kit and FcεRI

Mast cells are important effector cells which contribute to the body's ability to respond defensively to invading pathogens and parasites [1,2]. However, activation of mast cells can have disabling consequences by initiating allergic inflammatory reactions in response to antigen which are associated with atopic asthma, allergic rhinitis, eczema, and anaphylaxis [3]. In addition, mast cells have also been implicated in rheumatoid arthritis, multiple sclerosis, atherosclerosis, inflammatory bowel disease, and angiogenesis associated with tumor progression [4]. Such responses are the result of the generation and/or release of various inflammatory mediators including histamine, leukotriene C₄, prostaglandin D₂, cytokines, chemokines, and proteases, by mast cells following their activation [5,6]. Multiple processes are required for the mature tissue-localized mast cells to respond in this manner. These include growth, differentiation and maturation of the mast cells from their bone marrow-derived progenitor cells, homing to their target tissues, continued survival of the mature tissue-resident mast cells, and eventually, mast cell activation [1–5,7]. Of the various receptors expressed on mast cells, two key ones are the growth factor receptor, Kit (CD117), and the high affinity receptor for IgE, FcεRI (Box 1). Whereas Kit plays a major role in growth, development, and homeostasis of mast cells [7], the FcεRI is principally responsible for initiating the signaling events leading to inflammatory mediator release [8]. In this review we shall thus primarily

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Conflict of interest statement

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focus on the role of PI3K in mediating responses initiated by these two receptors. However, since specific G protein-coupled receptors (GPCRs) can either induce or modify FcεRI-dependent mast cell activation, the role of PI3K in specific GPCR-mediated mast cell responses will also be discussed.

The intricacies of the downstream signaling cascades initiated by Kit and FcεRI have been discussed in several recent review articles (for Kit signaling: [9]; and for FcεRI signaling: [6, 8,10]) and the readers are referred to these articles for further details. What is clear is that the majority, if not all, of the processes attributable to Kit, FcεRI, and other activating receptors, in mast cells are largely dependent upon PI3K activation. Thus, PI3K can be considered a central regulator of critical downstream signaling processes for receptor-mediated mast cell responses. How these multiple responses can be differentially regulated following PI3K activation has not been adequately delineated. However, recent studies are beginning to shed light on this topic. In this review, we thus discuss the signaling processes which regulate the activation of PI3K by FcεRI, Kit and other receptors expressed on mast cells and how the activation of PI3K regulates receptor-mediated mast cell function.

Expression and regulation of PI3K in mast cells

PI3Ks comprise a family of lipid kinases (Box 2) which play crucial roles in multiple biological responses [11–13]. Class 1 PI3Ks, the major haematopoietic forms of PI3K (Figure 1, Box 2), are heterodimeric comprising a regulatory and catalytic subunit. Mast cells express the class 1A p85α, p85β, and p50α regulatory subunit isoforms [14,15] in addition to all three class 1A PI3K catalytic subunit isoforms, p110α, p110β, and p110δ and the class 1B p110γ catalytic subunit [16,17] (Figure 1). As Kit and the FcεRI initiate their signaling processes via activation of tyrosine kinases, either intrinsically or by recruitment of cytosolic kinases [6,9], they utilize class 1A PI3Ks to mediate subsequent downstream signaling events, whereas GPCRs, such as those for adenosine, PGE₂, S1P, and C3a, mediate their responses via class 1B PI3K (Figure 2) [18].

The regulatory p85 subunits of Class 1A PI3Ks can directly bind to activated and phosphorylated (p) Kit (via -pY⁷²¹MDM-, in human Kit), however association with aggregated FcεRI requires indirect interaction via the cytosolic adaptor molecule, Gab2, following its phosphorylation by either Fyn [19] or Syk [20] (Figure 2A). It is also possible that the p85α subunit might directly bind to Syk following phosphorylation by Lyn, as suggested by studies conducted in the THP-1 monocytic cell line [21]. Such interactions are critical for propagation of signals initiated by Kit and the FcεRI. In this respect, disruption of the binding of PI3K to Kit abrogates Kit-dependent mast cell responses [22–25]. Similarly, the ability of dexamethasone to disrupt the FcεRI-induced interaction of PI3K with Gab2 in the rat RBL-2H3 mast cell line results in inhibition of PI3K and downstream signaling events [26]. The p110 catalytic subunit is maintained under constitutive inhibition by virtue of an inhibitory motif contained within the C-terminus of the p85 subunit [27,28]. Phosphorylation of a critical tyrosine residue (Y⁶⁸⁸) on the p85 subunit, by Src kinases alleviates this inhibition [27,28] which, together with the binding of the small GTP-binding protein Ras to the p110 subunit, results in enhancement of the catalytic activity associated with the p110 subunit [29]. In mast cells, Fyn is the major Src kinase involved in FcεRI-mediated PI3K activation [19]. The Ras guanyl nucleotide-releasing protein (RasGRP1) might also play an important role in FcεRI-mediated PI3K activation in mast cells and, it has been proposed that RasGRP1 might provide a link between the Lyn-Syk-LAT pathway and the Fyn-Gab2-PI3K pathway through binding to diacylglycerol generated by LAT-dependent phospholipase (PL)Cγ activation [30]. Thus, in both human [14,31] and rodent mast cells [16,32–35] triggered via Kit, FcεRI, or GPCRs, PI3K is rapidly activated resulting in the PI3K-dependent recruitment

of PH domain-containing signaling molecules, such as PLC γ_1 and γ_2 , AKT, PDK1, and BTK, to the receptor-signaling complex.

As will be discussed later, PI3K-dependent pathways in mast cells can however, also be negatively regulated by the inositol phosphatases PTEN (inositol phosphatase and tensin homologue deleted on chromosome 10), and SHIP (SH2-domain-containing inositol phosphatase) (Box 2, Figure 2B), the latter of which is recruited to the Fc ϵ RI in a Lyn-dependent manner (Box 1). The PI3K-signaling pathways in mast cells initiated upon Fc ϵ RI ligation therefore, represent a balance between Fyn-mediated positive regulation of PI3K and Lyn-dependent negative regulation, following activation of SHIP [19,36–38].

Functional responses of mast cells dependent on PI3K

Mast cell degranulation and cytokine production

A role for PI3K in mast cell activation has been revealed by a number of approaches as outlined in Box 3. The PI3K inhibitors, wortmannin and LY294002, have been widely reported to inhibit antigen-mediated degranulation and cytokine production in both rodent and human mast cells [14,32,39]. However, at least in human mast cells, these compounds fail to completely inhibit degranulation suggesting that, although PI3K is essential for optimal degranulation of mast cells, PI3K-independent pathways might also regulate this response. Studies utilizing mouse bone marrow-derived mast cells (BMMCs) expressing a kinase-inactive mutant isoform of the p110 δ catalytic subunit have revealed that p110 δ is the major isoform responsible for antigen-mediated degranulation and cytokine production in mast cells (Figure 1) [16,40]. This conclusion is further supported by the ability of the selective p110 δ inhibitor, IC87114, to inhibit antigen-mediated mast cell activation and by its ability to inhibit the enhancement of antigen-mediated degranulation by stem cell factor (SCF) [16]. By contrast, mast cells derived from the bone marrow of p85 α and p85 β knock out mice show normal antigen-mediated calcium flux and degranulation [14,15], suggesting that the p110 catalytic subunit can utilize alternative regulatory subunits for its interaction with phosphorylated Gab2.

Specific GPCR ligands including adenosine, PGE $_2$ and complement component C3A, can either enhance antigen-mediated mast cell activation (adenosine, PGE $_2$) or by themselves, directly induce mast cell degranulation and chemokine and cytokine production (C3A) [41]. A role for PI3K in GPCR-mediated responses in mast cells has been suggested by the ability of wortmannin to block C3a-induced formation of the chemokine, CCL2, in human mast cells [42], and by the reduced ability of adenosine to potentiate antigen-mediated degranulation in PI3K γ -deficient mast cells (Figure 1) [34,40]. Antigen-mediated degranulation is also attenuated in PI3K γ -deficient mast cells [34,40] and in wild type mast cells pretreated with the PI3K γ inhibitor, AS-252424 [40]. Similarly, the *in vivo* mast cell-dependent passive cutaneous anaphylaxis reaction has been reported to be virtually absent in mice deficient in the p110 γ PI3K catalytic subunit [34]. These data led to the conclusion that a component of antigen-mediated mast cell degranulation might be regulated by a positive feedback loop following the release of adenosine or other GPCR ligand from the mast cells. More recent studies, however, using both isoform-specific inhibitors and genetic approaches, have indicated that, although both p110 δ and p110 γ isoforms contribute to antigen-mediated degranulation *in vitro*, only the p110 δ isoform, is essential for the antigen-mediated mast cell-driven anaphylaxis response reactions *in vivo* [16,40]. The reasons for the differences between the *in vivo* data in the above studies are not clear. However, it is possible that differences in genetic backgrounds (129sv [34] vs C57BL/6 [40]) or differences in sensitization protocol (intravenous [34] vs intradermal [40]) could account for the discrepancies between the two studies [40]. The differences between the *in vitro* and *in vivo* responses might reflect a greater localized concentration of released adenosine or other GPCR agonist and/or reduced metabolism of the putative GPCR agonist *in*

vitro [41]. It is also possible that other regulatory factors or elements operating *in vivo*, but absent in cell cultures, may contribute to these differences.

Mast cell homing and homeostasis

In addition to its role in mast cell mediator release, PI3K is also critical for mast cell chemotaxis, adhesion and homeostasis. This has been evidenced by the ability of wortmannin and LY294002 to effectively inhibit SCF-mediated cell migration, adhesion to fibronectin coated plates, proliferation, and survival in human and mouse mast cell cultures [32]. In addition, reduced numbers of mast cells are observed in the peritoneal cavity, but not dorsal skin, of mice expressing a mutation in the PI3K binding site on Kit [25]. As with degranulation, mast cell chemotaxis, adhesion, and homeostasis appear to be mediated by specific PI3K isoforms. For example, in p110 δ inactive BMMCs, there is a dramatic defect in SCF-mediated mast cell adhesion and chemotaxis compared to the responses observed in wild type mast cells [16]. In addition, the ability of SCF to enhance mast cell growth is significantly reduced in cells expressing defective p110 δ [14]. These attenuated responses are similarly observed in wild type mouse mast cells incubated with the p110 δ -selective PI3K inhibitor, IC87114, but not in cells incubated with the p110 γ inhibitor, AS-252424 [16,40]. *In vivo*, there is a loss of peritoneal and gastrointestinal mast cells in p85 α -deficient mice [43] and reduced numbers of mast cells in the ear dermis, but not in the back skin, of p110 δ inactive mutant mice compared with wild type mice [16]. Furthermore, intratracheal administration of IC87114 to ovalbumin-sensitized mice, blocks ovalbumin-induced total leukocyte, eosinophil, neutrophil and lymphocyte infiltration, and the release of the TH2 cytokines, IL-4, IL-5, IL-13, and the chemokine, RANTES (CCL5) in the lungs in response to antigen challenge [44]. Taken together, the above data again provide evidence for a predominant role for p110 δ , compared to p110 γ , in antigen-mediated mast cell responses *in vivo* and furthermore suggest that GPCRs only play a minor role in mast cell homing and homeostasis *in vivo*.

In contrast to the regulation of antigen-mediated degranulation, Kit-mediated responses show selective regulation by specific p85 regulatory subunits. In this respect, Kit-dependent AKT phosphorylation and cell proliferation are markedly lower in p85 α ^{-/-} BMMCs compared to wild type cells [15]. Similarly, the aberrant growth of mast cell progenitor cells associated with a constitutively activated mutant form of Kit associated with the mast cell cancer, mastocytosis, also appears to be preferentially regulated by the p85 α isoform [45]. SCF-dependent chemotaxis also shows selective dependency on p85 α as demonstrated by the substantial reduction in the ability of SCF to cooperate with α 4 integrin to induce Rac activation and subsequent cell migration in p85 α ^{-/-} BMMCs [23].

Negative regulation of PI3K-dependent mast cell responses by PTEN and SHIP

PtdInsP₃ levels, and hence PI3K-regulated signaling pathways in mast cells and other cell types, are finely regulated by the balance between phosphorylation induced by PI3Ks and dephosphorylation regulated by the inositol phosphatases, PTEN and SHIP (Box 2 and Figure 2b). In quiescent human mast cells, shRNA-induced knock-down of PTEN results in increased basal PtdInsP₃ levels and constitutive phosphorylation of AKT and the MAP kinases, p38 and JNK resulting in enhanced cell survival and cytokine production [46]. Further augmentation of calcium mobilization, degranulation, and cytokine production is observed in the PTEN-knock-down human mast cells challenged with antigen [46]. These results suggest that PI3K-mediated responses are under tonic inhibition by PTEN.

Mast cells express both the SHIP-1 and SHIP-2 isoforms. Disruption of Lyn-dependent interaction of SHIP-1 with the Fc ϵ RI results in defective phosphorylation of SHIP-1, increased

PtdInsP₃ levels, and subsequently, inhibition of degranulation in antigen-challenged mast cells [37,47]. Mice deficient in SHIP-1 also have increased numbers of mast cells which exhibit elevated spontaneous degranulation [48]. It has been proposed that SHIP-1 suppresses degranulation, in part, by preventing the FcεRI-mediated cytoskeletal re-organization required for granule movement leading to exocytosis [49]. SHIP-2, also appears to negatively regulate FcεRI-mediated mast cell degranulation and cytokine production. However, in contrast to SHIP-1, FcεRI triggered SHIP-2-deficient BMMCs are not defective in FcεRI-mediated calcium mobilization or actin depolarization [50]. Depletion of SHIP-2 in BMMCs was shown to increase the activation of the GTPase Rac-1 as well as enhanced microtubule formation upon FcεRI activation [50]. Thus, SHIP-1 and SHIP-2 likely mediate their inhibitory responses by different mechanisms. It is possible, however, that PtdIns(3,4)P₂ generated by SHIP 1 and 2 may also contribute to the regulation of specific signaling events [51]. Whether this is true for mast cells is currently unclear.

SHIP-1 and -2 can associate with inhibitory ITIM (immunoreceptor tyrosine-based inhibitory motif)-containing receptors expressed on mast cells. FcγRIIb, MAFA (mast cell function-associated antigen), gp49B1, MAIR-I, PIR-A and -B, and CD200R have all been documented to be expressed on, and to down-regulate FcεRI-mediated responses in, mast cells [52,53]. Tyrosines within the ITIM sequences are phosphorylated by src kinases, thus allowing recruitment of SHIP-1 and -2, and the tyrosine phosphatases SHP-1 and -2, via their SH2 domains. SHIP-1 has been suggested to be the primary enzyme responsible for mediating MAFA's inhibitory responses [54] whereas, the ITIM of activated FcγRIIb recruits both SHIP-1 and -2. Fusion proteins which co-aggregate FcγRIIb and the FcεRI [55] have been demonstrated to block human antigen-induced mast cell mediator release and protect mice from IgE-dependent anaphylaxis [56] by a mechanism that partly involves SHIP reversal of PI3K-dependent signaling.

Downstream effectors regulated by PI3K

The above discussions demonstrate that PI3K regulates diverse responses in activated mast cells. From studies conducted in multiple cell types, it is evident that PI3K can influence a wide range of signaling molecules and pathways [57]. Systematic studies, utilizing a variety of approaches (Box 3), are now beginning to address which of these PI3K-regulated signaling processes regulate specific mast cell responses. In the following section and in Figure 3, we describe what is currently known about these downstream targets of PI3K activation in mast cells.

The role of PI3K in elevation of intracellular calcium concentrations

The ability of PI3K to control antigen-mediated mast cell degranulation, and in part, cytokine production, is likely linked to its capacity to regulate the calcium signal essential for these responses [46,58,59,60] (Figure 3). The initial calcium signal induced by FcεRI aggregation follows PLCγ-mediated PtdIns₂ hydrolysis and subsequent binding of the generated IP₃ to receptors on the endoplasmic reticulum resulting in liberation of calcium from these intracellular stores [61]. The calcium signal is maintained by subsequent influx of external calcium by store operated calcium entry (SOCE) as a consequence of intracellular storage depletion. In human mast cells, early FcεRI-mediated PLCγ₁ activation and resulting IP₃ production and calcium mobilization from intracellular stores appear to be PI3K independent [14]. In contrast, the subsequent SOCE-dependent maintenance phase of the calcium signal is, at least, partially PI3K dependent [14].

In mouse mast cells deficient in the Tec kinase, Btk, a similar partial reduction in antigen-mediated PLCγ₁ activation, calcium mobilization, degranulation, and cytokine production, to that produced by PI3K inhibitors is observed [62,63]. From these studies, and by extension of

studies conducted in B cells [64], it has been concluded that Btk is an essential intermediate in the ability of PI3K to regulate antigen-mediated calcium mobilization leading to mast cell degranulation [65]. Although the initiation of antigen-mediated signaling processes is PI3K-independent, it has been proposed that the PI3K-Btk axis might constitute a delayed maintenance and amplification pathway regulating calcium mobilization for degranulation, through continued PLC γ_1 activation (Figure 3) [6]. The ability of Kit to enhance Fc ϵ RI-mediated degranulation appears to be linked to its capacity to interact with this maintenance/amplification pathway [6]. Indeed there is more of an absolute requirement for PI3K and Btk in this response than for antigen-mediated degranulation [16,62]. Btk might also contribute to antigen-mediated cytokine production via activation of PKC β_1 and the JNK pathway [66]. However, the enhancement of antigen-mediated cytokine production by SCF appears to be less dependent on Btk [62].

In addition to the regulation of degranulation via Btk, PI3K can also regulate degranulation at other stages of the secretory process. For example, it has been proposed that PtdInsP $_3$, produced by PI3K, directly stimulates calcium transportation across the mast cell plasma membrane [59]. PI3K might also control the calcium signal in mast cells by the activation of phospholipase D (PLD) [67] which, by activating sphingosine kinase1 [68] may enhance sphingosine 1 phosphate (S1P) production, a putative regulator of calcium mobilization [69]. Studies in fMLP (f-methionine-leucine-phenylalanine peptide) - challenged RBL-2H3 cells [70] have also suggested that the phosphorylation of the Snare complex proteins Snap-23, Syntaxins 2 and 4, which are involved in the fusion of the granules with the plasma membrane and subsequent release of their contents, is dependent on PI3K.

PDK, AKT and FOXO

PI3K activation results in recruitment of the serine/threonine kinase PDK1, (3-phosphoinositide-dependent kinase 1) to the plasma membrane where PDK1 subsequently phosphorylates and activates AKT [71] (Figure 3). Thus, in mast cells, Fc ϵ RI aggregation, activation of Kit, and GPCR ligation, induce activation of PDK and AKT [35,72]. Although AKT, in other cell types, has been demonstrated to control multiple downstream targets [73, 74] the targets for AKT in mast cells are relatively unknown. Inhibitor and transfection studies have provided evidence that the PDK1-AKT interaction might contribute to the PI3K-dependent signaling events that regulate mast cell growth, homeostasis, and cytokine production. For example, Leflunomide, a drug which inhibits PDK1 activation and resulting AKT phosphorylation [31], was reported to induce apoptosis in SCF-maintained human mast cells [31]. Phosphorylation of glycogen synthase kinase 3 β (GSK3 β), a downstream target of AKT, was also reduced in the cells treated with leflunomide. Studies in mouse BMMCs have suggested that AKT activation leads to cytokine production by regulating transcription from the NF κ B, NFAT and AP-1 binding regions in the IL-2 and TNF- α promoter sites [75]. These studies also indicated that GSK3 β might be involved in the regulation of NFAT and AP-1 activity leading to mast cell cytokine production.

The forkhead box class O (FOXO) transcription factors family members, FOXO1a and FOXO3a, are phosphorylated in an AKT-dependent manner in SCF-stimulated BMMCs [76]. FOXO3a phosphorylation leads to its proteosomal degradation following ubiquitination thereby blocking its transcriptional control of the pro-apoptotic factor, Bim. SCF also induces the AKT- and MAPK-dependent phosphorylation of Bim in mouse bone marrow-derived mast cells and it has been proposed that this also leads to its proteosomal degradation [76]. Thus these observations would account for the ability of PI3K-AKT driven pathways to promote mast cell survival.

mTOR

mTOR is a conserved serine/threonine kinase which exists in two distinct multimolecular complexes, mTOR complex 1 (mTORC1) and mTORC2 [77] (Figure 3). PI3K regulates the mTORC1 pathway via the activation of AKT which directly phosphorylates the negative regulators of mTOR activation, TSC1 and TSC2 (tuberin), thereby inactivating its inhibitory activity. This allows mTOR activation resulting in the phosphorylation of p70 ribosomal S6 kinase (p70S6K) and eukaryotic initiation factor 4E-binding protein1 (4E-BP1) [78,79]. These events lead to mTOR-dependent gene transcription that regulates cell growth, protein synthesis, and metabolism in response to a variety of environmental stimuli [80].

A marked PI3K-dependent activation of the mTORC1 pathway is observed in human or mouse mast cells stimulated via FcεRI or Kit [32]. Rapamycin, a specific inhibitor of mTORC1, selectively and completely blocks the FcεRI- and Kit-induced mTORC1-dependent p70S6K phosphorylation and partially blocks the 4E-BP1 phosphorylation in both mouse and human mast cells [32]. This is associated with a significant inhibition of antigen- and SCF-mediated cytokine production, and SCF-mediated mast cell chemotaxis, growth, and survival, suggesting that these responses are at least in part regulated by the mTORC1 complex. However, in the case of cytokine production and chemotaxis, inhibition by rapamycin was not as marked as that produced by wortmannin, suggesting that PI3K might also regulate these responses independently of mTORC1. Interestingly, there is a marked enhancement in the activation of components of the mTORC1 complex in the LAD2 and HMC-1 mast cell tumor lines, even under resting conditions [32]. Rapamycin blocks this constitutive activation of the mTORC1 pathway and inhibits the survival of these cells. These data imply that the PI3K-mTORC1 axis contributes to the abnormal cell growth in human tumor mast cells.

Summary, conclusions, and future considerations

In summary, it is now clear that PI3K-regulated signaling events play a central role in mast cell biology. The downstream targets responsible for the receptor-mediated, PI3K-dependent responses in mast cells have still not been fully elucidated. However, PI3K-regulated degranulation, and to a certain extent cytokine production, appears to be linked to the regulation of a latent calcium signal, likely requiring activation of Btk. Multiple PI3K-regulated process appear to contribute to mast cell growth and survival including those requiring activation of PDK, AKT and the mTORC1 cascade, and inactivation of FOXO. It is less clear how PI3K can regulate mast cell chemotaxis and adhesion, but data suggest that mTORC1 might also play a partial role in the regulation of SCF-mediated mast cell chemotaxis.

Due to their central roles in the generation and activation of mast cells, PI3K-regulated pathways are attractive targets for the treatment of mast cell-related disorders, for example anaphylaxis, asthma and mastocytosis. The selective expression of the p110δ and p110γ catalytic subunit isoforms in cells of haematopoietic lineage, and the consensus of studies supporting a role for especially the p110δ isoform in both Kit- and FcεRI-mediated mast cell responses *in vitro* and *in vivo*, indicates the potential utility of isoform-selective PI3K inhibitors in mast cell-driven disease. Alternatively, downstream targets of PI3K might also lend themselves to pharmacological intervention. However, a major challenge would be the selective targeting of these molecules in mast cells. Although progress has been made in the identification of these downstream targets, much is still unknown regarding how these, and potentially other molecules, are differentially regulated following activation by individual surface receptors on mast cells. Further research is thus required to fully identify these targets and to precisely delineate their role in specific mast cell responses.

Box 1. Kit and FcεRI on mast cells

Kit is a single chain receptor with intrinsic tyrosine kinase activity [9,81]. The extracellular domain possesses 5 immunoglobulin-like regions which contain the binding site for its ligand, stem cell factor (SCF; alternatively termed steel factor or Kit ligand). Within the cytosolic tail, there is a split tyrosine kinase catalytic domain and multiple tyrosine residues which serve as auto-phosphorylation sites following Kit activation. These phosphorylated sites subsequently recruit specific signaling molecules which are crucial for Kit-mediated responses [9,81]. In contrast to Kit, the FcεRI is a multi-chain receptor complex consisting of an α chain, a β chain and a γ chain homodimer [8]. The FcεRIα chain possesses two extracellular immunoglobulin-like regions which bind IgE with high affinity. Both the β and γ subunits, possess immunoglobulin receptor-based tyrosine activation motifs (ITAMs) which are phosphorylated by the Src kinase, Lyn, upon antigen-dependent aggregation of IgE-occupied FcεRI [8]. The phosphorylated γ chains serve as the major initiators of signaling mediated by the FcεRI following recruitment of the tyrosine kinase Syk, whereas, the β chain might serve to modify these responses [8]. It should be noted that, an addition to its role in initiation of FcεRI-mediated signaling events, Lyn also appears to down-regulate FcεRI-mediated mast cell activation as a consequence of the recruitment of the inositol phosphatase, SHIP to the phosphorylated FcεRI β chain.

Box 2. The composition and activation of PI3Ks

The PI3K family has been grouped into three major classes (class I, class II, and class III) depending on their molecular structure, modes of activation, and substrate specificity [82]. Class I PI3Ks are the best characterized and studied with regards to the regulation of immunological processes [83–85]. To date, there is little evidence for a role for Class II and Class III PI3Ks in mast cell biology and indeed other cell types of haematopoietic lineage.

Class I PI3Ks are heterodimers, subdivided into two groups, class IA and class IB. The class IA PI3Ks consist of a regulatory subunit (p85s: p85α, p55α, p50α, p85β, and p55γ) and a catalytic subunit (p110α, p110β, and p110δ) [86]. The class IB PI3K p110γ catalytic subunit interacts with the p101 or the p87^{PIKAP} regulatory subunits [87]. Whereas the catalytic p110α and p110β subunits are ubiquitously expressed in many cell types, tissues, and organs, the p110δ and p110γ subunits are preferentially found at high levels in lymphocytes [88]. All class IA regulatory subunits have a p110-binding region as well as two Src-homology (SH)2 domains that bind a specific recognition sequence (-YXXM-) found in various receptors and adaptor molecules, following phosphorylation of the tyrosine contained within this sequence. In contrast to class IA PI3Ks, the class IB PI3Kγ is activated by heterotrimeric G-protein-coupled receptors (GPCR) and regulated by the free βγ subunits of G proteins following GPCR activation. PI3Ks phosphorylate the inositol ring of membrane-associated phosphoinositides at the D3 hydroxyl position [89], thereby converting phosphatidylinositol-(4,5)-bisphosphate (PtdInsP₂) to phosphatidylinositol-(3,4,5)-triphosphate (PtdInsP₃) (Figure 2A). PtdInsP₃ provides inducible docking sites for pleckstrin homology (PH) domains of associating signaling molecules allowing recruitment of these molecules to the receptor-signaling complex [89]. Responses initiated by PI3K are terminated following PTEN and SHIP-mediated de-phosphorylation of PtdInsP₃. PTEN removes the phosphate group from the D3 position of the inositol ring whereas SHIP removes the phosphate in the D5 position [90], thereby converting PtdInsP₃ to PtdIns(4,5)P₂ and PtdIns(3,4)P₂, respectively (Figure 2B).

Box 3. Approaches for the study of the role of PI3K in mast cell functions

A number of approaches have been utilized to explore the role of PI3K and its isoforms on mast cell functions. The most widely used approach has been through the use of the naturally occurring PI3K inhibitor wortmannin, and the synthetic inhibitor, LY294002. However, these inhibitors display non-selective inhibition of other kinases, for example, several PI3K-related kinases and PI4K [91,92] at similar or higher concentrations. Furthermore, they cannot discriminate between the different isoforms of PI3Ks. Recently, isoform-specific PI3K inhibitors have been employed to define the role of specific PI3K isoforms in mast cells; namely IC87114 which is a selective inhibitor of the p110 δ isoform and AS-252424 which is a selective inhibitor of p110 γ isoform [16,40,93]. Genetic approaches to examine the roles of specific isoforms in mast cell function have been somewhat hampered by the overlapping or redundant functions of specific subunit isoforms and the embryonic lethality associated with disruption of either p110 α or p110 β , or p85 α /p85 β double knock out mice [86]. However, mice lacking the p85 α or p85 β subunits individually, and mice lacking catalytic p110 γ , p110 δ subunits, or mice with a knock-in point mutation of p110 δ ^{D910A} have been generated, and utilized to examine the specific roles of these subunits in mast cell function. For further discussions on the PI3K gene-targeted murine models readers are referred to [86].

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Figure 1.

Structure and function of PI3K subunit isoforms expressed in mast cells. The emphasized catalytic subunit isoforms are those that have been documented to play specific roles in mast cell responses as outlined in the text column. Whereas there is little selectivity in utilization of the p85 subunits for Fc ϵ RI-mediated responses, Kit responses show selective requirements for the p85 α subunit (emphasized). Abbreviations: BH: Bcr/Rac GAP homology domain; C2: protein kinase C homology domain 2; GPCR; G protein-coupled receptor; SH: Src homology domain. ¹As determined by passive cutaneous anaphylaxis reaction. ²Likely indirectly following release of adenosine or other GPCR ligand. ³GPCRs binding adenosine, MIP-1 α (CCL3), Rantes (CCL5), or platelet activating factor. ⁴As determined by passive systemic anaphylaxis reaction.

Figure 2.

Positive and negative regulation of PI3K-dependent signaling pathways in activated mast cells. (A) Activation of class 1A PI3Ks by Kit and FcεRI and class 1B PI3K by GPCRs at the inner cell membrane. The SH2 domains of the p85 regulatory subunits of class 1A PI3K can directly bind to phosphorylated Kit, whereas the association of p85s with the FcεRI is indirect via the cytosolic adaptor molecule, Gab2. The p101 regulatory subunit of class 1B PI3K directly binds to free G protein βγ homodimers that are dissociated from heterotrimeric G-proteins after GPCR activation. The catalytic subunits of PI3K, p110δ and p110γ, phosphorylate the inositol ring of phosphoinositides at the D3 hydroxyl position, thereby converting phosphatidylinositol-(4,5)-bisphosphate (PtdInsP₂) to phosphatidylinositol-(3,4,5)-triphosphate (PtdInsP₃) at the inner plasma membrane. This leads to the regulation of the downstream signaling events in activated mast cells depicted in Figure 3. (B) Activation of PTEN and SHIP by FcεRI and inhibitory receptors. PTEN removes the phosphate group from the D3 position of the inositol ring whereas SHIP removes the phosphate from the D5 position of the inositol ring, converting PtdInsP₃ to PtdIns(4,5)P₂ and PtdIns(3,4)P₂, respectively. Thus, PtdInsP₃ levels decrease, and the effect of PI3K activation is negatively regulated by PTEN and SHIP. SHIP interacts with FcεRI in a Lyn-dependent manner and with inhibitory receptors such as FcγRIIb and MAFA, through association with their ITIM motifs.

Figure 3.

Downstream targets of PI3K in activated mast cells. Activation of class 1 PI3Ks generates the membrane-associated PtdInsP₃ at the inner membrane, which provides inducible docking sites for pleckstrin homology (PH) domains of associating signaling molecules. The Ser/Thr kinases, PDK1 and AKT are subsequently phosphorylated and activated after Kit and FcεRI activation. Major downstream targets of PI3K and AKT in mast cells, mTORC1, Btk, FOXO3a, and GSK3β pathways are shown (also see text) together with the specific responses in activated mast cells. Although depicted in the figure, and documented in other systems, it is not known whether AKT activation is regulated by the mTORC2 complex in mast cells. In addition, the function of GSK3β in activated mast cells still requires clarification. Whether these events are also initiated by GPCRs is currently unknown. For clarity other signaling events required for FcεRI- and Kit-mediated responses in mast cells are not depicted in this figure. Readers are referred to recent review articles for further details of these processes [6,10].