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Phosphatase regulation of immunoreceptor signaling in T cells, B cells and mast cells

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Abstract

Recent progress has begun to reveal the often complex and changing roles of phosphotyrosine and phosphoinositide phosphatases in regulation of immunoreceptor signaling. The resultant confusion has been further increased by discoveries of new players. Here we provide a review of recent progress in defining the roles of these enzymes in immunoreceptor-dependent mast cell, T cell and B cell activation.

Introduction

Cell activation results from the transient perturbation of an active balance between positive and negative signals that is consequent to engagement of membrane receptors. These include activating and inhibitory receptors. Prototypic activating receptors such as TCR, BCR and FcεRI, expressed by T cells, B cells and mast cells, respectively, contain Immunoreceptor Tyrosine-containing Activation Motifs (ITAMs). Inhibitory receptors, expressed by these cells, contain Immunoreceptor Tyrosine-containing Inhibition Motifs (ITIMs). ITAM-containing receptors trigger primarily positive signals, but also negative signals, while ITIM-containing receptors trigger only negative signals [1]. Some receptors contain both ITAMs and ITIMs presumably serving a dual role [2]. Classically, kinases and phosphatases have been viewed as the effectors of positive and negative signals, respectively. However, kinases can generate negative signals and phosphatases can generate positive signals.

Immunoreceptor signaling can be divided into three steps. Signal transduction is the initial process that transforms an extracellular mechanical perturbation, for example, receptor aggregation, into an intracellular chemical perturbation, for instance, ITAM phosphorylation by *src family tyrosine kinases* (SFKs). Phosphorylated ITAMs then nucleate signalosomes into which signaling molecules assemble, while others translocate to the membrane. From these signalosomes, biochemical pathways are launched which propagate intracellular signals that drive responses such as gene transcription. These three steps are differentially regulated by phosphatases.

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Several sets of phosphatases control cell activation. *Receptor Tyrosine Phosphatases* (RTP) are transmembrane molecules that contain intracytoplasmic catalytic domains. While Src Homology 2 (SH2) domain-containing cytosolic phosphatases can be recruited by phosphorylated ITIMs and ITAMs, phosphatases lacking SH2 domains can be recruited into signalosomes by various means. Both protein tyrosine and lipid phosphatases operate in ITAM and ITIM signaling. This review is focused on the functions of these enzymes in lymphocytes and mast cells. Table 1 summarizes the regulatory functions of the different phosphatases expressed in these cells. We will use SHP-1/2 and SHIP1/2 as examples to discuss the different ways phosphatases can regulate cell activation below. Owing to space limitations, additional recent advances in our understanding of these phosphatases will be highlighted in the reference section.

SHP-1 and SHP-2

The *SH2-containing phosphotyrosine phosphatase-1* **SHP-1** is a well-known effector of negative regulation of cell activation that is employed by the majority of inhibitory receptors. Its expression is restricted primarily to hematopoietic lineage cells. The recruitment of SHP-1 by ITIM-containing receptors is well-documented [3-5]. Binding to a phoshorylated ITIM via their SH2 domains enhances the phosphatase activity of SHP-1 [6], leading to dephosphorylation of tyrosines in signaling molecules. Interestingly, SHP-1 may dephosphorylate ITIMs, thus providing feedback regulation of inhibitory signaling.

SHP-1 appears to have distinct functions in different cells. SHP-1-dependent negative regulation of BCR signaling was first demonstrated by the observation that B cells from SHP-1-deficient *motheaten* mice have a hyperactive phenotype [7]. B cell-targeted SHP-1 deficient mice showed a broader range of defects, including increased B cell proliferation, altered B cell development and develop lupus-like autoimmunity [8]. Conditional deletion demonstrated that SHP-1 also controls antigen-induced proliferative responses of CD8 T cells [9]. Unlike the inhibitory effect of SHP-1 on BCR signaling, SHP-1 has both positive and negative effects on FcεRI-dependent responses of BMMC from motheaten mice. SLP76, LAT and MAP kinases are hyperphosphorylated and cytokine production is increased, while PLC-γ is hypophosphorylated and subsequent calcium mobilization and degranulation are reduced. In addition to its catalytic activity, which accounts for its suppressive effects, SHP-1 also appears to function as an adaptor that links PLC-γ to SLP76, thus facilitating its phosphorylation after FcεRI crosslinking [10].

The role of the ubiquitously expressed *SH2-containing phosphotyrosine phosphatase-2* **SHP-2** in the regulation of lymphoid and mast cell activation is less clear. SHP-2 can inhibit cell activation. Using molecular imaging, Yokosuka *et al.* showed that PD-1 recruits SHP-2 via its *Immunoreceptor Tyrosine-based Switch Motif* (ITSM) after it is recruited to ligand-engaged TCR molecules [11]. SHP-2 then inhibits T cell activation by dephosphorylating TCR-proximal signaling molecules. By contrast, SHP-2 appears to have a dominant positive regulatory role in mast cells. SHP-2 can promote SFK activation by dephosphorylating PAG and Cbp, restricting Csk access to target SFKs, thereby preventing phosphorylation of the inhibitory tyrosine [12]. In mast cells SHP-2 regulates Fyn activity by dephosphorylation its inhibitory tyrosine directly [13] Supporting a physiologic significance of this observation, SHP-2 deficient BMMC and SHP-2 knockdown RBL-2H3 cells have decreased Fyn activation following FcεRI crosslinking, and this lead to decreased PLC-γ and MAP kinase activation, affecting cytokine production and degranulation [13,14]. SHP-2 also signals downstream of Kit in mast cells, promoting Erk activation and cell survival by downregulating the pro-apoptotic molecule Bim. In fact, mice in which SHP-2 was deleted in mast cells have decreased numbers of skin mast cells [15].

SHIP1 and SHIP2

By generating phosphatidylinostol 3,4,5-trisphosphate [PI(3,4,5)P3], *Phosphatidylinositol 3-Kinase* (PI3K) causes the membrane recruitment of important signaling effectors that contain Plekstrin homology (PH) domains, including SOS, Vav, PLC-γ, Btk, PDK1 and Akt. The PI3K pathway is critical for immunoreceptor signaling, controlling both cell activation and cell survival. The *SH2-containing inositol 5-phosphatases* SHIP1 and SHIP2 regulate this pathway by hydrolyzing PI(3,4,5)P3 to PI(3,4)P2.

SHIP1 is best known as the effector of inhibitory signaling by FcγRIIB. It also negatively regulates BCR [16], FcεRI [17] and TCR signaling [18] in the absence of FcγRIIB engagement. SHIP1-mediated inhibition of signaling occurs by two mechanisms. SHIP1 is well known to hydrolyse PI(3,4,5)P3. It also recruits the adapter Dok-1 that activates RasGAP, inhibiting Ras and activation of MAP kinases such as Erk1/2 [20].

T cell-specific SHIP1 deletion had only minor effects *in vivo* [19]. By contrast, SHIP1-deficient mice have reduced antibody responses, but elevated basal immunoglobulin levels. They also develop autoantibodies and severe lupus-like disease. [22•,24,25••]. B cells lacking SHIP1 display enhanced BCR-signaling and proliferation [21,22•] and altered BCR clustering [23].

Germinal center (GC) B cells are subject to potent negative regulation by both SHP-1 and SHIP1 [26••]. Hyporesponsiveness is relieved transiently during G2-M cell cycle progression. This dynamic regulation of BCR signaling by phosphatases may be important in selection in GC of B cells expressing high affinity antibodies. Induced deletion of SHP-1 in an ongoing germinal center reaction strongly reduced the number of B cells selected. Furthermore, mice in which B cells lack SHIP1 develop fewer antigen-specific GC B cells upon immunization, and their antigen receptors have fewer mutations. Finally, affinity-based selection appears to fail in these mice [22•]. The need for both increased SHP-1 and SHIP1 activity may reflect the inherent difference in specificity and reach. While SHP-1 only inhibits molecules in its direct proximity [27], SHIP1-mediated inhibition of the PI3K pathway affects all cellular receptors which utilize this pathway, for example chemokine receptor signaling [28].

Recently, we showed that SHIP1 is important for maintaining tolerance in anergic B cells. Anergic B cells are autoreactive B cells which are present in the periphery in an antigen unresponsiveness state [29]. Their unresponsiveness is the consequence of chronic BCR signaling and it is rapidly reversed by removal of the autoantigen from receptors [30]. Anergic B cells have an elevated basal level of SHIP1 and Dok-1 phosphorylation, indicative of increased inhibitory signaling, and upon deletion of SHIP anergic B cells regain responsiveness [25••]. The driver of SHIP1 and Dok-1 activation in anergic B cells appears to be BCR ITAM monophosphorylation. ITAMs of Igα and Igβ are monophosphorylated in anergic B cells and additional monophosphorylation but not biphosphorylation is induced by additional stimulation. Biased SHIP activation is a direct consequence of this monophosphorylation. How the BCR ITAMs become monophosphorylated and how this results in SHIP1 activation is still unclear. Studies of TCR and Fc receptor signaling suggest that weak ligand binding favors monophosphorylation [31,32], although monophosphorylation could also be a consequence of differential dephosphorylation [33].

In addition to deletion of SHIP1 [25••], deletion of PTEN [34], another phosphatase which hydrolyzes PI(3,4,5)P3, can also result in the loss of tolerance. This strongly suggests that suppression of the PI3K pathway is key to the maintenance of B cell anergy. It is

noteworthy, however, that while SHIP1 activity is increased in both Ars/A1 and MD4/ML5 transgenic models of B cell anergy, increased PTEN expression and phosphorylation are seen only in the MD4/ML5 model [25••]. This distinction may reflect quantitative differences in the effects of these phosphatases on PI(3,4,5)P3 levels. SHIP1, however, uniquely generates PI(3,4)P2, which functions as allosteric activator of SHIP1 [35] and also recruits the adaptors TAPP1/2, which were recently shown to restrain B cell activation by reducing Akt activation [36•]. Female knock-in mice expressing TAPP1 and TAPP2 PH domain mutants that were unable to bind PI(3,4)P2 developed lupus-like autoimmunity, suggesting that besides reducing PI(3,4,5)P3 levels, SHIP1 may reinforce tolerance via TAPP proteins.

SHIP1 is also a major negative regulator of FcεRI signaling [17,37•]. The mechanism by which FcεRI recruits SHIP1 to accomplish this feedback-regulation is unclear, although SHIP1 has been shown to bind directly to the phosphorylated ITAM of the FcRβ subunit [38•] and to two phosphorylated tyrosines of LAT [76]. Using BMMC, connective tissue mast cells and mucosal mast cells from SHIP1$^{-/-}$ mice, SHIP1 was found to inhibit FcεRI-dependent secretory responses through its enzymatic activity, but unexpectedly, to promote TLR-dependent cytokine secretion through its adapter activity [39]. SHIP1$^{-/-}$ mice displayed systemic mast cell hyperplasia, increased cytokine levels and enhanced anaphylaxis [40]. Accordingly, small molecules that are specific SHIP1 agonists have been found to protect from anaphylaxis [35].

The role of the ubiquitously expressed *SH2-containing inositol 5-phosphatase-2* **SHIP2** in regulating lymphocyte and mast cell activation is less clear. SHIP2 excecutes FcγRIIB-mediated inhibitory signaling in activated B cells [41]. siRNA knock-down of SHIP2 in BMMC enhanced IgE-induced degranulation and cytokine secretion, but had no effect on Ca^{2+} responses, MAP kinase activation or actin depolarization, while enhancing Rac-1 activation and microtubule polymerization. Thus, like SHIP1, SHIP2 negatively regulates FcεRI signaling but apparently by a distinct mechanism [42•]. Interestingly, SHIP2 expression is reduced in mast cells of idiopathic urticaria patients, and this is associated with spontaneous degranulation upon IgE sensitization [43].

Conclusion

Phosphatases are critical in the control of immunoreceptor signaling in lymphoid and myeloid cells. While the regulatory effects of phosphatases depend primarily on their catalytic activity, their adaptor functions are also important. Furthermore, they sometimes display distinct functions in different tissues. A better knowledge of these molecules is crucial for understanding the physiopathology of immune disorders, and may reveal utility as therapeutic targets.

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Table 1
Phosphatases and their role in B cells, T cells and mast cells

Phosphatases	B cells	T cells	Mast cells
Tyrosine phosphatases			
Receptor protein tyrosine phosphatase			
CD45: *Receptor-type tyrosine-protein phosphatase C*	Positively regulates BCR signaling by dephosphorylating the inhibitory tyrosine of Lyn [45•] B cell activation is only partially impaired in CD45−/− B cells due to expression of CD148 [48]	Dual positive and negative regulatory role by being able to dephosphorylate both the inhibitory tyrosine and the activating loop tyrosine of Lck [44,45•] T cell activation is impaired in CD45−/− T cells	Dephosphorylates the inhibitory tyrosine (Y570) of Lyn [49] Degranulation and IL6 secretion are decreased while IL3 and SF dependent-proliferation are enhanced in CD45−/− BMMC [49]
CD148: *Receptor-type tyrosine-protein phosphatase eta*	Positively regulates BCR signaling by dephosphorylating the inhibitory tyrosine of Lyn [48] B cell activation is partially impaired in CD148−/− B cells due to expression of CD45 [48]	Dual positive and negative regulatory role by being able to dephosphorylate both the inhibitory tyrosine and the activating loop tyrosine of Lck [46,47] Not expressed in all T cells [46]	*Not reported*
PTPα: *Receptor-type tyrosine-protein phosphatase alpha*	*Not reported*	Lipid raft associated phosphatase that negatively regulates Fyn activity in unstimulated cells by preferentially dephosphorylating the activating loop tyrosine [58]	Lyn, Fyn, Hck, Syk, SHIP, PI3K and MAPK activations are impaired in PTPα−/− BMMC [50•] IgE- induced degranulation, cytokine and cysteinyl leukotriene secretion are enhaced in PTPα−/− [50•] PTPa−/−mice display enhanced IgE-dependent anaphylaxis [50•]
Non-receptor Protein Tyrosine Phosphatase			
PTNP22 (PEP/LYP): *Tyrosine-protein phosphatase non-receptor type 2*	Negatively regulates BCR signaling by directly dephosphorylating Syk. Also reduces PLCγ2, p38 and Akt phosphorylation [54] Autoimmune-associated Lyp620W is a gain of function variant [54] Pep619W knockin mice have hyperresponsive B cells [56••,57•]	LYP (human)/Pep (mouse) dampens TCR signaling by dephosphorylating activating tyrosine of Lck and Fyn and their substrates such as Zap70 [51,52,55] Autoimmune-associated Lyp620W is a gain of function variant [55] Pep619W knockin mice have hyperresponsive T cells [56••,57•]	IgE-dependent degranulation and Ca^{2+} reponses are reduced in PEP−/− BMMC [53] PLCγ1 Phosphorylation is reduced in PEP−/− BMMC [53] PEP−/− mice display decreased passive anaphylaxis [53]
PTPN2: *Tyrosine-protein phosphatase non-receptor type 2*	*Not reported*	Negatively regulates TCR signaling by dephosphorylating the activating loop tyrosine of Lck and Fyn [59•]	*Not reported*
SH2 domain-containing Tyrosine phosphatases			
SHP-1: *Tyrosine-protein phosphatase non-receptor type 6*	Negatively regulates BCR signaling by dephosphorylating Igab ITAMs, Lyn, Syk, SLP-65/ BLNK, Vav1 [7,8]	Negatively regulates TCR signaling by dephosphorylating CD3 ITAMs, Lck, Zap70, LAT, SLP-76, Vav1 [61,62•]	Positive and negative regulation of mast cell functions [10,60]
SHP-2: *Tyrosine-protein phosphatase non-receptor type 11*	SHP-2 negatively regulates B cell activation by inhibiting phosphorylation of Igb ITAM, Syk, PLCγ2 and Erk [63]	Indirect evidence suggest that SHP-2 negatively regulates TCR signaling by directly dephosphorylating or indirectly causing reduced	Positively regulates FcεRI-dependent degranulation and TNF-α secretion [13,14] Negatively regulates Kit signaling [15]

Phosphatases	B cells	T cells	Mast cells
		phosphorylation of Zap70, Vav, PLCγ1 and Erk [11]	
Cytosolic Tyrosine Phosphatases lacking SH2 domains			
PTP-PEST: *Tyrosine-protein phosphatase non-receptor type 12*	Negatively regulats B cell activation by dephosphorylation of Shc, Pyk2, Fak and Cas, and inactivating the Ras pathway [65]	PTP-PEST can dephosphorylate the activating tyrosine of Lck, reduce Zap70 phosphorylation and inhibit the MAP pathway [66]. It has also been reported to have positive effects by dephosphorylating Pyk2, promoting secondary responses [64•]	*Not reported*
PTPε *Receptor-type tyrosine-protein phosphatase epsilon*	*Not reported*	*Not reported*	IgE-dependent degranulation, cytokine secretion are enhanced in PEPε−/− BMMC [67•] LAT, SLP76 phosphorylation and MAP kinase activation are incresead in PEPε−/− BMMC [67•] PEPε−/− mice undergo increased IgE-induced passive anaphylaxis [67•]
TULA-2: *Tyrosine-protein phosphatase STS1/TULA2*	*Not reported*	*Not reported*	Negatively regulates FcεRI-dependent degranulation by dephosphorylating Syk and PLCγ2 [68•] Upon FcεRI engagement, TULA-2 forms complex that contains Nck, SHIP1, SLP76 and Grb2 [68•]
Phosphatidate phosphatases			
LIPIN-1: *Phosphatidate phosphatase LPIN1*	*Not reported*	*Not reported*	IgE-dependent degranulation and prostaglandin D2 production are enhanced in Lipin-1 −/− BMMC [69] PKC and SNPA-23 phosphorylation are increased in Lipin-1−/− BMMC [69] Lipin1 deficiency exacerbated passive anaphylaxis reaction *in vivo* [69]
Lipid Phosphatases			
SH2 domain-containing lipid phosphatases			
SHIP1: *Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1*	Negatively regulates BCR signaling by hydrolyzing PI(3,4,5)P3, counteracting the PI3K pathway, resulting in reduced activity of SOS, Vav, PLC-γ, Btk, Akt [16,21,22•]. Inhibits Ras and activation of MAP kinases by recruiting Dok-1 [20]	Negatively regulates TCR signaling by recruiting Dok-1 and Dok-2 to LAT, which in their turn inhibit Akt and Zap70 activity [18]. *In vivo* T cell specific SHIP-1 mice have relatively normal signaling [19]	Negatively regulates FcεRI-dependent degranulation [17,39,40]
SHIP2: *Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 2*	Negatively regulates BCR signaling by hydrolyzing PI(3,4,5)P3, counteracting the PI3K pathway, resulting in reduced PLCγ, Btk and Akt activity [41]	*Not reported*	Negatively regulates FcεRI-dependent degranulation and cytokine secretion [42•]
Lipid phosphatase lacking SH2 domains			
PTEN: *Phosphatase and tensin homolog*	Negatively regulates BCR signaling by hydrolyzing	Negatively regulates TCR signaling by hydrolyzing	Negatively regulates human mast cells activation [70,71]

Phosphatases	B cells	T cells	Mast cells
	PI(3,4,5)P3, counteracting the PI3K pathway, resulting in reduced Akt activity [72] Suppressor of B cell lymphoma [75•]	PI(3,4,5)P3, counteracting the PI3K pathway, resulting in reduced Akt and Erk activity [73] Suppressor of T lymphoma [74]	PTEN deficiency causes mast cell hyperplasia and mastocytosis [71]