

Chapter 17

Protein Tyrosine Phosphatases in Mast Cell Signaling

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Abstract

For a time, mast cells were viewed as simple granulocytic effector cells that mediate allergic symptoms. More recent discoveries show that mast cells can also function as potent pro- and anti-inflammatory immune regulators in a plethora of human diseases. Much of the current knowledge about mast cell functions comes from studies on rodent models. The membrane receptors for antigen/IgE and growth factors are the core initiators of signaling cascades that trigger various mast cell responses. Yet, the regulation and multifunctionality of key receptor-proximal protein tyrosine phosphorylation events are still not well understood. The roles of the members of the protein tyrosine phosphatase superfamily of enzymes in regulating mast cell development, survival, and immune activation will be reviewed in this chapter.

Key words Mast cells, PTPs, FcεRI, c-Kit, SFKs, Anaphylaxis, Degranulation, Phosphotyrosine

1 Tyrosine Phosphorylation in Mast Cell Signaling

Mature mast cells are characterized by the expression of the stem cell factor (SCF) receptor tyrosine kinase c-Kit and the high-affinity IgE receptor FcεRI. These transmembrane receptors initiate many of the crucial signaling pathways that determine the development, migration, and immune activation of mast cells. The earliest event in c-Kit and FcεRI-mediated signaling is the tyrosine phosphorylation of several sites on the receptors themselves. Indeed, regulated protein tyrosine phosphorylation and dephosphorylation is a key mechanism controlling signaling downstream of these receptors. This chapter focuses on known and emerging actions of specific protein tyrosine phosphatases (PTPs) in mast cell signaling to promote or limit cell movement and activation, outcomes that are key to physiological and pathological mast cell roles.

Detailed descriptions of FcεRI and c-Kit mast cell signaling events can be found in several comprehensive reviews [1–3]. Briefly, the binding of antigen to IgE/FcεRI complexes, or of SCF to c-Kit, induces the formation of phosphotyrosyl receptor or receptor-linked tyrosine kinase complexes. Aggregated FcεRI, phosphorylated on

ITAMs (immunoreceptor tyrosine-based activation motifs) in its β - and γ -chains by Lyn, is associated with the Src family tyrosine kinases (SFKs) Lyn and Fyn and with the non-SFK tyrosine kinase Syk. These kinases phosphorylate diverse scaffolding/adaptor proteins (such as the Syk and Lyn substrates LAT1 and LAT2) and enzymes to coordinate the formation of activated signaling complexes that promote Ca^{2+} influx and degranulation as well as gene transcription and secretion. An overlapping cast of molecular players is mobilized by SCF binding to c-Kit. In this case, receptor dimerization activates the intrinsic tyrosine kinase activity of c-Kit and its trans-autophosphorylation. Phosphotyrosyl-c-Kit serves as a platform to recruit signaling molecules, including the kinases Lyn and Fyn and other enzymes such as PI3K and PLC γ that are also involved in Fc ϵ RI signaling. SCF/c-Kit signaling regulates mast cell migration, proliferation, survival, differentiation, and maturation.

2 The Protein Tyrosine Phosphatase Superfamily

The human PTP superfamily contains 107 phosphatases that are grouped into four families based on evolutionary relationships and structure, and use of a cysteine- or aspartate-based catalytic mechanism [4–6]. The Class I family is by far the largest, comprising a subfamily of 38 classical tyrosine-specific PTPs (transmembrane and intracellular) and another large subfamily of 61 dual-specific (for phosphotyrosine and phosphoserine/threonine) PTPs. The sole member of the Class II family is the tyrosine-specific low molecular weight (LMW) PTP, while the Class III family contains the three dual-specific CDC25 enzymes. Class I, II, and III PTP catalysis is mediated by an invariant active site cysteine residue. In contrast, the 4 EyA (eyes absent) members of Class IV employ an aspartic acid-based mechanism of tyrosine dephosphorylation [7–9] and also catalyze threonine dephosphorylation through a distinct domain [10].

3 PTP Expression in Mast Cells

Much of the knowledge of the mast cell roles of PTPs, all of which belong to the Class I family (Fig. 1), has come from studies of PTP-deficient mice and bone marrow-derived mast cells (BMMCs) as described in more detail below (Table 1). Over sixty different PTPs are expressed in mast cells, all of which are also expressed in seven other immune cell lineages: T cells, NKT cells, NK cells, B cells, immature DCs, macrophages, and neutrophils [11]. Interestingly, up to eleven other PTPs show mast cell-restricted expression among these types of immune cells. These PTPs comprise a receptor-like (*Ptprg*) and three non-receptor tyrosine-specific

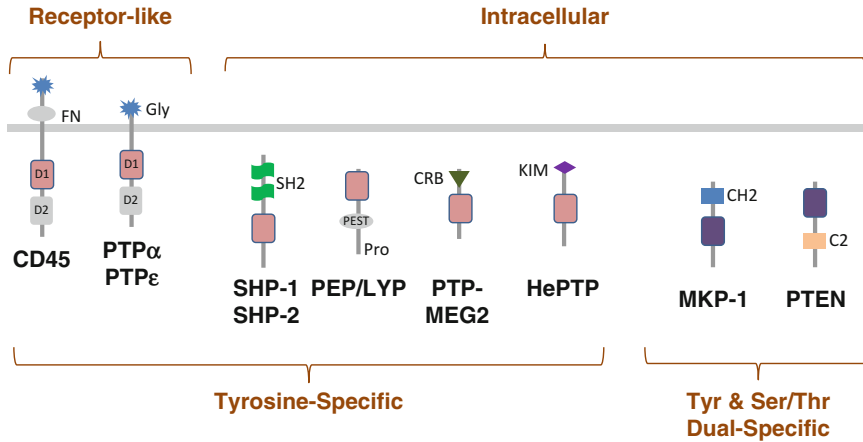


Fig. 1 Schematic representations of key protein tyrosine phosphatases affecting mast cell biology. Domain structures are abbreviated: *Gly* highly glycosylated; *D1* and *D2* membrane-proximal and membrane-distal receptor PTP domains; *FN* fibronectin-like; *SH2* Src homology domain 2; *PEST* rich in proline, glutamic acid, serine, and threonine; *Pro* proline-rich; *CRB* cellular retinaldehyde-binding protein-like; *KIM* kinase interaction motif; *CH2* Cdc25 homology domain 2; *C2* protein kinase C conserved region 2

Class I members (*Ptpn13*, *Ptpn20*, *Ptpn21*), six representatives of several types of dual-specific Class I enzymes (*Dusp3*, *Dusp9*, *Dusp23*, *Dusp26*, *Epm2a*, *Ssh3*), and a single Class IV PTP (*Eya4*) [11]. Their unique mast cell expression in this particular immune cell lineage panel suggests that at least some of these PTPs may function in specialized granulocytic processes in the mast cell context, although this remains to be determined.

4 Receptor-Like PTPs in Mast Cells

4.1 CD45

The leukocyte-specific receptor-like PTP CD45 (*Ptpnc*) is generally recognized as a positive regulator of Fc ϵ RI signaling and mast cell secretory responses [12–14]. Alternatively spliced exons encoding the extracellular domain allow the expression of several variants of CD45 in leukocytes [15], although murine BMMCs lack the larger B220, RA, RB, and RC isoforms [14]. The CD45 cytoplasmic region contains two tandem catalytic domains, and as is the case with most other receptor-like PTPs, the membrane-proximal D1 domain is responsible for catalytic activity while the membrane-distal D2 domain is inactive. Extensive investigation of the role of CD45 in T and B cells has identified members of the Src family of tyrosine kinases as key CD45 substrates, with CD45 regulating Lck in T cell receptor-mediated T cell activation and Lyn in B cell receptor-mediated B cell activation [16, 17]. CD45 activates these kinases by dephosphorylating an inhibitory tyrosine residue in the C-terminal tail region. CD45 can also repress SFK activity by dephosphorylating an activation-associated tyrosine residue in the kinase domain.

Table 1
PTP-deficient mast cell phenotypes

PTP deficiency	Signaling effects	Altered responses	Reference
CD45 ^{-/-} BMMCs	• Hyper-phosphorylation of Tyr-507 on Lyn • Enhanced IL-3-induced tyrosine phosphorylation of Jak2	• Decreased Ag-induced degranulation and release of IL-6 • Enhanced cell proliferation in response to cytokines IL-3 and SCF • Increased cell death upon withdrawal of cytokines • Enhanced IL-3-mediated cell proliferation	[14, 19]
PTP α ^{-/-} BMMCs and mice	• Decreased Ag-induced activation of Lyn and Fyn kinases • Reduced SCF-induced activation Fyn kinase	• Enhanced Ag-induced degranulation and release of IL-2, IL-6, TNFα, and leukotrienes • Enhanced Ag-induced anaphylaxis in mice • Altered tissue distribution of mast cells • Reduced spreading, polarization, and chemotaxis of SCF-treated BMMCs	[20, 28]
PTP ϵ ^{-/-} BMMCs and mice	• Enhanced Ag-induced activation of Syk	• Enhanced Ag-induced degranulation and release of IL-6 and TNFα • Enhanced Ag-induced anaphylaxis	[21]
SHP-1 ^{-/-} (<i>me</i>) BMMCs and mice	• Increased tyrosine phosphorylation of adaptor protein Shc after stimulation with SCF • Enhanced Ag-induced phosphorylation of LAT and SLP-76 • Reduced PLCγ1/2 activation, association with SLP-76	• Severe spontaneous lung inflammation and lethality of <i>me</i> mice • Increased mast cell numbers in c-Kit-deficient mice • Enhanced SCF-induced survival of BMMCs • Enhanced IgE-mediated release of IL-6 and TNFα • Reduced mast cell degranulation	[37, 43]
SHP-1 mutant (<i>mev</i>) mice and BMMCs	• Antigen stimulation induces SHP-1 to bind to NTAL, and without SHP-1 activity NTAL tyrosine phosphorylation is increased • Increased antigen-induced phosphorylation of Tyr-396 on Lyn and Tyr-1020 on SHIP	• <i>mev</i> mice have more mast cell progenitors in the bone marrow • <i>mev</i> BMMCs are more resistant to apoptosis, but proliferate more slowly than WT cells • <i>mev</i> BMMCs have enhanced spontaneous degranulation and in response to antigen • Enhanced release of cytokines with various stimuli • Reduced IgE-mediated release of IL-6 and TNFα cytokines	[35, 39]

SHP-1-deficient (<i>me</i>) and (<i>mer</i>) BMMCs	Increased IL-3-induced phosphorylation of Erk kinases	- Enhanced proliferation with low IL-3 doses and greater apoptosis with high IL-3 doses - Greater survival upon IL-3 cytokine starvation - Reduced proliferation of BMMCs with high IL-3 doses	[30]
BMMCs depleted of ~75 % of SHP-2	- Increased inhibitory phosphorylation of Tyr-531 on Fyn kinase upon Ag stimulation - Reduced association of SHP-2 with adaptor Gab2 and SCF-induced Rac/JNK activation	- Reduced secretion of TNFα, but not degranulation - Reduced SCF-induced proliferation of BMMCs	[50, 55]
PEP ^{-/-} BMMCs and mice	- Reduced Ag-induced phosphorylation of PLCγ1 and calcium mobilization - Increased activation of JNK MAP kinases	- Reduced IgE-mediated degranulation of mast cell and systemic anaphylaxis in mice - Reduced anti-anaphylactic effects of glucocorticoids	[56]
MKP-1 ^{-/-} BMMCs and mice	Enhanced Ag-induced activation of p38	Enhanced IgE-mediated degranulation and anaphylaxis in mice	[62]
PTEN ^{-/-} BMMCs and mice	Increased activating phosphorylation on STAT5 and Akt proteins	- Enhanced proliferation and survival of tissue mast cells and IgE-mediated anaphylaxis - Secretory hyperresponsiveness to antigen and SCF	[67]

CD45-deficient BMMCs exhibit almost no degranulation upon stimulation by Ag cross-linking, indicating an essential role for CD45 in this response [12, 14]. IL-6 secretion is similarly impaired [14] and CD45-deficient mice are resistant to IgE-mediated systemic anaphylaxis [12]. Consistent with SFKs being the major substrates of CD45 and with the interaction of CD45 and Lyn in BMMCs, the enhanced inhibitory phosphorylation of Lyn at Tyr-507 (and possibly of Hck at Tyr-519) is the most upstream defect detected in CD45^{-/-} BMMCs [14]. This is accompanied by a consequent reduction in tyrosine phosphorylation of the Lyn substrate Cbp/PAG and its reduced ability to recruit Csk. Other defects include reduced Akt, Erk1/2, p38, and SHIP-1 phosphorylation. Ag-induced calcium mobilization is also compromised. Parallel studies of BMMCs from mice expressing an activated mutant CD45 (E613R) that cannot form inhibitory dimers reveal a potential ability of CD45 to mediate activation of the Fyn-PI3K-Akt pathway [14].

The above findings provide evidence of essential positive roles for CD45 in mast cell activation. An additional complexity of CD45 action is indicated by findings in alternate experimental systems. For example, while CD45 inhibitors suppress Ag-induced degranulation by BMMCs and murine peritoneal mast cells and inhibit systemic anaphylaxis, treatment of BMMCs with a 30–200-fold lower concentration of inhibitor enhances activation responses [14, 18]. Rat basophilic leukemia (RBL) cell lines have been often used to model FcεRI-mediated mast cell events, and RBL-2H3 variant lines stably expressing a low level of CD45 exhibit reduced or delayed FcεRI-dependent responses at low doses of antigen but normal responses at high antigen dose, relative to cells stably expressing a high level of CD45 [13]. Taken in the contexts of the known ability of CD45 to repress and activate SFK activity in other immune cells, and of the positive and negative regulatory actions of the CD45 substrate Lyn in mast cell activation, it is probable that a spectrum of CD45-mediated Lyn-dependent and CD45-mediated Lyn-independent effects can be titrated by factors such as antigen dose and the population of responsive CD45.

CD45^{-/-} BMMCs develop normally *in vitro*, indicating that this PTP is not required for mast cell differentiation [12, 14, 19]. Proliferation of CD45-deficient BMMCs in response to IL-3 and/or SCF (murine SF) is increased [14, 19] or not affected [12], with differing observations perhaps due to differences in the amounts or nature (recombinant vs. conditioned medium) of the cytokine/growth factors used. Increased proliferation is accompanied by increased apoptosis upon IL-3 withdrawal [14]. The basis for the enhanced cytokine-stimulated proliferation of CD45^{-/-} BMMCs lies with the discovery of a non-SFK substrate of CD45. In IL-3 stimulated BMMCs, CD45 plays a negative regulatory role, dephosphorylating Jak2 to limit STAT3 and STAT5 tyrosine phosphorylation and repress cyclin D1 expression [19].

4.2 PTP α and PTP ϵ

PTP α (*Ptpra*) and PTP ϵ (*Ptpre*) are closely related receptor-like PTPs that feature short, glycosylated extracellular domains and tandem cytoplasmic catalytic domains. In response to antigen, BMMCs from mice lacking PTP α or PTP ϵ hyperdegranulate and have augmented cytokine production [20, 21]. The PTP α -null, but not the PTP ϵ -null, BMMC response also includes the elevated release of cysteinyl leukotrienes. Mice lacking either PTP α or PTP ϵ exhibit enhanced anaphylactic reactions. Thus, these two transmembrane PTPs negatively regulate IgE-/Fc ϵ RI-dependent mast cell activation.

PTP α characteristically functions as a Src family kinase (SFK) phosphatase in numerous cell types and tissues, dephosphorylating and activating various SFKs [22]. PTP α fulfills this role in antigen-stimulated BMMCs, activating Lyn and Fyn. PTP α also suppresses the activity of the SFK Hck, possibly through Lyn-mediated regulation of Hck [20]. The reduced Lyn activation in PTP α ^{-/-} BMMCs is likely responsible for reduced phosphorylation of Fc ϵ RI and the inhibitory inositol phosphatase SHIP, as both are substrates of Lyn. At low-intensity stimulation, Lyn positively regulates signaling events in BMMC activation [23]; however the hyperactive phenotype of Lyn^{-/-} BMMCs revealed the key role of this kinase in negatively regulating BMMC activation [23–26]. Overall, the role of PTP α in limiting mast cell activation and anaphylactic allergic reactions appears to be due to the action of PTP α in mediating this Lyn-dependent negative regulatory signaling [20]. PTP ϵ can also act as an activator of SFKs [27], but difficulty in detecting significant antigen-induced Lyn activation in wild-type BMMCs has precluded the determination of whether Lyn activation is affected in PTP ϵ -null BMMCs [21].

Antigen-induced activation of the tyrosine kinase Syk is enhanced in BMMCs lacking either PTP α or PTP ϵ , despite the reduced association of Syk with the hypophosphorylated Fc ϵ RI in PTP α ^{-/-} BMMCs [20, 21]. In the latter cells, Syk phosphorylation is increased at tyrosine residues associated with kinase activation and reduced at a site linked to kinase inhibition, reinforcing the notion that PTP α mediates negative regulatory signals to limit mast cell activation. Elevated phosphorylation of the Syk substrates Gab2, LAT, and SLP-76 correlates with Syk hyperactivation in the absence of PTP α or PTP ϵ , as do enhanced calcium mobilization and activation of downstream signaling molecules such as the MAPKs [20, 21]. These observations point to similar actions and regulatory roles of PTP α and PTP ϵ in signaling to control mast cell activation. Nevertheless, the defective IgE-/Fc ϵ RI-dependent phenotype of either the PTP α ^{-/-} or PTP ϵ ^{-/-} mice and BMMCs indicates that these PTPs are not, at least at physiological expression levels, redundant.

Altered numbers of resident mast cells were noted in certain tissues of PTP α -null mice but not in PTP ϵ -null mice [21, 28]. The PTP α -null phenotype could be due to defective signaling by

the SCF receptor c-Kit that regulates mast cell migration, since BMMCs lacking PTP α display reduced spreading, polarization, and chemotaxis towards SCF [28]. In SCF/c-Kit signaling, PTP α promotes the activation of receptor-proximal Fyn and c-Kit tyrosine phosphorylation. In the absence of PTP α , these upstream signaling events are impaired, with further defects in Gab2, SHP-2, and Vav1 phosphorylation and activation of Rac, Cdc42, PAK, and MAPKs [28].

5 SH2 Domain-Containing Intracellular PTPs in Mast Cells

5.1 SHP-1 and the Motheaten Phenotypes

The Src homology region 2 domain-containing phosphatase 1 (SHP-1, PTP1C) has been extensively studied as a crucial regulator of mast cell development, survival, and immune responses [29, 30]. SHP-1 is an intracellular, Class I tyrosine-specific PTP that is expressed mainly in hematopoietic cells [31] and contains two tandem SH2 domains that target it to specific phosphotyrosine protein sites [32]. Spontaneous loss-of-function mutation of its coding gene (*Ptpn6*) is responsible for the severe physiological aberrations in motheaten (*me*) mice, first described nearly four decades ago [33]. Young homozygous recessive *me* mice have stunted growth and patchy dermatitis and die by three weeks of age. Tissue analyses show spontaneous, vast accumulation of mast cells and other leukocytes in various organs, high levels of serum auto-antibodies with immune complex depositions, and a progressive development of hemorrhagic pneumonitis in the absence of any pathogens [34, 35].

A different mutation that causes the loss of phosphatase activity of SHP-1 protein produces a milder phenotype in motheaten viable (*mev*) mice and extends their life relative to SHP-1-null mice [36]. While the bone marrow of *mev* mice contains many more mast cell progenitors than in wild-type (WT) mice, SHP-1-deficient BMMCs actually proliferate more slowly when supplemented with the growth factor for mouse mast cells, IL-3 [35]. The increased numbers of mast cells in both motheaten phenotypes are believed to result from enhanced anti-apoptotic signaling in SHP-1-deficient BMMCs, prolonging their survival under various types of cell stress. Upon withdrawal of IL-3, SHP-1-null BMMCs are more resistant to apoptosis than WT cells, due to higher intrinsic activation of the Erk kinases and expression of the anti-apoptotic protein BCL-X_L [30]. Additionally, a high concentration of IL-3 induces apoptosis in *me* BMMCs, whereas a much lower dose of IL-3 enhances their proliferation relative to normal mast cells. This suggests that the strength of IL-3 receptor stimulation may alter the SHP-1-dependent temporal induction of Erk protein signaling and its influence on the mast cell survival and activation. It is plausible that SHP-1 directly dephosphorylates the β -subunit of the IL-3 receptor or its associated adaptor protein Shc, since either of

these events has been shown to reduce the activation of the Ras/MAPK pathway in other cell types [30].

Mast cells are indeed essential for the development of the severe motheaten phenotype. The loss of mast cells due to mutations in the growth factor receptor c-Kit greatly reduces the lethal autoimmunity in SHP-1-deficient mice [35, 37]. Interestingly, the loss of SHP-1 function also partially restores the reduced mast cell populations in c-Kit^{-/-} mice as well as increases the numbers of tissue-resident mast cells in c-Kit^{+/+} mice [37]. In accordance with previous reports [37], mast cell-deficient (Kit^{W^{Sh}}) *mev* mice are protected from the development of spontaneous lung inflammation caused by the lack of SHP-1 phosphatase activity [35]. This reciprocal alleviation of the SHP-1 and c-Kit-deficient phenotypes suggests that the WT SHP-1 protein may be inhibiting signaling downstream of the c-Kit receptor to limit the in vivo expansion of mast cells. As described in the following sections, regulatory phosphotyrosine sites on membrane receptors such as c-Kit and FcεRI, their associated tyrosine kinases (Lyn, Syk), and adaptor proteins may serve as targets of SHP-1 phosphatase activity in various cell models. However, whether SHP-1 directly dephosphorylates these proteins to regulate mast cell activation pathways requires further validation.

5.2 SHP-1 in FcεRI Signaling and Responses

Similarly to growth factor starvation, sustained antigen stimulation of mast cells can also promote cell death that is in part mediated by SHP-1 signaling. BMMC from *me* mice are more resistant to antigen-induced externalization of phosphatidylserine and fragmentation of DNA [38] that signal the induction of apoptosis. Loss of SHP-1 in these cells reduces the activity of the store-operated Ca²⁺ channel (SOC) and reduces pro-apoptotic mitochondrial permeability with cytochrome C release. SHP-1 also negatively regulates the pro-survival MAPK and BCL-X_L pathways [38], most likely via dephosphorylation of tyrosine residues on upstream adaptor/scaffolding proteins for the Ras/MAPK cascades such as LAT, as observed in other immune signaling pathways. SHP-1 may target proteins that control mitochondrial integrity and/or calcium channels, though such actions in Ag-stimulated mast cells have not yet been described. Taken together, these results show that enhanced cell survival is largely responsible for the abnormal increase in the numbers of tissue-resident mast cells that contributes to the spontaneous systemic inflammation in SHP-1-deficient mice [35, 38].

SHP-1 phosphatase activity-deficient *mev* mice have more mast cells in the lungs that store and spontaneously release higher levels of histamine than in WT mice. BMMCs from *mev* mice overall exhibit secretory hyperresponsiveness to LPS, oxidative stress, PMA, antigen, and/or SCF [35]. The heterozygous *me/+* mice are also susceptible to allergic and Th2-type immune activation, including ovalbumin-induced lung inflammation and

airway hyperresponsiveness to methacholine challenge [29]. Mast cells from *mc/+* mice spontaneously produce more IL-6 and IL-13 when treated with antigen, and T cells from these mice secrete much higher amounts of IL-4, IL-13, and IL-5 than wild-type cells upon T cell receptor stimulation and contribute to the moth-eaten-type phenotype [29].

The receptor-associated Lyn and SHP-1 proteins can reciprocally modulate each other's activation via their respective tyrosine kinase and phosphatase activities. Stimulation with low antigen concentrations (1 ng/ml) does not promote the recruitment of Lyn to FcεRI β-subunit and does not induce Lyn-mediated overall phosphorylation of tyrosine residues on SHP-1 [23]. Hypophosphorylation of SHP-1 is believed to reduce its inhibitory phosphatase activity in proximity to the FcεRI receptor, resulting in increased phosphorylation of FcεRI β-subunit ITAMs and Syk kinase-mediated induction of mast cell secretory responses. A high concentration of antigen (100 ng/ml) promotes Lyn-mediated recruitment and phosphorylation of SHP-1 and of the inhibitory inositol 5'-phosphatase SHIP-1, which together serve to down-regulate mast cell activation [23].

However, the activity of the SHP-1 protein may also positively regulate mast cell activation and secretion of inflammatory cytokines. Stimulation of FcεRI receptor enhances SHP-1-mediated dephosphorylation of Tyr-396 on Lyn and Tyr-1020 on SHIP-1 to reduce their inhibitory effects on FcεRI signaling [39]. This interaction between SHP-1 and Lyn is mediated by the adaptor functions of the phospholipase family protein PLCβ-3. Furthermore, in hematopoietic and mast cell progenitor cells, Lyn can phosphorylate Tyr-564 of SHP-1 to activate its phosphatase activity and suppress the activation of the transcription factor STAT5 to control the proliferation of myeloid cells. The activating phosphorylation of Tyr-536 on SHP-1 can be catalyzed by several kinases [40]. Interestingly, evidence from RBL-2H3 cells suggests that SHP-1 can auto-dephosphorylate Tyr-536, thus self-limiting the negative regulation of FcεRI signaling [41].

Evidence from several FcεRI-expressing cell systems suggests that SHP-1 differentially regulates the signaling pathways for mast cell degranulation and cytokine secretion [39]. In the RBL-2H3 cell line, exogenously expressed SHP-1 decreases the tyrosine phosphorylation of FcεRI subunits and Syk, promotes the Jnk-mediated secretion of TNFα, and has no effect on downstream antigen-induced release of histamine [42]. In another study, antigen-stimulated SHP-1-deficient BMMC were found to have higher levels of MAP kinase activation downstream of FcεRI, resulting in enhanced release of IL-6 and TNFα cytokines. Nevertheless, PLCγ-mediated calcium mobilization and degranulation are reduced in these cells [43]. Aggregation of FcεRI can also induce the phosphorylation of Tyr-564 on SHP-1 and facilitate its association with the adaptor protein

3BP2, which positively regulates the production of TNF α but not degranulation in RBL-2H3 cells [44]. The phosphorylation of Tyr-564 on SHP-1 may serve to recruit Syk, Lyn, LAT, and/or PLC γ -2 proteins, via 3BP2, allowing SHP-1 to regulate cytokine secretion via direct tyrosine dephosphorylation of these Fc ϵ RI pathway-associated signaling mediators.

Overall data suggest that Fc ϵ RI and Syk may be the key in vivo targets of SHP-1 phosphatase activity in antigen-stimulated mast cells that allow SHP-1 to inhibit downstream signaling activation. The ability of SHP-1 to transiently form various receptor-proximal protein complexes may also account for some of the positive regulatory effects of SHP-1 in mast cell activation. As described earlier, the intensity of stimulation of receptors for antigen and/or cytokines can further alter the regulatory roles of individual signaling proteins, such as Lyn and SHP-1. Additionally, the dissimilar origin of rat basophilic leukemia (RBL-2H3) cells from bone marrow-derived mast cells, the abnormal expression levels of SHP-1, and possibly other tyrosine phosphatases/kinases in this cell line could alter the effect of individual PTPs on Ag-induced mediator release. Thus, it remains to be proven whether any of the proteins that are direct targets of SHP-1 in leukocytes also play key roles in the regulation of Fc ϵ RI signaling in mast cells.

5.3 SHP-2 and SHP-1: Similarities and Differences

Both Src homology region 2 domain-containing phosphatases 1 and 2 feature tandem SH2 domains near the N-terminal tails and a protein tyrosine phosphatase domain near the C-terminus. Yet, these cytoplasmic PTPs share only 55 % overall amino acid sequence and play generally opposing roles in the activation of cell signaling [45]. Unlike the mainly inhibitory functions of SHP-1 in hematopoietic cells, SHP-2 (*Ptpn11*) is ubiquitously expressed and typically facilitates the signaling of growth factor receptors [46]. Dissimilarities in the SH2 and PTP domains allow SHP-1 and SHP-2 to associate with and dephosphorylate different regulatory tyrosine residues [32]. Interestingly, both SH2-containing PTPs are co-expressed in mast cells and other leukocytes, where they regulate the PI3K, Akt, Jak2/STAT, MAPK, and NF- κ B signaling cascades [47]. SHP-2 also facilitates the hematopoietic differentiation of murine stem cells that can give rise to mast cells [48].

Both SHP-1 and SHP-2 work in close proximity to regulate mast cell activation through c-Kit, Fc ϵ RI, and immune receptors with tyrosine-based inhibitory motifs (ITIMs). The binding of SHP-1 at Tyr-569 and SHP-2 at Tyr-567 of the tyrosine kinase receptor c-Kit inhibits SCF-induced signaling and proliferation of Ba/F3 Pro-B cells [49]. However, the association of SHP-2 with the adaptor protein Gab2 at c-Kit Tyr-567 also activates the Rac/Jnk pathway and proliferation of mouse mast cells [50]. Despite the ability of SHP-1 and SHP-2 to bind and/or dephosphorylate multiple tyrosine kinases and adaptor proteins in vitro, their direct

protein targets that allow both positive and negative regulation of c-Kit downstream signaling in mast cells have not been fully elucidated. Evidence from other cell types suggests that reciprocal regulation of tyrosine phosphorylation stimulus by Src family kinases and SHPs plays an important role in several mast cell activation pathways [40, 42].

The β -subunit of Fc ϵ RI constitutively associates with SHP-1, whereas SHP-2 is recruited upon aggregation of Fc ϵ RI. Receptor stimulation is followed by activating tyrosine phosphorylation of both PTPs, most likely by receptor-associated SFKs. Fc ϵ RI ITAMs can also be directly dephosphorylated by SHP-1 and SHP-2 in vitro [51]. Antigen stimulation of mouse BMMCs induces phosphorylation of Tyr-58 and Tyr-47 on Fc ϵ RI- γ . Tyr-58 is more important for the recruitment of Syk kinase and calcium signaling and is preferentially dephosphorylated by SHP-1 and SHP-2 over Tyr-47 [52]. The loss of SHP-2 from mast cells also reduces the activation of Fyn and Erk and the secretion of TNF α . SHP-1 and SHP-2 can inhibit the influx of calcium during mast cell activation through their recruitment to ITIMs in the immunoglobulin superfamily receptor gp49B1 [53], which functions to inhibit anaphylactic reactions in mice [54]. However, similarly to SHP-1, the loss of SHP-2 protein alone may not affect the overall antigen-induced degranulation of mast cells [55].

Given the complexity of the involvement of both SHP-1 and SHP-2 in multiple signaling pathways that control the differentiation, survival, and inflammatory activation of mast cells, it is unsurprising that both PTPs appear to have mixed effects on mast cell-mediated pathologies. Further research will elucidate the key direct binding partners and phosphatase substrates that allow SHP-1 and SHP-2 to exert both positive and negative regulation of important processes in mast cells.

6 Other Intracellular Mast Cell PTPs

6.1 Tyrosine-Specific PTPs: PEP, HePTP, and PTP-MEG2

The PEST domain-enriched tyrosine phosphatase (PEP, *Ptpn22*) in mouse mast cells (known as LYP in humans) promotes antigen-induced activation of PLC γ 1, calcium mobilization, degranulation, and IgE-mediated systemic anaphylaxis in mice [56]. Anti-inflammatory glucocorticoids can also upregulate the expression of PEP in BMMCs. Upregulated PEP acts to reduce the anti-anaphylactic effects of glucocorticoids, since direct chemical inhibition of PEP activity enhances the anti-anaphylactic effects of glucocorticoids, making it a potential drug target [56]. Mice lacking the expression of PEP phosphatase have a decreased capacity for anaphylactic reactions, but they do not gain additional protection from glucocorticoids. On the other hand, in T cell receptor signaling, PEP can physically associate with the inhibitory tyrosine kinase Csk to downregulate cell activation [57].

In general, Csk kinase antagonizes the activating dephosphorylations of SFKs by leukocyte PTPs.

In resting RBL-3H2 mast cells, the hematopoietic tyrosine phosphatase (HePTP, *Ptpn7*) is evenly distributed throughout the cytosol. FcεRI signaling induces HePTP to aggregate in many small, globular compartments in the cytoplasm and become tyrosine phosphorylated following the intracellular influx of calcium [58]. In other cell types HePTP can directly dephosphorylate Erk2 and possibly other MAP kinases to downregulate growth factor receptor signaling pathways [59]. However, it is not yet known whether HePTP protein itself significantly affects mast cell responses.

Expression of the megakaryocyte protein tyrosine phosphatase 2 (PTP-MEG2, *PTPN9*) in RBL-3H2 mast cells causes fusion and enlargement of vesicles near the *trans*-Golgi network. In Jurkat T leukemia cells, PTP-MEG2 tyrosine phosphatase activity can also inhibit the induced secretion of IL-2 [60]. Therefore, PTP-MEG2 may similarly regulate the formation or exocytosis of secretory vesicles in mast cells.

6.2 Dual-Specific PTPs: MKP-1 and PTEN

Dual-specific phosphatases are capable of catalyzing the removal of phosphates from tyrosine, serine, and threonine protein residues [61]. The MAP kinase phosphatase 1 (MKP-1) is a dual-specific phosphatase that regulates the phosphorylation and activation of MAPKs crucial for the activation of mast cells and other leukocytes [62]. In rodent mast cells, anti-inflammatory corticosteroid drugs increase the protein levels of MKP-1 (*Dusp1*) and other dual-specific PTPs by upregulating their gene transcription and inhibiting the proteosomal degradation of MKP-1 proteins [63]. MKP-1 then reduces the antigen-induced mast cell degranulation and cytokine production by dephosphorylating the activating tyrosine and threonine residues on Erk and p38 MAP kinases. MKP-1 also protects mice from IgE-mediated systemic anaphylaxis. However, MKP-1 can be dispensable for other anti-inflammatory functions of glucocorticoids in the mouse [62, 63]. In rat peritoneal mast cells, MKP-1 also reduces the SCF-induced phosphorylation of p38 to inhibit mast cell migration and release of cytokines [64, 65].

Perhaps the most unusual mast cell PTP is PTEN (phosphatase and tensin homolog deleted on chromosome ten), which was originally identified as a tumor suppressor gene mutated in many types of human cancers [66]. The PTEN protein contains domains homologous to protein tyrosine phosphatases and to the actin-binding protein tensin. PTEN was initially classified as a dual-specific phosphatase, due to its ability to dephosphorylate tyrosine, as well as serine and threonine, protein residues *in vitro*. However, the main *in vivo* function of PTEN is to antagonize cell-activating PI3K signaling by dephosphorylating the 3' position of the lipid second messenger PIP₃. Recently, mice lacking PTEN (*Pten*) were shown to have hyperproliferation of tissue-resident mast cells [67].

PTEN-deficient BMMCs are also more resistant to apoptosis due to elevated expression of pro-survival factors. PTEN^{-/-} BMMCs exhibit enhanced intrinsic and growth factor (SCF, IL-3)-induced activation of the transcription factor STAT5 for cell growth as well as PI3K-mediated activation of the kinase Akt. Loss of mast cell PTEN also contributes to secretory hyperresponsiveness to antigen and SCF and enhanced anaphylaxis in the mouse [67]. Though it is unknown whether the lack of tyrosine phosphatase activity of PTEN contributes to these phenotypes, its lipid phosphatase-dependent ability to downregulate PI3K signaling is crucial for controlling proliferation and immune activation of mast cells.

7 Oxidative Regulation of Mast Cell PTPs

The production of small reactive oxygen and nitrogen molecules is strongly associated with the inflammatory activation of mast cells and other leukocytes [68]. The amount of oxidative stress within mast cells may also influence their secretory responses [69]. Additionally, general inhibition of PTPs by peroxide and/or vanadate (pervanadate) ions induces the phosphorylation of tyrosine residues on the β - and γ -subunits of Fc ϵ RI, calcium influx, and degranulation of mast cells. However, treatment with pervanadate does not promote the movement of Fc ϵ RI complexes to lipid rafts, in contrast to the localization to rafts that occurs during aggregation of the Fc ϵ RI receptors by antigen [70, 71]. As with oxidizing reagents, antigen signaling leads to the reversible oxidation of active site cysteine residues of several PTPs (SHP-1, SHP-2, HePTP, PTP-MEG2) associated with the regulation of mast cell secretory responses. Furthermore, oxidation of mast cell PTPs can differentially alter their catalytic activities and induce their co-localization with the plasma membrane actin cytoskeleton [71]. In most cell systems, reversible oxidation generally reduces the catalytic activity of cysteine-based PTPs [72] and can promote the inhibitory dimerization of the receptor-like PTPs CD45, PTP α , and PTP ϵ [21, 73, 74]. Overall, this suggests that small oxidizing molecules produced by activated mast cells and/or nearby leukocytes may modulate Fc ϵ RI signaling and mast cell responsiveness by altering the catalytic activity and protein interactions of PTPs, thus affecting the tyrosine phosphorylation status of key signaling proteins.

8 Future Perspectives

In recent years, mast cells have been implicated in the pathogenesis of various forms of autoimmunity, cancer, and other severe chronic conditions [75, 76]. In addition to their pro-inflammatory effector

functions in allergies and infections, mast cells are also capable of downregulating several immune responses. In peripheral allografts, mast cells cooperate with regulatory T cells to suppress other leukocytes and promote allograft tolerance [77]. Additionally, mast cells can secrete the anti-inflammatory cytokine interleukin-10 during skin exposure to toxins and UV-B irradiation, reducing the extent of hypersensitivity dermatitis [78]. The abilities of mast cells to modulate immune environments, promote angiogenesis, and remodel the extracellular matrix is exploited by tumors to promote metastasis [79]. Colorectal tumors can recruit mast cells to systemically influence regulatory T cells to begin secretion of pro-inflammatory mediators that may promote cancer progression [80]. Additionally, in a mouse model of pancreatic tumor initiation, the release of angiogenic factors by recruited mast cells is an essential step for the growth of these tumors [81].

For such reasons, targeted regulation of mast cell responses may 1 day lessen the severity of human allergies, cancers, and autoimmune disorders. Knowledge gained from rodent mast cell models demonstrates that several PTPs are probably also crucial for the normal and aberrant functions of human mast cells.

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