Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Neuroimmunology, 33 (1991) 7–15

JNI 02007

T cell sensitization to proteolipid protein in myelin basic protein-induced relapsing experimental allergic encephalomyelitis

Linda L. Perry[1], Elena Barzaga-Gilbert[2] and John L. Trotter[3]

[1] *Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT 59840, U.S.A.,* [2] *Department of Microbiology, University of Virginia, Charlottesville, VA 22908, U.S.A., and* [3] *Department of Neurology and Neurological Surgery, Washington University, St. Louis, MO 63110, U.S.A.*

(Received 10 December 1990)
(Revised, received 28 January 1991)
(Accepted 28 January 1991)

Key words: Encephalomyelitis; Myelin basic protein; Proteolipid protein; Multiple sclerosis; (Mouse)

Summary

$(SJL/J \times PL/J)F_1$ mice immunized with myelin basic protein (MBP) develop an autoimmune demyelinating disease termed relapsing experimental allergic encephalomyelitis (rEAE). The acute stage of disease is mediated by $CD4^+$ T cells specific for MBP amino acids 1–9. To determine the immunologic bases for disease relapse, host sensitization to additional autoantigens of the central nervous system was measured. Results indicate that most animals develop T cell reactivity to endogenous myelin proteolipid protein (PLP) during rEAE. However, PLP-specific immunity does not appear to account for expression of relapse episodes of demyelination.

Introduction

Relapsing experimental allergic encephalomyelitis (rEAE) is an autoimmune T cell-mediated demyelinating disease (Alvord et al., 1984; Lublin, 1985) that can be induced in several experimental animal species by immunization with myelin basic protein (MBP) (Panitch and Ciccone, 1981; Fritz et al., 1983a) or proteolipid protein (PLP) (Cambi et al., 1983; Endoh et al., 1986; Yamamura et al., 1986; Trotter et al., 1987), the major protein components of central nervous system (CNS) myelin. Susceptibility to EAE (Fritz et al., 1984, 1985; Zamvil and Steinman, 1990) and to its human counterpart disease, multiple sclerosis (MS) (McDevitt et al., 1989; Oksenberg and Steinman, 1989; Haegert et al., 1990), is controlled predominantly by genes within the T cell receptor and major histocompatibility complex (MHC) loci. In murine studies, a permissive genotype has been shown to allow MHC-linked recognition of CNS autoantigen by host $CD4^+$ autoreactive T cells (Zamvil et al., 1985). Affected animals display one acute and 2–3 relapse episodes of flaccid limb paralysis sep-

Address for correspondence: Linda L. Perry, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT 59840, U.S.A.

arated by periods of spontaneous recovery, similar to the pattern of relapse and remission that occurs in MS. Regulation of the acute stage of EAE is achieved at least in part through the action of antigen-specific T suppressor cells, shown to influence the incidence, severity, and recovery from the initial episode of disease signs (Welch et al., 1980; Hinrichs et al., 1981; Perry and Barzaga, 1987; Perry et al., 1988). The immunologic mechanism(s) allowing the reappearance of autoreactivity during relapse stages of rEAE or MS is less well understood.

Detailed studies in a murine model of MBP-induced rEAE have defined precise epitopes recognized by T cells from mice of distinct genetic backgrounds. The response of SJL/J (H-2^s) mice is directed primarily against MBP epitopes located in the carboxy terminus at positions 87–98 and 91–104 (Pettinelli et al., 1982; Kono et al., 1988). In contrast, PL/J (H-2^u) and (SJL/J × PL/J)F_1 mice respond preferentially to an amino terminal epitope at amino acids 1–9 (Fritz et al., 1983b; Zamvil et al., 1986). Antigen recognition is I-A restricted in each case. The immunodominance of specific T cell epitopes, coupled with a high degree of receptor homogeneity among T cells responsive to these epitopes (Acha-Orbea et al., 1988; Urban et al., 1988), suggests that acute EAE is mediated by a predominant receptor population of $CD4^+$ T cells. Participation of this same cell population in relapse disease is supported by the demonstrated capacity of a single T cell clone to transfer rEAE (Zamvil et al., 1985), although the potential role of host T cells was not excluded in these studies. On the other hand, evidence also exists to support an increased level of heterogeneity among T cells involved in relapse as opposed to acute EAE. First, analyses of T cell proliferative responses to MBP at different stages of rEAE revealed a population of I-E^u-restricted T cells that were detectable only during relapse episodes of paralysis (Perry and Barzaga, 1987). Second, suppression of T cells active in acute EAE by anti-receptor monoclonal antibody (mAb) immunotherapy did not prevent the occurrence of relapse disease in a large proportion of treated animals, apparently due to the emergence of T cells expressing a distinct receptor and epitope specificity (Acha-Orbea et al., 1988). Finally, recent reports have identified several minor epitopes on the MBP molecule that could serve as targets of the relapse T cell response (Kono et al., 1988; Zamvil et al., 1988; Fritz et al., 1990).

The present studies were designed to examine an alternate explanation for the expression of relapse T cell autoreactivity in animals that have recovered from acute EAE. Based upon histologic evidence of macrophage accumulation and increased Ia expression at sites of demyelination (Sobel et al., 1984a, b; Butter et al., 1988), it was reasoned that myelin autoantigens in addition to the sensitizing MBP may become available for presentation to T cells as disease progresses. To test this possibility, the capacity of T cells from (SJL/J × PL/J)F_1 mice in various stages of MBP-induced rEAE to respond to CNS autoantigens such as PLP or myelin-associated glycoprotein (MAG) was analyzed. Results indicate that T cell sensitization to endogenous PLP, but not to MAG, does occur during the course of MBP-induced disease. However, PLP-specific autoimmunity does not appear to account for the expression of relapse episodes of demyelination and paralysis in this disease model.

Materials and methods

Animals

SJL/J, PL/J, and (SJL/J × PL/J)F_1 mice were bred at the Rocky Mountain Laboratory animal facilities from breeder stocks purchased from The Jackson Laboratory, Bar Harbor, ME, U.S.A. Animals were introduced into experiments at 8–12 weeks of age. Mice unable to reach ad libitum food and water stocks due to hind limb paralysis of rEAE were hand fed and watered daily.

Antigens

MBP was purified from guinea pig brains (Pel-Freez) according to the procedure of Chou et al. (1976). This protocol calls for the extraction of brain homogenates in chloroform-methanol, allowing an initial separation of the insoluble MBP-containing fraction from the chloroform-methanol-soluble PLP fraction. Nevertheless, the possibility of contamination of purified MBP with PLP was tested rigorously. PLP could not be

detected when MBP preparations were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis or by radioimmunoassay for PLP (Trotter et al., 1981). More importantly, T cell clones specific for PLP failed to respond in vitro to the MBP used in these studies, despite a strong response of these cells to purified PLP (Van der Veen and Trotter, unpublished data). Finally, the absence of PLP reactivity in some mice immunized with MBP indicates that sensitization to each antigen occurs independently.

PLP was purified as described previously (Hampson and Poduslo, 1986) and analyzed for the presence of contaminating MBP. MBP could not be detected in these preparations when analyzed by immunoblot (Macklin et al., 1984) or by radioimmunoassay (RIA) (lower limit of detection = 0.05% contamination) (Cohen et al., 1975). Further, T cell clones specific for MBP did not respond to the PLP preparations used in this study (Van der Veen et al., 1990). Thus, no evidence for contamination of either MBP or PLP with the opposing protein could be obtained when samples were analyzed biochemically or through recognition by antibody or T cells, the latter being most relevant to the cellular events analyzed in the present studies.

MAG was obtained through the generosity of Dr. John Roder, University of Toronto.

Immunizations

Mice were injected with 200 μg MBP in 0.1 ml incomplete Freund's adjuvant supplemented with 50 μg *Mycobacterium tuberculosis* $H_{37}RA$ (Difco Laboratories, Detroit, MI, U.S.A.). 24 and 72 h later, animals received 3×10^9 *Bordetella pertussis* organisms retroorbitally (Michigan Department of Health, Lansing, MI, U.S.A.). Animals were examined daily and scored for disease severity on the basis of the following objective criteria: 1 = flaccid tail, weak grip; 2 = mild limb weakness, difficulty turning over; 3 = severe limb weakness or mild paralysis; 4 = severe limb paralysis; 5 = total paralysis and wasting.

To allow direct comparisons of T cell reactivity over the course of rEAE, groups of 20–30 mice were immunized at 2-week intervals and animals from groups at the acute, first and second relapse episodes of disease were examined within the same experiment.

T cell proliferation assays

T cell sensitization to MBP or PLP was determined by measuring the proliferative response of lymph node T cells to antigens presented in vitro. Inguinal and axillary lymph nodes were removed from 2–3 MBP-immunized F_1 mice at varying times post-immunization and T cells were isolated using nylon wool columns as described by Julius et al. (1973). T cells (4×10^5/well) were cocultured in 96-well Linbro plates (Flow Laboratories, McLean, VA, U.S.A.) with purified MBP (2.5 μg/well), PLP (1, 2.5, or 10 μg/well), or MAG (1–10 μg/well) in the presence of 3×10^4 oyster glycogen-induced syngeneic peritoneal exudate cells as a source of antigen-presenting cells (1 ml 2.5% glycogen i.p. on day −3, Sigma Chemical Co., St. Louis, MO, U.S.A.) in a total of 200 μl. RPMI 1640 containing 10% fetal calf serum and 100 U L-glutamine. In certain experiments, the restriction element specificity of T cells responding to MBP or PLP in vitro was determined by the addition of one of a panel of purified mAbs (Perry and Williams, 1985) at the initiation of cultures at a final concentration of 50 μg/ml. After 3 days at 37°C, wells were pulsed with 2 μCi [^{3}H]thymidine and harvested 16–20 h later using a Skatron Cell Harvester (Skatron, Sterling, VA, U.S.A.). Antigen-specific responsiveness was determined by subtracting the mean cpm incorporated in the absence of added antigen (1–3000 cpm) from counts obtained in the presence of antigen. Samples were assayed in triplicate and usually varied less than 5% from the mean.

DTH responses

Delayed-type hypersensitivity (DTH) to MBP and PLP was measured by injection of 30 μl of a 1 mg/ml solution of protein into the left hind footpad of mice immunized with 100 μg PLP or 200 μg MBP in adjuvant. Footpad thicknesses of both hind feet were measured 24 h later using an engineer's micrometer. The difference in thickness of the injected versus the uninjected footpad was used as a measure of the swelling response. Specific swelling was determined by subtracting the swell-

ing response of nonimmunized control mice from that of immunized experimental animals.

Tolerization

Mice were tolerized using the procedure of Kennedy et al. (1990). Briefly, erythrocyte-depleted syngeneic spleen cells were incubated with antigen in the presence of ethylcarbodiimide (Pierce Chemical Co., Rockford, IL, U.S.A.) for 60 min and then washed extensively before injection of $8-10 \times 10^8$ cells i.v. into recipients. Mice received two i.v. injections of PLP- or control bovine serum albumin (BSA)-coupled spleen cells on days -7 and 0 relative to subcutaneous immunization with 100 μg PLP or 200 μg MBP.

Results

T cell sensitization to PLP

Initial experiments were designed to measure the capacity of T cells from MBP-immunized $(SJL/J \times PL/J)F_1$ mice to respond to PLP, either by expression of delayed-type hypersensitivity (DTH) responsiveness in vivo or T cell proliferative reactivity in vitro. Optimum concentrations of PLP for use in both of these assays were determined in preliminary experiments using mice immunized with purified PLP (data not shown). In vitro proliferative responses to MBP and PLP were measured in parallel using lymph node T cells derived from F_1 mice at various stages of rEAE. Pre-disease T cells (day 11 p.i.) showed no reactivity to the immunizing autoantigen, MBP, or to PLP (Fig. 1). MBP reactivity was evident on day 21 p.i. and peaked around day 35 p.i., while responses to PLP developed more slowly and peaked around days 35–50 p.i. without achieving the magnitude of the MBP response. Cells from nonimmune mice did not respond to PLP (data not shown). Neither was T cell reactivity to MAG detected at any stage of disease, as measured by in vitro proliferative responsiveness (data not shown).

Based upon the detection of in vitro T cell responsiveness to PLP, the ability of MBP-immune mice to develop PLP-specific DTH reactivity was also examined. Animals at different stages of rEAE were injected with purified PLP in the hind footpad and swelling responses measured 24

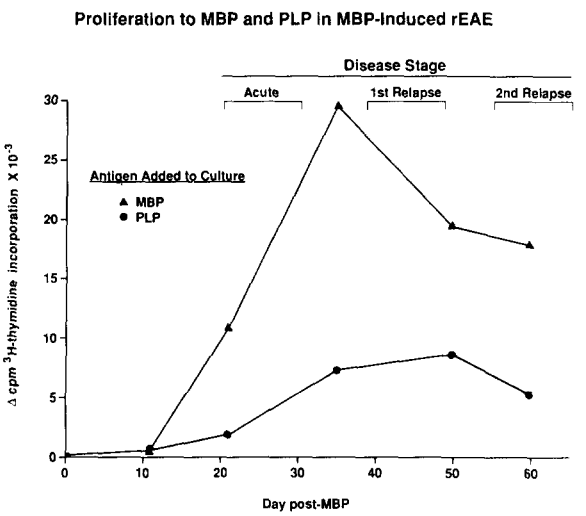

Fig. 1. Proliferative response of lymph node T cells to MBP (2.5 μg/well) and PLP (2.5 μg/well) during the progression of MBP-induced rEAE.

h later. Considerable variation was observed in the magnitude of individual DTH responses to PLP regardless of the disease stage, some animals displaying no evidence of reactivity while others developed significant footpad swelling after PLP challenge (Fig. 2). DTH responses to MBP in

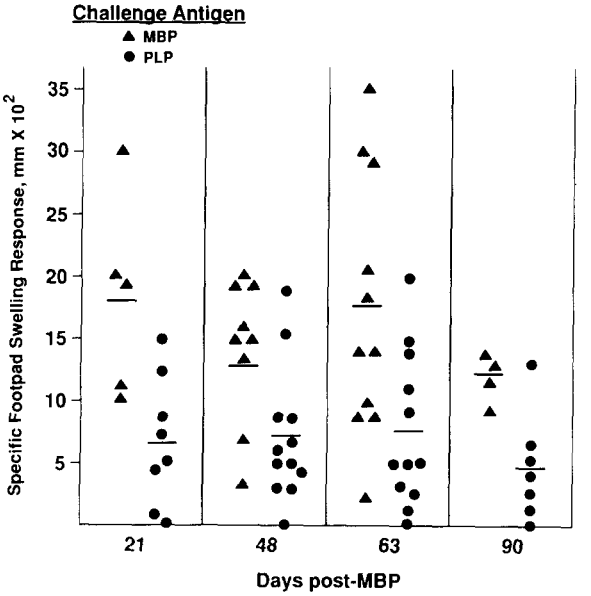

Fig. 2. DTH responses of individual mice to MBP and PLP at successive stages of rEAE. Horizontal bars indicate mean responses of each group.

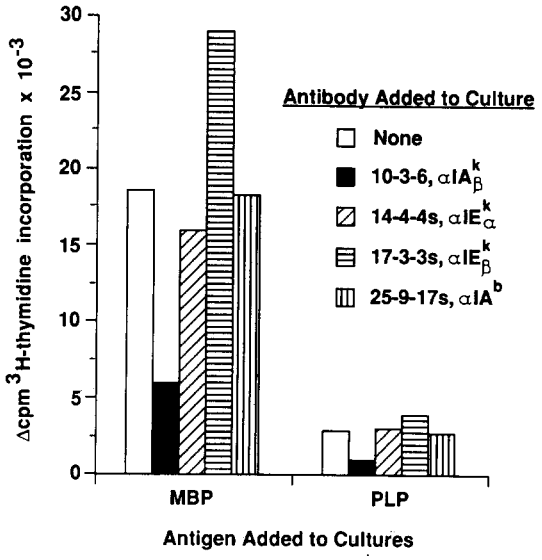

Fig. 3. Restriction element specificity of T cell recognition of MBP or PLP as determined by monoclonal antibody-mediated inhibition of T cell proliferative responses.

animals from the same experiments were uniformly positive, but also subject to quantitative variation between individuals. Responses to both autoantigens were detectable at least until day 90 p.i.

MHC restriction on T cell recognition of PLP

The restriction element specificity of T cells responsive to PLP was determined by monoclonal antibody-mediated inhibition of the antigen-specific in vitro proliferative response (Fig. 3). T cells derived from mice in the first relapse stage of rEAE were cultured in the presence of MBP or PLP. Proliferation to both antigens was suppressed by the addition of monoclonal anti-I-$A^{s,u}$ antibodies but not by mAbs specific for the α- or the β-chain of the I-E glycoprotein expressed by the PL haplotype or by mAb specific for an irrelevant I-A alloantigen (I-A^b). These results indicate that T cell recognition of PLP is I-A restricted in (SLJ/J × PL/J)F_1 mice.

TABLE 1

EFFICACY OF PLP TOLERIZATION REGIMEN

Immunization [a]	Tolerization [b]	Challenge [c]	Mean footpad swelling (mm × 10^{-2}) [d]
PLP/CFA	–	PLP	19.7 ± 1.8
PLP/CFA	BSA-sc i.v.	PLP	18.8 ± 2.1
PLP/CFA	PLP-sc i.v.	PLP	8.5 ± 1.3
–	–	PLP	8.1 ± 0.4

[a] Groups of seven (SJL/J × PL/J)F_1 mice were immunized s.c. with 100 μg PLP emulsified in complete Freund's adjuvant on day 0.

[b] Mice in certain groups also received 8×10^7 PLP- or BSA-coupled spleen cells (sc) i.v. on day −7 and day 0.

[c] Six days after immunization, mice were challenged with 30 μg soluble PLP in the hind footpad.

[d] Specific swelling responses were measured 24 h after challenge and are presented as the mean ± SE.

Role of PLP autoimmunity in MBP-induced EAE

In order to assess the relative contribution of PLP-specific autoimmunity to the progression of MBP-induced rEAE, F_1 mice were specifically tolerized to PLP prior to the induction of disease. The efficacy of a tolerization regimen defined by Kennedy et al. (1990) was verified in the present experiments by comparison of PLP-specific DTH responses in tolerized versus nontolerized mice. As indicated in Table 1, immunity to PLP was signifi-

TABLE 2

TOLERIZATION TO PLP FAILS TO INHIBIT EXPRESSION OF RELAPSE EAE

Immunization [a]	Tolerization [a]	Disease incidence	Mortality	Parameters of relapse [b]			PLP DTH [c]
				No. episodes	Severity	Duration (days)	
MBP	–	10/14	3/14	2.2 ± 0.3	1.8	6.6	9.0 ± 3.6
MBP	PLP	12/14	5/14	2.2 ± 0.2	1.8	6.7	1.7 ± 1.0

[a] Mice were immunized and tolerized as described under Materials and Methods.

[b] Numbers represent the mean number of relapse episodes of paralysis, the mean severity of disease signs during first relapse, and the mean duration (in days) of the first relapse episode.

[c] Specific footpad swelling response (mm × 10^{-2}) to PLP measured 35 days post-immunization (mean ± SE).

cantly depressed in mice that were tolerized by intravenous injection of PLP-coupled cells as compared to animals that received BSA-coupled cells or no tolerizing cells.

A similar protocol was then used to suppress PLP-specific immunity in F_1 mice immunized with MBP. Animals were injected intravenously with PLP-coupled cells on day −7 and day 0 relative to MBP immunization, in keeping with the protocol previously described (Kennedy et al., 1990), and monitored for clinical signs of rEAE over the next 90 days. Results of two separate experiments are summarized in Table 2. The incidence of disease, number and severity of relapse stages of paralysis, and incidence of mortality were found to be similar in PLP-tolerized versus nontolerized groups of mice. Efficacy of tolerization was again verified by the failure of tolerized mice to mount PLP-specific DTH during relapse disease (Table 2). These data indicate that reactivity to PLP, although detectable during the clinical course of rEAE, is not essential to the expression of relapse autoreactivity.

Discussion

The development of rEAE in (SJL/J × PL/J)F_1 mice immunized with purified MBP is shown herein to result in sensitization to additional autoantigens of the host CNS, most notably to PLP. T cell immunity to PLP, as measured in vitro by the antigen-induced proliferation of cells pooled from 2–3 donors, was first detected during acute EAE (3 weeks p.i.) and peaked during the first and second relapse stages of disease (5–7 weeks p.i.). PLP-specific DTH was detectable in some but not all MBP-immunized animals at least through 90 days p.i. Despite substantial variation in the magnitude of individual DTH reactions to either autoantigen, mean responses to PLP were consistently lower than responses to the immunizing MBP. The lack of sensitization to PLP in certain MBP-immune animals, coupled with the failure to detect PLP in preparations of purified MBP, provide compelling evidence for endogenous PLP as the only possible source of this antigen. Reactivity to myelin-associated glycoprotein was not detected at any stage of rEAE, as measured by T cell proliferation in vitro.

The relevance of PLP autoreactivity to the expression of relapse disease was also investigated. Acute EAE in MBP-immunized F_1 mice is known to be mediated by a population of I-A^u-restricted T cells specific for an amino terminal epitope comprised of amino acids 1–9 (Zamvil et al., 1986). Previous studies of the T cell proliferative response to MBP at different stages of rEAE revealed an additional population of I-E^u-restricted cells expressed only in animals experiencing relapse disease (Perry and Barzaga, 1987). To determine whether or not this population of relapse-specific I-E^u-restricted T cells could be responding to PLP that was carried into proliferation assays by contaminating host macrophages, the restriction element specificity of PLP-specific (SJL/J × PL/J)F_1 T cells was determined. Results indicated that recognition of PLP is I-A rather than I-E restricted, however, negating this possibility. A second I-E^u-restricted MBP epitope has since been identified as an encephalitogen in this F_1 animal (Zamvil et al., 1988).

Further attempts to discern a functional role for PLP-reactive T cells in the expression of relapse disease depended upon tolerization of F_1 mice to PLP prior to the induction of rEAE. Comparison of several parameters of disease expression in tolerized versus nontolerized mice failed to reveal any significant contribution of PLP responsiveness to the progression of rEAE, however. Thus, the incidence of acute paralysis, the severity of disease signs, and the number of relapse episodes were similar in both groups of animals. These results contrast the data obtained by Kennedy et al. (1990) using a model of mouse spinal cord homogenate-induced rEAE, where tolerization to PLP had a marked suppressive effect on disease expression. Their results suggest that PLP is the dominant encephalitogen in a spinal cord homogenate, as opposed to MBP as the encephalitogen in our system. The failure of PLP-specific tolerization to influence the course of MBP-induced disease in mice that normally exhibit at least some level of PLP-specific immunity probably reflects the fact that T cell recognition of epitopes on MBP dominate the autoimmune response at all stages of rEAE. At least three epi-

topes have now been identified as potential targets of the SJL/J (H-2^s) T cell response (Kono et al., 1988; Sakai et al., 1988a, b; Fritz et al., 1990) and two epitopes have been defined in the PL/J (H-2^u) strain or in the H-$2^{u/s}$ F_1 hybrid (Zamvil et al., 1988; Fritz and McFarlin, 1989). Apparently, suppression of the PLP-specific portion of relapse T cell reactivity is insufficient to alter significantly the overall course of disease.

The causative basis for immunological sensitization to myelin autoantigens in human MS has not been fully resolved. The epidemiology of the disease (Waksman, 1985) as well as the finding of cerebrospinal fluid (CSF) and serum immunoglobulins reactive with a variety of viruses such as measles, herpes simplex virus, paramyxovirus, rubella and coronaviruses suggest a possible viral etiology (Burks et al., 1980; Albrecht et al., 1983; Salmi et al., 1983; Cook et al., 1986; Coyle and Sibony, 1987; Goswami et al., 1987), although no consistent relationship between any particular agent and MS is apparent. Nevertheless, it has been postulated that CNS infection with such viruses in individuals possessing a permissive genetic background could lead to autoimmune demyelination either as a result of crossreactivity between epitopes on viral and myelin antigens or as a consequence of autoantigen presentation by macrophages and other class II-inducible cells present at sites of the anti-viral immune response (Waksman, 1985). Several laboratories have now identified class II-restricted proliferating T cells (Kitze et al., 1988; Richert et al., 1988; Baxevanis et al., 1989; Allegretta et al., 1990; Ota et al., 1990), cytotoxic T cells (Weber and Buurman, 1988; Martin et al., 1990; Zhang et al., 1990), and antibodies (Catz and Warren, 1986; Garcia-Merino et al., 1986; Olsson et al., 1990; Warren and Catz, 1990) reactive with MBP in MS patients. Reactivity to myelin-associated glycoprotein as well as to MBP has also been detected in certain individuals (Link et al., 1989), indicating the potential for host sensitization to multiple myelin antigens. Recently, peripheral lymphocyte reactivity to PLP has been described in association with MS (Trotter et al., 1990) and with other neurological diseases (Johnson et al., 1986) as well, demonstrating the potential for human T cell recognition of this CNS autoantigen.

The primary relevance of the present results to human MS is in the demonstration that sensitization to one myelin antigen can lead to the induction of reactivity towards additional CNS autoantigens. Thus, regardless of the initial antigenic target of a demyelinating response, the potential exists for subsequent involvement of T cells expressing a distinct antigen specificity. These findings, coupled with previous data demonstrating the development of reactivity towards minor MBP epitopes during relapse EAE (Perry and Barzaga, 1987), suggest that relapse episodes of demyelination may involve a greater array of T cell receptor and autoantigen epitopes than are active in the acute stage of disease. Demonstration of a similar phenomenon in the human patient would likely complicate immunotherapeutic approaches based upon the administration of monoclonal antibodies directed against a predominant T cell receptor population, as described recently in a murine model of EAE (Acha-Orbea et al., 1988; Urban et al., 1988). Nevertheless, it would seem to be a relevant issue to consider.

References

Acha-Orbea, H., Mitchell, D.J., Timmermann, L., Wraith, D.C., Tausch, G.S., Waldor, M.K., Zamvil, S.S., McDevitt, H.O. and Steinman, L. (1988) Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. Cell 54, 263–273.

Albrecht, P., Tourtellotte, W.W., Hicks, J.T., Sato, H., Boone, E.J. and Potvin, A.R. (1983) Intra-blood–brain barrier measles virus antibody synthesis in multiple sclerosis patients. Neurology 33, 45–50.

Allegretta, M., Nicklas, J.A., Sriram, S. and Albertini, R.J. (1990) T cells responsive to myelin basic protein in patients with multiple sclerosis. Science 247, 718–721.

Alvord, E.C., Kies, M.W. and Suckling, A.J. (1984) Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis, Alan R. Liss, New York.

Baxevanis, C.N., Reclos, G.J., Servis, C., Anastasopoulos, E., Arsenis, P., Katsiyiannis, A., Matikas, N., Lambris, J.D. and Papamichail, M. (1989) Peptides of myelin basic protein stimulate T lymphocytes from patients with multiple sclerosis. J. Neuroimmunol. 22, 23–30.

Burks, J.S., DeVald, B.L., Jankovsky, L.D. and Gerdes, J.C. (1980) Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients. Science 209, 933–934.

Butter, C., Healey, D.G., Agha, N. and Turk, J.L. (1988) An immunoelectron microscopical study of the expression of class II MHC and a T lymphocyte surface marker during chronic relapsing experimental allergic encephalomyelitis. J. Neuroimmunol. 20, 45–51.

Cambi, F., Lees, M.B., Williams, R.M. and Macklin, W.B. (1983) Chronic experimental allergic encephalomyelitis produced by bovine proteolipid apoprotein: immunological studies in rabbits. Ann. Neurol. 13, 303.

Catz, I. and Warren, K.G. (1986) Intrathecal synthesis of autoantibodies to myelin basic protein in multiple sclerosis. Can. J. Neurol. Sci. 13, 21–24.

Chou, F.C.-H., Chou, C.-H.J., Shapira, R. and Kibler, R.F. (1976) Basis of microheterogeneity of myelin basic protein. J. Biol. Chem. 251, 2671–2679.

Cohen, S.R., McKhann, G.M. and Guarnieri, M. (1975) A radioimmunoassay for myelin basic protein and its use for quantitative measurements. J. Neurochem. 25, 371.

Cook, S.D., Blumberg, B. and Dowling, P.C. (1986) Potential role of paramyxoviruses in multiple sclerosis. Neurol. Clin. 4, 303–319.

Coyle, P.K. and Sibony, P.A. (1987) Viral specificity of multiple sclerosis tear immunoglobulins. J. Neuroimmunol. 14, 197–203.

Endoh, M., Tabira, T., Kunishita, T., Sakai, K., Yamamura, T. and Taketomi, T. (1986) DM-20, a proteolipid apoprotein, is an encephalitogen of acute and relapsing autoimmune encephalitis in mice. J. Immunol. 137, 3832.

Fritz, R.B. and McFarlin, D.E. (1989) Encephalitogenic epitopes of myelin basic protein. Chem. Immunol. 46, 101–125.

Fritz, R.B., Chou, C.H. and McFarlin, D.E. (1983a) Relapsing murine experimental allergic encephalomyelitis induced by myelin basic protein. J. Immunol. 130, 1024–1026.

Fritz, R.B., Chou, C.H. and McFarlin, D.E. (1983b) Induction of experimental allergic encephalomyelitis in PL/J and (SJL/J×PL/J)F1 mice by myelin basic protein and its peptides: localization of a second encephalitogenic determinant. J. Immunol. 130, 191–194.

Fritz, R.B., Perry, L.L. and Chou, C.-H.J. (1984) Genetic control of myelin basic protein-induced experimental allergic encephalomyelitis in mice. Prog. Clin. Biol. Res. 146, 235–242.

Fritz, R.B., Skeen, M.J., Chou, C.H., Garcia, M. and Egorov, I.K. (1985) Major histocompatibility complex-linked control of the murine immune response to myelin basic protein. J. Immunol. 134, 2328–2332.

Fritz, R.B., Skeen, M.J., Chou, C.-H.J. and Zamvil, S.S. (1990) Localization of an encephalitogenic epitope for the SJL mouse in the N-terminal region of myelin basic protein. J. Neuroimmunol. 26, 239–243.

Garcia-Merino, A., Persson, M.A., Ernerudh, J., Diaz-Gil, J.J. and Olsson, T. (1986) Serum and cerebrospinal fluid antibodies against myelin basic protein and their IgG subclass distribution in multiple sclerosis. J. Neurol. Neurosurg. Psychiatry 49, 1066–1070.

Goswami, K.K., Randall, R.E., Lange, L.S. and Russell, W.C. (1987) Antibodies against the paramyxovirus SV5 in the cerebrospinal fluids of some multiple sclerosis patients. Nature 327, 244–247.

Haegert, D.G., Michaud, M., Schwab, C. and Francis, G.S. (1990) Multiple sclerosis and HLA class II susceptibility and resistance genes. J. Neurol. Sci. 26, 66–73.

Hampson, D.R. and Poduslo, S.E. (1986) Purification of proteolipid protein and production of specific antiserum. J. Neuroimmunol. 11, 117–129.

Hinrichs, D.J., Roberts, C.M. and Waxman, F.J. (1981) Regulation of paralytic experimental allergic encephalomyelitis in rats: susceptibility to active and passive disease reinduction. J. Immunol. 126, 1857.

Johnson, D., Hafler, D.A., Fallis, R.J., Lees, M.B., Brady, R.O., Quarles, R.H. and Weiner, H.L. (1986) Cell-mediated immunity to myelin-associated glycoprotein, proteolipid protein, and myelin basic protein in multiple sclerosis. J. Neuroimmunol. 13, 99–108.

Julius, M.H., Simpson, E. and Herzenberg, L.A. (1973) A rapid method for the isolation of functional thymus derived murine lymphocytes. Eur. J. Immunol. 3, 645–649.

Kennedy, M.K., Tan, L.-J., Dal Canto, M.C., Tuohy, V.K., Lu, Z., Trotter, J.L. and Miller, S.D. (1990) Inhibition of murine relapsing experimental autoimmune encephalomyelitis by immune tolerance to proteolipid protein and its encephalitogenic peptides. J. Immunol. 144, 909–915.

Kitze, B., Pette, M., Rohrbach, E., Stadt, D., Kappos, L. and Wekerle, H. (1988) Myelin-specific T lymphocytes in multiple sclerosis patients and healthy individuals. J. Neuroimmunol. 20, 237.

Kono, D.H., Urban, J.L., Horvath, S.J., Ando, D.G., Saavedra, R.A. and Hood, L. (1988) Two minor determinants of myelin basic protein induce experimental allergic encephalomyelitis in SJL/J mice. J. Exp. Med. 168, 213–227.

Link, H., Baig, S., Jiang, Y.P., Olsson, O., Hojeberg, B., Kostulas, V. and Olsson, T. (1989) B cells and antibodies in MS. Res. Immunol. 140, 219–226.

Lublin, F.D. (1985) Relapsing experimental allergic encephalomyelitis. An autoimmune model for multiple sclerosis. Springer Semin. Immunopathol. 8, 197.

Macklin, W.B., Oberfield, E. and Lees, M.B. (1984) Electroblot analysis of rat myelin proteolipid protein and basic protein during development. Dev. Neurosci. 6, 161.

Martin, R., Jaraquemada, D., Flerlage, M., Richert, J., Whitaker, J., Long, E.O., McFarlin, D.E. and McFarland, H.F. (1990) Fine specificity and HLA restriction of myelin basic protein-specific cytotoxic T cell lines from multiple sclerosis patients and healthy individuals. J. Immunol. 145, 540–548.

McDevitt, H.O., Wraith, D.C., Smilek, D.E., Lundberg, A.S. and Steinman, L. (1989) Evolution, function, and utilization of major histocompatibility complex polymorphism in autoimmune disease. In: Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, pp. 853–857.

Oksenberg, J.R. and Steinman, L. (1989) The role of the MHC and T-cell receptor in susceptibility to multiple sclerosis. Curr. Opin. Immunol. 2, 619–621.

Olsson, T., Baig, S., Hojeberg, B. and Link, H. (1990) Anti-myelin basic protein and antimyelin antibody-producing cells in multiple sclerosis. Ann. Neurol. 27, 132–136.

Ota, K., Matsui, M., Milford, E.L., Mackin, G.A., Weiner, H.L. and Hafler, D.A. (1990) T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. Nature 346, 183–187.

Panitch, H. and Ciccone, C. (1981) Induction of recurrent experimental allergic encephalomyelitis with myelin basic protein. Ann. Neurol. 9, 433.

Perry, L.L. and Barzaga, E. (1987) Kinetics and specificity of T and B cell responses in relapsing experimental allergic encephalomyelitis. J. Immunol. 138, 1434–1441.

Perry, L.L. and Williams, I.R. (1985) Regulation of transplantation immunity in vivo by monoclonal antibodies recognizing host class II restriction elements. I. Genetics and specificity of anti-Ia immunotherapy in murine skin allograft recipients. J. Immunol. 134, 2935–2941.

Perry, L.L., Barzaga, M.E. and Ferguson, T.A. (1988) Modulation of experimental allergic encephalomyelitis with anti-T suppressor factor antibodies. J. Immunol. 141, 3016–3021.

Pettinelli, C.B., Fritz, R.B., Chou, C.-H.J. and McFarlin, D.E. (1982) Encephalitogenic activity of guinea pig myelin basic protein in the SJL/J mouse. J. Immunol. 129, 1209.

Richert, J.R., Reuben-Burnside, C.A., Deibler, G.E. and Kies, M.W. (1988) Fine specificities of myelin basic protein-specific human T-cell clones. Ann. N.Y. Acad. Sci. 540, 345–348.

Sakai, K., Sinha, A.A., Mitchell, D.J., Zamvil, S.S., Rothbard, J.B., McDevitt, H.O. and Steinman, L. (1988a) Involvement of distinct murine T-cell receptors in the autoimmune encephalitogenic response to nested epitopes of myelin basic protein. Proc. Natl. Acad. Sci. U.S.A. 85, 8608–8612.

Sakai, K., Zamvil, S.S., Mitchell, D.J., Lim, M., Rothbard, J.B. and Steinman, L. (1988b) Characterization of a major encephalitogenic T cell epitope in SJL/J mice with synthetic oligopeptides of myelin basic protein. J. Neuroimmunol. 19, 21–32.

Salmi, A., Reunanen, M., Ilonen, J. and Panelius, M. (1983) Intrathecal antibody synthesis to virus antigens in multiple sclerosis. Clin. Exp. Immunol. 52, 241–249.

Sobel, R.A., Blanchette, B.W., Bhan, A.K. and Colvin, R.B. (1984a) The immunopathology of experimental allergic encephalomyelitis. I. Quantitative analysis of inflammatory cells in situ. J. Immunol. 132, 2393.

Sobel, R.A., Blanchette, B.W., Bhan, A.K. and Colvin, R.B. (1984b) The immunopathology of experimental allergic encephalomyelitis. II. Endothelial cell Ia increases prior to inflammatory cell infiltration. J. Immunol. 132, 2402.

Trotter, J.L., Lieberman, L., Margolis, F.L. and Agrawal, H.C. (1981) Radioimmunoassay for central nervous system myelin specific proteolipid protein. J. Neurochem. 36, 1256–1262.

Trotter, J.L., Clark, H.B., Collins, K.G., Wegeschiede, C.L. and Scarpellini, J.D. (1987) Myelin proteolipid protein induced demyelinating disease in mice. J. Neurol. Sci. 79, 173–188.

Trotter, J.L., Van der Veen, R.C. and Hickey, W.F. (1990) Is myelin proteolipid protein an MS antigen? Neurology 42 (Suppl.), 366.

Urban, J.L., Kumar, V., Kono, D.H., Gomez, C., Horvath, S.J., Clayton, J., Ando, D.G., Sercarz, E.E. and Hood, L. (1988) Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. Cell 54, 577–592.

Van der Veen, R.C., Trotter, J.L., Hickey, W.F. and Kapp, J.A. (1990) The development and characterization of encephalitogenic cloned T cells specific for myelin proteolipid protein. J. Neuroimmunol. 26, 139–145.

Waksman, B. (1985) Mechanisms in multiple sclerosis. Nature 318, 104–105.

Warren, K.G. and Catz, I. (1990) A myelin basic protein antibody cascade in purified IgG from cerebrospinal fluid of multiple sclerosis patients. J. Neurol. Sci. 96, 19–27.

Weber, W.E.J. and Buurman, W.A. (1988) Myelin basic protein-specific $CD4^+$ cytolytic T-lymphocyte clones isolated from multiple sclerosis patients. Hum. Immunol. 22, 97–109.

Welch, A.M., Holda, J.H. and Swanborg, R.H. (1980) Regulation of experimental allergic encephalomyelitis. II. Appearance of suppressor cells during the remission phase of the disease. J. Immunol. 125, 186.

Yamamura, T., Namikawa, T., Endoh, M., Kunishita, T. and Tabira, T. (1986) Experimental allergic encephalomyelitis induced by proteolipid protein in Lewis rats. J. Neuroimmunol. 12, 143.

Zamvil, S. and Steinman, L. (1990) The T lymphocyte in experimental allergic encephalomyelitis. Annu. Rev. Immunol. 8, 579–621.

Zamvil, S., Nelson, P., Trotter, J., Mitchell, D., Knobler, R., Fritz, R. and Steinman, L. (1985) T-cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination. Nature 317, 355.

Zamvil, S.S., Mitchell, D.J., Moore, A.C., Kitamura, K., Steinman, L. and Rothbard, J.B. (1986) T-cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. Nature 324, 258–260.

Zamvil, S.S., Mitchell, D.J., Powell, M.B., Sakai, K., Rothbard, J.B. and Steinman, L. (1988) Multiple discrete encephalitogenic epitopes of the autoantigen myelin basic protein include a determinant for I-E class II-restricted T cells. J. Exp. Med. 168, 1181–1186.

Zhang, J.W., Chou, C.H., Hashim, G., Medaer, R. and Raus, J.C. (1990) Preferential peptide specificity and HLA restriction of myelin basic protein. Cell. Immunol. 129, 189–198.