PROTECTION FROM EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS CONFERRED BY A MONOCLONAL ANTIBODY DIRECTED AGAINST A SHARED IDIOTYPE ON RAT T CELL RECEPTORS SPECIFIC FOR MYELIN BASIC PROTEIN

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The autoimmune disease experimental allergic encephalomyelitis (EAE)[1] can be induced by the injection of myelin basic protein (MBP), or by injection of MBP-specific Th into naive animals, and is considered to be a model for the study of neurodegenerative disease mechanisms. EAE is characterized by an acute phase of progressive hindlimb paralysis of varying severity. Histologically, autoimmune lesions of EAE resemble that seen in human multiple sclerosis, which is also characterized by acute neurological attacks (1–4).

Evidence that EAE is mediated by a T cell response subject to antiidiotypic modulation (5, 6), similar to that seen in other systems such as graft-vs.-host resistance (7, 8), suggests a limited idiotypic diversity for the disease inducing T cell repertoire, and as a corollary, the possibility of directed antiidiotypic therapy. We have examined this critical idiotype repertoire using a monoclonal anti-T cell antibody and have isolated and characterized the rat antigen-specific TCR. We found that most, if not all, of the antiencephalitogenic response bears an idiotype defined by this antibody (10.18). We furthermore tested the ability of this antibody to block the development of disease. Elimination of clinical disease was obtained.

Materials and Methods

Animals. Female Lewis rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA and were used at 8–12 wk of age. Female BALB/c mice were also obtained from Charles River Breeding Laboratories, Inc.

Antigen. MBP from the spinal cords of guinea pigs (GP MBP) or from brains of Lewis rats (rat MBP) was prepared as described previously (9). Synthetic peptides of varying lengths with sequences corresponding to the encephalitogenic determinant of GP MBP were used (9) and included the 73–88 sequence (GP14), the 71–88 sequence (GP16), and the 68–88 sequence (GP19) of GP MBP.

T Cell Hybridomas. The generation of T cell hybridomas specific for GP14 (5.10), for GP MBP (M3.35), or for OVA (B4) was carried out as described previously (9, 10) using popliteal and inguinal lymph node cells from antigen-primed (50 μmg/animal) Lewis rats. Hybridoma

This work was supported by grants from the National Multiple Sclerosis Society (NMS RG-15934A-1) and the U.S. Public Health Service (NS-11036). Address correspondence to Dr. E. Heber-Katz, The Wistar Institute, 36th Street at Spruce, Philadelphia, PA 19104.

[1] *Abbreviations used in this paper:* EAE, experimental allergic encephalomyelitis; GP, guinea pig; MBP, myelin basic protein; PPD, purified protein derivative of tuberculin; 2D, two dimensional.

5.10 responded to all the encephalitogenic peptides, to GP MBP, and to rat MBP, whereas M3.35 responded to GP MBP but not to the peptides or to rat MBP. B4 responded to OVA but not to the peptides nor to MBP.

The T cell hybridoma assays in which lymphokine production was measured were done according to the method described previously (9).

mAb. Establishment of an mAb to 5.10 was carried out according to the method, as described (11) with modifications. In short, BALB/c mice were immunized three times intraperitoneally with 10^7 5.10 T cell hybrids emulsified in CFA (Difco Laboratories, Detroit, MI) every other week. The spleens were removed 3 d after the last immunization and were hybridized to Sp2/0. Supernatants from growing wells were tested for Ig synthesis, inhibition of T cell responses as measured by IL-2, and binding to cell surface molecules by FACS analysis. The mAb was purified from the culture supernatant of hybridomas by using Affi-Gel Protein A (Bio-Rad Laboratories, Richmond, CA) for experimental use. The isotype of 10.18 was determined as IgG2a (kindly determined by Dr. J. M. Liptock, Wistar Institute, Philadelphia, PA).

Control antibodies included a mouse IgM specific for Thy-1.2 (12) and a mouse IgG2a (H37-43-1R1) specific for the hemagglutin of influenza virus (kindly provided by W. Gerhard, Wistar Institute).

FACS Analysis. 10^5 cells were incubated with 10.18 (0.1 mg/ml) in PBS containing 1% horse serum and 0.02% NaN_3 for 30 min at 4°C. After washing twice, the cells were incubated with FITC-labeled sheep $F(ab')_2$ anti-mouse IgG (Jackson Immunoresearch, Avondale, PA) precleared with rat Ig (Jackson Immunoresearch) for 30 min. The cells were washed twice and analyzed with an Ortho 50 HH Cytofluorograf, connected to an Ortho 2150 Data Handling System (Ortho Diagnostic Systems, Inc., Westwood, MA).

Radiolabeling. Labeling of the cell surface was carried out by the lactoperoxidase-catalyzed method as described (13). 10^7 cells were washed five times with PBS and suspended in 1 ml of 5 mM glucose in PBS containing 2 mCi ^{125}I. Then, 20 μl of enzyme cocktail (100 U/ml lactoperoxidase and 10 U/ml glucose oxidase) was added to the cell suspension and incubated at room temperature for 15 min with occasional mixing. The cells were washed four times with PBS containing 2 mM KI and 0.02% NaN_3. Cells were lysed with 1 ml of lysis buffer (1% NP-40, 0.05 M iodoacetamide, 50 μM PMSF, and 10 mM EDTA in PBS) at 4°C for 20 min, and the supernatant was cleared by centrifugation at 12,000 *g* for 20 min and then used for immunoprecipitation.

Immunoprecipitation. Aliquots of the cell lysate supernatant were incubated with appropriate mAbs at 4°C for 1 h, followed by precipitation of the immune complex using Affi-Gel protein A for 3 h. The agarose beads were then washed five times with 0.25% NP-40 in 0.45 M NaCl and once with 0.25% NP-40 in 0.15 M NaCl. The specifically bound material was removed by boiling in the sample buffer for SDS-PAGE. In some cases, anti-mouse Ig agarose beads (Sigma Chemical Co., St. Louis, MO) were used.

Gel Electrophoresis. Two-dimensional (2D) SDS-PAGE was carried out according to the method of Goding and Harris (14) using 12.5% acrylamide gel in both the first dimension (nonreduced) and second dimension (reduced).

T Cell Lines. T cell lines were selected from the popliteal lymph nodes of Lewis rats immunized in the hind footpads (0.2 ml total) with 20 μg GP MBP (MBP line and purified protein derivative of tuberculin [PPD] line) or OVA (OVA line) in H37Ra. 12 d later, the popliteal lymph nodes were removed, ground to single cell suspensions, and washed twice with PBS. The cells were cultured with 30 μg/ml of respective antigens at the cell concentration of 2×10^6/ml in ERPMI containing 1% fresh rat serum. Blast cells were separated on a Ficoll (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient and then cultured without antigens in ERPMI containing 10% horse serum supplemented with 5% rat growth factor for 7 d. The cells (5×10^5/ml) were restimulated with 10 μg/ml respective antigens in the presence of 5×10^6/ml x-irradiated thymocytes (3,000 rad). After five cycles of stimulation, the MBP line (MT11, M5) responded to GP MBP, rat MBP, GP16, and GP19, but not to PPD or OVA. The PPD line (PT7) or OVA line (OT4) responded only to respective-specific antigens, but not to MBPs or encephalitogenic peptides.

T Cell Proliferation Assay. Lymph node cells from immunized rats were tested for antigen

specificity by adding 4×10^5 cells to 2×10^5 x-irradiated spleen cells from normal rats in flat-bottomed 96-well plates (Costar, Cambridge, MA). Antigen was added at the indicated concentration. 72 h after the initiation of culture, [^{3}H]thymidine was added to measure the degree of DNA synthesis.

Induction of EAE. Animals were immunized in both hind footpads with a total of 0.2 ml of a 1:1 emulsion of the appropriate antigen in saline and H37Ra adjuvant (Difco Laboratories). Thus, each rat received a total of 100 μg of mycobacterium and 1–100 μg of protein. Rats were observed daily until clinical signs disappeared in affected animals, or for 3–4 wk if no clinical signs appeared.

Determination of Histological EAE. Animals were killed on day 18 after injection of MBP and whole spinal columns were removed by dissection. After fixing in 10% buffered formalin for 14 d, the cords were removed whole and sectioned into 7–10 fragments 0.7 cm in length. The sections were dehydrated with absolute ethanol followed by chloroform, arranged side by side, and embedded in paraffin. The paraffin blocks were sliced to reveal cross-sections of the cord segments, which were stained with hematoxylin and eosin. The slides were evaluated by Dr. L. Rourke (Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine).

Results

To produce the desired antiidiotypic antibody, we chose a rat-mouse T cell hybridoma, 5.10, specific for the encephalitogenic determinant of MBP (residues 68–88). This hybrid was made from a fusion of the mouse BW5147 AKR thymoma and an encephalitogenic peptide-stimulated Lewis rat T cell culture, which upon transfer could cause EAE, and was then used to immunize BALB/c mice.

Out of 400 mAbs tested, one, 10.18, showed the characteristics of an anticlonotypic antibody. As shown in Fig. 1 *A*, 10.18 bound only to the encephalitogenic peptide-specific T cell hybridoma 5.10, but not to the parental AKR thymoma BW5147, nor to an OVA-specific T cell hybridoma, B4, by FACS analysis. This binding specificity of 10.18 was confirmed by using ^{125}I-labeled 10.18 (data not shown).

One criterion for an antibody directed to a cell surface activation molecule such as the TCR is the ability to cause either (*a*) the inhibition of T cell activation by antigen or (*b*) the activation of T cells in the absence of antigen. The latter case has been shown to require multivalency for its effect (15). We first examined the effect of 10.18 on IL-2 production by antigen-stimulated cultures of B4, 5.10, or M3.35, (an MBP non-68–88-specific T cell hybrid) (Fig. 1 *B*). IL-2 production from B4 or M3.35 was not inhibited at any concentrations of 10.18 tested. On the contrary, 10.18 clearly inhibited IL-2 production from 5.10, the maximal inhibition being observed between 10 and 50 ng/ml. This inhibitory effect was lost at the highest concentration (10 μg/ml) of 10.18. A control γ_2a mAb, H37-43-1R1, did not inhibit IL-2 production from 5.10 (data not shown). The degree of IL-2 production in the presence of immobilized 10.18 was also examined as shown in Fig. 1 *C*. Serially diluted 10.18-coated 96-well flat-bottomed tissue culture plates were prepared and hybridomas were added in the absence of antigen and APC. No IL-2 production was observed from BW5147, B4, or M3.35. However, remarkable IL-2 production from 5.10 occurred even in wells that were coated with the lowest concentration (0.02 μg/ml) of 10.18. Similar results were obtained by using 10.18-coupled Sepharose beads (data not shown).

The ability of 10.18 to function as described above strongly indicated that it bound to a clonotypic marker, putatively the TCR. To demonstrate that this was indeed

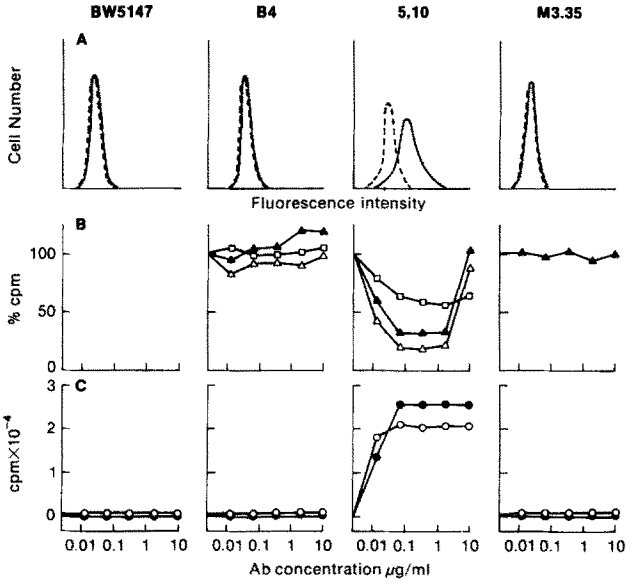

FIGURE 1. (*A*) Binding specificity of 10.18 to T cell hybridomas by FACS analysis. FACS analysis of parental thymoma BW5147, OVA-specific T cell hybridoma B4, MBP 68–88-specific T cell hybridoma 5.10, or MBP-specific T cell hybridoma M3.35 was done with (———) or without (----) 10.18. (*B*) Inhibition of IL-2 production from T cell hybridomas by 10.18. Various concentrations of 10.18 was added to B4 + OVA, 5.10 + GP MBP, or M3.35 + GP MBP at the antigen concentrations of (□) 10 μg/ml, (▲) 1 μg/ml, or (△) 0.1 μg/ml in the presence of APC. After a 24-h incubation, the supernatant was assayed for IL-2. (*C*) Production of IL-2 from T cell hybridoma by 10.18-coated plate. IL-2 production from BW5147, B4, 5.10, or M3.35 at the concentration of 10^5/ml (○) or 3×10^5/ml (●) was examined by using 96-well flat-bottomed microtiter plate coated with 10.18. After a 24-h incubation, the supernatant was assayed for IL-2.

the TCR and not another molecule expressed only on 5.10, we analyzed the structure of this cell surface molecule by 2D SDS-PAGE. Cell surface proteins were labeled with ^{125}I by the lactoperoxidase method (13) and a cell membrane lysate was precipitated with 10.18 and run on a 2D gel. As shown in Fig. 2 *A*, the precipitated molecule had an apparent mol wt of 76,000 in the nonreduced dimension (*horizontal*). This molecule was separated into 48,000- and 39,000-mol wt components after reduction. The spot on the diagonal line with some tailing of apparent mol wt of 44,000–46,000 could be the monomeric form of the TCR (16). SP2/0 culture supernatant did not precipitate these molecules (Fig. 2 *B*), nor did a control antibody, a murine IgM specific for mouse anti-Thy-1.2 (12) (data not shown).

Given the fact that previous work had suggested that EAE could be clonotypically modulated (5, 6), we were interested in the possibility that 10.18 recognized a receptor present, not only on 5.10, but on a representative T cell population that was antigen specific (68–88) and that could cause disease. We examined the binding specificity of 10.18 using four established rat T cell lines: PT7 (specific to PPD), OT4 (specific to OVA), MT11, and M5 (both specific to MBP). Long-term MBP-specific lines such as MT11 and M5 have been shown in our laboratory to be capable of inducing EAE when adoptively transferred to normal rats (9). As shown in Fig. 3, 10.18 did not bind to either PT7 or OT4. On the contrary, 10.18 bound to ~65% of MT11 and most, if not all, of the population of M5 that was generated independently from MT11.

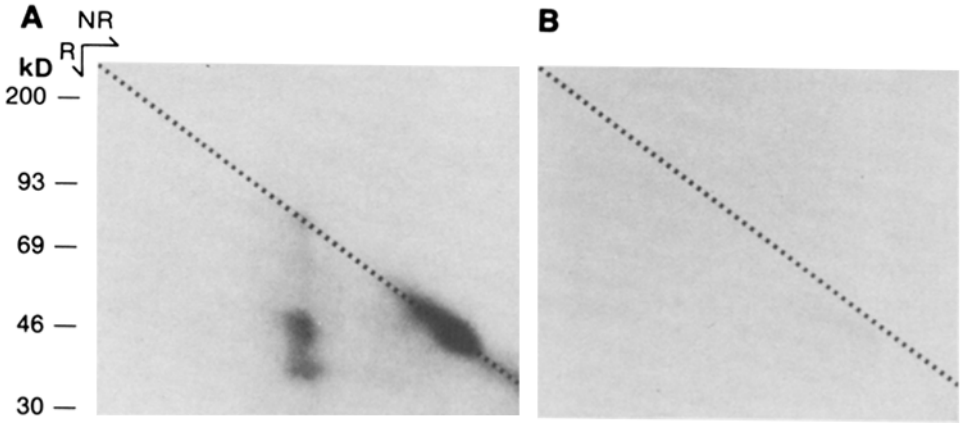

FIGURE 2. 2D SDS-PAGE analysis of ^{125}I-labeled surface proteins precipitated with 10.18. The lysates of ^{125}I-labeled cell surface proteins were precipitated with 10.18 (Fig. 2 *A*) or Sp2 0 culture supernatant (Fig. 2 *B*). The precipitate was subjected for 12.5% 2D SDS-PAGE under nonreducing conditions at first dimension and under reducing condition at second dimension.

We were of course interested in the expression of the 10.18 idiotype on in vivo T cell populations. Since 10.18 showed characteristics of an antiidiotypic antibody to a rat TCR specific for the encephalitogenic determinant of MBP, we examined the difference in expression between T cells from normal rats and those from paralyzed rats that were immunized with MBP. As shown in Table I, 10.18 cells are very rare in thymus and lymph node cell of normal rats. A modest increase in 10.18 cells was observed in thymocyte, spleen, and lymph node cells in the MBP-primed paralyzed rats, the greatest increase being observed in spleen. An augmentation of 10.18$^+$ cells was observed when cells from MBP-primed rats were cultured in vitro for 3 d with 10 μg/ml of GP MBP.

The critical test of the idiotypic nature of the autoimmune disease EAE is the ability of 10.18 to modulate disease. We therefore injected Lewis rats with GP MBP to induce EAE. As seen in Fig. 4, injection of 10.18 ascites into rats on day 0, 2, and 4 after MBP resulted in near ablation of clinical disease. This was not seen in rats injected with a control ascites or with saline.

To determine if this antibody was eliminating the cell populations involved in disease, we examined both the recirculation of cells into the spinal cord and the ability of T cells in the periphery to respond to antigen. Spinal cord and lymph nodes were removed from these rats on day 18 after MBP, a time in which the disease symptoms had just subsided. Upon examination of spinal cord sections (Fig. 5), we found to

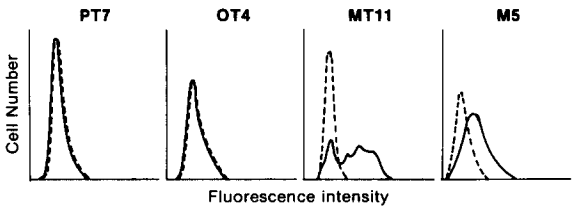

FIGURE 3. Binding specificity of 10.18 to Lewis rat T cell lines by FACS analysis. FACS analysis of (*A*) PPD-specific T cell line PT7, (*B*) OVA-specific T cell line OT4, (*C*) MBP-specific T cell line MT11, or (*D*) M5 was done with (———) or without (----) 10.18.

TABLE I

Binding of 10.18 to Thymocyte, Spleen, or Lymph Node Cell From Normal MBP-primed Lewis Rats Analyzed by FACS

	Percentage of 10.18^+ cell from:		
Rats	Thymus	Spleen	Lymph node
Normal	0.2	1.9	0
MBP primed	0.6	5.3	1.1
MBP primed + cultured	4.0	16.7	8.1

Paralyzed Lewis rats that were immunized with 25 μg GP MBP in H37Ra at 13 d before. Cells were cultured with 10 μg/ml GP MBP for 3 d,

our surprise that both the protected (10.18 injected) and unprotected (control ascites injected) animals showed similar perivascular cuffing, a hallmark of EAE.

On the other hand, these two groups of animals showed differences in proliferative T cell responses. As seen in Fig. 6, in the unprotected animals that just recovered from severe disease, there was reduced or no proliferation to either MBP or PPD. In the animals that received 10.18 and had no clinical symptoms, T cell proliferative responses were seen to both MBP and PPD; however, the response to the encephalitogenic determinant was absent.

We also examined the ability of varying amounts of the 10.18 antibody to protect from disease. In the first experiment, seen in Table II, one dose of antibody given either on day 0 or 5 after MBP caused reduction in the number of diseased animals, in the severity of disease, and delayed the day of onset. In the second experiment, also seen in Table II, very low doses of purified antibody caused reduction of disease.

Discussion

The antigen receptors of several T cell clones or hybridomas derived from mouse (16, 17) and human (18, 19) have been identified using anti-TCR antibodies. However, the rat TCR has not yet been characterized due to the difficulty of establishing stable T cell clones of this species. Recently, our laboratory established a stable rat-mouse T cell hybridoma, 5.10, by hybridizing MBP-primed rat lymph node cells with the BW5147 AKR mouse thymoma (9, 10). By using 5.10 as a immunogen, we have generated an mAb, 10.18, that shows clonotypic specificity.

The TCR consists of a disulfide-linked, glycosylated heterodimer of two chains, α and β. The mol wt of each chain is 49,000 and 43,000, respectively, in human

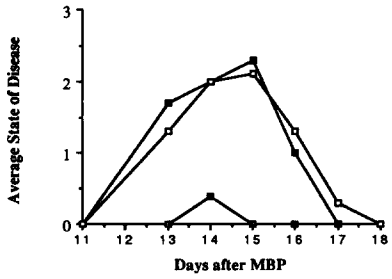

FIGURE 4. Kinetics of EAE symptoms in Lewis rats given GP MBP and treated with mAbs. Lewis rats (three animals/group) were injected with 25 μg GP MBP in CFA in the hind footpads and also were injected intraperitonealy with saline (□), a control monoclonal anti-influenza ascites (■), and 10.18 ascites (▲) on days 0, 2, and 4.

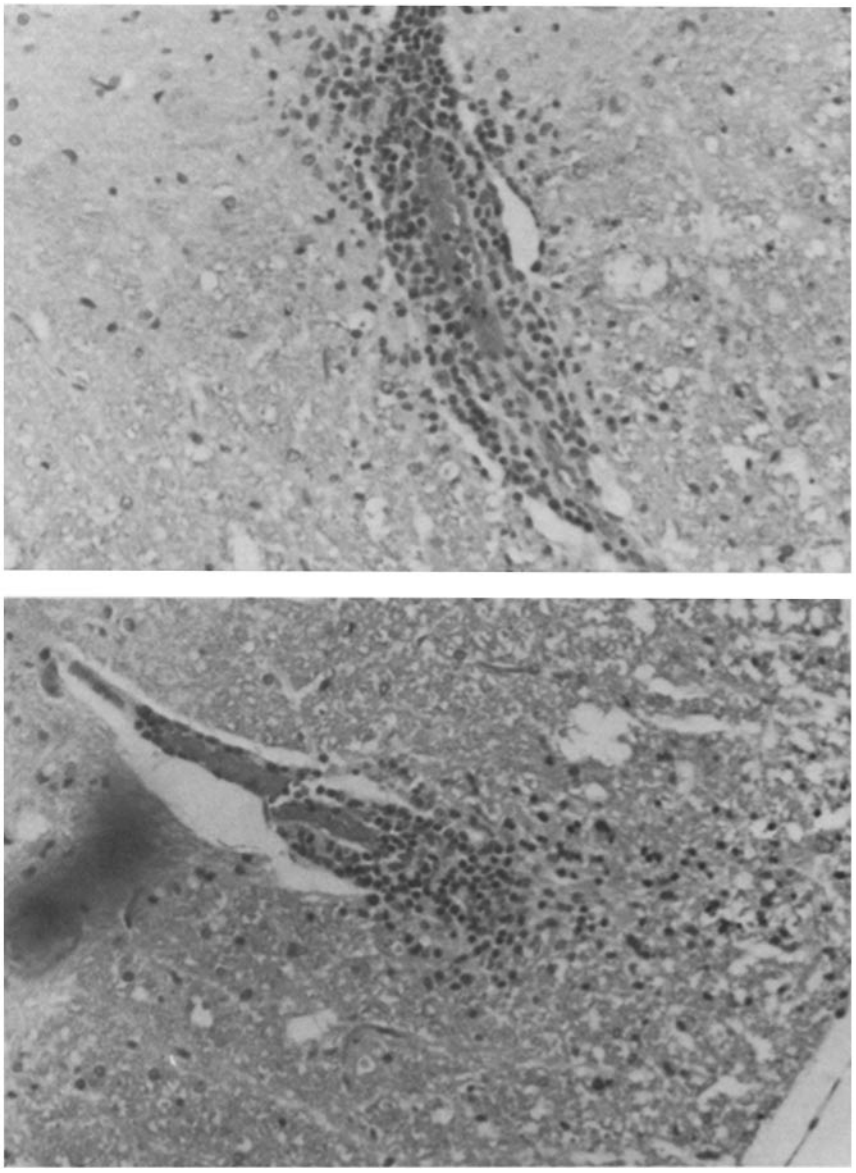

FIGURE 5. Hematoxylin- and eosin-stained sections of Lewis rat spinal cord taken at day 18 after MBP revealed inflammation with prominent perivascular cuffing in the white matter. This was seen in both (*A*) unprotected rats given a control antibody and having severe clinical symptoms and (*B*) protected rats given the mAb 10.18 and having no clinical symptoms. (× 200).

(19) and 40,000–44,000 in mouse (16, 17). In this study, we identified a clonotypic marker on MBP-specific rat T cells and on an MBP-specific rat-mouse T cell hybridoma with structure and biological activity similar to the mouse and human TCR. By 2D SDS-PAGE, it appears to be an α,β heterodimer consisting of chains of 48,000

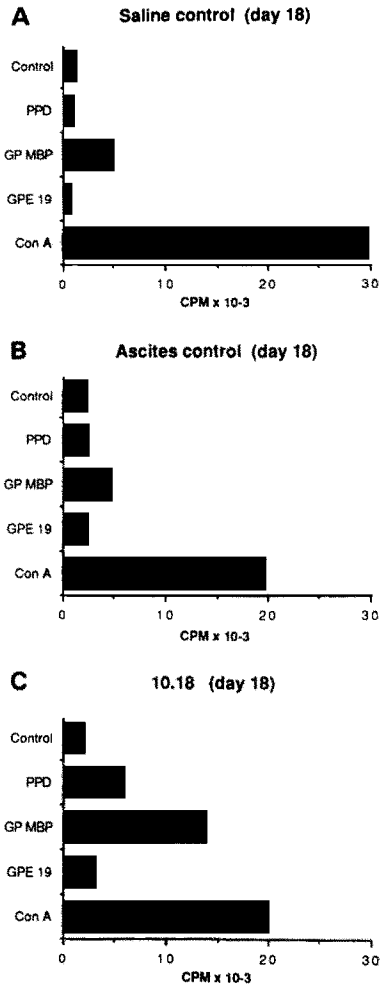

FIGURE 6. Proliferation of lymph node cells from Lewis rats given GP MBP and treated with (*A*) saline, (*B*) control ascites, and (*C*) 10.18 ascites. After disease symptoms had subsided (day 18, see Fig. 4), lymph nodes were removed and cells were assayed for proliferation to GP MBP (50 μg/ml), the synthetic encephalitogenic peptide 68–88 or GPE 19 (10 μg/ml), PPD (2 μg/ml), and Con A (4 μg/ml).

and 39,000 mol wt mAb 10.18, which precipitates this molecular complex, clearly inhibited IL-2 production by the MBP 68–88-specific T cell 5.10 but not an OVA-specific T cell. This inhibitory effect of soluble 10.18 was lost at the highest concentration of 10.18, possibly due to a stimulatory effect by the antibody (15). On the other hand, apparently low concentrations of plate-immobilized 10.18 stimulated the hybridoma 5.10 to produce IL-2 but did not stimulate the OVA hybridoma. These effects are characteristic of anti-TCR antibodies (15). A stimulatory action has been observed with anti-T cell antibodies directed to T3, T11 (20), T44 (21), and Thy-1 (22). However, stimulation in these cases is not clonotypically restricted, in contrast to 10.18.

We examined the binding (by FACS analysis) of 10.18 to not only T cell hybridomas and the mouse fusing line, but also to normal rat lymphocyte populations to confirm that the marker was not hybridoma specific. 10.18 clearly bound to the majority of the cells of the MBP-specific long-term T cell lines MT11 and M5 and not at

TABLE II

Blocking of EAE with the Anti-T Cell Idiotype 10.18

Exp.	GP MBP*	Antibody	Numbers of animals	Day of onset	Average state of disease
	μg/animal				
1‡	100	None	3/3	12	3
	100	10.18 d.0	1/3	18	0.3
	100	10.18 d.5	1/3	16	0.3
	100	IgG2a control	3/3	12	3
	0	10.18 da.0	0/3	None	None
2§	5	None	3/3	12	3.7
	5	0.3 μg (d0)	1/3	13	0.7
	5	0.03 μg (× 6)‖	0/3	None	None
	50	None	3/3	11	3
	50	0.03 μg (× 6)‖	1/3	13	1.3

* GP MBP was emulsified in CFA and 0.2 ml was injected into hind footpad of normal Lewis rats. Antibody was injected as indicated.

‡ 1:20 dilution of ascites, 0.5 ml intraperitoneally.

§ Purified antibody injected intraperitoneally.

‖ 0.03 μg antibody given on days 0, 2, 4, 6, 8, and 10 after MBP.

all to the OVA- and PPD-specific lines, indicating that the marker is present on antigen-specific normal cells and may function as a physiologically significant idiotype that is involved in the regulation of the encephalitogenic response. It is important to note that such long-term T cell lines are made up of predominantly 68–88-specific T cells that are of polyclonal origin as determined by TCR β chain constant region Southern blot analysis (23). Thus, in a fusion of BW5147 and a long-term T cell line, seven out of seven 68–88-specific hybridomas were of different clonal origin. The probability of drawing such a sample is 6% for a population of 10 different 68–88-specific clones and 49% for a population of 32 different clones, the majority of which also bear the idiotype recognized by 10.18. Therefore, we believe that we are not looking here at two independently derived cell lines that are all monoclonal and identical to 5.10, but rather that these lines are composed of diverse populations of MBP 68–88-specific T cells that are reasonably representative of the normal idiotypic diversity of the MBP 68–88 response in general. This interpretation is reinforced by the observation that antibody 10.18 blocks EAE in vivo. We conclude that the MBP-specific Lewis rat T cell lines, which are capable of inducing severe EAE in naive rats, express a specific TCR idiotype detectable with mAb 10.18. Though 10.18^+ T cells are rare in normal rats, an increase of 10.18^+ T cells was observed in thymocyte, spleen, and lymph node cells of paralyzed rats that were immunized with MBP. Clinical signs of EAE were eliminated using low doses of antibody (0.18–75 μg/animal), supporting the idea that the idiotype recognized by 10.18 is important in the disease-inducing lymphocyte repertoire. What was particularly interesting, however, was the fact that the 10.18-protected animals, which showed no signs of disease, and whose lymph node T cells showed no response to the encephalitogenic determinant of MBP, but normal responses to whole MBP and PPD, did have cells that migrated to the spinal cord in a manner indistinguishable from sick (unprotected) animals. One explanation is that the cells seen in the spinal cord are activated T cells of irrelevant

specificity and are not at all involved in the disease. A second explanation is that T cells that are MBP 68–88 specific are the cells found in the perivascular cuffs but that these cells are blocked in some way through their antigen receptors. A third explanation is that 10.18 is actually blocking the interaction with a regulatory T cell (10.18 like) that is specific for the anti-MBP 68–88 TCR and that perhaps, in an attempt to turn off the MBP-specific T cell, delivers a destructive signal in the spinal cord.

Any number of possibilities can be generated to explain this finding. However, at present it is known that there are other cases in which clinical symptoms and histology do not correlate (24, 25). One especially striking example in a different disease system is the nonobese diabetic mouse in which lymphocytes migrate to the pancreas resulting in insulitis though the animal remains symptom-free until 3 mo later when the cells become active and destroy the β cells causing diabetes (26), though similar findings have been seen in cell transfer experiments into normal animals (27).

It has also been shown in the PL/J, (PL × SJL)F_1, and B10.PL mouse that a commonly used V region of the TCR β chain, V β 8.2, is used by ~75% of clones that respond to the encephalitogenic determinant of MBP. A known anti-V β antibody, F23.1 (28), has been shown to block active disease (29, 30). This is very similar to our findings in the Lewis rat where all the T cells involved in EAE used a TCR β chain V region homologous to V β 8.2 (31). At present, however, we do not know if our antibody, 10.18, recognizes a V region or an idiotypic determinant.

Thus, we would conclude that it is probable that 10.18^+ T cells constitute the dominant pathogenic T cell repertoire of the autoimmune disease EAE in the Lewis rat. We would thus characterize EAE as a "mono-idiotypic" autoimmune disease.

Summary

Immunizing Lewis rats with guinea pig myelin basic protein (MBP) yielded an encephalitogen specific, Ia-restricted, rat-mouse T cell hybridoma 5.10, which was used to establish a clonotypic mAb (10.18) that binds to and precipitates the rat TCR. By two-dimensional gel electrophoresis, the rat TCR was shown to consist of two disulfide-linked peptide chains with mol wt of 48,000 and 39,000. 10.18 binds the majority of cells in MBP-specific T cell lines that are capable of transferring experimental allergic encephalomyelitis (EAE) to Lewis rat recipients, but does not bind to either a purified protein derivative of tuberculin-specific cell line or an OVA-specific line. Furthermore, soluble 10.18 can block antigen-specific stimulation of hybridoma 5.10 but cannot control hybridomas, while immobilized 10.18 stimulates 5.10, but cannot control the hybrids. Though 10.18^+ cells are very rare in normal rats, increase of 10.18^+ cells is observed in MBP-primed paralyzed rats. Finally, when 10.18 is injected into MBP-primed Lewis rats, EAE is abrograted. We have thus characterized EAE as a "mono-idiotypic" autoimmune disease.

We are grateful to Mr. J. S. Faust and J. M. Urffer for FACS analysis, and to Dr. J. M. Liptock for the determination of subclass of mAb. We thank Ms. S. Goldman for excellent technical assistance.

Received for publication 4 August 1988.

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