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Expression of the Tyrosine Phosphatase Src Homology 2 Domain-Containing Protein Tyrosine Phosphatase 1 Determines T Cell Activation Threshold and Severity of Experimental Autoimmune Encephalomyelitis[1]

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Experimental autoimmune encephalomyelitis (EAE) is a CD4 Th1-mediated inflammatory demyelinating disorder of the CNS and a well-established animal model for multiple sclerosis. Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) is a cytosolic tyrosine phosphatase that is involved in regulating the T cell activation cascade from signals initiated through the TCR. To study the role of SHP-1 in EAE pathogenesis, we immunized B10.PL mice heterozygous for deletion of the SHP-1 gene ($me^{v+/-}$) and B10.PL wild-type mice with the immunodominant epitope of myelin basic protein (MBP Ac1-11). T cell proliferation and IFN-γ production were significantly increased in $me^{v+/-}$ mice after immunization with MBP Ac1-11. The frequency of MBP Ac1-11-specific CD4 T cells, analyzed by staining with fluorescently labeled tetramers (MBP1-11[4Y]: I-A^u complexes), was increased in the draining lymph node cells of $me^{v+/-}$ mice compared with wild-type mice. In addition, $me^{v+/-}$ mice developed a more severe course of EAE with epitope spreading to proteolipid protein peptide 43-64. Finally, expansion of MBP Ac1-11-specific T cells in response to Ag was enhanced in $me^{v+/-}$ T cells, particularly at lower Ag concentrations. These data demonstrate that the level of SHP-1 plays an important role in regulating the activation threshold of autoreactive T cells. *The Journal of Immunology,* 2002, 168: 4511–4518.

E xperimental autoimmune encephalomyelitis (EAE),[3] a well-established demyelinating disease of the CNS that is similar to multiple sclerosis (MS) in its clinical course and pathology, has been a useful animal model for studying factors that influence autoimmune demyelination. Both EAE and MS are characterized by inflammation, demyelination, and a relapsing/remitting course (1). EAE is initiated by engagement of autoantigen with the TCR in the presence of costimulatory signals. Activated myelin-specific CD4$^+$ T cells initiate the attack on CNS myelin, which leads to demyelination in the CNS and produces signs of EAE.

Src homology 2 domain-containing protein tyrosine phosphatase (SHP-1) is a cytosolic protein tyrosine phosphatase that is expressed primarily in hemopoietic cells. SHP-1 is involved in negatively regulating T cell development and activation (2–5).

SHP-1 deficiency results in reducing the activation threshold of peripheral T cells and increasing T cell proliferative responses (6, 7). SHP-1-deficient mice, *motheaten* or *viable motheaten* (*me* or me^v) mice, have severe defects in immunity and hemopoiesis (8–16). The *me* or me^v mice have an overgrowth of macrophages and granulocytes (17–20), abnormal B cell development and polyclonal B cell activation (12, 21, 22), decreased NK cell activity (10, 23), and increased proliferative activity of thymocytes in response to TCR stimulation (24, 25). SHP-1 is also involved in the regulation of cytokine/chemokine signaling and function (26, 27). The me^v or *me* mice develop severe pneumonitis and autoimmunity in early life, leading to premature death (9, 14). Initially thought to be phenotypically normal, $me^{+/-}$ and $me^{v+/-}$ mice have about one-half the functional SHP-1 activity of wild-type (WT) mice (28).

Although SHP-1 plays a crucial role in the immune response, few studies have examined the role of SHP-1 in autoimmune diseases. To study the role of SHP-1 in a prototypical T cell-mediated autoimmune disease, EAE, we immunized H-2^u, $me^{v+/-}$ mice with myelin basic protein (MBP Ac1-11) and examined the T cell response to this autoantigen and the clinical signs of EAE. Our data show that a reduction in the level of SHP-1 enhances the T cell response to MBP Ac1-11, exacerbates clinical signs of EAE, and results in spreading to other myelin protein epitopes.

Materials and Methods

Mice

Vβ8.2 TCR, or Vα2.3Vβ8.2 transgenic (Tg), mice were kindly provided by Dr. J. Goverman (University of Washington, Seattle, WA) (29). The $me^{v+/-}$ B10.PL ($me^{v+/-}$) mice were generated by crossing $me^{v+/-}$ C57BL/6 ($me^{v+/-}$.B6) mice with B10.PL WT mice for more than nine generations. The $me^{v+/-}$.B6 and B10.PL mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The me^v TCR Vβ8.2 Tg

(Tg.$me^{v+/-}$) mice were generated by crossing $me^{v+/-}$ B10.PL with Vβ8.2 TCR mice. These mice were bred and maintained in our animal colony at the University of Texas Southwestern Medical Center (Dallas, TX) in compliance with the Animal Studies Committee. All mice were 7–10 wk of age when experiments were initiated. No spontaneous EAE was observed in the TCR Vβ8.2 mice bred onto the me^v background (Tg.$me^{v+/-}$).

Reagents

Whole MBP was prepared from guinea pig spinal cords as previously described, and purity was assessed by SDS-PAGE (30). MBP peptide Ac1-11, myelin oligodendrocyte glycoprotein (MOG) peptide 35-55, and proteolipid protein (PLP) peptide 43-64 were purchased from BioSource International (Camarillo, CA).

Immunization and evaluation of EAE

For induction of EAE, mice were immunized s.c. with MBP Ac1-11 (200 μg/mouse) in an emulsion with CFA. Pertussis toxin (200 ng/mouse) in PBS was injected i.p. at the time of immunization and 48 h later. EAE scoring was performed as previously described (31): 0, no abnormality; 1, a limp tail; 2, moderate hind limb weakness; 3, severe hind limb weakness; 4, complete hind limb paralysis; 5, quadriplegia or premoribund state; and 6, death. A relapse was defined as a sustained increase of at least one full grade in clinical score after the animal had previously improved at least a full clinical score and had stabilized. For short-term studies, the mice were immunized s.c. with MBP Ac1-11 (200 μg/mouse) in an emulsion with CFA. Ten days later, the draining lymph node cells (LNCs) were harvested for various assays.

Cell culture

Draining lymph nodes and/or spleens, as noted, from different mice were harvested, and single-cell suspensions were obtained by pressing the tissue through a wire mesh screen. The cells were cultured (4×10^6 cells/ml) in complete medium for the times specified in the text or figure legends. MBP, MBP peptide Ac1-11, or PLP peptide 43-64 (concentrations are indicated in the text or figure legends) were used as stimulating Ags.

Cytokine detection

IFN-γ, IL-4, and IL-10 were measured by ELISA. ELISA plates (Immunol 2; Dynatech Laboratories, Chantilly, VA) were coated with 2 μg/ml (50 μl/well) IFN-γ, IL-4, or IL-10 mAb (BD PharMingen, San Diego, CA) in 0.1 M carbonate buffer (pH 8.2) overnight at 4°C. The plates were blocked with 200 μl of 10% FBS in PBS for 2 h. A total of 100 μl of supernatant was added at various dilutions titered to the linear portion of the absorbance/concentration curve in duplicate and incubated overnight at 4°C. After the plates were washed four times with PBS and Tween 20 (0.05%), 100 μl of biotinylated anti-cytokine detecting mAbs (directed to a different determinant than the first Ab used to coat ELISA plates) at 1 μg/ml in PBS and 10% FBS were added for 45 min at room temperature. Then, 100 μl of avidin-peroxidase (2.5 μg/ml) was added and incubated for 30 min. Subsequently, the peroxidase substrate ABTS (6) in 0.1 M citric buffer (pH 4.35) in the presence of H_2O_2 was added, and the absorbance was measured at 405 nm.

Staining of splenocytes and LNCs with MBP1-11[4Y]:I-A^u tetramers

The generation of a soluble construct of I-A^u with covalently tethered -11[4Y] peptide has been described elsewhere (32). An analogous tetramer containing OVA 323-339 bound to I-A^u was also constructed (OVA 323-339 binds tightly to I-A^u) (33). Tetrameric complexes of MBP1-11[4Y]: I-A^u or OVA 323-339:I-A^u were prepared by incubation with PE-conjugated streptavidin (Sigma-Aldrich, St. Louis, MO) and used to stain splenocytes and LNCs as previously described (34), with slight modifications. Briefly, 3×10^6 cells were incubated with the tetrameric complexes in the presence of Ab mix for 30 min at 37°C. In every assay, CD80-FITC (16-10A1, Armenian hamster IgG), CD4-PerCP (RM4-5, rat IgG2a), CD62 ligand (CD62L)-APC (MEL-14, rat-IgG2A), and the corresponding Ig-isotype control were used (BD Biosciences, Mountain View, CA). Analysis of staining was performed using a FACScan (BD Biosciences) and the CellQuest (BD Biosciences) and WinMDI 2.8 analysis software (The Scripps Research Institute, La Jolla, CA).

Purification of CD4 T cells by negative selection

TCR Vβ8.2, Vα2.3 Tg splenocytes were purified by negative selection using R&D Systems (Minneapolis, MN) mouse T cell CD4 subset column kit (MCD4C-1000). Briefly, 2×10^8 splenocytes in 2 ml of column buffer

were incubated with 1 ml of mixture Ab for 15 min at room temperature. The cells were washed twice with column buffer and resuspended into 2 ml and loaded into the prewashed column. After the cells were suspended in the column and were incubated for 10 min at room temperature, a total of 10 ml of column buffer was used to elute the cells from the column. The cells were centrifuged at $250 \times g$ for 5 min and suspended into culture medium. The purity of CD4$^+$ T cells was determined by FACS.

Results

$me^{v+/-}$ mice develop more severe EAE than WT mice after Ac1-11 immunization

To study the effect of SHP-1 on the development of EAE, both male and female $me^{v+/-}$ and WT B10.PL mice were immunized s.c. with MBP Ac1-11 in CFA. Mice were examined daily for clinical signs of EAE. The mice initially developed EAE on day 10 after immunization. Overall, $me^{v+/-}$ mice had a higher incidence of disease and developed a more severe course of EAE (males, $p = 0.032$; females, $p = 0.045$; Fig. 1). The mean maximal EAE clinical score in $me^{v+/-}$ males was 5, whereas it was only 3.5 in WT mice. The $me^{v+/-}$ mice demonstrated more relapses, whereas WT mice generally exhibited a monophasic course of disease (Fig. 1). Male $me^{v+/-}$ mice experienced 17 relapses compared with 10 relapses for the WT mice. Female $me^{v+/-}$ mice experienced nine relapses compared with five for the WT mice. Thus, reduced levels of SHP-1 in $me^{v+/-}$ mice exacerbated the clinical course of EAE and increased the number of relapses.

In addition, we examined the role of SHP-1 in MOG-induced EAE in C57BL/6 $me^{v+/-}$ (B6.$me^{v+/-}$) and B6 WT mice. Ten mice in each group were immunized with MOG 35-55/CFA. Pertussis toxin was given on days 0 and 2. Onset of EAE was observed beginning 13 days after immunization. Nine of 10 $me^{v+/-}$ mice developed EAE, with all exhibiting a maximal clinical score of 3 or 4. Nine of 10 B6 mice also developed signs of EAE, however both maximal clinical score and disease duration were lower when compared with B6.$me^{v+/-}$ mice ($p = 0.0046$; Fig. 2).
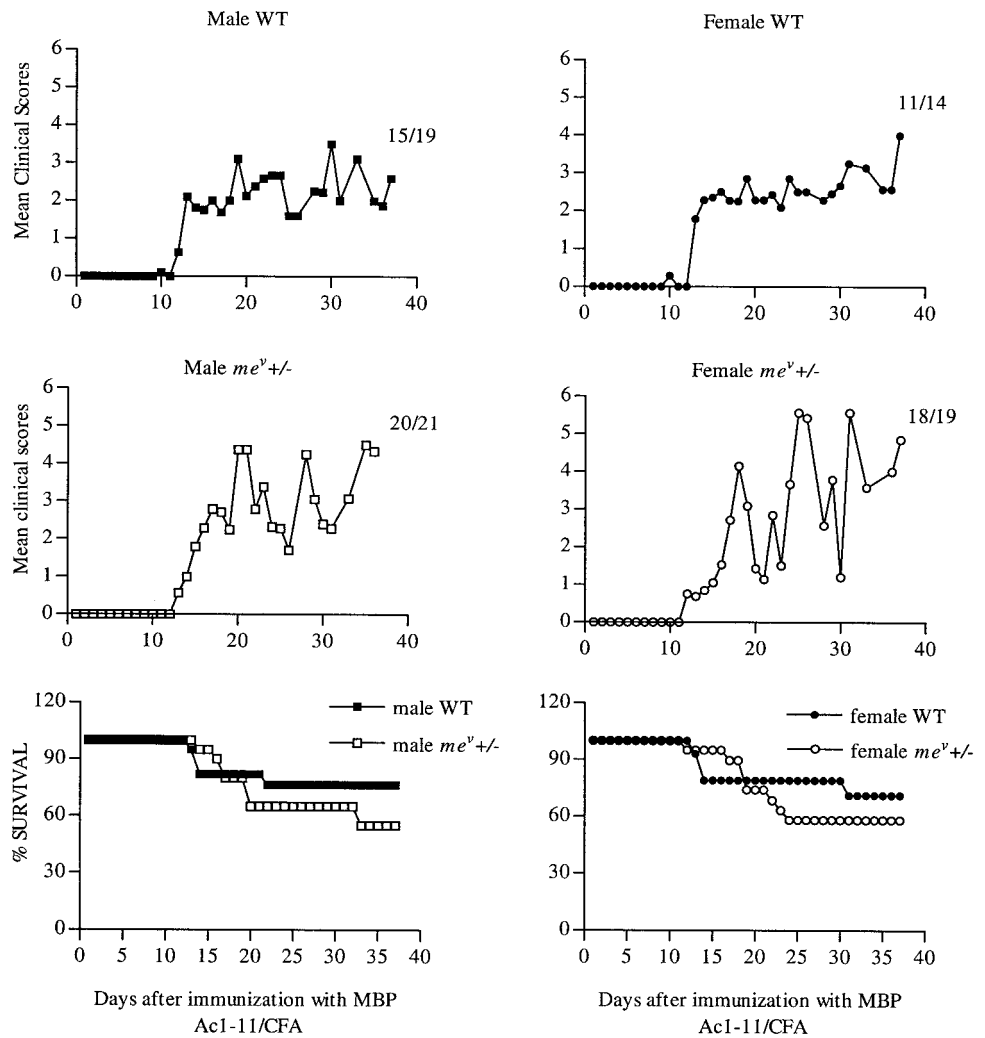
T cell proliferation and cytokine production are increased in $me^{v+/-}$ mice after antigenic challenge

Next, we examined T cell proliferation and cytokine production in $me^{v+/-}$ and WT mice in response to MBP Ac1-11 challenge. Both $me^{v+/-}$ and WT mice were immunized s.c. with MBP Ac1-11 in CFA. Ten days later, draining LNCs were collected and single-cell suspensions were made. LNCs were cultured in the presence of MBP Ac1-11. The $me^{v+/-}$ mice exhibited increased T cell proliferation to MBP Ac1-11 compared with WT mice (Fig. 3A). The difference was more apparent in males than in females. In addition, the production of IFN-γ was markedly increased in $me^{v+/-}$ mice compared with WT mice (Fig. 3B). Therefore, reduced SHP-1 levels in the heterozygous, $me^{v+/-}$ mice resulted in an increased T cell response to the priming Ag.

T cell responses are enhanced in $me^{v+/-}$ mice late in disease with epitope spreading to PLP 43-64

The $me^{v+/-}$ mice demonstrated more severe EAE and enhanced T cell responses to immunizing Ags. We examined whether increased T cell responses lasted over the course of EAE. To confirm our hypothesis, we tested T cell proliferation and cytokine production 8 wk after immunization with MBP Ac1-11. As expected, T cell proliferative responses to both MBP and MBP Ac1-11 were markedly increased in $me^{v+/-}$ mice compared with WT mice. Interestingly, we observed that T cells from $me^{v+/-}$ mice responded to PLP 43-64 peptide, whereas T cells from WT mice did not respond detectably to PLP 43-64 (Fig. 4A). In addition, $me^{v+/-}$

FIGURE 1. Decreased SHP-1 exacerbates EAE in B10.PL mice. Both male and female $me^{v+/-}$ and WT B10.PL mice were immunized with MBP Ac 1-11 (200 μg) in CFA on day 0. Pertussis toxin (200 ng) was injected i.p. on days 0 and 2. All mice were monitored daily for clinical signs of disease. The $me^{v+/-}$ mice had a more severe EAE with frequent relapses and decreased survival compared with WT mice. Twenty of 21 (95%) male and 18 of 19 (95%) female $me^{v+/-}$ mice vs 15 of 19 (79%) male and 11 of 14 (79%) female WT mice develop EAE. Nine of 21 male and eight of 19 female $me^{v+/-}$ mice vs six of 19 male and four of 14 female WT mice died due to severe EAE.

splenocytes produced more IFN-γ in response to MBP, MBP Ac1-11, and PLP 43-64 stimulation (Fig. 4B). This suggested that endogenous priming to PLP 43-64 had resulted in epitope spreading. We also examined whether a response to PLP 43-64 existed in $me^{v+/-}$ mice, which were immunized with MBP Ac1-11 for 10 days, and in naive Vβ8.2 TCR $me^{v+/-}$ (Tg $me^{v+/-}$) mice. T cells did not respond to PLP 43-64 peptide in these two situations (data not shown). Therefore, epitope spreading to PLP 43-64 occurred

FIGURE 2. C57BL/6.$me^{v+/-}$ mice developed more severe EAE than did B6 WT mice. The $me^{v+/-}$ C57BL/6 and C57BL/6 WT mice were immunized with 200 μg of MOG 35-55/CFA. Pertussis toxin was given on days 0 and 2. Mice developed EAE on day 13. The $me^{v+/-}$ mice developed more severe EAE compared with WT mice. The difference of mean EAE score between $me^{v+/-}$ and WT mice was statistically significant ($p = 0.004$).

during chronic EAE in $me^{v+/-}$ mice and may have contributed to the increased severity of disease and relapse rate.

MBP Ac1-11-specific cells are increased in $me^{v+/-}$ mice after immunization with Ac1-11/CFA

The $me^{v+/-}$ mice had shown an increased response to MBP Ac1-11 and enhanced signs of disease. Using MBP1-11[4Y]:I-A^u tetramers, which have been shown in previous studies (32, 34) to detect MBP Ac1-11-specific T cells, we next examined the frequency of MBP Ac1-11-specific T cells after immunization with MBP Ac1-11. Ten days later, the draining LNCs were harvested and stained with anti-CD4, CD62L, CD80 mAbs and MBP1-11[4Y]:I-A^u tetramers. The number of tetramer-positive cells was reproducibly higher in $me^{v+/-}$ mice compared with WT mice (Fig. 5). The percentage of CD62L^{low} cells and B7-1-positive cells in tetramer-positive cells was comparable between $me^{v+/-}$ and WT mice (data not shown). These data suggest that the increased T cell proliferation and IFN-γ production could be attributed to the higher frequency of MBP Ac1-11-specific T cells after immunization in $me^{v+/-}$ mice compared with WT mice. The precursor frequency of MBP Ac1-11-specific T cells in nontransgenic mice is undetectable. To analyze precursor frequencies in naive mice, it is therefore necessary to use mice Tg for the β-chain of a TCR specific for MBP Ac1-11. These Vβ8.2 Tg mice have a much higher frequency of MBP Ac1-11-specific cells ($\sim$0.5% of CD4$^+$ T cells; Fig. 6) than do nontransgenic mice. We analyzed the same cell surface
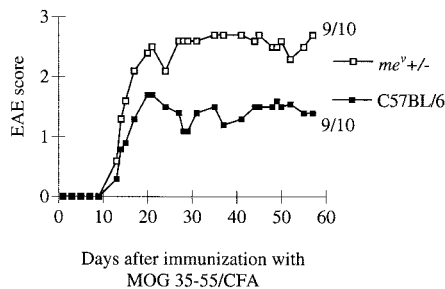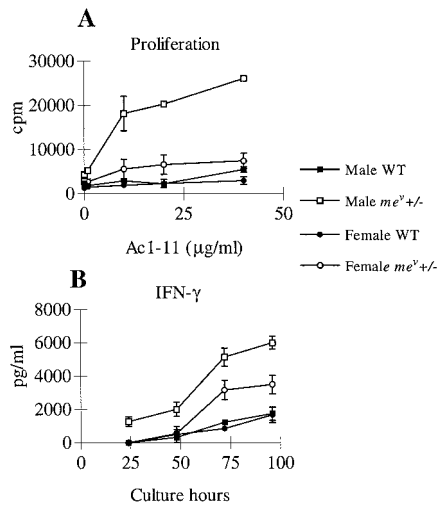
FIGURE 3. The effect of decreased SHP-1 on MBP Ac1-11-specific lymphocyte proliferation and cytokine production. The $me^{v+/-}$ and WT B10.PL mice were immunized with MBP Ac 1-11/CFA. Ten days later, draining LNCs were stimulated with MBP Ac1-11 for 4 days. Supernatants were collected at 24, 48, 72, and 96 h for cytokine assays. The proliferative response of LNCs from $me^{v+/-}$ mice was significantly higher than from WT mice ($p < 0.01$).

markers in naive Tg $me^{v+/-}$ and Tg WT mice. There was no difference in the number of tetramer-positive MBP Ac1-11-specific T cells, the expression of B7-1, or CD62L expression between naive Tg $me^{v+/-}$ and Tg WT mice (Fig. 6 and data not shown). These data demonstrate that the autoantigen-specific T cells are not activated in naive Tg $me^{v+/-}$ mice, and the baseline precursor frequency of MBP Ac1-11-specific T cells is the same between $me^{v+/-}$ and WT in the Vβ8.2 TCR Tg mice. Thus, the increased number of Ag-specific cells
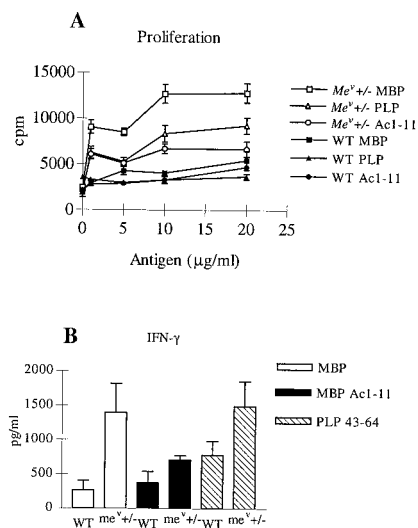

FIGURE 4. Decreased SHP-1 increases proliferative response and cytokine production, with epitope spreading response to PLP 43-64 peptide 8 wk after immunization with MBP Ac1-11/CFA. The splenocytes from mice with chronic EAE (40 days postimmunization) were cultured with different doses of MBP, MBP Ac1-11, and PLP 43-64. Proliferation to MBP and MBP Ac1-11 was markedly increased in $me^{v+/-}$ mice compared with WT mice ($p < 0.05$). MBP Ac1-11-primed splenocytes in $me^{v+/-}$ mice had a moderate response to PLP 43-64, whereas splenocytes from WT mice did not respond to PLP 43-64 ($p < 0.05$). The production of cytokine after 24-, 48-, and 72-h culture was measured by ELISA. Both IL-4 and IL-10 were undetectable in $me^{v+/-}$ and WT mice (data not shown).
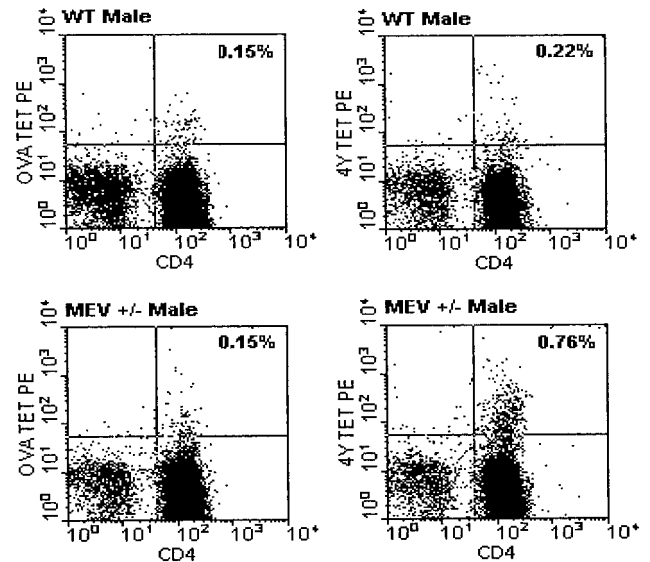

FIGURE 5. Decreased SHP-1 increases the number of MBP Ac1-11-specific T cells in B10.PL $me^{v+/-}$ mice after immunization with MBP Ac1-11. The $me^{v+/-}$ or WT B10.PL mice were immunized with 200 μg of MBP Ac1-11/CFA. Ten days later, LNCs were stained with MBP1-11[4Y]: I-A^u tetramer or control OVA323-339:I-A^u tetramer and anti-CD4 Ab. Percentages indicate the percentage of tetramer-positive cells within the CD4$^+$ population.

after immunization in $me^{v+/-}$ mice relative to WT mice (Fig. 5) is most likely because of increased expansion in vivo, rather than because of differences in precursor frequencies.
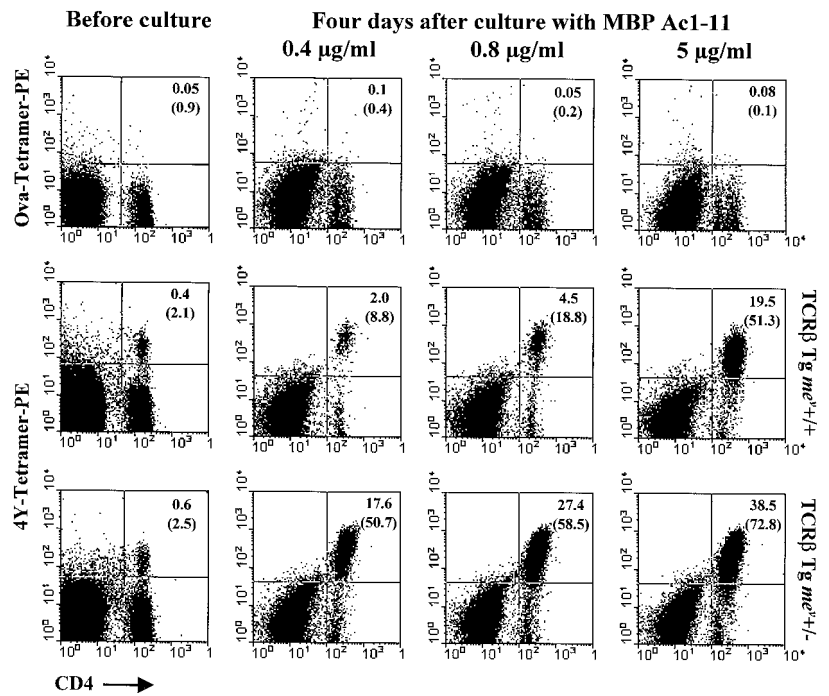
Activation threshold is reduced in Vβ8.2 TCR Tg $me^{v+/-}$ T cells compared with Vβ8.2 TCR Tg WT T cells

Naive LNCs and splenocytes from Vβ8.2 TCR Tg WT and Vβ8.2 TCR Tg $me^{v+/-}$ mice were stained with anti-CD4, B7-1, CD62L, and MBP Ac1-11[4Y] tetramer before and 4 days after culture with different concentrations of MBP Ac1-11. Before the cells were cultured with MBP Ac1-11, the number of tetramer-positive cells was comparable between Vβ8.2 TCR Tg $me^{v+/-}$ and Vβ8.2 TCR Tg WT mice (Fig. 6). After 4 days of culture with MBP Ac1-11, the tetramer-positive cells had expanded significantly more in $me^{v+/-}$ mice than those from WT mice. The tetramer-positive cells increased 9- and 6-fold in $me^{v+/-}$ mice when the MBP Ac1-11 concentrations were 0.4 and 0.8 μg/ml, respectively (Fig. 6), whereas there was only a 2-fold increase of tetramer-positive T cells in $me^{v+/-}$ mice compared with WT T cells when 5 μg/ml of MBP Ac1-11 was used for stimulation (Fig. 6). The number of CD62L^{low} tetramer-positive T cells was also increased in $me^{v+/-}$ mice compared with WT mice after stimulation with MBP Ac1-11 (data not shown). Similar data were obtained with LNCs (data not shown). These data indicate that a reduced level of SHP-1 leads to a reduction in T cell activation threshold, and they clearly demonstrate that at lower Ag concentrations, $me^{v+/-}$ T cells expand more readily than do WT T cells.

T cell proliferation and cytokine production are increased in heterozygous Vβ8.2 TCR Tg $me^{v+/-}$ mice

MBP Ac1-11-specific T cells were demonstrated to be at similar levels in naive $me^{v+/-}$ and WT Vβ8.2 TCR Tg mice (Fig. 6). We examined T cell proliferation and IFN-γ production by Vβ8.2 TCR Tg $me^{v+/-}$ and Vβ8.2 TCR Tg WT T cells after stimulation with different concentrations of MBP Ac1-11. LNCs and splenocytes from Vβ8.2 TCR Tg $me^{v+/-}$ or Vβ8.2 TCR Tg WT mice

FIGURE 6. T cell activation threshold is reduced in $me^{v+/-}$ mice. Splenocytes from Tg $me^{v+/-}$ and Tg WT mice were stained with MBP1-11[4Y]: I-A^u tetramer or control OVA323-339:I-A^u tetramer and anti-CD4 Ab before and 4 days after the cells were cultured with different concentrations of MBP Ac1-11. The baseline of MBP Ac1-11-specific cells (before culture) was similar between the groups of mice. After 4 days of culture, the percentage of MBP Ac1-11-specific cells was increased 9-, 6-, and 2-fold (of total cells) in $me^{v+/-}$ mice compared with WT mice for MBP Ac1-11 concentrations of 0.4, 0.8, and 5 $\mu g/ml$, respectively. The number in the *upper right quadrant* indicates the percentage of total cells. The number in parentheses indicates the percentage of gated CD4$^+$ T cells. OVA tetramer staining is shown for $me^{v+/-}$ T cells and was not different from WT (data not shown).

were cultured with MBP Ac1-11. T cell proliferation was measured by ^{3}H incorporation, and IFN-γ production was examined by ELISA. The T cell proliferation to MBP Ac1-11 was significantly increased in Vβ8.2 TCR Tg $me^{v+/-}$ compared with Vβ8.2 TCR Tg WT mice (Fig. 7A). IFN-γ secretion was markedly higher for Vβ8.2 TCR Tg $me^{v+/-}$ cells than for Vβ8.2 TCR Tg WT cells in response to MBP Ac1-11 (Fig. 7B). Our data indicate that Vβ8.2 TCR Tg $me^{v+/-}$ mice have a higher T cell proliferative response to MBP Ac1-11 compared with Vβ8.2 TCR Tg WT mice. Naive Vβ8.2 TCR Tg $me^{v+/-}$ mice have comparable MBP Ac1-11-specific cells compared with WT mice, and the T cells are not acti-
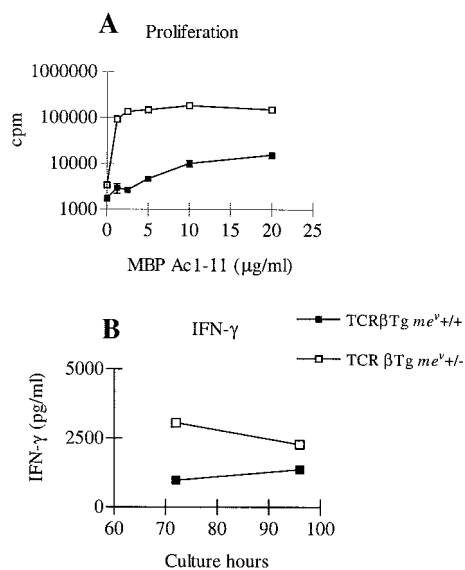
vated in naive mice. Therefore, the higher T cell responses observed in Vβ8.2 TCR Tg $me^{v+/-}$ mice are most likely because of the lower threshold for activation.

APCs do not contribute to the increased T cell response in $me^{v+/-}$ *mice*

To address whether APCs play a role in the difference of T cell activation between $me^{v+/-}$ and WT mice, we purified MBP Ac1-11-specific TCR $\alpha\beta$ Tg CD4 T cells by negative selection and stimulated CD4 T cells with MBP Ac1-11 in the presence of irradiated splenocytes from either WT or $me^{v+/-}$ mice. The T cell proliferation in response to MBP Ac1-11 was comparable between cultures with WT and $me^{v+/-}$ APCs (Fig. 8A). After 3 days of culture, the percentage of MBP Ac1-11-specific tetramer-positive CD4$^+$ cells was similar between the cultures with WT and $me^{v+/-}$ APCs (Fig. 8B). Interestingly, the production of IFN-γ was lower in the $me^{v+/-}$ APC culture compared with WT APC culture (Fig. 8C). Similar results were observed using Vβ8.2 TCR Tg T cells (Fig. 8D). These data clearly demonstrate that the increased T cell response in $me^{v+/-}$ mice is not because of alterations in APC function, with T cell proliferation and expansion being essentially identical. These data also would imply that the increased IFN-γ production noted in the $me^{v+/-}$ cultures must be because of effects related to the T cell, because $me^{v+/-}$ APC-stimulating WT T cells produced less IFN-γ than did WT APC-stimulating WT T cells.

Discussion

The me^v mice have impaired immunologic functions, including reduced proliferative response to B and T cell mitogens, absence of cytotoxic T cell responses, and severely reduced NK cell function (8, 35). In contrast, me^v mice also develop systemic autoimmunity (22, 35). They have polyclonal B cell activation accompanied by hyperimmunoglobulinemia and express multiple autoantibodies and widespread inflammatory lesions. The me^v mice die early with a 9- to 12-wk lifespan because of severe pneumonitis (5, 14). The heterozygous SHP-1-deficient $me^{v+/-}$ mice are physiologically normal, with ~50% of SHP-1 activity (28). Therefore, $me^{v+/-}$

FIGURE 7. Decreased SHP-1 increases T cell response to MBP Ac1-11 in TCR Vβ8 Tg mice. Splenocytes from Tg $me^{v+/-}$ and Tg WT mice were cultured with MBP Ac 1-11 (5 $\mu g/ml$) for 4 days. Supernatants were harvested at 72 and 96 h. The proliferative response to MBP Ac 1-11 in $me^{v+/-}$ mice was significantly higher than that in WT mice ($p < 0.005$). IFN-γ production was also higher in $me^{v+/-}$ mice compared with WT mice.
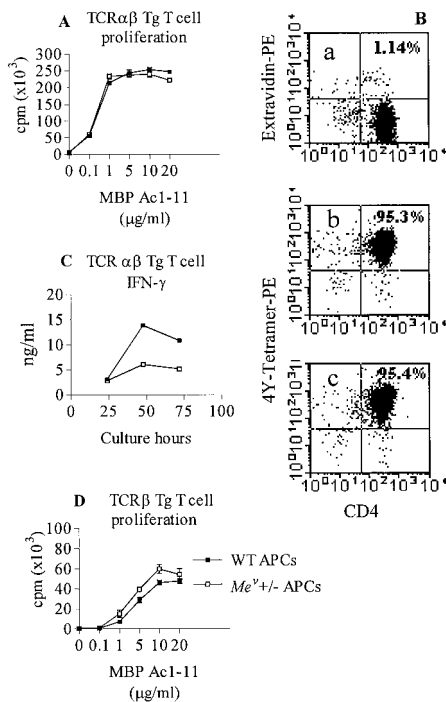
FIGURE 8. Increased proliferation and cytokine production are not because of reduced SHP-1 in APCs. TCR Vβ8.2, Vα2.3 Tg T cells specific for MBP Ac1-11 were purified and then cultured with 4-fold excess of either irradiated WT or $me^{v+/-}$ splenocytes as APCs. No difference in T cell proliferation was noted by [³H]thymidine incorporation (A) or with MBP1-11[4Y]:I-A^u tetramer staining (B). C, IFN-γ production was actually reduced in the cultures with me^v APCs. Similar proliferation results were observed using TCR Vβ8 Tg T cells (D).

mice provide a useful tool for studying the effect of reduced SHP-1 levels on the pathogenesis of EAE.

We examined the effects of decreased SHP-1 on the pathogenesis of EAE by immunizing $me^{v+/-}$ H-2^u mice with MBP Ac1-11 in CFA. The $me^{v+/-}$ mice had an exacerbated course of EAE that was associated with an increased T cell proliferative response to the priming Ag and increased IFN-γ production. Increased severity of EAE in $me^{v+/-}$ mice may be because of 1) an increased number of encephalitogenic MBP Ac1-11-specific T cells, 2) epitope spreading, or 3) a lower threshold for T cell activation. These are not mutually exclusive possibilities.

Cumulative data showed that SHP-1 negatively modulates the signaling function of the TCR. The proliferation and IL-2 production induced by TCR engagement are markedly increased in me^v T cells relative to WT T cells (3, 25). In mature T cells, downstream delivery of TCR stimulatory signals requires the initial activation of the Src family protein tyrosine kinases Lck and Fyn, with consequent tyrosine phosphorylation of the TCR CD3 and ζ subunits, recruitment of the ZAP-70 tyrosine phosphokinase, and the sequential activation of signaling effectors that transduce the signal to the nucleus. SHP-1 negatively regulates the activities of Lck, Fyn (25), and phosphatidylinositol 3-kinase (36), therefore regulating T cell activation. Our data clearly show that T cells from $me^{v+/-}$ mice have increased proliferative responses and IFN-γ production after MBP Ac1-11 stimulation compared with WT mice. Encephalitogenic T cells are typically of a Th1 phenotype, and IFN-γ is crucial for developing inflammatory lesions and demyelination in the CNS (37). Thus, $me^{v+/-}$ mice may have more severe EAE because of increased production of IFN-γ by encephalitogenic T cells.

The increased T cell proliferation and IFN-γ production in $me^{v+/-}$ mice may be because of the increased the number of MBP Ac1-11-specific T cells, decreased threshold of T cell activation because $me^{v+/-}$ mice have reduced SHP-1 activity, or both. Our data show that the number of MBP Ac1-11-specific T cells is higher in $me^{v+/-}$ mice after immunization with MBP Ac1-11/ CFA. The increased number of MBP Ac1-11-specific T cells could partially explain the enhanced proliferation and cytokine production in $me^{v+/-}$ mice after immunization with MBP Ac1-11. However, the number of MBP Ac1-11-specific T cells is comparable between naive Tg $me^{v+/-}$ mice and Tg WT mice, whereas T cell proliferation and IFN-γ production are higher in Tg $me^{v+/-}$ mice after MBP Ac1-11 stimulation. This suggests that even when starting with equal numbers of MBP Ac1-11-specific T cells, the $me^{v+/-}$ T cells have an inherent advantage in expanding in response to Ag. Johnson et al. (6) reported that SHP-1 contributes to establishing thresholds for TCR signaling in thymocytes and naive T cells. In their Tg MHC class I-restricted TCR system, both the number and the percentage of single positive CD8$^+$ thymocytes were significantly increased in SHP-1-deficient mice vs WT mice. In addition, expression of the activation marker CD44 was significantly higher in mutant mice than in WT mice, indicating that in vivo loss of SHP-1 leads to an increased basal level of activation of mature CD8 T cells. The CD8 cells showed hyperproliferation but an equivalent cytolytic activity in SHP-1-deficient mice in response to stimulation with cognate peptide. Similar data were reported by Carter et al. (7). Lack of SHP-1 revealed alterations in the percentages of thymocyte subpopulations; me/me thymocytes undergo negative selection to stimulation at lower concentrations of Ag compared with WT thymocytes and were hypersensitive to stimulation by specific Ag. In our system, the percentage of CD62L^{low} T cells was comparable between $me^{v+/-}$ and WT mice in the Vβ8.2 TCR Tg mice (data not shown). Our data indicate that T cells were activated at the same level before immunization in Tg $me^{v+/-}$ and Tg WT mice. Thus, prior activation of $me^{v+/-}$ TCR Tg T cells did not contribute to their proliferative advantage. Although we could not analyze the activation state of MBP Ac1-11-specific T cells in B10.PL WT or $me^{v+/-}$ mice for technical reasons, it is reasonable to assume that the observations in Vβ8.2 Tg mice concerning CD62L expression and cell number are relevant to nontransgenic mice. It is also clear from our data that $me^{v+/-}$ T cells expand better in response to antigenic stimulation both in vitro and in vivo (Figs. 3–7).

Our data showed that there was a difference in T cell expansion between $me^{v+/-}$ and WT mice, which we have interpreted to be because of decreased signaling threshold through the TCR in $me^{v+/-}$ T cells. Another explanation could be that there is a difference in apoptosis by activation-induced cell death between $me^{v+/-}$ and WT mice. Recently, Zhang et al. (38) reported that me^v T cells are more sensitive than WT T cells to induction of programmed cell death after TCR stimulation. The increased apoptosis in me^v T cells was mediated through up-regulated Fas-Fas ligand interaction and induction of the Fas signaling cascade. In their studies (38), the expression of Fas ligand on me^v T cells is markedly increased with anti-CD3 stimulation, whereas the Fas ligand expression is only mildly increased on WT T cells when stimulated through anti-CD3. In our study, if programmed cell death in $me^{v+/-}$ was increased compared with WT T cells, one would anticipate greater expansion of WT compared with $me^{v+/-}$ T cells. This was not the case. The other possibility is that programmed cell death is greater in $me^{v+/-}$ T cells than in WT cells, but that proliferation or expansion is dramatically greater in $me^{v+/-}$ T cells because of decreased signaling threshold, resulting in our present observations.

Costimulatory signals play a crucial role in T cell activation. Manipulation of B7 pathways could alter T cell activation, eventually influencing the outcome in animal models of autoimmunity. It has been shown that SHP-1 does not influence the functions of CTLA-4 and CD28 (3). Therefore, exacerbated EAE and increased T cell responses in $me^{v+/-}$ mice are unlikely because of influences of SHP-1 on costimulatory signals.

EAE has a relapsing-remitting course of paralysis that is very similar to the clinical profile observed in MS (39). MHC class II-restricted, Ag-specific T cells are crucial for the pathogenesis of EAE. Several studies have demonstrated that changes occur in the Ag specificity of neuroantigen-specific proliferative responses during the course of EAE. Proliferative responses to additional encephalitogenic myelin epitopes have been reported to arise after the initial acute phase of EAE (40). Relapses could result from activation of T cells specific for endogenous myelin epitopes released during the acute phase of disease, which was initiated by the priming encephalitogen (40–42). Thus, reactivity to neuroepitopes other than that used to induce the initial clinical episode, or epitope spreading, may contribute to the relapsing course of clinical relapsing EAE (43, 44). Epitope spreading has also been proposed to contribute to the pathogenesis of spontaneous autoimmune diabetes in nonobese diabetic mouse (45, 46). Recently, Karandikar et al. (47) reported that down-regulation of epitope spreading is mediated by CTLA-4 in relapsing EAE. Neville et al. (48) showed that treating Theiler's virus-induced demyelinating disease in SJL mice with CTLA-4 Ig or anti-B7-1 and B7-2 Abs significantly enhanced clinical disease severity. Epitope spreading to myelin epitopes was accelerated as a result of the increased availability of myelin epitopes, leading to a more severe chronic disease course (48). In our system, $me^{v+/-}$ mice have increased severity of EAE with more relapses. Eight weeks after immunization with MBP Ac1-11, the splenocytes from $me^{v+/-}$ mice responded not only to MBP Ac1-11 itself, but also to the PLP 43-64 peptide. However, there was no response to PLP 43-64 observed 10 days after $me^{v+/-}$ mice were immunized with MBP Ac1-11. This suggests that the response to PLP 43-64 was because of endogenous presentation of this epitope after the acute onset of CNS inflammation. Thus, reduced levels of SHP-1 result in a reduction in the threshold of activation for PLP 43-64-reactive T cells and enhancement of epitope spreading in $me^{v+/-}$ mice. Increased production of IFN-γ by the infiltrating MBP-Ac1-11-specific T cells may also play a role in enhanced epitope spreading.

In summary, our results show that SHP-1 activity plays an important role in EAE pathogenesis through regulation of autoreactive T cell activation. Reduction of the expression of SHP-1 leads to a lower T cell activation threshold, increases expansion of autoreactive T cells, and enhances processes such as epitope spreading. These factors result in $me^{v+/-}$ mice developing more severe clinical EAE with increased relapses. These results also suggest that factors that lower the T cell activation threshold may also have implications for human autoimmune diseases such as MS.

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