Brief Definitive Report

MULTIPLE DISCRETE ENCEPHALITOGENIC EPITOPES OF THE AUTOANTIGEN MYELIN BASIC PROTEIN INCLUDE A DETERMINANT FOR I-E CLASS II-RESTRICTED T CELLS

By SCOTT S. ZAMVIL, DENNIS J. MITCHELL, MARIANNE B. POWELL, KOICHIRO SAKAI, JONATHON B. ROTHBARD,* and LAWRENCE STEINMAN

*From the Department of Neurology, Stanford University, Stanford, California 94305; and the *Imperial Cancer Research Fund, London, United Kingdom WC2A3PX*

Experimental allergic encephalomyelitis (EAE) is a model for autoimmune disease mediated by antigen-specific, class II-restricted T cells. The autoantigen in EAE is myelin basic protein (MBP), a 17-kD multideterminant protein from CNS myelin (1). As for other murine T cell-mediated autoimmune diseases, susceptibility to EAE is associated with allelic I-A class II molecules (1-3). Initial investigations demonstrated that encephalitogenic determinants were located only within the NH_2-terminal 1-37 and COOH-terminal 89-169 MBP fragments (1). Encephalitogenic T cell epitopes within these two fragments have been identified. T cell recognition of MBP p1-11 is restricted by I-A^u (2), and recognition of MBP p89-101 is restricted by I-A^s (3). I-E-restricted antigen-specific T cells that participate in EAE or other murine autoimmune diseases have not been previously identified.

In this report, we have examined the specificity of a T cell clone that recognizes intact MBP only in association with hybrid I-E class II molecules. This clone does not recognize MBP 1-37 or MBP 89-169. Using a recently described method for predicting T cell epitopes of protein antigens (4), the epitope recognized by this clone has been identified. This determinant includes MBP residues 35-47. Reactivity to this portion of MBP has not been previously reported. When tested in vivo, MBP p35-47 causes EAE. T cell recognition of p35-47 is restricted by I-E molecules. This is the first example demonstrating that antigen-specific T cells restricted by I-E molecules participate in autoimmune disease. Furthermore, it is now clear that there are multiple (at least three) discrete encephalitogenic T cell epitopes within the autoantigen MBP, each recognized in association with separate allelic I-A and I-E molecules. These results may be relevant to human autoimmune disease whose susceptibility is associated with more than one allelic HLA-D molecule.

Materials and Methods

Mice. PL/J, SJL/J, and (PL/J × SJL/J)F_1([PLSJ]F_1) female mice were purchased from The Jackson Laboratory, Bar Harbor, ME.

Antigens. MBP peptides were synthesized by solid phase techniques (2) according to the sequences for mouse MBP (5). All peptides contained >90% of the desired product as determined by high pressure liquid phase column and amino acid (aa) analysis.

Address correspondence to Scott S. Zamvil, Dept. of Neurology A-363, Stanford Medical School, Stanford, CA 94305.

T Cell Clones. T cell clone F_1-28, isolated from a (PLSJ)F_1 mouse immunized with intact rat MBP, recognizes MBP in association with hybrid I-E($E_\alpha{}^u E_\beta{}^s$) and causes EAE in the same manner that we have described for other MBP-specific T cell clones (2). Other T cell clones were isolated from individual PL/J and (PLSJ)F_1 mice following the protocol described (2).

Proliferation Assay. As described previously (2), 10^4 T cells were cultured with 5×10^5 γ-irradiated (3,000 rad) PL/J splenic APC and the desired peptide in 0.2 ml culture media in 96-well flat-bottomed microtiter plates (model 3072; Falcon Labware, Oxnard, CA). At 48 h incubation, each well was pulsed with 1 μCi [^{3}H]thymidine and harvested 16 h later. Mean cpm thymidine incorporation was calculated for triplicate cultures. SD from replicate cultures were within 10% mean value.

Induction of EAE with MBP Peptides. Each peptide was given as an emulsion containing CFA and PBS in a 1:1 mixture with 4 mg/ml H37Ra (Difco Laboratories, Inc., Detroit, MI). Each mouse was injected with the peptide emulsion at the base of the tail. Heat-killed *pertussis* organisms (10^{10}) (Michigan Dept. of Health, lot 91B) were injected intravenously. 2 d later, a second i.v. injection of pertussis organisms was given.

mAbs. T cell clones were examined with TCR $V_{\beta 8}$-specific mAb by FACS analysis as described previously (6). mAb KJ16 binds a determinant associated with the expression of two members of the $V_{\beta 8}$ subfamily, $V_{\beta 8.1}$ and $V_{\beta 8.2}$, and F23.1 binds a determinant associated with expression of all three members of the $V_{\beta 8}$ subfamily (7). $V_{\beta 8}{}^+$ clones were stained positive with both antibodies.

Results and Discussion

T cell clone F_1-28, isolated from a (PLSJ)F_1 mouse immunized with intact rat MBP, proliferates to intact rat or mouse (self) MBP, and is restricted to hybrid I-$E_\alpha{}^u E_\beta{}^s$ molecules. F_1-28 causes EAE in (PLSJ)F_1 mice, as we have described for other MBP-specific T cell clones (2). However, this clone does not recognize any of the peptic MBP fragments. In a recent study, which examined >50 antigenic pep-

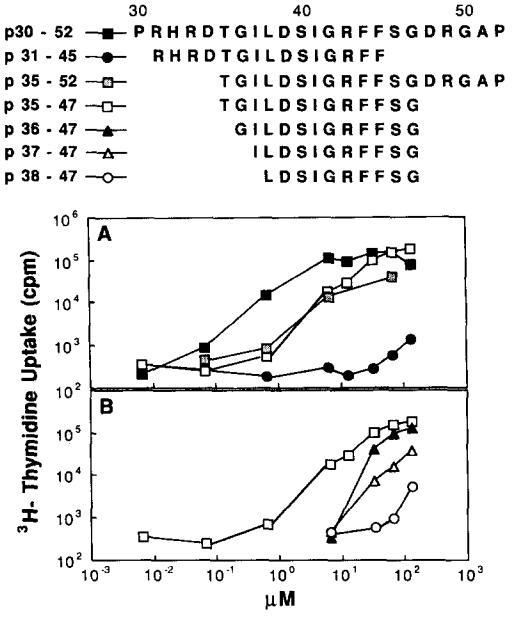

FIGURE 1. Specificity of an encephalitogenic MBP-specific T cell clone restricted by hybrid I-E ($E_\alpha{}^u E_\beta{}^s$) class II molecules. (*A*) Peptides p30-52, p31-45, and p35-47 were tested for their ability to stimulate proliferation of T cell clone F_1-28. (*B*) T cell clone F_1-28 proliferates to peptides p35-47, p36-47, p37-47, and p38-47. Proliferative responses were determined as described in Materials and Methods.

TABLE I
Induction of EAE with Synthetic MBP Peptides

Strain	Peptide	Incidence	Severity*	Day of onset
PL/J	p30-52	3/5	3.0	17
PL/J	p35-47	12/15	3.5	12
PL/J	p30-45	0/15	-	-
PL/J	Rat MBP	8/15	2.5	19
SJL/J	p35-47	0/20	-	-
SJL/J	p30-45	0/15	-	-
SJL/J	Rat MBP	6/10	2.5	20
(PLSJ)F_1	p30-52	3/5	4.1	14
(PLSJ)F_1	p35-47	11/15	4.3	12
(PLSJ)F_1	p30-45	0/15	-	-
(PLSJ)F_1	Rat MBP	14/20	3.8	17

EAE was induced with MBP peptides as described in Materials and Methods.

* Severity was graded as follows: 0, no sign of EAE; 1, decreased tail tone only; 2, mild paraparesis; 3, moderately severe paraparesis; 4, complete paraplegia; 5, moribund.

tides recognized by T cells, a sequence pattern common to a majority of epitopes was identified (4). This pattern includes a core of four contiguous amino acids, a charged residue or glycine, followed by two hydrophobic residues, and in the next position, a charged or polar amino acid (4). With this template, we predicted that F_1-28 would recognize peptides including the sequence 36-39 (GILD) or 42-45 (RFFS). When tested in vitro, MBP peptide p30-52, which encompasses these two tetramers, stimulates proliferation of F_1-28 (Fig. 1 *A*). F_1-28 also proliferates to peptides p35-52 and p35-47, but only weakly to p31-45. Although F_1-28 proliferates to p36-47, p37-47, and p38-47, progressively higher concentrations of peptide are required (Fig. 1 *B*). These results indicate that COOH residues 45-47 are necessary to complete the epitope recognized by this clone.

Peptide p35-47 was tested in vivo for induction of EAE. MBP p35-47 is encephalitogenic in homozygous PL/J (H-2^u) and (PLSJ)F_1 mice (Table I). Histologic signs of EAE, including perivascular infiltrates of mononuclear cells within the central nervous system were observed in PL/J and (PLSJ)F_1 mice immunized with p35-47. However, H-2^s strain mice, SJL/J or B10.S (0/10) immunized with p35-47, did not develop clinical or histologic signs of EAE (Table I). The overlapping peptide p31-45, which is weakly stimulatory (Fig. 1 *A*), did not cause EAE.

Lymphocytes isolated from PL/J and (PLSJ)F_1 mice immunized with p35-47 proliferate in vitro when cultured with p35-47. The proliferative response to MBP p35-47 is inhibited by mAbs specific for I-E, but not I-A, demonstrating that T cell recognition of p35-47 is restricted by I-E molecules. T cell clones specific for p35-47 that were isolated from (PLSJ)F_1 mice are restricted by either homozygous I-E($E_\alpha^u E_\beta^u$) molecules or hybrid I-E($E_\alpha^u E_\beta^s$) molecules. PL/J MBP p35-47-specific T cell clones are restricted by homozygous I-E molecules. Representative clones are shown in Table II. In contrast, MBP p35-47 primed lymphocytes isolated from SJL/J and B10.S (H-2^s[I-A^s]), strains that do not express I-E, did not proliferate in vitro

TABLE II

Peptide Specificity, Class II Restriction, and TCR Vβ8 Expression of MBP-specific T Cell Clones

Clone*	Origin	Immunization	MBP specificity	Class II restriction	TCR $V_{\beta 8}$‡
P1.5	PL/J	p30-52	p35-47	$E_\alpha^u E_\beta^u$	–
P2.1	PL/J	p30-52	p35-47	$E_\alpha^u E_\beta^u$	–
P6.6	PL/J	p30-52	p35-47	$E_\alpha^u E_\beta^u$	–
F3.3	(PLSJ)F_1	p30-52	p35-47	$E_\alpha^u E_\beta^u$	–
F_1-28	(PLSJ)F_1	Rat MBP	p35-47	$E_\alpha^u E_\beta^s$	–
PJR-25	PL/J	Rat MBP	p1-9	$A_\alpha^u A_\beta^u$	+
PJB-20	PL/J	Bovine MBP	p1-9	$A_\alpha^u A_\beta^u$	+
PJpR8.1	PL/J	pR1-11	p1-9	$A_\alpha^u A_\beta^u$	+
PJpR-9.6	PL/J	pR1-11	p1-9	$A_\alpha^u A_\beta^u$	–
PJpBR-6.3	PL/J	pR1-11	p1-9	$A_\alpha^u A_\beta^u$	+
PJp5-1.1	PRL/J	pR5-16	pR5-16	$A_\alpha^u A_\beta^u$	+
PJp5-2.1	PL/J	pR5-16	pR5-16	$A_\alpha^u A_\beta^u$	+
PJp5-3.7	PL/J	pR5-16	pR5-16	$A_\alpha^u A_\beta^u$	+
PJp5-4.6	PL/J	pR5-16	pR5-16	$A_\alpha^u A_\beta^u$	+
PJp5-5.2	PL/J	pR5-16	pR5-16	$A_\alpha^u A_\beta^u$	+
PJp5-5.9	PL/J	pR5-16	pR5-16	$A_\alpha^u A_\beta^u$	–
PJp5-6.2	PL/J	pR5-16	pR5-16	$A_\alpha^u A_\beta^u$	+

* All T cell clones were isolated from separate T cell lines except for PJp5-5.2 ($V_{\beta 8}$ +) and PJ5-5.9 ($V_{\beta 8}$ –), which were isolated from the same line. Clones PJR-25 and PJB-20 have been described (2).

‡ T cell clones were stained with $V_{\beta 8}$-specific mAb as described (6).

with p35-47. Thus, within these strains, T cell recognition of encephalitogenic determinant p35-47 is restricted by I-E molecules.

It has been suggested that TCR Vβ chain expression may correlate with MHC restriction (8). In one investigation, which examined Vβ expression of T cell clones isolated from DBA/2(H-2^d) mice that were specific for sperm whale myoglobin, it was demonstrated that most clones using a member of the $V_{\beta 8}$ subfamily were restricted by I-E, whereas $V_{\beta 8}^-$ clones were I-A restricted (8). Although only 20% PL/J peripheral T cells express $V_{\beta 8}$, we have observed that ~80% of PL/J MBP p1-11-specific clones, restricted by I-A, use TCR $V_{\beta 8}$ (6). None of 11 I-E-restricted p35-47-specific clones use TCR $V_{\beta 8}$. Six of seven I-A-restricted clones that recognize p5-16, a nonencephalitogenic MBP epitope, are $V_{\beta 8}^+$. Representative clones are shown in Table II. In contrast with Morel (8), if Vβ usage correlates with MHC restriction, in PL/J mice, $V_{\beta 8}$ expression may correlate with I-A restriction.

Encephalitogenic determinants within MBP 1-37 and 89-169 were recently identified (2, 3) (Table III). Since T cell clones have been isolated that recognize epitopes of mouse (self) MBP distinct from MBP 1-37 and 89-169, we have suspected that other "cryptic" encephalitogenic MBP determinants may exist. By examining the specificity of an encephalitogenic T cell clone that recognizes a distinct epitope of mouse (self) MBP, we have identified encephalitogenic epitope p35-47. It is clear that there are multiple (at least three) discrete encephalitogenic T cell epitopes of the autoantigen MBP.

In mice, two isotypic class II molecules can be expressed, I-A and I-E. I-A is the homologue of HLA-DQ and I-E is the homologue of HLA-DR (9). Susceptibility

TABLE III
Multiple Discrete Encephalitogenic T Cell Epitopes of MBP

Peptide	Encephalitogenic potential	Class II restriction
MBP 1-11	+	$A_\alpha^u A_\beta^u$
MBP 5-16	-	$A_\alpha^u A_\beta^u$
		$A_\alpha^s A_\beta^u$
MBP 35-47	+	$E_\alpha^u E_\beta^u$
		$E_\alpha^u E_\beta^s$
MBP 89-101	+	$A_\alpha^s A_\beta^s$

to several murine autoimmune diseases including EAE (1-3) and diabetes (10) is associated with specific allelic I-A class II molecules. Although certain investigations indicate that I-E expression is involved in susceptibility (11) or resistance (10) to certain diseases, antigen-specific I-E-restricted T cells that participate in the pathogenesis of murine disease have not been previously identified. In this report, we have identified encephalitogenic epitope, MBP p35-47, whose recognition is restricted only by I-E class II molecules. It is now clear that T cells restricted by I-E, the murine homolog of HLA-DR, participate in autoimmune disease.

Susceptibility to certain human autoimmune diseases is linked to more than one class II molecule (12). For example, susceptibility to insulin-dependent diabetes mellitis, a disease thought to involve T cells, is associated with both HLA-DR3 and HLA-DR4 (12). Although it is unclear why there are multiple class II associations with certain diseases, one possibility is that there are separate T cell antigens or separate determinants of a single autoantigen, each recognized in association with distinct class II molecules. Our studies demonstrate that there are multiple discrete T cell epitopes of the autoantigen MBP (Table III), each recognized in association with separate allelic class II molecules. Discrete T cell epitopes, as we have identified, could, in part, account for the association of more than one class II (HLA-D) molecule with susceptibility to certain autoimmune diseases.

Summary

Immunization with the autoantigen myelin basic protein (MBP) causes experimental allergic encephalomyelitis (EAE). Initial investigations indicated that encephalitogenic murine determinants of MBP were located only within MBP 1-37 and MBP 89-169. Encephalitogenic T cell epitopes within these fragments have been identified. Each epitope is recognized by T cells in association with separate allelic I-A molecules. A hybrid I-E-restricted T cell clone that recognizes intact mouse (self) MBP has been examined. The epitope recognized by this clone includes MBP residues 35-47. When tested in vivo, p35-47 causes EAE. T cell recognition of p35-47 occurs only in association with I-E molecules. These results provide the first clear example that antigen-specific T cells restricted by I-E class II molecules participate in murine autoimmune disease. Furthermore, it is clear that there are multiple (at least three) discrete encephalitogenic T cell epitopes of this autoantigen, each recognized in association with separate allelic class II molecules. These results may be relevant to human autoimmune diseases whose susceptibility is associated with more than one HLA-D molecule.

Received for publication 31 May 1988 and in revised form 29 June 1988.

References

1. Fritz, R. M., M. J. Skeen, C. H. Jen-Chou, M. Garcia, and I. K. Egorov. 1985. Major histocompatibility complex-linked control of the murine immune response to myelin basic protein. *J. Immunol.* 134:2328.
2. Zamvil, S. S., D. M. Mitchell, A. C. Moore, K. Kitamura, L. Steinman, and J. B. Rothbard. 1986. T cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. *Nature (Lond.).* 324:258.
3. Sakai, K., S. S. Zamvil, D. J. Mitchell, M. Lim, J. B. Rothbard, and L. Steinman. 1988. Characterization of an encephalitogenic T cell epitope in SJL/J mice with synthetic oligopeptides of myelin basic protein. *J. Neuroimmunol.* In press.
4. Rothbard, J. B. and W. R. Taylor. 1988. A sequence pattern common to T cell epitopes. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:93.
5. Martenson, R. E. 1984. A useful model for multiple sclerosis. *In* Experimental Allergic Encephalomyelitis. E. C. Alvord, Jr., editor. Alan R. Liss, Inc., New York.
6. Zamvil, S. S., D. J. Mitchell, N. E. Lee, A. C. Moore, M. K. Waldor, K. Sakai, J. B. Rothbard, H. O. McDevitt, L. Steinman, and H. Acha-Orbea. 1988. Predominant expression of a T cell receptor V_β gene subfamily in autoimmune encephalomyelitis. *J. Exp. Med.* 167:1586.
7. Behlke, M. A., T. J. Henkel, S. J. Anderson, N. C. Lan, L. Hood, V. L. Braciale, T. J. Braciale, and D. J. Loh. 1987. Expression of a murine polyclonal T cell receptor marker correlates with the use of specific members of the $V_{\beta 8}$ gene segment subfamily. *J. Exp. Med.* 165:257.
8. Morel, P. A., A. M. Livingstone, and C. G. Fathman. 1987. Correlation of T cell receptor V_β gene family with MHC restriction. *J. Exp. Med.* 166:583.
9. Kaufman, J. F., L. Auffray, A. J. Korman, D. A. Shackelford, and J. Strominger. 1984. The class II molecules of the human and murine major histocompatibility complex. *Cell.* 36:1.
10. Nishimoto, H., H. Kikutani, K.-I. Yamamura, and T. Kishimoto. 1987. Prevention of autoimmune insulitis by expression of I-E molecules in NOD mice. *Nature (Lond.).* 328, 432.
11. Wassom, D. L., C. J. Krco, and C. S. David. 1987. I-E expression and susceptibility to parasite infection. *Immunol. Today.* 8:39.
12. Stasny, P., E. J. Ball, P. J. Dry, and G. Nunez. 1983. The human immune response region (HLA-D) and disease susceptibility. *Immunol. Rev.* 70:113.