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SHP-1 and SHP-2 in T cells: two phosphatases functioning at many levels

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Summary

Tyrosine phosphorylation and dephosphorylation of proteins play a critical role for many T-cell functions. The opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) determine the level of tyrosine phosphorylation at any time. It is well accepted that PTKs are essential during T-cell signaling; however, the role and importance of PTPs are much less known and appreciated. Both transmembrane and cytoplasmic tyrosine phosphatases have been identified in T cells and shown to regulate T-cell responses. This review focuses on the roles of the two cytoplasmic PTPs, the Src-homology 2 domain (SH2)-containing SHP-1 and SHP-2, in T-cell signaling, development, differentiation, and function.

Keywords

SHP-1; SHP-2; T cells; motheaten mice; T-cell signaling; T-cell development

Introduction

Tyrosine phosphorylation and dephosphorylation of proteins comprise key regulatory events in many signal transduction pathways leading to proliferation, differentiation and death (1–3). The steady state level of tyrosine phosphorylation on any protein is determined by the opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). During the past two decades, much has been learned about the structure and physiological (and patho-physiological) functions of PTKs, while relatively less is known about the detailed roles of PTPs participating in signal transduction. Both transmembrane and cytoplasmic tyrosine phosphatases have been identified in T cells and shown to regulate T-cell responses. The two members of the Src-homology 2 domain (SH2)-containing PTPs, SHP-1 and SHP-2, and their role in T-cell function are the focus of this review.

General features of SHP-1 and SHP-2

Recent sequencing projects of the human genome have identified more than 100 putative protein tyrosine phosphatases (reviewed in 4). These PTPs can be sorted into four groups based on their substrate specificity. The largest group, the Cys-based class I PTPs, consists of the so-called ‘classical’ PTPs, which are true tyrosine-specific PTPs. This group can be further subdivided by their cellular localization into transmembrane (TM) and non-TM families. TM PTPs, like CD45 (reviewed in 5) and CD148 (reviewed in 6–9), are believed to be receptors for as yet undefined ligands. In T cells, there are only a few TM PTPs for which

a relatively clear role in signal transduction has been defined (reviewed in 10). One of the best studied is CD45, which is required for initiating and regulating T-cell receptor (TCR)-mediated signaling events (11). While a clear positive role for CD45 in TCR-mediated signaling has been established by many studies including homologous recombination experiments, in which abrogation of CD45 expression resulted in profound inhibition of T-cell development (12,13), a potential negative role in keeping Lck activity low prior to TCR activation has also been proposed (14–16).

In contrast to TM PTPs, many more non-TM PTPs have been identified and recognized for their regulatory roles (reviewed in 10,17). They contain a single catalytic PTP domain flanked by N- or C-terminal extensions important for localization and/or regulation. These domains have been shown to contain membrane-targeting functions (18–20), PEST sequences (21,22), and similarities to cytoskeletal proteins (23) and lipid binding domains (24). Moreover, two mammalian PTPs have been identified that contain SH2 domains (25), SHP-1 (21,26–28) and SHP-2 (29,30), which are the focus of this review.

SHP-1 or PTPN6 (previously also referred to as SH-PTP1, PTP1C, HCP, or Hcph) is expressed predominantly in hematopoietic cells of all lineages and all stages of maturation, while it is expressed at low levels in epithelial cells (21,26–28,31). In contrast, the structurally close relative SHP-2 or PTPN11 (previously called SH-PTP2, PTP1D, PTP2c, SHPTP-3, or SYP) is ubiquitously expressed including in cells that express SHP-1 (32,33). Both SHP-1 and SHP-2 are composed of a central catalytic domain (containing the characteristic PTP signature motif VHCSAGIGRTG), two SH2 domains at their N-termini, and a C-terminus (reviewed in 34) with potential tyrosine phosphorylation sites (35,36) (Fig. 1). The SH2 domains have been shown to be important for localization as well as for activity regulation (reviewed in 17,37). At the basal state, the N-terminal SH2-domain is intramolecularly associated with the PTP domain, thereby repressing its activity (38–40). Upon engagement of the SH2 domains, this suppression is released leading to a activation of the phosphatase (41,42). *In vitro* data suggest that besides the SH2 domains, the C-terminus may also be involved in regulation of the PTP activity of SHP-1 as shown by an increase in activity of SHP-1 proteins lacking the last 35 or 41 amino acids, respectively (41,43). In addition, two tyrosines in the C-termini of SHP-1 (Y536 and Y564) and SHP-2 (542 and 580) have been shown to become phosphorylated upon various stimuli, which might further influence the function and activities of these PTPs (36,44).

Regulation of tyrosine phosphatases through tyrosine phosphorylation

It has been difficult to evaluate the precise role of the tyrosine phosphorylation sites on these phosphatases, since these sites provide excellent substrates for the PTPs themselves and are very labile *in vitro* (35). Despite such hurdles, it has been shown that tyrosine phosphorylation of the C-terminal Y536 (upon insulin stimulation) in SHP-1 increases its PTP activity (45), while another study showed phosphorylation of Y564 by Lyn correlated with an increase in activity (46). To circumvent the problem of auto-dephosphorylation, non-hydrolysable phosphomimetic analogs have been used to replace the tyrosines, and replacement of Y542 or Y580 in SHP-2 caused an increase in basal activity and an overall more effective SHP-2 (47). Using an elegant *in vitro* system of protein ligation with incorporation of phosphonate analogues, the same group demonstrated that phosphorylation of Y536 increases SHP-1 activity about 4–8-fold, while phosphorylation of Y564 causes at the most a 1.6-fold increase (48). Additionally for SHP-2, it has been proposed that phosphorylated tyrosines generate docking sites for SH2-domain containing proteins, thereby providing either an adapter function for SHP-2 or a way for preventing auto-dephosphorylation (36).

Besides having a regulatory function for enzymatic activity, the C-terminus of SHP-1 but not SHP-2 has also been implicated in containing localization signals. A tightly regulated nuclear localization sequence has been identified in the most C-terminal end of SHP-1 that promotes nuclear localization upon cytokine (49) or EGF stimulation (50) in non-hematopoietic cells. Moreover, it has recently been shown that in T cells, the C-terminus mediates a constitutive lipid rafts localization of SHP-1 (51). Structure-function studies identified a novel 6 amino acid motif (SKHKED localized to aa 557–562) within the SHP-1 C-terminus that is necessary and sufficient for lipid rafts localization (52). A splice variant of SHP-1, SHP-1L, which differs in the C-terminus has also been identified (53). SHP-1L is longer, with 67 aa replacing the C-terminal 38 aa of SHP-1 (Fig. 1). SHP-1L lacks the potential Y564 phosphorylation site, but retains the Y536 site. In addition, SHP-1L gains a proline-rich motif in the C-terminus, which could mediate binding to SH3-domain containing proteins. A similar proline-rich motif is found in the C-terminus of SHP-2 but is absent in SHP-1. However, at this point no proteins have been identified that bind to SHP-2 or SHP-1L via their SH3 domains, and it remains unclear whether these proline-rich domains play any role in the function and/or localization of the PTPs.

Mouse models with SHP-1 or SHP-2 deficiency

The existence of a genetic model for SHP-1 deficiency has significantly aided our understanding of the biological function of SHP-1 (54,55). A splicing mutation in the SHP-1 locus causes the *motheaten* (*me/me*) phenotype. This mutation leads to a frameshift near the 5'-end of the SHP-1 coding sequence, resulting in no detectable protein. *me/me* mice are therefore effectively SHP-1 nulls. These mice display a variety of disorders affecting virtually all hematopoietic lineages, resulting in death two or three weeks after birth (56). Prominent amongst these defects are myeloid hyper-proliferation and inappropriate activation, which leads to the characteristic '*motheaten*' skin lesions and interstitial pneumonia that causes their early demise. A different splicing mutation in the SHP-1 locus, resulting in insertion or deletion of a few amino acids within the phosphatase domain, causes the *motheaten viable* (*me^v/me^v*) mouse (54,57). These mice display a similar but less severe phenotype than *me/me* mice, with an increased life span of about 8–12 weeks (58). Presumably, the major difference between the wildtype SHP-1 and the *me^v* form is the diminished (to about 20% of wildtype) PTP activity. This further supports the importance of the SHP-1 PTP activity in the biology of the *me/me* mouse. (59). Based on the current biochemical, functional, and genetic experiments using dominant negative mutants of SHP-1 and *motheaten* mice, SHP-1 has been implicated in negative regulation of signaling events induced by receptors for antigens, cytokines and growth factors (reviewed in 60,61). However, due to the complex phenotype in the whole mouse, it has been difficult to discriminate between primary defects caused directly by the loss of SHP-1 and secondary effects due to the dysregulation of one cell type influencing others. In support of models proposing a cell-or lineage-intrinsic effect of SHP-1, many of the findings describing signaling effects due to loss of SHP-1 have been confirmed in cell lines expressing putative dominant negative mutants of SHP-1. In addition, studies using the recently described conditional knockout of SHP-1 (62) should provide further clarification to questions regarding primary and secondary effects of SHP-1 deficiency.

Studies using mouse models for SHP-2 function were hampered by the embryonic lethality (at midgestation) after targeted homozygous mutation of the *SHP-2* allele (63). To examine the role of SHP-2 in lymphopoiesis, chimeric recombination-activating gene-2 (RAG-2)/SHP-2 double deficient mice were generated. No T or B lymphocytes developed in the SHP-2^{-/-}RAG-2^{-/-} chimeras, indicating an absolute requirement for SHP-2 during lymphocyte development (64). However, the absence of developing T and B cells prevented any further functional studies. Recently, a conditional knockout of SHP-2 has been

described (65), which should aid in further characterization of this phosphatase in specific tissues. In particular, the role of SHP-2 during T-cell development is discussed below.

While it has been clearly demonstrated that SHP-1 and SHP-2 regulate signaling events in all hematopoietic lineages (reviewed in 37,66), this review specifically focuses on T cells and how these PTPs affect T-cell development, differentiation, and function.

Signaling in T cells

Signaling via the TCR

The TCR is composed of antigen-specific α and β chains along with several invariant chains of the CD3 complex, the γ , δ , ϵ , and ζ chains. When the $\alpha\beta$ TCR binds to the antigen presented on major histocompatibility complex (MHC) molecules, intracellular signals are propagated via the cytoplasmic tails of the CD3 chains (reviewed in 67–69). MHC molecules also serve as ligands for the coreceptors CD4 and CD8 on T cells. Current models of T-cell activation suggest that many of the CD3 chains, most notably the ζ chain, become heavily tyrosine-phosphorylated upon receptor engagement by antigen plus MHC. A similar effect can be evoked by crosslinking of the TCR with antibodies. The non-transmembrane tyrosine kinases Lck and Fyn are implicated in tyrosine phosphorylation of the CD3 chains. These phosphotyrosines provide docking sites for the recruitment of several SH2 domain-containing proteins, including ZAP-70. The subsequent activation of ZAP-70 is considered to be responsible for much of the amplification of tyrosine phosphorylation of cellular proteins (reviewed in 70–72). In addition to the TCR/CD3 chains and the associated kinases, adapter proteins, such as LAT, SLP-76, GADS, and Shc, are also essential for propagating TCR signaling (reviewed in 73–75).

Although much is known about the initiation of TCR-mediated signaling events, relatively less is known about the mechanism(s) by which TCR signaling is negatively controlled or terminated. Dephosphorylation of tyrosine phosphorylated cellular substrates could be an important part of this process. Several lines of data suggest a role for SHP-1 in T cell signaling. Thymocytes and peripheral T cells from *me/me* mice hyperproliferate in response to TCR/CD3 stimulation and show increased IL-2 production as well as prolonged activation of Lck and Fyn kinases and increased intracellular tyrosine phosphorylation of several substrates compared to normal thymocytes (76,77). In contrast, over-expression of SHP-1 in T cell lines leads to inhibition of TCR-mediated phosphorylation of the TCR ζ chain, association of Zap-70 with the TCR ζ chain, phosphorylation of LAT, and IL-2 production (51,52). These data suggest a model, in which SHP-1 is a negative regulator of TCR-mediated signaling in T cells, acting, at least in part, directly or indirectly through the inactivation of *src*-family kinases. However, the mechanism of this regulation remains to be determined.

In most of the systems, where SHP-1 has been shown to be a negative regulator, a direct binding to either the regulated receptor itself (78–83) or an associated co-receptor (84,85) has been shown. In T cells, no clear binding partner for SHP-1 has been identified so far. Depending on the cell line and the model of activation, several potential binding partners have been proposed, such as ZAP-70 (86), Vav (87), Lck (88), and a number of coregulators of TCR-mediated signaling (discussed below in detail). However due to the variations in experimental design of the studies and the difficulties in reproducing the results between groups, the precise molecular mechanism of SHP-1 recruitment into the TCR/CD3 complex remains unclear. Direct substrate(s) of SHP-1 also remain to be identified. SLP-76 (89), ZAP-70 (86,90), and Lck (88) have been proposed. However, many of the studies are controversial due to the difficulty in reproducing the results (89,91).

While SHP-1 is widely accepted as a negative regulator of signaling events, SHP-2 is thought to positively promote signaling. However in T cells, studies on the role of SHP-2 have created considerable ambiguity. Although SHP-2 fails to become tyrosine phosphorylated in response to TCR stimulation, it could be found in a complex with the immunoreceptor tyrosine-based activation motifs (ITAMs) of the TCR ζ chain, indicating an involvement in TCR-mediated signaling (92). In a follow-up study, a positive regulatory role for SHP-2 in TCR signaling was proposed (93). In this study, expression of a putative dominant negative mutant (SHP-2 C/S) in Jurkat T cells caused a decrease in TCR-induced phosphorylation of Erk2 without affecting TCR ζ chain phosphorylation. However in this study, the SHP-2 C/S mutant was C-terminally deleted by about 30 amino acids. It is unclear whether this deletion had any additional effects that would explain differences in results with later studies. Recently, it was shown that generation of reactive oxygen species (ROS) in response to TCR/CD3 stimulation causes a temporary inactivation of SHP-2 but not SHP-1 (94). To mimic conditions of an inactive SHP-2, the SHP-2 C/S mutant was expressed in Jurkat cells. In contrast to the previous study, the SHP-2 C/S mutant was expressed as full-length protein. Under condition of SHP-2 C/S mutant protein expression, increased multimeric complex formation with LAT-GADs, SLP-76, Vav, and ADAP was observed. Moreover, expression of the putative dominant negative mutant caused an increase in LFA-1 clustering and adhesion to fibronectin suggesting that SHP-2 downregulates cell adhesion in response to TCR-mediated stimulation.

To assess SHP-2 function in a more physiological setting, transgenic mice were generated expressing the SHP-2 C/S mutant in a T-cell lineage specific manner. These mice display an overall normal thymic T cell development but exhibit an enhanced immune activation in the periphery in older mice (95). In these transgenic mice, SHP-2 C/S-expression does not affect the majority of TCR-induced tyrosine phosphorylation, ERK activation, and TCR-driven proliferation; however, LAT phosphorylation was consistently decreased and the magnitude of Ca^{2+} flux was also lowered. Considering that the overall TCR response was unchanged in SHP-2 C/S-expressing mice, it is likely that the phenotypes observed in the SHP-2 C/S transgenic mice may not be due to direct effects of SHP-2 on TCR-mediated signaling but rather on signaling pathways downstream of other receptors. In contrast to these results, mice with a SHP-2 deficiency in the T-cell lineage clearly show a decrease in TCR/CD3-driven proliferation and IL-2 production and also decreased induction of the activation markers CD69 and CD25 (96).

Taking all of these studies into consideration, there are numerous unresolved issues and contradicting results with many potential explanation for the observed discrepancies: (i) differences in expression levels, (ii) expression of a putative dominant protein vs. complete lack of SHP-2, (iii) the exact protein expressed (with or without C-terminal deletion), (iv) and/or compensatory phenotypes in transgenic mice, or (v) secondary effects on other signaling pathways. It is tempting to speculate that some of the difference between dominant negative mutant and lack of protein expression might be due to effects of SHP-2 that are independent of its PTP activity and are therefore not affected by a catalytically inactive protein. Such catalytically independent functions have been previously observed for other enzymes; for example, Lck can potentiate T-cell activation in a kinase-independent manner (97). Additional studies are needed to further address these issues; such as the generation of a mouse expressing a knockin SHP-2 C/S mutant, which should provide some clarification. Nevertheless, it is likely that SHP-2 has several biological functions within T cells. It might act both a signaling promoter as well as a negative regulator, especially in the context of inhibitory receptors of the TCR as discussed below.

SHP-1 localization to lipid rafts and its effect on T-cell signaling

The importance of the subcellular localization of signaling molecules during TCR activation has been re-emphasized recently. In particular, the critical role of specific membrane microdomains for optimal TCR signaling has been recognized (reviewed in 98–101). Cell membranes are composed of proteins and lipids, such as cholesterol and various glycopospholipids and sphingolipids that form microdomains within the membrane. Based on their biophysical properties, glycopospholipids tend to display a mobile fluid phase, whereas sphingolipids show a more tightly packed higher organization (reviewed in 102). Moreover, gaps between the fatty-acyl chains of the sphingolipids are filled with cholesterol, thereby forming a closely-packed lateral lipid cluster, the so-called lipid rafts, in an unsaturated glycopospholipid environment (reviewed in 103,104). Due to their biophysical properties, these cholesterol/sphingolipid rafts are insoluble in non-ionic detergent at 4°C and can be isolated as low-density complexes in sucrose gradients. They have also been referred to as detergent-insoluble glycolipid-enriched complexes (DIGs) (105), low-density Triton-insoluble fraction (LDTI) (106), or glycolipid-enriched membrane domains (GEMs) (107). Even though the initial models proposed a central role for lipid rafts in TCR-mediated signaling through mediating the assembly of complexes, there have been controversies regarding the definition and importance of lipid rafts (108). These controversies were comprehensively discussed in a review by Munro (109), where it was emphasized that the existence and function of lipid rafts in initiating and propagating signal transduction events downstream of receptors are less established than often assumed. However in a recent study, the condensation of plasma membrane at the site of TCR activation was visualized, which confirmed the presence of lipid rafts (110). One important novel contribution of the more recent studies has been that protein-protein complexes work in concert with lipid rafts to critically regulate early T-cell signaling and the proper formation of the immunological synapse (108,110).

Several key players in early signal transduction pathways downstream of the TCR, such as the ζ chain of the TCR/CD3 complex, Lck, Fyn, ZAP-70, Shc, LAT, SLP-76, and PLC γ 1, have been shown to localize either constitutively or upon stimulation to the rafts fraction (107,111–113). A critical role for both LAT and SLP-76 has been demonstrated by successfully reconstituting LAT-deficient Jurkat T-cell lines with LAT or SLP-76 deliberately targeted to the rafts (111,114). Interestingly, in T-cell lines as well as in primary thymocytes, 20–30% of SHP-1 localizes constitutively to lipid rafts (51). The functional importance for lipid rafts localization of SHP-1 was demonstrated by experiments showing that the expression of catalytically active mutants of SHP-1 lacking the lipid raft-targeting motif has no detectable effect on TCR-mediated signaling while expression of full length SHP-1 or mutants targeted to lipid rafts cause an inhibition of TCR-mediated signaling, as evidenced by loss of LAT phosphorylation and inhibition of IL-2 production (52). Somewhat surprisingly, the splicing alternative SHP-1L still localizes to lipid rafts although it lacks the targeting motif identified in SHP-1 (V. Fawcett and U. Lorenz, unpublished observation). The mechanism of this localization is unclear. It is possible that the alternative C-terminus contains another raft targeting motif or SHP-1L is targeted to lipid rafts via binding to another lipid rafts-localized protein, potentially via the Proline-rich motif unique to SHP-1L. The available data for SHP-2 and rafts localization are very limited. In one study, it was shown that SHP-2 can be found in a complex with the lipid rafts localized adapter protein LIME, which promotes T-cell activation (115). More recently, it has been reported that upon CD4 crosslinking a fraction of SHP-2 transiently localizes to lipid rafts (116). However, the exact mechanism of the translocation and any functional consequences remain to be tested.

Signaling via inhibitory receptors of TCR signaling

T-cell activation is a tightly controlled process with regulatory mechanisms in place not only during the initial activation but also later via the engagement and activation of inhibitory receptors. Inhibitory receptors are transmembrane proteins that inhibit signaling via immunoreceptor tyrosine-based inhibitory motifs (ITIMs; V/L/I X pY XX L/V) (reviewed in 117) or an immunoreceptor tyrosine-based switch motif (ITSM)(T X pY X X V/I) (118), which upon phosphorylation recruit proteins that mediate the inhibitory effect. Based on pY peptide library screenings, it is predicted that ITIMs and ITSMs provide good consensus motifs for the SH2 domains of both SHP-1 and SHP-2 (119–121). Consistent with the predictions, several of the inhibitory receptors have been shown to recruit SHP-1 and/or SHP-2. The below discussed examples of inhibitory receptors are thought to mediate their inhibitory function at least partially through SHP-1 and/or SHP-2. In addition to these reviewed examples, a number of additional inhibitory receptors have been found in a complex with SHP-1 and/or SHP-2. However, most of these receptors are only expressed in subsets of T cells or binding to the PTPs is only observed under certain conditions. Instead of listing every receptor, this review will concentrate on some of the more widely expressed receptors.

The carcinoembryonic antigen related cell adhesion molecule 1 (CEACAM1), an inhibitory receptor containing ITIM motifs, is induced upon activation of T cells (reviewed in 122). While it has previously been shown that CEACAM1 can bind to SHP-1 (123,124) and SHP-2 (124), it was recently demonstrated that SHP-1 is the mediator of the inhibitory function and acts on proximal TCR signaling by decreasing tyrosine phosphorylation of the TCR ζ chain and ZAP-70 (125,126).

CD5 is another inhibitory receptor expressed on T cells that has been shown to bind SHP-1 via association with ITIMs (127). However, a later study analyzing the phosphorylation of individual tyrosines suggests that the predicted binding site for SHP-1 is not phosphorylated by Lck and that the CD5/SHP-1 complex is therefore not likely forming at high stoichiometry (128). The role of SHP-1 in CD5-mediated inhibition remains therefore ambiguous.

The leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1) has been shown to be constitutively associated with SHP-1 in human T cells (129). Furthermore, it was proposed that due to the SH2-engagement, a fraction of SHP-1 protein was active basally in resting T cells. While association between human LAIR-1 and SHP-2 was not detectable (130), the murine homologue mLAIR-1 surprisingly recruits SHP-2 but not SHP-1 when tyrosine phosphorylated (131). Although the concept of mediating an inhibitory effect via the recruitment of a PTP is attractive, for most of the inhibitory receptors it is unclear whether binding to the PTPs is essential or alternative ways can be used to transmit the effect. In the case of LAIR-1, it was shown that in the chicken B-cell line DT-40 inhibition can be executed in the absence of any PTP. It was proposed that instead the kinase Csk mediates the inhibitory effect (132).

One of the best-studied inhibitory receptors is cytotoxic T-lymphocyte antigen-4 (CTLA-4) (reviewed in 133). CTLA-4 does not have ITIMs but contains two tyrosines that could provide potential SH2-domain binding sites. In fact, SHP-2 has been proposed to bind to CTLA-4 and mediate some of the inhibitory effect (134,135). However, later studies questioned the role of SHP-2 in CTLA-4 function (136). Moreover, depending on the experimental setting, the predicted binding site for SHP-2 on CTLA-4 is not always required for optimal CTLA-4 function (136–138). Whether SHP-1 plays any role in CTLA-4 function is unclear, although SHP-1 activity is found associated with CTLA-4 (139).

Two newer members of the CD28/CTLA-4 family are programmed death-1 (PD-1) and B and T-lymphocyte attenuator (BTLA), which are inducibly expressed on T cells (reviewed in 140). PD-1 has both an ITIM and an ITSM, which have been shown to bind SHP-1 and SHP-2 (118). In a similar fashion as the other inhibitory receptors, PD-1 acts on proximal TCR signaling by decreasing tyrosine phosphorylation of the TCR ζ chain and ZAP-70 (141). Two ligands, PD-L1 and PD-L2, have been identified for PD-1, which upon binding to PD-1 induce rapid phosphorylation of SHP-2 (142). In a study of human T cells, it was reported that upon PD-L2 binding, only SHP-2 was activated. In this study, additional inhibitory effects on integrin-mediated adhesion were observed (143). BTLA is unique with respect to its ligand. Although BTLA belongs to the CD28 family, its ligand, herpesvirus entry mediator (HVEM), belongs to the family of tumor necrosis factor receptors (TNFRs) (144). BTLA contains two ITIMs that have been shown to recruit SHP-1 and SHP-2 (145), which become phosphorylated themselves upon binding. BTLA engagement has an inhibitory effect on IL-2 production (146).

While binding of SHP-1 and/or SHP-2 to the various individual inhibitory receptors has been associated with biological functions and in some cases seems to be critical for the inhibitory function, none of the receptors by themselves are the major or dominant mediator of PTP function in T cells. Loss of function of any of these receptors causes phenotypes that differ from loss of PTP function. It is more likely that a combination of the inhibitory receptors provides the appropriate regulatory signal, and that the involvement of individual receptors depends on the stage of development, differentiation, activation, and lineage commitment of the T cell as well as whether the T cell is of human or murine origin.

SHP-1 and T-cell antagonism

Most events leading to T-cell activation are initiated by binding of the TCR complex to MHC/peptide presented by an antigen presenting cells. However, an existing threshold for TCR activation requires a certain minimal strength for the binding between the TCR and MHC/peptide complex. High affinity agonist peptides are able to induce a productive T-cell response. In contrast, low affinity antagonist peptides not only fail to induce a T-cell response but render the T cell resistant to stimulation by agonist peptides (reviewed in 147). Several studies have suggested a role for SHP-1 in the discrimination between agonist and antagonist signaling. Using a T-cell clone that expresses TCRs of two different specificities, it was shown that exposure to agonist peptide for one TCR and antagonist peptide for the other TCR caused a decreased proliferative response and tyrosine kinase activity compared to agonist alone suggesting a cross-talk between the two receptors (148). At the molecular level, it was shown that SHP-1 was rapidly recruited to the TCR exposed to antagonist peptide, whereas recruitment into the agonist-stimulated TCR complex was delayed. Furthermore, the data suggested that the recruited SHP-1 could spread to non-engaged TCRs, thereby preventing future signaling via this TCR. In a subsequent study (88), the following model for ligand discrimination was proposed: under conditions of weak ligand binding, Lck phosphorylates SHP-1 on Y564 resulting in the rapid recruitment of SHP-1 to the TCR complex via binding of the Lck SH2 domain to phosphorylated SHP-1. Once recruited, SHP-1 would then dephosphorylate and inactivate the Lck kinase, resulting in receptor desensitization. In contrast, under conditions of strong ligand binding, activated Erk kinase would phosphorylate Lck at Serine 59, which inactivates the SH2 domain and prevents recruitment of SHP-1 to the TCR complex thereby allowing signaling to continue. Another study also demonstrated that a combinatorial exposure of T cells expressing a Tg TCR to agonist and antagonist peptides causes an increase in SHP-1 activity compared to individual exposures. Moreover, the inhibitory function of the antagonist was dependent on a functional SHP-1 protein, as expression of a catalytically inactive SHP-1 mutant prevented any inhibitory effect mediated by the antagonist (149).

While these studies begin to address how T cells can discriminate between ligands, the proposed model of a negative feedback loop does not fully address how SHP-1 also plays a regulatory role under conditions of strong agonists. In the proposed model, SHP-1 is thought to be inactivated during the first 20 min, which would imply that any regulatory function would have to occur at a later time point. In a recent mathematical modeling paper, SHP-1 was classified as a digital regulator of the T-cell response, since there is a threshold for SHP-1 protein/activity above which the TCR-mediated signaling is inhibited (150). It was further suggested that until SHP-1 is expressed at a critical level (four times the average SHP-1 levels), strong agonists trigger the TCR-mediated signaling cascade without any inhibition. While this model is appealing, it is difficult to envision how this model could incorporate the hyper-responsiveness of SHP-1-deficient T cells.

Thymic T-cell development

A clear regulatory role for SHP-1 during T-cell development has been defined by several laboratories by studying either *motheaten* mice or transgenic mice expressing a dominant negative mutant of SHP-1 in the T-cell lineage (77,151–153). A brief summary of T-cell development in the thymus, as pertinent to this review is given below. The cell surface expression of the TCR, along with the co-receptors CD4 and CD8, the homing receptor CD44 and CD25 (the α chain of the IL-2 receptor) have been used to define the major differentiation stages of immature T cells in the thymus (reviewed in 154,155). T-cell progenitors (which originate in the bone marrow) colonize the thymus and first differentiate into TCR^{neg} double negative T cells (DN), named for their lack of CD4 and CD8 expression. DN thymocytes can be further subdivided into DN1, 2, 3, and 4, based on their CD44 and CD25 expression. During the DN2/DN3 stage, rearrangement of the TCR β chain begins, which upon successful rearrangement pairs with the surrogate α chain to form the preTCR. Surface expression and signaling via the preTCR/CD3 complex promotes DN3 to DN4 transition. DN4 cells differentiate into a double positive (DP) stage, expressing both CD4 and CD8. At the same time, the thymocytes start to rearrange the TCR α chain, which upon successful rearrangement will pair with the β chain to form the TCR $\alpha\beta$. While the majority of DP thymocytes die by neglect, the remaining DP cells will undergo either negative selection, which leads to their death, or positive selection, which via a TCR⁺CD4^{low}CD8⁻ transitional stage leads to further maturation and proliferation of selected cells, culminating in the production of either CD4⁺ or CD8⁺ single positive (SP) cells (reviewed in 156–160). After further maturation, the SP thymocytes leave the thymus and colonize the peripheral lymphoid organs such as spleen and lymph nodes.

Several studies have shown that modulation of the strength of signals from the TCR, for example by modifying the antigen dose or the antigen itself, can influence the outcome of positive and negative selection (161–163). Current models suggest that the TCR of an immature double positive thymocyte interacts with a putative endogenous antigen (presented by thymic epithelial cells or dendritic cells), and that the avidity of this T cell-antigen interaction determines the fate of the maturing thymocyte. Immature thymocytes expressing a TCR with a negligible or too low avidity will die due to neglect. Thymocytes that express a TCR of moderate avidity will be positively selected for further maturation, while those expressing a TCR of high avidity for the endogenous antigens will be negatively selected and deleted.

Initial analyses of the *me/me* mice had failed to detect any abnormalities in overall thymocyte composition as assessed by surface marker expression (76,164–166). However, since SHP-1 has been shown to control the strength of the TCR signal in the mature T cells, it was conceivable that the TCRs expressed on DP thymocytes in a *me/me* mouse would differ in their avidities from the ones selected in a normal mouse. If so, this might be better

revealed when the majority of the T cells expresses a specific transgenic TCR (Tg TCR). In fact, when MHC class II-specific DO11.10 TCR transgenic mice (167) are crossed onto the *motheaten* background, analyses of the progeny of these mice demonstrate that under conditions of SHP-1 deficiency negative selection is increased even in the absence of cognate peptide (77). Several other groups using different TCR transgenic systems have also reported regulatory effects of SHP-1 on positive and/or negative selection (151–153). These data clearly demonstrate a role for SHP-1 in the development of CD4⁺ and CD8⁺ T cells by controlling the thresholds for positive and negative selection.

As mentioned above, targeted mutation of the *SHP-2* allele is embryonic lethal at midgestation when homozygous (63). Moreover, no T or B lymphocytes developed in the *SHP-2*^{-/-}*RAG-2*^{-/-} chimeras, demonstrating an absolute requirement for SHP-2 during lymphocyte development (64). No cells of *SHP-2*^{-/-} origin could be detected in bone marrow or thymus of the *SHP-2*^{-/-}*RAG-2*^{-/-} chimeras, indicating the requirement for SHP-2 in early lymphopoiesis. To bypass the early developmental steps, transgenic mice were generated that express the putative dominant negative mutant SHP-2 C/S in the T-cell lineage at a later point of development under the control of the CD2 promoter (95). Analyses of these mice showed an overall normal thymic T-cell development both under conditions of endogenous TCR expression as well as under conditions of a transgenic TCR. A slight decrease in thymic cellularity in *SHP-2* C/S transgenic mice in the context of endogenous TCR expression became undetectable upon crossing onto a TCR transgenic background. Based on these results, it was concluded that SHP-2 does not play an essential role during T-cell development. However, this conclusion was challenged in recent study where SHP-2 was specifically deleted in the T-cell lineage. When mice carrying a conditionally targeted *SHP-2* allele were crossed to mice expressing Lck-Cre (which deletes floxed genes from the DN2/DN3 stages), the resulting T-cell-specific SHP-2 deficiency caused a defective T-cell development (96). The overall thymic cellularity was decreased by about 50%, which affected CD4⁺CD8⁺ DP, the SP subpopulations. In contrast, the DN population was increased by twofold. Further analysis of the DN population revealed that the lack of SHP-2 partially inhibited preTCR signaling as evidenced by a relative increase in the ratio of DN3:DN4 thymocytes. This block could not be overcome with anti-CD3 treatment, a measure to force thymocyte maturation at the DN3 stage indicating that SHP-2 is required for signaling downstream of the preTCR. At the molecular level, TCR/CD3 triggered ERK activation was decreased in thymocytes lacking SHP-2, providing further support for the positive regulatory role of SHP-2 protein. As discussed above, it is unclear whether some of the differences observed between mice expressing a putative dominant negative protein versus complete lack of SHP-2 expression might be due to certain functions of SHP-2 that are independent of its PTP activity.

Role of PTPs in Th1/Th2 T-cell differentiation

Upon activation of a naive T cell, and in the presence of appropriate other stimuli, T cells differentiate into Th1 or Th2 effector T cells, which are defined by their cytokine profiles and expression of lineage-specific transcription factors (168,169). It is thought that to a great extent the cytokine environment drives this decision, with IL-12 promoting a Th1 response and IL-4 supporting a Th2 response. Several studies have been reported that SHP-1 has an inhibitory effect on Th1 (170–172) and/or Th2 (173) differentiation. However, none of the studies show a change in skewing towards Th1 or Th2 as a consequence of SHP-1 activity, instead SHP-1 affects the degree of differentiation towards a Th1 or Th2 effector cell, under conditions that drive the respective Th cell differentiation. The inhibitory effects of SHP-1 are therefore most likely not at the level of lineage decision Th1 vs. Th2, but occur during the differentiation process. This inhibition could take place at several levels: (i) directly at the TCR-mediated signaling, which is required for differentiation, (ii) at the skewing

cytokine level, as it has been reported for T cells that SHP-1 is associated with the IL-4R and SHP-1 has been shown to function as negative regulator of IL-4 mediated signaling (173–176), or (iii) indirectly via an inhibitory cytokine such as TGF- β (170). Taken together, SHP-1 negatively regulates the differentiation process from a naive T cells to a Th1 or Th2 effector T cells and/or the expansion of differentiated T cells.

While there are fewer studies addressing any role of SHP-2 in the Th1/Th2 decision, there are indications that a Th2 phenotype is favored under conditions of decreased SHP-2 activity. In transgenic mice expressing the putative dominant negative SHP-2 C/S mutant in the T cell lineage, increased number of activated T cells have been observed (95). When SHP-2 C/S expressing T cells were cultured *in vitro* under non-skewing conditions, an increase in IL-4, IL-5, and IL-10 cytokine production was observed compared to wild type T cells. No difference was observed under Th1 conditions, but SHP-2 C/S T cells responded with increased Th2 differentiation to Th2 conditions, indicating that SHP-2 might have an inhibitory effect on Th2 skewing. It remains to be assessed whether SHP-2 deficient T cells show a similar preferential Th2 skewing.

SHP-1 and regulatory T cells

Immune tolerance is a complex process that requires a continuous balance between positive and negative signals. Over the last years, it has become increasingly clear that one of the important players involved in this process is a specialized subtype of T cells, the so-called regulatory T cell (Treg). Tregs have become one of the most studied subfields within immunology (reviewed in 177–189).

Immune tolerance and the prevention of autoimmune diseases are thought to occur at various levels of the immune system. During thymic T-cell development, most thymocytes expressing TCRs with high affinity for self-peptides undergo negative selection resulting in apoptosis of these cells, a process called central tolerance (reviewed in 190). Despite this elimination of auto-reactive T cells, every healthy adult still carries potentially harmful self-reactive T cells (191). In the periphery, such autoreactive T cells are rendered inert by mechanisms termed peripheral tolerance (192). A subpopulation of T cells, known as Treg cells, is thought to play a key role in tolerance and the prevention of autoimmunity. Treg cells have been shown to suppress the proliferation and function of effector T cells both *in vitro* and *in vivo*. In several mouse model systems, it has been demonstrated that functional Treg cells are involved in the suppression of an auto-immune response, while a depletion of this population promotes the development of autoimmune diseases (193–195).

Although the presence of Treg cells has been proposed for many years (196,197), their existence was rather controversial until very recently. This controversy was mostly due to contradicting observations based on the model system studied that postulated suppressive properties within different lymphoid populations (reviewed in 198). However, one type of Treg cells is now well accepted as a functionally suppressive T-cell subpopulation and is defined by expression of the transcription factors Foxp3 and surface expression of CD4 and CD25 (199). Naturally occurring CD4⁺CD25⁺Foxp3⁺ Treg cells are found at a relatively low frequency, 1.5–3% of total CD4⁺ T cells in human peripheral blood (200). In mice, CD4⁺CD25⁺ Treg cells compose ~5–10% of the splenic CD4⁺ T cells and ~2% of CD4⁺ T cells in the thymus (201). In addition to CD4 and CD25, a number of additional surface markers, such as GITR, CTLA-4, and CD103, have been associated with the Treg cell population (reviewed in 202). However, the precise mechanistic role these surface proteins play in Treg cell development and/or function is still under considerable debate. In addition, all of these marker proteins have been detected on non-regulatory T cells (203). Thus far, only the expression of the transcription factor Foxp3 has been shown to be restricted to Treg

cells. Moreover, expression of Foxp3 in T cells seems to be necessary and sufficient to induce the regulatory phenotype (204–206).

The precise developmental pathway of Treg cells still remains relatively unknown, although it is now well accepted that at least the so-called ‘natural’ Treg cells arise primarily in the thymus during the selection process (207). It has been shown that in TCR Tg mice, exposure of the developing T cells to the cognate peptide in the thymus caused an increase in the CD4⁺CD25⁺ Treg cell population. Interestingly, exposure to low affinity peptides failed to induce an increased Treg cell population (208), indicating that a strong TCR-mediated signal is required for the differentiation of Treg cells. Furthermore, expression of the specific peptide in the cortical epithelium resulted in an increase in the number of CD25⁺ Treg cells (209,210) consistent with the observation that radio-resistant cells direct CD25⁺ Treg cell development (208). A recent study additionally showed how the processing and presentation of a self-peptide could control the generation of Treg cells and that under conditions of optimal presentation, a substantial increase in the number Treg cells are achievable (211). While earlier studies proposed that Treg cells are induced to differentiate as a consequence of a strong positive selection signal, some more recent studies (212,213) suggest that Treg represent cells more resistant to negative deletion than developing conventional T cells and are therefore selectively enriched under conditions of increased negative selection. However, in all of these studies it has been hypothesized that Treg cells are selected in the thymus by high avidity interactions with a cognate peptide. Although there is a high interest in understanding the development of Treg cells, the knowledge at the molecular level is still mostly limited to the identification of a few proteins that are absolutely essential for the development of functional Treg cells.

The observation that *me/me* mice have increased percentages of CD4⁺CD25⁺Foxp3⁺ Treg cells identified SHP-1 as one of the first signaling molecules that influenced the ratio of conventional vs. regulatory T cells (213). In this study, it was shown that *me/me* mice displayed a 2–3-fold increase in percentage of Treg cells in the thymus and peripheral lymph organs, both in the context of endogenous TCR, as well as on a transgenic TCR background. Using fetal organ cultures derived from TCR transgenic mice, it was furthermore demonstrated that exposure to increasing amounts of cognate peptide caused deletion of developing conventional thymocytes with a concurrent enrichment of the CD4⁺CD25⁺ population, indicating a selective resistance to deletion. Fetal thymic organ cultures (FTOC) derived from *me/me* TCR-transgenic mice also displayed an enrichment of Treg cells compared to control FTOC upon exposure to the cognate peptide. These data suggest a pre-commitment/selection model for Treg cell development, where the developing thymocyte encounters a signal that pre-commits the cell towards the Treg lineage (Fig. 2). For positive selection and full differentiation into a functional Treg cell, a strong signal mediated via the TCR is required similar to the model of positive selection for conventional T cells. Whether SHP-1 directly affects this pathway remains to be seen, but it is clear that the percentage of Treg cells is increased in the absence of SHP-1. This could either be entirely due to increased deletion of conventional cells and a selective survival of Treg cells, or an increased deletion in combination with increased selection of Treg cells. SHP-1 is therefore one of the few proteins that seem to control the relative number of developing Treg cells. At this point, no role of SHP-2 in Treg development has been reported.

The functional definition of the CD4⁺CD25⁺ Treg cell is its ability to suppress proliferation and effector functions of other T cells while being anergic itself upon physiological, sub-optimal stimulation. Treg cells must be weakly stimulated in order to exert their suppressive effects, but once stimulated, Treg cells are thought to act in an antigen non-specific manner (193,214–216). However, a recent study suggests that under physiological conditions *in vivo*, Treg cells are antigen-specific and are only able to suppress effector T cells of the

same antigenic specificity (217), or are at least more efficient in the context of organ/antigen specificity (218). *In vitro*, the suppressive abilities of the Treg cells are connected to the anergic state, since addition of IL-2 not only breaks the anergy of the Treg cells but also abrogates their ability to suppress proliferation (203). Interestingly, strong activation of responder cells, e.g. via cross-linking of the TCR/CD3 complex or co-stimulation of CD28, renders them refractory to suppression (215). Although anergy is a hallmark of Treg cells *in vitro*, they are capable of considerable expansion *in vivo* without losing their suppressive ability (219,220). The mechanism by which Treg cells suppress the activation of other T cells is still unclear. *In vitro*, a requirement for cell-cell contact between the activated Treg cell and the suppressed effector T cell has been observed. CD4⁺CD25⁺ Treg cells produce IL-10 and TGF- β ; however at least in *in vitro* assays, cytokine secretion is not essential for suppressive activity (194). *In vivo*, Treg cells have been implicated in the control of a variety of autoimmune diseases, such as intestinal inflammation, autoimmune gastritis, diabetes, ovarian disease, arthritis and experimental autoimmune encephalomyelitis (193,221–225). While many studies have addressed the *in vivo* and *in vitro* function of Treg cells, the intracellular signaling of Treg cells are still relatively poorly understood. It has been shown that Treg cells have levels of SHP-1 comparable to conventional T cells (213). Moreover, SHP-1-deficient Treg cells are functional. Although SHP-1-sufficient and -deficient Treg cells are comparable in their overall gene expression profile as evidenced by microarray data, *me/me* and *me/+* Treg cells are more effective in their suppressive activities than *+/+* Treg cells indicating that SHP-1 may act as a ‘brake’ in Treg function (M. Sankarshanan, T. Iype, and U. Lorenz, manuscript in preparation). At this point, no studies have been reported assessing the role of SHP-2 in Treg function. Despite the popularity of regulatory T cells and the numerous papers published every week, very little progress has been made with respect to the role of signaling molecules for Treg cell development and/or function. The major reasons are the lack of a Treg cell line and the scarcity of primary Treg cells, which prevent most of the biochemical analyses that historically provided insights in our mechanistic understanding of signaling in conventional T cells. Insights into Treg signaling are therefore mostly limited to studies of wild type and genetically modified mice. Recently several groups developed Foxp3-specific Cre lines (226,227), which should allow to specifically target the Treg lineage, and are expected to dramatically improve the ability to study the role of individual signaling molecules including SHP-1 and SHP-2.

Conclusions: what we know and what needs to be explored

While it is widely accepted that both SHP-1 and SHP-2 are critical regulators of T-cell development, function, and differentiation, there are numerous outstanding questions at the molecular level that remain to be addressed. All of the available data support the notion that SHP-1 is a negative regulator of TCR-mediated signaling; however it remains unknown what specific substrates are targets for SHP-1 mediated regulation, how SHP-1 is brought into the signaling complex, and which other pathways important for T cell signaling are also regulated by SHP-1. Moreover our current understanding is very limited with respect to SHP-1-regulated pathways in T cells and the physiological consequences for the immune response.

It has been shown that mice with decreased SHP-1 activity are hypersensitive to MOG peptide-induced experimental autoimmune encephalitis (EAE) (172). For a long time, the model of EAE was thought to be mediated by Th1 T cells. It was therefore concluded that the EAE hypersensitivity observed in *motheaten* was due to an increase in Th1 T-cell differentiation and/or activation. However, recent studies have demonstrated that although Th1 cells are required for the development of EAE, the newly identified Th17 cells are major pathogenic T cell subpopulation during EAE progression (reviewed in 228). To fully understand why a decrease in SHP-1 activity causes a more severe case of autoimmunity, it

will be necessary to separate the various disease components. There are many potential reasons (acting alone or in combination) as to why a more severe form of autoimmunity might develop in the *motheaten* background: for example, there could be more Th1 or Th17 cells due to the increased signaling in the absence of SHP-1, with perhaps greater activity; alternatively, the number and activity of newly generated Treg cells could be decreased, or the effector cells could be more resistant to suppression by Treg cells; there could also be increased IL6 production by activated macrophages and/or dendritic cells that skew development towards Th17 cell type. None of the current studies have addressed these questions in the complexity of a whole animal. Moreover, while analyses of the *motheaten* mice have majorly contributed to our current understanding of SHP-1 function and biology, many of the above outlined details cannot be addressed in an animal where all lineages are affected. Future studies will therefore have to make use of lineage-specific knockouts or expression of mutant SHP-1 proteins, specifically in the T-cell lineage to pick apart specific mechanisms.

The role of SHP-2 in T cells is even less understood due to numerous contradicting reports. It is most likely that SHP-2 has different functions in T cells depending on the environment and signaling pathways involved, and also the developmental stage of the T cells. To dissect these pathways and to gain a true understanding of the function of SHP-2 during an immune response, studies will have to make use of the recently generated conditionally targeted mice; moreover, the development of new tools will likely be required, such as knockin mice expressing various SHP-2 mutant proteins. Nevertheless, the studies to date have clearly put SHP-1 and SHP-2 as key players in T cell development, and immune responses, and have highlighted the importance of non-transmembrane tyrosine phosphatases in T cells. Future studies using newer tools may really help us finely dissect the roles of these phosphatases, and in turn help us better understand how positive and negative signals may be carefully orchestrated to achieve an optimal immune response.

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Fig. 1. Model of the two SH2-domain containing PTPs, SHP-1 and SHP-2, and the splice variant SHP-1L

Defined domain within the proteins are indicated. Moreover, the PTP signature sequence, potential tyrosine phosphorylation sites, and the lipid rafts targeting peptide are marked. The differences in the C-termini between SHP-1 and SHP-1L are shown.

Fig. 2. Two alternative models depicting thymic development of natural $CD4^{+}CD25^{+}Foxp3^{+}$ Treg cells

In the model of increased selection (model 1), thymocytes undergoing the selection process at the DP stage are ‘undecided’ and can develop into Treg or conventional non-Treg cells depending on the strength of signal they encounter. $CD4^{+}CD25^{+}$ Treg cells are selected in response to a defined range of TCR signaling strength that falls between positive selection of non-Treg cells and negative selection/deletion (left panel). Under conditions of increased TCR signaling strength (right panel), such as exposure of the TCR to a high affinity ligand or loss of the negative regulator SHP-1, the selection process shifts towards positive selection of $CD4^{+}CD25^{+}$ Treg cells and greater deletion of non-Treg cells, resulting in an increase in the absolute number of $CD4^{+}CD25^{+}$ T cells. In contrast, in the model of pre-commitment/selection (model 2), a defined fraction of the $CD4^{+}CD8^{+}$ DP thymocytes is already committed to the $CD4^{+}CD25^{+}$ Treg cell lineage. Whether the pre-commitment is driven by encounter with a signal or by a stochastic process is unknown at this time. The remaining non-committed DP cells have the potential to develop into conventional non-Treg T cells upon passing of positive/negative selection, but lost the potential to develop into Treg cells. The cells pre-committed to the Treg lineage still require additional signals and might undergo a selection process similar to conventional T cell to acquire the Treg phenotype but their absolute numbers are unaffected by the TCR signal strength as long as it is above a certain threshold. Moreover, even when exposed to strong TCR-mediated signaling, these developing $CD4^{+}CD25^{+}$ Treg cells are highly resistant to deletion. Therefore under conditions of strong TCR signaling strength (right panel), the observed gain in the percentage of $CD4^{+}CD25^{+}$ Treg cells is due to increased negative selection and selective loss of the $CD4^{+}CD25^{-}$ conventional T cell population.