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Resistance to Experimental Autoimmune Encephalomyelitis and Impaired T Cell Priming by Dendritic Cells in Src Homology 2 Domain-Containing Protein Tyrosine Phosphatase Substrate-1 Mutant Mice

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Src homology 2 domain-containing protein tyrosine phosphatase (SHP) substrate-1 (SHPS-1) is a transmembrane protein that binds the protein tyrosine phosphatases SHP-1 and SHP-2 through its cytoplasmic region and is expressed on the surface of CD11c⁺ dendritic cells (DCs) and macrophages. In this study, we show that mice that express a mutant form of SHPS-1 lacking most of the cytoplasmic region are resistant to experimental autoimmune encephalomyelitis (EAE) in response to immunization with a peptide derived from myelin oligodendrocyte glycoprotein (MOG (35–55)). The MOG (35–55)-induced proliferation of, and most of the cytoplasmic region are resistant to experimental autoimmune encephalomyelitis (EAE) in response to immunization targeting in inflammatory disorders of the CNS and other autoimmune diseases. The Journal of Immunology, 2007, 179: 869–877.

Dendritic cells (DCs) are professional APCs and play a central role in the induction of immune responses to pathogens (1, 2). When immature DCs, such as Langhans cells, which reside in nonlymphoid tissues, encounter exogenous Ags, they engulf and process them for presentation of Ag-derived peptides in complexes with MHC molecules on the cell surface. The DCs subsequently migrate to draining lymph nodes (LNs) and make contact with naive T cells. During such migration, DCs mature and express costimulatory molecules such as CD80, CD86, and CD40 in addition to MHC class II molecules on their surface. The mature DCs thus present MHC-peptide complexes to naive T cells together with costimulatory molecules that are essential for priming of the T cells by DCs (3). However, the detailed molecular mechanism of priming of naive T cells by DCs remains largely unknown.

Src homology 2 domain-containing protein tyrosine phosphatase (SHP) substrate-1 (SHPS-1) (4), also known as signal-regulatory protein α (5, 6) or brain Ig-like molecule with tyrosine-based activation motifs (BIT) (7), is a transmembrane protein whose extracellular region comprises three Ig-like domains and whose cytoplasmic region contains four tyrosine phosphorylation sites that mediate the binding of the protein tyrosine phosphatases SHP-1 and SHP-2. Tyrosine phosphorylation of SHPS-1 is regulated by various growth factors and cytokines as well as by integrin-mediated cell adhesion to extracellular matrix proteins (8, 9). SHPS-1 thus functions as a docking protein to recruit and activate SHP-1 or SHP-2 at the cell membrane in response to extracellular stimuli. CD47 is a ligand for the extracellular region of SHPS-1 (10, 11). This protein, which was originally identified in association with \( \alpha_\beta_3 \) integrin, is also a member of the Ig superfamily, possessing an Ig-V-like extracellular domain, five putative membrane-spanning segments, and a short cytoplasmic tail (12). Among hemopoietic cells, SHPS-1 is especially abundant in DCs, macrophages, and neutrophils, being barely detectable in T or B lymphocytes (11, 13–16). In contrast, CD47 is expressed in a variety of hemopoietic cells, including RBCs and T cells (12). Indeed, the interaction of CD47 on RBCs with SHPS-1 on macrophages is thought to prevent phagocytosis of the former cells by the latter through activation of SHP-1, which forms a complex with SHPS-1 (17–19). Similarly, SHPS-1, through its interaction with CD47, is also thought to play a negative role in the immune response to pathogens.
Hybridoma cells producing the rat P84 mAb to SHPS-1 were provided by K. Okumura, Juntendo University, Tokyo, Japan. FITC-conjugated goat polyclonal Abs to mouse IgG (Zymed Laboratories), IgG1, and IgG2a (Bethyl Laboratories) were added (concentration 1/4,000 or 1/40,000 in PBS). Plates were incubated for 2 h at 37°C. Thereafter, HRP-conjugated goat polyclonal Abs to mouse IgG (Zymed Laboratories), IgG1, and IgG2a (Bethyl Laboratories) were added (concentration 1/4,000 or 1/40,000 in PBS). Plates were incubated for 2 h at 37°C. Tetramethylbenzidine substrate reagent set (BD Pharmingen) was used to develop the plates, and the reaction was stopped with stop solution (BD Pharmingen), and read at 450 nm.

Preparation of splenic DCs and T cells

Immature or mature splenic CD11c⁺ DCs were prepared from collagenase-digested spleen tissue, as described (25, 26), with minor modifications. In brief, spleen cells were released by homogenization of the spleen and subsequent exposure (with repeated passage through the tip of a pipette) of the tissue for 30 min at room temperature to collagenase (WAKO) at 400 U/ml to avoid nonspecific binding of labeled mAbs to FcR (24). The relative titers of Abs to MOG in serum samples were measured, as described previously (24). Serum samples were obtained from blood of MOG-primed WT and SHPS-1 mutant mice 40 days after immunization. A 96-well flat-bottom plate was coated with 5 μg/ml MOG (35-55) peptide at 4°C overnight. After washing the wells with PBS containing 0.05% Tween-20, the wells were blocked with 0.25% skim milk in PBS (pH 7.2) at 37°C. After washing, diluted serum samples (diluted 1/30 or 1/300 in PBS) were added and incubated for 2 h at 37°C. Thereafter, HRP-conjugated rat IgG to trinitrophenol (isotype control) and PE- or FITC-conjugated streptavidin were from BD Pharmingen. FITC-conjugated mAbs to mouse CD11c (HL3), CD4 (L3T4), or CD8α (Ly-2) as well as a biotin-conjugated mAb to mouse CD11c, biotin-conjugated rat IgG to trinitrophenol (isotype control), and PE- or FITC-conjugated streptavidin were from BD Pharmingen. FITC-conjugated mAbs to mouse NK1.1 (PK136) or B220 (RA3-6B2); biotin-conjugated mAbs to mouse MHC class I (28-14-8), MHC class II (M5/114.15.2), CD80 (16-10A1), CD86 (GL1), CD25 (PC61), or CD40 (3/23); and FITC-conjugated rat IgG to trinitrophenol (isotype control) were from eBioscience. Murine mAb to CD47 (2.4G2) were prepared from the culture supernatants of hybridoma cells (provided by K. Okumura, Juntendo University, Tokyo, Japan).

Flow cytometric and immunoblot analysis of DCs

For examination of the surface expression of SHPS-1 on DCs, immature splenic CD11c⁺ DCs (1 × 10⁶) were first incubated with a mAb to mouse CD16/32 (1 μg/ml) to avoid nonspecific binding of labeled mAbs to FcR. The cells were then washed and incubated consecutively with a biotin-conjugated mAb to SHPS-1 (1 μg/ml) and streptavidin FITC (0.2 μg/ml). The relative titers of Abs to MOG in serum samples were measured, as described previously (24). Serum samples were obtained from blood of MOG-primed WT and SHPS-1 mutant mice 40 days after immunization. A 96-well flat-bottom plate was coated with 5 μg/ml MOG (35-55) peptide at 4°C overnight. After washing the wells with PBS containing 0.05% Tween-20, the wells were blocked with 0.25% skim milk in PBS (pH 7.2) at 37°C. After washing, diluted serum samples (diluted 1/30 or 1/300 in PBS) were added and incubated for 2 h at 37°C. Thereafter, HRP-conjugated rat IgG to trinitrophenol (isotype control) and PE- or FITC-conjugated streptavidin were from BD Pharmingen. FITC-conjugated mAbs to mouse CD11c (HL3), CD4 (L3T4), or CD8α (Ly-2) as well as a biotin-conjugated mAb to mouse CD11c, biotin-conjugated rat IgG to trinitrophenol (isotype control), and PE- or FITC-conjugated streptavidin were from BD Pharmingen. FITC-conjugated mAbs to mouse NK1.1 (PK136) or B220 (RA3-6B2); biotin-conjugated mAbs to mouse MHC class I (28-14-8), MHC class II (M5/114.15.2), CD80 (16-10A1), CD86 (GL1), CD25 (PC61), or CD40 (3/23); and FITC-conjugated rat IgG to trinitrophenol (isotype control) were from eBioscience. Murine mAb to CD47 (2.4G2) were prepared from the culture supernatants of hybridoma cells (provided by K. Okumura, Juntendo University, Tokyo, Japan).

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before flow cytometric analysis with a FACSCalibur instrument and CellQuest software (BD Biosciences). For immunoblot analysis, immature splenic CD11c+ DCs were homogenized on ice in a solution containing 20 mM Tris-HCl (pH 7.6), 140 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 1 mM PMSF, aprotinin (10 μg/ml), and 1 mM sodium vanadate. The lysates were centrifuged at 10,000 × g for 15 min at 4°C, and the resulting supernatants were subjected to immunoblot analysis (18).

MLRs
Immature CD11c+ DCs prepared from WT or SHPS-1 mutant mice were exposed to γ-radiation (30 Gy) and then plated in 96-well, round-bottom microplates. CD4+ or CD8+ T cells were purified from BALB/c splenocytes by magnetic cell separation. Purified responder T cells (1 × 10^5 per well) were then cultured with the stimulator cells for 72 h, with [3H]TdR (1 μCi per well) being added for the final 12 h. Cells were harvested on glass fiber filters, and the incorporated radioactivity was measured. The culture supernatants were also assayed for IFN-γ, IL-2, and IL-10. In another set of experiments, irradiated immature CD11c+ DCs from BALB/c mice were incubated with CD4+ or CD8+ T cells from WT or SHPS-1 mutant mice, and proliferation of the responder cells was determined.

Proliferation of T cells from OT-II mice
For assay of the proliferation of OT-II CD4+ T cells in vitro (29), 8- to 12-wk-old male WT or SHPS-1 mutant mice were injected i.v. with 3 mg of OVA (Calbiochem) or vehicle. Twelve hours after the injection, immature CD11c+ DCs were purified from the spleen and cultured for 72 h at various densities with OT-II CD4+ T cells (1 × 10^5 per well) in 96-well, round-bottom microplates; the final 12 h of culture were performed in the additional presence of [3H]TdR (1 μCi per well), and the cell-associated radioactivity was subsequently measured with a scintillation spectrometer.

Assay of TLR- or IL-12-induced cytokine production
For the preparation of DCs derived from bone marrow (BMDCs), bone marrow cells were isolated from the femur and tibia of WT or SHPS-1 mutant mice with the use of a syringe fitted with a 23-gauge needle (30, 31). The cells (1 × 10^5/ml) were seeded onto a 24-well culture plate in RPMI 1640 complete medium supplemented with GM-CSF (10 ng/ml), and the culture medium was changed every 2 days to remove granulocytes. After culture for 6–7 days, loosely adherent and clustered cells were collected as immature BMDCs. For assay of cytokine production by BMDCs (30), the cells were stimulated for 20 h with LPS (Sigma-Aldrich) at 1 μg/ml or 1 μM phosphorothioate oligodeoxynucleotide (ODN) with a CpG motif (Cpg ODN 1826 5′-TCCATGACGTTCCTGACGTT-3′) (Japan Bio Services) in the absence or presence of IFN-γ (10 ng/ml). Culture supernatants were then assayed for IL-12 (R&D Systems) as well as TNF-α and IL-6 (BD Pharmingen) with ELISA kits. In other experiments, mature splenic CD11c+ DCs (1 × 10^5 per well) of WT or SHPS-1 mutant mice were cultured for 72 h in RPMI 1640 complete medium with various concentrations of IL-12. Culture supernatants were then assayed for IFN-γ with an ELISA kit.

Adoptive transfer
Adoptive transfer of T cells from WT mice was performed, as described previously (22). In brief, WT mice were immunized with 100 μg of MOG (35–55), and, after 10 days, draining LNs were harvested for isolation of lymphocytes. The cells were cultured with MOG (35–55) (10 μg/ml) and IL-12 (5 ng/ml) for 4 days, washed with PBS, and resuspended in PBS for transfer. WT or SHPS-1 mutant mice were injected i.v. with the lymphocytes (1 × 10^7) as well as with 200 ng of pertussis toxin (both immediately and 2 days after cell transfer). The animals were observed daily, and neurological effects were quantified, as described above.

Statistical analysis
Data are presented as means ± SE and were analyzed by Student’s t test with the use of Stat View 5.0 software (SAS Institute). A p value of <0.05 was considered statistically significant.

Results
Resistance of SHPS-1 mutant mice to EAE
We first examined the susceptibility of the SHPS-1 mutant mice to EAE, an animal model of multiple sclerosis. EAE was induced by immunizing mice; those were backcrossed onto the C57BL/6 background for five or six generations, with MOG (35–55) (32, 33). Fourteen of 15 (93%) WT mice developed typical EAE ~14 days after immunization with MOG (35–55) and pertussis toxin. In marked contrast, none of the 15 similarly immunized SHPS-1 mutant mice developed EAE. The time course and severity of EAE in WT mice compared with data for SHPS-1 mutant mice in a typical experiment are shown in Fig. 1A. Histological analysis of the spinal cord revealed pronounced infiltration of mononuclear cells around the blood vessels in WT mice, but not in the mutant mice. Scale bars: 200 μm (upper panels) and 50 μm (lower panels). C. Time course of the clinical score of WT and SHPS-1 mutant (KO) mice; those were backcrossed on the C57BL/6 background for nine generations and immunized with MOG (35–55). The boxed regions of the upper panels are shown in the lower panels. H&E staining shows marked infiltration of mononuclear cells around the blood vessels in WT mice, but not in the mutant mice. Scale bars: 200 μm (upper panels) and 50 μm (lower panels). R and D Systems as well as TNF-α and IL-6 (BD Pharmingen) with ELISA kits. In other experiments, mature splenic CD11c+ DCs (1 × 10^5 per well) of WT or SHPS-1 mutant mice were cultured for 72 h in RPMI 1640 complete medium with various concentrations of IL-12. Culture supernatants were then assayed for IFN-γ with an ELISA kit.

Adoptive transfer
Adoptive transfer of T cells from WT mice was performed, as described previously (22). In brief, WT mice were immunized with 100 μg of MOG (35–55), and, after 10 days, draining LNs were harvested for isolation of lymphocytes. The cells were cultured with MOG (35–55) (10 μg/ml) and IL-12 (5 ng/ml) for 4 days, washed with PBS, and resuspended in PBS for transfer. WT or SHPS-1 mutant mice were injected i.v. with the lymphocytes (1 × 10^7) as well as with 200 ng of pertussis toxin (both immediately and 2 days after cell transfer). The animals were observed daily, and neurological effects were quantified, as described above.

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mice developed EAE, and the clinical score for such SHPS-1 mutant mice was lower than that for WT mice. We therefore next compared the MOG-specific proliferative response of T cells derived from MOG-primed animals. The cell proliferation and production of IFN-γ in response to MOG were markedly reduced in lymphocytes derived from MOG-primed SHPS-1 mutant mice compared with the responses of WT cells (Fig. 2, A and B). The production of IL-2 in response to MOG was also reduced in splenic lymphocytes derived from MOG-primed SHPS-1 mutant mice compared with the responses of WT cells (Fig. 2C). In contrast, the production of IL-10 in response to MOG was minimal in splenic lymphocytes derived from MOG-primed WT or SHPS-1 mutant mice (data not shown). The production of IL-17 by MOG-primed Th17 cells was recently shown to be essential for development of EAE (34–36). The MOG-induced production of IL-17 was also greatly reduced in splenic lymphocytes derived from MOG-primed SHPS-1 mice compared with that apparent with WT cells (Fig. 2D). We also examined the relative titers of Abs to MOG in serum samples from MOG-primed WT and SHPS-1 mutant mice 40 days after immunization. However, no marked difference was observed in IgG, IgG1, and IgG2a titers between WT and mutant mice (data not shown).

**Reduced numbers of CD11c+ DCs as well as CD4+ or CD8+ T cells in SHPS-1 mutant mice**

Induction of EAE requires T cell responses that are initiated by priming of naive CD4+ T cells by DCs. SHPS-1 mutant mice express a mutant version of SHPS-1 that lacks most of the cytoplasmic region (Fig. 3A) (19, 21). Flow cytometry and immunoblot analysis with the P84 mAb to the extracellular region of SHPS-1 confirmed that SHPS-1 is expressed on CD11c+ DCs (Fig. 3B and C). The abundance of SHPS-1 was substantially reduced in CD11c+ DCs from the spleen of SHPS-1 mutant mice compared with that apparent for WT cells (Fig. 3B and C). In addition, immunoblot analysis with polyclonal Abs specific for the cytoplasmic region of SHPS-1 yielded no signal with splenic DCs from the homozygous mutant mice (Fig. 3C). These results suggested that the minimal susceptibility of SHPS-1 mutant mice to EAE may be attributable to a functional defect of DCs in priming of naive CD4+ T cells. We therefore characterized the functions of DCs in SHPS-1 mutant mice. We first showed that the number of CD11c+ DCs in the spleen was reduced in the mutant animals (Fig. 3D), although the total number of splenic cells was not altered (data not shown) (19). Small decreases in the numbers of CD4+ or CD8+ T cells were also apparent in the spleen of SHPS-1 mutant mice, whereas the numbers of B220+ B cells or NK1.1+ NK cells (or NK T cells) did not differ between the mutant and WT mice or for the indicated comparisons (Student’s t test).
Reduced abilities of DCs from SHPS-1 mutant mice to stimulate an allogenic MLR and the proliferation of Ag-specific CD4+ T cells

We next examined the ability of DCs from SHPS-1 mutant mice to stimulate alloreactive CD4+ or CD8+ T cells. In MLRs with CD11c+ DCs isolated from SHPS-1 mutant mice, the proliferation of CD4+ or CD8+ T cells from BALB/c mice was markedly reduced, compared with that apparent with DCs from WT mice (Fig. 4A). Consistent with this finding, the production of IFN-γ was also reduced on incubation of the CD4+ or CD8+ T cells with DCs from SHPS-1 mutant mice (Fig. 4A). In addition, the production of IL-2 was reduced on incubation of the CD4+ or CD8+ T cells with DCs from SHPS-1 mutant mice (Fig. 4B). The production of IL-10 was slightly reduced on incubation of the CD4+ T cells with DCs from SHPS-1 mutant mice, whereas it was not detected on incubation of the CD8+ cells with DCs from either WT or SHPS-1 mutant mice (Fig. 4B). In contrast, the proliferation of CD4+ or CD8+ T cells from SHPS-1 mutant mice was similar to that of the corresponding WT cells on incubation with DCs from BALB/c mice (Fig. 4C). These results suggested that DCs, but not CD4+ or CD8+ T cells, of SHPS-1 mutant mice are functionally defective in an allogenic MLR.

To characterize further the dysfunction of DCs in SHPS-1 mutant mice, we examined the proliferation of OVA-specific CD4+ T cells (prepared from OT-II transgenic mice (37)) in response to culture with OVA-pulsed CD11c+ DCs. The proliferation of OVA-specific CD4+ T cells cultured with DCs from SHPS-1 mutant mice was markedly impaired compared with that of those cultured with DCs from WT mice (Fig. 4D), suggesting that priming of Ag-specific T cells by DCs is indeed defective in SHPS-1 mutant mice.

Impaired TLR-dependent cytokine production and IL-12-induced IFN-γ release in DCs from SHPS-1 mutant mice

We therefore next examined what specific activity of DCs might be impaired in SHPS-1 mutant mice. For priming of naive T cells, DCs first capture exogenous Ags and then migrate from peripheral tissues to draining LNs, where they encounter naive T cells and present MHC-peptide complexes (1, 2). During this process, DCs also mature and express multiple costimulatory molecules, such as CD80, CD86, and CD40, on their surface. They also secrete IL-12, a key cytokine that promotes the production of IFN-γ not only by Th1 cells, but also by DCs themselves. However, neither the migratory response of, nor the uptake of FITC-labeled BSA by, BMDCs differed substantially between SHPS-1 mutant and WT mice (Fig. 5, A and B). In addition, the up-regulation of surface molecules, including MHC class I and II as well as CD80, CD86, and CD40, on their surface, mice. C, Cell proliferation in MLRs with various numbers of irradiated immature CD11c+ DCs of BALB/c mice and with splenic CD4+ or CD8+ T cells (1 × 10^5) from OT-II mice, after which cell proliferation was determined in triplicate determinations and are representative of three separate experiments. *, p < 0.05; **, p < 0.01 vs WT (Student’s t test).
We then evaluated the TLR-dependent production of cytokines by BMDCs. The production of 70-kDa heterodimeric IL-12 (IL-12p70) in response to either LPS or CpG ODN was slightly impaired in BMDCs from SHPS-1 mutant mice (Fig. 6A). Moreover, the production of TNF-α and IL-6 in response to either LPS or CpG ODN was markedly impaired in BMDCs from the mutant animals (Fig. 6A). The IL-12-induced production of IFN-γ by DCs is important for DC functions (25, 26, 38). We found that the IL-12-induced production of IFN-γ in splenic CD11c+ DCs from SHPS-1 mutant mice was greatly reduced compared with that

FIGURE 5. Migration, Ag uptake, and differentiation in DCs from WT and SHPS-1 mutant mice. A, BMDCs derived from WT or SHPS-1 mutant (knockout (KO)) mice were labeled with 10 μM CFSE for 10 min at 37°C in RPMI 1640, as described (31), washed three times, and then resuspended (5 × 10⁶ cells in 40 μl) in PBS and injected into a hind footpad of WT mice. Three days after injection, lymphocytes were isolated from the draining LNs and subjected to flow cytometric analysis for determination of the percentage of cells labeled with CFSE (gated area). Data are from a representative experiment. The mean ± SE values for the percentage of migrating cells were 0.17 ± 0.01 and 0.11 ± 0.05 for WT and SHPS-1 mutant mice, respectively (n = 3, p > 0.05). FSC, forward scatter. B, BMDCs from WT or SHPS-1 mutant (KO) mice were incubated with FITC-BSA (5 μg/ml) (Sigma-Aldrich) at 37°C or 4°C (control) for 15 or 30 min, washed, and then stained with biotin-conjugated Abs to mouse CD11c and PE-conjugated streptavidin. FITC-BSA uptake by CD11c+ DCs was then monitored by flow cytometry. Data shown in the left panels were obtained with cells incubated for 30 min and are representative of three separate experiments. The uptake of FITC-BSA by CD11c+ DCs at 15 or 30 min and 37°C was also determined as the percentage of CD11c+ DCs that had incorporated FITC-BSA (cells in the upper right (UR) quadrant) among total CD11c+ DCs (sum of the cells in the upper right and upper left (UL) quadrants), as shown in the right panel; data are means ± SE of values from three separate experiments. C, Immature or mature CD11c+ DCs prepared from the spleen of WT or SHPS-1 mutant (KO) mice were incubated first with biotin-conjugated mAbs to mouse MHC class I, MHC class II, CD80, CD86, or CD40 (thick traces), or with an isotype control mAb (thin traces) and then with FITC-conjugated streptavidin. They were then analyzed by flow cytometry. Data are representative of three separate experiments.
apparent with WT cells (Fig. 6B). These data thus suggested that SHPS-1 is essential for TLR-dependent cytokine production as well as the IL-12-induced production of IFN-γ by DCs.

**FIGURE 6.** Impaired TLR-dependent cytokine production and IL-12-induced IFN-γ production in DCs from SHPS-1 mutant mice. A, BMDCs from WT or SHPS-1 mutant (knockout (KO)) mice were stimulated with LPS (1 μg/ml) or CpG ODN (1 μM) in the absence or presence of IFN-γ (10 ng/ml) for 20 h, after which the levels of the indicated cytokines in culture supernatants were determined. B, Mature CD11c+ DCs from the spleen of WT or SHPS-1 mutant (KO) mice were cultured with various concentrations of IL-12 for 72 h, after which the concentration of IFN-γ in culture supernatants was determined. All data are means ± SE of values from triplicate determinations and are representative of three separate experiments. *p < 0.05; **p < 0.01 vs WT or for the indicated comparisons (Student’s t test).

Discussion

We have shown in this study that SHPS-1 mutant mice are resistant to EAE. Moreover, the MOG-induced proliferation of and production of IFN-γ as well as of IL-2 by T cells from immunized SHPS-1 mutant mice were markedly reduced compared with those apparent for WT cells. It was recently shown that IL-17 produced by Th17 cells in response to TGF-β, IL-6, or IL-23 is essential for development of EAE (34, 35). Indeed, IL-17-deficient mice also manifest minimal susceptibility to EAE (36). We found that the MOG-induced production of IL-17 by T cells from immunized SHPS-1 mutant mice was reduced compared with that apparent for WT cells. Induction of EAE requires T cell responses that are initiated by priming of naive CD4+ T cells by DCs. Given that SHPS-1 is expressed selectively in DCs, these results suggest that the resistance of SHPS-1 mutant mice to EAE is attributable to a defect in priming of CD4+ T cells by DCs.

This notion was further supported by the observations that, in an allogenic MLR with CD11c+ DCs from SHPS-1 mutant mice, the proliferation of and production of IFN-γ by CD4+ or CD8+ T cells from BALB/c mice were markedly reduced, compared with those apparent with DCs from WT mice. Furthermore, the proliferation of OVA-specific CD4+ T cells primed with DCs from SHPS-1 mutant mice was markedly impaired compared with that of those primed with DCs from WT mice. These results thus suggest that priming of either allogenic or Ag-specific T cells by DCs from SHPS-1 mutant mice is indeed defective. The interaction of SHPS-1 on macrophages with CD47 on RBCs prevents phagocytosis of RBCs by macrophages in a manner dependent on SHP-1, which binds the cytoplasmic region of SHPS-1 (17–19, 39). Moreover, SHPS-1 on DCs, through its interaction with CD47 on T cells, prevents activation of DCs (15), suggesting that SHPS-1 negatively regulates functions of macrophages and DCs. However, our present results demonstrate a positive regulatory role for SHPS-1 in efficient priming by DCs of naive T cells both in vivo and in vitro.

We investigated what specific function of DCs is actually impaired in SHPS-1 mutant mice. The abilities to migrate and to take up Ag were not markedly impaired in BMDCs from SHPS-1 mutant mice. In contrast, the TLR-dependent production of cytokines, including TNF-α and IL-6, was defective in BMDCs from the mutant animals. Moreover, the IL-12-stimulated production of IFN-γ by DCs from SHPS-1 mutant mice was greatly impaired compared with that in WT cells. This effect of IL-12 is thought to be important for priming and activation of naive T cells by DCs (25, 26, 38). The impairment in priming of T cells by DCs of SHPS-1 mutant mice may thus be attributable, at least in part, to the defects in IL-12-induced production of IFN-γ and in TLR-dependent cytokine production in DCs. The molecular mechanism by which SHPS-1 positively regulates the effect of IL-12 on IFN-γ production by DCs remains unknown. The JAK2-STAT4 signaling pathway and the p38 isoform of MAPK are implicated in this effect of IL-12 (26, 40). Given that SHP-2 positively regulates...
activation of the JAK-STAT pathway and MAPK signaling (41) and complex formation of SHPS-1 with SHP-2 is specifically defective in the SHPS-1 mutant mice, it is possible that the SHPS-1–SHP-2 complex positively regulates the action of IL-12 as well as that of other related cytokines that activate the JAK-STAT pathway and p38 MAPK in DCs.

Interaction of SHPS-1 (on DCs) with CD47 (on T cells) may contribute to the activation of T cells in a manner functionally similar to that apparent for costimulatory molecules on DCs such as B7 (which interacts with CD28 on T cells) and OX40 ligand (which interacts with OX40 on T cells). Mice deficient in either B7 or OX40 ligand indeed manifest a reduced susceptibility to EAE (22, 42). In addition, it was recently shown that delayed-type hypersensitivity to 2,4-dinitro-1-fluorobenzene, which is also thought to be mediated by Th1 cells, is markedly diminished in CD47-deficient mice (43). Conversely, we found that such delayed-type hypersensitivity is greatly reduced in extent in SHPS-1 mutant mice (44). Moreover, ligation of CD47 on T cells is thought to generate costimulatory signals for T cell activation (45). Together with the present results, these observations suggest a notion that interaction of SHPS-1 (on DCs) with its ligand CD47 (on T cells) is essential for priming of T cells by DCs.

We showed that induction of EAE by adoptive transfer of MOG-specific T cells from WT donor mice was impaired in SHPS-1 mutant mice, suggesting that these animals have a defect in the effector phase of EAE development as well as in the priming of T cells by DCs. It is possible that the production of chemokines or cytokines from various cell types, including macrophages and neutrophils, in the effector phase of EAE is impaired in SHPS-1 mutant mice. Secondary priming of T cells by APCs surrounding blood vessels of the CNS is also important for the development of EAE (46, 47). The reduced effect of adoptive transfer of MOG-specific T cells in SHPS-1 mutant mice may thus be attributable to a defect in secondary priming of the transferred T cells by host DCs.

Overall, our present study clearly indicates that SHPS-1 positively regulates the priming of T cells by DCs, and hence, that this protein is essential for the development of EAE. We found that SHPS-1 is required for multiple functions of DCs, including the IL-12-induced production of IFN-γ as well as the TLR-dependent production of cytokines by these cells. Our results also implicate SHPS-1 on DCs in the activation of T cells (presumably through interaction with CD47 on T cells) in a manner functionally similar to that apparent for other costimulatory molecules on DCs. We have recently shown that SHPS-1 mutant mice also manifest minimal susceptibility to collagen-induced arthritis, an animal model of rheumatoid arthritis (C. Okuzawa, Y. Kaneko, and T. Matozaki, unpublished observation), suggesting that SHPS-1 may contribute to development of various inflammatory autoimmune diseases. Given that the function of SHPS-1 is susceptible to regulation by ligands such as Abs, this protein is a potential therapeutic target for inflammatory autoimmune diseases in general.

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Disclosures

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References


