Th17 and Th1 Responses Directed Against the Immunizing Epitope, as Opposed to Secondary Epitopes, Dominate the Autoimmune Repertoire During Relapses of Experimental Autoimmune Encephalomyelitis

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Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease with similarities to multiple sclerosis (MS). It has been suggested that relapses of EAE and MS may be associated with, and even driven by, T cells specific for novel epitopes that are primed during the course of tissue destruction in the target organ or in secondary lymphoid tissues. We show, however, that IFN-γ and IL-17 responses against the immunizing epitope remain dominant throughout the course of multiphasic EAE. Furthermore, induction of tolerance against a putative secondary epitope did not prevent clinical relapses.

Key words: T lymphocyte; animal model; multiple sclerosis; autoimmunity; epitope spreading

Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS). It is widely believed to be mediated by CD4⁺ T cells reactive against myelin antigens. This theory is supported by striking histopathologic and clinical similarities between MS and the animal model, experimental autoimmune encephalomyelitis (EAE) (Martin and McFarland, 1995). SJL mice immunized with a peptide fragment of proteolipid protein that spans amino acids 139–151 (PLP₁₃₉–₁₅₁) follow a relapsing remitting course of EAE, reminiscent of the most common presentation of MS (McRae et al., 1992).

Although it is clear that the first episode of neurologic dysfunction in this model is driven by PLP₁₃₉–₁₅₁-specific T cells, a number of studies suggest that the myelin-specific T cell repertoire expands and diversifies thereafter in a process termed epitope spreading (McRae et al., 1995). It has been argued that T cells reactive against secondary and tertiary epitopes govern successive relapses (Vanderlugt et al., 2000). Similarly, an “epitope spreading cascade” has been observed in some individuals with MS (Tuohy et al., 1997). The phenomenon of epitope spreading holds important theoretic implications for the monitoring and treatment of MS. Drugs that target specific myelin determinants or T cell receptors might be rendered ineffective as the effector T cell pool diversifies (Fontoura et al., 2005). A number of researchers have questioned the prevalence as well as the physiologic significance of epitope spreading during the development of chronic or multiphasic autoimmune disease (Takacs et al., 1997; Lindsey, 1998; Takacs and Altmann, 1998). In fact, multiphasic EAE can be mediated by an immune response restricted to a single myelin epitope with no opportunity for the auto reactive T cell repertoire to evolve, as in SCID mice that express a transgenic T cell receptor specific for a peptide of myelin basic protein (Jones et al., 2003).

Another important issue involves the biologic properties assumed by autoimmune T cells that are generated in the context of spreading. Previous studies exclusively used tritiated thymidine incorporation, delayed type hypersensitivity (DTH) reactions, and IFN-γ production as measures of T cell reactivity against secondary and tertiary myelin epitopes. However, the recent recog-
nition that Th17, rather than Th1 cells are the key encephalitogenic effectors in EAE, suggests that the cells detected by the above assays might represent innocuous bystanders that are not engaged directly in the pathogenic process (Billiau et al., 1998; Segal et al., 1998; Langrish et al., 2005; Park et al., 2005; Chen et al., 2006). Such considerations raise the prospect that, even when epitope spreading occurs during autoimmune disease, it may simply represent an epiphenomenon. Indeed, spreading between myelin epitopes has been detected in MS and EAE during periods of clinical stability (Kumar, 1998; Ristori et al., 2000; Vergelli et al., 2001). It is even possible that spreading could give rise to the generation of regulatory cells that actually limit inflammation and trigger remissions. In support of this notion, IL-10-producing cells reactive against secondary epitopes prevent EAE relapses (Yin et al., 2001; Wildbaum et al., 2002).

In light of the unresolved issues discussed above, we re-examined the phenomenon of epitope spreading by carrying out highly sensitive ELISPOT assays against a broad panel of cytokines, including IL-17 and IL-10, with T cells harvested from the CNS, spleen, and draining lymph nodes of SJL mice at multiple time points after immunization with PLP peptides. Based on our findings, we conclude that in our model, memory Th17/Th1 CD4+ T cells responsive to the primary epitope targeted during the initiation of EAE comprise the immunodominant autoreactive effector cell population throughout the disease course.

**MATERIALS AND METHODS**

**Mice**

SJL/J mice were purchased from Jackson Laboratory (Bar Harbor, ME) and housed in microisolator cages. All animal protocols were approved by the University of Rochester Committee on Animal Resources.

**Peptides**

Peptides with the following sequences were obtained from Bio-Synthesis (Lewisville, TX): PLP\textsubscript{139–151} HSLGK-WLGHPDKF; PLP\textsubscript{178–191} NTWTTCCQIAFPSK; MBP\textsubscript{84–104} VHFFKNIIVTPRTPPSQKGR; and influenza nucleoprotein (NP)\textsubscript{260–283} RSALILRGSVAHKSCLPACVYG. Purity of >95% was confirmed by mass spectrometry. Peptides were suspended in PBS and sterile filtered (0.22 μm) before use.

**Monoclonal Antibodies**

The following hybridomas were used as capture antibodies in ELISPOT assays: JES6-1A12 (IL-2; eBioscience, San Diego, CA); A11B11 (IL-4; ATCC), TRFK5 (IL-5; eBioscience), JES5-2A5 (IL-10; eBioscience), TC11-18H10.1 (IL-17; Pharmingen), and AN18 for IFN-γ (ATCC). The following hybridomas were used as detection antibodies in ELISPOT assays: JES6-5H4 (IL-2; eBioscience), BVD6-24G2 (IL-4; eBioscience), TRFK4 (IL-5; eBioscience), SXC-1 (IL-10; Pharmingen), TC11-8H4.1 (IL-17; Pharmingen), and XMG1.2 (IFN-γ; eBioscience). All detection antibodies were biotinylated by the manufacturer.

**Induction and Rating of EAE**

SJL/J mice (6–8 weeks old) were immunized subcutaneously (s.c.) with 100 μg of PLP\textsubscript{139–151} or PLP\textsubscript{178–191} emulsified in CFA containing 250 μg Mycobacteria tuberculosis H37Ra (Difco, Detroit, MI). Mice were monitored daily and disability scored according to the following scale: 0, no clinical signs; 1, flaccid tail; 2, poor righting ability; 3, obvious hind limb weakness; 4, hind limb paralysis; 5, moribund state. To account for the severity and duration of neurologic deficits, a “cumulative disease score” was calculated for each mouse by summing daily disease scores over the entire observation period.

**Induction of Peptide-Specific Tolerance**

SJL mice were injected with 300 μg of PLP\textsubscript{178–191} peptide emulsified in IFA by the intraperitoneal (i.p.) route. Control mice were sham-tolerized with an equal volume of PBS in IFA. Two weeks later, mice were challenged with PLP\textsubscript{139–151} or PLP\textsubscript{178–191} emulsified in CFA for induction of EAE as described above.

**Cell Isolation and Culture**

Spleen and draining lymph nodes (axillary, inguinal, and brachial) were passed through a 70-μm cell strainer (BD Falcon, Bedford, MA) to create single cell suspensions. Red blood cells were removed with ACK lysing buffer (Quality Biologic, Gaithersburg, MD). The remaining cells were then washed twice and resuspended in HL-1 media (Cambrex Bio Science, Walkersville, MD) supplemented with 1% l-glutamine.

Spinal cord fragments were digested with DNase and collagenase for 1 hr at 37°C. Mononuclear cells were isolated via Percoll gradient centrifugation. On average, we isolated between 3 × 10^5–1 × 10^6 mononuclear cells per spinal cord from PLP\textsubscript{139–151} primed mice during clinical episodes. In some cases, CD4 T cells were purified from whole spinal cord mononuclear cells using APC-conjugated anti-CD4 antibody (eBioscience) and MACS anti-APC beads (Miltenyi, Auburn, CA). Depleted splenocytes were prepared by complement-mediated lysis of T cells with anti-Thyl.1 monoclonal antibody (clone J11).

**ELISPOT Assays**

Filtration plates (96-well) (MAIP N4550; Millipore, Bedford, MA) were coated with capture antibody (3 μg/ml; 100 μl/well) and incubated overnight at 4°C. Plates were washed three times with sterile PBS and blocked for 1 hr at room temperature with 1% BSA in PBS (Atlanta Biologics, Lawrenceville, GA). After three additional washes with PBS, lymph node cells were plated in triplicate (5 × 10^5 cells/ml in HL-1 media; 200 μl/well) with or without peptide (50 μg/ml). After a 24–48 hr incubation at 37°C/5% CO\textsubscript{2}, plates were washed before addition of detection antibodies (2 μg/ml in PBS-T containing 1% BSA;100 μl/well) and incubation at 4°C for 12 hr. Plates were
washed with PBS-T ×3, loaded with streptavidin-alkaline phosphatase (1:1,000 dilution in PBS-TB) and incubated for another 2 hr at room temperature. Plates were then washed 3 times with PBS and developed with alkaline phosphatase substrate Vector Blue (Vector Labs, Burlingame, CA). Spots were counted using an automated ELISPOT counter (CTL ImmunoSpot Analyzer with ImmunoSpot software, version 2.08; Cellular Technology, Cleveland, OH). Counts are shown as the mean ± SD for each set of triplicate wells.

**Lymphoproliferation Assays**

Splenocytes or draining lymph node cells were cultured in triplicate at 37°C with 5% CO₂ in 96-well plates (5 × 10⁵ cells in 200 µl/well) with or without peptides at the concentrations designated in the figure legends. Each well was pulsed with ³H thymidine (1 µCi/well) at 72 hr and harvested 16 hr later (TomTec). Incorporated radioactivity was read with a β-scintillation counter (Wallac MicroBeta Trilux).

**Statistical Analysis**

Differences in cytokine response, proliferation, and clinical score were assessed using the Student’s t-test. Values of P < 0.05 were considered significant.

**RESULTS**

**Th1 and Th17 Responses Are Directed Against the Immunizing Epitope PLP₁₃₉–₁₅₁, But Not Putative Secondary and Tertiary Epitopes, Throughout the Course of Relapsing EAE**

Previous analyses of T cell responses against the immunizing or spreading myelin epitopes during the course of relapsing EAE either failed to distinguish between different functional subsets (by simply measuring lymphoproliferation) or focused on Th1 responses. Consequently, pathogenic Th17 responses or regulatory Tr1 or Th2 responses might have been overlooked. Therefore, we carried out ELISPOT assays to measure the frequency of myelin-specific T cells producing IL-17, IL-10, IL-4, IL-5, and IFNγ in the CNS, draining lymph nodes, and spleens of SJL mice after immunization with PLP₁₃₉–₁₅₁ in CFA. In initial experiments mice were sacrificed on Day 35 post-immunization based on previous reports that epitope spreading is readily apparent by that time point (Vanderlugt et al., 2000). Lymph nodes and splenocytes were pooled from mice with the same clinical scores (n = 5–12).

As shown in Figure 1, we detected high frequencies of IL-17, IFNγ, and IL-2 producing cells in the spleen and draining lymph nodes on in vitro challenge with PLP₁₃₉–₁₅₁. We also detected significant Th1 and Th17 PLP₁₃₉–₁₅₁-specific responses in cervical lymph nodes, although at an approximately 10-fold lower magnitude (data not shown). PLP₁₃₉–₁₅₁ specific IL-4 responses were elicited routinely from splenocytes, but at approximately one-third to one-fifth the frequency of Th1 and Th17 cells. By contrast, we did not detect production of any of the cytokines in our panel in response to stimulation with PLP₁₇₈–₁₉₁ or MBP₈₄–₁₀₄. A similar pattern of antigenic responsiveness and cytokine production was observed when splenocytes and lymph node cells were analyzed from individual mice (Fig. 1C; unpublished observations).

Next we measured cytokine responses timed specifically to the first clinical relapse of EAE. Once again robust PLP₁₃₉–₁₅₁ specific IL-17 and IFNγ responses were
readily evident in spleen and lymph nodes as was a lower frequency IL-4 response in the spleen (Fig. 2B,C and data not shown). We did not, however, find any response to PLP 178–191 or MBP 84–104 irrespective of the cytokine measured. Published studies that have shown epitope spreading at relapse used tritiated thymidine incorporation as the read-out measure of antigen reactivity. Therefore, we measured lymphoproliferative responses to PLP139–151, PLP 178–191, and MBP 84–104 in parallel to the cytokine assays. Consistent with the ELISPOT data, only PLP 139–151 elicited significant thymidine incorporation by lymph node cells above background levels (Fig. 2D). 

We considered the possibility that, epitope spreading might emerge relatively late in the disease process. It was also possible that the anti-myelin cytokine response changes over time, with new Th lineage commitments arising in the aftermath of the first relapse and remission. Therefore, we repeated the above studies with lymphoid tissues harvested at the time of the second relapse as well as during remission. Once again, only responses against PLP139–151 were measurable (Fig. 3; data not shown). A similar pattern of cytokine production was observed at all time points, characterized by robust IL-17, IFNγ, and IL-2 responses in draining and cervical lymph nodes and spleen, a lower frequency IL-4 response in the spleen, and no antigen-specific IL-5 or IL-10 in any of the lymphoid tissues examined.

**IFNγ and IL-17 Responses Targeting the Primary Epitope Remain Dominant During Relapses of PLP178–191-Initiated EAE**

Because SJL mice preferentially express the DM2O isoform of PLP (that lacks residues 116–150) in the thymus (Klein et al., 2000), T cells specific for PLP139–151 evade negative selection and occur at an unusually high frequency in the naive peripheral repertoire (Anderson et al., 2000; Reddy et al., 2004). It was possible that the absence of epitope spreading documented in Figures 1–3 was particular to the model we used, consequent to an unparalleled immunodominance of PLP139–151 in the SJL strain (Whitman et al., 1991). We speculated that spreading might be conspicuous in SJL mice if PLP139–151...
acted as the secondary as opposed to the primary epitope. To test this hypothesis, we immunized SJL mice with an alternative encephalitogenic peptide, PLP<sub>178–191</sub>, and searched for reactivity against PLP<sub>139–151</sub> at several time points. PLP<sub>178–191</sub> immunized mice exhibited a severe relapsing course with mean clinical scores of 3.75 ± 0.5 at the peak of the first episode and 3.33 ± 1.15 at relapse.

Reminiscent of our findings with PLP<sub>139–151</sub>-induced disease, lymph node cells and splenocytes from mice immunized with PLP<sub>178–191</sub> mounted cytokine and lymphoproliferative responses exclusively against the primary epitope, both during the presenting episode and relapse (Figs. 4, 5; unpublished observations). As before, responses were skewed toward IFN<sub>γ</sub> and IL-17 producers.

**PLP-Specific T Cells in the CNS of Mice With Relapsing EAE Mirror Those in the Periphery With Regard to Antigen Specificity and Th Lineage**

Several studies have suggested that epitope spreading is initiated in the CNS during EAE (Targoni et al., 2001; McMahon et al., 2005). Therefore, we next turned our attention to CNS infiltrating cells. PLP<sub>139–151</sub>-specific IFNγ secreting cells occurred at a high frequency among spinal cord mononuclear cells that were isolated from PLP<sub>139–151</sub> primed mice during the first relapse, exceeding their frequency in the lymphoid tissues by over 100-fold (Fig. 6A). By contrast, IFNγ was...
not produced above background levels in wells pulsed with PLP178–191.

We also detected a high frequency of IL-17 producers, even in the absence of peptide challenge, presumably the result of presentation of endogenous myelin antigens by CNS resident APC (Fig. 6A). In an attempt to unmask PLP-specific IL-17 secretion, we repeated the ELISPOT assays using CD4+ T cells purified from the mononuclear cell fraction combined with T-depleted splenocytes from naive syngeneic donors as APC. Consequently, we were able to measure a significant PLP139–151-specific Th17 response (Fig. 6B). We did not, however, detect a PLP178–191-specific response.

Tolerance Against PLP178–191 Does Not Prevent Relapses of PLP139–151-Initiated EAE

To investigate directly the functional role of PLP178–191 reactive T cells in relapsing EAE in PLP139–151 immunized SJL mice, we used a well-established protocol to induce antigen-specific tolerance. Injection of PLP178–191 in IFA by the i.p. route prevents lymphoproliferative and cytokine recall responses as well as clinical EAE on subsequent challenge with PLP178–191 in CFA s.c. (Gaur et al., 1992; Marusic and Tonegawa, 1997; Ichikawa et al., 2002). Tolerance is maintained for 40 days or longer past the i.p. injection and it is not broken by s.c. injection with an immunogenic irrelevant peptide or PBS in CFA 2 weeks later (unpublished observations).

Despite pretreatment with PLP178–191 in IFA, 89% of mice underwent EAE relapses after active immuniza-

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**Fig. 6.** Myelin-specific IL-17 and IFN-γ responses in the CNS during the first relapse of PLP139–151-induced EAE. A, B: Mononuclear cells were isolated from the pooled spinal cords of 10–12 relapsing mice (mean clinical score = 2.5 ± 0.76) by Percoll gradient centrifugation. Whole mononuclear cells (A) or purified CD4+ T cells reconstituted with T-depleted naive splenocytes (B) were cultured with either PLP139–151 (black), PLP178–191 (white), MBP84–104 (diagonal lines), or cultured in media alone (gray). Frequencies of IL-17 and IFNγ producing cells were determined by ELISPOT. Only responses to PLP178–191 were higher significantly than background (P < 0.005). C: Lymphoproliferative responses of pooled splenocytes (black) and lymph node cells (white) were measured in parallel (C). Proliferation in response to PLP178–191 was greater significantly than proliferation in response to media alone (P < 0.005) or alternative myelin peptides (P < 0.005). The data shown is representative of three independent experiments.
tion with PLP<sub>139–151</sub> in CFA (Table I). This was comparable to the relapse rate of control mice pretreated with PBS in IFA. Furthermore, cumulative disease scores did not differ significantly between mice pretreated with PLP<sub>178–191</sub> in IFA and those pretreated with PBS in IFA ($P = 0.24$). Hence, consistent with our earlier data showing the immunodominance of PLP<sub>139–151</sub> throughout the disease course, this result suggests that clinical relapses in our model are not dependent on T cell reactivity against PLP<sub>178–191</sub>.

### DISCUSSION

Recent clinical studies have shown that broadly acting immunosuppressive agents, including drugs that inhibit hematopoiesis, globally deplete T and B lymphocytes, or block lymphocyte and monocyte trafficking, can be of therapeutic benefit in MS (Moreau et al., 1994; Polman et al., 2006). The occurrence of serious and sometimes life-threatening adverse events, such as opportunistic infections and neoplasia, provide a clear incentive for the development of more focused, antigen-specific strategies (Kleinschmidt-DeMasters and Tyler, 2005).

The literature on myelin reactivity during the course of EAE and MS is controversial. Whereas many studies have detected the emergence of novel T cell autoreactivities after the onset of clinical EAE, others have found that the autoimmune response remains focused on the disease-inducing epitope throughout the disease course. Some of these discrepancies could be attributed to differences in murine strain (or even colony), the immunogenicity of the priming epitope, the method of disease induction (i.e., passive transfer vs. active immunization), environmental conditions (housing, exposure to pathogens), and the timing or location of T cell harvest (Voskuhl et al., 1996). In addition, the use of T cell assays with various degrees of sensitivity and that measure different biologic functions have added to the complexity. A number of investigators have noted that reactivity against the priming, or initially immunodominant myelin epitope begins to wane at the time point when epitope spreading surfaces during the progression of both EAE and MS (Tuohy et al., 1999; Tarzgoni et al., 2001). Conversely, in our study as well as in others that failed to detect spreading, T cell responses against the priming epitope remained robust, and even became stronger, with successive relapses (Takacs and Altmann, 1998). One interpretation of these observations is that spreading is most likely to occur in those instances where the disease-initiating T cell subpopulation either contracts or becomes anergic after the peak of the presenting episode. Alternatively, T cells recruited against novel epitopes might either regulate or out-compete those T cell clones reactive to the primary epitope for access to myelin-bearing APC.

We expanded on previous studies by conducting an exhaustive search for emerging T cell reactivities in lymphoid tissues and the CNS over multiple time points (based on stage of disease as well as absolute time from immunization) using highly sensitive ELISPOT assays that encompassed a broad range of cytokines. Based on our results, we can be numbered among those investigators who find no evidence for recruitment of Th1 cells reactive against secondary or tertiary epitopes during the course of multiphasic disease. In addition, we were unable to detect spreading to alternative Th lineages that have been associated with regulatory subsets (such as Th1 cells) or pro-inflammatory subsets (i.e., Th17 cells) recognized more recently.

As alluded to earlier, EAE in PLP<sub>139–151</sub>-immunized SJL mice is not a typical model of progressive or relapsing autoimmune demyelinating disease in that T cells reactive against the initiating epitope occur at an unusually high precursor frequency. Nevertheless, we were also unable to detect spreading in SJL mice immunized with the subdominant epitope, PLP<sub>179–191</sub> (Figs. 4, 5). We can not rule out the possibility that epitope spreading is a feature of progressive EAE induced in alternative strains with other encephalitogenic peptides, or in MS, in which the target epitope(s) remain unknown. It is also possible that, in our model, spreading occurred to myelin epitopes that we did not investigate.

Although EAE and MS have been portrayed classically as Th1-driven diseases, a growing body of data suggest that this characterization is overly simplistic. Th17 polarized cells have emerged as critical effector cells in EAE pathogenesis (Park et al., 2005; Chen et al., 2006). In contrast, IFNγ plays a paradoxic protective role in some models of EAE and can inhibit Th17 differentiation (Ferber et al., 1996; Willenborg et al., 1996; Billiau et al., 1998; Segal et al., 1998; Harrington et al., 2005). Such observations suggest that PLP-specific Th1 cells act to regulate the pathogenic Th17 effector response, thereby limiting, rather than amplifying, neuroinflammation. In vitro stimulation of myelin-specific T cells from

### TABLE I. Tolerization to PLP<sub>178</sub> Does Not Affect Relapse Rate in PLP<sub>139</sub> Immunized Mice

<table>
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<tr>
<th>Pretreatment</th>
<th>PBS + IFA</th>
<th>PLP&lt;sub&gt;139&lt;/sub&gt; + CFA</th>
<th>PLP&lt;sub&gt;178&lt;/sub&gt; + IFA</th>
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<th>PLP&lt;sub&gt;178&lt;/sub&gt; + CFA</th>
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<td>Challenge</td>
<td>PLP&lt;sub&gt;139&lt;/sub&gt; + CFA</td>
<td>PLP&lt;sub&gt;139&lt;/sub&gt; + CFA</td>
<td>PLP&lt;sub&gt;139&lt;/sub&gt; + CFA</td>
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<td>PLP&lt;sub&gt;178&lt;/sub&gt; + CFA</td>
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<tr>
<td>Relapse incidence</td>
<td>8/8</td>
<td>8/9</td>
<td>3/3</td>
<td>0/3</td>
<td>3/4</td>
<td></td>
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<tr>
<td>Mean cumulative disease score&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58 (30)</td>
<td>44 (14)</td>
<td>46 (9)</td>
<td>0 (0)</td>
<td>23&lt;sup&gt;a&lt;/sup&gt; (5)</td>
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Mice were injected with either PBS or PLP<sub>178–191</sub> in IFA i.p. 2 weeks before challenge with PLP<sub>139–151</sub> in CFA. Standard deviations are shown in parentheses.

<sup>a</sup>Mice were sacrificed early (Day 23 post-immunization).

<sup>b</sup>Cumulative scores were calculated by summing daily scores of individual mice over the observation period.
innately resistant or tolerized mice with recombinant IL-12 enhances their encephalitogenic properties (Segal and Shevach, 1996; Ichikawa et al., 2002). Thus IL-12 induces characteristics, distinct from IFNγ production, that facilitate the CNS migration or effector functions of auto reactive T cells. Future experiments will determine the role of myelin-specific Th1 cells in our model, which we recognize might vary based on location and the stage of disease.

We found that mice pretreated with PLP178–191 in a tolerogenic context (i.e., injected at a high dose i.p. without adjuvants), nevertheless succumbed to relapsing EAE after challenge with PLP139–151 in CFA at an incidence comparable to controls (Table I). Mice that received the same pre-treatment were protected from disease comparable to controls (Table I). Mice that are capable of mediating bystander suppression. Therapeutic modalities that promote the development of regulatory T cells against novel neuroantigens could be

remitting MS and other chronic autoimmune diseases. Reactivities might be useful in some cases of relapsing–

2000). By extension, therapies that target specific T cell reactivities might be useful in some cases of relapsing–remitting MS and other chronic autoimmune diseases. Therapeutic modalities that promote the development of regulatory T cells against novel neuroantigens could be effective in disease driven by a focused response if they are capable of mediating bystander suppression.

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REFERENCES


Th17 Cells and EAE Relapses 1693


