High Frequency of Autoreactive Myelin Proteolipid Protein–specific T Cells in the Periphery of Naïve Mice: Mechanisms of Selection of the Self-reactive Repertoire

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Abstract

The autoreactive T cells that escape central tolerance and form the peripheral self-reactive repertoire determine both susceptibility to autoimmune disease and the epitope dominance of a specific autoantigen. SJL (H-2s) mice are highly susceptible to the induction of experimental autoimmune encephalomyelitis (EAE) with myelin proteolipid protein (PLP). The two major encephalitogenic epitopes of PLP (PLP 139–151 and PLP 178–191) bind to IAα with similar affinity; however, the immune response to the PLP 139–151 epitope is always dominant. The immunodominance of the PLP 139–151 epitope in SJL mice appears to be due to the presence of expanded numbers of T cells (frequency of 1/20,000 CD4+ cells) reactive to PLP 139–151 in the peripheral repertoire of naïve mice. Neither the PLP autoantigen nor infectious environmental agents appear to be responsible for this expanded repertoire, as endogenous PLP 139–151 reactivity is found in both PLP-deficient and germ-free mice. The high frequency of PLP 139–151-reactive T cells in SJL mice is partly due to lack of thymic deletion to PLP 139–151, as the DM20 isoform of PLP (which lacks residues 116–150) is more abundantly expressed in the thymus than full-length PLP. Reexpression of PLP 139–151 in the embryonic thymus results in a significant reduction of PLP 139–151-reactive precursors in naïve mice. Thus, escape from central tolerance, combined with peripheral expansion by cross-reactive antigen(s), appears to be responsible for the high frequency of PLP 139–151-reactive T cells.

Key words: autoimmunity • EAE • T cell receptor repertoire • thymic selection • major histocompatibility complex and disease

Introduction

Most autoreactive T cells are deleted in the thymus during T cell development, reducing both the frequency and affinity of the autoreactive T cells in the peripheral repertoire. However, not all autoreactive T cells are deleted, and those cells that do not undergo thymic (central) deletion are seeded to the peripheral immune compartment and form the self-reactive repertoire necessary for inducing autoimmune diseases. Several mechanisms have been proposed by which autoreactive T cells can escape thymic deletion. For myelin antigens, it was initially suggested that sequestration of myelin antigens behind the blood–brain barrier precludes central tolerance. The anatomy of the blood–brain barrier and the lack of lymphatic drainage from the central nervous system (CNS) have been cited to support this hypothesis (1, 2). However, recent data indicates that expression of myelin basic protein (MBP) and myelin proteolipid protein (PLP) is not limited to the CNS. Transcripts for MBP have been detected in both the human (3) and mouse (4). Moreover, there is now evidence for expression of MBP protein in the thymi (5) and peripheral lymphoid organs (6) of mice. PLP transcripts and protein also have been reported in human thymus (7), murine thymus (8), and myocardial cells (9). The discovery of myelin pro-

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1Abbreviations used in this paper: CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; LDA, limiting dilution analysis; LN, lymph node; MBP, myelin basic protein; MOG, oligodendrocyte glycoprotein; NASE, neuraminidase; PLP, myelin proteolipid protein.
tein expression outside the CNS has led to the reevaluation of immune tolerance to myelin antigens.

Studies with MBP-deficient (shiverer) mice on the Balb/c and C3H backgrounds have shown that these mice respond well to MBP, whereas wild-type Balb/c and C3H mice cannot mount proliferative responses to MBP and are resistant to MBP-induced experimental autoimmune encephalomyelitis (EAE; 10, 11). In addition, the MBP-reactive T cells from shiverer mice on the Balb/c background are highly encephalitogenic (11). Collectively, these data suggest that the expression of MBP results in tolerance to MBP in wild-type C3H and Balb/c mice. However, whether this tolerance is mediated in the thymus or periphery could not be established in these studies. A similar study of MBP-deficient mice on the B10.PL background has shown that the immunodominant epitopes of MBP in MBP-deficient mice are different from those in wild-type mice, indicating that there is tolerance to some MBP epitopes. This study concluded that the MBP-reactive T cells that form the dominant autoreactive repertoire in the periphery recognize epitopes that bind with low affinity and form unstable complexes with the self-MHC molecule, whereas T cells that bind to high-affinity epitopes are tolerized (12).

For PLP, a number of epitopes have been identified that bind to self-MHC molecules and induce EAE in different strains of mice. In the SJL (H-2d) strain, EAE can be induced by immunization with PLP 139–151 and PLP 178–191 (13–15). Both of these epitopes bind with high affinity to the IA^K MHC class II molecule (15), suggesting that low-affinity binding of these autoantigenic peptides to self-MHC molecules (or formation of unstable complexes) may not be responsible for the escape of PLP-reactive T cells, which form the autoreactive repertoire in the periphery. In addition, several lines of evidence demonstrate that of the two encephalitogenic epitopes of PLP, PLP 139–151 and 178–191, the immune response to PLP 139–151 is always dominant. First, SJL mice immunized with whole spinal cord homogenate, which contains multiple myelin antigens, respond selectively to PLP 139–151 (16). Second, if PLP 139–151-specific cells are tolerized in SJL mice, disease induction by whole spinal cord homogenate is abrogated (17). Lastly, in SJL mice that have recovered from a mild acute EAE after adoptive transfer of MBP-reactive T cell lines, the first relapse is concomitant with the development of delayed-type hypersensitivity responses to PLP 139–151 (18). The severity of this relapse is consistent with PLP 139–151 being the dominant encephalitogenic epitope in SJL mice. These results raise two important issues: (a) Why is there such a dominant autoimmune response to PLP 139–151 in SJL mice? and (b) How do PLP 139–151-reactive T cells escape thymic deletion even when the epitope binds to the IA^K class II molecule with high affinity?

To address these issues, we investigated the mechanism underlying the dominance of the PLP 139–151 epitope in SJL mice. The results presented in this study demonstrate that lymph node cells (LN Cs) from unimmunized SJL mice show a specific proliferative response to the PLP 139–151 but not to the PLP 178–191 epitope and that the frequency of PLP 139–151-reactive T cells in the peripheral repertoire is at least 1/20,000 CD4^+ T cells. This reactivity is present in all H-2d strains but differs in magnitude between EAE-susceptible (SJL) and EAE-resistant (B10.S) strains. Using PLP-deficient and germ-free mice, we demonstrate that selection or expansion of this repertoire is not dependent on PLP expression or cross-reactive infectious agents. Instead, it appears that failure of negative selection combined with peripheral activation/expansion by a cross-reactive antigen is responsible for the high frequency of PLP 139–151-reactive cells in naive SJL mice.

Materials and Methods

Animals. Female SJL/J, Balb/c, and C57BL/6 mice were purchased from The Jackson Laboratory. B10.S mice were obtained from the M. Claughlin Research Institute. Balb/c mice are from a Balb/c strain that has been bred. Balb/c mice were obtained from Dr. D. Murphy (New York State Health Labs, Albany, N.Y.). PLP-deficient mice on the 129 background were generated by Dr. K. Armin-Nave (University of Heidelberg, Heidelberg, Germany) (19). PLP-deficient mice were backcrossed onto both the SJL and Balb/c strains for at least five generations before being used in experiments. PLP-deficient, Balb/c, and B10.S mice were bred and maintained at the Eunice Kennedy Shriver Center. Defined flora SJL mice were purchased from Harlan Sprague Dawley Inc. Germ-free SJL mice were generated by Taconic Farms, Inc. from SJL/J stock obtained from The Jackson Laboratory.

Antigens. PLP 139–151 (HSLGKWLGHPDKF) and neurominidase (NASE) 101–120 (EALVRGGLKAVYVYPKNT) were synthesized by Dr. R. Laursen (Boston University, Boston, MA) on a Milligen model 9050 synthesizer using F-moc chemistry. PLP 138–191 (NTWTTCCQAIFPSK), MBP 84–104 (VHFFKNIIVTPRTTSSQGKVR), and myelin oligodendrocyte glycoprotein (MOG) 92–106 (DEGGYTCFFRDSYQ) were synthesized by Quality Controlled Biochemicals, Inc. Hemagglutin (HA) peptide 110–120 (SFERFEIPKPI) was synthesized by Research Genetics. All peptides were HPLC purified, and peptide identity was confirmed by mass spectroscopy.

In vitro Proliferation Assays. Lymph nodes were harvested from naive mice. LN Cs (4 × 10^6 per well) were cultured in serum-free media (HL-1) supplemented with l-glutamine (2 mM; BioWhitaker Inc.) in triplicate in 96-well round-bottomed plates in the presence of various concentrations of peptide for 48 h and pulsed with 1 μCi of [3H]thymidine per well for the last 16 h. [3H]Thymidine incorporation was determined in a Wallac scintillation counter (model 1250). For CD4 fractionation assays, CD3^+ T cells were purified from LN Cs using CD4 enrichment columns (R & D Systems, Inc.). CD3^+ T cells were then stained with anti-CD4 antibody (PharMingen) and separated into CD4^+ and CD4^- populations using MACS microbeads (Miltenyi Biotec). Fractionated T cells (2 × 10^5 per well) were incubated with irradiated syngeneic spleen cells (2 × 10^5 per well) in the presence of various concentrations of peptide for 48 h and pulsed with 1 μCi of [3H]thymidine per well for the last 16 h. [3H]Thymidine incorporation was determined as described above.

Limiting Dilution Analysis. Limiting dilution analysis (LDA) was performed on LN Cs from naive mice in the presence of 50 μg/ml of PLP 139–151 or NASE 101–120 and irradiated syngeneic spleen cells (5 × 10^5 cells per well) in 96-well round-bot-
toned plates. After 72 h, plates were pulsed with 1 μCi of [3H]thymidine per well and harvested 16 h later. [3H]Tymidine incorporation was determined as described above. Wells with cpm's that were three SD over the mean cpm of control wells (NASE 101–120) were counted as positive. Input cell number incorporation was determined as described above. Wells with [3H]Tymidine were then counted. Conditions were 94°C for 2 min (94°C for 1 min, 55°C for 1 min, 72°C for 1 min) for 40 cycles, with a final extension at 72°C for 5 min. β-Actin was amplified as above, except that 1.25 U of Taq polymerase, 200 nM 5’ primer (5’-TGG AAT CCT GTG GCA TCA C-3’), and 2.5 U of Taq polymerase (Promega Corp.) (20). Hot start PCR was performed: 95°C for 2 min (94°C for 1 min, 55°C for 1 min, 72°C for 1 min) for 40 cycles, with a final extension at 72°C for 5 min. β-Actin amplification was performed: 95°C for 2 min (94°C for 1 min, 55°C for 1 min, 72°C for 1 min) for 40 cycles, with a final extension at 72°C for 5 min. β-Actin amplification was performed: 95°C for 2 min (94°C for 1 min, 55°C for 1 min, 72°C for 1 min) for 40 cycles, with a final extension at 72°C for 5 min. PCR products were visualized on 1.5% agarose gels.

Figure 1. Naive SJL mice show a significant T cell response to PLP 139–151 but not to other myelin antigens. LN Cs were harvested from 9-wk-old naive SJL mice and tested in triplicate for reactivity to various myelin antigens over a dose-response of 0.1–100 μg/ml of peptide. [3H]Tymidine was added at 48 h, and plates were harvested 16 h later. The data is shown as mean ± SEM (CPM) of triplicate wells, where CPM = mean CPM in test wells – mean CPM in wells with media only. An experiment representative of at least four independent experiments is shown.

Results

To investigate the mechanism underlying the dominance of the PLP 139–151 epitope in SJL mice, we first tested the proliferative responses of LN Cs from naive SJL mice to a panel of myelin antigens known to induce EAE in the SJL strain: PLP 139–151, PLP 178–191, MOG 92–106, and MBP 84–104 (13, 15, 23, 24). LN Cs from naive SJL mice proliferated well to PLP 139–151 but not to any of the other myelin antigens tested (Fig. 1). This suggested that PLP 139–151-reactive T cells are present in expanded numbers in the peripheral repertoire of naive SJL mice such that they show a proliferative response in vitro without prior immunization.

To further analyze this phenomenon, we tested the proliferative responses of LN Cs from mice that are congenic at the MHC with SJL, Balb/s, and B10.S but that differ in their susceptibility to PLP 139–151-induced EAE. Whereas SJL and Balb/s mice both develop EAE, B10.S mice are relatively resistant to the development of disease (25). As shown in Fig. 2, LN Cs from naive SJL, Balb/s, and B10.S mice responded to PLP 139–151 but not to a control antigen, NASE. The T cell response to PLP 139–151 in Balb/s mice is comparable to that of SJL. Interestingly, however, the response in B10.S mice, which are resistant to PLP 139–151-induced EAE (25), is reduced when compared with SJL and Balb/s mice. We have compared in parallel the T cell response of LN Cs of a number (six to seven) of individual unimmunized SJL and B10.S mice to PLP 139–151. The data demonstrate that endogenous reactivity to PLP 139–151 is significantly reduced (P < 0.004) in the resistant B10.S mice (data not shown). LN Cs taken from naive non-H-2b (Balb/c and C57BL/6) mice did not exhibit any proliferative response to the PLP 139–151 peptide, indicating that the proliferation observed in the LN Cs of SJL, Balb/s, and B10.S mice is linked to H-2 and is not due to a nonspecific mitogenic effect of the PLP 139–151 peptide used in the assays (Fig. 2). Furthermore, this endogenous reactivity to PLP 139–151 has been observed with several batches of PLP 139–151 peptide made by different vendors. Taken together, these data demonstrate that all H-2b strains tested have a relatively high frequency (and/or high affinity) of PLP 139–151-reactive T cells in the periphery. There may also be differences in the size and/or affinity of this repertoire in susceptible versus resistant strains, supporting a functional role for this repertoire in autoimmune disease. These data suggest that the expanded endogenous
PLP 139–151-reactive repertoire in unimmunized SJL mice may be responsible for the immunodominance of the PLP 139–151 epitope in this strain.

Frequency of PLP 139–151-reactive T Cells Increases with Age. To determine the frequency of PLP 139–151-reactive T cells present in naive SJL mice, we performed LDA. In adult mice (6 wk of age), the frequency of PLP 139–151-reactive T cells is \( \frac{1}{43,700} \) CD4+ T cells (Table I). This is significantly higher than the previously reported frequency of \( \frac{1}{20,000} \) for T cells specific for MBP or for a foreign antigen in the peripheral repertoire of naive animals (26). When we analyzed the frequency of PLP 139–151-reactive T cells at 36 wk of age, the frequency was \( \frac{1}{19,100} \) CD4+ T cells, suggesting an increase over time. This could be due to constant seeding of naive PLP 139–151-reactive T cells to the periphery or to expansion of these cells once they have reached the peripheral immune compartment.

PLP 139–151 Reactivity in Memory versus Naive T Cells. To determine whether the endogenous PLP 139–151-reactive T cells observed in naive SJL mice are activated in the periphery, we tested whether PLP 139–151 reactivity resides in the naive or memory subset of T cells. To do this, we purified T cells from the lymph nodes of SJL mice and separated them into CD44hi (memory) and CD44lo (naive) populations. As shown in Fig. 3, PLP 139–151 reactivity is enriched in the CD44hi subset, and there was a commensurate decrease in PLP 139–151 reactivity in the CD44lo population. This indicates that PLP 139–151-reactive T cells are being stimulated in vivo. As CD44hi cells have a lower activation threshold and thus would proliferate more readily, this raised the possibility that there may not be real differences in the number of PLP 139–151-reactive cells that reside in the CD44hi versus CD44lo populations.

Table I. Frequency of PLP 139–151-reactive T Cells in SJL Mice

| Age (wk) | Mean frequency | Range |
|---------|----------------|-------|
| 6       | 1/43,700       | 1/20,000–1/73,500 |
| 36      | 1/19,100       | 1/12,000–1/25,600 |

LDA was performed on LNCs from naive SJL mice with 5 × 10^5 irradiated syngenic spleen cells and 50 μg/ml PLP 139–151 or NASE 101–120 in 96-well plates. Input cell number was corrected for percent of CD4+ T cells in the initial LNC population. The results show the mean of three 36-wk-old and four 6-wk-old mice. The range represents the lowest and highest frequencies observed in any of the mice.
address this issue, we undertook precursor frequency analysis to determine whether endogenous PLP 139–151-reactive cells would preferentially reside in the CD44hi population. Limiting dilution experiments comparing CD44hi and CD44lo cells confirmed that the frequency of responding cells was approximately two times higher in the CD44hi compartment than in the CD44lo compartment (data not shown). In spite of their high levels of CD44 expression, these cells do not appear to be differentiated to any effector phenotype, because when they are activated with PLP 139–151, they produce low levels of IL-2 but not IFN-γ, IL-4, IL-10, or TNF (data not shown).

Neither PLP A autoantigen nor Infectious Environment Is Required for Generation of the Endogenous PLP 139–151 Repertoire. As the data suggested that PLP 139–151-reactive T cells are present in high frequency and expand with age, this raised the question of what is responsible for inducing and expanding the PLP 139–151-reactive T cell repertoire in naive SJL mice. Answering this issue is critical, because autoreactive T cells to MBP, PLP, MOG, and other self-antigens are also seen in expanded numbers in the peripheral repertoire of normal humans. PLP itself, cross-reactive self-antigen, or the infectious environment could be responsible for the induction and/or expansion of the endogenous PLP 139–151-reactive repertoire. To address these possibilities, we analyzed PLP-deficient and germ-free SJL mice.

To address whether PLP itself is selecting or expanding the PLP 139–151-reactive T cells, we analyzed the response of LNCs from naive PLP-intact and PLP-deficient mice to PLP 139–151 peptide. PLP-deficient mice have been generated by introduction of the neomycin resistance gene in exon 1 of the genomic sequence of PLP, resulting in loss of expression for PLP and the DM20 isoform of PLP. The responses of LNCs from germ-free and defined flora mice to Con A, which activates all T cells in an unbiased manner, were not significantly different from the Con A response in wild-type SJL (data not shown). The heightened response in the defined flora and germ-free SJL mice may be due to lack of an effect (peripheral tolerance: deletion or anergy) of cross-reactive microbial antigens on PLP 139–151-reactive cells. Taken together, these results suggest that a cross-reactive antigen (or antigens) other than PLP is responsible for the positive selection of PLP 139–151-reactive cells and the low-level activation of PLP 139–151-reactive T cells in the periphery.

Thymic Selection of PLP 139–151-reactive T Cells. The fact that we detect a significant endogenous response to PLP 139–151 and not to PLP 178–191, both of which bind to IAa with similar affinity, led us to postulate that there might be differential negative selection of the repertoires for these two PLP epitopes in the thymi of SJL mice. The PLP gene has two isoforms, PLP and DM 20. The DM 20 form is generated by alternate splicing of full length PLP message and lacks residues 116–150, thereby eliminating the PLP 139–151 epitope, whereas the PLP 178–191 epitope remains intact (Fig. 6 A and reference 28). Previous studies have demonstrated mRNA and protein expression of PLP and PLP-free mice were rederived by cesarean section and maintained under germ-free conditions. As shown in Fig. 5, the endogenous PLP 139–151-reactive repertoire is detectable in all three groups of mice. In fact, LNCs from both the germ-free and defined flora mice have a significantly higher response to PLP 139–151 when compared with cells from wild-type SJL mice, P < 0.014 and P < 0.03, respectively. The responses of LNCs from germ-free and defined flora mice to Con A, which activates all T cells in an unbiased manner, were not significantly different from the Con A response in wild-type SJL (data not shown). The heightened response in the defined flora and germ-free SJL mice may be due to lack of an effect (peripheral tolerance: deletion or anergy) of cross-reactive microbial antigens on PLP 139–151-reactive cells. Taken together, these results suggest that a cross-reactive antigen (or antigens) other than PLP is responsible for the positive selection of PLP 139–151-reactive cells and the low-level activation of PLP 139–151-reactive T cells in the periphery.

Endogenous PLP 139–151-reactive repertoire is present in PLP-deficient mice. LNCs were harvested from PLP+/+ and PLP−/− mice on the BALB/c background and tested in triplicate for reactivity to PLP 139–151 and to a control antigen, NASE, over a dose-response of 0.1–100 μg/ml of peptide. [3H]Thymidine was added at 48 h, and plates were harvested 16 h later. The data is shown as mean ΔCPM of triplicate wells, where ΔCPM = mean CPM in test wells – mean CPM in wells with media only.
DM 20 in the thymi of humans (7) and PLP in mice (8), suggesting that PLP may play a role in thymic deletion and central tolerance. In these studies, the expression of PLP and DM 20 mRNA and protein in the thymi was shown at a single time point and therefore did not address the question of whether both isoforms are always expressed and whether they are expressed at the same ratios during embryonic thymic development, particularly when TCR rearrangements are occurring in the thymus. To address this, we examined the expression of transcripts for full length PLP and DM 20 in the thymi of SJL mice by RT-PCR at different ages (starting day 16 fetal to 36 wk of age) and compared this with the expression of these transcripts in the brains of the same mice. We used primers that can amplify both PLP and DM 20 cDNA (20). As previously reported, we amplified both PLP and DM 20 from brain cDNA, and the expression of PLP but not DM 20 increased in the brain with age (Fig. 6 B). In contrast, DM 20 was preferentially amplified from the thymic cDNA, and its expression was seen as early as day 16 of fetal life. The expression of the upper PLP band was difficult to detect and was only seen between 20 and 36 wk of age. The preferential and early expression of DM 20 in the thymi of SJL mice may explain the inefficient negative selection of PLP 139-151 but not 178-191-reactive T cells in the thymi of SJL mice. If low or absent expression of PLP 139-151 in the thymus is responsible for the lack of negative selection to PLP 139-151, reexpression of PLP 139-151 in the embryonic thymus should result in negative selection and loss of the endogenous repertoire to PLP 139-151.

Introduction of PLP 139-151 into the thymi of SJL mice leads to deletion of the endogenous PLP 139-151-reactive repertoire. Because the lack of mRNA expression for full length PLP in the thymus during early age suggested that the protein may not be available to mediate negative selection of PLP 139-151 precursors, we introduced the PLP 139-151 epitope into the embryonic thymi of SJL mice. To do this, we used an Ig chimera in which the CDR3 region of the antiarsonate antibody, 91A3, is replaced with the PLP 139-151 epitope, IgPLP 139-151 (21). When administered to pregnant SJL mice on days 16, 17, and 18 of gestation, the chimeric Ig crosses the placental barrier and is expressed in the thymi of newborn mice, where it can interact with PLP 139-151 but not PLP 178-191 precursors (22). We analyzed the thymocytes from neonates born of IgPLP 139-151-treated, control Ig (IgCTRL)-treated, or untreated females for reactivity to PLP 139-151 to determine whether IgPLP 139-151 treatment would result in the loss of endogenous PLP 139-151-reactive cells in the thymus itself. As shown in Fig. 7 A, the thymocytes of neonate SJL mice born of untreated females exhibit significant reactivity to PLP 139-151 and not to a control peptide. This confirms that PLP 139-151-reactive cells are present at detectable frequencies in the thymocyte population of SJL mice and excludes the possibility that these cells only become detectable after expansion in the periphery. When pregnant female mice are treated with IgPLP 139-151, PLP 139-151 reactivity is dramatically reduced, as demonstrated by a significant decrease in the proliferative response to PLP 139-151 in the thymocytes of pups born of IgPLP 139-151-treated mice when compared with pups born of untreated mice. IgCTRL treatment had no significant effect on PLP 139-151 reactivity in the thymus. This directly shows that presentation of the PLP 139-151 epitope in the thymus results in the tolerance of endogenous PLP 139-151-reactive cells by PLP 139-151. Furthermore, when we analyzed endogenous PLP 139-151 reactivity in adult mice born of IgPLP 139-151- or IgCTRL-treated females, we found, as shown in Fig. 7 B, that mice that were exposed to IgPLP 139-151 in utero have an ~75% reduction in endogenous PLP 139-151 reactivity when compared with mice exposed in utero to control Ig. These mice also do not develop significant EAE when immunized with PLP 139-151 peptide at 8-10 wk of age (22). We conclude that the PLP 139-151 epitope is not expressed in the thymi of SJL mice in a manner that is conducive to negative selection of PLP 139-151-reactive T cells. Therefore, exogenous expression of PLP 139-151 in utero results in a dramatic reduction in the precursors of PLP 139-151-reactive cells and a decrease in the endogenous PLP 139-151-reactive repertoire in adult mice. The lack of negative selection in the thymus in concert with expansion in the periphery by a cross-reactive self-antigen other than PLP results in a high frequency of PLP 139-151-reactive T cells in this autoimmune-prone strain.

Discussion

We have shown that a high frequency of PLP 139-151-reactive T cells in naive mice underlies the susceptibility of
the SJL strain to CNS autoimmunity and explains the dominance of this epitope in this strain. The high frequency of PLP 139–151-reactive cells is the result of at least two mechanisms: lack of negative selection in the thymus and expansion in the periphery by a cross-reactive antigen. The presence of PLP 139–151-reactive cells in the naive T cell repertoire appears to be linked to H-2, as it is shared among strains of the same MHC haplotype (H-2b) and not others. Moreover, the size of this repertoire may correlate with disease susceptibility (SJL and B10.S strains) and resistance (B10.PL strain), raising the possibility that background genes may influence this endogenous repertoire.

Role of Self-Antigen in Thymic Selection and Peripheral Expansion of the Autoreactive Repertoire. The study of MBP-deficient mice on the B10.PL background has demonstrated one mechanism for the escape from tolerance of autoreactive cells (10–12). Expression of MBP was shown to result in tolerance of T cells specific for MBP epitopes that bind with high affinity to class II molecules and form stable peptide–MHC complexes. Therefore, only those T cells reactive to epitopes that bind weakly to the MHC, i.e., MBP 1–11/I-A^d (IC_{50} of 7.4 μM; reference 29) are found in the periphery and form the dominant repertoire in the normal adult mouse (12). In contrast, both PLP 139–151 and PLP 178–191 epitopes bind with high affinity to the I-A^d class II molecule, with an IC_{50} of 40 and 740 nM, respectively (15), and therefore it is unlikely that this mechanism for escape from immune tolerance is operative in the PLP/H-2^d system.

Furthermore, our observations that SJL mice have a high frequency of PLP 139–151- but not PLP 178–191-reactive T cells in the naive repertoire suggests that there is differential tolerance and/or expansion of PLP 139–151-reactive T cells in H-2^b mice. This prompted us to further examine the mechanism that may be responsible for escape from immune tolerance and expansion of PLP 139–151-specific cells. Our data suggests that PLP 139–151-reactive T cells escape thymic deletion due to differences in the relative abundance and expression of D M 20 (which lacks residues 116–150) versus full length PLP in the thymus. If the amounts of PLP 139–151 are insufficient to delete PLP 139–151-reactive thymocytes, introduction of the PLP 139–151 epitope into the thymus during embryonic development should result in the deletion of PLP 139–151 precursors and loss of the endogenous PLP 139–151 repertoire. We demonstrated this by introducing the PLP 139–151 epitope as part of an Ig chimera. This resulted in significant loss of PLP 139–151-reactive thymocytes in neonate SJL mice. These animals were also resistant to the development of EAE with the PLP 139–151 peptide when tested as adults (22), suggesting that the endogenous PLP 139–151-reactive cells are the precursors of pathogenic cells. Thus, lack of thymic deletion may be one critical factor leading to the high precursor frequency of PLP 139–151-reactive cells in the periphery.

Although lack of negative selection to PLP 139–151 in the thymus is responsible for increased seeding of precur-
sors to the periphery, it is unlikely that this is solely responsible for the high frequency of PLP 139–151-reactive cells observed in the adult SJL mouse. The fact that reactivity for these cells is enriched in the CD44 hi T cell population and that the repertoire size for PLP 139–151 increases with age argues strongly for in vivo expansion of these T cells in the peripheral immune compartment. The recent description of CD44 upregulation on CD4 + cells transferred into lymphopenic mice (30, 31) suggests that CD44 may be a marker for cells that have undergone one or two divisions in the periphery but have not developed an effector phenotype. This is consistent with our finding that the relative frequency of reactive cells is higher in the CD44 hi compartment but that we do not detect effector cytokines from bulk cultures. It implies that even in mice with a full complement of lymphocytes, naive cells of some specificities can divide in the periphery. As neither PLP-deficient nor germ-free mice lose the endogenous PLP 139–151-reactive repertoire, we conclude that neither the PLP autoantigen nor the infectious environment are necessary for the positive selection or the peripheral activation/expansion of this repertoire. PLP 139–151-reactive T cells are most likely positively selected by cross-reactive self-antigen(s). Whether this same antigen or antigens present in diet are responsible for the expansion of these cells in the periphery remains to be seen.

A ssociation of MHC Class II with Autoimmunity. One important implication of this study is that it provides another explanation for the common finding of MHC class II associations with specific autoimmune diseases. One hypothesis for the association of autoimmune disease with particular MHC haplotypes is simply that only certain MHC molecules can bind and present self-peptides. A second hypothesis is that some MHC molecules (e.g., IAg7) are globally poor at mediating negative selection, leading to a peripheral T cell repertoire biased toward self-reactivity (32, 33).

W e have now shown that cells with pathogenic potential may arise in large numbers and in a peptide-specific/class II–associated fashion. Furthermore, our demonstration that such cells arise in PLP-deficient mice suggests that the autoantigen is not required for the positive selection of these cells. Rather, the IA a molecule presenting other cross-reactive antigens selects a very high frequency of PLP 139–151-reactive T cells that further expand in the periphery.

A s we observe the expanded repertoire to PLP 139–151 but not to other myelin antigens, this indicates that this is antigen specific and not a global effect of IA a due to loose peptide binding, as has been proposed for IA 97 (32, 33). Thus, our results imply that H-2 a-bearing mice, because of the inherent ability of IA a to expand PLP 139–151-reactive cells, will be more susceptible to CNS autoimmune disease and that PLP 139–151 will be the immunodominant epitope for disease induction. These data provide a cellular basis for the previous observations made in SJL mice that the PLP 139–151 epitope is the most dominant encephalitogenic epitope for EAE induction and for the induction of tolerance (16, 17).

E ffects of Environmental Microflora on the Autoreactive Repertoire. We had expected that the infectious environment might be responsible for the expanded PLP 139–151-reactive repertoire in unimmunized mice. Our finding that germ-free mice have a higher (and not lower) frequency of PLP 139–151-reactive cells than wild-type controls is consistent with the observation that NOD mice develop diabetes with higher frequency when they are maintained in clean animal facilities (34). The higher frequency of PLP 139–151-reactive T cells in germ-free SJL mice could be due to the elimination of competing T cell specificities, which allows for further expansion of PLP 139–151-reactive cells in the periphery. Alternatively, cross-reactive microbial antigens may be inducing peripheral tolerance of some of the PLP 139–151-specific T cells. Although to date no germ-free SJL mice have developed spontaneous disease, with a larger pool of circulating autoantigen-specific cells they may have a lower threshold for disease induction. Our observation is different from that of Goverman et al., who showed that mice with a TCR transgenic specific for MBP Ac1–11 develop more disease in dirty facilities (35). This difference may be due to the differences in affinity of MBP and PLP epitopes for their respective restriction elements, which consequently affects the selection of their respective T cell repertoires. Alternatively, the differences in the two systems may reflect a differential balance between factors that predispose toward disease and factors that initiate disease. Nonetheless, the data presented here show a significant difference in the selection and bias for the epitope dominance of MBP and PLP.

R egulation of Autoreactive Cells in the Periphery. As SJL mice have a very high frequency (at least 1/20,000 CD4 + T cells) of PLP 139–151-reactive T cells in the naive repertoire, the LNCs from unimmunized mice show a specific and significant proliferative response to PLP 139–151. This measurement of frequency by LDA is likely to be an underestimate; however, at the time of this writing, more sensitive techniques to measure frequency of PLP 139–151-specific cells such as PLP 139–151/IA a tetramers are unavailable. Two previous studies using in vitro proliferative assays have also suggested that there may be a higher precursor frequency of PLP 139–151-reactive T cells in the peripheral repertoire of SJL mice (36, 37), thus supporting the data presented here. Interestingly, by determining the precursor frequency of PLP 139–151-reactive T cells in the immune repertoire of SJL mice, the studies of Miller et al. (37) also reported a precursor frequency of 1/20,000, an estimate similar to that which we have made for the endogenous PLP 139–151-reactive repertoire in naive SJL mice. These results are reminiscent of data obtained with normal human volunteers, where it has been shown that PBMCs from healthy individuals can respond to several myelin antigens, including PLP and MBP (38). What induces this autoreactive antemyelin repertoire and what its function may be in the induction or regulation of autoimmunity has been debated. Our observation that unimmunized SJL mice show a specific proliferative response to PLP 139–151 provided us with a unique opportunity to address many of these issues. As shown by our results, neither the autoantigen nor the infectious environment is necessary for the ex-
panded PLP 139–151-reactive repertoire to arise in unimmunized SJL mice. So why don’t all normal individuals bearing an expanded autoreactive repertoire and normal SJL mice develop spontaneous disease? We believe that either these cells are not activated strongly enough to differentiate into a pathogenic phenotype, or these cells are kept under check by other endogenous regulatory mechanisms that inhibit/control the development of spontaneous autoimmunity.

In summary, we have found that the predisposition of H-2s strains toward CNS autoimmunity is partly due to the very high frequency of autoreactive T cells specific for a known encephalitogenic epitope of PLP present in naïve mice. This high frequency is the result of at least two mechanisms: lack of negative selection in the thymus and further expansion by cross-reactive antigen(s) in the periphery. In contrast to MBP or other self-antigens, the mechanism by which PLP-reactive T cells escape thymic tolerance and expand in the periphery is quite different. Thus, the mechanisms that underlie epitope dominance and susceptibility to autoimmune disease may vary depending on the antigen involved and the genetic background of the individual. Moreover, we have described for the first time a significant T cell reactivity to a CNS autoantigen in unprimed mice of a defined MHC haplotype. Yet these mice do not develop spontaneous autoimmune disease. We can now use this model to explore the peripheral mechanisms that prevent these T cells from becoming pathogenic in vivo. In doing so, we now have the opportunity to better understand what mechanisms regulate the autoreactive T cells present in normal, healthy individuals with defined disease-associated MHC haplotypes.

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