Targeted loss of SHP1 in murine thymocytes dampens TCR signaling late in selection

Ryan J. Martinez, Emory University
Anna B. Morris, Emory University
Dennis K. Neeld, Emory University
Brian Evavold, Emory University

Journal Title: European Journal of Immunology
Volume: Volume 46, Number 9
Publisher: Wiley: 12 months | 2016-09-01, Pages 2103-2110
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1002/eji.201646475
Permanent URL: https://pid.emory.edu/ark:/25593/s4rq7

Final published version: http://dx.doi.org/10.1002/eji.201646475

Copyright information:
© 2016 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Accessed December 26, 2018 5:11 PM EST
Targeted loss of SHP1 in murine thymocytes dampens TCR signaling late in selection

Ryan J Martinez¹, Anna B Morris¹, Dennis K Neeld¹, and Brian D Evavold¹
¹Emory University, Department of Microbiology and Immunology, 1510 Clifton Rd NE, Atlanta GA, 30322, Phone#: +1-404 -727-1546, Fax#: +1-404-727-8250

Abstract

SHP1 is a tyrosine phosphatase critical to proximal regulation of TCR signaling. Here, analysis of CD4-Cre SHP1fl/fl conditional knockout thymocytes using CD53, TCRβ, CD69, CD4 and CD8α expression demonstrates the importance of SHP1 in the survival of post selection (CD53+), single-positive thymocytes. Using Ca²⁺ flux to assess the intensity of TCR signaling demonstrated that SHP1 dampens the signal strength of these same mature, post-selection thymocytes. Consistent with its dampening effect, TCR signal strength was also probed functionally using peptides that can mediate selection of the OT-I TCR, to reveal increased negative selection mediated by lower-affinity ligand in the absence of SHP1. Our data show that SHP1 is required for the survival of mature thymocytes and the generation of the functional T-cell repertoire, as its absence leads to a reduction in the numbers of CD4⁺ and CD8⁺ naïve T cells in the peripheral lymphoid compartments.

Keywords

SHP1; thymocyte development; T-cell selection; T-cell homeostasis; TCR signaling

Introduction

T-cell development and function is dependent on the regulation of T cell receptor (TCR) signaling as well as downstream transcriptional alterations [1]. Early in the TCR signaling pathway, protein kinases and phosphatases regulate the functionality of signaling intermediates to either modify or enhance downstream effects [2]. SHP1, encoded by Ptpn6, is expressed in hematopoietic cells and is an important phosphatase in many cellular signaling pathways [3]. In naturally occurring SHP1 deficient mice (me/me), groups have found SHP1 to be important in the processes of thymocyte development (both positive and negative selection), naïve T-cell homeostasis, T helper (T_H) differentiation, functionality and Treg suppression [4]–[12]. These T-cell defects occurred in me/me mice with the global loss of SHP1 in all hematopoetic cells. Therefore, specific T cell intrinsic effects of SHP-1 remain to be determined.

Corresponding Author: Brian Evavold, bevavol@emory.edu.

Conflict of interest

The authors declare no financial or commercial conflict of interest.
The conditional deletion of SHP1 has not been reported to effect thymocyte development [13,14]. As SHP1 is a key proximal regulator in TCR signaling, its lack of participation in thymocyte selection is surprising as thymocyte development is highly reliant upon the strength of TCR signaling in discriminating weak from strong peptide-MHC (pMHC) interactions [8,15]. SHP1 interacts with many different proteins in a host of signaling pathways. For example, SHP1 is recruited to LAT via GRB2 to interact with the protein Themis, which is involved in a negative feedback loop to alter the threshold of thymocyte positive and negative selection [16]–[21]. The lack of Themis prevents the recruitment of SHP1 to the GRB2:LAT complex, correlating with excessive signaling in response to lower-affinity ligands [16]. The outcome of the Themis:GRB2:SHP1 interactions is to preserve lower-affinity TCRs in the repertoire by generating a negative feedback loop to limit thymocyte deletion [22]. Yet, global analysis of the thymic compartment in SHP1 deficient mice did not reveal any effects on thymocyte development [13]. Here we have probed thymocyte development in greater detail and contrary to previous reports, find that SHP1 is necessary to reduce TCR signaling and prevent the deletion of mature thymocytes. Furthermore, the absence of SHP1 during development leads to an altered peripheral T-cell repertoire with a loss of naïve T cells and over representation of memory-like T cells. Therefore, SHP1 expression in developing thymocytes is intrinsically essential for the generation of a normal naive T-cell repertoire and the rescue of developing thymocytes from negative selection.

**Results**

Thymocytes selection is dependent upon TCR binding to self-pMHC, with the translation of biophysical TCR:pMHC interactions dictating signal strength and thymocyte fate [15,23], [24]. Defects in the proximal TCR signaling intermediates have been demonstrated to alter T-cell selection and therefore the repertoire of T cells in the periphery [15,16,25],[26]. To determine if SHP1 alters thymocyte selection, we interrogated polyclonal CD4-Cre SHP1flo/flo thymocyte development based on expression of TCRβ and CD69, with no differences noted in the absence of SHP1 (Figure 1A). Thymocytes were further analyzed based on defined maturation phases using TCRβ and CD69, with thymocytes progressing through selection by upregulating TCR and CD69 before down regulating CD69 upon completion of selection [27]. Within the TCR and CD69 subsets, distinct thymocyte developmental stages were identified using CD4 and CD8α that included double negative (DN), double positive (DP), post selection DP, CD8α intermediate (CD8α int), CD4 single positive (CD4SP) and CD8α single positive (CD8SP) (Figure 1B) [27]. When the individual subsets of maturing thymocytes were analyzed the most mature (TCRβHiCD69−) stage revealed significantly reduced numbers of SHP1 deficient cells in the post-selection CD8α Int, CD4SP and CD8αSP populations (Figure 1C). Comparison of the maturation stages before expression of CD4 (before Cre expression) and selection (DN and pre selection DP) were not found to be different (Supplemental Figure 1). Thus, SHP1 controls the survival of thymocytes during late stages of development.

As SHP1 deficiency revealed a defect in post-selection thymocytes, we next probed thymocytes for CD53 expression, a marker that distinguishes pre (CD53−) and post (CD53+) selected cells (Figure 2A) [28]. Loss of SHP1 in CD4-Cre SHP1flo/flo mice revealed a reduced...
frequency of post-selection (CD53+) thymocytes as compared to WT mice (Figure 2A). Enumeration of thymocyte subsets based on CD4 and CD8 expression in the CD53+ post-selection population of SHP1$^{fl/fl}$ and CD4-Cre SHP1$^{fl/fl}$ mice revealed a 2.9 fold and 2 fold reduction in CD8αSP and CD4SP thymocytes respectively (Figure 2B). These data confirmed that SHP1 controls the survival of thymocytes at later developmental stages. To corroborate the survival defect of mature thymocytes was SHP1 dependent, mixed bone marrow (BM) chimeras with CD4-Cre SHP1$^{fl/fl}$ (Thy1.2/CD45.2) and WT (Thy1.2/CD45.1) were created. After reconstitution, total thymocytes from BM chimeras were analyzed for maturation using TCR$\beta$ and CD69 expression and the contribution of WT and SHP1 KO thymocytes were compared at each developmental stage (Figure 3A,B). Initially, the frequency of SHP1 deficient thymocytes was significantly higher than WT thymocytes (Figure 3B,C). However, once the thymocytes matured to the TCR$\beta^{Hi}$CD69$^{Hi}$ stage, no significant differences were noted (Figure 3B,C). Upon completion of the final maturation stage, WT thymocytes were found significantly over-represented (Figure 3B,C). This suggests that loss of SHP1 initially impacts survival of TCR$\beta^{Hi}$CD69$^{Hi}$ thymocytes, as this stage is where WT thymocytes begin to show a survival advantage even though there are no significant differences between the cell types. Thus, even in the same developing environment, thymocytes deficient in SHP1 have a defect in survival.

The reduced levels of thymocyte survival could result from either a decrease in positive selection or an increase in negative selection or a mixture of both. Decreased positive selection could arise from decreased TCR signaling strength, while increased negative selection would occur if there was increased signaling caused by TCR selection events [26]. Both of these possibilities have been reported using me/me mice [7],[29]. To distinguish these possibilities, thymocyte subsets were assayed for their ability to flux calcium (Ca$^{2+}$), a measurement that is positively correlated with TCR signal strength [30]. Using the ratiometric Ca$^{2+}$ dye Indo-1, post-selection DP and CD8α intermediate thymocytes deficient in SHP1 were found to flux greater amounts of Ca$^{2+}$, as measured by area under the curve (AUC) and peak value measurements (Figure 4A). Interestingly, Ca$^{2+}$ flux in SHP1 deficient thymocytes was not increased until after selection (CD53+) and then returned to levels similar to SHP1$^{fl/fl}$ mice as thymocytes matured (Figure 4B). These findings demonstrate the deficiency of SHP1 during selection permits increased TCR signaling. As greater negative selection would be predicted by greater TCR signaling, this data indicates the lack of SHP1 dominantly affects negative selection of the developing thymocytes, thereby causing the decrease in thymocyte cell number (Figure 1C).

To further study the development of thymocytes lacking SHP1, we created OT-I TCR-Transgenic (Tg) mice with T cells lacking SHP1 (OT-I CD4-Cre SHP1$^{fl/fl}$). The OT-I TCR-Tg system allows for the study of SHP1’s influence on selection of thymocytes with differing selecting affinities, as altered peptide ligands (APLs) have been defined for the native SIINFEKL:H2-Kb (N4) epitope. A range of APLs have been defined that cause positive or negative selection of the OT-I thymocytes [31]–[33]. To determine how SHP1 affects the selection of OT-I thymocytes, OT-I and OT-I CD4-Cre SHP1$^{fl/fl}$ were mixed with a fluorescent nucleic acid stain impermeant to viable cells (Sytox Green) and OVA:H2-Kb or APL:H2-Kb tetramer to induce thymocyte selection as read out by apoptosis (affinity hierarchy for ligands used is N4> Q4R7) [32]–[34]. Q4R7 has been previously shown to be
the lowest-affinity APL that can negatively select the OT-I TCR[30]. These stimulated thymocytes were then analyzed using the IncuCyte Zoom system to quantitate dead (Sytox Green+) thymocytes by imaging the cells every hour (Figure 4C). Comparison of N4 and Q4R7 between WT OT-I thymocytes revealed affinity-dependent cell death, while OT-I’s lacking SHP1 were not able to display a similar dependence (Figure 4C). Quantification of cellular death was calculated by taking the area under the curves (AUC) for the H-2Kb-tetramer treatments and normalizing the data to the WT N4 ligand (Figure 4D). SHP1 was not needed to discriminate high-affinity ligands (N4), but was essential for separating the effect of the lower-affinity ligand (Q4R7) that has been used to map selection events for the OT-I TCR. In this regard, our data is similar that reported for Themis−/− mice (Figure 4D) [16]. Therefore, SHP1 is essential in discriminating high- and low-affinity pMHC ligands during thymocyte selection.

Since the number of SP thymocytes is decreased in the absence of SHP1, CD4 and CD8 T cell counts could also be reduced as naïve peripheral T cells are populated by emigrating SP thymocytes [35]. However, previous work using the same CD4-Cre model reported increased frequency of memory (CD44+) T cells without taking into account potential T cell numerical differences[13]. Analysis of splenic CD4 and CD8 T cell numbers demonstrated a reduction of both cell types in the absence of SHP1 (Figure 5A, 5B). In contrast to the previous work, enumeration of naïve (CD44−) and memory (CD44+) T cells found the reduction of CD4s and CD8s in the absence of SHP1 originated from the loss of naïve T cells (Figure 5A,B) [13]. Of note, SHP1 deletion caused changes in CD8, but not CD4 memory T cells. The lack of naïve T cells was confirmed to be driven by SHP1 expression in T cells by using mixed BM chimera experiments as described above. When WT and SHP1 deficient T cells were analyzed and counted in the same animals, a reduction in the number of T cells was appreciated, which was driven by the loss of naïve (CD44−) T cells (Figure 5C). In parallel, analysis of memory (CD44+) T-cell compartments in the BM chimeras showed no enhancement of CD8 memory generation in the absence of SHP1, but did show significant differences for CD4 T cells (Figure 5C). Contrary to previous reports[13], our results demonstrate SHP1 controls selection of thymocytes and in the peripheral compartment predominantly effects the maintenance of the naïve T-cell population with only minimal alterations to memory T cells.

Discussion

Here we demonstrate the phosphatase SHP1 dampens TCR signaling of developing thymocytes and is critical for the development of mature, single positive thymocytes. As predicted by Themis−/− experiments, SHP1 functions to discriminate lower-affinity TCR signaling and dampen the negative selection to weaker ligands [16]. SHP1’s negative regulation was found to be key in early post-selection thymocytes, as defined by CD53, but was not found to be necessary for controlling TCR signaling of later-stage, SP thymocytes. Conversely, thymocyte enumeration showed delayed effects of the increased TCR signaling with similar numbers of early selection thymocytes but differences in later stages. The decrease in late-stage thymocytes extended into the peripheral T-cell compartment, showing a decrease in the number of naïve T cells. By defining SHP1s role during thymocyte
development, this work validates findings from Themis\textsuperscript{−/−} mice and reiterates SHP1s is an essential phosphatase for the generation of functional T cells.

The conclusion that SHP1 is necessary for thymocyte development and functionality demonstrates distinct roles for SHP1 throughout the life cycle of a T cell. This is apparent in the comparison of experiments where SHP1 is deleted in DP thymocytes (CD4-Cre) \cite{13} or in mature SP thymocytes (dLck-Cre), leading to distinct outcomes \cite{14}. Comparisons of these models reveal several key differences. First, when SHP1 deletion occurs in immature DP thymocytes, no proliferative differences of peripheral T cells are noted, whereas SHP1 deletion in mature thymocytes produced a proliferative advantage of these T cells. Second, SHP1 deletion during thymocyte development caused the enrichment of memory-like CD8 T cells and IL-4 producing CD4 T cells, with few naïve T cell alterations identified when SHP1 was deleted post-thymocyte selection \cite{13}. Yet, it was previously unexplained why these two Cre systems gave different results, as it was incorrectly established that use of the CD4-Cre system did not cause thymocyte alterations \cite{13}. With the new understanding that SHP1 plays a role in thymocyte selection, these two findings can be reconciled. The absence of SHP1 during thymocyte development causes alterations to the peripheral T-cell repertoire by reducing the number of naïve T cells, but leaving the memory T-cell compartment unaltered or increased. This implies the naïve populations of antigen-specific T cells are reduced in number and diversity, potentially altering the functionality of the immune response to pathogens. Therefore, fine tuning of TCR signals executed by SHP1 is important to prevent the reduction of naïve T cells and to generate a T-cell repertoire with the capacity to fully protect the host.

Our work on the role of SHP1 during thymocyte selection has revealed disparities with other work \cite{13}. Several factors may be playing a role in these discrepancies. The previous study only reported CD4 and CD8 expression of thymocytes as opposed to our more extensive analysis \cite{13}. Furthermore, the numbers of peripheral lymphocytes was not calculated in the previous work\cite{13}, leading the incorrect conclusion of increases in memory T-cell populations. Even with genetically identical mice in both experiments, factors such as age, sex and housing conditions could impact the findings. This could be due to SHP1 functioning differently due to sex/age of mice, or due to difference in microbiota between facilities \cite{36}. It is also possible that deletion of SHP1 may be incomplete, or with different levels of redundancy with SHP2 between laboratories. Therefore, several unknown confounding factors may exist, but our more detailed analysis revealed SHP1s role in selection and repertoire generation.

The role of SHP1 in the generation and maintenance of memory T cells is complex. Even with the decreased number of naïve T cells, we find SHP1 acts to maintain or increase numbers of memory CD4 and CD8 T cells. SHP1 could potentially be controlling memory T-cell differentiation in several ways. SHP1 could prevent the steady state naïve to memory transition by reducing the signaling generated by tonic TCR:pMHC interactions needed for T cell maintenance and survival \cite{37},\cite{38}. Alternatively, SHP1 may control the activation sensitivity of naïve T cells, allowing them to proliferate to larger populations and generate greater numbers of antigen-experienced T cells. Excessive generation of antigen-experienced T cells in the absence of SHP1 has been shown previously \cite{14}, but it is unclear if it can
explain our observations in the CD4-Cre model as we are measuring memory T-cell contribution at the steady state. Either way, both hypotheses would end in the generation of a memory T-cell population with reduced TCR clonotype diversity, reiterating SHP1 acts to diversify the T-cell immune population.

Identifying the role of SHP1 during selection corroborates data from mice lacking Themis, as Themis−/− thymocytes are not able to recruit SHP1 to GRB2 or phosphorylate SHP1 [16], [20]. These changes in SHP1 associated with a loss of Themis lead to altered negative selection thresholds, allowing lower-affinity TCR:pMHC interactions that normally positively select thymocytes to result in deletion [16]–[19]. This change in the threshold between positive and negative selection is likely due to an alteration in the negative feedback loop generated by Themis/SHP1. The negative feedback loop allows for the generation of constant TCR signaling output, even with increases in TCR affinity, and is essential to demarcate the sharp, digital response between positive and negative selection[16],[22]. Interestingly, the peripheral T-cell phenotype found in CD4-Cre SHP1fl/fl mice and Themis−/− mice is very similar, with loss of naïve T cells and enhanced survival of memory-like T cells [18],[19]. However, the CD4-Cre SHP1fl/fl mouse does not exactly phenocopy Themis−/− thymocytes. Themis−/− thymocytes are noted to have a selection defect at the CD3hiCD69+ (TCRHiCD69+) stage of development, while we do not find any difference using this gating strategy (Figure 1A). As well, signaling defects were found in Themis−/− thymocytes before selection[16], but we only found differences in TCR induced Ca2+ flux after positive selection (Figure 4A,B). We believe two factors could be playing a role in these differences. First, SHP2 can bind to Themis similar to SHP1 and potentially regulate signaling in a similar fashion and partially rescue the phenotype [20]. Generation of SHP1/SHP2 double knockout thymocytes would address this issue. Second, as the system we studied is a conditional knockout where SHP1 is deleted upon CD4 upregulation, SHP1 may still be partially present and functional during the initial selection. The half-life of SHP1 after Cre-induced excision is unknown, though previous work suggested SHP1 is undetectable via western blot by the DP thymocyte stage[13]. Therefore, the timing of SHP1 deletion is likely important for the generation of this selection phenotype. As for the IL-4 producing CD4 T cells found in the CD4-Cre SHP1fl/fl mice [13], we hypothesize these cells arise due to an altered TCR repertoire caused by excessive negative selection. Of interest TCRs with lower interactions with antigen tend to develop into T_{H2} cells [39]. Nonetheless, the role of SHP1 is fundamental in discriminating TCR strength of thymocytes and generation of a functional naïve TCR repertoire.

Materials and Methods

Mice

CD4-Cre SHP1fl/fl and SHP1fl/fl mice have been described previously and were purchased from Jackson Laboratories and bred on site [13]. OT-I mice were purchased from Jackson Laboratories and the OT-I CD4-Cre SHP1fl/fl mice were bred on site. C57BL/6 (WT) mice were purchased from the National Cancer Institute. All mice in experiments were 6–8 weeks old except for those used in the IncuCyte assay, which were 4–6 weeks old, and both males and females were used in experiments. All animals were housed in an Emory University

Eur J Immunol. Author manuscript; available in PMC 2017 September 01.
Flow Cytometry

Mouse thymus or spleens were processed into a single cell suspension and stained with fluorochrome-labeled antibodies for 30 min on ice. Antibodies used are listed per manufacturer: CD3ε (145-2C11), CD8α (53-6.7) (Tonbo biosciences), CD4 (RM4-5), CD69 (H1.2F3), Thy1.2 (30-H12), CD45.1 (A20) (Biolegend), CD11b (M1/70), CD11c (HL3), CD19 (1D3), CD53 (OX-79) (BD Pharmigen), CD44 (IM7), F4/80 (BM8), TCRβ (H57-597) (eBioscience). For thymocytes, viable cells (as identified by FSC and SSC) were gated and then analyzed as described in the figures. For splenocytes, viable cells (as identified by FSC and SSC) were gated on and T cells (CD3ε+) were identified that were lineage (CD19, CD11b, CD11c, F4/80) negative and then analyzed as described in the figure captions. Cells were counted using AccuCheck microbeads (Invitrogen). Data was acquired on a LSRII (Becton Dickinson) and analyzed using FlowJo (Treestar).

Calcium Flux Measurements

Calcium flux measurements were performed as previously described. Briefly, thymocyte cell suspensions from SHP1\textsuperscript{fl/fl} or CD4-Cre SHP1\textsuperscript{fl/fl} mice were made and rested for 60 min at 37°C. One of the samples was stained with Cell Trace Violet (CTV) for 10 min at room temperature while the other sample received equal amounts of vehicle-only control (DMSO). Cells were then washed and equal number of cells were mixed together at a density of 2×10\textsuperscript{6} cells/ml in R10 media (RPMI1640, 10% (v/v) FCS, 2mM L-glutamine, 0.05mM 2-mercaptoethanol and 0.05mg/ml gentamicin sulfate). The mixed thymocytes were incubated with the calcium indicator Indo-1 AM (Molecular Probes; 1mM final concentration) for 15 min at room temperature. Cells were washed once in R10 and stained for 15 min at room temperature with fluorescently conjugated antibodies and biotinylated anti-CD3ε (Tonbo Biosciences; 145-2C11) antibody. Cells were washed once in cHBSS (Ca\textsuperscript{2+} and Mg\textsuperscript{2+} free Hank’s balance salt solution supplemented with 1% (v/v) FCS, 1mM MgCl\textsubscript{2}, 1mM EGTA and 10mM HEPES, pH 7.3) and resuspended in cHBSS. Samples were warmed to 37°C for 2 min and then analyzed on a LSRII (Becton Dickinson). CaCl\textsubscript{2} (2mM) was added 30 seconds into analysis followed by streptavidin (10 mg/ml) at 60 seconds to activate the samples.

Bone Marrow Chimeras

Bone marrow cells harvested from the femur, tibia and sacrum were processed into a single cell suspension and depleted of T cells using Thy1.2-PE conjugated antibody and anti-PE magnetic microbeads as per manufacturer protocol (Miltenyi Biotec). Recipient mice (Thy1.1/CD45.2) were irradiated (950 rad) and injected i.v. with 1×10\textsuperscript{7} bone marrow cells from C57BL/6 (Thy1.2/CD45.1) and CD4-Cre SHP1\textsuperscript{fl/fl} (Thy1.2/CD45.2) mice mixed at a 50:50 ratio. Recipient mice were treated with drinking water containing 2% sucrose, 0.5 mg/ml Neomycin, 0.0125 mg/ml Polymyxin B for 7 days after irradiation and were analyzed 6–8 weeks after irradiation.
**Incucyte Sytox Assay**

Thymocytes from OT-I or OT-I CD4-Cre SHP1^{fl/fl} mice were harvested and processed into a single cell suspension in complete R10 media without phenol red (recipe above). Cells were enriched for viable cells by density centrifugation using Lymphocyte Separation Medium (density= 1.077–1.080 g/mL at room temperature) by overlaying 2mL of room temperature cell suspension over 1mL of room temperature Lymphocyte Separation Medium and centrifuging for 15min at 400 × g. The interface was removed and washed in complete RPMI. A 96 flat-bottom plate was prepared for the assay by adding 25µl CellTak (BD) per well at a concentration of 22.4µg/ml diluted in PBS. The CellTak was incubated for 20 minutes at room temperature, and then washed using dH2O. Next, cells were resuspended at 1.5x10^6 cells per mL in complete RPMI media supplemented with 125nM Sytox Green (Life Technologies). 100µl of cells were plated on the pretreated plate and mixed with 100µl of complete RPMI media with H-2Kb tetramers (1µl of tetramer per well) or left unstimulated. Cells were then tacked to plate using centrifugation (acceleration: 5, deceleration: 5) by accelerating the plate to 450rpm, stopping the spin, rotating the plate 180°, and then spinning again to 450rpm. Cells were next placed into IncuCyte (Essen Bioscience) machine and imaged once every hour using the 20x phase and fluorescent channel 1 objectives. Analysis of images was performed after experiments using the following settings. Phase: Background=0.3, Cleanup (Holefill=0 µm², Adjust Size=−3 pixels). Green: TopHat parameters (radius=100µm, Threshold=2.000 GCU), Edge split on (Edge sensitivity=4), Cleanup (Holefill=0 µm², Adjust Size=−3 pixels), Filters (Max=650 µm², Mean Intensity=5.000 µm²). Unstimulated samples were analyzed for Sytox^+ cells and this background was subtracted from H-2Kb treated Sytox^+ events before analysis.

**Statistical Analysis**

All data were analyzed using Prism (Graphpad) Software. Outliers were excluded if identified by Grubs Test.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

The authors would like to thank Laurel Lawrence for maintaining the mice used in these experiments as well as Shayla Shorter, Hunter Martinez and Emily Cartwright for helpful discussion. This work was supported by NIH grant T32 AI007610, RO1 AI096879, RO1 AI110113, F31 NS086130 and T32 AI070081.

**References**


Figure 1. SHP1 is necessary for thymocyte development
(A) Representative flow cytometry and gating from WT (top) and CD4-Cre SHP1^{fl/fl} (bottom) thymocytes showing TCRβ and CD69 expression. Enumeration of TCRβ and CD69 thymocyte subsets in WT and CD4-Cre SHP1^{fl/fl} mice shown as mean+/−SEM, n=6–10 mice pooled from three independent experiments. (B) CD8α and CD4 expression of thymocyte subsets by the maturation markers TCRβ and CD69 with WT (top) and CD4-Cre SHP1^{fl/fl} (bottom). (C) Enumeration of the developing thymocytes according to TCRβ, CD69, CD4 and CD8α expression shown as mean+/−SEM, n=6–10 pooled from 3 independent experiments. CD8α int p=0.01, CD8αSP p=5.1×10^{-5}, CD4SP p=7.51×10^{-5}, Multiple Sample T-test with correction for multiple comparisons using the Holm-Sidak method.
Figure 2. Thymocyte survival is decreased in the absence of SHP1
CD53 was analyzed on bulk SHP1^{fl/fl} and CD4-Cre SHP1^{fl/fl} thymocytes. (A) Representative histograms of CD53 expression from SHP1^{fl/fl} and CD4-Cre SHP1^{fl/fl} mice are shown (left). Symbols represent individual mice, data pooled from 3 independent experiments (right). *p<0.0001, Student’s T-test. (B) Numerical comparison of CD53\(^+\) thymocyte CD4/CD8 subsets as evaluated by flow cytometry, is shown as mean±SEM of n=6–10 mice pooled from three independent experiments. CD8α SP *p=0.0001, CD4SP *p=0.00015, Multiple T-test with correction for multiple comparisons using the Holm-Sidak method.
Figure 3. Mature thymocytes intrinsically depend on SHP1 to survive
Thymocyte maturation was analyzed in mixed BM chimeras generated from WT and CD4-Cre SHP1^fl/fl^ BM. (A) Representative dot plot of TCRβ and CD69 expression of total thymocytes in mixed BM chimera. (B) The contribution of WT (Thy1.2^+^/CD45.1^+^) and CD4-Cre SHP1^fl/fl^ (Thy1.2^+^/CD45.1^-^) at maturation stages, as defined by TCRβ and CD69 expression, was evaluated by flow cytometry. Representative plots of n=10 mice pooled from two independent experiments. (C) Tabulated frequency of the thymocyte contribution at each stage in thymocyte development, shown as mean+/−SEM of n=10 mice pooled from two independent experiments. TCRβ^-^CD69^-^ *p=0.0009, TCRβ^int^CD69^int^ *p=2.6×10^-6, TCRβ^Hi^CD69^-^ *p=3.14×10^-8, Multiple T-test with correction for multiple comparisons using the Holm-Sidak method.
Figure 4. Increased thymocyte TCR signaling in the absence of SHP1
TCR reactivity was measured in SHP1$^{fl/fl}$ and CD4-Cre SHP1$^{fl/fl}$ thymocytes. (A) Ratiometric calcium changes of Indo-1 dye of post selection (CD53$^+$) DP SHP1$^{fl/fl}$ and CD4-Cre SHP1$^{fl/fl}$ thymocytes after Ca$^{2+}$ and streptavidin (SA) addition. (B) Normalized Indo-1 calcium flux calculated by dividing the area under the curve (AUC) and peak value from SHP1$^{fl/fl}$ and CD4-Cre SHP1$^{fl/fl}$ thymocytes. Solid black line designates equal Ca$^{2+}$ flux between phenotypes. Symbols represent individual mice n=6 pooled from three independent experiments. AUC: CD53$^+$DP *p=0.009, CD8α.int *p=0.01, Peak Value: CD53$^+$DP *p=0.035, CD8α.int *p=0.04, One-sample T-test for each point with a reference value of 100. (C) IncuCyte analysis of cell death after incubation with high-affinity (N4), lower-affinity (Q4R7) and no stimulation in the OT-I TCR Tg system. Total Sytox green$^+$ cells per image were counted every hour and graphed as a function of time, one representative sample, two independent experiments. (D) Normalized AUC data from (C) shown as mean±SEM of two experiments, means calculated from combined technical replicates from two independent experiments. Two-Way ANOVA with Sidak’s multiple comparison test, *p<0.05.
Figure 5. SHP1 controls the homeostasis of naïve T cells
Analysis of frequency and number of CD4+ and CD8α+ T cells in WT and CD4-Cre SHP1^{fl/fl} mice. (A, B) Flow cytometry was used to evaluate the expression of CD44+ T cells in CD4-Cre SHP1^{fl/fl} (filled gray histogram) to WT (open black histogram) of both (A) CD8+ and (B) CD4+ T cells (left). Enumeration of flow cytometry data are shown as mean±SEM of n=6–10 mice pooled from three independent experiments (right). CD4: CD4+ total *p<0.0001, CD44− *p<0.0001, CD8: CD8+ total *p<0.0001, CD44− *p<0.0001, CD44+ *p=0.0037, Multiple T-tests with correction for multiple comparisons using the Holm-Sidak
method. (C) Peripheral T cell enumeration of flow cytometry data from CD4-Cre SHP1^{fl/fl} and WT mixed bone marrow chimeras. Data are shown as mean+/−SEM of n=10 pooled from two independent experiments. CD4^{+} *p=0.00469, CD44^{−} *p=0.0088, CD44^{+} *p=0.00034, Multiple T-tests with correction for multiple comparisons using the Holm-Sidak method.