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SHP-1 in T Cells Limits the Production of CD8 Effector Cells without Impacting the Formation of Long-Lived Central Memory Cells

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During responses against viruses and malignancies, naive CD8 T lymphocytes expand to form both short-lived effector cells and a population containing cells with the potential to be long-lived and participate in memory responses (memory precursor effector cells). The strength of antigenic, costimulatory, and cytokine signals during responses impacts the magnitude and type of CD8 populations formed. In vitro studies have revealed that the tyrosine phosphatase Src homology region 2 domain-containing phosphatase-1 (SHP-1) regulates signal transduction from receptors on T cells including the TCR, helping set the activation threshold, and therefore may shape responses of mature CD8 T cells in vivo. Analysis of CD8 T cells from moth eaten mice, which are globally deficient in SHP-1, proved problematic due to cell-extrinsic effects of SHP-1 deficiency in non-T cells on CD8 T cells. Therefore, a conditional knockout of SHP-1 in mature single-positive T cells was developed to analyze cell-intrinsic consequences of complete and partial SHP-1 deficiency on CD8 T cell responses to acute viral infection. The results demonstrated that SHP-1 has disparate effects on subpopulations of responding cells, limiting the magnitude and quality of primary and secondary responses by reducing the number of short-lived effector cells generated without affecting the size of the memory precursor effector cell pool that leads to formation of long-term memory. The Journal of Immunology, 2010, 185: 3256–3267.

CD8 CTLs play an important role in the control of viral infections and tumor surveillance and eradication. Following Ag encounter, naive CD8 T cells expand to form two major specialized subpopulations. These include short-lived effector cells (SLECs) that produce effector cytokines and lyse target cells, but have a limited life span and die during the contraction phase following peak response, and memory precursor effector cells (MPECs) that contain cells that survive after the primary response to form the long-lived memory population (1). Cell-extrinsic environmental signals modulate both the magnitude of the response and the relative abundance of MPECs and SLECs generated. High Ag loads (1, 2), costimulatory signals from APCs (3), and exposure to IFN-α/β (4, 5) all increase the magnitude of CD8 SLECs and MPECs generated at the peak of the response. The inflammatory cytokine IL-12 also increases the size of the total effector response, but promotes differentiation, leading to greater increases in SLEC compared with MPECs (1, 6, 7). The inhibitory cytokine TGF-β has the opposite effect on the magnitude of the primary response to a pathogen, selectively decreasing the survival of SLECs during the expansion phase (8).

The strength of signals induced within responding T cells also contributes to the magnitude of the SLEC and MPEC responses and reflects not only extrinsic events but also cell-intrinsic events. For instance, CD8 T cells with higher affinity TCRs for a defined amount of peptide–MHC complex will receive greater signal strength and produce responses of greater magnitude than CD8 T cells bearing TCRs of lower affinity (9). The activity of transcriptional factors can alternatively influence the response, such as the transcriptional repressor methyl-CpG-binding domain protein 2, which impacts the quality of response by regulating the generation of MPECs (10). Thus, perturbation of molecules in cell-intrinsic signaling pathways can influence the overall magnitude of the response as well as the relative balance between SLECs and MPECs by modulating CD8 T cell perception of the signals received from the environment.

Src homology region 2 domain-containing phosphatase-1 (SHP-1) is a tyrosine phosphatase expressed by hematopoietic cells (11) that regulates TCR signal transduction and the T cell activation threshold (12–15). SHP-1 is activated after TCR engagement by Lck-mediated phosphorylation and is then recruited to the TCR complex, where it can dephosphorylate molecules, such as Lck (16, 17), ZAP-70 (18, 19), PI3K (20), Vav (21), and potentially many other signaling molecules including linker for activation of T cells (22), Src homology 2 domain-containing leukaocyte protein of 76 kDa (23), and CD3ζ (24). The speed of SHP-1 recruitment to the TCR complex and dephosphorylation of Lck depends on the potency of T cell activation. Sufficiently strong TCR signals lead to rapid and sustained ERK activity, which phosphorylates Lck to induce a conformation resistant to dephosphorylation by SHP-1 (25). Weaker or antagonistic TCR signals produce only transient or limited downstream ERK activation, permitting SHP-1 to inactivate
Lck and other components of the TCR complex, thereby terminating the signal. These activities of SHP-1 are particularly evident in thymic development, in which SHP-1 contributes to setting the thresholds for positive and negative selection (12, 13, 26, 27). SHP-1 also regulates T cell activation by mediating inhibitory effects of cytokines, such as IL-10 (28) and TGF-β (29), and of the receptors programmed death-1 (30), CD5 (31), and carcinoembryonic Ag-related cell adhesion molecule 1 (32) following ligand engagement. SHP-1 may also play a role in regulating the action of IL-2 and IFN-α/β via dephosphorylation of downstream signaling molecules, such as Jak1, Jak3, and Tyk2 (33–35).

As a pleiotropic regulator interfacing with multiple signaling pathways, SHP-1 likely serves an important role in shaping how mature CD8 T cells perceive environmental signals and respond to Ag in vivo, but its regulatory functions have not been studied in the physiological context of pathogen infection. Additionally, the role of SHP-1 regulation has not been evaluated during secondary responses by memory cells, which typically respond with greater magnitude and rapidity than naive cells, in part due to changes in the signaling required for activation (36–38). Memory cells have a higher phosphoprotein content in membrane lipid rafts, which facilitates the amplification of proximal signals and makes memory cells more poised to respond to a stimulus than naive cells (37). Memory cells are also less reliant on the signaling molecule Lck for secondary response (36). Thus, SHP-1 may have distinct regulatory impacts on primary and secondary responses.

Much of what is known about SHP-1 regulation of CD8 T cells has been derived from in vitro studies using cells from motheaten mice that contain a null mutation resulting in truncation of SHP-1 mRNA and no expression of the SHP-1 protein (11). However, because SHP-1 has regulatory roles in multiple hematopoietic lineages, mice homozygous for the mutant allele (SHP-1<sup>−/−</sup>) display abnormalities in the function/development of macrophages, granulocytes, T cells, B cells, and NK cells, develop autoimmune disease and systemic inflammation, and generally die at 3 to 4 wk of age from pneumonitis (11). This severe phenotype of complete SHP-1 deficiency on CD8 T cell responses. Our results demonstrate that SHP-1 limits the magnitude and alters the quality of both primary and secondary responses by significantly reducing the number of SLECs generated without affecting the size of the MPEC pool that subsequently leads to formation of long-term memory.

### Materials and Methods

**Mice**

Floxed SHP-1 mice (SHP-1<sup>lox/lox</sup>) have been described (40) and were obtained from L. Pao and B. Neel (Beth Israel Deaconess Medical Center, Boston, MA) with permission from K. Rajewsky (Harvard Medical School, Immune Disease Institute, Boston, MA). Distal Lck-Cre (dLck-Cre) and Rosa-EYFP mice have been described (41, 42) and were a gift from P. Fink (University of Washington, Seattle, WA) with permission from N. Killeen (University of California, San Francisco, San Francisco, CA). P14 transgenic mice (43) [that recognize the lymphocytic choriomeningitis virus (LCMV) gp33 presented by H-2<sup>D</sup>] were a gift from K. Murali-Krishna (University of Washington). SHP-1<sup>+/−</sup> breeders, Thy1.1<sup>+</sup> breeders, and naive C57BL/6 (B6) hosts were purchased from The Jackson Laboratory (Bar Harbor, ME). Motheaten mice (SHP-1<sup>Me/Me</sup>) were generated by crossing SHP-1<sup>−/−</sup> mice. SHP-1<sup>−/−</sup> mice were bred to TCR specific for an epitope derived from the Friend murine leukemia virus (TCR<sup>Fm</sup>) mice [previously described (44)], and positive double-heterozygous F1 mice were backcrossed to generate TCR<sup>Fm</sup> SHP-1<sup>−/−</sup>MeMe mice. P14<sup>+</sup> and Thy1.1<sup>+</sup> mice were crossed and maintained in our colony. SHP-1<sup>lox/lox</sup> mice were bred to P14<sup>+</sup> Thy1.1<sup>+</sup> and dLck-Cre<sup>+</sup> mice individually. Positive F1 offspring from each breeding were then crossed to generate P14<sup>+</sup> Thy1.1<sup>+</sup> Cre<sup>+</sup> SHP-1<sup>−/−</sup>F<sup>lox/lox</sup> or <sup>F<sub>lox/lox</sub></sup> mice for use in adoptive transfer experiments (Fig. 1C). These experimental mice are referred to as P14<sup>+</sup> Thy1.1<sup>+</sup> SHP-1<sup>−/−</sup>F<sub>lox/lox</sub> or F<sub>lox/lox</sub> mice for use in adoptive transfer experiments (Fig. 1C).

**Surface staining and Abs**

FACS staining for surface Ags took place at 4°C in PBS plus 1% FBS followed by analysis on an FACScalibur or FACSCanto (BD Biosciences, San Jose, CA). Abs were purchased from BD Biosciences (CD8α (53-6-7), Thy1.1 (OX-7), CD4 (RM4-5), 1/36 (AL-21), CD52 (PC61), CD69 (HL-2F3), and CD62L (MEL-14A), eBioscience [San Diego, CA], CD127 (A7R34), killer cell lectin-like receptor subfamily G, member 1 [KLRG1] (2F1)), or R&D Systems (Minneapolis, MN; IL-15Rα). Where indicated, matched isotype control Abs (BD Biosciences) were used to determine background staining. The GP33 (KAVYNFATM) D<sup>9</sup> MHC class I tetramer was generated by the Immune Monitoring Lab at Fred Hutchinson Cancer Research Center (Seattle, WA) and tetramer staining took place at 4°C.

**Isolation of naive CD8 T cells for in vitro stimulation and adoptive transfer**

Peripheral lymphocyte cell populations were obtained from spleens and/or lymph nodes (as indicated in figure legends) by physical disruption followed by RBC lysis with ACK buffer. Naive CD8<sup>+</sup> T cells for in vitro stimulations and adoptive transfers were isolated using Dynal Mouse CD8 Cell Negative Isolation Kits (Invitrogen, Carlsbad, CA) per the manufacturer’s instructions. Based on calculations from the postisolation purity (generally >90% as assessed by FACS analysis), 10<sup>4</sup> naive CD8<sup>+</sup> lymphocytes were transferred i.v. into normal B6 hosts for primary infection experiments with the Armstrong strain of LCMV (LCMV<sub>Arm</sub>). For experiments with motheaten mice, the transfer numbers are indicated in the legend for Supplemental Fig. 1.

**LCMV<sub>Arm</sub> infection**

LCMV<sub>Arm</sub> was grown on BHK cells and titered on Vero cells. For induction of primary and secondary CD8 T cell responses, LCMV<sub>Arm</sub> was administered by i.p. route at a dose of 2 × 10<sup>3</sup> PFU/mouse 1 to 2 d after adoptive transfer of T cells.

**In vitro T cell stimulation**

CD8<sup>+</sup> cells isolated from P14<sup>+</sup> Thy1.1<sup>+</sup> SHP-1<sup>−/−</sup> F<sub>lox/lox</sub>, F<sub>lox/lox</sub>, or F<sub>lox/lox</sub> mice were mixed with Thy1.2<sup>+</sup> B6 splenocytes (at a ratio of 1:10) and then labeled with 10 μM CFSE in serum-free HBSS for 10 min at 37°C. The reaction was quenched with pure FCS and the cells washed twice and then plated in 96-well round-bottom plates (5 × 10<sup>5</sup> donor cells/4.5 × 10<sup>5</sup> B6 splenocytes/well). Cells were stimulated with the indicated concentrations of GP33 peptide (KAVYNFATM) and analyzed 48 and/or 72 h later.

**Annexin V/7-aminoactinomycin D staining for cell apoptosis**

In vitro-stimulated T cells or splenocytes obtained at days 7–10 postinfection with LCMV<sub>Arm</sub> were stained to detect cell apoptosis using the Annexin V PE Apoptosis Detection Kit 1 (BD Biosciences) per the manufacturer’s instructions.

**Degranulation assay and intracellular cytokine staining**

Intracellular cytokine staining was performed on splenocytes from day 8 postinfection using the Cytofix/Cytoperm Plus kit (BD Biosciences) per the
manufacturer’s instructions. Briefly, $10^6$ splenocytes were stimulated ex vivo with the indicated concentrations of GP33 peptide for 5 h in the presence of GolgiPlug (brefeldin A). Following surface staining, cells were fixed, made permeable, and stained with Abs to IFN-γ (XMG1.2), IL-2 (JES6-5H4), and TNF (MP6-XT22) from BD Biosciences. For simultaneous assessment of degranulation, Abs to CD107α (1D4B; BD Biosciences) and CD107β (ebioABL-93, ebioscience) were included in the culture media during the 5-h peptide stimulation to stain the surface of cells prior to fixation and intracellular staining for cytokine production.

**Sorting of central memory CD8 T cells for adoptive transfer**

For secondary adoptive transfer of central memory cells (TCMs), donor mouse P14-Thyl.1+ CD62L+ T cells were sorted from spleen and inguinal lymph nodes of previously infected Thyl.2+ primary hosts using a BD Aria 1 cell sorter (BD Biosciences). A total of $3 \times 10^6$ P14+ TCMs were then i.v. transferred into naive B6 hosts 1 to 2 d prior to infection with LCMVarm.

**Western blot analysis of SHP-1 protein expression**

CD8+ T cells were isolated from naive P14+ Thyl.1+ SHP-1+/+, +/-, or /-/- mice by staining and sorting for CD8+ Thyl.1+ cells using the BD Aria 1 cell sorter (BD Biosciences). Cells were lysed in standard RIPA lysis buffer (at a concentration of 10^7 cells/ml) for 30 min and the nuclear debris and unlysed cells removed by centrifugation. Equal volumes of lysate ($\sim 10^6$ cells) were subjected to SDS-PAGE and then transferred to a polyvinylidene fluoride membrane. The membrane was stained with primary Abs to SHP-1 (C-19; Santa Cruz Biotechnology, Santa Cruz, CA) and actin (C-2; Santa Cruz Biotechnology) diluted 1:200 in TBST 0.1% followed by secondary staining with fluorescence-conjugated Abs, IRDye800-conjugated donkey anti-mouse IgG (Rockland Immunochemicals, Gilbertsville, PA), and Alexa Fluor 680-conjugated goat anti-rabbit IgG (Invitrogen), diluted 1:10,000 in TBST 0.1%. The membrane was visualized, and the integrated intensity of bands was determined using the LI-COR Odyssey Infrared Imaging System and software (LI-COR Biotechnology, Lincoln, NE).

**Statistical analysis**

Result graphs express data as mean ± SEMS. Statistical analyses of the data were performed using either a one-way ANOVA followed by Tukey post hoc testing or unpaired two-tailed Student t test with Bonferroni correction for multiple comparisons. The p values <0.05 were considered significant for the ANOVA/Tukey testing. In the cases in which a Bonferroni correction was applied, the adjusted threshold of statistical significance has been indicated in the figure legend. Statistical testing was performed using the computer programs GraphPad Prism (GraphPad, La Jolla, CA) and Excel (Microsoft, Redmond, WA).

**Results**

**Conditional rather than global deficiency of SHP-1 distinguishes cell-extrinsic and intrinsic regulation of CD8 T cell phenotype by SHP-1**

To define the cell-intrinsic and extrinsic roles of SHP-1 in generation of SLEC and MPEC T cell responses, we initially generated SHP-1+/- Me/Me TCR-transgenic mice expressing TCRGag (44). Analysis of TCRGag-transgenic motheaten CD8 T cells revealed, as previously reported (12), expression of increased levels of the activation markers CD44 and CD25. Additionally, we observed upregulation of Ly6C and IL-15Rα, molecules suggesting a prior Ag encounter by the T cells (Supplemental Fig. 1A). However, no upregulation of CD69 or downregulation of CD127 and CD62L expression was detected (data not shown), suggesting the cells were not being actively stimulated. As these phenotypic changes could represent either effects of SHP-1 deficiency intrinsic to T cells or extrinsic effects of inflammation in the motheaten host, wild-type virus-specific CD8 T cells were transferred into 3-wk-old motheaten hosts, which already exhibit systemic inflammation and autoimmunity due to the loss of SHP-1 regulation in non-T cells (11). The transferred T cells upregulated CD44 and Ly6C expression, despite having replete SHP-1 levels in the T cells (Supplemental Fig. 1B). By contrast, adoptive transfer of wild-type virus-specific TCR-transgenic CD8 T cells into wild-type hosts resulted in no alteration of the naive phenotype. The upregulation of CD44 and Ly6C by motheaten TCR-transgenic CD8 T cells remained unchanged following transfer into a wild-type environment (Supplemental Fig. 1C), suggesting either cell-intrinsic SHP-1 deficiency can maintain these changes independently of the motheaten environment or the effects of cell-extrinsic SHP-1 deficiency are imprinted on CD8 T cells. Thus, extrinsic and intrinsic regulation of T cells by SHP-1 cannot readily be distinguished using the motheaten model.

To study cell-intrinsic regulation of mature CD8 T cell responses independent of environmental imprinting and the impact of SHP-1 deficiency on thymic development, we developed a model in which SHP-1 is conditionally deleted in mature single-positive (SP) T cells. Mice generated with a SHP-1 gene flanked by loxP sites (floxed) were crossed to mice expressing Cre recombinase (Cre) under control of the dLck promoter, which initiates Cre expression in SP mature T cells (41). Mice floxed at both SHP-1 alleles and expressing Cre showed almost complete loss of SHP-1 expression in mature CD8 T cells from the spleen (Fig. 1A). Efficiency of Cre expression in this system was analyzed by measuring expression of EYFP in peripheral CD8 T cells of mice containing an EYFP reporter in the Rosa26 locus preceded by a floxed stop codon (41, 42). A small fraction of the CD8 T cells, ~7%, failed to express EYFP, suggesting residual SHP-1 expression we detected likely reflected incomplete excision of SHP-1 in a small percent of cells rather than low-level expression in a high percent of cells (Fig. 1B). As contamination of transferred SHP-1-deficient CD8 T cell populations with a small number of cells expressing residual SHP-1 would only minimize differences observed between SHP-1-deficient and wild-type groups, we reasoned that any observed effects of SHP-1 deficiency would be underestimates of the actual effect. Mice with only one floxed SHP-1 allele expressed 50–60% of wild-type levels of SHP-1 protein (Fig. 1A) and were used as a model of partial SHP-1 deficiency in this study.

Similar to our strategy above for motheaten mice, mice were bred to have conditional deletion of SHP-1 (dLck-Cre+ SHP-1+/+, Flox+/+, or Flox/Flox) as well as expression of a transgenic TCR, which in this case was the P14 TCR recognizing an epitope (gp33–41) derived from the glycoprotein of LCMV presented by H2-D^d (Fig. 1C). For simplicity, these mice are referred to as P14+ Thyl.1+ SHP-1+/+, /-/-, or /-/- in the text. Surface staining of CD8 T cells from P14+ SHP-1+/+ mice showed upregulation of Ly6C and CD44 (Fig. 1D), similar to what was observed in motheaten T cells. The level of CD44 upregulation, which was less than what we typically detect in cells following productive Ag encounter (data not shown), and the absence of downregulation of CD62L and CD127 suggest these cells have largely retained a naive phenotype (Fig. 1D). Additionally, the absence of upregulation of CD69 and CD25 suggests that there is no ongoing/persistent activation (Fig. 1D). These mice remain healthy and exhibit neither chronic inflammation nor evidence of autoimmunity. Thus, SHP-1 may play a direct cell-intrinsic role in regulating basal expression of CD44 and Ly6C. The observed phenotypic changes were not detected in CD8 T cells with partial deficiency of SHP-1.

SHP-1 raises the threshold for in vitro proliferation and expansion in a cell-intrinsic manner

SHP-1 was previously demonstrated to regulate the activation threshold for induction of proliferation by CD8 T cells (13, 15). To determine the extent to which this is a cell-intrinsic effect of SHP-1, P14+ Thyl.1+ CD8 T cells (SHP-1+/+, SHP-1+, or SHP-1−/−) were CFSE-labeled and stimulated with titrating doses of GP33 peptide. After 72 h, SHP-1−/− cells showed significantly greater expansion at
low concentrations of peptide ($10^{-4}$ and $10^{-3}$ μg/ml) than SHP-1+/+ cells (Fig. 2A). Additionally, SHP-1+/− cells reached peak expansion at peptide doses of 10- and 100-fold less than those required for maximum expansion by SHP-1+/− and SHP-1+/− cells, respectively. The data suggest SHP-1 negatively regulates both the degree and the threshold for CD8 T cell expansion in a cell-intrinsic manner. Analysis of CSFE dilution suggests that more SHP-1− cells enter the cell cycle at low peptide concentrations than either SHP-1+/− or SHP-1+/− cells (Fig. 2B). Staining for Annexin V and 7-aminoactinomycin D (7-AAD) also revealed a lower proportion of Annexin V+ or 7-AAD+ cells in the SHP-1−/− compared with the SHP-1+/+ and SHP-1+/− CD8 T cell populations ($p < 0.01$) at a peptide dose of $10^{-4}$ μg/ml GP33 peptide (Fig. 2C, 2D). Thus, SHP-1 appears to regulate activation and the magnitude of expansion by increasing the threshold required for cell cycle entry and by decreasing CD8 T cell survival.

**SHP-1 negatively regulates the peak magnitude of the primary response in vivo**

To elucidate the role of SHP-1 in regulating the physiological expansion of CD8 T cells during a primary response to infection, small numbers ($10^5$) of CD8 T cells from P14+ Thy1.1+ SHP-1−/−, SHP-1+/−, or SHP-1−/− mice were adoptively transferred into wild-type littermate control. Partial deficiency of SHP-1, however, did not alter the expansion of CD8 T cells during in vivo infection. Kinetic analysis confirmed that all donor groups peaked at day 8 (Fig. 3D). The endogenous GP33-specific CD8 T response of all host mice also peaked at day 8 (data not shown) and was of similar magnitude in all groups, suggesting the increased expansion of SHP-1− cells represented a cell-intrinsic effect of SHP-1 deficiency and not differences in the extent of infection between groups (Fig. 3A–C).

To determine if SHP-1 regulates effector functions of cells at the peak of the primary response, splenocytes from mice immunized 8 d prior were stimulated ex vivo with GP33 peptide and stained for intracellular production of IFN-γ and TNF and surface expression of CD107 (to detect degranulation). SHP-1+/+, SHP-1+/−, and SHP-1−/− CD8 T cells were equivalently poised to mediate effector functions, with peak effector cells from all groups showing similar degranulation and cytokine production to a broad range of peptide doses (Fig. 3E). Thus, the effector activities of acutely generated CD8 T cells at the peak of the response appear to proceed independent of SHP-1 regulatory effects.

**SHP-1 negatively regulates the generation of SLECs without affecting the generation of MPECs at the peak of response**

At the peak of response, Thy1.1+ donor CD8 T cells from all experimental groups expressed a phenotype consistent with productive Ag encounter, with upregulation of CD44 and Ly6C and downregulation of CD62L and CD127 expression (Fig. 4A). None of the groups expressed CD69, suggesting the cells were no longer being triggered by Ag. However, an increased frequency of SHP-1−/− peak effector cells expressed high amounts of the NK cell marker, KLRG1 (Fig. 4A, 4B). KLRG1 expression with loss of CD127 expression at the peak of response marks terminally...
differentiated effector cells or SLECs. In contrast, low expression of KLRG1 and high expression of CD127 marks MPECs, a subpopulation with the potential to contribute to long-lived memory (1). The expanded SHP-1−/− peak effector population contained an increased proportion of SLECs (KLRG1high CD127high), resulting in an average 3.5-fold increase in the absolute number of SLECs compared with the SHP-1+/+ effector population, as determined in three independent experiments (p < 0.01) (Fig. 4C–E). Notably, the naive SHP-1+/+, SHP-1+/−, and SHP-1−/− cell populations were equivalently KLRG1low and CD127low (Fig. 1D). Despite the relative decrease in the frequency of MPECs (KLRG1low CD127high) in the SHP-1−/− peak population, there was a trend toward a slight increase in total number of MPECs, although this increase was not statistically significant (Fig. 4E). Partial deficiency in SHP-1 did not significantly increase the frequency or number of SLECs formed during the primary response (Fig. 4E). Thus, SHP-1 has a cell-intrinsic role in regulating the magnitude and phenotype of CD8 peak expansion during the primary response, manifest most prominently in limiting the generation of SLECs.

Because normal MPECs, as opposed to most SLECs, would be expected to retain the ability to produce IL-2 (1), SHP-1+/+, SHP-1+/−, and SHP-1−/− CD8 cells at the peak of the response were also assessed for IL-2 production following ex vivo stimulation with GP33 peptide. The absolute number of SHP-1−/− cells capable of producing IL-2 was not decreased compared with wild-type, although the frequency was lower due to the expansion of more differentiated effector cells or SLECs. In contrast, low expression of KLRG1 and high expression of CD127 marks MPECs, a subpopulation with the potential to contribute to long-lived memory (1). The expanded SHP-1−/− peak effector population contained an increased proportion of SLECs (KLRG1high CD127high), resulting in an average 3.5-fold increase in the absolute number of SLECs compared with the SHP-1+/+ effector population, as determined in three independent experiments (p < 0.01) (Fig. 4C–E). Notably, the naive SHP-1+/+, SHP-1+/−, and SHP-1−/− cell populations were equivalently KLRG1low and CD127low (Fig. 1D). Despite the relative decrease in the frequency of MPECs (KLRG1low CD127high) in the SHP-1−/− peak population, there was a trend toward a slight increase in total number of MPECs, although this increase was not statistically significant (Fig. 4E). Partial deficiency in SHP-1 did not significantly increase the frequency or number of SLECs formed during the primary response (Fig. 4E). Thus, SHP-1 has a cell-intrinsic role in regulating the magnitude and phenotype of CD8 peak expansion during the primary response, manifest most prominently in limiting the generation of SLECs.

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FIGURE 2. Cell-intrinsic SHP-1 deficiency lowers the threshold for and increases the magnitude of in vitro expansion by CD8 T cells. A, A total of 5 × 10^5 purified P14+ Thy1.1+ CD8 T cells was mixed with 4.5 × 10^5 B6 splenocytes, CFSE-labeled, stimulated with the indicated GP33 peptide dose (μg/ml), and analyzed for expansion 72 h later. Cell numbers were calculated from the percent CD8+ Thy1.1+ cells in the live gate of the FACS analysis and the total count of cell numbers in each well. Chart shows average of 3 wells (± SEM)/dose from a representative experiment of two total similar experiments (n = 2 and n = 3). Statistical analysis by one-way ANOVA indicated a significant difference between the means of the donor responses at the 10^{-4} and 10^{-3} μg/ml peptide doses (p < 0.006 and p < 0.009, respectively). Significant differences from post hoc (Tukey) testing are indicated on the chart. B, Plots were gated on CD8+ Thy1.1+ cells and show CFSE dilution at the indicated GP33 peptide dose (μg/ml) after 48 h stimulation. Values represent the percent of cells that have divided at least once. The data are representative of two independent experiments. C, Cells were stained for 7-AAD and Annexin V after 72 h of stimulation with GP33 at the indicated concentration (μg/ml). Plots were gated on CD8+ Thy1.1+ cells. Values in quadrants show the percent of the donor population in that quadrant, and the top values represent the total percent of cells that were 7-AAD+. D, Chart shows the total percent of cells that were Annexin V+ and/or 7-AAD+ for each donor cell type over the indicated range of GP33 doses. C and D show data from one representative experiment (n = 3) of two total experiments (n = 1 and n = 3, respectively). Statistical analysis between the groups was conducted using unpaired, two-tailed Student t tests with the level of statistical significance set at p < 0.016 per Bonferroni correction for the multiple comparisons (three t tests) performed.
SHP-1+/– day 7 to 8 by SHP-1 a role in the increased accumulation of SLEC cell numbers from lowest in the SHP-1 However, the percent of viable cells during this transition period was over the next 2 d for all groups as the number of SLECs contracted. the peak of response, the percent of viable cells gradually increased to the changes in the response by SHP-1–deficient cells. Following panded population of MPECs to become SLECs, likely contribute creased proliferation of SLECs and/or differentiation of an ex- changes in SLEC magnitude. Thus, other processes, such as in- is unlikely to be the only mechanism leading to the observed changes in SLEC population was observed in all groups concurren- with the rapid increase in SLEC numbers from day 7 to 8. SHP-1–/– SLECs, however, displayed a smaller decline between day 7 and 8 in the fraction of viable cells than wild-type SLECs (Fig. 5B, 5C). This trend suggests that improved survival may play a role in the increased accumulation of SLEC cell numbers from day 7 to 8 by SHP-1–/– CD8+ populations. Because the percent of viable cells was relatively similar on day 7, when SHP-1–/– SLECS were already significantly increased in number, enhanced survival is unlikely to be the only mechanism leading to the observed changes in SLEC magnitude. Thus, other processes, such as in- creased proliferation of SLECs and/or differentiation of an expanded population of MPECs to become SLECs, likely contribute to the changes in the response by SHP-1–deficient cells. Following the peak of response, the percent of viable cells gradually increased over the next 2 d for all groups as the number of SLECs contracted. However, the percent of viable cells during this transition period was lowest in the SHP-1–/– SLEC population (Fig. 5B, C), consistent with the greater rate of contraction (Fig. 5A). Thus, the observed improvement of SLEC survival is temporary, and SHP-1 deficiency does not protect these cells from death during contraction after the peak. SHP-1–/– SLECs exhibited slightly better relative survival than wild-type cells at all time points, but this effect of reduced SHP-1 expression did not significantly change the numbers of SLECs compared with wild-type controls at any time point, suggesting a greater reduction of SHP-1 than occurs in SHP-1+/– mice is required to impact SLEC accumulation.

SHP-1 does not regulate the formation or magnitude of long-lived memory populations

SHP-1 deficiency did not diminish the generation of MPECs at the peak of response, but only a small fraction of these cells survive to form long-term memory, and we therefore stained splenocytes at 58 d postinfection with LCMVarm with Abs to CD8 and Thy1.1 as well as with GP33 tetramer to enumerate the persisting memory populations. The frequency and absolute numbers of persisting memory cells derived from SHP-1–/– cells were always equal to or slightly higher than wild-type controls (Fig. 6A, 6B), suggesting SHP-1 deficiency, despite producing greater numbers of SLECs at the peak of the response, did not decrease the formation of long-lived memory cells. The host endogenous GP33-specific memory responses were comparable in all recipient mice, affirming that the host environment for memory formation postinfection was similar in all groups (Fig. 6A, 6B).

During the formation of persistent memory populations, a subset of memory cells re-expresses the surface molecule CD62L (46). These cells make up the Tcm population and are unique from CD62L– cells (effector memory [TEM]) in production of IL-2,
A total of $10^5$ P14+ Thy1.1+ CD8+ T cells (SHP-1+/+, SHP-1−/−, or SHP-1−/−) was transferred into wild-type B6 hosts that were infected with LCMV_Arm 48 h later and the responses analyzed 8 d postinfection. Data for A–E are representative of at least three independent experiments with three to four mice per group. Splenocytes were stained for the indicated surface molecule followed by FACS analysis. Histograms were gated on CD8+ Thy1.1+ cells. The shaded histograms represent staining of splenic CD8+ cells from an uninfected B6 mouse. B. Chart shows average percent (± SEM) of CD8+ Thy1.1+ donor cells that expressed high levels of KLRG1. Data represent combined results from four independent experiments, 17 to 18 mice total per group. Statistical analysis by one-way ANOVA showed a significant difference between the donor groups ($p < 0.004$). Post hoc (Tukey) results are indicated on the chart. C. Plots were gated on CD8+ Thy1.1+ cells that were simultaneously stained for KLRG1 and CD127. Values represent percent of donor cells in the quadrant. Splenic CD8+ cells from an uninfected B6 mouse were used for the naive control shaded histograms. D. D. Chart shows the average number (± SEM) of CD8+ Thy1.1+ donor cells that express an SLEC (KLRG1<sup>high</sup>CD127<sup>+/-</sup>) or MPEC (KLRG1<sup>low</sup>CD127<sup>high</sup>) phenotype, respectively. Cell numbers were calculated from the total number of spleen cells and the percent of donor cells determined by FACS analysis. E. E. Chart shows average fold change (± SEM) in SLEC and MPEC cell numbers at the peak of response from three independent experiments. Fold change was calculated for SHP-1+/+ and SHP-1−/− groups relative to wild-type within each of the three experiments. Wild-type values were then normalized to 1.0 for relative comparisons, and fold change was averaged for each group. Statistical analysis by one-way ANOVA showed a significant difference in the magnitude of SLEC generation between the donor groups ($p < 0.004$). Post hoc (Tukey) results are indicated on the chart. No significant difference was noted between the groups in MPEC generation. F. Splenocytes harvested 8 d after LCMV_Arm infection were incubated with the indicated dose of GP33 peptide for 5 h in the presence of brefeldin A and then stained for intracellular IL-2. The chart on the left shows the average percent (± SEM) of the Thy1.1+ donor population producing IL-2 at the indicated peptide dose. Absolute numbers of donor cells producing IL-2 are shown in the chart on the right. These data were calculated from the percent of donor cells determined by FACS analysis and the total spleen cell counts. The data represent two independent experiments with three to four mice per group.

**FIGURE 4.** SHP-1 deficiency increases the generation of SLECs at the peak of response without diminishing the size of the MPEC population. A–F. A total of $10^5$ P14+ Thy1.1+ CD8+ T cells (SHP-1+/+, SHP-1−/−, or SHP-1−/−) was transferred into wild-type B6 hosts that were infected with LCMV_Arm 48 h later and the responses analyzed 8 d postinfection. Data for A–E are representative of at least three independent experiments with three to four mice per group. A. Splenocytes were stained for the indicated surface molecule followed by FACS analysis. Histograms were gated on CD8+ Thy1.1+ cells. The shaded histograms represent staining of splenic CD8+ cells from an uninfected B6 mouse. B. Chart shows average percent (± SEM) of CD8+ Thy1.1+ donor cells that expressed high levels of KLRG1. Data represent combined results from four independent experiments, 17 to 18 mice total per group. Statistical analysis by one-way ANOVA showed a significant difference between the donor groups ($p < 0.004$). Post hoc (Tukey) results are indicated on the chart. C. Plots were gated on CD8+ Thy1.1+ cells that were simultaneously stained for KLRG1 and CD127. Values represent percent of donor cells in the quadrant. Splenic CD8+ cells from an uninfected B6 mouse were used for the naive control shaded histograms. D. D. Chart shows the average number (± SEM) of CD8+ Thy1.1+ donor cells that express an SLEC (KLRG1<sup>high</sup>CD127<sup>+/-</sup>) or MPEC (KLRG1<sup>low</sup>CD127<sup>high</sup>) phenotype, respectively. Cell numbers were calculated from the total number of spleen cells and the percent of donor cells determined by FACS analysis. E. E. Chart shows average fold change (± SEM) in SLEC and MPEC cell numbers at the peak of response from three independent experiments. Fold change was calculated for SHP-1+/+ and SHP-1−/− groups relative to wild-type within each of the three experiments. Wild-type values were then normalized to 1.0 for relative comparisons, and fold change was averaged for each group. Statistical analysis by one-way ANOVA showed a significant difference in the magnitude of SLEC generation between the donor groups ($p < 0.004$). Post hoc (Tukey) results are indicated on the chart. No significant difference was noted between the groups in MPEC generation. F. Splenocytes harvested 8 d after LCMV_Arm infection were incubated with the indicated dose of GP33 peptide for 5 h in the presence of brefeldin A and then stained for intracellular IL-2. The chart on the left shows the average percent (± SEM) of the Thy1.1+ donor population producing IL-2 at the indicated peptide dose. Absolute numbers of donor cells producing IL-2 are shown in the chart on the right. These data were calculated from the percent of donor cells determined by FACS analysis and the total spleen cell counts. The data represent two independent experiments with three to four mice per group.

**SHP-1 limits the generation of SLECs during the secondary response of primed cells without affecting the formation of memory precursor cells**

Similar to naive precursors in a primary response, T<sub>CM</sub> CD8 cells must rapidly proliferate to produce expanded populations of reactive cells that again include SLECs and cells capable of sustaining the memory population. Memory T cells compared with naive T cells exhibit a reduced activation threshold (36–38), suggesting memory cells may have acquired means to bypass SHP-1 regulatory activities. SHP-1+/+, SHP-1−/−, and SHP-1−/−<sup>+</sup> T<sub>CMs</sub>, generated by transfer of $10^5$ naive T cells into B6 hosts followed by LCMV_Arm infection, were isolated by sorting at 300 d postinfection. A total of $3 \times 10^5$ T<sub>CMs</sub> was then transferred into naive hosts and subjected to secondary exposure to LCMV_Arm infection. The response of wild-type donor cells in the spleen reached peak magnitude at day 5 postinfection, and the cell numbers remained relatively constant through days 6 and 7. At day 5, SHP-1−/−<sup>+</sup> T<sub>CMs</sub> were already present at larger numbers than SHP-1+/+ cells (Fig. 7A, 7B). Both SHP-1−/−<sup>+</sup> and SHP-1−/−<sup>+</sup> cells exhibit a reduced activation threshold (36–38), suggesting memory cells may have acquired means to bypass SHP-1 regulatory activities. SHP-1+/+, SHP-1−/−, and SHP-1−/−<sup>+</sup> T<sub>CMs</sub>, generated by transfer of $10^5$ naive T cells into B6 hosts followed by LCMV_Arm infection, were isolated by sorting at 300 d postinfection. A total of $3 \times 10^5$ T<sub>CMs</sub> was then transferred into naive hosts and subjected to secondary exposure to LCMV_Arm infection. The response of wild-type donor cells in the spleen reached peak magnitude at day 5 postinfection, and the cell numbers remained relatively constant through days 6 and 7. At day 5, SHP-1−/−<sup>+</sup> T<sub>CMs</sub> were already present at larger numbers than SHP-1+/+ cells (Fig. 7A, 7B). Both SHP-1−/−<sup>+</sup> and SHP-1−/−<sup>+</sup> cells

**self-renewal capacity, and expansion upon secondary encounter with Ag. To determine the relative proportion of T<sub>EM</sub> and T<sub>CM</sub> in the SHP-1+/+, SHP-1−/−, and SHP-1−/−<sup>+</sup> memory populations, splenocytes were stained for CD62L expression 58 d after primary infection. At this time, ∼20% of memory cells from all groups had upregulated expression of CD62L (Fig. 6C, 6E), a marked increase from the uniformly low levels in all groups at the peak response (Fig. 4A). The absolute numbers of T<sub>CM</sub> and T<sub>EM</sub> were also not significantly different between groups (Fig. 6D). As the conversion from T<sub>EM</sub> to T<sub>CM</sub> is a gradual process (46), SHP-1+/+, SHP-1−/−, and SHP-1−/−<sup>+</sup> memory populations were also stained for CD62L expression at 110 d postinfection, and all experimental groups showed similar increases in the frequencies of T<sub>CM</sub>, from day 58 (Fig. 6E). The SHP-1−/−<sup>+</sup> memory population did appear to have a slightly increased frequency of T<sub>CM</sub> at the later time point, but the small difference was not significant. These results suggest that SHP-1 deficiency does not diminish the formation of stable memory populations or the differentiation of memory cells to T<sub>CMs</sub> within these populations following primary infection.
The magnitude of CD8 T cell expansion following Ag recognition is influenced by the strength of signals perceived through the TCR and costimulatory and cytokine receptors, all of which are potentially subject to regulation by SHP-1. Insights into the regulatory role of SHP-1 following T cell activation have largely been derived from in vitro studies (12–14, 25, 27), but elucidating the physiological cell-intrinsic activities of SHP-1 during in vivo responses can provide more detailed insights into the sequential events following activation and differentiation and the associated changes in function, survival/apoptosis, and memory formation. Therefore, we expressed a transgenic TCR in congenically distinct wild-type mice and mice deficient in SHP-1 selectively in the T cell compartment and directly assessed the impact of SHP-1 deficiency on CD8 T cell responses to a viral challenge. The rapid and extensive expansion of wild-type CD8 T cells that occurs in response to LCMV infection, reaching peak response at 8 d post-infection, has been extensively described (45). Although SHP-1 deficiency did not impact the kinetics of this expansion, it did dramatically increase the magnitude of the peak primary response of naïve T cells (3.5-fold). This likely resulted from a composite of influences, as the size of the peak CD8 T cell response from a defined number of reactive precursors is determined by many factors, including the signal strength resulting from the avidity of TCR–peptide–MHC interactions and the amount of Ag available, factors that support CD8 T cell expansion, such as IL-2, which is known to regulate the TCR signaling pathway (47). However, in contrast to the primary response, a deficiency of ~50% of SHP-1 protein appears sufficient to result in an increased formation of SLECs compared with wild-type cells, with complete deficiency resulting in even greater numbers of SLECs. Thus, the secondary CD8 T cell response appears more sensitive to regulation by SHP-1 than the primary response.

Discussion

SHP-1, which has broad activity in many hematopoietic cell types, is known to regulate the TCR signaling pathway (47). However, defining the distinct role of SHP-1 in T cells in physiologic responses was not possible from previous studies with T cells from mice globally deficient in SHP-1, as we observed that the entire CD8 T cell compartment was modified by the inflammatory host environment independent of a cell-intrinsic SHP-1 deficiency. Therefore, to overcome these cell-extrinsic effects of SHP-1 deficiency, we built a model of cell-intrinsic SHP-1 deficiency by crossing mice with floxed alleles of SHP-1 to mice that expressed Cre under the control of the dLck promoter. This strategy resulted in excision of SHP-1 in mature SP T cells, making it possible to evaluate the role of SHP-1 in T cell responses without confounding influences of SHP-1 during T cell development or from cell-extrinsic environmental effects.

The magnitude of CD8 T cell expansion following Ag recognition is influenced by the strength of signals perceived through the TCR and costimulatory and cytokine receptors, all of which are potentially subject to regulation by SHP-1. Insights into the regulatory role of SHP-1 following T cell activation have largely been derived from in vitro studies (12–14, 25, 27), but elucidating the physiological cell-intrinsic activities of SHP-1 during in vivo responses can provide more detailed insights into the sequential events following activation and differentiation and the associated changes in function, survival/apoptosis, and memory formation. Therefore, we expressed a transgenic TCR in congenically distinct wild-type mice and mice deficient in SHP-1 selectively in the T cell compartment and directly assessed the impact of SHP-1 deficiency on CD8 T cell responses to a viral challenge. The rapid and extensive expansion of wild-type CD8 T cells that occurs in response to LCMV infection, reaching peak response at 8 d post-infection, has been extensively described (45). Although SHP-1 deficiency did not impact the kinetics of this expansion, it did dramatically increase the magnitude of the peak primary response of naïve T cells (3.5-fold). This likely resulted from a composite of influences, as the size of the peak CD8 T cell response from a defined number of reactive precursors is determined by many factors, including the signal strength resulting from the avidity of TCR–peptide–MHC interactions and the amount of Ag available, factors that support CD8 T cell expansion, such as IL-2, which

continued to expand after day 5, however, and reached peak response during the next 48 h (Fig. 7B). Data from days 6 and 7 postinfection were combined to increase the power of statistical comparisons and revealed significantly increased numbers of SLECs in both SHP-1−/− and, to a lesser degree, SHP-1+/− populations compared with wild-type cells (Fig. 7C). No significant increase in the numbers of cells with an MPEC phenotype was observed between the groups (Fig. 7D). A decrease in the number of MPECs was noted in all groups from day 5 through days 6 to 7, which may reflect not just contraction but some degree of continued conversion to SLEC during this time period. A similar experiment was performed with secondary transfer of a smaller number of donor TCMs isolated from mice at later times (110 d) after primary infection that again revealed an increase in the generation of SLECs from SHP-1−/− and SHP-1+/− transferred cells compared with wild-type cells, but no differences in the formation of MPECs (data not shown). These results suggest that SHP-1 regulates the expansion of memory cells similar to the regulation of naïve cells. However, in contrast to the primary response, a deficiency of ~50% of SHP-1 protein appears sufficient to result in an increased formation of SLECs compared with wild-type cells, with complete deficiency resulting in even greater numbers of SLECs. Thus, the secondary CD8 T cell response appears more sensitive to regulation by SHP-1 than the primary response.

FIGURE 5. SHP-1−/− deficient SLECs show increased survival at the peak of primary expansion. A–C. A total of 10^7 P14 Thyl.1+b+ CD8+ T cells (SHP-1+/+, SHP-1−/−, or SHP-1+/−) were transferred into wild-type B6 hosts that were infected with LCMV Armstrong 48 h later. Figure shows data from four mice per group on days 7–9 and three mice per group on day 10. A, Splenocytes were stained for CD8, Thy1.1, KLRG1, and CD127 to identify donor SLEC populations (KLRG1^b+ CD127^low), with average cell numbers (± SEM) calculated from total spleen counts on days 7–10 of infection and the percent of donor cells determined by FACS analysis. B, 7-AAD and Annexin V staining of splenocytes on days 7–10 postinfection was performed to assess cell survival. Plots show data from day 8 postinfection gated on CD8+ Thyl.1+b+ SLECs (KLRG1^b+ CD127^low). Values represent percent of gated cells within each quadrant. C, Chart shows the average percent of 7-AAD− Annexin V− cells of the total donor SLEC population (± SEM) on days 7–10 postinfection with four mice per group on days 7–9 and three mice per group on day 10. Statistical analysis between the groups on day 8 was conducted using unpaired, two-tailed Student t tests with the level of statistical significance set at p < 0.016 per Bonferroni correction for the multiple comparisons (three t tests) performed.
promotes proliferation of the responding CD8 T cells, and type I IFNs, which promote survival, may also contribute (4, 5, 50). SHP-1 regulates IL-2 signaling through its recruitment to the IL-2R and subsequent dephosphorylation of IL-2Rβ, Jak1, and Jak3 (34). SHP-1 is also found associated with the IFN-α receptor (33) and with the downstream signaling molecule Tyk2 (35). The loss of SHP-1 or its activity from T cell and myeloid cell lineages results in increased Jak1 and Tyk2 phosphorylation (33, 35). Thus, the enhanced survival of SHP-1–deficient CD8 T cells that we observed following in vitro stimulation and the known involvement of SHP-1 in regulating signaling from IL-2 and type I IFNs suggests that the effects of SHP-1 deficiency on in vivo responses may reflect in part an increase in the perceived strength of cytokine signals.

SHP-1 deficiency not only impacted the total size of the responding population, but also its composition and quality, with the higher peak of primary response reflecting almost entirely an increase in the numbers of SLECs formed and very little change in the size of the longer-lived MPEC population. This finding could result from a generalized increase in the magnitude of the response by the population of MPECs as well as SLECs with a simultaneous increase in the differentiation of early effector cells and MPECs into SLECs, a process known to be promoted by inflammatory cytokines, such as IFN-γ (1, 51, 52). IFN-γ signaling is regulated by SHP-1, and SHP-1 deficiency in T cells and macrophages is associated with a higher degree of phosphorylation of Jak2 and Stat1 (29, 53–55). Thus, an increased intracellular perception of inflammation as mediated by IFN-γ signaling may push the responding CD8 T cell populations to further differentiate and enter the short-lived effector pool.

The observed increase in SLECs at the peak of the response could also represent a selective effect of SHP-1 deficiency on the generation of SLECS by increasing the survival and/or proliferation of already formed SLECs. During the expansion of CD8 T cells responding to an in vivo infection, the effector cells being generated are not only proliferating, but also a subset of the expanding effector cells are concurrently undergoing apoptosis (8). TGF-β selectively induces apoptosis of SLECs during the expansion of CD8 T cells in the context of a primary in vivo response to *Listeria* infection, and disruption of TGF-β signaling leads to the generation of increased numbers of SLECs at the peak of the response (8). We observed increased survival of SHP-1–deficient SLECs following LCMV infection, and, as SHP-1 is activated downstream of TGF-β signaling in T cells (29), the increased survival might represent in part a decrease in susceptibility to TGF-β–induced apoptosis.

The analysis of apoptotic and preapoptotic cells at different time points during the in vivo response suggested that increased survival contributes to, but cannot totally account for, the observed increase in SHP-1–deficient SLECS during the expansion phase of the primary response, implying there may also be a selective increase in proliferation of SLECS. KLREG1<sup>high</sup> SLECS have been proposed to have a lower proliferative potential than the KLREG1<sup>low</sup> MPECS, in part due to expression of higher levels of the cell cycle protein that regulates proliferation. Thus, SHP-1 deficiency may affect the balance between survival and proliferation, leading to an overall increase in the size and quality of the SLEC population.
inhibitor p27 (56). Studies of SHP-1 regulation of epithelial cell growth have correlated decreases in SHP-1 activity (or inhibition by a dominant-negative form of SHP-1) with reduced levels of p27 and increased entry into S phase of the cell cycle, even in the absence of mitogenic stimuli (57). In CD8 T cells, IL-2 signaling induces proliferation in part by downregulating expression of p27 (58), and, as a regulator of IL-2 signaling (34), SHP-1 likely directly or indirectly maintains p27 expression. Thus, SHP-1–deficient SLECs may acquire increased proliferative potential secondary to decreases in p27 levels.

Although SHP-1 deficiency resulted in no decrease in the size of the MPEC population at the peak of the primary response, the increase in TCR signal strength associated with SHP-1 deficiency could change the survival and ability of this precursor population to form long-lived TCMs, which characteristically retain the ability to proliferate following secondary encounter with the Ag. Although the total population of SHP-1−/− cells present at the peak of the response underwent proportionally greater contraction than wild-type cells following the peak, there was no decrease in the size of the TCM population formed. This finding suggests that once a cell in the MPEC population has been programmed to become a memory cell, SHP-1 has little effect on the transition to become a mature central memory cell. Many extrinsic factors, such as signaling through the IL-7 and IL-15 receptors and intracellular pathways, for example, modulation of antiapoptotic molecules, such as Bcl-2, are known to impact the survival and homeostasis of long-lived memory cells, and our observations suggest that SHP-1 is not critical to the regulation of these specific signaling pathways during memory formation and maintenance.

Memory cells have a decreased threshold for activation and enter the cell cycle more rapidly than naïve cells, in part because of enrichment of phosphoproteins in lipid rafts that facilitate the amplification of TCR signals (37, 38). As these rafts contain molecules influenced by SHP-1 during primary responses, it was uncertain if the acquisition of enhanced reactivity by memory cells would make secondary responses less subject to regulation by SHP-1. Despite SHP-1−/− and wild-type TCM populations appearing phenotypically similar, our results revealed that SHP-1 regulates secondary responses in much the same way as primary responses. The higher peak of secondary response once again reflected the generation of a greater number of effector cells with an SLEC phenotype and likely limited survival potential, but no change in the numbers of cells that contain the precursors of putative long-term secondary memory. Moreover, heterozygous SHP-1+/− memory cells, which are only partially deficient in SHP-1, exhibited enhanced responsiveness very similar to cells completely lacking SHP-1. This advantage of partial SHP-1 deficiency had not been apparent in the MPEC population at the peak of the primary response, and wild-type TCM populations appear-
SHP-1 activity in CD8 T cells for use in adoptive therapy may influence not only CD8 T cell activation but also the magnitude and deficient CD8 T cells likely reflects a composite of these pathways, and the enhanced functions observed in SHP-1–defined and tested (32).

Using knockdown of SHP-1 levels in adoptively transferred T cells, a threshold for activation (data not shown). The observation that Ag-tumor Ag, exhibit enhanced proliferative responses and a reduced magnitude of effector CD8 T cells generated during an immune response, and increase the magnitude of effector CD8 T cells activated CD8 T cells.

Bcl-3 up-regulation by signal 3 cytokine (IL-12) prolongs survival of antigen-activated CD8 T cells. Bcl-2 protein-tyrosine-phosphatase deficiency.

7769.


A. SHP-1+/+ and SHP-1Me/Me CD8 T cells expressing a transgenic TCR (TCR\textsuperscript{Gag}) recognizing the Gag protein from the Friend Murine Leukemia Virus were stained for CD8 and surface expression CD44, Ly6C, CD25, and IL-15Rα followed by FACS analysis. Histograms were gated on CD8\textsuperscript{+} cells and the values represent the percent of the total CD8 population within the gate defined as positive for the marker. The shaded histograms show the background staining with isotype control antibodies. B. 5x10\textsuperscript{6} naive CD44\textsuperscript{Low} Ly6C\textsuperscript{-} SHP-1+/+ CD8 T cells expressing TCR\textsuperscript{Gag} and Thy1.1 were transferred into SHP-1+/+ or SHP-1Me/Me hosts for 7 days, followed by staining of splenocytes for CD8, Thy1.1, CD44 and Ly6C. Histograms were gated on CD8\textsuperscript{+} Thy1.1\textsuperscript{+} cells. The values represent the percent of donor cells with positive staining for the indicated molecule, and the shaded histograms represent background staining with isotype control antibodies. C. 2x10\textsuperscript{6} TCR\textsuperscript{Gag} CD8\textsuperscript{+} Thy1.1\textsuperscript{+} T cells were isolated from SHP-1+/+ and SHP-1Me/Me mice and transferred into B6 hosts. 3 weeks later, splenocytes were harvested and stained for CD8, Thy1.1, CD44, and Ly6C. Histograms were gated on CD8\textsuperscript{+} Thy1.1\textsuperscript{+} cells. The values represent the percent of donor cells with positive staining for the indicated marker and the shaded histograms represent background staining with isotype control antibodies. The data are representative of at least two independent experiments for panels A and B and one experiment for panel C.