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ENCEPHALITOGENIC T CELL CLONES SPECIFIC FOR MYELIN BASIC PROTEIN

An Unusual Bias in Antigen Recognition

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Experimental allergic encephalomyelitis (EAE)[1] is a clear example of an autoimmune disease mediated by class II–restricted T lymphocytes (1–5). Certain forms of EAE are characterized by relapsing paralysis, with histopathology revealing both perivascular lymphocytic cuffs and demyelination. Because of these features, chronic relapsing EAE is often cited as a model for the human disease multiple sclerosis (MS) (6). Further similarities exist between EAE and MS, including the presence of T helper cells in the inflammatory lesions (7, 8), and the linkage of susceptibility for both EAE and MS to immune response genes (9, 10). With the capability for cloning antigen-specific T cells (11), it is now possible to analyze the precise cellular and immunogenetic mechanisms involved in EAE.

Recently, it has been shown (12, 13) that the *N*-terminal 1–37 amino acid (aa) peptide of rat or guinea pig myelin basic protein (MBP) can induce EAE in PL/J ($H\text{-}2^u$) mice, while the *C*-terminal 89–169 aa peptide is encephalitogenic in SJL/J ($H\text{-}2^s$) mice. The (PL/J × SJL/J)F_1 [(PLSJ)F_1] mice do not respond to MBP in a codominant manner. Instead, only the *N*-terminal peptide of MBP induces EAE in the (PLSJ)F_1 mouse (12, 13).

To characterize the T cells involved in the encephalitogenic response, MBP-specific T cell lines and clones have been isolated from (PLSJ)F_1 mice after immunization with rat or bovine MBP. The separate determinants recognized by these clones have been investigated using peptides derived from MBP. In addition, the pattern of restriction to I-A or I-E class II molecules has been analyzed for each clone. With both the initial MBP-primed T cell lines and the

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[1] *Abbreviations used in this paper:* aa, amino acid; APC, antigen-presenting cell; CNS, central nervous system; EAE, experimental allergic encephalomyelitis; mAb, monoclonal antibody; MBP, myelin basic protein; MS, multiple sclerosis.

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clones isolated from these lines, we have observed a paucity of proliferative T cells recognizing MBP in association with parental I-A^s class II molecules.

The majority of the clones recognize an epitope within the *N*-terminus of rat MBP, although not all of these recognize a determinant shared with mouse (self) MBP. By in vivo administration of individual clones, we have shown that only those T cell clones that recognize mouse (self) MBP are encephalitogenic.

Following in vivo administration of these T cells, various clinical forms of EAE have been observed, including acute-fulminant paralysis, chronic relapsing paralysis, and chronic unremitting paralysis. Histopathologic examination of this paralytic disease induced with cloned T cells at the light and electron microscopic levels has revealed features shared with MS, including perivascular lymphocytic infiltrates, primary demyelination, and remyelination within the central nervous system (CNS).

Materials and Methods

Mice. PL/J, B10.PL, SJL/J, and (PL/J × SJL/J)F_1 mice were purchased from The Jackson Laboratory, Bar Harbor, ME. BALB/c, B10, B10.S, C3H/HeJ, (BALB/c × SJL)F_1, (B10 × SJL/J)F_1, and (C3H × SJL/J)F_1 mice were bred in our animal facilities in the Departments of Genetics and Medical Microbiology, Stanford University School of Medicine. Adult female mice, aged 12–20 wk, were used for all experiments.

Culture Medium. Complete culture media consisted of RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% fetal calf serum (M. A. Bioproducts, Walkersville, MD), 5×10^{-5} M 2-mercaptoethanol, 2 mM glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin.

Preparation of Antigens. Rat, mouse, and guinea pig spinal cords were purchased from Pel-Freeze, Rogers, AR. Bovine spinal cord was purchased from Ferraro Meat Co. (San Jose, CA). Myelin basic protein was isolated from spinal cord tissue as previously described (14). Preparation and isolation of peptic peptides have been described previously (12).

T Cell Lines and Clones. Isolation and maintenance of proliferative T cell lines and clones specific for MBP were carried out as described previously (5, 11). T cell clones PJR-25 and PJB-20 were derived from separate T cell lines, which were generated from homozygote PL/J mice following immunization with rat and bovine MBP, respectively. Following immunization with rat MBP, a T cell line and F_1 clones were derived from (PLSJ)F_1 mice.

Proliferation Assay. To assay for proliferative response to MBP and MBP peptides, 10^4 T cells were cultured with 5×10^5 irradiated APC from PL/J, SJL/J, or (PLSJ)F_1 mice in 0.2 ml of culture media in 96-well flat-bottomed microtiter plates. Each clone was tested with and without rat MBP. Cultures were pulsed using 1 μCi/well of [^{3}H]thymidine at 48 h, and harvested 16 h later. The mean cpm of [^{3}H]thymidine incorporation were calculated for triplicate cultures. SD calculated from replicate cultures were within 10% of the mean value.

Monoclonal Antibodies (mAb). All mAb have been described previously. mAb 10-2.16 binds determinant Ia17 on certain I-A chains, including Aα^s and Aβ^u (15). mAb 40M binds Ia.1, a determinant on I-Aβ^u, but not I-Aβ^s (16). mAb 14-4-4 binds determinant Ia7, expressed on I-E chains (17, 31). mAb Y-17 binds determinant Iam.44 on certain hybrid I-E molecules, including Eα^uEβ^s (18). In these blocking studies, 1 μg of each mAb was added at the beginning of culture to those wells containing cloned T cells, antigen-presenting cells (APC), and rat MBP. mAb 10-2.16, 14-4-4, and Y-17 were provided by P. P. Jones (Stanford University, Stanford, CA).

In Vivo Administration of MBP-specific T Cell Clones. 14 d after previous stimulation with rat MBP and irradiated APC, viable T cells were separated by centrifugation through a Ficoll gradient. These cells were then stimulated for 24 h in complete media containing 10% supernatant from concanavalin A–stimulated rat spleen cells.

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Recipient mice were given low-dose whole body irradiation (350 rad) before intraveneous injection of the T cell clones. We (5) and others (20, 21) have shown that low-dose x-irradiation facilitates induction of EAE when given before injection of 10^6 cloned T cells. Inactivated *Bordetella pertussis*, which is necessary for inducing EAE with MBP or MBP peptides (12), is not necessary in this model of T cell clone–mediated paralysis (5).

Histopathologic Techniques. Mice were first anesthetized with carbon dioxide, and then perfused by intracardiac injection of 3.5% glutaraldehyde in phosphate-buffered saline. The brain and spinal cord were dissected free and segments from multiple levels along the neuraxis were embedded in paraffin or Epon. Paraffin-embedded tissues were sectioned and stained with hematoxylin and eosin alone, or in combined with Luxol Fast Blue. Epon-embedded 1-μm sections were stained with toluidine blue. Thin sections were stained with alcoholic uranyl acetate, and reviewed in a Hitachi II-uc transmission electron microscope at 70 kV.

Results

Bias Against Rat MBP-specific Responses in Association With H-2^s in (PLSJ)F_1 T Cell Clones. A proliferative T cell line specific for MBP was established after immunization of (PLSJ)F_1 mice with rat MBP. 17 clones were then derived from this line. These clones were analyzed for their ability to recognize rat MBP in association with either parental or (PLSJ)F_1 APC. In Table I, the proliferative responses of these clones to rat MBP are shown using APC from parental PL/J (H-2^u), SJL/J (H-2^s), or (PLSJ)F_1 mice. 5 of the 17 clones proliferate in response to rat MBP when cultured with PL/J and (PLSJ)F_1 APC. This indicates that these five clones are H-2^u restricted. Only 1 of the 17 clones responds to rat MBP in association with SJL/J, B10.S (data not shown), and (PLSJ)F_1 APC. This clone is restricted to H-2^s. 11 clones respond to rat MBP only when provided with (PLSJ)F_1 APC. These clones must rcognize rat MBP in association with hybrid (PLSJ)F_1 determinants. Thus, recognition of rat MBP occurs in association with H-2^u or H-$2^{(u \times s)}$ F_1 determinants with a paucity of H-2^s-restricted T cell clones.

To confirm that this bias was not an artifact that arose from cloning, but instead reflected the characteristics of the population of T cells primed to MBP, the MBP-specific (PLSJ)F_1 T cell line from which these clones were derived was also analyzed. Fig. 1 shows that the (PLSJ)F_1 T cell line gives a significantly stronger response to rat MBP when cultured with PL/J or (PLSJ)F_1 APC than when cultured with SJL/J APC. This does not appear to be due to an intrinsic defect in either H-2^s-restricted T cells or SJL/J APC. It is possible to expand the population of H-2^s-restricted T cells by repeated stimulation on SJL/J APC. These results confirm other observations (22) that there is a lack of priming to MBP in association with H-2^s in (PLSJ)F_1 mice.

Response to Mouse (self) MBP Occurs Primarily with T Cell Clones Restricted to H-2^u. The results in Table II show that all five H-2^u-restricted clones (pattern I) respond to mouse (self) MBP as well as to rat MBP. The one H-2^s restricted clone (pattern II) does not respond to mouse MBP. Of the 11 H-$2^{(u \times s)}F_1$-restricted T cell clones (patterns III and IV), only 1 clone (pattern IV) proliferates in response to mouse MBP.

The inability of H-2^s-restricted T cells to respond to mouse MBP can be demonstrated in the (PLSJ)F_1 T cell line from which the 17 clones were derived. In Fig. 2, it can be seen that (PLSJ)F_1 T cells respond to mouse MBP when

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TABLE I
Determination of Parental or F_1 H-2 Restriction Pattern for MBP-specific T Cell Clones

Clone	[^{3}H]Thymidine incorporation (Δcpm) by APC in response to MBP			Restriction
	PL/J	SJL/J	(PLSJ)F_1	
F_1-5	62,133	130	47,121	PL/J
F_1-11	4,239	361	4,150	PL/J
F_1-12	182,910	761	186,173	PL/J
F_1-21	24,086	62	28,589	PL/J
F_1-24	34,922	241	69,405	PL/J
F_1-13	312	91,324	49,272	SJL/J
F_1-2	24	79	125,114	F_1
F_1-8	5	94	96,041	F_1
F_1-14	262	214	62,909	F_1
F_1-15	112	294	237,455	F_1
F_1-16	206	64	35,047	F_1
F_1-17	31	17	9,276	F_1
F_1-18	17	36	14,047	F_1
F_1-20	23	83	11,589	F_1
F_1-22	34	85	23,503	F_1
F_1-27	200	130	107,987	F_1
F_1-28	43	307	153,113	F_1
PJR-25	88,065	−53	76,627	PL/J
PJB-20	76,489	−4	69,154	PL/J

All T cell clones are derived from (PLSJ)F_1 mice, except PJR-25 and PJB-20, which were derived from homozygous PL/J mice. 10^4 cloned T cells were cultured with 5×10^5 PL/J, SJL/J, or (PLSJ)F_1 irradiated (3,000 rad) splenic APC. Rat MBP was added at the beginning of culture to give a 6.7 μM final concentration. Cultures were labeled with 1 μCi [^{3}H]-thymidine at 48 h, and harvested 16 h later. Mean [^{3}H]thymidine incorporation was determined for triplicate cultures. For cultures incubated with rat MBP, the SD from replicate cultures were within 10% of the mean value. For background cultures incubated without rat MBP, SD were within 15% of the mean. In all cases, background proliferation was <800 cpm. Uptake (Δcpm) was determined by subtracting the mean cpm of background cultures from the mean cpm of cultures incubated with 6.7 μM rat MBP. ND, not determined. Positive proliferative responses are underscored.

cultured with PL/J or (PLSJ)F_1 APC, but do not respond to mouse MBP when cultured with SJL/J APC. (PLSJ)F_1 T cells do respond to rat MBP in association with SJL/J APC, when tested at the same time.

Specificity of Response of (PLSJ)F_1 Clones to Peptide Fragments of MBP. In Table III, the proliferative response to individual MBP peptides is shown for the (PLSJ)F_1 MBP-specific T cell clones. Rat and guinea pig MBP have identical aa sequences for residues 1–37, and differ from mouse MBP only at two residues (Fig. 3). In mouse MBP, there is a deletion of the His_{10} and Gly_{11} residues (23). Bovine 1–37 aa sequence of MBP also has this deletion, but differs from rat, guinea pig, and mouse MBP at residues 2 and 17 (Fig. 3).

All five clones restricted to H-2^u determinants (pattern I) recognize mouse

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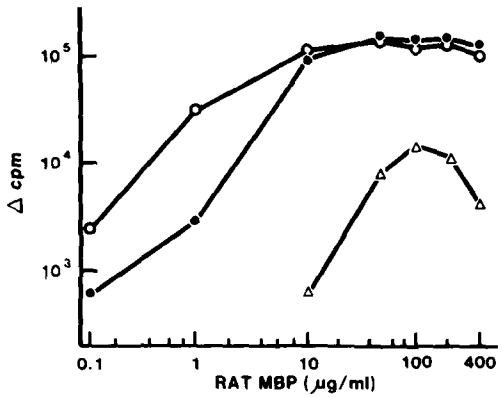

FIGURE 1. Proliferative response of a (PLSJ)F_1 MBP-specific T cell line to rat MBP with PL/J (open circles), SJL/J (open-triangles), and (PLSJ)F_1 (closed circles) APC. 10^4 (PLSJ)F_1 cells were cultured with 5×10^5 APC. Rat MBP was added at the beginning of culture. T cell clone SJ1.3, an MBP-specific T cell clone derived from SJL/J mice, was tested on SJL/J APC as a positive control for SJL/J APC. The proliferative response for 10^4 SJ1.3 cells cultured with 5×10^5 SJL/J APC and 100 μg/ml rat MBP gave 109,316 Δ cpm [^{3}H]thymidine incorporation (data not shown). Proliferative responses were determined as described in Table I.

MBP and respond to the *N*-terminal peptide of guinea pig or bovine MBP. The single clone, restricted to H-2^s (pattern II), responds to a unique determinant of rat MBP (Tables II and III). All eight H-$2^{(u \times s)}F_1$-restricted T cell clones tested (pattern III) respond to the *N*-terminal 1–37 aa peptide of guinea pig MBP, but do not proliferate in response to intact mouse or bovine MBP. This indicates that these clones may recognize a determinant of MBP requiring residues His_{10} and Gly_{11}, which are the only differences between rat or guinea pig MBP, and mouse MBP within the *N*-terminal 1–37 aa region. The pattern IV clone, which is hybrid H-$2^{(u \times s)}F_1$, recognizes a determinant that is common to all forms of MBP tested (Table II and III). Clones in pattern II and IV do not respond to any *N*-terminal MBP peptides tested, or to bovine MBP peptide 89–169, which shares complete homology with large rat MBP for this region (23). In contrast, certain MBP-specific T cell clones derived from SJL/J mice (19) do proliferate in response to peptide 89–169 when tested at the same time (data not shown).

Ia Restriction Pattern of (PLSJ)F_1 Clones. Using mAb directed to specific I-A and I-E gene products, we have determined the class II molecule used by each clone for recognition of MBP (24, 25). Clones in pattern I are inhibited from proliferating by mAb binding to I-A^u, (Table IV). The single pattern II clone that responds to rat MBP on SJL/J or F_1 APC is not blocked by either of the anti I-A mAb. Clones in pattern III can be blocked by an mAb that recognizes I-$A\beta^u$ but not I-$A\beta^s$ chains. The only hybrid H-$2^{(u \times s)}F_1$-restricted T cell clone that recognizes mouse MBP (pattern IV), can be completely inhibited from proliferating to MBP by a mAb that binds a determinant on I-Eα chains. A summary of the class II restriction and MBP recognition pattern is shown in Table V.

I-A^s Can Be Expressed on (PLSJ)F_1 APC. We studied whether the bias against I-A^s-restricted responses in the (PLSJ)F_1 T cell line and the (PLSJ)F_1 T cell clones

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TABLE II

Response of MPB-specific T Cell Clones to Rat, Bovine, or Mouse (self) MBP

Pattern	Restriction	Clone	[^{3}H]Thymidine incorporation in response to MBP			
			No antigen	Rat MBP	Bovine MBP	Mouse MBP
I	H-2^u	F_1-5	146	70,215	16,747	50,737
I	H-2^u	F_1-11	315	20,402	4,905	4,172
I	H-2^u	F_1-12	251	182,424	42,147	209,622
I	H-2^u	F_1-21	149	34,861	6,703	5,691
I	H-2^u	F_1-24	163	53,044	39,048	41,904
II	H-2^s	F_1-13	152	49,929	267	708
III	H-2$^{(u \times s)}F_1$	F_1-2	158	125,266	171	146
III	H-2$^{(u \times s)}F_1$	F_1-8	129	96,170	120	118
III	H-2$^{(u \times s)}F_1$	F_1-14	99	63,008	233	144
III	H-2$^{(u \times s)}F_1$	F_1-15	271	157,700	245	392
III	H-2$^{(u \times s)}F_1$	F_1-16	126	35,173	138	206
III	H-2$^{(u \times s)}F_1$	F_1-17	115	9,391	122	147
III	H-2$^{(u \times s)}F_1$	F_1-18	78	14,125	75	109
III	H-2$^{(u \times s)}F_1$	F_1-20	128	11,717	250	115
III	H-2$^{(u \times s)}F_1$	F_1-22	126	23,733	211	177
III	H-2$^{(u \times s)}F_1$	F_1-27	97	108,084	86	97
IV	H-2$^{(u \times s)}F_1$	F_1-28	196	244,854	183,371	160,736
I	H-2^u	PJR-25	484	95,736	4,602	76,006
I	H-2^u	PJB-20	379	111,485	128,510	117,411

Rat, bovine, or mouse MBP were added to cultures at a final concentration of 100 μg/ml. 5×10^5 (PLSJ)F_1 APC were cultured with all (PLSJ)F_1 T cell clones and 5×10^5 PL/J APC were cultured with clones PJR-25 and clones PJB-20. The [^{3}H]thymidine incorporation was determined as described in Table I. Positive proliferative responses are underscored.

was due to a deficiency in expression of I-A^s molecules in (PLSJ)F_1 APC. We tested the ability of (PLSJ)F_1 APC to present rat, bovine, and mouse MBP to four T cell clones derived from SJL/J mice immunized with bovine MBP (19). All four clones respond to rat, bovine, or mouse MBP on APC of the following genotypes: SJL/J, (B10 × SJL/J)F_1, (BALB/c × SJL/J)F_1, (C3H × SJL/J)F_1, and (PLSJ)F_1. The results for a representative clone are shown in Fig. 4.

Induction of Autoimmune Relapsing Paralysis with MBP-specific T Cell Clones. Three MBP-specific T cell clones isolated from three independent T cell lines have induced autoimmune paralysis in 100% (>100) (PLSJ)F_1 mice tested. Two of these T cell clones, PJR-25 and F_1-12, were derived from PL/J and (PLSJ)F_1 mice, respectively, following immunization with rat MBP. T cell clone PJB-20 was derived from PL/J mice immunized with bovine MBP, a form of intact MBP that does not induce EAE in homozygote PL/J mice. All three encephalitogenic T cell clones recognize the *N*-terminal peptide of MBP (Table III) in association with I-A^u class II determinants (Table IV).

Classic histologic features of EAE are present, including perivascular infiltration in the white matter but not the gray matter of the central nervous system

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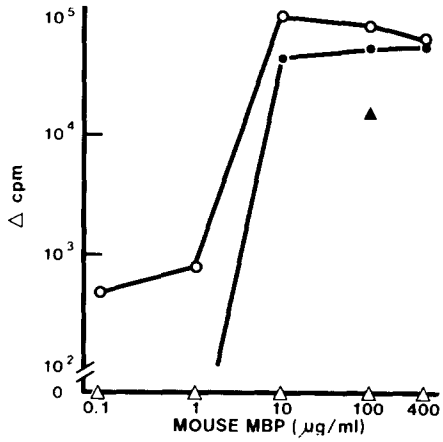

FIGURE 2. Proliferative response of a (PLSJ)F_1 MBP-specific T cell line to mouse (self) MBP with PL/J (open circles), SJL/J (open triangles), and (PLSJ)F_1 (closed circles) APC. 10^4 F_1 T cells were cultured with 5×10^5 APC. Mouse MBP was added at the beginning of culture. The response of the F_1 T cell line to rat MBP with SJL/J APC is shown (filled triangle). Proliferative responses were determined as described in Table I.

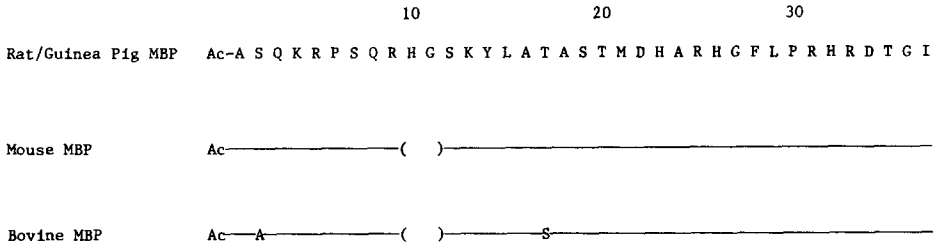

FIGURE 3. Amino acid sequences of MBP from various species.

(Fig. 5*A*). These infiltrates consist of mononuclear cells (Fig. 5*B*). Meningeal inflammation, demyelination, and a variable degree of axonal degeneration has also been observed (Fig. 6*A*). Primary demyelination is found in areas of cellular infiltration (Fig. 7*A*). Remyelination by oligodendrocytes is also observed (Fig. 7, *B* and *C*). T cell clones that recognize the encephalitogenic *N*-terminal peptide of MBP, but not mouse (self) MBP, have neither produced clinical disease nor any of these histologic features, even when injected with 5×10^6 cloned T cells (Fig. 6*B*).

The clinical course of representative individual (PLSJ)F_1 mice following injection of an encephalitogenic T cell clone is shown in Fig. 8. The onset of paralysis is dependent upon the number of cloned T cells injected. With injection of greater numbers of cells, clinical signs appear earlier and death is more likely (5). Of 12 (PLSJ)F_1 mice injected with 5×10^6 cloned T cells, all developed acute EAE within 10–14 d, and died 1–16 d after the onset of paralysis.

Chronic relapsing paralysis can occur after the injection of smaller numbers of encephalitogenic cloned T cells (Fig. 8 *A*, *B*, *D*, and *E*). When injected with 10^5 cells, not only is onset delayed, but the chronic relapsing paralysis is less severe (Fig. 8*A*). In addition to relapsing paralysis, one-third of mice injected with 5 ×

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TABLE III

Specificity of Response of MBP-specific T Cell Clones to Peptide Fragments of MBP

Pattern	Clone	[^{3}H]Thymidine incorporation in response to MBP					
		No antigen	Rat MBP	Guinea pig MBP	GP1-37	GP43-88	Bovine 43-88
I	F_1-5	469	7,624	6,783	10,569	542	668
I	F_1-11	143	777	904	1,079	164	ND
I	F_1-12	441	28,894	25,372	35,746	303	270
I	F_1-21	162	11,090	2,899	15,881	341	91
I	F_1-24	84	28,023	31,178	49,713	247	193
II	F_1-13	45	95,448	104	121	144	183
III	F_1-2	244	78,430	77,155	ND	ND	ND
III	F_1-8	255	23,217	26,242	32,015	1.314	484
III	F_1-14	286	7,434	8,102	ND	ND	ND
III	F_1-15	252	94,670	106,008	98,317	816	433
III	F_1-16	238	74,286	83,687	95,650	213	230
III	F_1-17	ND	ND	ND	ND	ND	ND
III	F_1-18	95	7,624	6,783	10,569	542	668
III	F_1-20	399	1,573	970	ND	ND	ND
III	F_1-22	ND	ND	ND	ND	ND	ND
III	F_1-27	295	143,795	129,501	ND	ND	ND
IV	F_1-28	91	67,288	56,114	149	105	63
I	PJR-25	213	108,007	83,489	108,853	577	388
I	PJB-20	379	111,485	121,621	207,516	ND	ND

All MBP peptides are products from pepsin cleavage of either intact guinea pig MBP or bovine MBP. These peptides have been isolated and purified as previously described (11, 12). All peptides were added initially to cultures to give a final concentration of 50 μg/well. Intact proteins were added likewise, but at a concentration of 100 μg/ml. (PLSJ)F_1 APC were cultured with all (PLSJ)F_1 T cell clones and PL/J APC were cultured with clones PJR-25 and PJB-20. Proliferation assay was done as described in Table I. ND, not determined. Positive proliferative responses are underscored.

10^5 or 10^6 encephalitogenic cloned T cells developed chronic unremitting paraplegia, a form not usually described (Fig. 8*C*).

A subclone of PJR-25, derived by sorting the parent clone at one cell/well, also causes paralysis (5). Thus, the encephalitogenic potential of this T cell clone has not diminished by subcloning or continuous culture in vitro.

Two MBP-specific T cell clones derived from two other independent T cell lines also cause paralysis in (PLSJ)F_1 mice. Clone F_1-12, derived from (PLSJ)F_1 mice immunized with rat MBP, causes chronic relapsing paralysis (5). T cell clone PJB-20 was derived from homozygote PL/J mice after immunization with intact bovine MBP, a form of MBP that does not induce EAE in homozygote PL/J mice. When (PLSJ)F_1 mice are injected with 5×10^6, 10^6, or 5×10^5 PJB-20 T cells, all mice (12 of 12) have developed paralysis in the manner described (Fig. 8, *B*, *D*, and *F*).

MBP-specific T cell clones that respond to rat but not mouse MBP, were also tested for encephalitogenic activity by injection into (PLSJ)F_1 recipient mice.

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TABLE IV
Determination of Ia Restriction of MBP-specific T Cell Clones

Pattern	Clone	[^{3}H]Thymidine incorporation in response to:					
				Rat MBP plus:			
		No antigen	Rat MBP alone	Anti-I-A$\beta^{u,s}$ (10-2.16)	Anti-I-Aβ^{u} (40M)	Anti-I-Eα^{u} (14-4-4)	Anti-I-E$^{u\times s}$ (Y-17)
I	F_1-5	135	67,743	1,339	327	45,686	63,078
I	F_1-11	ND	ND	ND	ND	ND	ND
I	F_1-12	125	48,328	8,828	2,474	47,324	47,461
I	F_1-21	162	5,666	81	54	4,459	5,408
I	F_1-24	69	34,692	25,578	613	31,567	46,791
II	F_1-13	361	33,002	28,025	27,303	34,635	38,253
III	F_1-2	244	78,430	197	201	60,125	80,410
III	F_1-8	117	24,272	925	299	22,447	30,851
III	F_1-14	286	7,434	222	314	4,688	5,869
III	F_1-15	311	204,273	782	353	176,244	194,155
III	F_1-16	319	99,157	282	329	80,699	71,526
III	F_1-17	ND	ND	ND	ND	ND	ND
III	F_1-18	1,201	47,168	112	137	43,251	48,378
III	F_1-20	399	1,573	283	356	1,403	1,447
III	F_1-22	ND	ND	ND	ND	ND	ND
III	F_1-27	295	143,785	333	332	104,871	132,296
IV	F_1-28	224	52,438	33,721	46,757	146	29,009
I	PJR-25	1,497	66,208	2,985	4,362	55,272	52,606
I	PJB-20	379	111,475	749	4,481	77,629	ND

All mAb have been described previously. mAb 10-2.16 binds determinants Ia.17 on certain I-Aβ chains, including I-Aβ^u and I-Aβ^s (15). mAb 40M binds Ia.1, a determinant on I-Aβ^u, but not I-Aβ^s (16). mAb 14-4-4 binds determinant Ia.7 expressed on Eα chains including Eα^u (17, 31). mAb Y-17 binds determinant Iam.44 on certain hybrid I-E molecules, including Eα^uEβ^s (18). In these blocking studies, 1 μg of each mAb was added at the beginning of culture. Each clone was cultured with 5×10^5 (PLSJ)F_1 APC. (PLSJ)F_1 APC were cultured with all (PLSJ)F_1 T cell clones and PL/J APC were cultured with clones PJR-25 and PJB-20. Proliferative responses were determined as described in Table I. ND, not determined. For all T cell clones tested, significant inhibition of proliferative responses to rat MBP by mAb are underscored.

TABLE V
Patterns of Antigen Specificity and Ia Restriction of 17 (PLSJ)F_1 MBP-specific T Cell Clones

Pattern	Class II restriction	Recognition of MBP 1-37	Recognition of mouse MBP
I	I-A (Aα^uAβ^u)	+	+
II	I-A (Aα^sAβ^s)	−	−
III	I-A (Aα^sAβ^u)	+	−
IV	I-E (Eα^uEβ^s)	−	+

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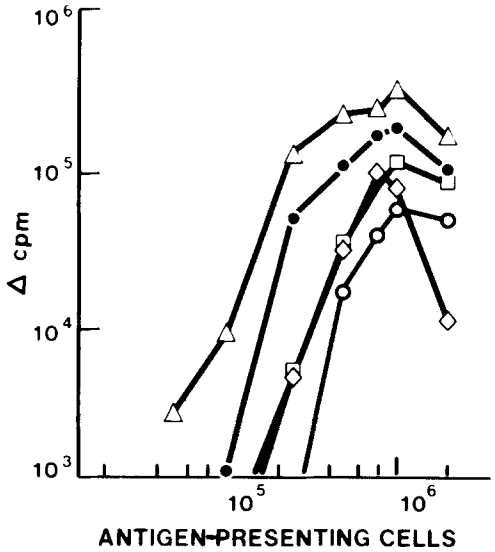

FIGURE 4. Proliferative response of an I-A^s-restricted MBP-specific T cell clone to rat MBP with SJL/J (open triangles); (B10 × SJL/J)F_1 (open diamond); (BALB/c × SJL/J)F_1 (open circle); (C3H/HeJ × SJL/J)F_1 (open square); and (PLSJ)F_1 (closed circle) APC. T cell clone SJ1.3 was derived from SJL/J mice primed to bovine MBP (21). 10^4 SJ1.3 T cells were cultured with 100 μg/ml rat MBP and APC. Proliferative responses were determined as described in Materials and Methods. For each concentration of APC used, background (without rat MBP) proliferations and proliferations with rat MBP were determined. Δ cpm was determined by subtracting the mean cpm of background cultures from the mean cpm of cultures incubated with rat MBP at each respective concentration of APC.

These clones were administered to (PLSJ)F_1 mice in the same manner as the encephalitogenic T cell clones PJR-25, PJB-20, and F_1-12. Five individual clones, F_1-8, F_1-13, F_1-15, F_1-16, and F_1-18 were injected into (PLSJ)F_1 recipient mice. Four of these T cell clones, F_1-8, F_1-15, F_1-16, and F_1-18, recognize a determinant within the encephalitogenic *N*-terminal peptide of rat and guinea pig MBP not shared with mouse (self) MBP (Tables II and III). Clone F_1-13 recognizes a determinant that is unique to rat MBP (Tables II and III). Even though injected with 5×10^6 T cells, no recipient mice developed clinical paralysis.

None of the five control clones induce EAE despite a strong in vitro proliferative response to MBP. This argues against passive transfer of MBP as a mechanism for EAE induction. If any trace amount of rat MBP is passively transferred with PJR-25, PJB-20, or F_1-12, one would expect similar amounts to have been transferred with the control clones handled in the same manner. Furthermore, viability of encephalitogenic T cell clones appears to be essential for induction of paralysis. None of six recipient (PLSJ)F_1 mice developed paralysis after injection of 10^6 lethally x-irradiated (3,000 rads) PJR-25 T cells.

T cell clones F_1-21 and F_1-24 have also been tested in vivo. These two clones show the same recognition pattern as PJR-25, PJB-20, and F_1-12 (Tables I–IV). 24 (PLSJ)F_1 recipient mice have been injected with 5×10^6 F_1-21 or F_1-24 cells each. None of these recipient mice have developed any signs of paralysis, even 5 mo after injection.

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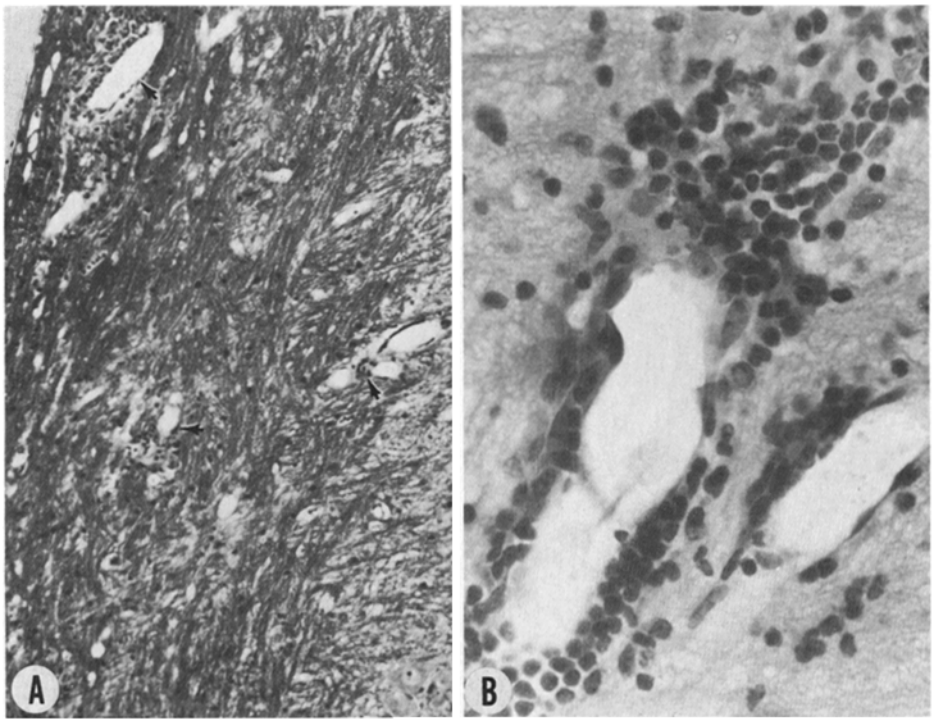

FIGURE 5. Histologic evidence of EAE induced by administration of clone PJR-25 (5×10^5 cells). Perivascular infiltration is present in the white matter but not the gray matter of the CNS. In this photomicrograph of a longitudinal section of spinal cord (*A*), three perivascular cuffs are indicated in the white matter (arrows). They are surrounded by more pale demyelinated areas. The gray matter, in the lower right hand corner, shows intact neurons (× 160). The cells in a representative perivascular cuff (*B*) are lymphocytes and macrophages (× 700). Hematoxylin and eosin stains were used.

Discussion

Our experiments show that EAE can be induced with clonal populations of T cells. Depending on the number of cloned T cells that are administered, different clinical forms of EAE are observed, including acute paralysis, chronic relapsing paralysis, and chronic unremitting paralysis. Histopathology reveals perivascular infiltration, demyelination, and remyelination. These observations establish EAE induced with T cell clones as a pertinent model, with distinct features resembling MS. Like clinical MS, relapsing and chronic unremitting paralysis are seen. Demyelination, a hallmark of MS, and remyelination are also observed with paralysis induced by MBP-specific T cell clones. In contrast, antigen-induced EAE does not frequently cause demyelination (26). Further, restriction of autoimmune T cell responses to certain Ia molecules can be demonstrated, providing a model for studying how human demyelinating diseases like MS might be linked to class II molecules of the major histocompatibility complex.

These results confirm previous studies (2, 3) which have indicated that T cells restricted by class II molecules of the major histocompatibility complex, bearing the L3T4 phenotype, play a central role in the induction of EAE. Previously, it

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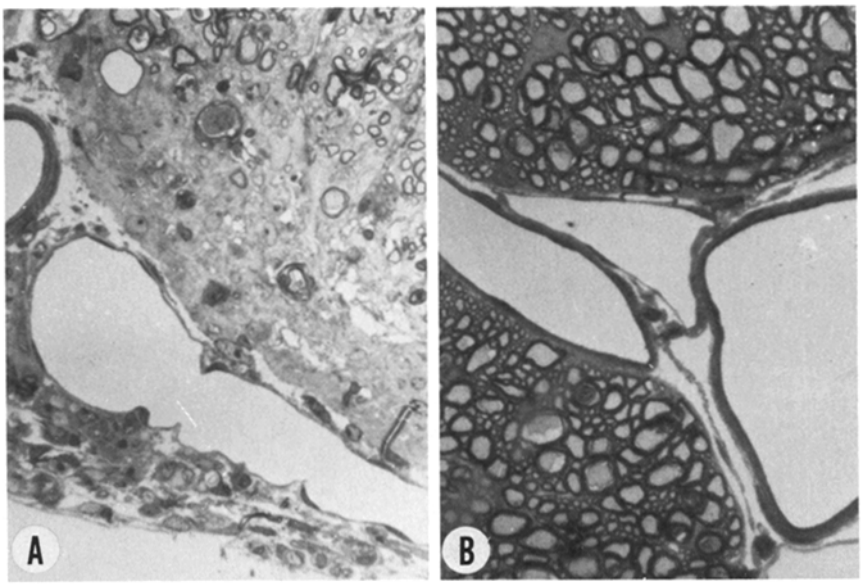

FIGURE 6. Meningeal inflammation, demyelination, and axonal degeneration are demonstrated (*A*) following chronic paralysis induced by clone PJR-25 (5×10^5 cells). These histologic features were not present (*B*) following injection of clone F_1-15 (5×10^6 cells). Toluidine blue–stained, Epon-embedded, 1-μm sections ($\times$ 700).

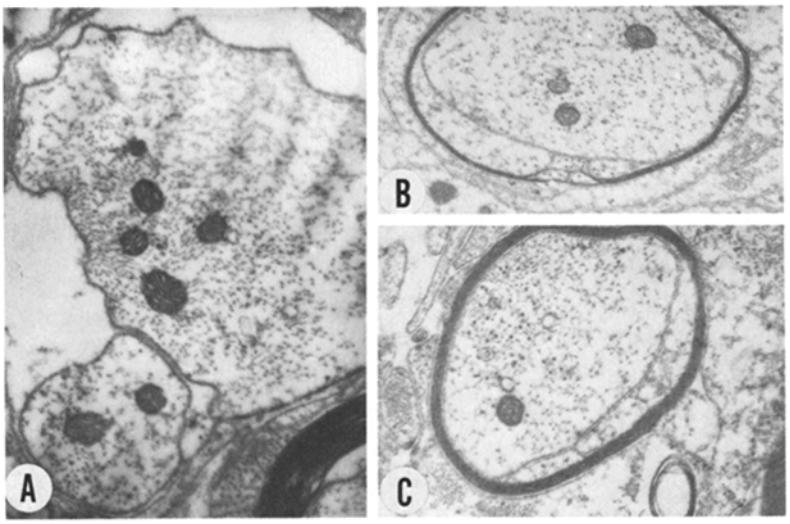

FIGURE 7. Primary demyelination, characterized by an intact axon that has lost its myelin sheath (*A*), was found in areas of cellular infiltration by electron microscopy ($\times$ 35,000). Remyelination by oligodendrocytes (*B* and *C*) also can be found ($\times$ 30,000). This mouse showed chronic paralysis after receiving PJR-25 (5×10^5 cells).

has been shown that the predominant lineage of T cells in EAE lesions is Ly-1^+, Lyt-2^- (6), class II–restricted cells (7). It has been shown (27) that class II antigens appear on vascular endothelium within the CNS early in the development of EAE. Astrocytes isolated from the CNS have been shown to express class II

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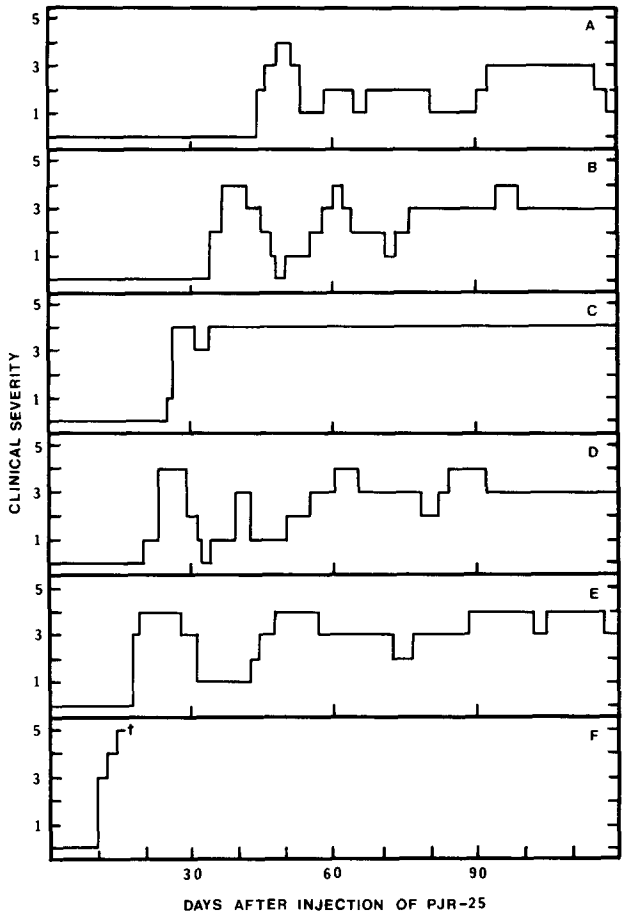

FIGURE 8. Clinical observation of individual (PLSJ)F_1 recipient mice following injection of T cell clone PJR-25. Representative mice from each of the following groups are shown: *A* (10^5 cells); *B* and *C* (5×10^5); *D* and *E* (10^6); *F* (5×10^6). Clinical disease was graded as follows: 0, no observable signs of clinical disease; 1, loss of tail tone only; 2, mild paraparesis; 3, moderately severe paraparesis; 4, complete paraplegia; 5, moribund; †, death. In vivo administration of encephalitogenic MBP specific T cell clones is described in Materials and Methods.

antigens in vitro, and are capable of presenting MBP to MBP-specific T cell lines (18). Thus, T cell clones may recognize MBP in association with particular Ia molecules present in brain and spinal cord, or on vascular endothelial cells within these organs.

One prerequisite for MBP-specific T cell clones to induce autoimmune paralysis is their ability to recognize mouse (self) MBP. These T cell clones isolated from independently-derived MBP-specific T cell lines all recognize an epitope within the *N*-terminus of mouse (self) MBP, and are 100% encephalitogenic at the cell concentrations tested. In contrast, two other clones, F_1-21 and F_1-24, which have a similar recognition pattern as encephalitogenic T cell clones, do not cause paralysis, even when injected with as many as 5×10^6 T cells. Thus, although recognition of mouse (self) MBP appears to be a prerequisite, it is not

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a sufficient condition for these T cell clones to cause paralysis in (PLSJ)F_1 mice. It is possible that these two clones may respond to a nonencephalitogenic determinant within the *N*-terminus which is distinct from that recognized by encephalitogenic T cell clones. This possibility is currently being tested with synthetic peptides derived from the aa sequence for the *N*-terminal 1–37 aa peptide of MBP.

Other properties of encephalitogenic T cells, in addition to recognizing an encephalitogenic epitope of MBP, are probably important for induction of EAE. Clones that cause paralysis may have a greater capability to migrate to the CNS. When analyzed by fluorescence-activated cell sorter analysis, encephalitogenic T cell clone PJR-25 was negative for the expression of MEL-14 (data not shown), a cell surface marker that is associated with lymphocyte homing to high endothelial venules (29). Further, it is not known whether injected MBP-specific T cells directly mediate the observed pathogenesis within the CNS. These T cells may interact with other cell types, including macrophages, B cells, and plasma cells, which have been observed in EAE lesions (7). Thus, the nonencephalitogenic T cell clones may differ from encephalitogenic T cells in their response to or release of certain lymphokines, a possibility that is currently being investigated.

A clear bias in the pattern of responsiveness to self-MBP emerged in the T cell lines, and the group of T cell clones derived from the (PLSJ)F_1 mice. The results revealed a deficiency of priming to MBP in the context of I-A^s (Tables I–IV and Figs. 1 and 2) in (PLSJ)F_1 mice. Further, T cells that do respond to rat MBP in association with I-A^s do not recognize a shared determinant with mouse MBP. These results were evident when examining the responses of individual T cell clones, and were confirmed when analyzing the MBP-primed T cell line from which these clones were derived. In contrast, lymph node cells removed from SJL/J mice primed to rat MBP proliferate in response to either rat or mouse MBP (data not shown). Thus, it appears that (PLSJ)F_1 and SJL/J mice may differ in the repertoire of I-A^s-restricted MBP-specific T cells.

One possibility that may have influenced the relative lack of I-A^s-restricted MBP-specific T cells in (PLSJ)F_1 mice is that these T cells may have been lost when generating a continuous T cell line. This possibility seems unlikely for several reasons: first, the T cells were cloned at an early stage, namely at the time of the second stimulation with splenic APC. These results were observed again with another independently generated (PLSJ)F_1 MBP-specific T cell line. Furthermore, Fritz et al. (22) observed a lack of priming to MBP in association with H-2^s in primary cultures following immunization of (PLSJ)F_1 with MBP in adjuvant.

It is interesting that MBP-specific T cell clones restricted to $A\alpha^u A\beta^u$ and $A\alpha^s A\beta^u$ class II molecules, which constitute a majority of the clones analyzed, all recognize the *N*-terminus. These results could reflect the possibility that a certain dominant epitope(s) exists within the *N*-terminus. However, the deficiency of proliferative response that occurs in association with I-A^s in (PLSJ)F_1 mice is not specific for MBP. A deficient response has also been demonstrated (22) in primary cultures for antigens such as ovalbumin, purified protein derivative (PPD), and *Listeria monocytogenes*, which can elicit proliferative responses in homozygous SJL/J mice.

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A more plausible explanation for this bias in (PLSJ)F_1 mice is that the level of expression of I-A^s is insufficient (at a certain site) for effective antigen priming to proliferative T cells. A different expression of I-A molecules has been reported (30) to occur in (C57Bl/6 × A/J)F_1 mice in which I-A^b expression is deficient. In another study, a relative lack of expression of certain combinatorial Ia molecules was observed in various hybrid F_1 mice when one of the parents is B10.PL or PL/J, both H-2^u, and the other parent is either H-2^b, H-2^k, or H-2^s. In these studies, it was shown (31, 32) that there is a preferential *cis* association of Eα^u with Eβ^u to form Eα^uEβ^u parental heterodimers. Differential expression of I-E complexes has also been demonstrated in other F_1 hybrids using cloned alloreactive T cells (33) and antigen-specific T cell clones (34).

It is intriguing that (PLSJ)F_1 APC can express a functional level of I-A^s in vitro. I-A^s-restricted MBP-specific T cell clones proliferate in response to mouse, rat, and bovine MBP associated with (PLSJ)F_1 spleen cells given as a source of APC. This experiment has been repeated with other I-A^s-restricted T cell clones derived from SJL/J mice recognizing different epitopes on MBP. In each case, (PLSJ)F_1 APC can present MBP to these I-A^s-restricted T cell clones, as well as the other hybrid F_1 APC (Fig. 4). With mAb that discriminate between I-A molecules of each parental haplotype, we are currently investigating the possibility of a differential expression of class II major histocompatibility complex molecules.

A quantitative difference in expression of class II molecules has been found in EAE. Although strain 2 guinea pigs are resistant to EAE, strain 13 and hybrid strain (2 × 13)F_1 are susceptible. It has been observed (35) that the vascular endothelial cells in strain (2 × 13)F_1 guinea pigs express quantitatively greater strain 13–specific Ia than strain 2 Ia antigens, whereas normal tissues do not show this difference. Thus, although it can be demonstrated in vitro that (PLSJ)F_1 APC can express a functional level of I-A^s, in vivo there is clearly a bias against I-A^s restriction. Identifying a bias in antigen responsiveness might be exploited in the treatment of autoimmune disease with anti-Ia antibodies directed against the Ia molecule associated with disease susceptibility (36). It has been demonstrated (37) that in vivo treatment with anti-I-A antibodies is haplotype-specific. Thus, it is possible to inhibit the response to an antigen under the control of a particular Ia molecule, while immune responses linked to other Ia molecules are left intact.

The results from this study and others (31, 32, 38) demonstrate the inability to predict immune responses based on major histocompatibility complex genotype. A deficient response to MBP in association with H-2^s could not be predicted on the basis of the Ia genotype of the parental strains. This example emphasizes one of the difficulties in studying associations between diseases and HLA, relying solely on techniques for analysis of genomic DNA. It is intriguing that the analysis of these T cell clones derived from (PLSJ)F_1 mice demonstrated that I-A^u, not I-A^s, is the restriction element associated with responsiveness to the encephalitogenic *N*-terminus of mouse (self) MBP. It is clear that using antigen-specific T cell clones will complement studies of genomic DNA in the analysis of the functional role of Ia antigens in autoimmunity. Such studies may help to clarify

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associations between HLA-D region genes and certain autoimmune diseases in humans.

Summary

Class II–restricted T cell clones specific for myelin basic protein (MBP) have been generated from PL/J and (PL/J × SJL/J)F_1 [((PLSJ)F_1] mice following sensitization to rat MBP. Of 17 T cell clones generated from (PLSJ)F_1 mice, 5 are I-A^u($A\alpha^uA\beta^u$) restricted, one is restricted to I-A^s($A\alpha^sA\beta^s$), 10 are restricted to hybrid I-$A^{(u \times s)}F_1$ ($A\alpha^sA\beta^u$) determinants, and one clone is restricted to hybrid I-$E^{(u \times s)}$ ($E\alpha^uE\beta^s$) molecules. Thus, of 16 I-A-restricted T cell clones generated from (PLSJ)F_1 mice, only one is I-A^s-restricted, reflecting a lack of priming to MBP in association with I-A^s. T cell clones restricted to I-A^u and to I-E ($E\alpha^uE\beta^s$) molecules recognize mouse (self) MBP. Furthermore, only the five T cell clones restricted to I-A^u molecules recognize a determinant in common with mouse (self) MBP within the encephalitogenic *N*-terminal peptide. Three such I-A^u restricted T cell clones, derived independently, cause paralysis in 100% of (PL/J × SJL/J)F_1 mice tested. Acute, chronic unremitting, and chronic relapsing paralysis are all induced following injection of these clones. Administration of greater numbers of cloned T cells causes acute and fatal experimental allergic encephalomyelitis, while administration of lower numbers of cloned T cells is associated with chronic unremitting and relapsing paralysis. Paralysis induced with T cell clones shares many clinical, immunologic, and histologic aspects with human demyelinating diseases such as multiple sclerosis. Histopathology reveals perivascular lymphocytic infiltration, demyelination, and remyelination. These studies demonstrate the utility of T cell clones for analyzing the association between class II major histocompatibility complex molecules and disease susceptibility.

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