Reactivity to Myelin Antigens in Multiple Sclerosis

Peripheral Blood Lymphocytes Respond Predominantly to Myelin Oligodendrocyte Glycoprotein

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Abstract

Although T cell responses to the quantitatively major myelin proteins, myelin basic protein (MBP) and proteolipid protein (PLP), are likely to be of importance in the course of multiple sclerosis (MS), cell-mediated autoimmune responses to other myelin antigens, in particular quantitatively minor myelin antigens, such as myelin-associated glycoprotein (MAG) and the central nervous system–specific myelin oligodendrocyte glycoprotein (MOG), could also play a prevalent role in disease initiation or progression. Highly purified myelin antigens were used in this study to assess cell-mediated immune response to MOG in MS patients, in the context of the reactivity to other myelin antigens, MBP, PLP, and MAG. The greatest incidence of proliferative response by MS peripheral blood lymphocytes was to MOG, as 12 of 24 patients tested reacted and, of these, 8 reacted to MOG exclusively. In contrast, only 1 control individual of 16 tested reacted positively to MOG. The incidence of responses to MBP, PLP, and MAG did not differ greatly between MS patients and control individuals. A predominant T cell reactivity to MOG in MS suggests an important role for cell-mediated immune response to this antigen in the pathogenesis of MS. (*J. Clin. Invest.* 1993. 92:2602–2608.) Key words: multiple sclerosis • myelin oligodendrocyte glycoprotein • myelin antigens • cell-mediated immune response • experimental autoimmune encephalomyelitis

Introduction

Multiple sclerosis (MS),[1] a disease of the human central nervous system (CNS) characterized by perivascular inflammation accompanied by primary demyelination, is believed to result from autoimmune mechanisms leading to myelin destruction (1). Accumulation of activated T cells in early MS lesions, as well as at the periplaque area and in surrounding normal-appearing white matter (2–4), seemingly points to the importance of cell-mediated immune reaction in the pathogenesis of the disease (1, 5). In view of the restricted localization of MS lesions to the white matter (6), it is generally accepted that autoimmune-activated T cells in MS CNS recognize components of the myelin sheath. In this context, a purported model for MS, experimental autoimmune encephalomyelitis (EAE), was shown to be a cell-mediated disease that could be caused in susceptible animals by intravenous injection of activated CD4^{+} T cells specific for the quantitatively major myelin proteins: myelin basic protein (MBP) (7, 8) and proteolipid protein (PLP) (9–11). In MS, reactivity to these myelin antigens has been extensively investigated and controversial results indicate that, although specific responses to these antigens are likely to be of importance in the course of the disease, they may not represent the primordial response involved in the pathogenesis (12). It has recently been acknowledged that autoimmune recognition of quantitatively minor myelin autoantigens in MS could also play a prevalent role in disease initiation or progression. Quantitatively minor myelin antigens include myelin-associated glycoprotein (MAG) and the CNS-specific myelin oligodendrocyte glycoprotein (MOG). In contrast to MBP and PLP, which account for most of the protein fraction of CNS myelin (~ 30 and 50%, respectively; 13), MAG and MOG constitute only ~ 1 and 0.05% of CNS myelin proteins, respectively (14, 15). Because of their low abundance, it is difficult to obtain these antigens in a highly purified form and in sufficient quantities, hence the paucity of reports investigating T cell reactivity to MAG and MOG. Although autoimmune response to MAG may be more important in demyelinating disorders of the peripheral nervous system (16), immune responses to MOG may be highly relevant to CNS demyelination. Thus, despite the fact that only very small amounts of MOG are found in the CNS (17), antibodies to MOG can be detected in the sera of guinea pigs with EAE induced with whole spinal cord homogenate (18). Furthermore, the demyelinating activity of such sera has been attributed to anti-MOG antibodies (19). Corroborative evidence that the presence of anti-MOG antibodies within CNS tissue leads to extensive demyelination has been obtained both in vivo (20, 21) and in vitro (22) with a monoclonal anti-MOG antibody (17). If MOG is indeed a target antigen for autoimmune attack, the possible involvement of cell-mediated immune response to MOG is also likely to be an important factor in the pathogenesis of MS.

In this study, the relevance of MOG as a T cell autoantigen was investigated by assessing the reactivity to MOG by PBLs of MS patients and control individuals, in the context of the reactivity to other myelin antigens: MBP, PLP, and MAG.

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1. *Abbreviations used in this paper:* CNS, central nervous system; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; MAG, myelin-associated glycoprotein; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; PLP, proteolipid protein; SI, stimulation index.

Methods

Myelin antigens. Human MBP was purified from neurologically normal human brain according to the procedure of Dunkley and Carnegie

(23). This highly purified preparation comprises the 18.5- and the 17.3-kD MBP isoforms observed in adult human CNS (Fig. 1 A; 24).

Human MOG was purified from neurologically normal brain white matter or spinal cord according to the procedure of Webb (personal communication; 17) modified as detailed previously (24a). Briefly, membrane proteins were extracted by addition of deoxycholate to human white matter homogenate and MOG was isolated from the solubilized protein fraction by immunoaffinity chromatography on an anti-MOG IgG (see below) column. It was eluted, in the absence of detergent, with 50 mM diethylamine, pH 11.5, which was then dialyzed out or removed on a desalting column. Human extracellular derivative of MAG (dMAG) was purified as described previously (25).

PLP was obtained from a washed total lipid extract of bovine white matter (26) and lipid was removed by chromatography on a Sephadex LH60 column (27). It was kept as a chloroform/methanol soluble preparation. On the day PLP was to be used in the proliferation assay, an aliquot of the solvent-soluble preparation was converted to a water-soluble form and dialyzed extensively against large volumes of water for 10–12 h. All preparations contained the two PLP isoforms, PLP and DM-20.

SDS-PAGE and immunoblotting. SDS-PAGE and immunoblotting analyses of the MOG and MBP preparations were carried out on 15% gels as described previously (24). SDS-PAGE analyses of MAG and PLP were performed on 10 and 12% gels, respectively (28). Immunoblotting of PLP was performed as described by Yamamura et al. (29) with the following modifications: the blocking solution consisted of PBS containing 2% nonfat dry milk and the hybridoma supernatant was used undiluted; the second antibody was goat anti-rat IgG conjugated to alkaline phosphatase (Fisher Scientific Co.), and an alkaline phosphatase conjugate substrate kit (Bio-Rad Laboratories, Richmond, CA) was used for detection.

Antibodies. Mouse monoclonal anti-rat MOG antibody (clone 8-18C5; 17) was purified from ascites fluid by affinity chromatography on a protein G Sepharose 4 Fast Flow column (Cat. no. 17-0618-01; Pharmacia LKB, Uppsala, Sweden) according to the manufacturer's specifications. The cells producing 8-18C5 antibody were a gift from Dr. M. Webb (Sandoz Institute of Medical Research, London).

Mouse monoclonal anti–human MBP antibody (clone 65 [2-1]; 30) was a gift from Prof. P. R. Carnegie (Murdoch University, Murdoch, Western Australia). It was purified by chromatography on protein G Sepharose 4 Fast Flow.

Mouse monoclonal anti-HNK1 antibody (clone 6-3-19-1) (Cat. no. MON 1010; Sanbio BV-Biological Products, the Netherlands) was used to detect possible contamination of MOG and MBP preparations with MAG, the carbohydrate moiety of which contains the HNK1 epitope (26, 31).

Rat monoclonal anti-PLP antibody (clone AB3) was obtained as described (29). Polyclonal anti-PLP antibodies were kind gifts from Prof. W. B. Macklin (UCLA, Los Angeles, CA) and Dr. E. Trifilieff (Centre de Neurochimie du CNRS, Strasbourg, France). All three antibody preparations were used at various times to assess possible contamination of the MOG preparations with PLP.

Patients and blood samples. Venous heparinized blood samples were obtained from patients and staff of the Department of Neurology (Assaf Harofeh Hospital, Zerifin, Israel). The MS group was comprised of 22 consecutive outpatients and 2 inpatients (18 females, 6 males) who agreed upon request to be tested and were not under steroid treatment or other medication known to affect the immune system for at least 1 mo before the time of testing. 21 patients had definite MS and 3 had probable MS (32), and their Kurtzke's Expanded Disability Status Scale (EDSS) ranged from 2 to 8 (33) (Table II). The 16 patients and healthy individuals who comprised the control group (9 females, 7 males) were not under medications known to affect the immune system. None of the patients had other inflammatory diseases of the CNS.
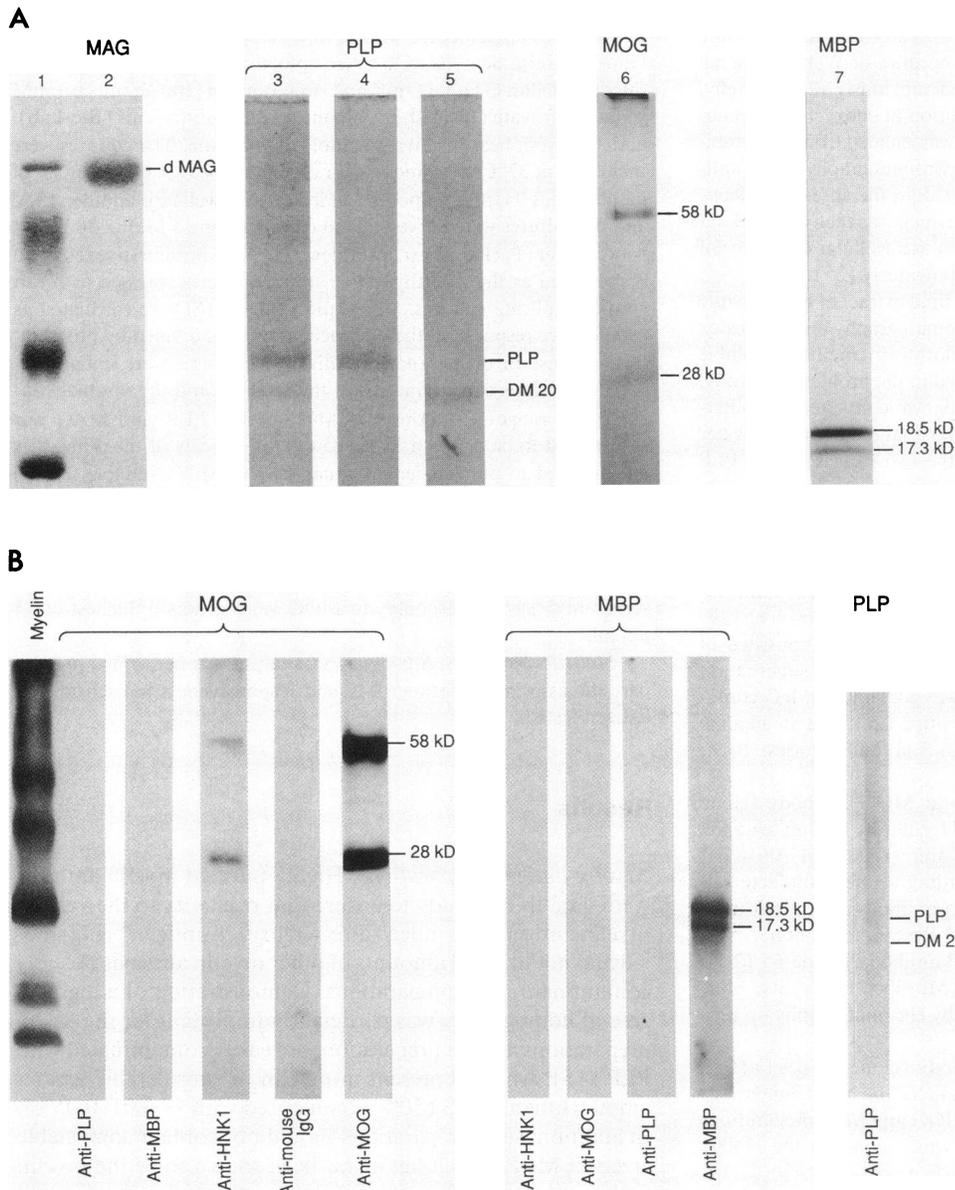
Proliferation assays. PBLs were separated from 20 ml of MS or control blood using Leucosep tubes as described by the manufacturer (Cat. no. ES 030 PP; Esquire Chemie, Switzerland). Isolated PBLs were cultured in microtiter wells in the absence or presence of the relevant antigens (5–25 μg/ml), essentially as described previously (34). Briefly, 2×10^5 cells/well were incubated in triplicate cultures in RPMI 1640 supplemented with 10 mM Hepes buffer, 2 mM glutamine (Bio-Lab, Jerusalem, Israel), 2-mercaptoethanol (5×10^{-5} M), antibiotics (penicillin G [100 U/ml] and streptomycin [100 μg/ml]), 1 mM sodium pyruvate (Bio-Lab), 1% nonessential amino acids (Bio-Lab), and 10% fresh heat-inactivated autologous plasma. The cultures were incubated at 37°C in humidified air containing 7.5% CO_2. After 5 d of incubation, [^{3}H]thymidine was added (1 μCi/well) for another 18 h, and the cultures were harvested and counted using a Matrix 96 Direct beta counter (Packard Instr., Meriden, CT). The proliferative response is measured as the [^{3}H]thymidine incorporation expressed as mean cpm of triplicate cultures. Stimulation indices (SI) are calculated as proliferative response in the presence of antigen, divided by proliferative response in the absence of antigen. Responses were initially assayed at various concentrations of the antigens and the optimal concentration range of responses to MOG, MBP, PLP, and MAG was determined to be between 5 and 25 μg/ml. In view of the difficulties encountered to purify adequate quantities of MOG suitable for cell culture, the samples were subsequently tested only at two protein concentrations (5 and 25 μg/ml) of the optimal concentration range; only the highest response observed is given in Results for each patient and control tested. Highest responses to MBP and PLP were generally observed at 5 μg/ml; responses to MOG were generally highest at 25 μg/ml.

Statistical analysis. A two-way χ^2 analysis was performed to compare the association between MS and responsiveness to each myelin antigen tested.

Results

Myelin antigen preparations. Highly purified myelin antigens were used in this study to ensure that reactivity to the various myelin antigens was indeed directed to each antigen being studied and not to small amounts of other myelin antigens possibly contaminating the preparations. Demonstration of a high degree of homogeneity was particularly important for the MOG preparations as such preparations are easily contaminated with PLP (15), which is present in myelin in considerably greater amounts than MOG (50% as compared with $\sim$ 0.01–0.05%). In addition, such preparations should not contain appreciable levels of MBP, which has so far been shown to be the myelin antigen towards which most of the T cell reactivity is directed (12). As demonstrated by immunoblotting with highly specific antibodies, the MOG preparations were not contaminated with even very low levels of PLP or MBP (Fig. 1 B). Anti-HNK1 antibody–reactive bands could not be detected in MOG preparations at molecular masses expected for MAG (90–100 kD); however, such reactivity was observed for bands corresponding to MOG (Fig. 1 B), the carbohydrate moiety of which contains the HNK1 epitope (35). Similarly, human MBP purified by the method of Dunkley and Carnegie (23) does not contain detectable levels of MOG, PLP, or MAG (Fig. 1 B). Such a procedure yields an undegraded preparation, as indicated by immunoblotting with anti-MBP antibodies (Fig. 1 B). Coomassie blue staining of a gel lane overloaded with purified human MAG reveals a single band (Fig. 1 A) of a molecular mass compatible with that of dMAG ($\sim$ 90 kD; 25). No protein band could be detected at molecular masses expected for MBP ($\sim$ 17–19 kD), PLP ($\sim$ 30 and 24 kD), or MOG (28 and 58 kD) (Fig. 1 A). Because of availability, bovine, rather than human, PLP was used. It was felt that such a species difference

A

B

was unlikely to have a marked effect on the potential T cell reactivity, in view of the very high homology between PLPs from various species (three amino acid differences between bovine and human PLP [36]). To ensure that we could indeed detect reactivity to bovine PLP with cells from another species and that conversion of PLP from solvent-soluble form to water-soluble form did not interfere with possible T cell epitopes, the freshly converted PLP preparation was tested on a mouse T cell line specific for a PLP peptide spanning amino acid residues 139–151, prepared as detailed previously (7). As shown in Table I, the water-soluble preparation of bovine PLP stimulated the PLP-specific mouse T cell line, even when amounts as low as 1 μg of the whole protein were used in the assay.

Proliferative responses to the myelin antigens by PBLs from MS patients and control subjects. Levels of reactivity to myelin proteins in standard proliferation assays are notoriously low

(12, 37). Low, albeit reproducible, proliferative responses were also observed in the present study. Accordingly, a SI threshold of 2 was arbitrarily selected as representing a positive response. Table II shows the proliferative responses to the various myelin

Table I. Bovine PLP Preparation Stimulates Mouse T Line Cells Specific for PLP Peptide 139–151

Antigen	Dose	Proliferative response	SI
	μg	*cpm*	
None	–	678	–
PLP peptide	2.0	48,607	71
PLP	1.0	5,506	8.12
PLP	2.5	6,938	10.0

Table II. Proliferative Responses to Myelin Antigens by PBLs from MS Patients

			Clinical profile					Proliferative response* of PBLs to:				
Patient	Sex	Age	Diagnosis	Duration	Type‡	Activity	EDSS (K.S)	None	MOG	MBP	MAG	PLP
		yr										
MS1	F	43	Definite	20 yr	RR	Rem.	2.5	2,065±244	10,008±372 (4.8)	1,874±257	906±159	2,095±21
MS2	F	33	Definite	8 yr	RR	Rem.	2.5	460±103	1,697±228 (3.6)	972±34 (2.1)	298±83	475±66
MS3	F	41	Probable	1 wk	?	First attack	5.5	5,491±3,469	7,369±926	10,079±2,146	3,304±90	7,301±2,997
MS4	F	48	Definite	16 yr	CP	–	6.0	1,411±67	19,540±638 (13.8§)	906±76	ND	499±79
MS5	F	55	Probable	19 yr	RR	Rem.	3.5	1,061±161	1,475±894	1,485±99	1,122±105	765±209
MS6	F	66	Definite	3 yr	RR	Rem.	3.0	588±66	4,482±1,479 (7.6)	2,481±475 (4.2)	426±17	1,408±339 (2.4)
MS7	F	46	Definite	10 yr	RR	Rem.	7.0	304±114	402±135	329±36	297±111	224±108
MS8	F	46	Definite	9 yr	RR	Rem.	2.5	2,056±499	1,955±5	2,549±95	1,356±171	1,585±99
MS9	F	40	Definite	7 yr	RR	Rem.	2.5	439±247	1,090±285 (2.5)	367±152	843±158	374±4
MS10	M	44	Definite	12 yr	RR	Rem.	6.0	444±27	819±113	1,028±181 (2.3)	599±70	638±70
MS11	F	17	Definite	2 yr	RR	Rem.	3.0	3,235±737	6,868±567 (2.1)	7,336±1,693 (2.2)	2,379±158	9,935±1,497 (3.0)
MS12	M	55	Definite	19 yr	CP	–	8.0	288±35	562±113	432±55	317±61	364±74
MS13	F	42	Definite	2 yr	RR	Rem.	5.0	103±5	256±105	104±4	ND	202±19
MS14	F	40	Definite	6 yr	RR	Rem.	2.0	1,808±426	6,151±108 (3.4)	2,019±219	940±212	3,343±298
MS15	F	46	Definite	26 yr	RR	Rem.	4.0	1,249±322	670±277	1,443±433	236±37	888±635
MS16	F	51	Definite	16 yr	RR	Rem.	7.0	393±25	764±164	438±59	485±101	435±101
MS17	F	42	Definite	15 yr	RR	Rem.	2.5	336±61	386±20	432±165	246±31	331±80
MS18	F	36	Definite	12 yr	RR	Rem.	2.0	594±103	1,313±39 (2.2)	908±104	490±258	615±199
MS19	M	33	Definite	9 yr	RR	Rem.	3.5	198±18	1,471±387 (7.4)	238±86	ND	201±93
MS20	F	43	Definite	21 yr	RR	Rem.	4.0	211±73	1,077±125 (5.1)	302±43	263±153	330±86
MS21	M	52	Definite	24 yr	RR	Rem.	2.5	1,318±288	1,534±777	945±98	277±40	507±134
MS22	M	47	Definite	16 yr	RR	Relapse	2.5	1,022±12	914±39	949±162	482±31	530±153
MS23	M	56	Definite	21 yr	CP	–	8.0	477±115	1,438±454 (3.0)	503±121	392±63	456±143
MS24	F	50	Probable	20 yr	RR	Rem.	ND	452±162	3,318±457 (7.3)	1,394±625 (3.0)	ND	377±96

* Expressed as mean cpm of incorporated [³H]thymidine±SD. Where significantly increased (≥2), SI is given in parentheses.
‡ RR, relapsing-remitting; CP, chronic progressive; Rem., remission.
§ Tested on fetal calf serum.

antigens tested by PBLs from MS patients. As can be seen, the greatest incidence of response observed in MS patients was to MOG. Of the 24 MS patients tested, 12 showed a positive response to this antigen, whereas only 5 and 2 responded to MBP and PLP, respectively (Table II). It is interesting to note that reactivity to MOG was not only more frequent in MS patients than reactivity to MBP and PLP, but the extent of reactivity was also generally higher (see Table II). In this population of MS patients, only one patient (MS9) responded to MAG; however, the stimulation (SI = 1.92) did not reach our threshold of significance. Although reactivity to MOG of PBLs from MS patients was also seen in combination with reactivity to other myelin antigens, > 66% (8/12) of the MS patients responding to MOG reacted to this antigen exclusively (Table II). Of the five MS patients that showed a positive response to MBP (Table II), only one (patient MS10) reacted with MBP exclusively; the other four (patients MS2, MS6, MS11, and MS24) responded to MOG also and two of these (patients MS6 and MS11) had PLP-reactive PBLs as well (Table II). Reactivity to PLP by PBLs from MS patients was only observed in combination with reactivity to other myelin antigens, MOG and MBP (patients MS6 and MS11; Table II). Thus, only two MS patients reacted to a combination of three myelin antigens, and reactivity to all four myelin antigens tested was not observed.

The incidence of MOG reactivity measured in individuals of the control group (Table III) was significantly lower than that measured in MS patients as, of the 16 controls tested, only 1 responded (Table III, subject C11). It should be noted that PBLs from this control individual (C11) also reacted to MBP.

Reactivity to MBP was also observed in two other control individuals (Table III, C8 and C15). Positive responses (SI ≥ 2) to PLP and MAG were not observed with control PBLs (Tables III and IV).

Statistical analysis of the association between MS and responsiveness to each myelin antigen tested indicated that the association between MS and responsiveness to MOG was highly significant (χ^2 = 8.37, degree of freedom [d.f.] = 1, p < 0.004; Table IV). There was no statistically significant association between MS and responsiveness to the other myelin antigens in the MS population tested in this study.

Discussion

In view of the encephalitogenic activity of purified MBP and PLP (reviewed in reference 38) and, more specifically, because MBP- or PLP-specific T cells are sufficient to cause EAE (7–11), these two most abundant myelin proteins have been regarded as prime candidate antigens to elicit relevant immune responses in MS. However, and in contrast to this widely accepted notion, our present analysis shows that the quantitatively minor myelin protein, MOG, may be at least as important an antigen in MS as MBP or PLP. Indeed, the fact that 12 of 24 patients (50%) reacted to MOG while only 5 and 2 patients reacted to MBP and PLP, respectively, may point to MOG as a primary candidate antigen in MS. That only 1 of the 16 control individuals reacted to MOG further supports the postulate that reactivity to MOG plays a role in the pathogenesis of MS.

Table III. Proliferative Responses to Myelin Antigens by PBLs from Control Individuals

Control	Sex	Age	Health status	Proliferative response* of PBLs to:				
				None	MOG	MBP	MAG	PLP
		yr						
C1	F	47	Epilepsy	2,203±86	516±91	1,182±99	178±31	1,478±241
C2	M	18	Brachial plexitis	6,532±1,029	6,981±1143	6,795±1,228	709±64	4,546±140
C3	M	43	Epilepsy	1,552±8	2,409±59	1,864±307	911±183	1,666±98
C4	M	61	CVA	1,237±34	703±74	1,108±176	345±65	527±168
C5	M	46	Epilepsy	5,103±740	5,364±587	6,529±130	3,327±577	4,400±215
C6	F	45	Dismyelinating disease	3,231±191	4,764±46	1,268±267	666±106	1,728±298
C7	F	19	Healthy	2,505±269	2,025±790	4,695±484	482±37	867±125
C8	F	20	Healthy	690±144	986±6	1,457±284 (2.1)	581±52	949±145
C9	F	70	Parkinson's disease	236±58	370±104	367±201	307±103	239±18
C10	M	42	Healthy	1,149±389	1,157±1	1,031±161	381±77	604±53
C11	F	46	Tension headache	342±34	790±32 (2.3)	797±164 (2.3)	ND	651±701
C12	M	42	Tension headache	11,482±3,252	9,510±743	14,258±905	ND	7,757±572
C13	M	45	Meningioma	5,128±1,826	4,905±79	4,891±109	ND	4,288±183
C14	F	58	Healthy	1,420±329	1,438±191	2,027±301	ND	1,124±135
C15	F	42	Epilepsy, brain atrophy	1,248±154	1,065±207	2,287±179	454±130	1,547±137
C16	F	45	Healthy	1,412±157	1,260±126	833±67	562±48	1,552±212

* Expressed as mean cpm of incorporated [^{3}H]thymidine. Where significantly increased (≥ 2), SI is given in parentheses.

In this study, few MS patients (5/24; 20.8%) were found to show positive cell-mediated responses to MBP, and a similar proportion (2/16; 12.5%) of control individuals also reacted to this antigen. Although a low frequency of MBP-responsive MS patients was unexpected, in view of the data of some previously published studies (39–43) and the assumption that MS is associated with the response to MBP, these results are in line with other studies where PBL sensitization to MBP could be detected in some MS patients, but also in patients with other neurological diseases and in healthy controls (37, 44–50). Similarly, our data on PLP reactivity, whereby 2 of 24 MS patients reacted positively to this antigen, are in agreement with some studies but in disagreement with others, as diverging results have been obtained by various groups assessing PLP sensitization in MS. Thus, data reported by Trotter et al. (48) showed sensitization to PLP in 6 of 16 patients with chronic progressive MS and 3 of 15 patients with relapsing remitting MS; Sun et al. (51), using an immunospot assay to detect IFN-γ secretion by antigen-specific lymphocytes, reported increased numbers of PLP-reactive T cells in both blood and cerebrospinal fluid

Table IV. Summary of Proliferative Responses to Myelin Antigens

Stimulating antigen	Frequency of positive response by:	
	MS PBLs (%)	Control PBLs (%)
MOG	12/24 (50)*	1/16 (6.25)
MBP	5/24 (20.8)	2/16 (12.5)
MAG	0/20 (0)	0/12 (0)
PLP	2/24 (8.3)	0/16 (0)
MOG and MBP	4/24 (16.6)	1/16 (6.25)
MOG and PLP	2/24 (8.3)	0/16 (0)

* $P < 0.004$ compared with control group.

(CSF) of most MS patients tested. Although no differences in frequencies of circulating T cells to PLP were found by Chou et al. (49) between MS patients and control subjects, the same study showed a significantly higher frequency of PLP-reactive T cells in the CSF of MS patients than in the CSF of individuals with other neurological diseases (49). In contrast, Hafler et al. (45) could not detect PLP reactivity by any of 57 T cell clones derived from autopsied MS plaque tissue, nor by any of 235 clones derived from the CSF and 126 clones from the peripheral blood of other subjects with MS. In the study of Johnson et al. (37), reactivity to PLP could only be uncovered in a few MS patients after deletion of the T8 (cytotoxic/suppressor) lymphocyte subset from PBLs; however, a significantly elevated number of patients with other neurological diseases reacted to this antigen whether or not T8-positive cells had been removed (37). Ota et al. (47) observed more reactivity to PLP, albeit of no statistical significance, in patients with MS compared with patients with other neurological diseases. Very little reactivity to MAG was observed in our study as PBLs from only one MS patient (MS9) responded, albeit not significantly (SI = 1.92). This is in contrast with the study of Johnson et al. (37), where low-level MAG sensitization was seen to occur in some active MS patients, and the study of Zhang et al. (52), where 7 of the 11 MS patients tested responded to this antigen, 2 of them exclusively. Higher numbers of MAG-reactive cells were also reported in blood and CSF of MS patients by Link et al. (53) using the immunospot assay. Controversial results obtained with MAG as well as with the other myelin antigens tested may reflect not only differences in antigenic preparations and/or amounts of antigen used in the assays (12), but also experimental differences in protocols followed to detect sensitization by assessing proliferative responses to purified antigens. An alternative explanation for variable results is that MS may in fact be associated with more than one myelin antigen so that the primary target may be different in different patients. Further-

more, and as demonstrated in EAE induced with purified MBP, sensitization to one myelin antigen can lead to the induction of reactivity towards additional CNS autoantigens (54), which could perhaps override reactivity to the primary target.

MOG has recently attracted a lot of interest in view of its possible role as a target antigen in immune-mediated demyelinating diseases (reviewed in reference 55). Thus, humoral immunity to MOG has been correlated with demyelinating activity in chronic relapsing EAE (18), and the presence of antibodies to MOG within CNS tissue results in extensive demyelination in vivo (19–21) and in vitro (22, 56). In addition to the possible role of anti-MOG antibodies in mediating demyelination, these studies demonstrated the immunogenic potential of MOG, which, despite being present in very low amounts in the CNS (15, 17), can induce important, detectable immune responses in animals injected for EAE with spinal cord or brain homogenates (18, 57). That MOG may be a primary target for autoimmune responses associated with demyelinating diseases is also strongly suggested by our preliminary results whereby Lewis rats injected with purified human MOG develop a demyelinating and relapsing EAE-like disease with concomitant production of increasing levels of anti-MOG antibody in the serum, to the exclusion of detectable humoral immune response to other myelin proteins (Kerlero de Rosbo, N., T. Johns, K. Menon, S. Abo, and C. Bernard, manuscript in preparation). Because MOG, a highly hydrophobic molecule present in very small quantities in CNS tissue (15, 58), is extremely difficult and cumbersome to purify in quantities and quality required to analyze specific T cell reactivity by standard assays, studies to assess sensitization to this antigen have not been forthcoming. However, in one study, where problems associated with purification of MOG were circumvented by using the immunospot assay, MOG-reactive T cells were observed in peripheral blood of most of the 14 MS patients studied, whereas only 25% of the 28 controls showed reactivity (59). Nevertheless, this study is, to the best of our knowledge, the first report of an investigation of the proliferative response to highly purified MOG by PBLs from MS patients. As yet, it is difficult to correlate reactivity to MOG with a particular status of the disease. Neither average duration of the disease (12.08±6.9 and 15.2±6.5 yr for responders and nonresponders, respectively) nor average age of onset (30±11 and 32.5±5.4 yr for responders and nonresponders, respectively) appear to be factors influencing the response to MOG of PBLs from MS patients. Most importantly, the responsiveness to MOG by MS PBLs was not restricted to a particular subgroup of MS patients as it was observed in definite as well as probable MS, in either the relapsing remitting or the chronic-progressive type. Two of the three chronic progressive MS patients (Table II, MS4 and MS23) responded to MOG, but not to the other three myelin antigens tested, and the two patients with an acute attack (Table II, MS3 and MS22) did not show any reactivity to MOG. However, due to the small number of patients in each subgroup, it is difficult to draw any conclusion correlating MOG responsiveness with disease stage. Nevertheless, our study demonstrates not only that assessment of the reactivity to minor myelin antigens may be highly relevant to the understanding of MS pathogenesis, but also emphasizes the importance of testing reactivity to all potential antigens in parallel, in the attempt to assign a primary role to one or the other possible targets. In conclusion, predominance of reactivity to MOG in MS in the context of comparatively rarer reactivity to the other

myelin antigens tested concomitantly in the same patients shows that MOG may be as important as MBP or PLP in disease pathogenesis and may indeed be the primary target myelin antigen in at least some MS patients. Our observation that MOG itself may also be encephalitogenic (Kerlero de Rosbo, N., T. Johns, K. Menon, S. Abo, and C. Bernard, manuscript in preparation) strongly supports this postulate and emphasizes the relevance of immune responses to MOG in MS. Persistence of MOG reactivity together with reactivity to other myelin antigens could point to a greater role for immune recognition of MOG in disease initiation with broadening of the autoimmune response to additional determinants on this and/or other autoantigens as the disease progresses. Further studies are in progress to investigate this hypothesis.

Acknowledgments

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