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 NH2-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\(^\text{b}\) (2), and recognition of MBP p89-101 is restricted by I-A\(^\text{a}\) (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 x 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 F1-28, isolated from a (PLSJ)F1 mouse immunized with intact rat MBP, recognizes MBP in association with hybrid I-E(EαEγEγ') 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)F1 mice following the protocol described (2).

Proliferation Assay. As described previously (2), 10⁴ T cells were cultured with 5 x 10⁷ 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 (10⁵) (Michigan Dept. of Health, lot 9113) were injected intravenously. 2 d later, a second i.v. injection of pertussis organisms was given.

mAbs. T cell clones were examined with TCR Vα-specific mAb by FACS analysis as described previously (6). mAb KJ16 binds a determinant associated with the expression of two members of the Vα subfamily, Vα1 and Vα2, and F23.1 binds a determinant associated with expression of all three members of the Vα subfamily (7). Vα+ clones were stained positive with both antibodies.

Results and Discussion

T cell clone F1-28, isolated from a (PLSJ)F1 mouse immunized with intact rat MBP, proliferates to intact rat or mouse (self) MBP, and is restricted to hybrid I-E(EαEγEγ') molecules. F1-28 causes EAE in (PLSJ)F1 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-
The severity of EAE 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.

Peptide p35-47 was tested in vivo for induction of EAE. MBP p35-47 is encephalitogenic in homozygous PL/J (H-2b) and (PLSJ)F₁ 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)F₁ mice immunized with p35-47. However, H-2b 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)F₁ 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)F₁ mice are restricted by either homozygous I-E(E₄₊E₅⁺) molecules or hybrid I-E(E₄⁺E₅⁺) 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-2b[I-A⁺]), strains that do not express I-E, did not proliferate in vitro.

### Table I

<table>
<thead>
<tr>
<th>Strain</th>
<th>Peptide</th>
<th>Incidence</th>
<th>Severity*</th>
<th>Day of onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL/J</td>
<td>p30-52</td>
<td>3/5</td>
<td>3.0</td>
<td>17</td>
</tr>
<tr>
<td>PL/J</td>
<td>p35-47</td>
<td>12/15</td>
<td>3.5</td>
<td>12</td>
</tr>
<tr>
<td>PL/J</td>
<td>p30-45</td>
<td>0/15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PL/J</td>
<td>Rat MBP</td>
<td>8/15</td>
<td>2.5</td>
<td>19</td>
</tr>
<tr>
<td>SJL/J</td>
<td>p35-47</td>
<td>0/15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SJL/J</td>
<td>p30-45</td>
<td>0/15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SJL/J</td>
<td>Rat MBP</td>
<td>6/10</td>
<td>2.5</td>
<td>20</td>
</tr>
<tr>
<td>(PLSJ)F₁</td>
<td>p30-52</td>
<td>3/5</td>
<td>4.1</td>
<td>14</td>
</tr>
<tr>
<td>(PLSJ)F₁</td>
<td>p35-47</td>
<td>11/15</td>
<td>4.3</td>
<td>12</td>
</tr>
<tr>
<td>(PLSJ)F₁</td>
<td>p30-45</td>
<td>0/15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(PLSJ)F₁</td>
<td>Rat MBP</td>
<td>14/20</td>
<td>3.8</td>
<td>17</td>
</tr>
</tbody>
</table>
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-2d) mice that were specific for sperm whale myoglobin, it was demonstrated that most clones using a member of the Vβ8 subfamily were restricted by I-E, whereas Vβ8- clones were I-A restricted (8). Although only 20% PL/J peripheral T cells express Vβ8, we have observed that ~80% of PL/J MBP pl-11-specific clones, restricted by I-A, use TCR Vβ8 (6). None of II I-E-restricted p35-47-specific clones use TCR Vβ8. Six of seven I-A-restricted clones that recognize p5-16, a nonencephalitogenic MBP epitope, are Vβ8-. Representative clones are shown in Table II. In contrast with Morel (8), if Vβ usage correlates with MHC restriction, in PL/J mice, Vβ8 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
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 mellitus, 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.

### Table III

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Encephalitogenic potential</th>
<th>Class II restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP 1-11</td>
<td>+</td>
<td>Aα<em>Ab</em></td>
</tr>
<tr>
<td>MBP 5-16</td>
<td>-</td>
<td>Aα<em>Ab</em></td>
</tr>
<tr>
<td>MBP 35-47</td>
<td>+</td>
<td>Eα<em>Eβ</em></td>
</tr>
<tr>
<td>MBP 89-101</td>
<td>+</td>
<td>Aα<em>Ap</em></td>
</tr>
</tbody>
</table>

Peptide Encephalitogenic potential Class II restriction

MBP 1-11 + A α Ab
MBP 5-16 - A α Ab
MBP 35-47 + E α E β
MBP 89-101 + A α Ap

Published September 1, 1988
Received for publication 31 May 1988 and in revised form 29 June 1988.

References


