Experimental Autoimmune Encephalomyelitis Induced with a Combination of Myelin Basic Protein and Myelin Oligodendrocyte Glycoprotein Is Ameliorated by Administration of a Single Myelin Basic Protein Peptide

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Experimental Autoimmune Encephalomyelitis Induced with a Combination of Myelin Basic Protein and Myelin Oligodendrocyte Glycoprotein Is Ameliorated by Administration of a Single Myelin Basic Protein Peptide

Elizabeth A. Leadbetter,1 Cheryl R. Bourque, Brigitte Devaux,2 Carl D. Olson, Geoffrey H. Sunshine,3 Shirish Hirani,4 Barbara P. Wallner,5 Dawn E. Smilek, and Mary Pat Happ6

Multiple sclerosis is an autoimmune disease of the central nervous system in which T cell reactivity to several myelin proteins, including myelin basic protein (MBP), proteolipid protein, and myelin oligodendrocyte glycoprotein (MOG), has been implicated in the perpetuation of the disease state. Experimental autoimmune encephalomyelitis (EAE) is used commonly as a model in which potential therapies for multiple sclerosis are evaluated. The ability of T cell epitope-containing peptides to down-regulate the disease course is well documented for both MBP- and proteolipid protein-induced EAE, and recently has been shown for MOG-induced EAE. In this study, we describe a novel EAE model, in which development of severe disease symptoms in (PL/J × SJL)F1 mice is dependent on reactivity to two different immunizing Ags, MBP and MOG. The disease is often fatal, with a relapsing/progressive course in survivors, and is more severe than would be predicted by immunization with either Ag alone. The MOG plus MBP disease can be treated postinduction with a combination of the MOG 41–60 peptide (identified as the major therapeutic MOG epitope for this strain) and the MBP Ac1–11[4Y] peptide. A significant treatment effect can also be obtained by administration of the MBP peptide alone, but this effect is strictly dose dependent. This MBP peptide does not treat the disease induced only with MOG. These results suggest that peptide immunotherapy can provide an effective means of mitigating disease in this model, even when the treatment is targeted to only one component epitope or one component protein Ag of a diverse autoimmune response. The Journal of Immunology, 1998, 161: 504–512.

Multiple sclerosis (MS)7 is an inflammatory disease of the central nervous system (CNS) white matter that results in areas of demyelination with disruption of neurologic function. In MS patients, white matter inflammatory infiltrates contain numerous T lymphocytes (1), and specific immune recognition of myelin components by these T lymphocytes has long been thought to contribute to the pathogenesis of MS (reviewed in Ref. 2). Several myelin proteins have been implicated as targets of autoreactive T cells, including myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), and myelin-associated glycoprotein (3–9). It is not clear which of these myelin-specific T cell reactivities plays a more critical role in the initiation or perpetuation of the disease state in MS, and it is likely that more than one Ag is involved.

The most widely used animal model of MS is experimental autoimmune encephalomyelitis (EAE), in which disease is induced by immunization with myelin proteins in adjuvant. EAE has been induced with MBP, PLP, and MOG (10–14), and the syndromes that develop bear varying degrees of resemblance to human MS. These models have been used to test the feasibility of treatment of MS with soluble peptides containing major T cell epitopes of the inducing myelin Ag (14–24). MBP-, PLP-, and MOG-induced EAE all have been prevented or treated postdisease induction by administration of MBP, PLP, or MOG peptides, respectively. These experiments suggest that it may be possible to develop a therapy for human MS based on T cell epitope-containing myelin peptides.

In several different experimental systems, regulatory cells specific for one Ag have been observed to down-regulate responsiveness to a second Ag in a bystander fashion (20, 25–28). In all of these experiments, pretreatment with the first or tolerizing Ag induced a suppressor or Th2 response, and this population was then able to negatively influence the outcome of the subsequent priming event to the second Ag. This down-regulation of priming appears to depend on recognition of the tolerizing Ag and the priming Ag occurring in the same microenvironment, or on the surface of the same APC, a phenomenon known as linked recognition. In the
EAE model, the tolerizing Ag has been either an exogenous, non-self protein (26) or a myelin protein distinct from that used to induce EAE (20, 25). In either case, subsequent disease induction with the priming myelin Ag was blocked. However, in the human autoimmune disease situation, the opportunity to intervene occurs in the postpriming phase of the disease. It is therefore important to determine whether the tolerance that can be induced to single Ags after disease induction would mediate bystander effects and down-regulate other preprimed encephalitogenic responses via a linked recognition type of event.

EAE induced with a homogenate of spinal cord has been used as a model of an antigenically complex disease in which peptide therapy can be assessed. Indeed, it has been shown that a peptide derived from a single myelin protein will treat EAE in this model (17, 24, and D. Smilek, unpublished observation). However, it could not be demonstrated that more than one myelin component in the homogenate had encephalitogenic activity in the mouse strains used in these experiments. Recently, adoptive transfer models of EAE have been used in an attempt to address the question as well, and the conclusions have been contradictory (21, 29).

We describe in this work a novel EAE model in which two different myelin proteins, MBP and MOG, administered in combination, induce a more severe form of EAE than that generated by either protein alone. In this model, lymphocytes specific for each of the two proteins appear to act together to produce the disease state. Furthermore, we show that this disease can be reduced significantly postinduction by parenteral administration of myelin epitope-containing peptides, either from one or both of the disease-inducing proteins. The results suggest that it may be possible to down-regulate a complex autoimmune disease by parenteral administration of T cell epitope peptides, even if the T cell response to one of the initiating Ags in that disease has been identified and targeted for therapy.

Materials and Methods

**Mice**

Experiments were initiated with 8- to 12-wk-old female (PL/J × SJL/J)F1 mice purchased from The Jackson Laboratory (Bar Harbor, ME), and housed in microisolator cages in the animal facility at ImmunoLogic Pharmaceutical (Waltham, MA).

**Peptides and proteins**

MBP Ac1–11[4Y] (ASQYRPSQRHG), and the peptides corresponding to human MOG amino acids 1–20, 11–30, 21–40, 31–50, 41–60, 51–70, 61–80, 71–90, 81–100, 91–110, 101–120, and 111–130 were prepared by standard 9-fluorenylmethoxycarbonyl chemistry. The peptide corresponding to human MOG amino acids 41–60 was also synthesized using tert-butoxycarbonyl chemistry, which resulted in higher peptide yields. Peptides were purified by reverse-phase HPLC (>80%), and amino acid composition of each peptide was confirmed by amino acid analysis.

A truncated form of human recombinant MOG (thr-MOG), spanning the first 120 amino acids lacking the transmembrane regions, and containing a His 6 segment, was purified from supernatant of insect Hi-5 cells infected with high-titered stock of plaque-purified baculovirus, was purified from supernatant of insect Hi-5 cells containing a His 6 segment, was purified from supernatant of insect Hi-5 cells infected with high-titered stock of plaque-purified baculovirus. The His-tagged protein was recognized by the antiserum against human MOG. The protein was purified from supernatant of insect Hi-5 cells infected with high-titered stock of plaque-purified baculovirus.

**MBP was prepared from guinea pig (gpMBP) spinal cords (17, 24, and D. Smilek, unpublished observation). MBP was prepared from guinea pig (gpMBP) spinal cords (17, 24, and D. Smilek, unpublished observation).**

**Statistical analysis**

Significant differences in daily MCS and disease index were determined by ANOVA ($p \leq 0.001$) or repeated measures ANOVA ($p \leq 0.001$), respectively, with post hoc comparisons by the Scheffé’s F test ($p \leq 0.05$).

**Results**

**Induction of EAE and treatment with peptide**

EAE was induced by s.c. immunization at the base of the tail with 75 to 200 μg gpMBP, or 100 to 400 μg thr-MOG, or the combination of both 75 μg gpMBP and 100 μg thr-MOG, emulsified in CFA (Life Technologies, Grand Island, NY). Emulsions were supplemented with 400 μg Mycobacterium tuberculosis H37Ra (Difco, Detroit, MI) per mouse. Three hundred nanograms of pertussis toxin (List Biologic Laboratories, Campbell, CA) was administered i.v. at the time of immunization, and again 48 h later.

Therapeutic peptides or PBS were administered at the times indicated in the figure legends. Lyophilized peptides were reconstituted in PBS (Life Technologies, Grand Island, NY) to the appropriate concentration and were administered i.v. in a volume of 0.2 ml. A total of 250 nmol equals 332 μg of MBP Ac1–11[4Y] or 589 μg of MOG 41–60.

Mice were scored daily for clinical signs of disease based on the following scale: 0, normal; 1, limp tail; 2, partial hind limb paralysis or ataxic gait; 3, complete hind limb paralysis; 4, partial or complete forelimb paralysis; and 5, moribund or dead. Food was made accessible to immobile animals, and moribund animals were sacrificed on the third day with a score of 5. A score of 5 was included in the calculation of daily mean clinical score only during periods of morbidity and on day of death, and was thereafter excluded from these calculations. In some experiments, mice were weighed daily, at the time their clinical scores were assessed.

Several parameters of disease were examined to evaluate the severity of EAE and the success of peptide therapy: mean clinical score (MCS), the mean of clinical scores for all mice within a group on a given day; mean day of onset, the mean day that affected mice within a group first developed clinical signs of disease; percentage of mortality, the number of mice within a group that died or were sacrificed as a result of severe EAE, expressed as a percentage of the starting number of mice in that group; percentage of incidence, the number of mice within a group that developed a clinical score of 1 or greater, expressed as a percentage of the starting number of mice in that group; mean maximum severity, the mean of the maximum daily score that each mouse in a group developed over the course of the experiment; and disease index, the sum of the daily mean clinical scores for a group over a given number of days, divided by that number of days. The percentage of observations with a score of 0 or percentage of observations with a score ≥3 was calculated as the total number of such observations over a given period of time, divided by the total number of live animal observations recorded in that time period.

**T cell proliferation assays**

Draining lymph nodes (LN; inguinal, popliteal, and paratrophic) were removed from mice 10 days after s.c. immunization with myelin proteins, as described above, in CFA supplemented with 400 μg M. tuberculosis H37Ra. Tissue was forced through fine wire mesh to form single cell suspensions into RPMI 1640 supplemented with 10% heat-inactivated FCS, penicillin-streptomycin, l-glutamine, and 5 × 10^-3 M 2-ME. The LN cell suspensions were cultured in 96-well flat-bottom microtiter plates at 4 × 10^5 cells/well with the peptide or protein Ags listed in the figure legends. Controls included cells stimulated with media, Con A, and purified derivative of mycobacteria. Proliferation assay microwells were pulsed on day 4 with 1 μCi of [3H]TdR for 12 h. Cells were harvested onto glass fiber filters, and [3H]TdR incorporation was assessed by scintillation counting. The stimulation index was calculated by dividing the number of counts incorporated in response to Ag by the number of counts incorporated in the absence of any Ag.

**Statistical analysis**

Significant differences in daily MCS and disease index were determined by ANOVA ($p \leq 0.001$) or repeated measures ANOVA ($p \leq 0.001$), respectively, with post hoc comparisons by the Scheffé’s F test ($p \leq 0.05$). Differences in mean maximum severity were determined using the Mann-Whitney U test. Differences in mean day of onset were determined using Student’s t test. Differences in all percentages were determined using the χ² test, or Fisher’s exact test when cell sizes lower than 5 were observed. Because all non-ANOVA comparisons involved repeated pairwise comparisons within a data set, a lower p value ($p < 0.01$) was required for significance. Data analysis was performed using Abacus Concepts, Statview software (Abacus Concepts, Berkeley, CA).
induced by the combination of 50 μg thr-MOG plus 50 μg gpMBP was more severe than that induced by 200 μg of either Ag alone. When mice were immunized with suboptimal doses of either thr-MOG or gpMBP alone, the contribution of the other to the combination disease was made quite apparent (Fig. 1B). Table I illustrates the higher incidence, severity, and mortality that resulted from immunization with 100 μg thr-MOG plus 75 μg gpMBP compared to immunization with either of those Ags alone, indicating that reactivity to both components contributed to the underlying pathology of the combination disease process.

Table I also confirms, in additional experiments, that the severity of the disease induced by the combination of MOG plus MBP exceeded that induced by the same total dose of either immunizing Ag alone. The higher incidence associated with gpMBP EAE and the earlier onset associated with thr-MOG EAE appear to combine to produce a higher disease index in the thr-MOG plus gpMBP EAE than that found in disease induced with 200 μg of either protein alone. This disease index is not simply a reflection of the significantly higher mortality that accompanied the combination disease, as clinical scores of mice that die are dropped from the calculation of disease index after the day of death.

The disease induced by the combination of Ags displayed a relapsing/progressive character. Mice that survived the unusually high mortality associated with the acute phase of this disease went on to demonstrate remissions and relapses of variable duration, as assessed by both disease score and animal weight. Clinical scores and weights from three representative animals are illustrated in Figure 2, A–C. At disease onset, increases in symptom scores were always accompanied by substantial decreases in weight. Although lost weight was recovered within 2 wk, indicating a remission period, disease scores showed varied improvement from animal to animal in the same time frame. Eventual progression to persistently high disease scores was observed in 19 of 28 surviving mice that were followed for 45 days postimmunization. Continued fluctuations in weight revealed additional disease remissions and relapses in these mice that were not evident from examination of clinical scores alone.

The recurring weight fluctuations in the combination disease resemble the relapsing-remitting weight changes that accompany the disease induced with optimal doses of gpMBP (11). In contrast, disease induced with thr-MOG is monophasic in this time period (23, and data not shown). The observation that mice with MOG plus MBP EAE seldom reached baseline scores of 0 in the remission phase suggests a more aggressive development of persistent...

### Table I. Severity of EAE induced with thr-MOG, gpMBP, or MOG plus MBP

<table>
<thead>
<tr>
<th>Immunizing Ag</th>
<th>n</th>
<th>% Incidence</th>
<th>Mean Day of Onset</th>
<th>Disease Index</th>
<th>MMS</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 μg MBP + 100 μg MOG</td>
<td>59</td>
<td>100</td>
<td>12.6</td>
<td>2.3</td>
<td>4.5</td>
<td>56</td>
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<tr>
<td>75 μg MBP</td>
<td>50</td>
<td>78</td>
<td>15.9</td>
<td>0.7</td>
<td>2.5</td>
<td>16</td>
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<td>200 μg MBP</td>
<td>38</td>
<td>97</td>
<td>14.0</td>
<td>1.2</td>
<td>3.3</td>
<td>18</td>
</tr>
<tr>
<td>100 μg MOG</td>
<td>52</td>
<td>60</td>
<td>12.6</td>
<td>0.6</td>
<td>1.8</td>
<td>8</td>
</tr>
<tr>
<td>200 μg MOG</td>
<td>25</td>
<td>84</td>
<td>12.1</td>
<td>1.6</td>
<td>2.8</td>
<td>4</td>
</tr>
<tr>
<td>400 μg MOG</td>
<td>40</td>
<td>100</td>
<td>11.0</td>
<td>2.0</td>
<td>4.1</td>
<td>32</td>
</tr>
</tbody>
</table>

* (PL/J × SJL) F1 mice were immunized for induction of EAE with the doses of the proteins indicated and observed for 25 days. Clinical parameters and statistics were calculated for days 7 through 25, as described in Materials and Methods. Calculation of mean day of onset includes only those mice that developed disease by day 25. The data are compiled from 15 individual experiments and do not include the data illustrated in Figure 1. The total number of mice in each group is indicated as n. Not all groups were represented in all experiments. Underlined numbers are values different from the MBP plus MOG-immunized group (p < 0.01) by Fisher’s exact test (percent incidence and mortality), Student’s t test (mean day of onset), or the Mann-Whitney U test (mean maximum severity). Statistical analysis of disease index was by repeated measures ANOVA across all scores for all groups during the observation period. Underlined values in this category indicate groups different from PBS with p < 0.05 in the Scheffé procedure for post hoc comparisons.
neurologic deficit in this combination disease than that seen in gpMBP-induced EAE.

T cell responsiveness in mice immunized with a combination of MOG and MBP

The results above suggest that both myelin proteins induce an immune response in MOG plus MBP EAE. Before the specificity of this response could be assessed, MOG T cell epitopes had to be defined in the (PL/J × SJL)F1 strain. Peptide-specific proliferation of LN cells from thr-MOG-primed mice was examined using a panel of MOG peptides 20 amino acids long, spanning the thr-MOG sequence and overlapping each other by 10 amino acids. Figure 3A illustrates that peptides from at least three separate regions of the MOG protein induced T cell proliferation. Those regions were located within amino acids 1–20, 31–60, and 81–110. The same regions were identified when IL-2 production was used as an assay of T cell responsiveness (data not shown).

Each of the three regions of reactivity corresponds to a previously reported T cell epitope region in either the PL/J (MOG 31–60; 31) or SJL (MOG 1–20 and 81–110; 12, 23) mouse strains. The presence of the PL/J H-2u MHC in the (PL/J × SJL)F1 has been shown to reduce the contribution of H-2s molecules to Ag presentation in the F1 (32), and so it was predicted that the H-2u-restricted response to the 31–60 region would predominate. The dominant response observed to the 1–20 peptide (Fig. 3A) was unexpected. Examination of the amino acid sequence of this peptide reveals three substitutions in the human sequence at residues 9, 10, and 20 compared with native mouse MOG (Gly, Tyr in mouse to Arg, His in human at 9 and 10; Ala in mouse to Val in human at 20) (33, 34). Figure 3B illustrates that the 1–20 peptide-specific response in mice immunized with thr-MOG did not cross-react on the murine MOG 1–20 sequence. Therefore, the dominant response to human MOG 1–20 was not an autoimmune response in the mouse, but rather a response to the non-self human MOG substitutions.

Mice immunized with the combination of thr-MOG plus gpMBP mounted proliferative responses to both proteins (Fig. 4A). These data confirm that both proteins induce T cell reactivity in this model system. The dominant epitopes in these responses matched those found in responses to either immunizing Ag used alone (Fig. 4, B and C).

FIGURE 2. Disease course in individual animals with MOG plus MBP EAE. A–C, EAE was induced by immunization with a combination of 75 μg gpMBP and 100 μg thr-MOG. Clinical scores (solid diamonds) and animal weights (open diamonds) are plotted for three representative individual mice that survived the first acute phase of the disease.

FIGURE 3. MOG peptide responsiveness in (PL/J × SJL)F1 mice. LN proliferation was assessed 10 days after immunization with thr-MOG in CFA. A, Pooled LN cell suspensions from 10 mice were cultured in vitro with 100 μg/ml (solid bar), 10 μg/ml (hatched bar), or 1 μg/ml (open bar) of the indicated human sequence MOG peptide, thr-MOG protein, or media. Results are expressed as stimulation index calculated from the means of triplicate wells. Media control wells produced 5,565 ± 3,396 cpm. Similar results were obtained in a second experiment. B, Pooled LN cell suspensions from five mice were cultured in vitro with the indicated doses of thr-MOG (diamonds), human sequence MOG 1–20 (squares), murine sequence MOG 1–20 (triangles), or media. Results are expressed as stimulation index calculated from the means of triplicate wells. Media control wells produced 19,098 ± 1,949 cpm. Similar results were obtained in two additional experiments.
Reactivity to thr-MOG was dominated by the high response to the human sequence MOG 1–20 peptide (again with no cross-reactivity on murine 1–20; data not shown), with small, but detectable (stimulation index greater than 2) responses to peptides in the 31–60 region. The response to gpMBP was directed primarily to the acetylated amino terminus of that molecule (35, 36), with no detectable reactivity to the minor determinant reported in the 31–47 region (16) or the H-2s-restricted determinant in the 89–101 sequence (14, 37). Therefore, immunization with the two Ags together induces T cells of comparable specificity with those induced by immunization with each Ag alone.

In addition to these T cell responses, mice with the combination disease developed serum IgG titers greater than 1/10,000 to both MOG and MBP by day 15 after disease induction, while control mice immunized with either protein alone developed Ab titers only to the immunizing Ag (data not shown).

**Treatment of EAE induced with MOG in (PL/J × SJL)F₂**

It was of interest to evaluate the ability of peptides derived from both thr-MOG and gpMBP to treat the symptoms associated with the combination disease. Although the MOG peptide 91–110 has been shown to be effective in treating MOG-induced EAE in SJL mice (23), treatment of MOG-induced EAE has not been studied previously in (PL/J × SJL)F₂ mice. The peptides identified as the major epitopes of thr-MOG in the studies described above were used to treat disease induced with thr-MOG in (PL/J × SJL)F₂ mice. Figure 5A shows that MOG 41–60 effectively treated thr-MOG-induced EAE, while the other major epitope peptides did not. The success of treatment with the 41–60 peptide was somewhat variable, with Figure 5B illustrating a less optimal therapeutic effect. Five experiments produced treatment effects similar to those seen in Figure 5, while two additional experiments demonstrated no discernible benefit from MOG 41–60 using the same protocol. The success of therapy in this disease appeared to be more dependent on the severity of disease in the control group (the most severe disease predicting a poor treatment outcome), rather than the number or schedule of the peptide injections. The MBP peptide Ac1–11[4Y], although effective at preventing gpMBP-induced EAE in this strain (19, 22), did not have a measurable effect on thr-MOG-induced disease in any experiment (Fig. 5B).

**Treatment of EAE induced with MOG plus MBP**

Intravenous administration of the MBP peptide Ac1–11[4Y] and the MOG peptide 41–60 together was effective in treating MOG plus MBP EAE. Figure 6A illustrates the reduction in daily MCS obtained upon administration of 125 nmol of each of these peptides in combination, starting 9 days after the disease-inducing immunization with both myelin proteins in adjuvant. Data from three experiments, including the experiment illustrated in Figure 6, were compiled for the purpose of statistical analysis. The daily clinical scores of mice in the group treated with the MBP/MOG peptide combination were significantly different from those of the PBS-treated controls on 15 of the 25 observation days following the initiation of peptide treatments. Table II illustrates that reductions in clinical incidence, disease index, and mean maximum score in this peptide-treated group were all statistically significant when compared with the PBS-treated control group. Additional parameters to assess disease burden included the calculation of percentage of observations with high clinical scores (3 or greater) or with clinical scores of 0 (no symptoms). These reveal a dramatic and significant reduction in the number of days with severe disease, with a corresponding increase in the number of disease-free days, in MBP/MOG peptide-treated mice.

The MBP Ac1–11[4Y] peptide alone was also capable of treating the combination disease, although with a lower therapeutic effect than was seen with the combined peptides. When 125 nmol of MBP Ac1–11[4Y] was administered to mice with MOG plus MBP EAE, daily mean clinical scores were reduced compared with PBS-treated controls. However, these reductions were less than those seen when both MBP and MOG peptides were administered together (Fig. 6A). In the statistical analysis, mice treated with the 125 nmol dose of MBP Ac1–11[4Y] alone had daily clinical scores significantly different from PBS-treated controls on only 7 of the 25 observation days following the initiation of peptide treatments. This dose of MBP Ac1–11[4Y] alone did not produce statistically significant reductions in clinical incidence, disease index, or mean maximum score compared with PBS-treated controls (Table II). These results suggest that both the MBP Ac1–11[4Y] and MOG 41–60 peptides contributed to the treatment effect observed when 125
nmol of each peptide was used in combination to treat MOG plus MBP EAE.

To adequately compare the efficacy of treatment with a single peptide with treatment with a peptide combination, however, it is important to consider the total peptide dose, as we have observed that peptide treatment of MBP-induced EAE is dose dependent (22, and data not shown). Figure 6 illustrates the effects of administration of either the MOG 41–60 or the MBP Ac1–11[4Y] peptide alone when the dose of peptide was increased to 250 nmol (same total nmol dose as the MBP Ac1–11[4Y] + MOG 41–60 peptide at 125 nmol each). Daily MCS obtained with the 250 nmol dose of the MBP peptide alone were similar to those obtained using the combination of 125 nmol each of MBP and MOG peptides, with no statistically significant differences between these two groups on any of the 25 observation days following the initiation of peptide treatment. In Table II, it can be seen that all measures of disease were reduced in mice treated with 250 nmol of MBP Ac1–11[4Y] compared with 125 nmol of the same peptide, indicating a dose-dependent therapeutic effect. Although treatment with 250 nmol of MOG 41–60 alone significantly reduced the number of days with severe disease, with a corresponding increase in disease-free days, significant reductions in clinical incidence, overall disease index, and mean maximum score were not apparent (Table II).

All of the same disease outcome measures that were reduced by the MBP/MOG combination peptide treatment were also significantly impacted by administration of 250 nmol of MBP Ac1–11[4Y] alone. Although mortality was reduced by both of these
treatment protocols, the reductions were not statistically significant in either case. In addition, the day of disease onset in mice that did develop symptoms was not delayed significantly by either protocol (data not shown). Thus, the MBP peptide alone was capable of reducing the incidence and severity of EAE induced with a combination of two myelin proteins in a dose-dependent manner.

**Discussion**

In this study, we describe a novel model of EAE induced by a combination of two myelin proteins, MOG and MBP, and investigate the capacity of peptides derived from MOG and MBP to treat this multicomponent disease. As the pathogenesis of human MS is likely to be driven by immune responses to multiple myelin proteins, a murine EAE model involving defined but multiple myelin proteins represents an invaluable tool for the study of therapeutic effects of individual peptides. We found that EAE induced by the combination of MOG and MBP was more severe than that induced with either protein alone, indicating a role for both proteins in the pathogenesis of the disease. MOG plus MBP EAE was characterized by rapid onset of severe disease, associated with a high mortality and a relapsing/progressive neurologic deficit in surviving mice. Ab and LN proliferative responses to both proteins were observed during the course of disease, with recognition of the same dominant T cell epitopes identified by immunization with either Ag alone.

An interesting feature of the disease induced by the combination of thr-MOG plus pMBP was that severity by any measure was greater than would be produced by the same total dose of either Ag alone. A synergy of effects is inferred. It previously has been shown that Ab to MOG can induce demyelination in vivo when administered to animals that have been immunized with MBP, or that have received T cells specific for MBP or MOG (38–40). In the new EAE model described in this work, it is possible that the primary contribution of the MOG immunization is the induction of anti-MOG Abs that act in combination with T cells specific for MBP to cause the severe, and often fatal, disease that was observed. Alternatively, the pathogenic potential of two nonoverlapping T cell populations may be greater than that of a larger single population.

The primary goal of this study was to determine the capacity of immunodominant MOG and MBP peptides, administered together or individually, to treat EAE induced with the MOG plus MBP Ag combination. This required some initial studies in the MOG EAE system, as MOG peptides have not been assessed previously for treatment of MOG-induced EAE in (PL/J × SJL)F1 mice, although the MOG 91–110 peptide has been shown to reduce disease in SJL mice (23). The MOG T cell epitopes identified using a T cell proliferation assay and thr-MOG-immunized F1 mice corresponded to epitopes previously identified in the parent strains (12, 23, 31). The dominance of the MOG 1–20 epitope was surprising, as it has been reported previously to be an epitope in SJL (23, 31, and the H-2d of the PL/J parent was expected to dictate the dominant epitopes in the F1 (32). Further analysis of this response in thr-MOG-primed mice revealed no cross-reaction to the mouse MOG 1–20 sequence, which differs from human at residues 9, 10, and 20. The response to human MOG 1–20 therefore should have no pathologic consequences, consistent with the observation that human MOG 1–20 did not treat thr-MOG-induced EAE (Fig. 5) and, like the corresponding mouse MOG sequence (12), was not encephalitogenic (B. Devaux and D. Smilek, unpublished observation).

Of the remaining antigenic MOG peptides identified in the F1 (Fig. 3), MOG 41–60, but not 31–50, was used successfully to treat MOG-induced EAE in this strain. An encephalitogenic H-2d-restricted determinant has been reported previously by Kerlero de Rosbo et al. (31) in the 35–55 region of MOG, although it was proposed that the T cell epitope resided in the amino-terminal half of that peptide. It is not clear why the human sequence MOG 31–50 peptide used in the current study did not confer protection from disease; a difference in the human to mouse MOG sequences in that region (Pro to Ser at residue 42) may again be significant. Finally, although MOG 91–110 reduced MOG EAE in SJL mice, this peptide was not effective in treating MOG-induced disease in (PL/J × SJL)F1 mice. This H-2d-restricted epitope is a minor one in the F1 strain, confirming earlier observations in the MBP disease model that major epitopes are relatively better therapeutics than minor epitopes (16).

With this knowledge, it was possible to determine the capacity of MOG and MBP peptides, administered together or individually, to treat EAE induced with the MOG plus MBP Ag combination. Based on the data discussed above, the MOG 41–60 peptide was chosen for these studies. With respect to MBP peptides, both Ac1–11 and Ac1–11[4Y] have been shown to be effective in treating MBP-induced EAE (19, 22). MBP Ac1–11 is a dominant T cell epitope in the PL/J and (PL/J × SJL)F1 mouse strains (35, 36). The substituted MBP peptide Ac1–11[4Y], in which tyrosine replaces lysine at residue 4, is a strong agonist peptide, demonstrating improved antigenicity compared with Ac1–11 (41, 42), most likely because the tyrosine at residue 4 enhances binding of the peptide to the I-Aa molecule (42, 43). Of these two peptides, MBP Ac1–
11[4Y] was selected for these experiments because it is also more potent as a therapeutic (19, 22), and was expected to be more effective in treating the severe MOG plus MBP EAE.

A combination of the two therapeutic peptides, MBP Ac1–11[4Y] and MOG 41–60, successfully treated MOG plus MBP EAE, with statistically significant reductions in daily and maximum clinical scores, clinical incidence, and disease index. Both peptides appeared to contribute to the optimal therapeutic effect, because MBP Ac1–11[4Y] alone, administered at the same 125 nmol dose used in the combination, was not as effective as the combination of the two peptides. This result provides further evidence that T lymphocytes responsive to both MBP and MOG are involved in the propagation of EAE induced with the combination of these two myelin proteins. When the dose of MBP Ac1–11[4Y] was increased to 250 nmol, therapeutic benefit was increased, and approached that observed with the combination of MOG and MBP peptides at 125 nmol each. Even this higher dose of MBP Ac1–11[4Y] was not effective in treating EAE induced with MOG alone, indicating that the treatment effect of Ac1–11[4Y] in MOG plus MBP disease was not due to cross-reactivity between these two epitopes at the T cell level.

The most significant benefit for any of the peptide treatment groups with the combination disease was a reduction in disease severity, assessed by a number of different measurements. Although clinical incidence was not reduced to zero in the PBS control group. Reductions in disease-associated mortality, although obvious, were not statistically significant in any peptide treatment group. The mean day of death was not delayed by peptide treatment (range of mean 14.7 to 17.2), generally occurring 3 to 4 days after disease onset. Despite the persistence of deaths in peptide treatment groups, measures of clinical severity showed the same statistically significant improvements over PBS-treated controls whether these measures were calculated with a score of 5 recorded on the day of death only, or retained through the length of the experiment.

The results of these studies suggest that a sufficiently high dose of a peptide derived from one myelin protein can effectively treat established EAE that results from an immune response to multiple myelin components, when the peptide is derived from one of the immunizing myelin components. The failure to completely reduce the combination disease is consistent with the results of Brocke et al. (21) in the treatment of disease induced by adoptive transfer of two different T cell clones. The mechanism of the therapeutic effect we have generated is not clear. It is significant, in this respect, that the MBP peptide was not capable of alleviating EAE induced only with MOG. This stands in contrast to the findings of Nicholson et al. (20) and Al-Sabbagh et al. (25), in which preimmunization or feeding with one myelin Ag led to protection from EAE induced with a second unrelated myelin Ag, due to the generation of regulatory Th2 cells capable of influencing the outcome of the disease-inducing immunization by virtue of colocalization to the CNS. Either Th2 cells are not induced utilizing the treatment protocol used in the current studies, or such cells do not have an impact on encephalitogenic responses postpriming.

It is possible that the treatment protocol used in the current studies produces a state of anergy in the targeted cells (16, 44, 45). This does not rule out a role for suppression through linked recognition, as energized cells have been shown to serve as cytokine sinks for concurrent responses (46). The observation that the MOG plus MBP disease itself was significantly more severe than that induced by the individual components makes it difficult to distinguish the lower level of disease in successfully treated animals, from EAE induced with only one component Ag. Removal of the contribution of a single Ag to the synergistic processes in disease induction may be the most significant impact of this therapy, and such an effect may also prove significant in human disease.

In comparison with MBP Ac1–11[4Y], the MOG 41–60 peptide demonstrated an inferior capacity to treat MOG plus MBP EAE when administered alone at a dose of 250 nmol. There are several potential explanations for the difference between the therapeutic potentials of these two peptides. It is possible that the MOG-specific T cell component contributes substantially less to the generation of the disease pathology than does the MBP-induced component, making treatment of the combination disease less potent with this Ag. Alternatively, 250 nmol of MOG 41–60 might not be a sufficiently high dose to achieve the treatment effect observed with 250 nmol of MBP Ac1–11[4Y]. It has been shown that effectiveness of MBP peptide therapy is highly dependent on the dose of peptide used for each injection, and that the strong agonist peptide MBP Ac1–11[4Y] is at least 100-fold more potent than MBP Ac1–11, both as a stimulator of in vitro proliferation and as a therapeutic in vivo (19, 22, 41). In support of this hypothesis, the LN proliferative response to MBP Ac1–11[4Y] was clearly better than the response to MOG 41–60 in MOG plus MBP-immunized mice (Fig. 4).

A marmoset model of MOG-induced EAE was recently described (47), in which disease onset could be delayed by administration of soluble MOG, using a treatment schedule very similar to the studies reported in this work. Despite the protective effect of MOG administration, a striking finding in the marmoset model was the onset of a very severe, and even fatal, episode of EAE within 1 wk of therapy completion. This exacerbation was not observed in control animals. The MOG therapy-treated animals showed elevated Ab titers to several different peptides of MOG. We have not observed such a disease exacerbation following peptide treatment in either the SJL or the (PL/J × SJL)F1 murine models of MOG-induced disease. While it is true that mice with MOG plus MBP-induced disease did exhibit a rise in daily MCS after MOG peptide treatments were withheld (generally within 3 to 15 days after the last peptide injection), these disease relapses were never observed to be more severe than ongoing disease in the PBS-treated control group. It is possible that treatment with the MOG protein is more likely to boost titers of pathogenic autoantibodies than is administration of T cell epitope-containing peptides.

In summary, we describe a new model of EAE induced with a combination of the myelin proteins MBP and MOG, in which clinical severity is greater than predicted by immunization with either Ag alone. This disease can be treated postinduction with a combination of MBP and MOG peptides, which individually have been shown to be therapeutic for EAE induced with either MBP or MOG, respectively. Both peptides appear to be necessary for optimal therapy of disease when administered at a limiting dose. However, a single MBP peptide administered at a higher dose also provides significant reductions in disease severity. This is the first demonstration that parenteral administration of a T cell epitope peptide can suppress disease symptoms in an EAE model in which it is clear that reactivity to more than one myelin component plays a role in generation of the disease pathology. These results support the hypothesis that a human autoimmune disease, such as MS, may be down-regulated by administration of peptides derived from a single myelin protein, despite the presence of an immune response to multiple myelin proteins.

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References


