Journal of Neuroscience Research 45:680–689 (1996)

Male SJL Mice Do Not Relapse After Induction of EAE With PLP 139-151

B.F. Bebo, Jr., A.A. Vandenbark, and H. Offner
Neuroimmunology Research, Department of Veterans Affairs, (B.F.B., A.A.V., H.O.) and Department of Neurology (A.A.V., H.O.) and Microbiology and Immunology (A.A.V.), Oregon Health Sciences University, Portland Oregon

SJL mice immunized with proteolipid protein (PLP) develop relapsing experimental autoimmune encephalomyelitis (R-EAE). R-EAE is a CD4$^+$, Th1 cell-mediated demyelinating disease of the central nervous system (CNS) that is used as a model for the human disease multiple sclerosis (MS). Previous studies showed that young (<8 weeks) male SJL mice were resistant to active induction of EAE with CNS homogenate, while female mice were susceptible. We have recently observed that young male SJL mice immunized with a major encephalitogenic peptide of myelin, PLP 139-151, developed initial clinical and histological symptoms of EAE with a severity similar to age-matched females; however, unlike females, male mice did not relapse. Significant T cell proliferation to PLP 139-151, but not to other PLP and myelin basic protein (MBP) epitopes, was observed in both males and females during the initial episode, recovery, and first relapse of clinical disease. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of lymphokine mRNA revealed differences in IFN-γ and IL-4 synthesis consistent with the hypothesis that Th2 T cells develop in young male SJL mice that regulate the relapsing phase of the disease. These data suggest that immunization of young male SJL mice with PLP 139-151 overrides a defect in antigen presentation responsible for the previously observed resistance to EAE, and that natural processing and presentation of neuroantigens during the course of acute EAE induces Th2 cells that prevent the relapse of disease. © 1996 Wiley-Liss, Inc.

Key words: relapsing experimental autoimmune encephalomyelitis, proteolipid protein, cytokine, sex differences in autoimmunity

INTRODUCTION

Relapsing experimental autoimmune encephalomyelitis (R-EAE) is an inflammatory demyelinating disease of the central nervous system that is induced by immunization of SJL mice with the myelin proteins, or peptides (Fritz et al., 1983; Trotter et al., 1987; McRae et al., 1992). R-EAE is a useful experimental model, and has provided considerable insights into the pathogenesis of the human disease multiple sclerosis (MS). Immunization with myelin proteins or peptides induces autoreactive CD4$^+$, Th1 cells that home specifically to the central nervous system (CNS) and regulate the accumulation of inflammatory mononuclear cells, resulting in demyelination and clinical disease (Paterson and Swanborg, 1988). Encephalitogenic T cell lines or clones derived from PLP 139-151 immunized mice can be stimulated in vitro with specific antigen and are capable of transferring relapsing disease to naive hosts (Kuchroo et al., 1992). Analysis of inbred mouse strains demonstrates that specific major histocompatibility complex (MHC) haplotypes (H-2^{p,q,r,s,u}) confer susceptibility to EAE (Bernard, 1976; Arnon, 1981; Linthicum and Frelinger, 1982). In addition, vascular sensitivity to vasoactive agents, sex hormones, or sex-linked genes are factors outside the MHC implicated in controlling genetic susceptibility to EAE (Linthicum and Frelinger, 1982; Montgomery and Rauch, 1982).

Differences in the immune response of males and females have been studied extensively. Females have an enhanced immune response, a more developed thymus, better humoral immunity, stronger primary and secondary immune responses, are more resistant to the induction of tolerance, and have a greater ability to reject tumors (Homo-Delarche et al., 1991). The heightened immune response observed for females may explain their increased incidence of autoimmune diseases. For MS, the ratio of females to males is approximately 2:1. Dur-

Received May 14, 1996; accepted May 21, 1996.

Address reprint requests to Bruce F. Bebo Jr., Ph.D., Department of Veterans Affairs, Neuroimmunology Research 151-D, 3710 SW US Veterans Hospital Rd., Portland, OR 97201.

© 1996 Wiley-Liss, Inc.

ing pregnancy the clinical symptoms of MS are often ameliorated, but are exacerbated postpartum (Davis and Maslow, 1992). The levels of estrogen and progesterone rise sharply during pregnancy and may be important for regulating the autoimmune response. Estrogen or synthetic estrogen analogs have been shown to suppress EAE in both mice and rats (Trooster et al., 1993; Jansson et al., 1994). Further evidence for the influence of sex hormones on the immune response comes from reports demonstrating differences in susceptibility to EAE between males and females (Keith, 1978; Griffin et al., 1993). Susceptibility to EAE in Lewis rats was altered by restraint stress, which is thought to induce hormonal suppression of immunity (Griffin et al., 1993). Clinical signs of EAE were significantly reduced in stressed female rats relative to males. Moreover, restrain stress, did not alter the course of EAE in rats immunized with the encephalitogenic myelin basic protein (MBP) peptide 68–88, suggesting that hormonal regulation of EAE may affect processing/presentation of MBP.

Recent studies have demonstrated that young male (4–8 weeks) SJL mice have a developmental defect in their ability to initiate a delayed type hypersensitivity (DTH) response to neuroantigens (i.e. MBP and PLP) and are resistant to the active induction of R-EAE with CNS homogenate, while older males (>10 weeks) and young female mice are DTH responders and susceptible to R-EAE (Cua et al., 1995a). It was further shown that this defect occurred during the induction phase of the immune response and could be reversed by the transfer of Mac–1^+, Mac–2^-, Mac–3^+, I–A^+ macrophages from either young female or older (>10 weeks) male mice (ibid.). In a subsequent report, immunization of young male and female mice with MBP induced significant T cell proliferation. However, male T cells produced Th2 cytokines in contrast to female T cells which produced Th1 cytokines (Cua et al., 1995b). Adoptive transfer of MBP-activated female Th1 cells resulted in clinical R-EAE, while transfer of male Th2 cells produced no clinical disease. These data suggest that sex-linked factors influence macrophage APC in polarizing T-helper cell responses.

These studies prompted us to immunize young male and female SJL mice with the immunodominant PLP peptide, PLP 139–151. Male SJL mice immunized with PLP 139–151 in complete Freund's adjuvant (CFA) developed EAE with a severity similar to age-matched females; however, unlike the females, male mice did not relapse. Although no significant differences in antigen specific T-cell proliferation were noted between males and females, differences in IFN-γ and IL-4 synthesis were found consistent with the hypothesis that Th2 cells develop in young male SJL mice that regulate the relapsing phase of the disease.

MATERIALS AND METHODS

Mice

Male and female SJL mice were purchased from Jackson Laboratory (Bar Harbor, ME) at 5 to 6 weeks of age, and were housed in the Animal Resource Facility at the Portland Veterans Affairs Medical Center in accordance with institutional guidelines.

Antigens

Mouse PLP 139–151 (HCLGKWLGHPDKF), 43–64 (EKLIETYFSKNYQDYEYLINVI), and myelin basic protein 87–99 (PQKSQRTQDENPVVHF) were synthesized using solid phase techniques and HPLC purified (Beckman Institute, Stanford University, Palo Alto, CA). Mouse PLP 178–191 (NTWTTCQSIAFPSK) was a gift from S. Miller, Northwestern University, Chicago, IL. Mouse myelin basic protein (MoBP) was extracted from mouse brains (Pel-Freeze Biologicals, Rogers, AK) and purified as previously described (Diebler, et al., 1972). All antigens were diluted to 1 mg/ml in RPMI, except PLP 178–191, which was diluted in 0.035 M acetic acid as previously published (McRae et al., 1995).

Active Induction of EAE

To induce disease with PLP peptide, mice were inoculated subcutaneously in the flanks with 0.2 ml of an emulsion containing 150 μg of PLP 139–151 in saline and an equal volume of CFA containing 200 μg of Mycobacterium tuberculosis H37RA (Difco Laboratories, Detroit, MI). Mice were examined daily for clinical signs of disease and scored according to the following scale: 0, normal; 1, minimal or mild hind limb weakness; 2, moderate hind limb weakness or mild ataxia; 3, moderately severe hind limb weakness; 4, severe hind limb weakness or moderate ataxia; 5, paraplegia with no more than moderate forelimb weakness; 6, paraplegia with severe forelimb weakness or severe ataxia. A clinical relapse was defined as an increase of at least one full grade in clinical score developing after a period of stabilization or improvement and lasting at least 24 hr.

Preparation of Lymph Node and Spleen Cells

Draining lymph node cells were recovered from PLP 139–151 immunized male and female SJL mice at onset (d11–12), peak (d14–15), recovery (d19–22), and relapse (d24–35) of R-EAE. Inguinal lymph nodes were dissected and a single cell suspension prepared by passage through a wire mesh screen. Cells were washed twice in RPMI 1640 medium (GIBCO/BRL) and resuspended at 2×10^6 cells/ml in stimulation media containing 1% syngeneic mouse serum, 2-ME (5×10^{-5}

M), L-glutamine (2 mM), sodium pyruvate (1 mM), and antibiotics in RPMI.

Preparation of Spinal Cord Mononuclear Cells

Spinal cord mononuclear cells were isolated by using a previously described technique (Whitham et al., 1991). Briefly, spinal cords were removed by insufflation, washed three times in RPMI in order to remove any contaminating blood cells, and homogenized by passage through a wire mesh screen. The homogenate was then washed and resuspended in isotonic percoll (80%). The homogenate from three animals was layered onto a 10 ml step gradient. Each step gradient contained 100% (2ml), 80% (4ml), and 40% (4ml) isotonic percoll, with the cells suspended in the 80% fraction. The gradients were centrifuged at 1200 × g for 30 min and the cells were harvested from the 80%/40% interface. The cells were subsequently washed in RPMI and resuspended in growth media (stimulation media with 10% FBS and 120 U/ml of rhIL–2). The cells were expanded in growth media for 7–10 days before in vitro proliferation assays were performed.

Proliferation Assays

The in vitro proliferative responses of lymph node, spleen, and spinal cord T cells were determined using 96 well flat-bottom microtiter plates as previously described (Bourdette et al., 1988). Briefly, 4×10^5 lymph node or spleen cells were incubated in stimulation media alone (control), or specific antigens at 37°C in 7% CO2. The cultures were incubated for 72 hr, the last 18 hr in the presence of 0.5 μCi [^{3}H]-TdR. The cells were harvested onto glass fiber filters and TdR uptake was measured by liquid scintillation chromatography. Mean cpm were calculated from triplicate wells. For spinal cord proliferation assays, 2×10^4 spinal cord cells and 5×10^5 syngeneic, irradiated thymocytes were incubated with stimulation media alone, or specific antigens, and proliferation measured as above. The stimulation index was determined by calculating the ratio of antigen specific cpm to background cpm.

Lymphokine mRNA Analysis

For lymph node and spleen cell preparations 4×10^6 cells were cultured in 1 ml of stimulation media alone, or with antigens (50 μg/ml) and incubated at 37°, 7% CO_2 for 24 hr. 0.5×10^5 spinal cord cells were combined with 10×10^6 irradiated thymocytes and treated in a similar manner. Total cellular RNA was isolated by a modified guanidinium thiocyanate-phenol-chloroform single extraction method. (Chomczynski and Sacchi, 1987). First strand cDNA was synthesized from total cellular RNA preparations using MLV-RT (BRL, Bethesda, MD). Cell equivalent amounts of cDNA, approximately 10,000 cells, were used per PCR reaction. Each sample was then normalized by PCR amplification with control primers specific for mouse actin. The normalized amount of cDNA was added for each sample and amplified with mouse cytokine specific primers for IFN-γ, and IL-4. The linear portion of the amplification curves were determined empirically to be between 25–35 cycles. The PCR reactions for actin and IFN-γ were run for 30 cycles, and IL-4 for 32 cycles.

Phenotyping

Analysis of spinal cord mononuclear cells was performed on a FACScan (Becton Dickinson, Mountain View, CA). Cells were isolated from the spinal cord as previously described, and incubated with specific antibodies for 30 min at room temperature in RPMI with 2% FBS. The cells were washed twice and directly analyzed. The CD3 antibody (Pharmigen, San Diego, CA) was directly conjugated to PE, the TCR specific antibodies were unconjugated and required a FITC labeled detection antibody. All of the cells were stained with isotype control antibodies to set the quadrants, and to calculate the percent positive cells.

Histology

Mice were sacrificed by CO_2 inhalation, spinal cords were dissected and immersed in 10% phosphate buffered formalin. The organs were paraffin-embedded and sections were stained with luxol fast blue-periodic acid schiff-hematoxylin for light microscopy.

RESULTS

Clinical Course of EAE in PLP 139-151 Immunized Male and Female SJL Mice

Prior studies have demonstrated that young male SJL mice are resistant to active induction of R-EAE induced by immunization with spinal cord homogenate (Cua et al. 1995a). We sought to determine if young male SJL mice were resistant to R-EAE induced by active immunization with the immunodominant peptide PLP 139–151. Young, age-matched (5–6 weeks), male and female SJL mice were actively immunized with PLP 139–151 in CFA, and observed for clinical signs of EAE. Both male and female mice developed paralytic EAE between days 10–16 post-immunization (p.i.), with no significant difference in the day of onset (Fig. 1 and Table I). Female mice consistently developed a slightly more severe acute attack of disease than males (Fig. 1 and Table I). A significant difference in acute cumulative disease index (CDI) was observed for experiment 1 ($P = 0.005$), but there was no significant difference in the acute CDI averaged over three different experiments ($N = 35$). Both male and female mice recovered from acute

TABLE I. Active Induction of R-EAE in Young Male and Female SJL Mice Immunized With PLP 139-151

		Incidence of EAE			Incidence of Relapsing Disease		
Experiment	Sex	# of Mice (%)	Mean Day of Onset	CDI[a] Acute	# of Mice (%)	Mean Day of Relapse	CDI[b] Relapse
1	Male	6/10 (60)	12.8	12.7	0/5 (0)		3.8
	Female	9/10 (90)	12.2	23.5	4/9 (44)	33.8	19.4
2	Male	8/10 (80)	12.3	10.3	0/6 (0)		3.6
	Female	10/10 (100)	13.1	12.3	5/6 (83)	29.6	14.1
3	Male	14/15 (93)	12.6	13.1	0/5 (0)		3.9
	Female	15/15 (100)	12.4	19.0	7/8 (88)	23.8	16.2
Total	Male	28/35 (80)	12.2	12.0	0/16 (0)[c]		3.8[d]
	Female	34/35 (97)	12.7	18.2	16/23 (70)	29.1	16.5

[a]Cumulative disease index for days 12–21 post-immunization (acute disease).
[b]Cumulative disease index for days 24–33 post-immunization (relapsing disease)
[c]Significant difference between males and females ($P < 0.0001$)
[d]Significant difference between males and females ($P = 0.007$)

disease between days 19–25 p.i. Female mice developed paralytic relapses between day 24–35 p.i., while male mice failed to relapse over a 90-day observation period (Fig. 1 and Table I.).

Proliferative Responses of Spleen and Lymph Node T Cells From Male and Female SJL Mice Immunized With PLP 139–151

It has been suggested that relapses in EAE might be due to induction of T cells specific to secondary encephalitogenic epitopes as a result of CNS damage from the initial episode of disease (epitope spreading). To evaluate the specificity of T cell responses during the course of R-EAE, we quantitated differences in proliferation responses in male and female mice that might explain the lack of relapses in males. Significant proliferation to PLP 139–151 was observed for male and female mice at peak (days 13–15 p.i.), recovery (days 19–21 p.i.), and relapse (days 24–35 p.i.) of clinical EAE. However, no significant proliferative responses were seen against any of the other antigens tested at any time during the course of EAE (Fig. 2). No significant differences were observed in T-cell responses between male and female mice, except at recovery of disease when male lymph node T cells had a significantly lower level of proliferation to PLP 139–151 than female cells. ($P = 0.06$).

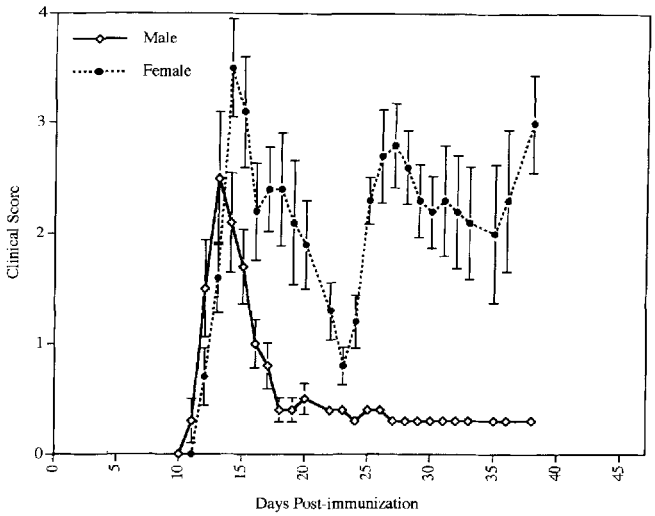

Fig. 1. Sex differences in susceptibility to relapsing EAE induced by PLP 139–151 in young SJL mice. Mice were immunized with PLP 139–151 and CFA at day 0, and monitored daily for clinical disease as described in materials and methods. Mean clinical score ± SEM was calculated from a representative experiment (experiment #3, Table I). Open diamonds, males; closed circles, females.

Characterization of CNS Mononuclear Cells

CNS mononuclear cells were isolated from the spinal cord of male and female SJL mice during clinical disease. At the peak of the initial episode of EAE there were no significant differences in the total number of cells isolated from male and female spinal cords (Table II) although there were significantly more T cells in female spinal cords as demonstrated by staining with anti-CD3 antibody (Table II). However, consistent with clinical and histological observations, there were significantly fewer mononuclear cells in male spinal cords than female cords at relapse of disease (Table II).

The potential of spinal cord T cells isolated from male and female mice to proliferate to PLP 139–151 was examined. After isolation from the spinal cords, cells were grown in IL-2 containing media for 7–10 days, as it was previously determined that a period of growth in IL-2 is required before proliferation of CNS lymphocytes can be measured (Whitham et al., 1991). Both male and female spinal cord T cells isolated at the peak of disease had significant proliferative responses to PLP 139–151 (Table II), with females showing a small, but consis-

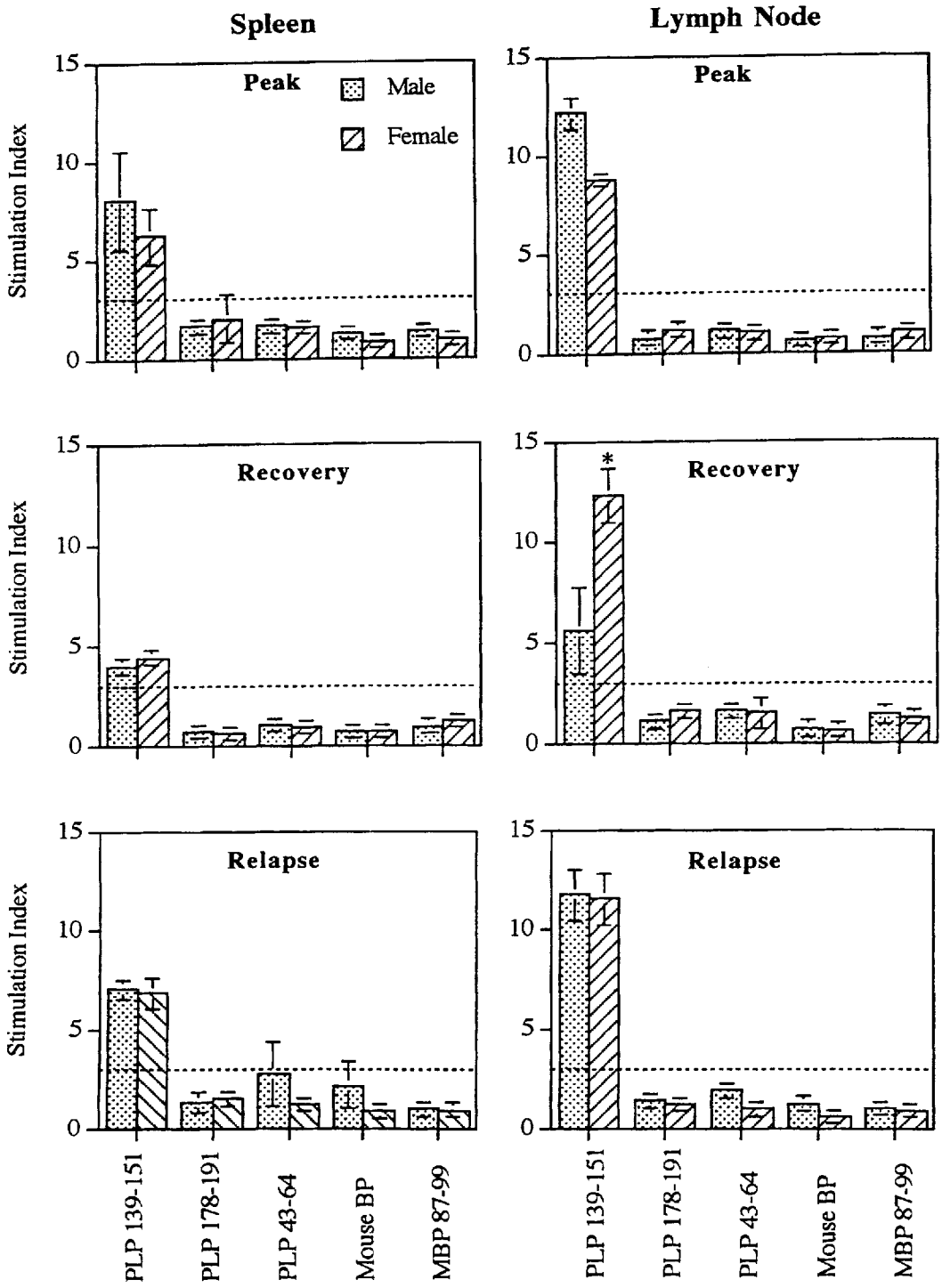

Fig. 2. Lymph node and spleen cell proliferation during the course of R-EAE. Male and female SJL mice were immunized with PLP 139–151 in CFA. Spleen and lymph node cells were recovered at indicated times, peak (d14–15), recovery (d19–22), relapse (d24–35), and proliferation to specific antigens determined using a standard ^{3}H-thymidine incorporation assay. The data are presented as stimulation indices ± SEM from 2 experiments (3 mice pooled for each experiment). The horizontal line indicates a stimulation index of 3, which is the cutoff for significance. *Significant difference between male and female (P = 0.06).

TABLE II. Characterization of Spinal Cord Cells Isolated From Male and Female SJL Mice at Peak and Relapse of Clinical Disease Induced by Immunization With PLP 139–151

Cell Source	Cell #/SC	Stimulation Index[a]	% CD3	% TCR expression on CD3+ cells Vβ2	Vβ4	Vβ17
Peak						
Male	10×10^4	7.6	47	22	15	17
Female	7.1×10^4	9.5	76[c]	17	14	18
Relapse						
Male	1.8×10^4	nd	40	nd	nd	nd
Female	17×10^4[b]	3.6	70[c]	nd	nd	nd

[a]Stimulation index for PLP 139–151
[b]Significant difference between males and females ($p = 0.001$)
[c]Significant difference between males and females ($p \leq 0.001$)

tently greater stimulation index than males. Females also demonstrated significant proliferation at relapse of disease; the proliferative response of male spinal cord cells at relapse was hindered by the low cell numbers.

T-cell receptor β chain expression of $CD3^+$ spinal cord T cells isolated at peak of disease was examined using chain specific antibodies and FACS analysis. Approximately 50% of the $CD3^+$ cells in the spinal cord at the peak of disease stained positive for Vβ2, Vβ4, or Vβ17 (Table II). No significant differences were noted between male and female Vβ TCR expression.

RT-PCR Analysis of Cytokine mRNA Synthesized by Lymph Node and Spinal Cord T Cells

Expression of cytokine mRNA was examined in lymph node (LN) and spinal cord (SC) cells isolated at onset, peak, and relapse in order to determine the pattern of lymphokine production in PLP 139–151-immunized male and female mice. LN and SC cells isolated from female mice demonstrated a profound Th1 type response at onset, peak and relapse, with production of mRNA coding for IFN-γ and TNF-α, but did not possess IL-4 specific transcripts (Fig. 3 and Table III). In contrast, LN cells from male mice had a mixed response at onset and peak, characterized by synthesis of IFN-γ, TNF-α and IL-4 (Fig. 3 and Table III). SC cells at onset did not make IL-4 mRNA, although IL-4 mRNA was detected at peak of disease. At relapse of disease only TNF-α specific transcripts were detected in male LN cells. Analysis of SC cells was precluded by low cell recovery.

Histology

Pathological examination of brain and spinal cord sections was performed at peak and relapse of clinical EAE. Perivascular inflammation with mild demyelination was observed in the meninges and white matter in the brain and spinal cord of both male and female mice at the peak of clinical disease (Fig. 4A and B). Profound

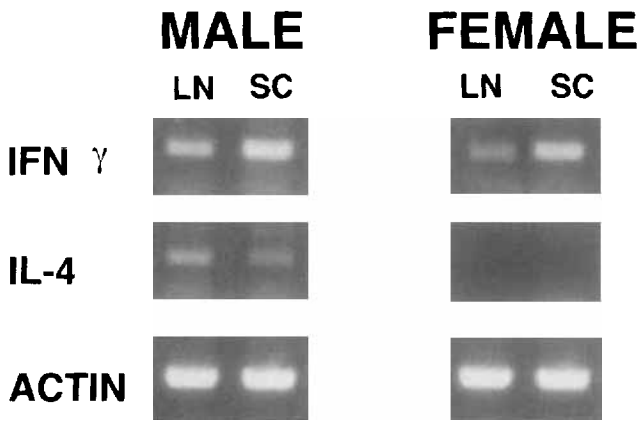

Fig. 3. RT-PCR analysis of lymph node (LN) and spinal cord (SC) cells isolated at peak of disease from male and female SJL mice immunized with PLP 139–151.

inflammation and demyelination was observed in females at relapse of clinical disease, in contrast to nonrelapsing male mice which had only residual demyelination (Fig. 4C and D).

DISCUSSION

Previous studies demonstrated that young male SJL mice were resistant to the active induction of EAE with CNS homogenates, due apparently to their inability to develop proinflammatory Th1 cells (Cua et al., 1995a,b). These intriguing results prompted us to investigate gender differences in susceptibility to EAE in young SJL mice immunized with an immunodominant myelin peptide, PLP 139–151. Our results demonstrate that both young males and females developed a similar severe initial episode of clinical and histological EAE. However, unlike females, young male mice did not develop clinical relapses (Fig. 1 and Table I). This pro-

TABLE III. RT-PCR Analysis of Lymphokine mRNA at Onset, Peak, and Relapse of EAE Induced With PLP 139–151 and CFA

		IFN-γ	TNF-α	IL-4
Onset				
Male	lymph node	+	+	+
	spinal cord	+	+	−
Female	lymph node	+	+	−
	spinal cord	+	+	−
Peak				
Male	lymph node	+	+	+
	spinal cord	+	±	+
Female	lymph node	+	+	−
	spinal cord	+	±	−
Relapse				
Male	lymph node	−	+	−
	spinal cord	nd	nd	nd
Female	lymph node	+	+	−
	spinal cord	±	+	±

found difference during the relapsing phase of EAE in young males was reflected by greatly reduced inflammation and demyelination (Fig. 4), and by almost ten-fold reduction in recovery of infiltrating SC lymphocytes (Table II), essentially precluding further functional studies of male CNS T cells. Both male and female T cells proliferated in response to the PLP 139–151 peptide, but males preferentially developed a Th2-like response during the peak of the initial clinical episode and thereafter, potentially diluting or regulating the encephalitogenic Th1 response that appeared in relapsing females.

Recent results suggest that one consequence of inflammation in the CNS during the acute phase of EAE is exposure of secondary or cryptic encephalitogenic determinants that subsequently stimulate additional pathogenic T cell specificities. This phenomenon, termed "epitope spreading," has been implicated as a major mechanism for the induction of relapses in EAE (McCarron et al., 1990; Perry et al., 1991; Lehmann et al., 1992; Cross et al., 1993; McRae et al., 1995; Yu et al., 1996). In the SJL mouse, induction of EAE with PLP 139–151-specific T cell lines induced recognition of a second encephalitogenic epitope, PLP 178–191, but not a third encephalitogenic determinant, PLP 104–117, or to the major MBP epitope 84–104 (McRae et al., 1995). In the current study we observed significant responses to PLP 139–151 by both male and female lymph node and spleen cells, but we did not detect epitope spreading to PLP 178–191, PLP 43–64, whole MBP, or MBP 87–99 (Fig. 2). These results differ from others, but might be explained by differences in immunization protocol, immunizing peptide, or source of SJL mice. These data suggest that immunization of young mice may not incite epitope spreading, and imply that proliferation to additional self-epitopes may not be required to generate relapses.

Differences in T-cell proliferation between males and females was observed only during recovery of disease, when male LN cells had a significantly reduced stimulation index compared to females (Fig. 2). The decreased proliferation to PLP 139–151 may be a result of increased immune regulation in males, possibly mediated by sex steroids. The role of sex hormones in the immune regulation of males and females has not been well studied. We hope to clarify the role of sex hormones on immune regulation in R-EAE using T cell transfers from castrated and hormone-treated animals. In addition, sex hormone treatment of PLP 139–151-specific T cell lines will be evaluated for effects on encephalitogenicity.

Two major subsets of T-helper cells, Th1 and Th2, have been defined based on biological activity and the cytokines they produce (Mossmann and Coffman, 1989). The inflammatory CD4+ T cells that mediate EAE have been shown to produce Th1 cytokines including IFN-γ, IL-2, LT and TNF-α (Khoury et al., 1992; Merrill et al., 1992; Baron et al., 1993). In contrast, Th2 cells produce IL-4, IL-5, IL-6, IL-10, and TGF-β, and are thought to down-regulate EAE (Racke et al., 1994; Kuchroo et al., 1995). Differential activation of Th1 or Th2 cells may play a critical role in the induction and regulation of autoimmune disease. In this study we examined the production of cytokine mRNA in LN and SC cells from PLP 139–151 immunized male and female mice. At onset and peak of clinical disease, female LN and SC cells had a Th1 phenotype, while male cells had a mixed Th1 and Th2 response (Fig. 3 and Table III). This mixed cytokine response may explain the modest but consistent reduction in acute clinical score for male mice. At relapse of clinical disease, female LN cells continued to produce a Th1-like phenotype. However, female SC cells showed reduced amounts of IFN-γ and low levels of IL-4 mRNA, which may be indicative of episodic cytokine regulation in the spinal cord. In contrast, male LN cells did not produce mRNA for IFN-γ or IL-4, but did synthesize TNF-α mRNA. The absence of IFN-γ in males during this time may account for the lack of clinical relapse in these mice.

Current evidence suggests that Th1 and Th2 cells differentiate from a common precursor (Th0) cell, and factors present during activation and differentiation determine the phenotype of the Th cell response (Rocken et al., 1992). Receptors for sex hormones are present on leukocytes, and estrogen and testosterone are known to modulate T cell cytokine secretion patterns (Homo-Delarche et al., 1991). Steroid mediated regulation of cytokine production could serve to dictate the type of immune effector response that can be initiated upon exposure to antigen, and may be responsible for gender

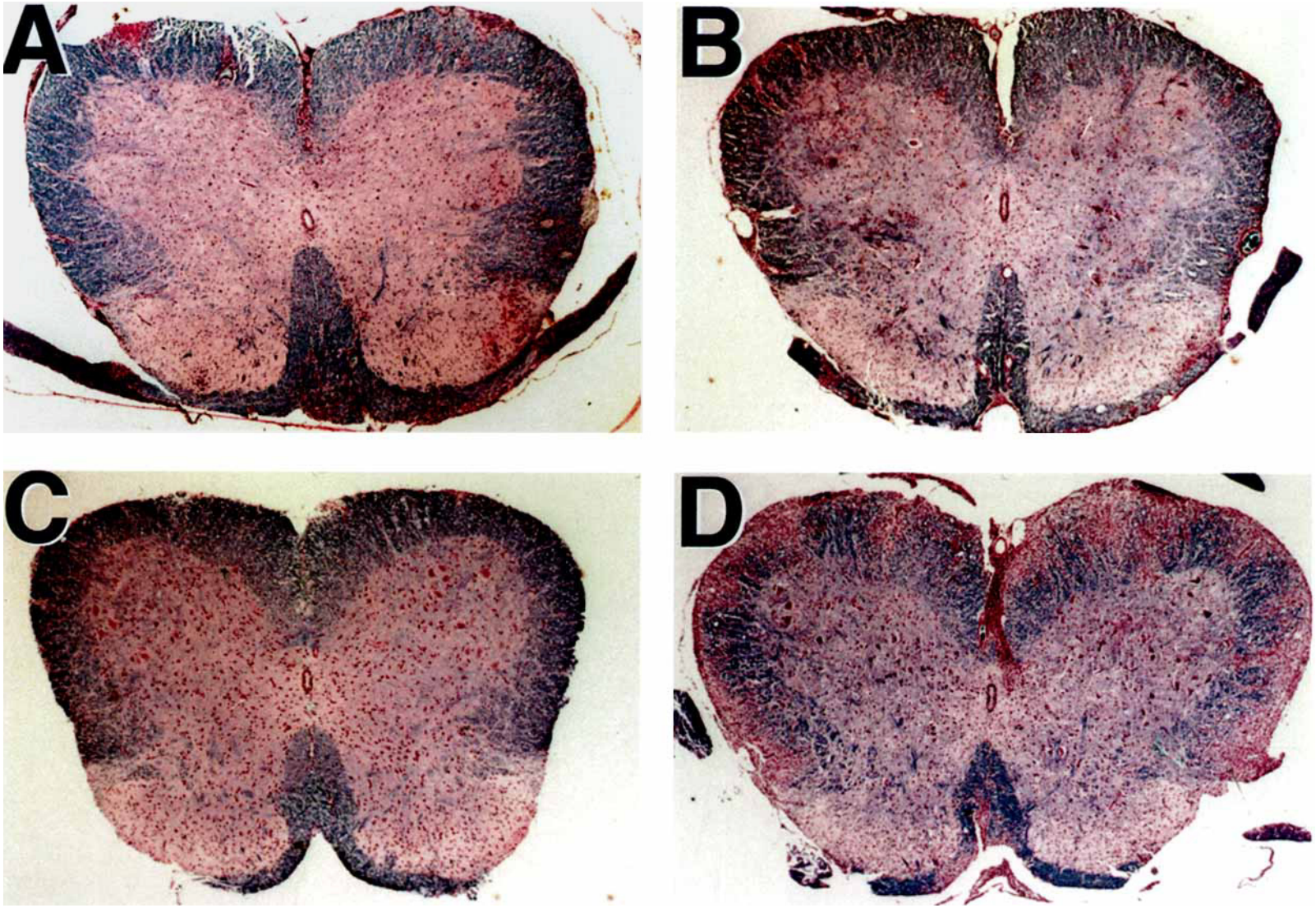

Fig. 4. Paraffin-embedded transverse spinal cord sections isolated at peak and relapse of PLP 139–151 induced R-EAE. **A:** Male at peak of disease; **B:** Female at peak of disease; **C:** Male at day 35; **D:** Female at relapse (d35). Luxol fast blue-periodic acid schiff-hematoxylin stain, 50 × magnification.

differences described above and elsewhere, in susceptibility to R-EAE. Preliminary data suggest that castration of male and female SJL mice influences R-EAE susceptibility (unpublished observations). In addition, recent reports have demonstrated gender differences in adoptively transferred R-EAE in the mature SJL mouse (Pitchekian-Halabi et al., 1996; Smith and Eller, 1996). The role of gender in the pathogenesis of R-EAE is not clear and more information is needed.

Because CNS lymphocytes are in close proximity to the sites of disease, and peripheral immune cells likely migrate to the CNS at disease onset, we felt it was important to study these cells. Significant T cell proliferation to PLP 139–151 was observed in both males and females at peak of disease (Table II). Females consistently had a higher stimulation index; females also had higher background (unpublished observations). These observations may indicate increased inflammatory cell activity in the CNS of female mice, and may be indicative of gender differences in immune regulation. Limitations in cell number kept us from evaluating proliferation to other encephalitogenic epitopes. However, we hope to study epitope spreading in the CNS in subsequent experiments. Significant differences in the numbers of mononuclear cells were observed only during relapse of clinical disease, which is in agreement with clinical and histopathological observations. In addition, females had a consistently higher percentage of T cells in the inflammatory CNS infiltrate. These observations may indicate gender differences in inflammatory cell migration into the CNS, an area that requires more study.

Previous studies demonstrated that young male SJL mice were resistant to both acute and relapsing EAE induced with whole CNS homogenate. In contrast, our results show that young male SJL mice immunized with PLP 139–151 are susceptible to acute EAE, but do not relapse. One possible explanation for these differing results is that peptides are presented by a different subset of antigen presenting cells (APC) than intact proteins and bypass the defect in antigen presentation previously described for young male mice. This idea is consistent with previous studies which showed that stress attenuated the clinical course of whole MBP-induced EAE in the Lewis rat, but did not affect the course of EAE in rats immunized with the encephalitogenic peptide MBP 68–88 (Griffin et al., 1993). If this explanation is correct, we would predict that PLP or MBP peptides (i.e. PLP 178–191, MBP 89–101), but not intact PLP or MBP molecules, would induce acute disease in young male SJL mice.

A related question raised by our results is why young male SJL mice immunized with PLP 139–151 develop acute disease, but fail to relapse? One possible explanation is that during acute disease, myelin proteins are processed and presented in the CNS by the subset of APC, described in Cua et al. (1995a) that selectively induces a Th2 response. Data from our study showing IL-4 mRNA in male LN and SC T cells at peak of disease is consistent with this hypothesis. Thus, we propose that initial induction of acute EAE in young male mice requires sensitization with encephalitogenic peptides, not proteins, and that the absence of relapses can be attributed to endogenous induction of Th2 responses, an event possibly influenced by sex hormones

ACKNOWLEDGMENTS

This work was supported by the Department of Veterans Affairs, and by NIH grants NS23221 and NS23444.

REFERENCES

Arnon R (1981): Experimental allergic encephalomyelitis-susceptibility and suppression. Immunol Rev 55:5–30.

Baron JL, Madri JA, Ruddle NH, Hashim G, Janeway CA (1993): Surface expression of α4 integrin by CD4 T cells is required for their entry into brain parenchyma. J Exp Med 177:57–68.

Bernard CCA (1976): Experimental autoimmune encephalomyelitis in mice: genetic control of susceptibility. J Immunogenetics 3:263–274.

Bourdette DB, Vandenbark AA, Meshul C, Whitham RH, Offner H (1988): Basic protein specific T-cell lines that induce experimental autoimmune encephalomyelitis in SJL mice: comparison with Lewis rats. Cell Immunol 112:351–363.

Chomczynski P, Sacchi N (1987): Single step method of RNA isolation by acid quanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159.

Cross AH, Tuohy VK, Raine CS (1993): Development of reactivity to new myelin antigens during chronic relapsing autoimmune demyelination. Cell Immunol 146:261–269.

Cua DJ, Hinton DR, Kirkman L, Stohlman SA (1995a): Macrophages regulate induction of delayed type hypersensitivity and experimental allergic encephalomyelitis in SJL mice. Eur J Immunol 25:2318–2324.

Cua DJ, Hinton DR, Stohlman SA (1995b): Self antigen-induced Th2 responses in experimental autoimmune encephalomyelitis (EAE)-resistant mice: Th2 mediated suppression of autoimmune disease. J Immunol 155:4052–4059.

Davis RK, Maslow AS (1992): Multiple sclerosis in pregnancy: a review. Obstet Gynecol Surv 47:290–296.

Deibler GE, Martenson RE, Kies MW (1972): Large-scale preparation of myelin basic protein from central nervous system tissue of several mammalian species. Prep Biochem 2:139–165.

Fritz RB, Chou CH, McFarlin DE (1983): Relapsing murine experimental allergic encephalomyelitis induced by myelin basic protein. J Immunol 130:1024–1026.

Griffin AC, Lo WD, Wolny AC, Whitacre CC (1993): Suppression of experimental autoimmune encephalomyelitis by restrain stress: sex differences. J Neuroimmunol 44:103–116.

Homo-Delarche F, Fitzpatrick F, Christeff N, Nunez EA, Bach JF, Dardenne M (1991): Sex steroids, glucocorticoids, stress and autoimmunity. J Steroid Biochem Molec Biol 40:619–637.

Jansson L, Olsson T, Holmdahl R (1994): Estrogen induces a potent

suppression of experimental autoimmune encephalomyelitis and collagen-induced arthritis in mice. J Neuroimmunol 53: 203–207.

Khoury SJ, Hancock WW, Weiner HL (1992): Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth factor β, interleukin 4, and prostaglandin E expression in the brain. J Exp Med 176:1355–1364.

Keith A (1978): Sex differences in Lewis rat in the incidence of recurrent experimental allergic encephalomyelitis. Nature 272: 824–825.

Kuchroo VK, Sobel RA, Laning JC, Martin CA, Greenfield E, Dorf ME, Lees MB (1992): Experimental allergic encephalomyelitis mediated by cloned T cells specific for a synthetic peptide of myelin proteolipid protein. Fine specificity and T cell receptor V beta usage. J Immunol 148:3776–3782.

Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, Weiner HL, Nabavi N, Glimcher LH (1995): B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 development pathways: application to autoimmune disease therapy. Cell 80:707–718.

Lehmann PV, Forsthuber T, Miller A, Sercarz EE (1992): Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. Nature 358:155–157.

Linthicum DS, Frelinger JA (1982): Acute autoimmune encephalomyelitis in mice. II. Susceptibility is controlled by the combination of H-2 and histamine sensitization genes. J Exp Med 156:31–40.

McCarron RM, Fallis RJ, McFarlin DE (1990): Alterations in T cell antigen specificity and class II restriction during the course of chronic relapsing experimental allergic encephalomyelitis. J Neuroimmunol 29:73–79.

McRae BL, Kennedy MK, Tan LJ, Dal Canto MC, Miller SD (1992): Induction of active and adoptive chronic-relapsing experimental autoimmune encephalomyelitis (EAE) using an encephalitogenic epitope of proteolipid protein. J Neuroimmunol 38: 229–240.

McRae BL, Vanderlugt CL, Dal Canto MC, Miller SD (1995): Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis. J Exp Med 182:75–85.

Merrill JE, Kono DH, Clayton J, Ando DG, Hinton DRR, Hofman, FM (1992): Inflammatory leukocytes and cytokines in the peptide-induced disease of experimental allergic encephalomyelitis in the SJL and B10.PL mice. Proc Natl Acad Sci 89:574–578.

Montgomery IN, Rauch HC (1982): Experimental autoimmune encephalomyelitis (EAE) in mice: primary control of EAE susceptibility is outside the H-2 complex. J Immunol 128:421–425.

Mossman TR, Coffman RL (1989): Th1 and Th2 cells: Different patterns of lymphokine secretion lead to different functional properties. Ann Rev Immunol 7:145–173.

Paterson PY, Swanborg RH (1988): Demyelinating diseases of the central and peripheral nervous systems. In Sampter M, Talmage DW, Frank MM, Austen KF, Claman HN (eds): "Immunological Diseases." Boston: Little, Brown and Co., pp 1877–1916.

Perry LL, Barzaga-Gilbert E, Trotter JL (1991): T cell sensitization to proteolipid protein in myelin basic protein-induced experimental allergic encephalomyelitis. J Neuroimmunol 33:7–16.

Pitchekian-Halabi H, Kim S, Mackenzie-Graham A, McFarland HF, Raine CS, Voskuhl, RR (1996): Gender differences in autoimmune demyelination in the mouse: implications for multiple sclerosis. FASEB J 10:A1352.

Racke MK, Bonomo A, Scott DE, Canella B, Levine A, Raine CS, Shevach EM, Rocken M (1994): Cytokine-induced immune deviation as a therapy for inflammatory autoimmune disease. J exp Med 180:1961–1966.

Rocken M, Saurat JH, Hauser C (1992): A common precursor for CD4+ T cells producing IL-2 and IL-4. J Immunol 148:1031–1036.

Smith ME, Eller NL (1996): Gender influences EAE both before and after sexual maturation. FASEB J 10:A1353.

Trooster WJ, Teelken AW, Kampinga J, Loof JG, Nieuwenhuis P, Minderhoud JM (1993): Suppression of acute experimental allergic encephalomyelitis by the synthetic sex hormone 17-alpha-ethinylestradiol: an immunological study in the Lewis rat. Int Arch Allergy Immunol 102:133–140.

Trotter JL, Clark HB, Collins KG, Wegeschiede CL, Scarpellini JD (1987): Myelin proteolipid protein induces demyelinating disease in mice. J Neurol Sci 79:173–188.

Whitham RH, Bourdette DN, Hashim GA, Herndon RM, Ilg RG, Vandenbark AA, Offner H (1991): Lymphocytes from SJL/J mice immunized with spinal cord respond selectively to a peptide of proteolipid protein and transfer relapsing demyelinating experimental autoimmune encephalomyelitis. J Immunol 146: 101–107.

Yu MY, Johnson JM, Tuohy VK (1996): A predictable sequential determinant spreading cascade invariably accompanies progression of experimental autoimmune encephalomyelitis: a basis for peptide-specific therapy after onset of clinical disease. J Exp Med 183:1777–1788.