SHIP Family Inositol Phosphatases Interact with and Negatively Regulate the Tec Tyrosine Kinase*

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The Tec family of protein-tyrosine kinases (PTKs), that includes Tec, Itk, Btk, Bmx, and Tsk, plays an essential role in phospholipase Cγ (PLCγ) activation following antigen stimulation. This function requires activation of phosphatidylinositol 3-kinase (PI 3-kinase), which promotes Tec membrane localization through phosphatidylinositol 3,4,5-trisphosphate (PtdIns 3,4,5-P₃) generation. The mechanism of negative regulation of Tec family PTKs is poorly understood. In this study, we show that the inositol 5’ phosphatases SHIP1 and SHIP2 interact preferentially with Tec, compared with other Tec family members. Four lines of evidence suggest that SHIP phosphatases are negative regulators of Tec. First, SHIP1 and SHIP2 are potent inhibitors of Tec activity. Second, inactivation of the Tec SH3 domain, which is necessary and sufficient for SHIP binding, generates a hyperactive form of Tec. Third, SHIP1 inhibits Tec membrane localization. Finally, constitutively targeting Tec to the membrane relieves SHIP1-mediated inhibition. These data suggest that SHIP phosphatases can interact with and functionally inactivate Tec by de-phosphorylation of local PtdIns 3,4,5-P₃ and inhibition of Tec membrane localization.

Antigen receptor signal transduction is central to the development and function of T and B lymphocytes in generating a productive immune response. Key features of antigen receptor signaling include rapid activation of non-receptor protein tyrosine kinases (PTKs),1 of the Src, Syk, and Tec families, followed by their rapid inactivation to terminate the signal. The activation mechanisms for these PTKs have been well characterized by their rapid inactivation to terminate the signal. The mechanisms of negative regulation of Tec family PTKs is poorly understood. Since the inactivation process is rapid, it is likely that negative regulatory phosphatases are directly recruited to activated PTKs. Few such PTK-phosphatase interactions have been reported, suggesting that they may be relatively weak and transient.

The Tec family of PTKs, comprising Tec, Btk, Itk, Bmx, and Tsk (Rlk in mouse), perform an essential role in antigen receptor signaling of T and B lymphocytes (reviewed in Refs. 6 and 7). Tec family PTKs consist of an N-terminal pleckstrin homology (PH) domain, a proline-rich region (10–12). However this mechanism has not been proved. A number of distinct mechanisms by which Tec family PTKs are negatively regulated have been described. These include direct inhibition by PI 3-kinase (13), by the SH3 domain-binding protein Sab (14). The mechanisms by which Tec family PTKs are inactivated are relatively poorly understood. Structural studies have lead to the hypothesis that Tec, Btk, and Itk are negatively regulated by an intramolecular interaction between the adjacent SH3 domain and proline-rich region (10–12). However this mechanism has not been proved. A number of distinct mechanisms have been reported for Btk inactivation. These include direct inhibition by Btk activity by the PH domain-interacting protein IBtk (13) and the SH3 domain-binding protein SAB (14). In addition, Btk membrane localization can be inhibited by protein kinase C-β-mediated phosphorylation of the proline-rich region (15), or by the 5’-inositol phosphatase SHIP1, which hydrolyzes PtdIns 3,4,5-P₃ (16, 17).

The SHIP family of inositol phosphatases, comprising the hematopoietic cell-specific SHIP1, and the ubiquitous SHIP2, are characterized by an N-terminal SH2 domain, a central inositol phosphatase domain and a C-terminal tail containing...
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protein interaction motifs for SH3, SH2, and phosphotyrosine binding (PTB) domains (reviewed in Refs. 18 and 19). SHIP phosphatases de-phosphorylate the 5 position on inositol rings of PtdIns 3,4,5-P3 and inositol-(1,3,4,5)-tetrakisphosphate (IP4). PtdIns 3,4,5-P3 is a ligand for the PH domains of Tec PTKs and several other PH domain-containing signaling proteins, and is required for membrane localization and function of Btk and Itk (16, 17) and Tec-(178–245), Btk-(212–282), and Itk-(171–238), and human Bmx-phosphatases de-phosphorylate the 5 position on inositol rings and are required for membrane localization and function of Tec, like Btk and Itk, can function in antigen recognition (28) and in platelets (29), which may be caused by its relatively low level of expression (30). Tec is believed to play a relatively minor role alongside Btk in PLCγ activation in B cells (28) and in platelets (29), which may be caused by its relatively low level of expression (30). Tec is believed to play a more important role in activated and Th2 effector T cells, where Tec protein is substantially up-regulated (30). In a recent study, we demonstrated that Tec is unique among Tec family PTKs in its capacity to induce PLCγ1 phosphorylation and NFAT activation when overexpressed in T and B cell lines (30). These data suggest that Tec is regulated in a manner that is distinct from other Tec family PTKs. Indeed, we hypothesize that Tec overexpression overcomes the effects of an endogenous Tec-specific negative regulator. The Tec SH3 domain is a candidate binding partner for such a negative regulator, because a Tec SH3 point mutant is hyperactive, unlike point mutants of other Tec domains (30). In this study we have identified a novel interaction between SHIP family phosphatases and the SH3 domain of Tec, but not other family members. This interaction inhibits Tec membrane localization and effector function.

EXPERIMENTAL PROCEDURES

Cells—The Jurkat and HUT78 T cell lines were cultured in RPMI supplemented with 5% fetal bovine serum, penicillin, streptomycin, and glutamine. The human kidney 293T cell line was cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, penicillin, streptomycin, and glucose.

Plasmids—Glutathione S-transferase (GST)-tagged SH3 domain constructs were generated by PCR from mouse Tec, isoform IV (amino acids 178–245), Btk-(212–282), and Itk-(171–238), and human Bmx-(186–297) and Itk-(82–151) cDNA (26) and cloned into pGEX-2T (Amersham Biosciences, Piscataway, NJ). Tec family expression constructs, HA-tagged at the N terminus, were described before (30). The human SHIP1 and SHIP2 cDNAs were gifts from L. Rohrschneider (Fred Hutchinson Cancer Research Center, Seattle, WA) and S. Moodie (Metabolix Inc., Hayward, CA), respectively. SHIP1 and SHIP2 were PCR-amplified and subcloned into the pEF6 vector (Invitrogen, Carlsbad, CA), in-frame with the C-terminal Myc tag. Truncation mutants of full-length SHIP1, containing amino acids 1–173, 1–370, 364–1192, and 900–1192, were generated by PCR. The SHIP1 yeast two-hybrid construct was generated by PCR amplification of SHIP1 followed by cloning into the pGBT9 vector (BD Biosciences), in-frame with the N-terminal GALA DNA binding domain. The NFAT luciferase reporter contained three copies of the distal NFAT site from the IL-2 promoter (31). The pEF6-lacZ expression construct was from Invitrogen.

Antibodies—The anti-SHIP1 rabbit antiserum was kindly provided by M. Coggeshall (University of Oklahoma, Oklahoma City, OK) and the anti-Tec rabbit antiserum was from Upstate Biotechnology (Charlottesville, VA). The anti-HA tag mAb was 16B12 (Covance Research Products, Berkeley, CA), the anti-Myc tag mAb was 9B11 (Cell Signalling Technology, Beverly, MA), and the anti-GST mAb was from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-TRC4 mAb C305, for Jurkat stimulations, was described previously (32).

Biochemical Analyses—Recombinant GST-SH3 fusion proteins were made in the BL21(DE3)pLysS strain of Escherichia coli (Invitrogen) according to the manufacturer’s protocol (Amersham Biosciences). For pull-downs, Jurkat and HUT78 cell lysates were prepared by lysing 1 × 106 cells in 1 ml of 1% Nonidet P-40 lysis buffer containing protease inhibitors, precleared three times with 25 μl of glutathione-Sepharose (Amersham Biosciences), and centrifuged at 100,000 × g for 30 min. GST pull-downs were done from these lysates using 10 μg of recombinant GST-SH3 fusion protein coupled to 12.5 μl of glutathione-Sepharose. Pull-downs were washed twice with 1 ml of Nonidet P-40 lysis buffer, twice with centrifuging through 1 ml of 1% sucrose in 1% Nonidet P-40 lysis buffer, and twice more with 1 ml of 1% Nonidet P-40 lysis buffer. Proteins were separated by SDS-PAGE and visualized by SimplyBlue Coomassie staining (Invitrogen). Protein bands were excised, the gel sliced into 1-mm square pieces and subjected to an in-gel digestion with endoprotease Lys-C (Roche Diagnostics, Indianapolis, IN) (33). Peptides were extracted from the gel pieces and cleaned up using a gel-loading pipette tip filled with 100 μl of POROS C18 resin (Applied Biosystems, Foster City, CA). The peptide mixture was eluted into a nanospray glass capillary (Protana, Odense, Denmark) using 500 nl of 60% methanol, 5% formic acid. After mounting the capillary onto the nanospray source (Protana), peptide solutions were infused into an LCQ Classic Iontrap mass spectrometer (ThermoFinnigan, San Jose, CA) at a flow rate of 10 nlm. Individual peptide mass fragments were isolated and subjected to MS/MS analysis using an automated “zoomscan walking” method (34). The acquired MS/MS spectra were subjected to a non-redundant protein database search using the SEQUEST program (Jimmy Eng and John Yates III, University of Washington, Seattle, WA) (35). For GST pull-downs from SHIP-transfected 293T cells, several modifications were made to the above protocol; the preclearing and 100,000 × g centrifuge steps were replaced by pull-down using 5 μg of recombinant GST-SH3 fusion protein, and samples were washed four times with 1 ml of 1% Nonidet P-40 lysis buffer. Immunoprecipitations and Western blotting were performed as described previously (36). Western blots were visualized using Western Lightning chemiluminescence reagents (PerkinElmer Life Sciences) in combination with either a Kodak Image Station 4000SR (Raytest, Rochester, NY) or with Hyperfilm (Amersham Biosciences) developed using a Compact X4 film processor (Xograph Imaging Systems, Gloucestershire, UK).

Yeast Two-hybrid—Yeast strain and media culture conditions were as previously described (37), except that in synthetic media, twice the level of amino acids and nucleotides were used. The strain PJ69–4A (MATa trp1–901, leu2–3,112, ura3–52, his3–200, gal4–lacZ1, his1–11002) was transformed with combinations of Gal4p activation and binding domain fusions. Yeast two-hybrid assays were performed as previously described (39). Briefly, cultures were grown in selective media to stationary phase, diluted to OD600 of 1 and spotted on to synthetic media either containing or lacking histidine. Plates were incubated for 5 days at room temperature.

luciferase activities—Jurkat and HUT78 cells were transfected in a volume of 0.4 ml of RPMI (without serum) by electroporation using a Gene Pulser Electroporator (Bio-Rad) set at 250 V, 960 μF (Jurkat) or 240 V, 960 μF (HUT78). 293T cells were transfected by the calcium phosphate method using the CalPhos mammalian transfection kit (BD Biosciences).

Luciferase Assays—Jurkat and HUT78 cells were transfected, as described above, with the expression construct of interest, in addition to 2 μg of pEF6-lacZ to control for transfection efficiency. Sixteen hours after transfection, live cells were counted by trypan blue exclusion and samples divided for luciferase assay, β-gal assay, and Western blotting, as described (30). All luciferase assay data were normalized to β-gal values. Expression of each construct was confirmed by Western blotting.

Microscopy—Transfected Jurkat cells were sorted for Tec-GFP using a MoFlo cell sorter (DakoCytomation, Glostrup, Denmark) and pre-
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The Tec SH3 Domain Interacts with SHIP1 and SHIP2—Structural studies have lead to the hypothesis that Tec, Btk, and Itk are negatively regulated by an intramolecular interaction between the adjacent SH3 domain and proline-rich region (10–12). A prediction of this model is that inactivating mutations of either the SH3 or proline-rich region would result in a hyperactive molecule. However, using a Jurkat T cell line model system to investigate this, we have found that a SH3 point mutant is more active than wild type, but that the proline mutant is not (30). This suggests that the initial model is incorrect for Tec, which instead may be regulated by a novel mechanism that involves its SH3 domain interacting with a negative regulator.

In order to identify a putative negative regulator, we used a proteomic approach to identify proteins that interact with recombiant GST fusion proteins of the SH3 domain of Tec. A GST fusion protein of the SH3 domain of Itk was included to enable identification of proteins that interact uniquely with Tec. GST-SH3 pull-downs were carried out using lysates of Jurkat and HUT78 T cell lines, and interacting proteins were detected by Coomassie Blue staining (Fig. 1). Six protein bands that were present in Tec pull-downs, but not in control GST pull-downs, were excised and identified by mass spectrometry. The cytoskeleton-related proteins α-actin, Wiskott-Aldrich Syndrome protein (WASP), and WASP-interacting protein (WIP) were found to interact with both Tec and Itk SH3 domains (Fig. 1), indicating that they are unlikely to be Tec-specific negative regulators. The identification of WASP in Itk SH3 pull-downs confirms a previous report (40) and is consistent with a role for Itk in regulating actin polymerization (41). In contrast, dynamin (a GTPase), SOS1 (a guanine nucleotide exchange factor for Ras), ASAP1 (a GTPase-activating protein for Arf), and SHIP1 were observed to bind selectively to Tec (Fig. 1).

SHIP1 is known to negatively regulate Btk membrane localization by de-phosphorylating PtdIns 3,4,5-P3 (16, 17). Therefore we focused on SHIP1 as a potential negative regulator of Tec. To confirm that the SH3 domain interaction with SHIP1 was specific to Tec, the samples shown in Fig. 1 were Western-blotted with a SHIP1 antiserum. SHIP1 was detected only in Tec SH3 pull-downs from HUT78 cell lysates (data not shown), which is consistent with SHIP1 expression in HUT78 but not Jurkat (42).

To determine whether the SH3 domains of the other Tec family PTKs could interact with SHIP1, GST-SH3 pull-downs were performed with lysates of 293T cells transiently transfected with Myc-tagged SHIP1, followed by anti-Myc Western blotting (Fig. 2A). The Tec SH3 domain interacted relatively strongly with SHIP1, confirming the observations made using HUT78 cells (Fig. 1). SHIP1 interacted relatively weakly with the Btk SH3 domain, but not with Bmx, Itk, Txk, or mutant Tec SH3 domains (Fig. 2A). To address whether a similar relationship holds for SHIP2, GST-SH3 pull-downs were performed using lysates from 293T cells transiently transfected with Myc-tagged SHIP2 (Fig. 2B). SHIP2 was detected relatively efficiently in Tec SH3 pull-downs, weakly in Btk SH3 pull-downs, and not at all in Bmx, Itk, Txk, or mutant Tec SH3 pull-downs (Fig. 2B). To confirm that similar quantities of each GST-SH3 fusion protein were used in these experiments, the pull-downs were Western-blotted with an anti-GST mAb (Fig. 2C).

These data show that the Tec SH3 domain interacts with SHIP1 and SHIP2 more efficiently than do other Tec family SH3 domains, supporting the possibility that SHIP1 and SHIP2 selectively regulate Tec.

The Tec SH3 Domain Can Interact with Two Distinct Regions of SHIP1—In order to further characterize the interaction between Tec and SHIP1, we attempted to map the SH3 binding site on SHIP1. Six canonical SH3-binding motifs with the se-
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**SH3-dependent manner.** Yeast two-hybrid analyses were performed using a bait of SHIP1 fused to the GAL4 DNA-binding domain and a prey of Tec, Itk, or Btk fused to the GAL4 activation domain. On histidine-deficient media (−His, right panel), a SHIP1 interaction with Tec reconstituted the activity of the GAL4 transcription factor, leading to His transcription and growth of the yeast. No growth was observed for Tec with an inactivating mutation of the SH3 domain, for Itk or Btk, or in the absence of SHIP1. As a control, on His-sufficient media (+His, left panel), similar growth was observed for each yeast strain.

**domain of Tec is sufficient to interact with SHIP phosphatases.** To determine whether full-length Tec interacts with SHIP1 in intact cells, we performed immunoprecipitation using antibodies that would precipitate endogenous proteins in cell lines, or epitope-tagged forms of Tec and SHIP1 in transfected cells. An interaction could not be detected (data not shown), which suggests that Tec and SHIP1 do not interact in intact cells or that the interaction is not maintained during cell lysis and immunoprecipitation. To distinguish between these possibilities we performed a yeast two-hybrid assay, as this is a more sensitive way to detect weak and transient interactions in living cells (44). The bait was SHIP1 fused to the GAL4 DNA-binding domain and the prey was Tec, or other family members, fused to the GAL4 activation domain. In this system, a SHIP1-Tec interaction would reconstitute the activity of the GAL4 transcription factor, leading to histidine (His) transcription and growth of the yeast on His-deficient media. In Fig. 4, each yeast strain grew on control His-sufficient media (left panel), but on His-deficient medium growth was only observed in the presence of both SHIP1 and Tec (right panel). An intact Tec SH3 domain was required for growth and, importantly, neither Itk nor Btk could rescue growth (Fig. 4). These data suggest that Tec, but not Itk or Btk, can interact with SHIP1 in vivo and that this interaction requires the Tec SH3 domain.

**SHIP1 and SHIP2 Inhibit Tec Function**—SHIP1 is known to negatively regulate Btk by indirectly preventing its membrane localization through de-phosphorylation of PtdIns 3,4,5-P3 (16, 17). It is not known if SHIP1 can negatively regulate Tec in the same way, and possibly also through a novel mechanism mediated by direct interaction with the SH3 domain. To address this, Jurkat T cells, which do not express SHIP1 (42), were used as a model system to measure Tec function in the presence or absence of transfected SHIP phosphatases. In Fig. 5, Jurkat cells were co-transfected with HA-tagged Tec (5 μg) and two different doses (1.25 or 5 μg) of Myc-tagged forms of wild-type SHIP1, phosphatase inactive SHIP1, or wild-type SHIP2, and an NFAT-luciferase reporter construct. Expression of each construct was confirmed by Western blotting with anti-Myc and anti-HA mAbs (Fig. 5, lower panel). In unstimulated cells (Fig. 5, upper panel), Tec-induced NFAT activation was potently inhibited by SHIP1 and SHIP2 (94 and 92% inhibition with 5 μg of SHIP1 and SHIP2, respectively). This inhibitory effect required phosphatase activity, as the phosphatase inactive form of SHIP1 did not inhibit Tec. To address whether SHIP
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Fig. 5. SHIP1 and SHIP2 inhibit Tec-induced NFAT activation. Jurkat T cells were transfected with an NFAT-luciferase reporter construct, a β-gal construct, and Myc-tagged wild-type (WT) or point-mutated Tec. The slightly lower level of Tec activation was used as a readout for Tec function. Inactivation of the SH3 Domain Generates a Hyperactive Form of Tec—Since SHIP phosphatases can negatively regulate Tec (Fig. 5) and the Tec SH3 domain is both necessary (Fig. 4) and sufficient (Figs. 1–3) for interaction with SHIP, it is not known if SHIP1 also affects Tec in this manner. To address this issue, GFP-tagged Tec was expressed in Jurkat T cells in the presence or absence of SHIP1, and the cells were visualized by fluorescence confocal microscopy. In the absence of SHIP1, Tec was largely membrane-localized (Fig. 7A), as previously reported (27, 30), which is consistent with the relatively high level of PtdIns 3,4,5-P3 in Jurkat (42). As we have shown before (30), Tec exhibited a punctate localization pattern at the cell surface, which was most apparent when the z-sections for each image were merged to generate a two-dimensional projection image (Fig. 7A). In the presence of SHIP1, however, Tec was predominantly localized to the cytoplasm and the punctate localization pattern largely disappeared (Fig. 7B). These data show that SHIP1 inhibits membrane localization of Tec.

A Constitutively Membrane-targeted Form of Tec Is Refractory to Inhibition by SHIP1—Since SHIP1 inhibits both Tec membrane localization (Fig. 7) and Tec function in a phosphatase-dependent manner (Fig. 5), these effects are potentially mediated by de-phosphorylation of PtdIns 3,4,5-P3. A prediction of this model is that artificially targeting Tec to the membrane should relieve SHIP1 inhibition. To test this hypothesis, we examined the effect of SHIP1 on the function of HA-tagged versus membrane-targeted Tec (Myr-Tec, containing the Src myristylation sequence at the N terminus). In Fig. 8, Jurkat cells were transfected with HA-Tec or Myr-Tec in the absence or presence of Myc-tagged SHIP1 and NFAT-luciferase activation was used as a readout for Tec activity. As shown previously (Fig. 5), SHIP1 potently inhibited NFAT activation by HA-Tec. In contrast, SHIP1 did not substantially inhibit NFAT activation by Myr-Tec. The slightly lower level of NFAT activity induced by Myr-Tec relative to HA-Tec is likely caused by lower expression levels, as measured by Tec Western blotting. These data suggest that SHIP phosphatases interact with and inhibit Tec by impairing membrane targeting to PtdIns 3,4,5-P3.

DISCUSSION

We have previously found that Tec is unique among Tec family PTKs in its capacity to signal constitutively when over-
mechanisms are probable and the hypothesis that underlies this study is that Tec is negatively regulated by a novel Tec-interacting protein.

Using a proteomic approach we identified the inositol phosphatases SHIP1 and SHIP2 as Tec SH3 domain-interacting proteins. We further demonstrated that the Tec SH3 domain is necessary for the SHIP1-Tec interaction in intact cells and that other Tec family PTKs do not interact with SHIP1. Since SHIP1 can negatively regulate Btk membrane localization by de-phosphorylation of PtdIns 3,4,5-P$_3$ (16, 17), SHIP phosphatases are attractive candidate negative regulators of Tec. A number of predictions of this hypothesis were tested in a T cell line model system for Tec function. The predictions were that SHIP phosphatases would inhibit Tec membrane localization and Tec function, an SH3 domain-mutated form of Tec would be hyperactive, and that artificially targeting Tec to the membrane would bypass the inhibitory effect of SHIP1. Each of these predictions was confirmed experimentally, thereby confirming that SHIP phosphatases interact with and negatively regulate Tec in vivo.

We speculate that the interaction between Tec and SHIP phosphatases is regulated by auto-phosphorylation of a tyrosine residue in the Tec SH3 domain, which is thought to occur upon Tec activation (6, 7). Importantly, the predicted auto-phosphorylation site is within the ligand-binding pocket of the SH3 domain (12, 45) and phosphorylation is proposed to modulate its binding specificity (46). Thus upon activation, phosphorylated Tec may exhibit reduced binding affinity for SHIP1, and the proteins may dissociate, allowing Tec translocation to PtdIns 3,4,5-P$_3$ in the membrane. Tec may subsequently be down-regulated by de-phosphorylation of its SH3 domain by a phosphatase such as PTP20, which interacts with the Tec SH2 domain and can de-phosphorylate Tec (47). This would allow re-association of Tec with SHIP phosphatases and their de-phosphorylation of local PtdIns 3,4,5-P$_3$, thus inhibiting Tec membrane localization to complete the cycle of regulation. This is unlikely to be the only mechanism of Tec regulation. Indeed, proteins that regulate Btk, such as IBtk (13), Sab (14), and PKC$\beta$ (15), may also regulate Tec. Furthermore, negative regulation is not the sole function of the SH3 domain of Tec, since it can also mediate recruitment to activated CD28 during T cell signaling (48, 49).

A striking feature of the Tec-SHIP1 interaction is that two distinct regions of SHIP1 are sufficient to interact with the Tec SH3 domain. These are the SH2-phosphatase interdomain region and the C terminus. We did not map the precise SH3-binding motifs, although multiple PXXP motifs are present within these two regions of both SHIP1 and SHIP2, some of which are canonical K/RXXPXXP or PXXPXXK/R sequences (43). We cannot rule out the possibility that non-PXXP motifs are involved (43). This may be relevant to the SH2-phosphatase interdomain region, since neither of the two canonical PXXP motifs were sufficient for SH3 binding (data not shown). Regardless of the precise binding motifs, our data suggest that one SHIP1 molecule has the potential to interact with two Tec molecules. Such an interaction is not without precedent in cell signaling. For example, the guanine nucleotide exchange factor SOS interacts with two Ras molecules, one of which (Ras-GTP) activates SOS and the other of which (Ras-GDP) is a SOS substrate (50). Thus it is possible that an interaction of two Tec molecules with SHIP1 is functionally relevant. Indeed, structural studies have suggested that Tec family PTKs can form homodimers (10–12), and although the functional consequences of dimerization are not known, the interaction with SHIP1 may promote Tec dimerization.

Interestingly, Tec and SHIP1 are both known to interact expressed in lymphocyte cell lines (30). Therefore it is likely that regulatory mechanisms have evolved to prevent unwanted activation of endogenous Tec in resting cells. One such mechanism appears to operate at the level of Tec protein expression, since Tec is expressed at substantially lower levels than Itk and Btk in primary lymphocytes, but is up-regulated upon T cell activation and in effector T cells (30). However, additional...
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with Dok family adapter proteins. The Tec SH2 domain interacts with phosphotyrosine motifs within Dok-1 and Dok-2 and Tec is functionally inhibited by these adapters (51, 52), whereas NPXY motifs within the SHIP1 C terminus interact with the phosphotyrosine binding domains of Dok-1, Dok-2, and Dok-3 (53, 54). These findings suggest the existence of a Dok/SHIP1/Tec negative regulatory complex, or signalosome, that is regulated by tyrosine phosphorylation in a comparable manner to the LAT/Gads/SLP76/PLCγ1 signaling in TCR signaling (reviewed in Ref. 55). Recently a Dok-3/SHIP1 complex was shown to inhibit BCR signaling in a manner that did not involve inhibition of Btk (54). The possibility that Tec is the target of inhibition in this system has yet to be examined.

The interaction with Tec may provide answers to key questions concerning the mechanisms of SHIP1 inhibitory function. For example, SHP1 can negatively regulate BCR signaling even in the absence of FcyRIIB co-ligation with the BCR (56, 57), and SHIP1 inhibits signaling of cytokine receptors for IL-3 (22) and stem cell factor (SCF) (58) in myeloid cells. For each of these signaling responses the mechanism of SHIP1 receptor to the receptors is not clear. However, Tec is proposed to play a role in signaling from the BCR (28, 59), IL-3 (60), and SCF (61) receptors, suggesting that SHP1 may inhibit signaling by these receptors via an effect on Tec. A second question concerns the role of the relatively poorly characterized regions of SHIP1, namely the SH2-phosphatase interdomain region and the C-terminal tail. In particular, the C terminus is required for the inhibition of SCF-induced mast cell activation (58) and for FcyRIIB-mediated inhibition of BCR-induced calcium mobilization (62), but the mechanism is unclear. Since Tec is thought to positively regulate these signaling pathways (62), but the mechanism is unclear. Since Tec is expressed in myeloid cells (59, 60, 61), the Tec interaction with the SHIP1 C terminus may, in part, explain these findings.

In summary, we have shown that SHP1 and SHP2 preferentially interact with Tec, compared with other Tec family PTKs, and that SHIP phosphatases negatively regulate Tec by inhibiting membrane localization. A prediction of this model is that Tec would be hyperactive in cells that are deficient in SHIP family phosphatases. Interestingly, the SHP1*−/− mouse dies at a young age because of myeloid cell infiltration of the lungs (22, 23). Since Tec is expressed in myeloid cells (59, 60, 63), hyperactive Tec may be partly responsible for this aberrant myeloid activation. The future generation of SHP1*−/− mice will help to address this issue.

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