Effective Antigen-Specific Immunotherapy in the Marmoset Model of Multiple Sclerosis

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Mature T cells initially respond to Ag by activation and expansion, but high and repeated doses of Ag cause programmed cell death and can suppress T cell-mediated diseases in rodents. We evaluated repeated systemic Ag administration in a marmoset model of experimental allergic encephalomyelitis that closely resembles the human disease multiple sclerosis. We found that treatment with MP4, a chimeric, recombinant polypeptide containing human myelin basic protein and human proteolipid protein epitopes, prevented clinical symptoms and did not exacerbate disease. CNS lesions were also reduced as assessed in vivo by magnetic resonance imaging. Thus, specific Ag-directed therapy can be effective and nontoxic in primates. ***The Journal of Immunology*****, 2001, 166: 2116–2121.**

Multiple sclerosis (MS)[4] is a paralytic disease involving destruction of myelin sheaths surrounding axons in the CNS (1, 2). MS affects young adults, most often women residing in northern latitudes. The disease exhibits relapsing and remitting symptoms including disturbances in vision, speech, coordination, and cognition as well as weakness, spasticity, and paralysis (1, 2). Lymphocytic infiltration in the CNS white matter and immune reactions against myelin Ags indicate an autoimmune etiology for MS (1–8). Allergic encephalomyelitis was first observed as a side effect of the rabies vaccine prepared from rabbit brains by Pasteur in the 1880s (see Ref. 3). Rivers and others showed that the CNS inflammation was caused not by the rabies virus but by immune sensitization to the combination of adjuvant and brain tissue contaminating the vaccine (3, 4). Experimental allergic encephalomyelitis (EAE) models in various animal species, typically rodents, were later developed by immunization with myelin proteins in adjuvant or by the adoptive transfer of myelin-reactive T cells, causing inflammatory damage to the white matter (1–6). Rodent EAE is the most widely used disease model despite important differences from MS (2).

Encephalitogenic $CD4^+$ T cells are believed to initiate and perpetuate EAE and MS and thus constitute a therapeutic target (1–8). Abundant myelin protein Ags, including myelin basic protein (MBP) and proteolipid protein (PLP) as well as the less abundant Ags, myelin oligodendrocyte glycoprotein (MOG) and myelin-associated glycoprotein (MAG), are recognized by T cells in MS patients (9–11). T cell responses against MBP and PLP may occur at an increased frequency in MS patients compared with controls (1, 2, 11, 12). Ag-specific immunotherapies directed at T cells could avoid the harmful side effects of general immunosuppressive treatments. We have investigated a potential immunotherapy for MS based on our observation that T cells undergo apoptosis both in vitro and in vivo when exposed to high or repeated doses of their cognate Ag (13, 14). Antigenic restimulation of T cells cycling in IL-2 up-regulates CD95 (Fas/Apo-1), TNF receptor and their respective ligands causing apoptosis (15–17). MBP administration to mice with EAE can ameliorate disease by deleting specifically reactive T cells (14, 18, 19).

To present a broad array of potential epitopes to reactive T cells, we constructed MP4, a protein chimera of the 21.5-kDa isoform of human MBP, and a modified form of human PLP, termed PLP4, that lacks the hydrophobic domains of the protein but includes all of the known T cell epitopes (19–21). MP4 is processed into multiple determinants and can eliminate rodent EAE by promoting tolerance to different epitopes (19, 20). This is important in view of epitope or determinant "spreading" in MS and EAE (19, 20, 22–28). We previously documented epitope spreading in EAE in marmosets (29). Epitope spreading poses a challenge for Ag-specific therapies, but even single epitopes can be effective in treating disease in some circumstances (27, 28). For example, severe EAE induced in $(PL/J \times SJL)F_1$ mice by immunization with $MOG_{41–60}$ and MBP $Ac_{1–11}$ peptides can be treated effectively by the MBP peptide alone (28).

In a few instances, EAE and Ag treatments have been studied in nonhuman primates. EAE was originally induced in rhesus macaques using CNS homogenates or purified MBP (3, 4, 30–32). It was also found that repeated injections of MBP could arrest EAE

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[4] Abbreviations used in this paper: MS, multiple sclerosis; MBP, myelin basic protein; PLP, proteolipid protein; EAE, experimental allergic encephalomyelitis; MOG, myelin oligodendrocyte glycoprotein; MAG, myelin-associated glycoprotein; rMOG, rat myelin oligodendrocyte glycoprotein; MT, magnetization transfer; MRI, magnetic resonance imaging; HPRT, hypoxanthine phosphoribosyltransferase.

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in the macaque model (31, 32). However, EAE in macaques involves hemorrhagic lesions with neutrophil infiltrates that are not characteristic of MS (7, 30–32). A new nonhuman primate model of EAE in *Callithrix jacchus jacchus* (common marmoset) has been developed that has clinical and pathological features closely resembling those of MS (7, 8). Disease in marmosets involves predominantly perivascular lymphocytic infiltrates and demyelination. The marmoset model has advantages over rodent EAE models in that a rigorous neurological examination and the evaluation of lesions by high resolution magnetic resonance imaging (MRI) are possible (8, 29, 33). Only a single study of Ag treatment of EAE, which used the low abundance MOG protein, has been conducted in marmosets, with the ominous finding that acute disease was suppressed but severe fatal disease rebounded after treatment cessation (34). However, the previous study did not resolve the important question that remains, whether a defined protein representing the abundant myelin epitopes could have a significant and durable therapeutic effect in primates. Here we show that Ag treatment of marmoset EAE achieves a clinical benefit without severe late toxicity.

Materials and Methods

Animals

Nine *C. jacchus jacchus* marmosets were obtained from a colony maintained by the National Institute for Child Health and Human Development at the National Institutes of Health Primate Unit (Poolesville, MD). The animals, all males, ranged in age from ~1 year, 8 mo to 2 years, 2 mo of age and were cared for under an approved protocol in accordance with the guidelines established by the National Institutes of Health Animal Care and Use Committee.

Antigens

MP4 was prepared by metal affinity chromatography and reversed phase HPLC as previously described (20). The recombinant extracellular domain of rat MOG (rMOG), was prepared as described (35).

Induction of EAE

MP4 was emulsified 1:2 in TiterMax adjuvant (Vaxcel, Norcross, GA). Animals received 100 μl intradermal injections containing a total of 1 mg MP4 at four sites on the back. On the day of immunization and again 2 days later, all immunized animals were given an i.v. injection of 5 ml sterile normal saline containing 10^{10} killed *Bordetella pertussis* organisms. The *B. pertussis* was kindly provided by Dr. Pat Van Zandt (Wyeth-Lederle Vaccines, Madison, NJ).

Ab responses

Serum Ab titers were tested in duplicate by ELISA (36). ELISA plates (Pierce, Rockford, IL) were coated overnight with 1 μg/well rMOG or MBP in 0.25 M carbonate buffer (pH 8.6), washed with PBS containing 0.05% Tween 20, and blocked with 1% BSA in the same buffer. After washing, 100 μl of a 1:200 or appropriate dilution of immune sera were incubated in the wells for 2 h at 37°C, followed by immunoperoxidase-conjugated anti-monkey IgG (Sigma, St. Louis, MO; 1:6,000) for 1 h at 37°C. Plates were developed with *o*-phenylenediamine dihydrochloride in 0.05 M phosphate-citrate buffer (pH 5.0; Sigma) for 30 min and read at 490 nm in a Vmax ELISA reader (Molecular Devices, Sunnyvale, CA).

T cell proliferation assays

PBL were separated using Lymphocyte Separation Medium (Organon Teknika, Durham, NC) density gradients according to the manufacturer's instructions and plated in U-bottom 96-well microtiter plates (Becton Dickinson, Lincoln Park, NJ) at 1×10^5 cells/well in a total volume of 0.2 ml of AIM V serum-free medium (Life Technologies, Gaithersburg, MD). Test wells were prepared in triplicate with medium only, 50 or 100 μg/ml MP4, or 1 μg/well Con A (Boehringer Mannheim, Indianapolis, IN) and pulsed with 1 μCi [*methyl*-^{3}H]thymidine (Amersham, Arlington Heights, IL). After 3 days of incubation, plates were harvested on a Tomtec MachII96 (Wallac, Gaithersburg, MD), and analyzed using a Betaplate 1205 scintillation counter (Wallac).

Cytokine RT-PCR

PCR were conducted as previously described (34) for primers (5′ to 3′ sequences): HPRT 5′, TGACCAGTCAACAGGGGAC; HPRT 3′, GCTCT ACTAAGCAGATGGC. IFN-γ 5′, CTGTTACTGCCAGGACCCAT; IFN-γ 3′, CGTCTGACTCCTTCTTCGCTT. IL-10 5′, GGTTACCTGGGTTGC CAAGCCT; IL-10 3′ (37), CTTCTATGTAGTTGATGAAGATGTC. TGF-β 5′, GCCCTGGACACCAACTACTGC; TGFβ 3′, GTCGCATTTGCAGGA GCGCAC. IL-4 5′, TGTCCACGGACACAAGTGCGA; IL-4 3′, CATGAT CGTCTTTAGCCTTTCC.

Clinical and pathological evaluation of EAE

Marmosets were observed daily, and clinical symptoms were scored as previously described (Table I) (7). At 105 days after immunization, animals were euthanized, and the brain and spinal cord were removed and fixed in Formal-Fixx (Shandon, Pittsburgh, PA). Sections of 3 mm were prepared in coronal, transverse, or longitudinal orientations using tissue fragments embedded in paraffin. The sections were stained using hematoxylin and eosin, Luxol fast blue, or Bodian's silver stain techniques (American Histolabs, Gaithersburg, MD). Histopathological sections of CNS were scored in a blinded manner as previously described (7) with minor changes as described in Table I. Typically, seven to nine coronal and transverse 3-mm sections of the entire spinal cord were evaluated. Photomicrographs were taken on a Axiophot microscope (Carl Zeiss, Thornwood, NY).

Magnetic resonance imaging

Scans were performed in the coronal plane with 2-mm interleaved slices on a Signa 1.5 T unit (General Electric, Milwaukee, WI) and included a T2-weighted spin echo pulse sequence SE 2000/20/80 and T1-weighted sequences SE 450/13 with and without a magnetization transfer (MT) pulse, using a 3-inch surface coil (29, 33). T1-weighted and MT images were performed before and after i.v. administration of the contrast agent gadopentetate dimeglumine 0.3 mmol/kg (Magnevist; Berlex Laboratories, Cedar Knolls, NJ). Scans were interpreted in a blinded manner.

Results

High dose i.v. MP4 administration abrogates clinical symptoms

Nine male marmosets were randomly assigned to three treatment groups. Siblings (two sets) were placed in different groups. The groups received 1 ml 5% dextrose in sterile water containing 6 mg MP4 (high dose), 0.6 mg MP4 (low dose), or 0 mg MP4 (sham). The 6-mg high treatment dose was based on a body weight scale-up from a dose that eliminated disease in rodents (14, 18–21). The treatments were administered through an indwelling venous catheter in the tail twice daily at ~10 a.m. and 6 p.m., on days

Table I. *Summary of clinical and histopathological data*

Animal	Treatment	Clinical[a]	Onset[b]	Inflammation[c]	Demyelination[c]
J43	Sham	2	14	2	0
J81	Sham	2	7	2	1
L9	Sham	1	29	1	0
J80	Low dose	0	None	1	0
J106	Low dose	2	24	3	0
J54	Low dose	2	42	1	2
J97	High dose	0	None	0	1
J88	High dose	0	None	2	1
J42	High dose	0	None	0	0

[a] Maximum observed clinical disease score defined as: 0 = normal; 1 = lethargy, anorexia, weight loss; 2 = para- or monoparesis, ataxia, sensory loss, incontinence, anisocoria; and 3 = para- or hemiplegia.

[b] Onset of clinical symptoms (days postimmunization).

[c] Inflammation and demyelination were assessed on microscopic sections taken postmortem at the end of the 105-day clinical observation period, and the slides were scored in a blinded manner separately for inflammation and demyelination as follows: 0 = no inflammation present; 1 = minimal (1–3 lesions/average section); 2 = moderate (3–10 lesions/average section); 3 = extensive; 0 = no demyelinating lesions; 1 = minimal demyelination (1–3 lesions/average section); 2 = moderate demyelination (3–10 lesions/average section); and 3 = widespread demyelination with large confluent lesions.

5, 7, and 9 after immunization. Neurological evaluation, weight, and temperature measurements were performed daily for 105 days by observers unaware of the treatment groups. All animals in the sham group showed moderate clinical symptoms of EAE including weight loss, ataxia, incontinence, and mono- or paraparesis with onset ranging from 7 to 29 days (Fig. 1, Table I). By contrast, no clinical symptoms were observed in the high dose group. In the low dose group, moderate clinical disease was observed in two of three animals with onset delayed to day 24 for one animal and day 42 for the other animal. No symptoms were seen in 10 unimmunized control marmosets. Single episodes of seizures were observed in a pair of siblings, one in the high dose group and one in the sham group. Idiopathic sporadic seizures have been described in unmanipulated marmosets and are not characteristic of marmoset EAE (35, 40). In one unimmunized animal given the 6-mg dose of MP4 i.v., a solitary cerebral inflammatory lesion was observed, however, this animal did not manifest any other CNS changes and had no T cell or Ab responses to MP4 (data not shown). In further control experiments, 12 unimmunized marmosets received daily doses of MP4 for one month and no CNS disease was observed (data not shown).

MP4 treatment delays white matter disease evident on MRI

We used serial MRIs performed throughout the 105-day observation period to evaluate white matter disease in real time. MRI changes occurred in all animals with clear differences between the treatment groups (Fig. 2, Table II). First, the onset of MRI changes for the sham-treated animals was at least 2 wk earlier than that of the treatment groups. White matter disease on MRI correlated well with clinical symptoms for the sham and low dose groups. Second, despite the lack of clinical symptoms, animals in the high dose group exhibited MRI changes indicating that the disease process was not completely abolished. Third, severe white matter disease (score, 3) was reached by two of three sham-treated animals, by one of the low dose animals, and in none of the high dose animals. Moreover, only one of three animals in the high dose group reached a score of 2, and the mean MRI score for the high dose group was reduced at all time points relative to the sham-treated animals. Thus, serial MRI imaging demonstrated ameliorative effects of both doses of MP4 treatment in live animals.

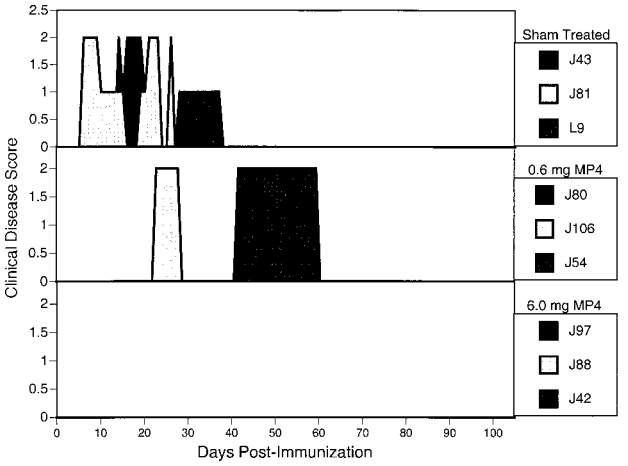

FIGURE 1. Abrogation of clinical symptoms of EAE by high dose MP4 immunotherapy. Histograms of clinical disease scores for the sham treatment group (*top*), 0.6-mg/dose MP4 treatment group (*center*), and 6.0-mg/ml treatment group (*bottom*) as a function of days postimmunization. Individual animal identification numbers are indicated at the right of each treatment panel.

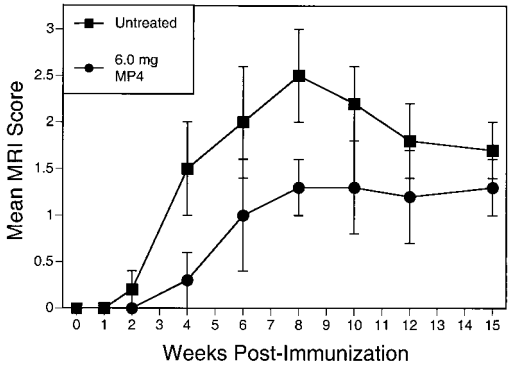

FIGURE 2. Delayed onset of white matter disease by MRI in high dose MP4 treatment group. Mean MRI scores for the sham (■) and high dose (6 mg/dose) (●) treatment groups. Error bars show SEM for the three animals in each treatment group. When given as a range (e.g., 0/1) in Table II, the mean of the raw scores was used for purposes of calculation of treatment group mean and SEM.

Histopathology shows reduced CNS disease in the high dose group

After 105 days, we evaluated pathology in coronal brain and spinal cord sections (Table I). Significant inflammatory infiltrates were found in all animals in both the sham and low dose groups, but only one animal in the high dose group showed perivascular infiltrates in the CNS. Relatively little demyelination was observed in any of the animals, possibly because of the large time interval between the early occurrence of severe clinical/MRI findings and the time of the pathological analyses. Remyelination obscures evidence of early demyelinating disease (1–5).

Decreased proliferation responses and antimyelin Ab production

PBL proliferative responses to the MP4 Ag were also evaluated (Table III). At 18 days postimmunization (9 days post treatment), proliferative responses were seen in all groups, but the mean stimulation index for the sham-treated animals was 21.0 as compared with a mean of 8.3 for the high dose group. Control proliferative responses to Con A did not differ between groups (data not shown). At later time points, significant differences in MP4-specific responses between treated and untreated animals were not observed (data not shown), but were similar to levels shown at 18 days postimmunization.

ELISA was used to measure humoral responses induced to the myelin proteins MPB, PLP, and MOG. Our interest here was to evaluate the effect of treatment on immune responses to the individual marmoset myelin components and not just to the sensitizing Ag MP4. ELISA detected MBP-specific Abs as early as wk 2 postimmunization but anti-MBP titers were decreased in the high dose group (Fig. 3). One sham-treated marmoset, J81, but no other animals showed clear evidence of epitope spreading with markedly elevated anti-MOG titers that we have previously correlated with demyelination in marmosets (29).

No evidence of immune deviation in MP4-treated marmosets

Previously, MOG treatment of marmoset EAE was suggested to deviate T cells from a Th1 to a Th2 response (34). Cytokine mRNA production was therefore evaluated by semiquantitative RT-PCR using mRNA samples obtained at day 16 (1 wk after the final treatment) from either the sham-treated or the high dose MP4-treated animals (34, 38). Relative mRNA amounts of the Th1 cytokine, IFN-γ, the Th2 cytokines, IL-4 and IL-10, and TGF-β are shown in Fig. 4 as a fraction of HPRT control mRNA. Only IFN-γ

Table II. *MRI scores*

		MRI Examinations[a]								
Animal	Group	Examination Week	1 1	2 2	3 2.5	4 4	5 6	6 7.5	7 10	8 12	9 15
J43	Sham		0	0	ND	1	2	3	3	2.5	2
J81	Sham		0	0	1	1	1	1.5	1.5	1	1
L9	Sham		0	0.5	ND	2.5	3	3	2	2	2
J80	Low dose		0	0	ND	0	0	0	0	0	1
J106	Low dose		0	0	ND	3	2	0	0/1	1	1.5/2
J54	Low dose		0	0	ND	1	2	2	2	2	3
J97	High dose		0	0	ND	0	0	1	1.5	1	1
J88	High dose		0	0	ND	1	2	2	2	2	2
J42	High dose		0	0	ND	0	1	1	0/1	0/1	1

[a] Maximum observed MRI score: 0 = normal; 1 = 1–5 T2 lesions; 2 = 5–10 T2 lesions; 3 = >10 lesions or focal and diffuse abnormalities; 4 = extensive diffuse white matter abnormalities. The scores were increased if contrast-enhancing lesions indicative of active disease and blood brain barrier disruption were detected (0.5 point for one lesion present and 1 point for two or more lesions). Scores separated by a slash (e.g., 0/1) signify examinations in which it was difficult to assign an exact score.

was modestly induced after a 4-h incubation with 25 μg/ml MP4. No differences were found between sham and high dose animals, and the mRNA levels of the Th2 cytokines IL-4 and IL-10 were lower than the levels of IFN-γ or TGF-β. Thus, immune deviation did not occur as a result of MP4 treatment and could not account for disease amelioration.

Discussion

MS is a potentially severe paralytic disease for which no cure is presently known. Much research has focused on new therapeutic approaches to inhibit the immune processes that are believed to initiate CNS damage in MS. The importance of local immune responses in the brain for disease has been demonstrated by the fact that immune modulation by nerve growth factor can ameliorate EAE when administered in the cerebral ventricles (40). Considerable progress has been made in Ag-specific immunomodulation of rodent EAE. For example, Ag-induced T lymphocyte apoptosis can abort the encephalitogenic process (14). Very little has been done to extend these studies to nonhuman primates despite the fact that Kabat and Morgan in the 1940s showed that immunization could induce hemorrhagic EAE and Eylar and Brostoff showed that MBP could ameliorate this form of the encephalitic disease (31, 32). The marmoset model of EAE has clinical and pathological features (7, 41) that more closely resembles those of MS, yet only a single study has previously examined Ag treatment with unsuccessful results (34). It was therefore of great interest to explore whether different Ag preparations could achieve a beneficial effect without toxicity. In particular, it was important to examine abundant myelin Ags, because these might be targets of the encephalitogenic immune responses in MS patients (9–12, 42). Despite the restricted availability of *C. jacchus jacchus*, the species used for marmoset EAE, we conducted a pilot study to evaluate the feasibility of Ag therapy. The results we have obtained differ markedly from the previous study reporting treatment with repeated doses of MOG (34), and several conclusions can be made.

First and foremost, it was clear that high dose Ag treatment decreased disease as judged by clinical and MRI evaluation. Histopathology also showed that Ag treatment was associated with less lymphocyte infiltration. We also found decreased T cell proliferative responses and Ab production in Ag-treated animals. Taken together, these results are consistent with previous observations in rodent EAE that soluble i.v. Ag decreases rather than enhances cognate immune reactions (14) and suggest that Ag therapy could be safe and effective for T cell-mediated autoimmune diseases. One of the most important outcomes of our study is that we did not find the emergence of severe demyelination or fatalities after Ag administration as previously observed with MOG treatment (34).

The hyperacute disease observed in the previous marmoset study could pose a very serious drawback to using Ag treatment in human MS (34). The difference between the previous study and our own likely lies in the fact that different Ags were used for treatment. In the prior study, marmosets were immunized with MOG and then treated with an 11-day course of i.p. injections of MOG every other day (34). A hallmark of the disease in that study was severe demyelination possibly due to activated B cells that caused an Ab response against MOG (29, 34, 39). Severe demyelinating disease can also be achieved by immunizing marmosets with total white matter which contains a small fraction of MOG (7, 8). MOG has been consistently shown to provoke strong Ab responses that cause severe demyelination and in the previous study, anti-MOG Ab levels were higher in the treated animals than in the placebo controls at day 21 (29, 34, 42). In contrast, using MBP/PLP epitopes, we found that disease was potently suppressed and that Ab levels against MBP were lower rather than higher in the treated animals at numerous points throughout 105 days of observation. It is also important that MOG treatment in the previous study caused immune deviation of T cells to a Th2 phenotype, which would promote B cell activity and Ab production (34). With MBP/PLP treatment, no such deviation was evident.

High dose MP4 treatment eliminated clinical disease, but it was evident from the MRI scans that CNS tissue effects, although reduced, were not completely abolished. However, the mean MRI scores for the treated animals never reached the levels observed in the untreated animals, which peaked at ~8 wk and then fell to

Table III. *Proliferative responses to MP4*

Group	Animal	Unstimulated cpm[a]	MP4 cpm	(SI)
Sham	L9	329	8,824	(27)
	J81	1,689	51,722	(31)
	J43	9,096	41,530	(5)
Low dose	J80	4,569	24,834	(5.4)
	J106	352	2,317	(6.6)
	J54	360	8,867	(25)
High dose	J97	11,286	43,681	(3.8)
	J42	709	5,321	(7.5)
	J88	3,813	51,578	(13.5)

[a] Proliferation as measured by [*methyl*-^{3}H]thymidine incorporation after a 3-day incubation with 0 or 50 μg/ml MP4. Cells were obtained from peripheral blood at 18 days after immunization. SI, Stimulation index.

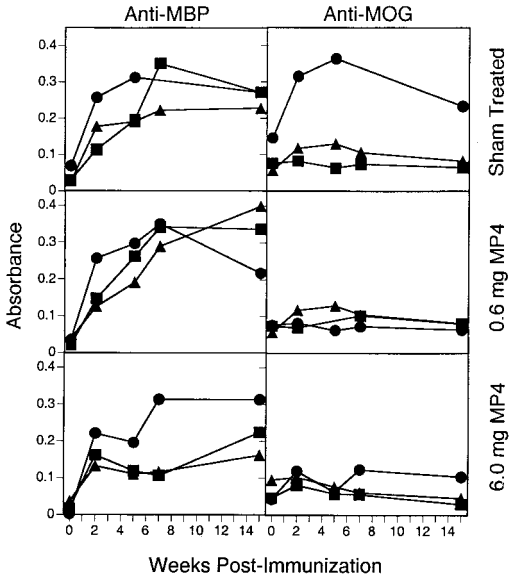

FIGURE 3. Kinetics of myelin Ag-specific Ab responses in MP4 and sham-treated marmosets. Serum anti-MBP and anti-MOG Ab responses were determined by ELISA. Symbols represent individual animals: sham animals = J43 (■), J81 (●), L9 (▲); 0.6 mg/dose MP4-treated animals = J80 (■), J54 (●), J106 (▲); 6.0-mg/dose MP4-treated animals = J42 (■), J97 (●), J88 (▲).

levels similar to those of the high dose MP4-treated animals by 15 wk. This lower level of CNS disease intensity on MRI in treated animals is reflected in the absence of clinical symptoms. There is a general correlation in the untreated group between trends in clinical symptoms and MRI scores. The appearance of disease symptoms in some cases preceded evidence of CNS disease as demonstrated by MRI. This is likely due to technical limitations in our ability to visualize tiny, early lesions of <0.5 mm (33). In some animals, CNS disease was observed in the absence of clinical symptoms. This is a common observation in EAE and MS and may reflect the fact that most lesions detected by MRI are clinically silent, perhaps because of functional redundancy in the brain or lack of involvement of vital neural pathways to clinically apparent levels (33, 43). A larger lesion load, as was observed in the untreated animals, may increase the likelihood that a lesion will appear in an area in the CNS in which damage can produce an observable clinical outcome. There is also a general correlation between the inflammation demonstrated by histopathology and the MRI and clinical score. Because of the many changes that may have occurred over time including decreased inflammation and remyelination, the histopathology may only dimly reflect the original appearance and number of the lesions, some of which may have been 100 days old. The limitations of histopathology in assessing the efficacy of a CNS immunotherapy highlight the value of MRI in studies of this kind. With these considerations in mind, the clinical, histopathological, and MRI data tell a consistent story: that high dose Ag immunotherapy was effective in reducing the lesion load and eliminating the clinical symptoms of EAE.

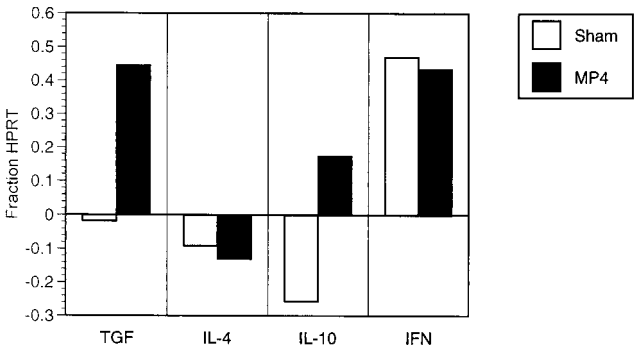

FIGURE 4. Cytokine mRNA expression assessed by semiquantitative PCR. Lymph node cells were stimulated for 4 h in the presence of 0 or 50 μg/ml MP4. Results for each cytokine are presented as the fraction of the band intensity of the relatively noninducible housekeeping gene *HPRT* mRNA above unstimulated baseline values by RT-PCR. Data are presented as the mean of values of the three marmosets in either the high dose MP4 treatment group (MP4, ■) or the sham treatment group (sham, □).

Previously, we documented determinant spreading to MOG in three of four animals immunized with MP4 in adjuvant (29). Anti-MOG Abs occurred in only one of the three untreated animals, J81, which was the only animal to show persisting demyelination. This supports the association of determinant spreading to MOG with demyelination in MP4-immunized marmosets (29, 36). We observed no determinant spreading in MP4-treated animals by anti-MOG Ab titers; thus, abolition of immune responses to the abundant myelin Ags might have prevented demyelination by limiting determinant spreading. Additional studies with a greater number of animals are necessary to validate these trends. Nevertheless, our results suggest that the choice of Ag for immunomodulation may be critical for successful treatment and provide new hope for Ag-specific therapy in humans.

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