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The Phosphatase Src Homology Region 2 Domain-Containing Phosphatase-1 Is an Intrinsic Central Regulator of Dendritic Cell Function

Indu R. Ramachandran,* Weitao Song,† Natalia Lapteva,* Mamatha Seethammagari,* Kevin M. Slawin,‡ David M. Spencer,*∥ and Jonathan M. Levitt*†

Dendritic cells (DCs) initiate proinflammatory or regulatory T cell responses, depending on their activation state. Despite extensive knowledge of DC-activating signals, the understanding of DC inhibitory signals is relatively limited. We show that Src homology region 2 domain-containing phosphatase-1 (SHP-1) is an important inhibitor of DC signaling, targeting multiple activation pathways. Downstream of TLR4, SHP-1 showed increased interaction with several proteins including IL-1R–associated kinase-4, and modulated LPS signaling by inhibiting NF-κB, AP-1, ERK, and JNK activity, while enhancing p38 activity. In addition, SHP-1 inhibited prosurvival signaling through AKT activation. Furthermore, SHP-1 inhibited CCR7 protein expression. Inhibiting SHP-1 in DCs enhanced proinflammatory cytokines, IL-6, IL-12, and IL-1β production, promoted survival, and increased DC migration to draining lymph nodes. Administration of SHP-1–inhibited DCs in vivo induced expansion of Ag-specific cytotoxic T cells and inhibited Foxp3* regulatory T cell induction, resulting in an enhanced immune response against pre-established mouse melanoma and prostate tumors. Taken together, these data demonstrate that SHP-1 is an intrinsic global regulator of DC function, controlling many facets of T cell-mediated immune responses.

Dendritic cells (DCs) are specialized APCs that initiate and direct innate and adaptive immune responses upon recognition of foreign or self-danger signals (1, 2). They do so by undergoing maturation, resulting in the upregulation of adhesion molecules, MHC molecules, costimulatory molecules, and the lymph node (LN) chemotactic receptor CCR7. CCR7 upregulation allows DCs to migrate to local LNs where they encounter T cells (3), and depending on their activation state, they can induce a variety of immunogenic or tolerogenic responses (4, 5). Signals induced by ligation of pattern recognition receptors, MHC molecules, costimulatory molecules (e.g., CD40 and CD80), and inflammatory cytokine receptors (e.g., IFN-γR and TNFR) activate DCs, enabling them to drive immunogenic T cell responses. In contrast, signals induced by ligation of inhibitory and coinhibitory receptors (e.g., the inhibitory Ig-like transcript family members) and suppressive cytokine receptors (e.g., IL-10R and TGF-β receptors) can result in DC-mediated regulatory T cell responses (6). Despite a solid understanding of activating receptor signaling within DCs, the mechanisms of inhibitory signaling that modulate DC activation are less clear.

The Src homology region 2 domain-containing tyrosine phosphatase-1 (SHP-1) is expressed in a wide variety of immune cells where it plays a largely inhibitory role in cell signaling initiated through a range of cytokine, chemokine, growth factor, and AgR stimuli (7). Although SHP-1 signaling has been studied in lymphocytes and monocytes, its DC-specific role has not been well characterized. Studies addressing SHP-1 function in DCs have typically been performed using the motheaten (me/me) and motheaten viable (me/me′) mouse strains, which are spontaneously arising SHP-1–deficient strains. These mice display global hematopoietic compartment dysregulation, resulting in severe autoimmunity within the first few weeks of life (8). DCs from me/me′ mice are hyperresponsive to GM-CSFR signaling (9) and accumulate extensively in the lungs, contributing to increased asthma susceptibility (10). In addition, myeloid cells (DC precursors) from me/me′ mice show enhanced survival, cell growth, activation, and chemotactic responses (11). In these studies, however, it is difficult to distinguish DC-intrinsic and -extrinsic effects because SHP-1 is deficient in all somatic cells.

Evidence for an intrinsic role for SHP-1 in DCs comes from the observations that several DC inhibitory receptors (e.g., Ig-like transcript 3, FCγRIIB, signal regulatory protein-1α, and sialic acid-binding Ig-like lectins) recruit SHP-1 to their cytoplasmic domains (7, 12). Despite the implications of such recruitment, the specific signaling and functional consequences of SHP-1 activation in DCs is unclear. Recently, a role for SHP-1 in regulating TLR4 activity in DCs has been reported previously (13). In that study, the authors showed that SHP-1 inhibited TLR4-induced NF-κB signaling while promoting a TLR4-induced switch to type I IFN production. In addition, in lymphocytes, NK cells, and macrophages, SHP-1 can regulate pathways such as PI3K/AKT, JAK/STAT, chemokine receptor signaling, and NF-κB activation (7, 14), all of which are functionally important in DCs. Furthermore, SHP-1 can modulate MAPK signaling, both positively and negatively.
negatively, depending on the cell type (15–17). These observations have led us to hypothesize that SHP-1 may be a key DC-intrinsic regulator and thereby may control downstream T cell responses.

In this study, to investigate its DC-intrinsic role, we modulated SHP-1 activity in wild-type (WT) DCs by RNA interference (RNAi), chemical inhibition, and overexpression of an enzymatically dead SHP-1 mutant. We show that in DCs, SHP-1 modulated MAPK signal transduction and inhibited NF-kB and AP-1 activity. SHP-1 inhibition in DCs also led to increased proinflammatory cytokine production and Akt activation, corresponding to increased DC survival. Although inhibiting SHP-1 did not affect most DC maturation markers, CCR7 was upregulated and led to enhanced DC migration to draining LNs in vivo. Importantly, mice vaccinated with SHP-1–inhibited DCs mounted effective immune responses against both melanoma and prostate tumors. Our studies demonstrate that SHP-1 is an intrinsic inhibitor of DC signaling controlling a wide range of functions required for the initiation of T cell immunity.

Materials and Methods

Mice and cell lines

Three- to 5-wk-old WT C57BL/6, me/mε, and OVA-specific TCR (OT-I)–expressing transgenic mice (18) were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were housed in the pathogen-free transgenic mouse facility at the Baylor College of Medicine (Houston, TX). Mice were humanely killed before necropsy. Bone was harvested in accordance with protocols approved by the institutional animal care and use committees. HEK293, RAW 264.7, B16-F10 melanoma, and TRAMP-C2 prostate tumor cell lines were obtained from the American Tissue Culture Collection (Manassas, VA). The D2SC/1 mouse DC line was obtained from Dr. S.-M. Kang (University of California, San Francisco, CA) (19).

Reagents, cytokines, and Abs

For use in cell culture, sodium stibogluconate (SSG), LPS, and MG132 (Sigma-Aldrich, St. Louis, MO) were diluted in sterile PBS and used at a final concentration of 10 μg/ml, 0.1–1 μg/ml, and 1 μM, respectively. Mouse cytokines CCL21, IL-4, GM-CSF, IL-10 (PeproTech, Rocky Hill, NJ), and IFN-γ (BD Biosciences, San Jose, CA) were used for culturing or stimulating bone marrow-derived DCs (BMDCs). Phospho-Western blots were done using Abs to phospho (p)-SAPK/JNK, SAPK/JNK, p-p38, p-ERK, ERK, p-PAK (S473), p-PAK (T380), AKT, and p-3-phosphoinositol-dependent protein kinase-1 (PDK1) (S244) (Cell Signaling Technology, Danvers, MA). Other Western blotting Abs used were mouse anti–hemagglutinin (HA) (Covance, Emeryville, CA), goat anti-HA (Genscript, Piscataway, NJ), and those specific to SHP-1, GAPDH, IL-1R–associated kinase (IRAK4) (Santa Cruz Biotechnology, Santa Cruz, CA), phosphotyrosine (clone 4G10; Millipore, Billerica, MA), and β-actin (Sigma-Aldrich). Fluorochrome-conjugated mAbs against mouse CD11c, CD40, CD80, CD86, CD54 (ICAM-1), F(ab′)2 (BD Pharmingen), CD4, CD8, CD25, IFN-γ, Foxp3, CD3, IL-4, IL-17, CD44, CD-62L, and CCR7 (eBioscience, San Diego, CA) were used for flow cytometry.

BMDC culture

Bone marrow cells were flushed from the femurs and tibias of 6- to 8-wk-old WT mice and cultured for 7–5 d in RPMI 1640 medium containing 10% fCSF and 10% FBS, 50 μM beta-mercaptoethanol, as described previously (20). On day 6, cells were either sorted by MACS using anti-CD11c-conjugated magnetic beads (Miltenyi Biotec, Auburn, CA) or left unsorted prior to adenosine infection for use as in vivo vaccines studies or various in vitro assays.

Cloning of full-length SHP-1 and generating a dominant-negative SHP-1 mutant

Full-length mouse SHP-1 (WT-SHP-1) was cloned from mouse spleen cDNA into the pAdTrack expression vector (Stratagene, La Jolla, CA) by PCR using an N-terminal HA tag and XhoI restriction site-containing forward primer 5′-GGCCATGGAATTCGAGCTCCTGTTCTC3′ and a HindIII restriction site-containing reverse primer 5′-CCGAACGTTTCTAACCTCTCTTGGAGAAACCTTTGTTC3′. A dominant-negative (DN) SHP-1 (C455S) mutant was generated by PCR using the point mutation containing forward primer 5′-CATTGTTGCAATCTCCAGCCGCTGAGTCGCG3′ and the point mutation containing reverse primer 5′-CCGATGGCAAGCGCT- GGAATGCAACAATG-3′ in combination with the described cloning primers.

Adenoviruses

Mouse SHP-1–specific short hairpin RNA (shRNA) containing the sequence 5′-GGAGGATGACAGACGAGAATA3′ was designed and cloned into an adenoaviral vector using the pAd-BLOCK-iT-DEST RNAi gateway vectors (Invitrogen, Carlsbad, CA). HA-tagged WT-SHP-1 (Ad5-WT-SHP-1) and HA-tagged DN-SHP-1 (Ad5-DN-SHP-1) were cloned into adenoviral vectors using the Ad-Easy system (Stratagene). All adenoviruses were generated in HEK293 cells, and their large-scale preparations, including those of controls Ad5-scrambled-shRNA and Ad5-CMV-Empty, were done at the Baylor College of Medicine vector core.

Reporter assays

A total of 1–10 μg pSH1-NF-κB-SEAP or pSH1-AP-1-SEAP reporter plasmids (20) were transfected into 5–10 × 10^3 RAW 264.7 cells or BMDCs. In some assays, cells were cotransfected with WT-SHP-1 or DN-SHP-1 expression plasmids or pretransduced with Ad5-WT-SHP-1 or Ad5-DN-SHP-1 24 h prior to reporter transfection. RAW 264.7 cells were transfected by electroporation using the following conditions: 250 V, 950 μF. BMDCs were transfected using the nucleofection technology (Amaxa, Walkersville, MD) (protocol Y-001). Twenty-four hours posttransfection, 10^3 cells/group were treated in triplicates with various stimuli overnight, after which, the cells were harvested at 68°C for 1 h, and the supernatants were incubated with 4-methylumbelliferone phosphate substrate (Sigma-Aldrich) for 3–24 h. Fluorescence was detected using a plate reader.

DC survival assays

CD11c+ MACS sorted or unsorted bulk BMDCs were transduced with either Ad5-SHP1-shRNA or Ad5-scrambled-shRNA, treated with SSG, or left untreated as a control. Virus was washed away in 2 h. A total of 2 × 10^5 BMDCs/group were used to determine cell survival by dual propidium iodide and annexin V staining, following by flow cytometric analysis over a 2-wk period.

In vitro DC migration assays

Overnight SSG-treated or untreated BMDCs were washed and resuspended in serum-free RPMI 1640 medium (SMF) at 5 × 10^5 cells/100 μl. Five hundred microliters of SMF containing 200 ng/ml CCL21 (PeproTech) and 20 µg/ml of either CCR7-neutralizing Ab (clone 4B12; eBioscience) or a rat isotype control Ab (eBioscience) was added to the bottom well of a 24-well Transwell plate (Corning, Corning, NY) as trafficking media. SMF without CCL21 were used as control. One hundred microliters of the cell suspension was loaded in each 5-µm-pore Transwell chamber that was placed on the wells with appropriate trafficking media. The plate was incubated at 37°C for 24 h. Migrated cells were counted using a hemocytometer.

Western blots and immunoprecipitation assays

For Western blotting, cell lysates were prepared in a lysis buffer (0.002 M Tris, 0.14 M NaCl, and 0.025% NaN_3 containing 1% Nonidet P-40 and a protease and phosphatase inhibitor mixture (Roche, Florence, SC). For phospho-Western blots, BMDCs were serum starved for 16 h in the presence or absence of SSG prior to stimulation. The following day, 1 × 10^6 cells/group were treated with LPS for varying time points or left untreated as a control. Stimulations were stopped by adding ice-cold PBS, cells were collected, and lysates were prepared. For immunoprecipitation assays, D2SC/1 cells were transfected with control Ad5-CMV-empty, Ad5-DN-SHP-1, or Ad5-WT-SHP-1 at 40,000 viral particles/cell. Twenty-four hours posttransduction, cells were either stimulated with LPS for 10 min or left unstimulated. Ice-cold PBS was used to stop the stimulations. Cells were collected and washed three times with ice-cold PBS, and lysates were prepared using a lysis buffer containing a protease and phosphatase inhibitor mixture, 2 mM EDTA, 20 mM HEPEs, and 0.5% Triton X-100. Precleared lysate (1500–2000 µg) from each sample was incubated with 3–4 µg of the immunoprecipitating Ab on a rocking platform for 30 min and 4°C. Eighty microliters of protein G-agarose beads (Roche) was added to the mixture and incubated for an additional 2 h at 4°C. The beads were washed five times with the lysis buffer and boiled with 2× laemmli buffer. The lysates were run on an SDS-PAGE gel, transferred, and blotted using standard Western blotting procedures.
Real-time quantitative PCR analysis

To quantify CCR7 and SHP-1 mRNA levels in BMDCs, mRNA was purified using TRIzol (Invitrogen) and reverse transcribed using standard protocols before performing real-time TaqMan PCR on the cDNA using primer/probe sets specific for CCR7, SHP-1, and GAPDH (Applied Biosystems, Foster City, CA) using an ABI/PRISM7000 sequence detection system (Applied Biosystems). All samples were run in duplicate, and CCR7 and SHP-1 mRNA levels in each sample were normalized to the GAPDH internal control by using the 2^(-ΔΔCT) method of analysis (21).

In vitro T cell proliferation assays

Modified or unmodified BMDCs were pulsed overnight with 25 μCi of either OVA257–264 (SIINFEKL) or tyrosinase-related protein-2 (TRP-2)(181–188 (SYVDFFVWVL) and cocultured with 10^6 OT-1 transgenic responder RBC-labeled splenocytes at varying ratios. Twenty-four hours later, 1 μCi thymidine was added to these cultures. Another 16 h later, thymidine incorporation was measured by counting radioactivity using a liquid scintillation counter.

In vivo T cell analysis

Spleens were collected from experimental mice 1 wk after vaccination, single-cell suspensions were prepared, and RBCs were lysed using ACK buffer. T cell phenotype was analyzed by flow cytometry with staining for intracellular cytokines IL-4, IL-17, and IFN-γ and Foxp3, after permeabilizing cells using the BD Cytoperm Cytofix kit (BD Biosciences).

In vivo tumor vaccine experiments

BMDCs were transduced with Ad5-SHP-1 shRNA, Ad5-DN-SHP-1, or control viruses or left untransduced and matured with 1 μg/ml LPS. Twenty-four hours after the transductions, BMDCs were pulsed with 25 μM TRP-2 peptide for the B16 tumor vaccine experiments or pulsed with TRAMP-C2 whole-cell lysates for the TRAMP-C2 tumor vaccine experiments. The TRAMP-C2 whole-cell lysates were prepared by rapidly freeze-thawing the cells three times, vortexing, and centrifugation. The supernatant from lysates was collected and used to pulse BMDCs at a 3:1 TRAMP-C2:BMDC ratio. Ectopic tumors were established in WT mice by injecting 10^4 B16 cells or 10^6 TRAMP-C2 cells. Tumor-bearing mice were vaccinated with 2–3 × 10^5 prepared BMDCs each via i.p. or s.c. routes 3 d posttumor inoculation. Tumor growth was measured every 3–4 d using calipers. Tumor volume was calculated using the formula m1^2 × m2 × 0.5236, where m2 is the larger dimension of the tumor.

Statistical analysis

All statistical analyses were performed using the Student t test or one-way ANOVA, followed by Tukey-Kramer honestly significant difference post hoc test using JMP IN 5.1.2 software (SAS, Cary, NC).

Results

SHP-1 inhibits NF-κB and AP-1 activation and cytokine production in DCs

We first asked whether SHP-1 regulated signaling through one (or more) of four major DC receptors: IFN-γR, IL-10R, CCR7, and TLR4. Because these receptors can activate NF-κB and AP-1 (22–25), we initially determined the effect of the small molecule SHP-1 inhibitor SSG (26) on ligand-mediated NF-κB and AP-1 reporter induction (Fig. 1A, 1B). SHP-1 inhibition in primary mouse BMDCs enhanced LPS-, CCL21-, IFN-γ-, and IL-10–induced NF-κB activity by 5-, 2.6-, and 2-fold, respectively (Fig. 1A). SSG treatment enhanced CCL21- and IFN-γ-induced AP-1 activity by 2-fold, whereas it had no effect on LPS- and IL-10–induced AP-1 activity (Fig. 1B). These results show that SHP-1 inhibits signaling through multiple receptors and regulates NF-κB and AP-1 signaling in DCs.

Because NF-κB and AP-1 are both important in cytokine regulation (27), we next asked whether SHP-1 modulated cytokine production in DCs. To test this, we used three methods of SHP-1 inhibition in BMDCs: 1) SSG treatment; 2) overexpression of a DN-SHP-1; and 3) SHP-1–specific RNAi (Fig. 1E). DN-SHP-1 was generated by creating a point mutation, C453S, in the phosphatase domain of WT-SHP-1 to render SHP-1 catalytically inactive (28). SHP-1 functionally enhanced NF-κB activity in BMDCs, and this level was comparable to that achieved by SSG treatment (data not shown). The DN-SHP, WT-SHP-1, and SHP-1 shRNA were cloned into adenoviral vectors, and their expression (Fig. 1C) and SHP-1–specific knockdown (Fig. 1D) were determined by titration of viral particles on BMDCs. Although SHP-1 inhibition had a minimal effect on basal cytokine production, SHP-1 inhibition by SSG, shRNA, or DN-SHP-1 enhanced LPS-induced IL-12 p70, IL-1β, and IL-6 production while decreasing LPS-induced TNF-α production unchanged (Fig. 1E). This effect of SHP-1 inhibition on LPS-induced cytokine production was also observed at lower concentrations of LPS (10 ng/ml to 1 μg/ml) (Supplemental Fig. 1). CCL21 treatment moderately enhanced LPS-induced IL-6 production, which was further induced by SSG treatment (Supplemental Fig. 2A). However, CCL21 did not alter the effects of LPS or SSG on IL-12 p70 (Supplemental Fig. 2B). Although IFN-γ alone did not affect the production of any of the cytokines tested, IFN-γ synergistically enhanced the effect of SHP-1 inhibition on LPS-induced IL-6 (Supplemental Fig. 2C) and IL-12 p70 (Supplemental Fig. 2D) production but did not affect LPS-induced TNF-α (Supplemental Fig. 2E) or IL-1β (Supplemental Fig. 2F) production. Taken together, these data show that SHP-1 inhibition enhances proinflammatory cytokine production by LPS, IFN-γ, and CCL21 signaling, potentially because of the ability of SHP-1 to inhibit NF-κB and AP-1 signaling.

SHP-1 can also regulate signaling through the DC differentiating cytokines IL-4 and GM-CSF by dephosphorylating JAK/STATs downstream of their receptors (9, 29). To determine whether SHP-1 affected IL-4 and GM-CSF signaling in DCs, we stimulated BMDCs with IL-4 or GM-CSF and measured TNF-α expression. As previously reported (30), IL-4 treatment inhibited TNF-α production, and GM-CSF enhanced TNF-α production. SHP-1 inhibition synergized with both IL-4 and GM-CSF to further inhibit or enhance TNF-α production, respectively (Fig. 1F). Collectively, our results show that in DCs, SHP-1 can modulate signaling downstream of major DC receptors TLR4, IFN-γR, IL-10R, CCR7, IL-4R, and GM-CSFR and regulate proinflammatory cytokine production.

SHP-1 is involved in the negative feedback of LPS-induced signaling in DCs

To prevent autoimmunity, excessive activation by TLR4 signaling is regulated by the induction of inhibitory molecules (31). We have shown that SHP-1 can inhibit NF-κB and AP-1 downstream of TLR4 in DCs (Fig. 1A, 1B). But interestingly, NF-κB itself binds the SHP-1 promoter (14), suggesting a negative feedback mechanism regulating TLR4 signals. We therefore asked whether LPS altered SHP-1 expression in BMDCs. LPS stimulation rapidly induced native SHP-1 protein expression over basal levels, starting within 5 min of stimulation and continuing to at least 1 h poststimulation (Fig. 2A). This rapid induction of SHP-1 protein occurs at a rate not likely achieved through de novo expression, suggesting that SHP-1 levels may be posttranscriptionally regulated. To determine this, we asked whether LPS changed the rate of SHP-1 protein degradation or the steady-state concentrations of SHP-1 mRNA. Incubation of BMDCs with the proteasomal inhibitor MG132 stabilizes SHP-1 protein within 5 min of its addition (Fig. 2A). This stabilization is further enhanced by the concurrent addition of LPS to the media (Fig. 2A). Addition of MG132 or the combination of MG132 and LPS, conversely, had no effect on the steady-state levels of SHP-1 mRNA (Fig. 2B). Taken together, these data suggest that SHP-1 protein levels are undergoing rapid turnover within the cells and that protein levels...
are rapidly stabilized on LPS stimulation, at least in part, by a decrease in the rate of proteasomal degradation.

In DCs, TLR4-mediated functional changes are regulated in part by the activation of the MAPKs (2). Although MAPKs ERK and JNK primarily promote DC maturation and proinflammatory cytokine production (32, 33), p38 promotes maintenance of immature phenotype and regulatory cytokine production in DCs (34), suggesting that the collective activation status of each individual MAPK determines the balance between pro- and anti-inflammatory responses by DCs. Because SHP-1 modulates MAPK signaling in lymphocytes and macrophages (13, 16), and SHP-1 inhibited LPS-induced BMDC cytokine production (Fig. 1E–G), we examined the role of SHP-1 in TLR4-induced MAPK activation in DCs. Untreated or SSG-treated BMDCs were stimulated with LPS and analyzed for ERK, JNK, and p38 phosphorylation. As expected, LPS stimulation in untreated BMDCs led to an increase in phosphorylation of all three MAPKs. Although SSG treatment alone did not change basal ERK and JNK phosphorylation, LPS stimulation in SSG-treated BMDCs led to an increase in ERK and JNK phosphorylation kinetics (Fig. 2C). Conversely, basal levels of p-p38 were decreased in SSG-treated BMDCs, although LPS stimulation did induce p38 phosphorylation with similar kinetics to untreated cells. This suggests that under resting conditions, SHP-1 enhances p38 phosphorylation in DCs helping to maintain an immature phenotype, which is overcome by stimuli such as LPS that drive DC maturation.

To identify target proteins of SHP-1 in LPS-mediated TLR4 signaling, we expressed HA-tagged WT-SHP-1 or an HA-tagged

**FIGURE 1.** SHP-1 regulates DC activation of NF-κB and AP-1 and cytokine production. NF-κB (A) or AP-1 (B) reporter assays were performed in SSG-treated or untreated BMDCs stimulated with LPS (1 μg/ml), IFN-γ (500 U/ml), CCL21 (200 ng/ml), IL-10 (100 ng/ml), or left unstimulated. The y-axes show fold difference over the untreated group. Histograms are representative of two to three independent experiments and are shown as mean ± SEM of triplicates within the same experiment. C, Immunoblot analysis of Ad5-DN-SHP-1 and Ad5-WT-SHP-1 expression in BMDCs with an Ab specific to the HA tag. Western blots were repeated twice. D, Immunoblot analysis of SHP-1 protein knockdown by Ad5-mSHP-1 shRNA or control Ad5-scrambled shRNA in BMDCs 48 h post viral or mock (no virus) transduction. Western blots were repeated at least three times. E, IL-12 p70, IL-1β, IL-6, and TNF-α ELISAs performed on supernatants from untreated or SSG-treated BMDCs, Ad5-SHP-1 shRNA (SHP-1 shRNA)- or Ad5-scrambled shRNA (scram shRNA)-transduced BMDCs or Ad5-WT-SHP-1–, Ad5-DN-SHP-1–, or control Ad5-CMV-empty–transduced BMDCs in the presence or absence of 1 μg/ml LPS for 24 h. Data are shown as mean ± SEM of duplicates within the same experiment. Experiments were repeated three times. F, TNF-α ELISA performed on supernatants from BMDCs cultured in cytokine-free media overnight in the presence or absence of SSG and stimulated overnight with IL-4 or GM-CSF. Data are shown as mean ± SEM of duplicates within the same experiment. Experiments were repeated twice.
substrate trapping DN-SHP-1 mutant in the mouse DC line D2SC/1. Following LPS stimulation, we used an anti-HA Ab to immunoprecipitate our transfected constructs and looked for tyrosine-phosphorylated targets that were differentially coimmunoprecipitated with SHP-1. Although few tyrosine phosphorylated proteins coprecipitated with WT-SHP-1 in the absence of stimulus, this basal coprecipitation was higher with DN-SHP-1, suggesting that DN-SHP-1 was indeed trapping its substrates (Fig. 2D). In the presence of LPS, several tyrosine-phosphorylated proteins showed increased interaction with WT-SHP-1, and this increased interaction was noticeably higher with DN-SHP-1. To identify some of these candidates, we screened for proteins that are known to be involved in TLR4 signaling and whose molecular weights correspond to those of the LPS-induced phospho-tyrosine bands following immunoprecipitation with the anti-HA (Fig. 2D). We observed that LPS stimulation induced SHP-1 interaction with IRAK4 (Fig. 2E) but not with MAL/TIRAP, MyD88, SYK, VAV1, PYK2, or BTK (data not shown). To confirm this observation, we performed the reciprocal immunoprecipitation with anti-IRAK4 on LPS-stimulated cells and probed for HA. We observed, as before, that SHP-1 coprecipitated with IRAK4 (Fig. 2E). These findings indicate that LPS rapidly induces SHP-1 protein expression by preventing its proteasomal degradation, and SHP-1, in turn, dampens DC activation by negatively regulating LPS-mediated ERK and JNK signaling and positively regulating basal p38 phosphorylation. At least one mechanism by which SHP-1 can modulate TLR4 signaling is through IRAK4 interaction and dephosphorylation.

SHP-1 inhibits CCR7 upregulation and CCR7-induced DC migration

Maturation and migration to LNs in response to Ag acquisition by DCs in combination with immunological danger signals are required for initiating T cell responses (3). We asked whether SHP-1 was involved in the regulation of DC maturation, which is marked by upregulation of costimulatory receptors MHC and CCR7.
BMDCs were treated with LPS, SSG, or both or left untreated, and the expression of CD40, CD80, MHC class II, CD54, and CCR7 was determined after 24 h. As expected, LPS enhanced surface expression of all five receptors on BMDCs compared with untreated cells (Fig. 3A, 3B). SHP-1 inhibition upregulated CCR7 expression that was further induced in combination with LPS stimulation. SHP-1 inhibition, however, had no effect on the expression of CD40, CD80, MHC class II, and CD54. To investigate the mechanism by which SHP-1 affects CCR7 expression in DCs, we measured CCR7 mRNA levels following SHP-1 inhibition alone or in combination with LPS stimulation. SHP-1 inhibition alone did not change CCR7 mRNA levels in BMDCs compared with untreated cells (Fig. 3C). CCR7 transcription was induced by LPS, but the combination of SHP-1 inhibition and LPS did not change CCR7 message levels compared with LPS alone. These data suggest that although LPS regulates CCR7 expression by inducing transcription, regulation by SHP-1 likely occurs by a posttranscriptional mechanism.

Because SHP-1 inhibition induced CCR7 protein levels, we asked whether SHP-1 played a role in DC migration to LNs. Two lines of evidence suggest that SHP-1 might be involved in CCR7-mediated DC chemotaxis. First, SHP-1 can dephosphorylate the nucleotide exchange factor VAV1 (35), which transduces signals downstream of CCR7 (36). Second, our data show that SHP-1 inhibits CCL21-induced CCR7 signaling, leading to IL-6 expression in BMDCs (Supplemental Fig. 2A). To examine whether SHP-1 affected CCR7-mediated DC chemotaxis, we first performed in vitro migration assays using CCL21 as the chemotactic agent. SHP-1 inhibition enhanced CCL21-induced chemotaxis of BMDCs by ~2-fold (Fig. 3D, 3E). This enhancement of CCL21-induced BMDC migration by SHP-1 inhibition, as well as basal CCL21-induced BMDC migration, was inhibited by a CCR7-neutralizing Ab but not by an isotype control. This demonstrates that the observed migration was indeed CCR7 mediated. To determine whether upregulated CCR7 expression on DCs is physiologically relevant, we studied the effect of SHP-1 inhibition on DC migration in vivo. BMDCs were labeled with CFSE, treated with SSG, and injected into the hind footpads of WT mice. Twenty-four hours postinjection, popliteal LNs were removed and examined for the presence of CFSE-labeled cells. SHP-1 inhibition in BMDCs resulted in a significant increase (>4-fold) in their migration to draining LNs compared with no SHP-1 inhibition (Fig. 3F). Corresponding to their enhanced LN migration, fewer SHP-1–inhibited BMDCs remained in the footpads compared with untreated BMDCs. Taken together, these results demonstrate that SHP-1 plays an inhibitory role in CCR7 expression and can regulate CCR7-mediated migration in vitro and in vivo.

**FIGURE 3.** Effect of SHP-1 on DC maturation and migration. A. Maturation marker expression on CD11c+ BMDCs treated with SSG, 1 μg/ml LPS, or both or left untreated for 24 h. Histograms represent BMDCs from one of six mice. B. CCR7 expression on CD11c+ BMDCs treated with SSG, LPS, or both or left untreated for 24 h. Data are represent the combined mean ± SEM of six experiments. Statistical significance was determined by nonpaired Student t test. C. CCR7 mRNA analysis from untreated or BMDCs treated with SSG, 1 μg/ml LPS, or both as a fold change over untreated BMDCs. Data represent the combined mean ± SEM of three experiments. D. In vitro Transwell migration assay determining total SSG-treated or untreated BMDCs migrated in the presence or absence of CCL21 (n = 4) along with CCR7-neutralizing Ab (anti-CCR7) or isotype control (ISO). E. BMDC migration index, calculated by dividing the total number of cells migrated in the presence of CCL21 by the total number of cells migrated in the absence of CCL21. Data represent mean ± SEM of four combined experiments (n = 4). Statistical significance was determined by nonpaired Student t test. F. In vivo migration assay performed by injecting CFSE-labeled, unmodified, or SSG-treated BMDCs into footpads of WT mice. Twenty-four hours post-BMDC injection, popliteal LNs and corresponding footpads were removed, and single-cell suspension were made and analyzed by flow cytometry. Data represent nine mice per group pooled from two independent experiments (n = 9). Nonpaired Student t test was used to determine statistical significance.
**SHP-1 inhibits DC survival and AKT activation**

DCs are terminally differentiated cells that have a limited lifespan. To mount T cell responses, they need to present acquired Ag in LNs for a sufficient length of time (37). SHP-1 deficiency improves T and B cell survival in mev/mev mice (7), suggesting that SHP-1 might regulate DC survival. To test this hypothesis, BMDCs were maintained in growth factor-free minimal serum media in the presence or absence of SSG and analyzed for survival for a period of 2 wk by annexin V staining. SSG-treated BMDCs showed enhanced survival compared with untreated cells (Fig. 4A, 4B). The increase in survival of SHP-1–inhibited BMDCs was equivalent to that seen in the presence of LPS during the first 7 d, but inhibiting SHP-1 increased BMDC survival significantly over LPS treatment at day 12 (Fig. 4A). In addition to SHP-1 inhibition with SSG, we observed that reducing SHP-1 in BMDCs with Ad5-SHP-1 shRNA reduced cell death over a 72-h time period compared with treatment with control Ad5-scrambled shRNA (Fig. 4C). In these experiments, treatment with adenovirus alone caused increased cell death in comparison with untreated BMDCs, an observation that has been previously noted (38). Overall, these results indicate that in DCs, SHP-1 is an inhibitor of survival.

Although DC survival can be promoted by NF-κB and MAPKs activation (33), the AKT pathway has an important and nonredundant role in promoting DC survival (39). In T cells, SHP-1 can dephosphorylate the AKT activator PI3K (40). In other systems, SHP-1 can dephosphorylate and activate phosphatase and tensin homolog, thereby enabling it to inhibit PI3K activation (41). Thus, we hypothesized that SHP-1 inhibition might increase DC survival through the activation of AKT. To study AKT activation, we examined its phosphorylation at T308 and S473 in lysates of BMDCs stimulated with LPS in the presence or absence of SHP-1 inhibition. SHP-1 inhibition led to an increase in LPS-induced AKT phosphorylation kinetics at both T308 and S473 (Fig. 4D). Because PDK1 is the upstream kinase responsible for AKT T308 phosphorylation, we measured PDK1 activation (autophosphorylation of its activation loop at S244) in the same lysates. Basal and LPS-induced phosphorylation of PDK1 (S244) was enhanced by SHP-1 inhibition (Fig. 4D). These data show that SHP-1 inhibits DC survival, and this effect of SHP-1 is, at least in part, due to the inhibition of AKT activation.

**SHP-1 inhibition enhances DC stimulation of T cells**

Having observed that SHP-1 affected DC signaling, cytokine production, migration, and survival, all functions that affect T cell priming by DCs (4), we investigated the functional consequences of SHP-1 inhibition on T cell responses. To determine the effect of SHP-1 inhibition on DC-induced T cell proliferation, we initially performed in vitro DC:T cell coculture experiments in which transgenic OT-1 T cells (specific for Kb-OVA) were used as responders, and untreated BMDCs or SSG-treated BMDCs were used as stimulators. Pulsing untreated BMDCs with OVA peptide alone induced OT-1 cell proliferation (Fig. 5A), where as pulsing BMDCs with TRP-2, an irrelevant peptide for OT-1 responders, did not induce proliferation. When BMDCs were treated with SSG and OVA peptide, OT-1 cell proliferation was enhanced at lower DC:T cell ratios than was observed in untreated BMDCs with OVA (Fig. 5A). We repeated these experiments using Ad5-DN-SHP-1–transduced BMDCs and observed comparable results (Fig. 5B). These observations demonstrate that SHP-1 inhibits the ability of DCs to induce Ag-specific T cell proliferation.

Next, to study the effect of SHP-1–inhibited DCs on T cell responses in vivo, WT mice were vaccinated with TRP-2–pulsed BMDCs transduced with Ad5-SHP-1 shRNA, a control Ad5-scrambled shRNA, or an empty vector (Ad5-CMV-empty). One week postvaccination, spleens were collected, splenocytes were stimulated with PMA and ionomycin, and intracellular IFN-γ or Foxp3 was analyzed in CD3+ cells. We observed a significant increase in the proportion of IFN-γ–producing CD8+ T cells from

![Image 1](https://www.jimmunol.org/)

**FIGURE 4.** SHP-1 inhibits DC survival and AKT activation. A and B, Survival of untreated, SSG-treated, or 1 μg/ml LPS-treated BMDCs measured by annexin V staining. Plot (A) represents mean ± SEM of pooled data from three individual experiments (n = 3). Nonpaired Student t test was used to determine statistical significance. *p ≤ 0.05, **p ≤ 0.005. B shows representative flow cytometry histograms of one of the three experiments shown in A. C, Survival of untreated, Ad5-SHP-1 shRNA-treated, or Ad5-scrambled shRNA-treated BMDCs measured by annexin V staining. Data are representative of two individual experiments. D, Immunoblot analysis of AKT activation in untreated BMDCs or SSG-treated BMDCs stimulated with 1 μg/ml LPS as indicated. Lysates were prepared and probed for p-AKT (T308 and S473). Same blots were stripped and reprobed for total protein. Dividing lines indicate different parts of the same gel. Lysates of untreated BMDCs or SSG-treated BMDCs stimulated with 1 μg/ml LPS were also probed for p-PDK1 and loading control (β-actin). All blots represent results from two to three independent experiments.
mice vaccinated with Ad5-SHP-1 shRNA-transduced BMDCs compared with mice vaccinated with BMDCs treated with vehicle alone (Fig. 5C). No difference was seen in the proportion of IFN-γ producing between CD8+ T cells from vehicle- or control vector-vaccinated mice (Fig. 5C). In addition, Ad5-SHP-1 shRNA-transduced BMDC-vaccinated mice showed a significant in the proportion of IFN-γ–producing CD8+ T cells (C), IFN-γ–producing CD4+ T cells (D), Foxp3-expressing CD4+ T cells (E), and TRP-2–specific CD8+ T cells (F) by flow cytometry. F is representative data from one of five mice. One-way ANOVA, followed by Tukey-Kramer HSD post hoc test, was used to determine statistical significance in C–E where n = 2–7 mice/group, as represented by individual data points. Groups marked with similar symbols (*) or #) were statistically different from each other with p ≤ 0.05.

FIGURE 5. SHP-1 inhibits DC-induced T cell responses. A and B, In vitro T cell proliferation assays performed using OVA or TRP-2 peptide-pulsed SSG-treated (SSG) (A) and Ad5-DN-SHP-1 (DN-SHP-1)- or Ad5-Luciferase (Luc)-transduced or untreated WT BMDCs as stimulator cells (B). These cells were cocultured with OT-1 responder splenocytes, followed by the measurement of thymidine incorporation. Error bars represent SEM between replicates in the same experiment. Data represent two independent experiments with similar results. C–F, T cell responses measured in WT mice vaccinated i.p. with vehicle (PBS) or TRP-2–pulsed Ad5-SHP-1 shRNA-, Ad5-scrambled shRNA-, or Ad5-CMV-empty–transduced BMDCs. One week postvaccination, splenocytes were analyzed for IFN-γ–producing CD8+ T cells (C), IFN-γ–producing CD4+ T cells (D), Foxp3-expressing CD4+ T cells (E), and TRP-2–specific CD8+ T cells (F) by flow cytometry. F is representative data from one of five mice. One-way ANOVA, followed by Tukey-Kramer HSD post hoc test, was used to determine statistical significance in C–E where n = 2–7 mice/group, as represented by individual data points. Groups marked with similar symbols (*) or #) were statistically different from each other with p ≤ 0.05.
tion, and T cell stimulation responses, we investigated whether SHP-1–inhibited DCs could enhance vaccines against the nonimmunogenic mouse B16F10 melanoma and TRAMP-C2 prostate cancer models. Mice with pre-established ectopic tumors were vaccinated with Ad5–SHP-1 shRNA-transduced BMDCs, control Ad5–scrambled shRNA-transduced BMDCs, or vehicle alone. We observed significantly slower tumor growth in mice vaccinated with Ad5–SHP-1 shRNA-transduced BMDCs compared with mice vaccinated with the control groups in both tumor models (Fig. 6A, 6C). Similar results were observed in B16 melanoma-bearing mice vaccinated with DN–SHP-1–expressing BMDCs (Fig. 6B). Furthermore, in the B16 melanoma-bearing mice, a corresponding increase in survival was observed in the Ad5–SHP-1 shRNA-transduced BMDC-vaccinated mice (data not shown). These experiments demonstrate that inhibiting SHP-1 in DCs can improve their efficacy in vivo as antitumor vaccines.

Discussion

Our results establish that SHP-1 is an intrinsic central regulator of DC signaling and function. We show that SHP-1 inhibits DC activation, TLR4, cytokine and chemokine receptor signaling, survival, and LN migration. Thus, inhibiting SHP-1 in DCs enhances their overall activation and survival, leading to their enhanced ability to stimulate Ag-specific T cell proliferation and effector function.

The majority of SHP-1 functional studies to date used the globally SHP-1–deficient mev/mev mice (8, 11, 14). Our analysis of mev/mev BMDCs showed that they are defective in the expression and induction of MHC class II, and when mev/mev BMDCs were used as vaccines, this functional defect failed to elicit antitumor immune responses (data not shown). Because we did not observe a similar MHC class II defect in SHP-1–inhibited WT BMDCs, the observed mev/mev BMDC phenotype is likely to involve DC extrinsic compensatory mechanisms in response to lymphocyte hyperactivation. A similar MHC class II defect has been observed on BMDCs from paired Ig-like receptor-B (PIR-B)–deficient mice (44) that display autoimmunity as a result of B cell hyperactivation. Although PIR-B signals through SHP-1, it is again unclear whether the observed BMDC phenotype is DC intrinsic or DC extrinsic. Therefore, to overcome the disadvantages of using the mev/mev system, we have used alternate strategies to inhibit SHP-1 in developmentally normal BMDCs, including use of 1) DN–SHP-1, 2) SHP-1–specific shRNA, and 3) the small molecule SHP-1 inhibitor SSG. It is important to note the differences in the mechanism of SHP-1 inhibition by these strategies, because recent studies have shown that SHP-1 has phosphatase-independent functions (13). Although DN–SHP-1 and SSG function by inhibiting SHP-1 catalytic function (28), SHP-1 shRNA diminishes SHP-1 protein expression. Using multiple SHP-1 inhibition strategies, we show that SHP-1 negatively regulates DC NF-κB and AP-1 activation and DC cytokine production, indicating that SHP-1 phosphatase activity is required for these functions. An additional advantage of using multiple SHP-1 inhibition strategies is addressing potential off-target activity of SSG, which is highly effective irreversible inhibitor of SHP-1 activity (99%) at the dose used (10 μg/ml), but can also partially inhibit SHP-2 and PTP1B at this same concentration (26).

Although LPS-triggered TLR4 signaling can lead to DC activation through multiple signaling pathways, inhibitory molecules are induced that limit this activation as a negative feedback mechanism (31). We show in this paper that SHP-1 is one such inhibitory molecule that is rapidly induced by TLR4 signaling in DCs. Although SHP-1 mRNA and protein can be induced by these and other stimuli within 4 h (45), we show that LPS induces SHP-1 protein stabilization rapidly (within 5 min) by inhibiting its proteasomal degradation and not by mRNA induction. A high turnover of SHP-1 protein mediated by the proteasome has been recently demonstrated by others as well, where MG132 treatment induced SHP-1 expression rapidly (46).

SHP-1 participated in LPS-negative feedback in DCs not only by inhibiting NF-κB and AP-1 but also by modulating MAPK activation. We have shown that SHP-1 inhibits ERK1/2 and JNK activation while simultaneously promoting p38 activation in DCs. Our results differ from those of An et al. (13) where SHP-1–deficient DCs show increased LPS-induced p38 activation. We believe this discrepancy is due to differences in methodology. An et al. (13) use BMDCs derived from mev/mev to study the effects of LPS stimulation on SHP-1 signaling. In our experiments, we use WT-BMDCs, serum starved, and incubated for 16 h in SSG.
prior to LPS exposure. In our experience, me/mε BMDCs are significantly less responsive to LPS than WT-BMDCs inhibited for SHP-1 (I. Ramachandran, unpublished data). We see a decrease in basal p-p38 at time 0 (Fig. 2C) and the same time course for its induction (beginning at 30 min) as does An et al (13). We interpret this to mean that under resting conditions, SHP-1 enhances p38 phosphorylation. Despite conflicting literature about the specific roles of the MAPKs in DCs, a large body of evidence suggests that ERK and JNK roles in DCs are important in DC maturation, proinflammatory cytokine production, and survival (32, 33). Although p38 family proteins are known to contribute to IL-12 production (47), an excess of p38 activity inhibits DC maturation and induces anti-inflammatory cytokines such as IL-10 and TGF-β in (34, 48). We show that at least one mechanism by which SHP-1 can modulate TLR4 signaling is by causing IRAK4 dephosphorylation. IRAK4 is indispensable in the activation of the MyD88 arm of TLR4 signaling (49). MyD88 recruits IRAKs including IRAK4 and IRAK1, leading to their phosphorylation and association with TNFR-associated factor-6. TNFR-associated factor-6 then activates downstream MAPks and NF-κB by activating MAPK kinase kinase TAK1. Other studies have shown that SHP-1 can bind IRAK1 through an ITIM-like domain on IRAK1 (50, 51). IRAK4, however, lacks this domain. It is possible that SHP-1 is recruited by IRAK4 to a complex containing IRAK4, which is then dephosphorylated by SHP-1. Although IRAK4 is known to be phosphorylated on serine and threonine residues, our data suggest that IRAK4 is tyrosine phosphorylated as well. In addition, we show that besides IRAK4, there are several other tyrosine-phosphorylated SHP-1 targets in the TLR4 pathway, which we are currently working to identify. SHP-1’s function in the TLR4 pathway results in the inhibition of TLR4-induced cytokines IL-12, IL-6, and IL-1β. Thus, SHP-1 is an important global regulator of the TLR4 pathway. Overall, our data show that SHP-1 is induced by TLR4 stimulus and can participate in negative feedback signaling to dampen DC activation.

In addition to inhibiting TLR4-induced cytokines, SHP-1 regulates TNF-α production by the IL-4R and GM-CSFR. Both GM-CSFR and IL-4R signal through JAK/STAT pathways several members of which are direct substrates for SHP-1. IL-4 signaling leads to activation of STAT-6, which is required for IL-4-mediated inhibition of TNF-α production (30, 52). Furthermore, SHP-1 has been shown to directly inhibit Stat-6 phosphorylation (29, 53), suggesting that this may be the mechanism by which SHP-1 inhibition augments IL-4-mediated TNF-α inhibition.

In contrast to IL-4R, GM-CSF stimulation has been shown to induce TNF-α expression (54, 55). One known mechanism by which this occurs is through GM-CSF–induced activation of STAT3, which in turn binds to and induces the TNF-α promoter (56). Several studies have shown that STAT3 and the GM-CSF proximal kinase JAK2 are direct substrates for SHP-1 (45, 57, 58). Thus, we believe it is likely that the enhancement of GM-CSFR–induced TNF-α production in DCs is mediated at least in part by increased JAK2 and STAT3 phosphorylation when SHP-1 is inhibited.

LPS strongly induces TNF-α production in BMDCs (Fig. 1E) at levels between 2 and 3 ng/ml. LPS signaling through TLR4 can drive TNF-α expression through activation of NF-κB, IFN regulatory factor-3, and AP-1 binding sites for which are all present in the TNF-α promoter (59). The observation that SHP-1 inhibition does not enhance the already high levels of TNF-α (>10-fold higher than GM-CSFR–induced levels), combined with the fact that there are at least three pathways through which TLR4 can drive TNF-α expression, suggests that SHP-1 may not be the rate limiting step.

CCR7-mediated DC migration to the LN is crucial in priming T cell responses (3). We show that SHP-1 is also a negative regulator of CCL21-triggered CCR7 signaling, because SHP-1 inhibition led to CCL21-mediated enhancement of LPS-induced IL-6. CCR7 is a G protein-coupled receptor expressed on mature DCs. SHP-1 can bind and dephosphorylate VAV1 (35), a key signaling molecule that functions as a cytoplasmic guanine nucleotide exchange factor for Rho-family GTPases found downstream of several chemokine receptors (36). VAV1 dephosphorylation might explain the observed effect of SHP-1 on CCR7-induced IL-6. In addition, we show that inhibiting SHP-1 enhances the overall CCR7 protein levels on BMDCs in 24 h; however, it does not alter SHP-1 mRNA levels. This suggests that SHP-1 regulates CCR7 expression in DCs through a posttranscriptional mechanism. In other cell types, SHP-1 can regulate protein stability by promoting their proteasome-mediated degradation (60). It is also possible that SHP-1 uses a similar mechanism to posttranscriptionally inhibit CCR7 protein levels. Functionally, the ability of SHP-1 to inhibit CCR7 signaling and protein up-regulation resulted in reduced CCL21-dependent BMDC migration in vitro and reduced footpad to LN BMDC migration in vivo. Taken together, these data show that SHP-1 is a key inhibitor of CCR7-regulated DC migration.

Although DC activation and maturation determine the quality of T cell responses, DC lifespan determines the magnitude of T cell responses (37). Mature DCs are thought to be terminally differentiated, with a relatively short lifespan (61). DC survival can be promoted by activation of several independent signaling modules including MAPks, NF-κB, and the PI3K/AKT pathway (33, 39). Our studies show that SHP-1 is an inhibitor of DC survival. Although NF-κB and MAPks regulation may be involved in the mechanism by which SHP-1 inhibits DC survival, we show that SHP-1 also inhibits the AKT pathway in DCs. In lymphocytes and macrophages, SHP-1 is known to inhibit AKT signaling by directly inhibiting PI3K or promoting phosphatase and tensin homolog activation (40, 41). In this paper, we showed that SHP-1 inhibited LPS-induced AKT phosphorylation in BMDCs at both S473 and T308. SHP-1 also inhibited LPS-induced autophosphorylation of AKT T308 upstream kinase PDK1. Taken together, our data suggest that SHP negatively regulates DC survival by targeting prosurvival pathways.

Although increased rate of DC migration to lymph nodes (Fig. 3F) and their longevity (Fig. 4) may contribute to increased T cell stimulation by SHP-1–inhibited DCs in vivo, these factors likely do not play a role in the observed increase in T cell proliferation induced by SHP-1–inhibited DCs in vitro. As seen in Fig. 3A, CD40, MHC class II, CD86, and ICAM-1 surface expression is present at some measurable level on BMDCs, even in the absence of any treatment. Although SSG treatment does not itself enhance the expression of these proteins, it can still affect the ability of DCs to stimulate T cell proliferation through alterations in the cytokine milieu. As shown in Fig. 1E, SHP-1 inhibition results in increased expression of IL-12, IL-1β, and IL-6, all of which are known to be able to enhance TCR-driven T cell proliferation. Other investigators have described a similar phenomenon where BMDCs deficient for PIR-B, a SHP-1 recruiting receptor, are able to enhance T cell stimulation without increasing surface costimulatory molecule expression (13). In addition, we cannot rule out that additional DC surface proteins or cytokines that we have not measured might also contribute to increased stimulation of T cells.

Overall, in this study, we have shown that SHP-1 negatively regulates multiple DC functions including activation, cytokine production, migration, and survival, all of which significantly influence DC ability to stimulate T cell responses. Indeed, we show
that SHP-1 inhibits DC-induced T cell proliferation in an Ag-specific fashion in vitro and in vivo. In addition, SHP-1 inhibits DC-induced Th1 responses and IFN-γ–producing CD8+ T responses while concurrently promoting Foxp3+ T cell proliferation. These observations correspond with SHP-1–inhibited BMDCs inhibiting tumor growth in the mouse B16 melanoma and TRAMP-C2 prostate cancer models. A single vaccine of SHP-1–inhibited DCs also enhanced survival of tumor-bearing mice. Thus, as a proof of principle, we have shown that SHP-1 inhibition is an effective strategy to enhance the effectiveness DC-based vaccines against pre-established tumors. These data suggest that manipulating SHP-1 in DCs could potentially be used as a platform to improve vaccines not only against tumors but also against infectious diseases as well. A further implication of SHP-1 as a central intrinsic regulator of DC function is the potential for developing methods to enhance SHP-1 activity, which could modulate DC-induced tolerance and thus help control autoimmune diseases.

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Disclosures
The authors have no financial conflicts of interest.

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The Phosphatase SHP-1 is an Intrinsic Central Regulator of Dendritic Cell Function

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Supplemental Figures

Figure S1. SHP-1 inhibition by SSG enhances LPS-induced IL-6 production in DCs. IL-6 ELISA performed on supernatants from vehicle (PBS)-pre-treated or SSG-pre-treated (16h) BMDCs stimulated with varying concentrations of LPS for 24 hours. Data are shown as mean +/- SEM of two independent experiments.
Figure S2. Regulation of CCL21 and IFN-γ-induced cytokine production by SHP-1 in DCs.

ELISAs were performed to determine (A) IL-6 and (B) IL-12 p70 production in supernatants from untreated or SSG-treated BMDCs that were further stimulated with LPS and/or CCL21 for 24 hours. Data are shown as mean +/- SEM of duplicates within the same experiment. ELISAs were performed to determine (C) IL-6, (D) IL-12 p70, (E) TNF-α and (F) IL-1β production in supernatants from untreated or SSG-treated BMDCs that were further stimulated with LPS and/or IFN-γ for 24 hours. Data are shown as mean +/- SEM of duplicates within the same experiment. All experiments were repeated 3 times.