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Intravenous synthetic peptide MBP8298 delayed disease progression in an HLA Class II-defined cohort of patients with progressive multiple sclerosis: results of a 24-month double-blind placebo-controlled clinical trial and 5 years of follow-up treatment

K. G. Warren[a], I. Catz[a], L. Z. Ferenczi[b] and M. J. Krantz[b]

[a]*Multiple Sclerosis Patient Care and Research Clinic, Division of Neurology, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada; and* [b]*BioMS Medical Corp., Edmonton, Alberta, Canada*

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MBP8298 is a synthetic peptide with a sequence corresponding to amino acid residues 82–98 of human myelin basic protein (DENPVVHFFKNIVTPRT). It represents the immunodominant target for both B cells and T cells in multiple sclerosis (MS) patients with HLA haplotype DR2. Its administration in accordance with the principle of high dose tolerance results in long-term suppression of anti-myelin basic protein (MBP) autoantibody levels in the cerebrospinal fluid (CSF) of a large fraction of progressive MS patients. MBP8298 was evaluated in a 24-month placebo-controlled double-blinded Phase II clinical trial in 32 patients with progressive MS. The objective was to assess the clinical efficacy of 500 mg of MBP8298 administered intravenously every 6 months, as measured by changes in Expanded Disability Status Scale (EDSS) scores. Contingency analysis for all patients at 24 months showed no significant difference between MBP8298 and placebo-treatments ($n = 32$, $P = 0.29$). Contingency analysis in an HLA Class II defined subgroup showed a statistically significant benefit of MBP8298 treatment compared with placebo in patients with HLA haplotypes DR2 and/or DR4 ($n = 20$, $P = 0.01$). Long-term follow-up treatment and assessment of patients in this responder group showed a median time to progression of 78 months for MBP8298 treated patients compared with 18 months for placebo-treatment (Kaplan–Meier analysis, $P = 0.004$; relative rate of progression = 0.23). Anti-MBP autoantibody levels in the CSF of most MBP8298 treated patients were suppressed, but antibody suppression was not predictive of clinical benefit. Anti-MBP autoantibodies that reappeared in the CSF of one patient at 36 months, whilst under treatment with MBP8298, were not reactive with the MBP8298 peptide *in vitro*. The identification of a responder subgroup (62.5% of the patients in this study) enables a more efficient design of a large confirmatory clinical trial of MBP8298. The probability that patients with other less common HLA-DR haplotypes will respond to this treatment should not be ignored.

Introduction

Multiple sclerosis (MS) is a chronic, inflammatory disease of the central nervous system, with onset in early adulthood. Most commonly, initial stages of the disease involve acute attacks followed by full or partial recovery [1,2]. In the majority of patients, unpredictable episodes of paralysis and visual and/or other sensory disturbances are eventually replaced by progressive deterioration with loss of ambulation, upper body function and autonomic functions such as bowel and bladder control. MS is generally considered an autoimmune disease triggered by environmental factors in genetically susceptible individuals. Disease modifying drugs have become available in recent years, but an unmet need for new treatments remains because the existing drugs have not shown a clinically and statistically significant impact on disease progression.

Autoimmune diseases such as MS involve the aberrant activation of T cells and/or B cells with epitope specificities that are ordinarily excluded from the repertoire as the result of self-tolerance which is established during early development of the immune system [3]. It is reasonable to expect that restoration of immunologic tolerance with respect to these inappropriate specificities would have an impact on the autoimmune disease

Correspondence: Ingrid Catz, MS clinic, University of Alberta, 9-101 Clinical Science Building, Edmonton, Alberta, Canada, T6G 2G3 (tel.: 780 492-6409; fax: 780 492-1365; e-mail: ingrid.catz@ualberta.ca).

process. Induction of tolerance by administration of high doses of soluble myelin antigens or peptides containing immunodominant epitopes has been an effective treatment in animal models of MS many of which employ inbred rodent strains [4–9]. In contrast, MS is a complex disease, highly variable and unpredictable, and the reported specificities of undesired autoreactive T cells in humans involve several target antigens within the myelin sheath and different epitope specificities within these antigens [10]. B cells and autoantibodies are also thought to be involved in the pathogenesis of MS [11,12].

In practice, effective treatment of human autoimmune diseases by tolerance induction with high dose soluble peptide is limited by the diversity of immune specificities involved. Nevertheless, an immunodominant disease-associated T-cell epitope in the central region of the myelin basic protein (MBP) molecule has been recognized in patients with HLA haplotype DR2 [13–15]. This immune response genetic marker is associated with increased susceptibility to MS and it is found in 50–70% of MS patients, compared with 20–30% of individuals in the general population [16–19]. The same centrally located MBP domain is an immunodominant target of autoantibodies in the cerebrospinal fluid (CSF) and central nervous system tissue [20–22] of MS patients. It was further demonstrated that key contact residues of the B- and T-cell epitopes are identical [23]. It has been suggested that normal proteolytic processing of MBP destroys this epitope, thus limiting the establishment of central tolerance which could predispose autoimmune attack in individuals who present this peptide on high affinity HLA molecules [24,25]. The identity of the B- and T-cell epitope defined by residues 85–96 suggest that it may play an important role in the disease process in the majority of MS patients. Therefore, this epitope is a prime candidate for specific restoration of immunological tolerance which may have an impact on the disease process in an easily identified subset of MS patients. The MBP8298 peptide sequence includes amino acid extensions at both ends of the minimal B-cell epitope; these improve the solubility characteristics of the drug and optimize the potential for interaction with T cells [14].

Clinical studies have shown that MBP autoantibody (anti-MBP) levels in CSF rise and fall with the course of relapsing disease, but remain elevated and relatively stable in patients with primary progressive MS (PPMS) or secondary progressive MS (SPMS) [11,26,27]. CSF autoantibody levels in patients with progressive MS were therefore used as an indicator of immune tolerization as a result of treatment with MBP8298. Monitoring of CSF anti-MBP antibody was used to identify the preferred route of administration, an effective dose, the fraction of responsive patients and an effective dosing schedule for administration of MBP8298 to MS patients [26–29]. No safety issues were identified in the course of these studies. More durable suppression of anti-MBP autoantibody was reported in patients who expressed HLA molecules with high affinity for the administered MBP8298 peptide (DR2, DR4 and DR7) [29].

In the present study, we examined the effect of intravenous (iv) administration of MBP8298 synthetic peptide on clinical progression in patients with progressive MS, and its relationship to HLA-DR haplotype. Long-term clinical outcome is reported for patients who have continued to receive treatment on an ongoing compassionate use program.

Methods

Study objectives

The study objectives were to: (i) determine if iv MBP8298 had an effect on the rate of disease progression compared with placebo, (ii) determine if suppression of CSF anti-MBP levels in response to iv MBP8298 was associated with disease stabilization, and (iii) confirm safety of iv MBP8298.

Study design

This was a Phase 2 single center, double-blind, placebo-controlled clinical study of administration of synthetic peptide MBP8298 to patients with progressive MS carried out at the MS Clinic of the University of Alberta, Edmonton, Canada. The placebo-controlled study was followed by an indefinite open label compassionate use treatment which continues to provide safety and clinical data. Thirty-two patients with progressive MS were entered as placebo/peptide pairs matched as closely as possible according to gender, age, disease severity and CSF anti-MBP levels. This led to two closely matched groups of 16 patients. MBP8298 was administered as a 500 mg dose of soluble synthetic peptide in 10 ml normal saline. The matching placebo was 10 ml normal saline. Doses were administered according to principles of 'high zone tolerance', intravenously by slow push over 2–5 min, at 6-month intervals. All patients received the same amount of peptide (500 mg) per injection, therefore the administered dose ranged between 5 and 10 mg/kg (assuming that patients weighed between 100 and 50 kg, respectively). All patients were offered the option to continue on peptide at the end of the double-blind phase and they received injections at 4-month intervals for the

next 2 years. At that time, patients who showed clinical benefit continued to receive peptide injections at 6-month intervals. Follow-up is still in progress and the last measurement included in this report was taken at the 84 month mark.

Synthetic peptide MBP8298 was manufactured under GMP conditions by Peninsula Laboratories (San Carlos, CA, USA) and by UCB Bio Products (Braine l'Alleud, Belgium).

Patients

Thirty-two volunteers with clinically definite, magnetic resonance imaging (MRI) confirmed progressive MS were enrolled in the study. All patients gave informed consent and the study was approved by the Research Ethics Board of the University of Alberta and by the Therapeutic Drugs Division of the Government of Canada under a Compassionate Use Protocol. Inclusion criteria were: (i) clinical neurological disability because of primary or secondary progressive MS, (ii) absence of relapses in the previous 2 years, (iii) Expanded disability status scale (EDSS) scores between 3.0 and 7.5, (iv) MRI of the brain and spinal cord with lesions characteristic of MS, (v) CSF positive IgG oligoclonal banding, (vi) CSF autoantibodies to MBP and not to proteolipid protein (PLP) [30], (vii) free/bound anti-MBP ratios of ≤1.0 characteristic of progressive MS, and (viii) cognitive ability to authorize/sign an Informed Consent. Exclusion criteria included: (i) CSF autoantibodies reactive with PLP and not reactive with MBP [30], (ii) one or more relapses in the previous 2 years, (iii) a second disease, (iv) personality or cognitive impairment, or (v) inability to authorize/sign an Informed Consent.

Patients were entered without regard to the HLA DR haplotype. However, in view of the more sustained duration of MBP autoantibody suppression reported in patients with HLA DR2 and HLA DR4 [29], molecular typing of DRB1* alleles (CIRION BioPharma Research Inc., Laval, PQ, Canada) was performed after the completion of the double-blind phase.

Clinical assessments

Quantitative neurological assessments and EDSS scores were determined two times pre-entry and at 6-month intervals during treatment. The results in this report represent an independent assessment and assembly of data collected by the study investigators. Compilation of EDSS scores was performed by an independent neurologist experienced in MS clinical trials. The following safety data was recorded: (i) spontaneous patient calls to the MS Clinic, (ii) periodic scheduled calls by the research nurse to patients who participated in the study, (iii) MRI of the brain with and without Gadolinium enhancement, once during pre-entry and at the end of each year during the double-blind phase, (iv) CSF analyses every 2 months during the double-blind phase, for occurrence of meningoencephalitis (lymphocytosis or abnormal protein and/or glucose levels), and (v) blood analyses including complete blood count (CBC), electrolytes, cardiac, liver and kidney panels, every 6 months during the double-blind phase and periodically during the follow-up. In addition, information was recorded if patients complained of acute worsening of their disease, if they were clinically diagnosed as having an MS relapse, or if they requested corticosteroids.

Analysis of clinical results

Statistical methods used in this study included descriptive statistics, contingency analysis, Fisher's exact test, Kaplan–Meier survival analysis and the two-sided Mantel–Cox log rank test. Statistical software used in this study included JMP(SAS), Version 5.0.1a, StatView (SAS), Version 5.0.1 and Microsoft Excel 2002. The pair-wise entry of patients required analysis of a single sample ($n = 16$) of differences between placebo and peptide patients which failed to show statistical significance ($P = 0.29$). The hypothesis-forming aspect of this study lead to re-analysis of clinical results in two other ways for: (i) all patients ($n = 32$) at 24 months assuming complete randomization to MBP8298 or placebo, and (ii) a subgroup of patients with HLA haplotypes DR2 and/or DR4 ($n = 20$) at 24 months and at 84 months. Clinical progression was defined as a sustained (6 months) increase in EDSS scores of 1 unit if the baseline score was < 5.5 or of 0.5 units if the baseline was 5.5 or higher.

Anti-MBP levels and fine specificity

Anti-MBP autoantibody levels in CSF were determined by a solid phase radioimmunoassay (RIA) as previously described [27]. *In vitro* inhibition assays were carried out retrospectively with CSF samples from certain patients, to determine epitope specificity [22].

22-m timed walk test

Because preservation of the ability to walk is a major concern of MS patients, results of a 22-m timed walk test were recorded and evaluated, specifically: (i) time required to complete 22-m, (ii) failure to complete 22-m, and (iii) a requirement for a more advanced physical aid.

Results

Demographics

Baseline demographics of individual patients are illustrated in Table 1 and baseline characteristics for treatment groups are illustrated in Table 2. The male to female ratio was not representative of a typical MS population. The number of SPMS/PPMS patients was equally distributed between placebo and MBP8298 treatment groups. The mean disease duration and the median EDSS scores are indicative of a population with advanced disease. Relapse rates have not been reported because patients with relapses were excluded from the study. Baseline characteristics and CSF anti-MBP autoantibody levels were similar regardless of MS type. None of the patients were treated with beta interferons or with glatiramer acetate. Entry of patients as matched pairs resulted in relatively well-matched characteristics for the MBP8298 and placebo patient groups. There were no significant differences (unpaired *t*-test) regarding age ($P = 0.26$), disease duration ($P = 0.38$) and EDSS scores ($P = 0.38$) between MBP8298 and placebo-treated patients in the HLA-defined subgroup.

EDSS scores

Contingency analysis of the disease progression data at 24 months as two independent samples, assuming completely random assignment of all patients to MBP8298 or placebo, is shown in Table 3. Overall the relative rate of progression for the MBP8298 treatment group compared with the placebo-group overall was 0.56, but the difference was not significant ($P = 0.29$,

Table 1 Demographics of individual patients at baseline

Patient ID#	Gender	Age	HLA Type[a] DRB1*	HLA Type[a] DR	MS Type	Disease duration (years)	EDSS score	Cerebrospinal fluid anti-myelin basic protein level (Ru) Free	Cerebrospinal fluid anti-myelin basic protein level (Ru) Bound	Randomization
1	F	50	01,04	1,4	SP	9	6.5	5.4	6.5	Placebo
2	M	39	08,11	8,5	SP	19	3.5	5.1	6.2	Placebo
3	M	38	01,13	1,6	SP	10	6.5	8.2	11.5	MBP8298
4	M	47	11,15	5,2	SP	13	6.5	9.5	8.7	Placebo
5	M	60	03,15	3,2	PP	8	7.0	9.3	7.7	Placebo
6	F	42	04,15	4,2	SP	13	4.0	5.4	9.5	Placebo
7	M	54	04,13	4,6	PP	22	7.0	4.0	6.5	MBP8298
8	M	48	03,15	3,2	SP	20	4.0	5.1	7.6	Placebo
9	M	45	13,15	6,2	PP	4	6.5	9.2	9.1	Placebo
10	F	36	03,-	3,-	SP	11	7.5	7.5	7.5	MBP8298
11	F	60	03,13	3,6	SP	27	6.5	6.5	6.0	MBP8298
12	M	45	04,11	4,5	SP	10	6.5	5.0	11.2	Placebo
13	M	49	03,-	3,-	SP	17	6.5	9.0	5.8	Placebo
14	F	41	04,07	4,7	SP	17	6.5	5.3	11.2	MBP8298
15	M	34	03,11	3,5	PP	14	7.5	10.1	9.2	MBP8298
16	F	52	01,04	1,4	PP	11	6.5	7.3	10.6	Placebo
17	F	37	04,11	4,5	SP	12	6.5	9.4	7.1	MBP8298
18	F	40	15,16	2,2	PP	3	6.5	4.9	7.2	MBP8298
19	F	37	01,15	1,2	SP	12	5.5	6.9	10.9	Placebo
20	M	46	15,-	2,-	PP	4	6.0	9.8	5.4	MBP8298
21	M	50	03,11	3,5	PP	16	5.5	5.3	6.3	Placebo
22	M	50	11,13	5,6	PP	4	5.0	3.6	10.9	Placebo
23	M	32	03,16	3,2	SP	16	6.5	8.1	6.6	MBP8298
24	M	33	03,07	3,7	SP	5	6.0	6.5	9.3	MBP8298
25	F	39	0103,13	1,6	SP	12	6.5	9.7	7.5	Placebo
26	M	51	11,15	5,2	SP	17	6.0	6.0	7.3	Placebo
27	F	43	13,15	6,2	SP	8	6.5	5.4	4.0	MBP8298
28	F	44	13,14	6,6	SP	24	6.0	3.3	11.2	Placebo
29	M	41	0103,15	1,2	SP	9	6.0	9.7	6.1	MBP8298
30	M	46	04,08	4,8	SP	23	5.0	5.3	7.0	MBP8298
31	M	49	11,13	5,3	PP	10	6.0	5.5	11.8	MBP8298
32	F	52	11,15	5,2	SP	32	6.5	10.0	11.2	MBP8298

[a]Only the first two numbers of the DRB1* allele group were identified.

Table 2. Baseline characteristics of patient groups

Characteristic	Patient groups				
	Entire cohort	Placebo	MBP8298	HLA-DR2 and/or DR4 Placebo	HLA-DR2 and/or DR4 MBP8298
Number	32	16	16	10	10
Gender, M/F	19/13	10/6	9/7	6/4	5/5
Age (years)					
Mean	45	47	43	47	44
Range	33–60	37–60	33–58	37–60	33–54
Weight (kg)					
Mean	75	79	70	83	70
Range	52–111	52–111	52–87	62–111	52–84
MS type					
SPMS/PPMS	22/10	11/5	11/5	7/3	7/3
Disease duration (years)					
Mean	14	13	14	12	15
Range	3–32	4–24	3–32	4–20	3–32
EDSS scores					
Median	6.5	6.3	6.5	6.5	6.5
Range	3.5–7.5	3.5–7.0	5.0–7.5	4.0–7.0	5.0–7.0
Free cerebrospinal fluid (CSF) anti-myelin basic protein (MBP) autoantibody (Ru)					
Median	6.5	5.9	7.3	6.5	7.8
Range	3.2–10.1	3.3–9.7	3.2–10.1	5.2–9.3	3.2–9.8
Bound CSF anti-MBP autoantibody (Ru)					
Median	8.0	7.8	8.6	8.3	9.7
Range	4.0–11.8	4.0–11.8	5.8–11.8	6.0–11.8	5.8–11.2

Table 3 Contingency analysis of EDSS progressions at 24 months

	All patients ($n = 32$, $P = 0.29$)		DR2 and/or DR4 patients ($n = 20$, $P = 0.01$)	
Treatment	MBP8298	Placebo	MBP8298	Placebo
Not progressed	11	7	10	4
Progressed	5	9	0	6
Total	16	16	10	10

Fisher's exact test). Statistical significance was achieved in the HLA-defined subgroup ($P = 0.01$). Analysis of the same 24 month data in terms of the time to first confirmed disease progression, for the peptide and placebo-groups overall, likewise did not show statistical significance (Fig. 1); the *P*-value (Kaplan–Meier curve, two-sided Mantel–Cox log rank test) was 0.31. A *P*-value for the Kaplan–Meier curve could not be calculated for the HLA-defined subgroup at 24 months because no progression events had yet occurred in the MBP8298 treatment arm. Progression events in this group did occur during the 5 year follow-up treatment period, as shown in Fig. 2. The median time to first confirmed progression in the MBP8298 treated group was 78 months compared with 18 months for placebo-treated patients, and the *P*-value (two-sided Mantel–Cox log rank test) was 0.004 The relative rate of disease progression for the MBP8298 treatment group was 0.23.

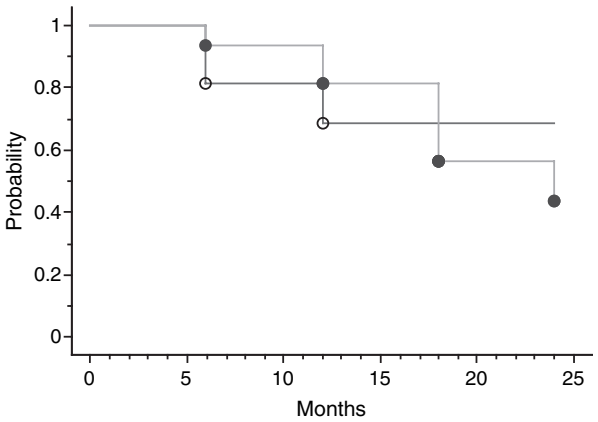

Figure 1 Time to first confirmed disease progression for all patients at 24 months. Event times for MBP8298 treated patients are shown in open symbols and for placebo-treated patients in filled symbols ($n = 32$, $P = 0.31$).

CSF anti-MBP autoantibody levels

Changes in CSF anti-MBP autoantibody levels were not predictive of clinical outcome. A substantial and durable reduction in the CSF anti-MBP autoantibody level was observed for all but three of the patients who were treated with MBP8298, regardless of HLA-DR haplotype or type of MS, whilst autoantibody levels remained elevated in the CSF of all placebo-treated patients. Peptide inhibition assays carried out

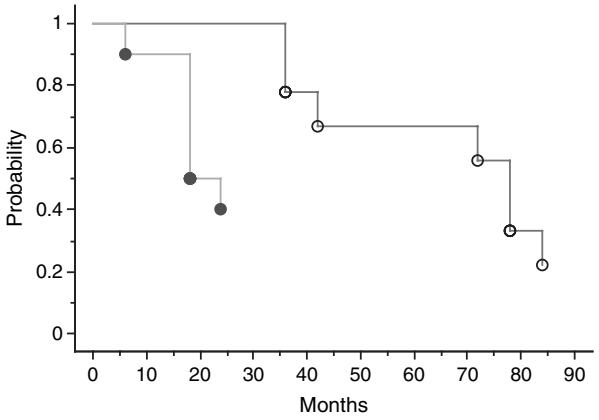

Figure 2 Time to first confirmed disease progression in a retrospectively identified group of patients with HLA-DR2 and/or DR4 haplotypes at 84 months. Event times for MBP8298 treated patients are shown in open symbols and for placebo-treated patients in filled symbols ($n = 20$, $P = 0.004$). Data from 24 to 84 months is from the open label follow-up treatment of patients from the original MBP8298 treatment group. One patient in this group was discontinued at 24 months before confirmed disease progression; therefore, data from this patient is considered censored.

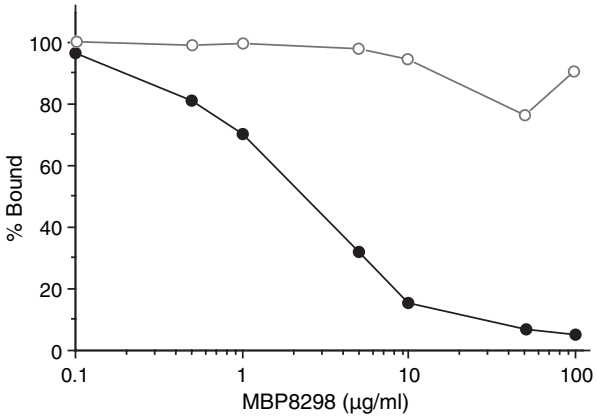

Figure 4 *In vitro* inhibition of cerebrospinal fluid (CSF) anti-myelin basic protein (MBP) autoantibody from patient 10 by MBP8298 synthetic peptide. Filled symbols, pre-treatment CSF; open symbols, CSF after 36 months of MBP8298 treatment. Anti-MBP autoantibody in the CSF of this patient were completely suppressed for nearly 3 years by MBP8298 treatment, but returned to the pre-treatment level at 36 months despite ongoing treatment.

retrospectively showed that the three poor responders, in terms of autoantibody suppression, expressed CSF antibodies with specificity for MBP epitopes other than MBP8289. Alternative specificities were present in the pre-treatment CSF of two patients (Fig. 3), and appeared in the CSF of the third patient after 36 months of treatment with MBP8298 (Fig. 4). These results are consistent with the proposed mechanism of action of MBP8298, namely, the induction of a state of immunologic tolerance with respect to the administered antigen, without affecting immune responses to unrelated antigens.

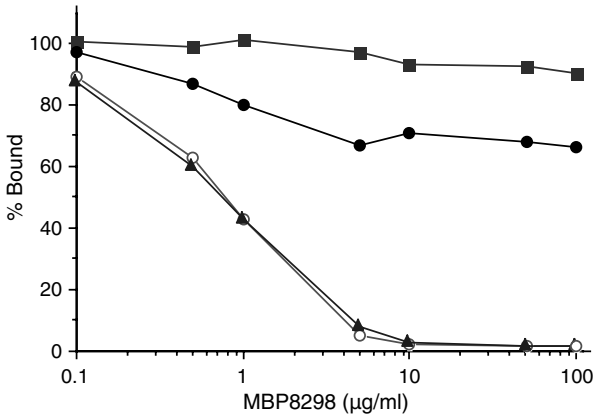

Figure 3 *In vitro* inhibition of pre-treatment cerebrospinal fluid (CSF) anti-myelin basic protein (MBP) autoantibody by MBP8298 synthetic peptide. Open circles, patient 20; triangles, patient 30; filled circles, patient 23; squares, patient 24. Patients 20 and 30 responded to MBP8298 treatment with complete and durable suppression of CSF anti-MBP autoantibody and are included as controls, patient 23 had partial antibody suppression and patient 24 showed no antibody suppression.

Safety

There were no serious adverse events that could be directly linked with MBP8298 peptide injections in this study, or in any previous MBP8298 peptide study. Local injection site reactions if the peptide was injected interstitially and occasional facial flushing sometimes associated with mild blood pressure decrease were the most commonly seen drug related adverse events. Routine clinical laboratory testing (complete blood panels, heart panels, liver enzymes, kidney panels, CSF analyses) as well as MRI studies did not reflect any medically adverse responses after single or multiple dosing with MBP8298. The usefulness of MRI measurements in patients who no longer experience relapses is considered minimal; MRI changes measured during the study included unique new, unique enlarging, new proton density, enlarging proton density, new enhancing and spinal cord lesions. The results showed no statistically significant difference between MBP8298 and placebo-patients for any of these variables.

22-m timed walk test

Contingency analysis showed no impact of MBP8298 treatment on outcomes in the 22-m timed walk test

when analyzed as described in Methods. At baseline, 27 of the 32 entered patients were able to complete the 22-m timed walk test. One of these patients died (placebo-group) during the 24 month evaluation period. At 24 months there was no significant difference in disease progression between MBP8298 and placebo-treatment groups ($n = 26$, $P = 0.99$) when progression was defined as a requirement for a more advanced physical aid or failure to complete 22-m. Most of the 15 patients who had not progressed by these criteria did not have a significant reduction in walking speed, which precluded further meaningful statistical analysis. Similarly, lack of statistical significance was observed in the subset of patients with HLA haplotypes DR2 and/or DR4 ($n = 16$, $P = 0.60$).

Discussion

Antigen-based approaches for the treatment of autoimmune diseases are particularly attractive because modifications of essential immunological activities unrelated to the disease can be avoided. In this report we show a significant delay of disease progression by MBP8298 treatment in a major subgroup of MS patients with HLA haplotypes DR2 and/or DR4. Future trials with more extensive analysis of HLA haplotype are needed to identify additional responders.

This study is the first MBP8298 clinical trial which provides an opportunity to relate changes in CSF anti-MBP autoantibody levels in MS patients to clinical outcome. Whilst clinical benefit was significantly associated with HLA-DR haplotype, anti-MBP autoantibody suppression was observed more generally and appeared to be largely independent of HLA haplotype. The use of a 500 mg dose and a twice yearly dosing frequency for MBP8298 were based on the assumption that CSF anti-MBP autoantibody suppression is a reliable indicator of tolerization. Fine specificity analysis of CSF antibodies from several patients confirmed that the dosing schedule maintained complete tolerization with respect to the MBP8298 epitope. *In vitro* inhibition assays with the MBP8298 peptide showed that in each of three cases where anti-MBP autoantibodies were not suppressed by MBP8298 *in vivo*, the antibodies were not reactive withMBP8298. Thus, no evidence for the breaking of drug induced tolerance to MBB8298 was seen in this study, but an epitope shift could be inferred in one case.

The timed walk test used in this study was not the conventional 20 foot walk which is a component of the multiple sclerosis functional composite (MSFC) test. Our intention was to capture information bearing on patients' concern to maintain their ability to walk. The outcome showed that most patients either maintained their walking ability or progressed to require a more advanced physical aid; no significant difference between the treatment groups could be demonstrated. A timed walk test may be more useful in a patient group with similar walking abilities when measured over a period of time which would not involve a change in the physical aid.

The therapeutic approach we have taken with MBP8298 is substantially different from those reported for other antigen-based MS treatments that have been evaluated in clinical trials.

Synthetic peptides that cover nearly the same region of MBP as MBP8298 but have strategically substituted amino acids [altered peptide ligands (APL)] have been the subject of several clinical trials. APLs have been administered subcutaneously in relatively low doses, and have induced novel immune responses that may regulate the immune system in ways that are beneficial for MS patients [31,32]. However, encephalitogenic potential was noted in one APL study [33]. The potential for safe and effective immunotherapy by immunization with APLs has recently been reviewed [34]. A solubilized complex of HLA-DR2 with MBP peptide 84–102 (AG284) has been administered intravenously to DR2-heterozygous secondary progressive MS patients in a dose escalation safety study [35]. The formulation was deemed to be safe at doses of up to 150 μ/kg but the study was not able to establish that T cells were tolerized by the treatment, and it was not designed to demonstrate clinical efficacy. Oral bovine myelin showed promise in animal models [36], and a subset of apparently responding patients was identified in a Phase II clinical trial [37], but a larger study failed to confirm clinical benefit. In retrospect, it seems probably that digestion of the myelin epitope which is immunodominant in patients with HLA-DR2 would preclude exposure of this particular peptide to the mucosal immune system in the gut. It has been suggested that proteolysis of this epitope may compromise the establishment of central tolerance by limiting its display in the thymus [25].

T cells and their by-products have been extensively used as indicators for evaluation of potential new approaches to MS therapy and the presence of myelin reactive T cells in normal individuals continues to complicate this approach [10]. Circulating antibodies specific to MS patients have been equally elusive. A sensitive enzyme linked immunosorbent assay for circulating anti-MBP antibodies in MS patients has been reported recently, but the signal levels compared with background imply that circulating antibody levels in MS patients are quite low or that they have low affinities [38]. Low levels of antibodies to MBP and myelin oligodendrocyte glycoprotein (MOG) have also

been detected by Western blot analysis of serum samples from patients with a clinically isolated syndrome suggestive of MS [39]. Because our efforts to develop a reliable serum-based assay had failed to meet our needs, we have continued to use a CSF-based assay of anti-MBP autoantibodies as a more robust and reliable method to monitor the effect of MBP8298 on autoimmune activity in patients with progressive MS [27,29].

MBP8298 represents the first in a new class of drug candidates for the treatment of MS. Its benign safety profile in this study confirms previous observations in patients with chronic progressive MS [27–29] and in patients with acute MS relapses [26], and is not surprising in view of its composition and mode of delivery. As a small peptide with natural amino acid sequence administered intravenously, MBP8298 is cleared rapidly from the circulation. A large trial with MBP8298 in SPMS is underway to confirm the encouraging clinical results obtained with this novel treatment approach. If MBP8298 is proven effective, the approach of high dose tolerization may also be taken with other therapeutic peptides that specifically target patients who do not respond clinically to MBP8298. Although clinical benefit to the responder group of patients in this study appears to be unprecedented in MS drug development, the long-term results show that the disease process does continue. In the probable scenario that residual disease involves autoimmunity to other epitopes, there may be opportunities to develop peptides that complement or synergize with the activity of MBP8298.

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