Oligodendrocyte-Specific Protein Peptides Induce Experimental Autoimmune Encephalomyelitis in SJL/J Mice

David B. Stevens, Kendall Chen, Robert S. Seitz, Eli E. Sercarz and Jeff M. Bronstein

*J Immunol* 1999; 162:7501-7509;
http://www.jimmunol.org/content/162/12/7501

---

**References**

This article cites 57 articles, 17 of which you can access for free at:
http://www.jimmunol.org/content/162/12/7501.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Oligodendrocyte-Specific Protein Peptides Induce Experimental Autoimmune Encephalomyelitis in SJL/J Mice

David B. Stevens,*† Kendall Chen,* Robert S. Seitz,‡ Eli E. Sercarz,† and Jeff M. Bronstein 2*

Oligodendrocyte-specific protein (OSP) is a recently isolated and cloned, 207-aa, hydrophobic, four-transmembrane protein found in CNS myelin. It represents ~7% of total myelin protein. The OSP cDNA sequence has no significant homology with previously reported genes, but the predicted protein structure suggests that OSP is a CNS homologue of peripheral myelin protein-22. We previously reported the presence of anti-OSP Abs in the cerebrospinal fluid of relapsing-remitting multiple sclerosis (MS) patients, but not control patient groups. In this study, we tested the ability of a panel of 20-mer peptides with 10-aa overlaps, representing the sequence of murine OSP, to induce experimental autoimmune encephalomyelitis (EAE), an animal model for MS. SJL mice challenged with murine OSP peptides 52–71, 82–101, 102–121, 142–161, 182–201, and 192–207 exhibited clinical EAE. OSP:52–71 elicited severe relapsing-remitting EAE in some individuals. All other encephalitogenic peptides elicited, at most, a loss of tail tonicity from which the mice most often completely recovered. Mononuclear cell infiltrates and focal demyelination characteristic of EAE were evident. T cell proliferative responses were seen with all encephalitogenic peptides except 142–161 and 182–201. OSP peptides 72–91 and 132–151 did not cause clinical EAE, but did elicit robust proliferative responses. B10.PL and PL/J mice challenged with the same OSP peptide doses as SJL mice did not exhibit clinical EAE. These results in the SJL EAE model, together with the results from MS patient clinical samples, make OSP a promising candidate for autoantigenic involvement in MS. The Journal of Immunology, 1999, 162: 7501–7509.

Multiple sclerosis (MS) is a paralytic disease of the CNS characterized histologically by perivascular mononuclear cell infiltration and focal areas of myelin destruction. Although the etiology remains elusive, the pathology of MS bears attributes indicative of autoimmune disease (1). Many candidate molecules for autoantigenic involvement in MS have been put forth, including: myelin basic protein (MBP) (2) and myelin oligodendrocyte glycoprotein (MOG) (7, 8), myelin-associated glycoprotein (MAG) (9–13), transaloidase (14), S100β (15), α B-crystallin (16), and 2',3' cyclic nucleotide 3' phosphodiesterase (CNP) (17, 18). Among these, only MBP, PLP, and MOG, and MOG peptides together with the results from MS patient clinical samples, make OSP a promising candidate for autoantigenic involvement in MS. The Journal of Immunology, 1999, 162: 7501–7509.

Received for publication January 25, 1999. Accepted for publication March 25, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 Address correspondence and reprint requests to Dr. Jeff M. Bronstein, Department of Neurology, 710 Westwood Plaza, Reed Neurological Research Center, UCLA School of Medicine, Los Angeles, CA 90095-1769. E-mail address: jbronste@ucla.edu

*† Abbreviations used in this paper: MS, multiple sclerosis; AcI-9, the N-terminal acetylated first nine amino acids of MBP; CNP, 2',3' cyclic nucleotide 3' phosphodiesterase; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; MAG, myelin-associated protein; MBP, myelin basic protein; MOG, myelin oligodendrocyte protein; OSP, oligodendrocyte-specific protein; mOSP, murine OSP; PLP, proteolipid protein; SI, stimulation index.

Copyright © 1999 by The American Association of Immunologists 0022-1767/99/$02.00
induced a progressive relapsing-remitting EAE that clinically and histologically resembles MS.

**Materials and Methods**

**Mice**

Female SJL/J (H-2b), B10.PL H-2b H2-T18(73NS)Sn (H-2b), and PL/J (H-2b) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and housed in the U.S.D.A. and Association for the Assessment and Accreditation of Laboratory Animal Care, International Approved Animal Facilities of the La Jolla Institute for Allergy and Immunology using National Institutes of Health Guidelines. This conventional specific pathogen-free facility has been maintained in an exceptional hygienic state, as evidenced by the early onset and greater severity of disease seen in several experimental autoimmune disease models. Mice were maintained on autoclaved autoclavable rodent breeder diet 5013 (PMI Nutrition International, St. Louis, MO) and acidified water ad libitum. Mice were used in EAE induction experiments at 6 wk of age.

**Peptides**

Twenty-mer peptides with 10-aa overlaps, representing the sequence of mOSP (Table I), were synthesized at Research Genetics (Huntsville, AL). The 13-mer PLP:139–151 peptide and the 9-mer MBP:Ac1–9 were synthesized at the UCLA Peptide Core Facility. The PLP:139–151 peptide contained a serine substituted for cysteine to serine at position 140.

**Preparation of encephalitogenic emulsions**

The extreme hydrophobicity and adherence properties of many of the OSP peptides demanded exacting technique for the preparation of consistent homogenous CFA emulsions. Emulsions were prepared as follows: The aqueous phase was prepared by dissolving 5 mg of lyophilized peptide per 1 ml of sterile, autoclaved autoclavable medium (BioWhittaker, Walkersville, MD) plus 5 µl 2-ME (BioWhittaker, Walkersville, MD) plus 5 × 10⁻³ M 2-ME for 4 days at 37°C in 200 µl total culture volumes. The concentration of peptide Ag used for restimulation in culture and the cell culture density were both titrated as specified in the figure legends. In direct experimental comparisons between the 13-mer PLP:139–151 peptide and 20-mer OSP peptides, the approximate molar equivalence of the PLP:139–151 peptide was calculated by multiplying the gram amount of OSP peptide(s) used by 0.65 (13-mer peptide ÷ 20-mer peptide = 0.65).

**Spleen cell proliferation assays**

Spleen cell proliferation assays were performed on all surviving SJL mice. Spleen cells were cultured in 96-well culture plates in X-VIVO 10 synthetic medium (BioWhittaker, Walkersville, MD) plus 5 × 10⁻³ M 2-ME for 4 days at 37°C in 200 µl total culture volumes. The concentration of peptide Ag used for restimulation in culture and the cell culture density were both titrated as specified in the figure legends. The shorter PLP:139–151 peptide was added to wells at an approximate molar equivalence. A total of 1 µCi [³H]thymidine was added to each well in 50 µl medium for the last 18 h of culture. Relative [³H]thymidine incorporation was determined by harvesting the cells onto a glass fiber filtermat (Wallac, Turku, Finland) using a semiautomated cell harvester and liquid scintillation counting. Results are reported as stimulation indices (SI) calculated using average counts of triplicate wells. SI were calculated as: (counts with peptide in medium) ÷ (counts with medium alone).

In a separate effort to determine whether prior dissolution of the OSP peptides in DMSO would effect proliferative responses, 1 mg of peptide dissolved in 50 µl of DMSO, then medium was added to a total volume of 1 ml to make a resulting 1 mg/ml stock solution. Peptides from these stock solutions were used for stimulation of cells in culture at a final

---

**Table I. Murine OSP synthetic peptides used in this study**

<table>
<thead>
<tr>
<th>Murine OSP Amino Acids</th>
<th>Amino Acid Sequence</th>
<th>Solubility in Aqueous Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>12–25</td>
<td>VTSFVGWIG11VTTTSNDWV</td>
<td>−</td>
</tr>
<tr>
<td>22–41</td>
<td>IVTNTSNDDVVTCSYIPTC</td>
<td>+</td>
</tr>
<tr>
<td>32–51</td>
<td>VTSYIPTCRKMDLGSKG</td>
<td>+</td>
</tr>
<tr>
<td>42–61</td>
<td>RKMDELGSKGLWADVMATG</td>
<td>+</td>
</tr>
<tr>
<td>52–71</td>
<td>LWDVCMATGLYHCPLVD1</td>
<td>+</td>
</tr>
<tr>
<td>62–81</td>
<td>LHCPLVDIILPLGYQAC</td>
<td>+</td>
</tr>
<tr>
<td>72–91</td>
<td>LILPGYQACRAALMIAASVL</td>
<td>+</td>
</tr>
<tr>
<td>82–101</td>
<td>RALMIAASVLRPLAI111</td>
<td>+</td>
</tr>
<tr>
<td>92–111</td>
<td>GLPAI111TLTVLPCIRMGE</td>
<td>−</td>
</tr>
<tr>
<td>102–121</td>
<td>VLPICRIMGHEFVGAVYRRQA</td>
<td>+</td>
</tr>
<tr>
<td>112–131</td>
<td>PGVAKYRRQAQLAVGLLILA</td>
<td>+</td>
</tr>
<tr>
<td>122–141</td>
<td>LCAIVATIFPPVCAHERIT</td>
<td>+</td>
</tr>
<tr>
<td>132–151</td>
<td>P V C A H R E I T S F G Y S L Y A G</td>
<td>+</td>
</tr>
<tr>
<td>142–161</td>
<td>GCYIVCSCSDAQSFGENRFY</td>
<td>+</td>
</tr>
<tr>
<td>152–171</td>
<td>AVC1–9</td>
<td>+</td>
</tr>
<tr>
<td>162–181</td>
<td>HSLGKWLGHPDFK</td>
<td>+</td>
</tr>
<tr>
<td>172–191</td>
<td>Y SSGSSSPThAKSAHV</td>
<td>+</td>
</tr>
<tr>
<td>PLP:139–151</td>
<td>MBP:Ac1–9</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>acetyl-AsQKRPSQR</td>
<td>+</td>
</tr>
</tbody>
</table>

* Underlined amino acids are in hydrophobic regions as determined by Kyte-Doolittle analysis. PLP:139–151 contains a cysteine to serine substitution at position 140.
**Table II. Expt. 1: Induction of EAE with many OSP peptide pools indicates a multiplicity of determinants**

<table>
<thead>
<tr>
<th>Peptide Pool No.</th>
<th>mOSP Peptides Injected</th>
<th>EAE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Incidence</td>
</tr>
<tr>
<td>I</td>
<td>12–25</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>22–41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32–51</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>42–61</td>
<td>3/4</td>
</tr>
<tr>
<td></td>
<td>52–71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>62–81</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>72–91</td>
<td>4/6</td>
</tr>
<tr>
<td></td>
<td>82–101</td>
<td></td>
</tr>
<tr>
<td></td>
<td>92–111</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>102–121</td>
<td>4/6</td>
</tr>
<tr>
<td></td>
<td>112–131</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>132–151</td>
<td>3/6</td>
</tr>
<tr>
<td></td>
<td>142–161</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>172–191</td>
<td>2/6</td>
</tr>
<tr>
<td></td>
<td>182–201</td>
<td></td>
</tr>
<tr>
<td></td>
<td>192–207</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>PLP 139–151 (positive control)</td>
<td>6/6</td>
</tr>
<tr>
<td>VIII</td>
<td>No peptide, adjuvants only (negative control)</td>
<td>0/4</td>
</tr>
</tbody>
</table>

*Six-week-old, female SJL/J mice were challenged with OSP peptide pools containing 250 μg of each component OSP peptide. PLP:139–151 peptide served as a positive control. EAE induction and scoring were as described in Materials and Methods. Peptide pool II originally had six mice, two of which died early from apparent adjuvant-induced shock. OSP:42–61, a component of peptide pool II, had physicochemical properties that inhibited emulsification. At 25 days (two individuals from each group) or 35 days (all remaining individuals), brains and spinal cords were harvested for histological analysis and proliferation assays were performed (Fig. 1).*

**Results**

On the basis of the deduced amino acid sequence of mOSP (23), 20-mer peptides with a 10-aa overlap were synthesized to span the 207-aa molecule. The peptides used are listed in Table I. Gaps in the peptide scan represent regions of extreme hydrophobicity that proved to be too difficult to synthesize using standard solid-phase synthesis technology.

**Experiment 1: Induction of EAE with many OSP peptide pools indicates a multiplicity of determinants**

In our first attempt to induce EAE in SJL mice, pools of two to three contiguous overlapping peptides were used (Table II). Peptide pools contained 250 μg of each component OSP peptide. In addition, one group of mice was injected with PLP:139–151, the most encephalitogenic peptide known to induce EAE in the SJL mouse strain, as a positive control. Another group of mice was injected with only the adjuvants, the CFA emulsion alone (without any added peptide) and pertussis toxin, as a negative control. Adjuvant alone has never been shown to induce EAE, but this negative control group was nevertheless included to eliminate the possibility that the relatively high adjuvant doses used in this study might cause neurologic impairment. The mice were observed for 25 days (two individuals per group) or 35 days (the remaining mice from each group) for clinical signs of EAE before harvesting their spleens for T cell proliferation assays and their brains and spinal cords for histologic analyses.

Surprisingly, mice from five of the six peptide pool groups (groups II, III, IV, V, and VI) displayed frank signs of EAE (Table II), indicating that there are many T cell determinants on the OSP...
molecule. The clinical score for four of the five peptide pools that elicited frank EAE was at most a grade 1, a loss of use of the tail. Severe hind limb paralysis was seen in group II (mice injected with mOSP peptides 42–61, 52–71, and 62–81). Four of six mice injected with PLP:139–151, together with the same relatively high adjuvant doses given to all of the other experimental groups, died from fulminating acute phase EAE.

**Experiment 2: Challenge with individual mOSP peptides reveals six that cause EAE**

Since five of six of the peptide pools in experiment 1 (Table II) induced frank EAE and we wished to confirm that the three constituent peptides of the one remaining peptide pool were incapable of inducing clinical disease, we went on to test all of the OSP peptides individually for their ability to induce EAE. Four mice per group were challenged with 250 μg of each individual peptide (Table III), and the clinical course of EAE was scored daily. Six of the OSP peptides, OSP:52–71, 82–101, 102–121, 142–161, 182–201, and 192–207, and the PLP:139–151 positive control peptide induced frank clinical EAE with a day of onset ranging from day 8 to 22. OSP:52–71 was the most encephalitogenic of the OSP peptides, inducing sometimes severe clinical paralysis in all immunized individuals. All other encephalitogenic OSP peptides elicited, at most, a loss of tail tonicity from which the mice most often completely recovered after a period of days. The initial episode of EAE, most often commencing day 8 to 12, was more severe when induced with PLP:139–151 than with OSP:52–71, but subsequent relapses, which commenced at about day 20 or thereafter, were equally severe. Two of the OSP:52–71-injected individuals displayed no clinical signs of EAE between days 8 and 12, around the time that their cagemates were exhibiting clinical acute phase symptoms. However, these same two individuals later exhibited severe clinical signs on day 21, at about the same time that the first relapse commenced in their cagemates. Thus, the temporal alignment of these events suggests that the initial episode of OSP:52–71-induced EAE may have been subclinical in these two individuals, but nevertheless led to a progressive relapsing-remitting clinical disease course. Interestingly, 102–121, the peptide that encompasses the 114–120 region against which anti-OSP Ab binding is focused in relapsing-remitting MS patients (28), was encephalitogenic in SJL mice (Table III) and elicited T cell proliferative recall responses (Figs. 1 and 2, discussed below).

### T cell proliferative responses from mice challenged with OSP peptide pools (Expt. 1)

Spleens were harvested from surviving mice challenged with OSP peptide pools at 25 days (two mice per group) or 35 days postchallenge (remaining mice) for use in T cell proliferation assays. Spleen cells from mice from each peptide pool were restimulated for in vitro recall with each individual peptide component of the pool separately (Fig. 1). Proliferation was elicited by OSP:52–71, 72–91, 82–101, 102–121, 132–151, and 192–207. The one surviving mouse challenged with PLP:139–151, which was tested on day 35, also elicited a proliferative recall response.

### T cell proliferative recall in mice challenged with individual OSP peptides (Expt. 2)

Spleen cells from mice that were challenged with individual OSP peptides were tested for in vitro T cell proliferative recall with the same peptide used for challenge (Fig. 2). One individual mouse from each group was tested on days 30, 36, 42, and 48 postinjection. Both the cell density and the peptide concentration in culture were titrated. Proliferative recall responses were obtained to the same peptide used for challenge (Fig. 2). Proliferation was seen to all encephalitogenic peptides except 142–161 and 182–201. These two peptides, as well as others in our panel, formed conspicuous, insoluble aggregates in culture. Two OSP peptides, 72–91 and 132–151, did not cause EAE, but did elicit robust proliferative
recall responses. The proliferative responses to OSP peptides often exceeded the proliferative response of the positive control peptide, PLP:139–151. The proliferative response to restimulation with OSP peptides waned rapidly after 36 days postchallenge, with a marked diminution in proliferative response in assays performed on days 42 and 48. The proliferative response obtained did not always correlate with the clinical status of the mouse. In this experiment, restimulation in culture was also performed using peptides that shared a region of overlap with the immunizing peptide in an effort to determine whether any T cell determinants might reside on the overlapping region. In no case did an overlapping flanking peptide elicit proliferative recall (data not shown).

**T cell proliferative recall using poorly soluble peptides**

Since many of the peptides in the panel were poorly soluble in aqueous media, the performance of highly hydrophobic peptides in immunologic assays was a concern throughout this study. However, it became evident in the course of our experiments that extreme hydrophobicity does not necessarily interfere with a peptide’s ability to elicit good immune responses either in vivo when delivered as a component of an oil and water emulsion (compare Table I to Tables II and III) or in vitro in aqueous media in T cell proliferation assays (compare Table I to Figs. 1 and 2). Two peptides, OSP:142–161 and 182–201, contained T cell determinants, as evidenced by their ability to induce EAE (Table III), but did not elicit T cell proliferative responses (Figs. 1 and 2). We initially attributed the lack of proliferative responses to the readily observed insoluble aggregates of these two peptides in culture. However, these two peptides also failed to elicit proliferation even after complete dissolution in DMSO before addition to culture. The presence of small quantities of DMSO in the cultures (0.10–0.15%) somewhat diminished the proliferative responses to the entire panel of OSP peptides, but did not significantly alter the relative proliferative responses to the OSP peptides (data not shown).

![FIGURE 1](http://www.jimmunol.org/)

**FIGURE 1.** T cell proliferative recall responses to OSP peptides in SJL mice immunized with OSP peptide pools (Expt. 1, Table II). Spleen cell proliferation assays were performed on two mice from each group on day 25 and on the surviving mice on day 35 postinjection. Spleen cells were cultured at 4 x 10^6 and 2 x 10^6 cells/ml. OSP peptides were added to a final concentration in culture of 10, 20, and 30 μg/ml. The best SIs were obtained at 20 or 30 μg/ml. The PLP:139–151 peptide was used at approximate molar equivalence. The highest SI obtained from each two-dimensional titration of cell density and peptide concentration is plotted. Each column represents the response of an individual mouse. Peptide pools that induced clinical EAE are indicated.

![FIGURE 2](http://www.jimmunol.org/)

**FIGURE 2.** T cell proliferative recall responses to OSP peptides in SJL mice immunized with individual OSP peptides (Expt. 2, Table III). Spleen cell proliferation assays were performed on one mouse from each group on days 30, 36, 42, and 48 postinjection. OSP peptides were added to a final concentration in culture of 5, 20, and 40 μg/ml. The best SIs were obtained at 20 or 40 μg/ml. The PLP:139–151 peptide was used at approximate molar equivalences. The highest SI obtained for each individual mouse’s spleen cells cultured at 4 x 10^6 and 2 x 10^6 cells/ml is plotted as pairs of adjacent like-patterned bars. The left-hand bar of each pair represents the highest SI obtained from the OSP peptide titration at 4 x 10^6 cells/ml; the right-hand bar represents the highest SI obtained at 2 x 10^6 cells/ml. Peptides that induced clinical EAE are indicated with an E; peptides that elicited proliferative recall, but no clinical EAE are indicated with a P. Note that OSP peptides 142–161 and 182–201 induced EAE, but did not elicit proliferation.
addition, some OSP peptides that formed visible aggregates in aqueous media did elicit good proliferative responses either with or without prior dissolution in DMSO. Since addition of weak acid or base did not significantly increase the solubility of any of the OSP peptides in aqueous solution, this method of dissolving peptides was not employed in experiments.

OSP peptides fail to induce EAE in B10.PL and PL/J mouse strains

Although the SJL mouse strain was susceptible to EAE induced with many OSP peptide determinants, we were unable to induce EAE with OSP peptides in the commonly used, MBP-susceptible B10.PL (H-2^d) strain. B10.PL mice injected with the same encephalitogenic emulsions of OSP peptide pools described in experiment 1 (Table II) displayed no overt signs of EAE over a 3-mo observation period (data not shown). In contrast, mice injected with 50 μg of the dominant determinant peptide, MBP:Ac1–9, together with the same relatively high adjuvant dosages used throughout this study as a positive control, exhibited an often lethal acute episode of EAE. Spinal cords and brains taken from selected B10.PL individuals from each OSP peptide pool group on day 62 postinjection showed no signs of histologic EAE.

In a separate effort, groups of SJL, B10.PL, and PL/J mice were challenged with a mixture-emulsion containing 50 μg of each of the 16 OSP peptides in our panel plus 300 ng of pertussis toxin administered i.p. on day 1 and again on day 3. The SJL mice in this experiment exhibited severe relapsing-remitting EAE, while the B10.PL and PL/J mice displayed no signs of clinical EAE (data not shown). The unpredictability of the EAE disease course after the first relapse and mounting disability with each subsequent relapse was evident in the SJL mice in this experiment. Spinal cords and brains of these individuals were taken for extensive histologic analyses (Fig. 3).

Histology of OSP peptide-induced EAE

The perivascular mononuclear cell infiltrates and focal demyelination characteristic of EAE (Fig. 3A) were evident in SJL mice challenged with OSP peptides. In general, more extensive demyelination was seen during clinical relapses. In experiment 2, in which we have data for SJL mice challenged with OSP peptides individually, the histologic score for mononuclear cell infiltration into the spinal cord correlated with clinical EAE scores (Table III). As is seen in other murine models of EAE, the pathology of OSP peptide-induced EAE was also observed in the brain. We also identified cellular infiltration into the forebrain of some individuals (Fig. 3C), as is also seen in MS. Immunohistochemical staining demonstrated a loss of binding by Abs specific for MBP (Fig. 3, C and D) and OSP (Fig. 3B) in the demyelinated lesions. Infiltrates at the pia mater were common with OSP peptide-induced EAE. A few individuals that never displayed overt clinical signs of EAE had slight infiltrates at the pia mater of the brain.

Discussion

In this study, we demonstrate the presence of multiple T cell determinants within the OSP molecule that can induce EAE in SJL mice. One of the peptides tested, OSP:52–71, was capable of inducing a progressively severe relapsing-remitting disease.
Both OSP peptides and the PLP:139–151 peptide produce extensive mononuclear cell infiltration with severe demyelination in the spinal cord and brain. More extensive demyelination was seen during clinical relapses. Infiltrates at the pia mater were common. This contrasts with MOG peptide-induced EAE, in which cellular infiltration at the meninges was reported as being rare compared with spinal cord homogenate-induced disease (32). A few individuals that never displayed overt clinical signs of EAE in experiment 2 (Table III) had slight cellular infiltrates at the pia mater of the brain. This underscores the importance of performing histopathologic analysis on brain sections when screening candidate autoantigens in disease models intended to reflect the pathology of MS. The classic ascending paralysis of EAE is almost certainly biased toward spinal cord involvement.

OSP appears to be essentially a CNS myelin protein with concentrated expression in the spinal cord and brain (23). OSP comprises approximately 7% of total CNS myelin protein (24), making it the third most abundant CNS myelin protein yet discovered, behind MBP and PLP. Standard Northern blots used to screen a panel of mouse tissues, including sciatic nerve, liver, lung, skeletal muscle, kidney, spleen, and heart, with a radiolabeled OSP cDNA probe did not detect OSP RNA transcripts in these tissues (23). Efforts are underway in this laboratory to determine whether low level OSP expression can be detected in lymphoid and other non-CNS tissues using more sensitive methods. MBP and PLP have been shown to be components of both CNS and peripheral nervous system myelin (33–35). MOG expression appears to be essentially a CNS glycoprotein (36–39). Thus, the CNS-restricted pattern of expression of OSP and MOG corresponds to the localization of the immunohistopathology of MS, which resides within the compact myelin tracts of the CNS.

According to the predicted structure of OSP, OSP:52–71 lies within the most prominent extracellular region of the four-transmembrane domain OSP molecule (23). This is akin to the situation seen with MOG, in which the most encephalitogenic determinant is also in an extracellular region. The close proximity of both T and B cell determinants on an extracellular domain is thought to be a potentiating factor in the induction of pathogenic humoral responses to MOG (40). According to the predicted structure of the PLP molecule (41), the dominant PLP:139–151 determinant is not in an extracellular domain of the PLP molecule, but the secondary encephalitogenic determinant, PLP:178–191, does lie within an extracellular domain. MBP is entirely compacted within myelin and is not exposed to the extracellular surface.

Six different mOSP peptides were able to prime T cells to cause EAE. This suggests that these peptides contain determinants that can be processed and presented from whole OSP by APCs residing in the spinal cord and brain. The ability of two other peptides, OSP:72–91 and 132–151, to elicit vigorous T cell proliferative responses, but not EAE, could be due to either a lack of processing and presentation of the determinants within these peptides from whole OSP by CNS APCs (42–44), or possibly, by the balance of Th2 versus Th1 cells induced by these peptides.

When the T cell proliferative assays were performed on mice immunized with individual peptides (Fig. 2), recall responses were tested not only to the immunizing peptide, but to its flanking peptides (which possessed a 10-aa overlap with the immunizing peptide) to determine whether the overlapping peptides shared a determinant. In no case did immunization with a peptide elicit a proliferative response in an adjacent overlapping peptide, indicating that each individual peptide tested possessed distinct T cell determinants.

Although efforts were made in this study to identify all of the T cell determinants on the OSP molecule in the SJL mouse strain, it is possible that determinants remained undetected for a number of reasons. First, peptides representing some of the most highly hydrophobic regions of the OSP molecule proved too difficult to synthesize. The presence of determinants within these regions thus went untested. Second, the alignment of the panel of 20-mer peptides with 10-aa overlaps may have truncated some determinants. Third, because differences in Ag processing and the display of peptides versus the whole Ag have been observed in other systems, determinants that may be processed and presented from whole OSP may not be similarly processed and presented from the peptide containing the determinant (42–44). Fourth, a lack of T cell proliferation may have been due to the relative insolubility, toxicity, instability, or other properties of some of the peptides in culture.

Many autoantigens have been previously put forth as candidates for autoantigenic involvement in MS based upon evidence of humoral and/or cellular autoreactivity in MS patient samples. These include MBP (2) and golli-MBP (3), PLP (4–6), MOG (7, 8), MAG (9–13), transaldolase (14), S100β (15), α B-crystallin (16), and CNP (17, 18). Classically, the defining criteria for cellular autoimmune diseases requires that candidate autoantigens also be capable of inducing an analogous autoimmune response when injected into a laboratory animal (19). Of the previously proposed autoantigens, MBP (45, 46), PLP (47, 48), and MOG (32, 49, 50) are the ones that, in addition to showing evidence of autoantigenicity in MS patient samples, are capable of inducing active EAE. The T cell determinant structure of MBP (51), PLP (52, 53), and MOG (32, 50, 54) has been characterized extensively in a number of mouse models of EAE using peptide scans of the respective molecules. OSP can now be added to this list of most promising candidate myelin autoantigens that both elicit evidence of autoreactivity in MS patient samples and whose EAE-inducing determinant structure has been analyzed. Evidence of autoreactivity to MAG has been reported in MS patient specimens (55) and animals with EAE (56). However, neither purified MAG nor MAG peptides have yet been shown to directly induce EAE. Two astrocyte proteins, S100β and glial fibrillary acidic protein, have been shown to induce a panencephalomyelitis, which differs from classical EAE in laboratory animals, by adoptive transfer of in vitro activated cell lines, but not by active immunization (57). T cell reactivity to S100β has also been reported in MS patient samples (15). Several autoantigens whose expression is not restricted to myelin-producing cells have also been implicated in MS. Evidence of autoreactivity to α B-crystallin (16) and CNP (17, 18), both members of the heat-shock family of proteins, has been reported in MS patient clinical samples, but these two molecules have not been shown to be capable of inducing EAE in a laboratory animal model (58). Similarly, evidence of autoreactivity to transaldolase, a housekeeping gene product, has been reported in MS patient clinical samples (14), but transaldolase has not been shown to induce EAE. Thus, if autoreactivity to these molecules actually plays a part in MS, it may be through a variety of determinant-spreading mechanisms.

Observation of the determinant-spreading phenomenon in experimental autoimmune disease models such as EAE has provided vital insight into the underlying mechanism of disease chronicity and a rationale for the limited success seen in past attempts at immunotherapy using single autoantigens. It has also provided a working conceptual framework that raises possibilities for the design of future immunotherapeutic strategies, most of which hinge upon the identification, and the ranking of the relative importance of the T cell autoreactivity directed against additional target autoantigens. Much effort has recently been focused upon the use of
synthetic peptides representing autoantigenic determinants or rationally designed peptide analogues of the known neuroantigens in immunotherapy in the EAE model (59, 60). It may be that assemblages of peptides representing all relevant determinants, or at least the ones against which the most potent T cell autogressivity is focused, may succeed immunotherapeutically where similar strategies targeting only a single determinant have failed. The possibility that autogressivity may begin with initiator determinants (61, 62) and the observation that determinant-spreading may be unidirectional (21, 22) may further circumscribe the number of peptides needed for effective immunotherapy. At present, we do not know whether a substantial number of target autoantigens that are relevant to demyelinating disease remain at large or whether, with this study of OSP, the major players may now have been identified.

We have established a model of OSP peptide-induced EAE in the SJL mouse, whose progressive relapsing-remitting clinical course closely resembles the clinical course of MS. The vigorous T cell proliferative responses to multiple determinants of OSP in SJL mice make for a good system to explore questions regarding intramolecular determinant spreading. Of six determinants that elicit vigorous proliferative responses on the OSP molecule, four are capable of inducing EAE (one of which encompasses the focus of Ab binding in humans), and two are determinants that elicit strong proliferative responses, but not EAE. Thus, the OSP molecule has determinants displaying a number of different attributes, and studies are ongoing in this laboratory to determine the role that OSP determinants may play in demyelinating autoimmune disease. The results of this study, taken together with the previous evidence of anti-OSP Abs in the CSF of relapsing-remitting MS patients (28), make OSP an excellent candidate protein for autogressive involvement in MS.

Acknowledgments
We thank Brian A. Pedersen and Darren Sigel for excellent laboratory assistance, Denise O’Masters for excellent management of the LIIA Lab, and Henrietta Salazar for efficient administrative assistance.

References


