Antigen-specific therapy of experimental allergic encephalomyelitis by soluble class II major histocompatibility complex–peptide complexes
(autoimmunity/immunotherapy)

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ABSTRACT Experimental allergic encephalomyelitis is a T-cell-mediated, major histocompatibility complex (MHC) class II gene-linked autoimmune demyelinating disease of the central nervous system. To develop therapies that will specifically inactivate only the autoantigen-reactive T cells, mice were treated with soluble MHC class II molecules that had been complexed with encephalitogenic peptides. Intravenous injections of 300 μg of complexes consisting of encephalitogenic peptide 91–103 of myelin basic protein plus I-A<sup>+</sup> protein on day 0, 4, and 7 were effective in preventing experimental allergic encephalomyelitis. Similarly, administration of 45 μg of I-A<sup>+</sup> protein complexed to peptide 139–151 from proteolipoprotein on day 1, 4, and 7 prevented mortality and significantly reduced paralysis induced by immunization with the encephalitogenic proteolipoprotein peptide. Histological examination of sections of animal brains revealed that treatment with I-A<sup>+</sup> protein plus myelin basic protein 91–103 peptide prevents the development of inflammatory lesions characteristic of experimental allergic encephalomyelitis. Thus, treatment with MHC-self-peptide complexes could serve as a highly specific therapeutic modality in treating autoimmune disease when the putative autoantigen and the MHC restricting elements are known.

The T-cell receptor is composed of an individually specific heterodimeric glycoprotein receptor complex (αβ) that binds to antigenic fragments associated with major histocompatibility complex (MHC) molecules (1–4). The signals generated by the molecular triad formed by the T-cell receptor, antigenic peptide, and MHC antigens are critical in understanding the mechanism(s) of T-cell activation. Delivery of at least two separate signals is required to activate T cells (5, 6). (i) The T-cell receptor must bind to the complex of antigenic peptide bound to self-MHC protein, and (ii) simultaneous delivery of an as-yet-undefined costimulatory signal by antigen-presenting cells seems necessary for T-cell activation (5, 6). Prior studies have suggested that binding of T cells to the MHC–peptide complex on planar membranes in the absence of costimulatory signals(s) leads to T-cell inactivation and the subsequent development of antigen-specific tolerance, referred to as clonal anergy (7). This situation suggests that occupancy of antigen-specific T cells with soluble MHC–antigen (peptide) complexes will lead to the development of antigen-specific inactivation in vitro and in vivo. If true in vivo, then one might be able to inactivate autoantigen-specific T cells in vivo during an autoimmune disease for which the MHC locus and putative disease-inducing autoantigens were known. We chose murine experimental allergic encephalomyelitis (EAE), which shows clinical and pathological similarities to human multiple sclerosis, as an experimental model system to test this hypothesis.

EAE is a T-cell-mediated, paralytic, autoimmune demyelinating disease of the central nervous system that can be induced in a number of susceptible strains of mice by the injection of either myelin basic protein (MBP) or proteolipoprotein (PLP) (8–12). In SJL mice the T-cell responses to peptide determinants are associated with the class II (I-A<sup>+</sup>) allele of the MHC class II complex. Encephalitogenic regions of MBP and PLP correspond to peptides 91–103 of MBP and 139–151 of PLP (13, 14). Furthermore, transfer of activated CD4<sup>+</sup> T-cell lines or clones derived from SJL mice that recognize amino acids 91–103 of MBP or amino acids 139–151 of PLP are sufficient to cause EAE (15, 16). Therefore, SJL mice are an ideal host in which to test the efficacy of soluble MHC–peptide complexes in the therapy of EAE. We show here that administration of I-A<sup>+</sup> protein plus MBP 91–103 or I-A<sup>+</sup> protein plus PLP 139–151 reduces the incidence and severity of EAE.

MATERIALS AND METHODS

Mice. Female SJL/J mice were purchased from The Jackson Laboratory and were used between 8 and 12 weeks of age.

Antigens. Peptides of MBP and PLP corresponding to amino acids 1–14, 91–103, and 139–151 were synthesized by solid-phase 9-fluorenylmethoxycarbonyl (FMOC) procedures. Peptides were purified by reverse-phase HPLC and were characterized by both HPLC and mass spectroscopy.

Purification of class II MHC. I-A<sup>+</sup> protein was purified from a Nonidet P-40 extract of spleen cell membranes from SJL/J mice by affinity chromatography using the monoclonal antibody 10-2.16 (specific for I-A<sup>+</sup> and I-A<sup>+</sup>), coupled to Sepha-rose 4B beads. Extracted lysate from the high-speed (100,000 × g) membrane fraction in a buffer of 10 mM TrisHCl, pH 8.3/0.5% Nonidet P-40/0.1 M sodium chloride/5 mM EDTA/0.02% sodium azide/1 mM phenylmethysulfonyl fluoride was recycled over the preequilibrated column at 4°C for 16 hr. The column was washed first with 10 bed volumes of deoxycholate buffer/10 mM TrisHCl, pH 8.3/0.5% deoxycholate/0.1 M sodium chloride/5 mM EDTA/0.02% sodium azide/1 mM phenylmethysulfonyl fluoride and then by 5 bed volumes of phosphate-buffered saline (PBS)/1% 1-octyl β-d-glycopyranoside buffer. Finally, I-A<sup>+</sup> was eluted with 20 mM phosphate buffer, pH 11/0.1 M sodium chloride/1% octyl glucoside/0.02% sodium azide/1 mM phenylmethysulfonyl fluoride. Fractions were neutralized with acetic acid to a final

Abbreviations: EAE, experimental allergic encephalomyelitis; MBP, myelin basic protein; PLP, proteolipoprotein; MHC, major histocompatibility complex.

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concentration of 12 mM, and I-A<sup>+</sup> was concentrated by using an Amicon Centriprep-10 concentrator. Purified I-A<sup>+</sup> was >98% pure based on silver staining of SDS/PAGE gels.

**Preparation of MHC–Peptide Complexes.** I-A<sup>+</sup> protein at 1 mg/ml was incubated with 50-fold molar excess of MBP or PLP peptides at 37°C for 48 hr. The excess peptide was removed by extensive dialysis of the complex against PBS/0.1% octyl glucoside, a concentration easily tolerated by mice. There was no significant aggregation of complex preparations in 0.1% octyl glucoside as assessed by gel-filtration studies. Percent of I-A<sup>+</sup> occupancy with peptide ranged from 20 to 50 as determined by a parallel pilot experiment using radiolabeled peptide as described in detail elsewhere (17). The absence of endotoxin in the final complex preparation was confirmed by *Limulus* amebocyte lysate procedure as described by Whittaker Bioproducts.

**T-Cell Clone.** HS1 cells were cloned from MBP 91–103-reactive encephalitogenic T-cell line as described (18). Briefly, draining lymph node cells from SJL mice immunized with 400 μg of MBP 91–103 in complete Freund’s adjuvant were harvested and stimulated with MBP 91–103 at 5 μg/ml in RPMI medium/10% fetal calf serum. After 10 days in culture, the T cells were harvested and cloned by limiting-dilution method with 0.3 cell per well.

**Induction of in Vitro Nonresponsiveness.** To ensure that no residual antigen-presenting cells were present, HS1 T cells were subjected to two rounds of Ficoll/Hypaque density-gradient centrifugation. Cells (1 x 10<sup>6</sup>) were cultured with 10 μg of I-A<sup>+</sup> protein plus MBP 91–103, I-A<sup>+</sup> protein plus PLP 139–151, or medium alone for 24 hr. The cells were washed, and 1 x 10<sup>6</sup> T cells were cultured with 5 x 10<sup>5</sup> fresh irradiated spleen cells and MBP 93–101 at 10 μg/ml or recombinant interleukin 2 at 20 units per ml. During the last 18 hr of a 72-hr incubation, 1 μCi (1 Ci = 37 GBq) of [<sup>3</sup>H]thymidine was added, and the degree of radioactivity incorporated was determined by liquid scintillation counting. Data shown are means of triplicate determinations.

**Induction of EAE.** Two distinct protocols were used to induce EAE. (i) Mice were immunized with 400 μg of MBP 91–103 in complete Freund’s adjuvant on the dorsum. Ten to 12 days later, regional draining lymph node cells were harvested and cultured in 24-well plates at a concentration of 6 x 10<sup>5</sup> cells per well in 1.5 ml of RPMI 1640 medium/10% fetal bovine serum/1% penicillin/streptomycin/MBP at 50 μg/ml.

**Fig. 1.** Induction of in vitro nonresponsiveness by MHC–peptide complexes. Data shown are mean of triplicate determinations. ■, Interleukin 2; □, MBP 91–103.

After a 4-day *in vitro* stimulation, MBP 91–103-reactive T-cell blasts were harvested via Ficoll/Hypaque density gradient, washed twice in PBS, and 1.3 x 10<sup>6</sup> cells were injected into each mouse.

(ii) Animals were immunized with the PLP peptide 139–151 dissolved in PBS and mixed with complete Freund’s adjuvant containing *Mycobacterium tuberculosis* H37Ra at 4 mg/ml in a 1:1 ratio. Mice were injected with 152 μg of peptide adjuvant mixture, a dose found to induce EAE in 100% of animals, s.c. in both flanks. On the same day and 48 hr later, all animals were given 400 ng of pertussis toxin (List Biological Laboratories, Campbell, CA).

**Clinical Evaluation.** Animals were observed daily and graded for clinical signs of EAE: grade 1, loss of tail tone; grade 2, hind leg weakness; grade 3, hind leg paralysis; grade 4, moribund; grade 5, death. In accordance with the regulations of the Animal Care Committee, mice that could not feed themselves were sacrificed.

**Histological Evaluation.** The brains were removed after perfusion in PBS, fixed in formalin, and stained with hema-
RESULTS AND DISCUSSION

To determine the effect of soluble MHC-peptide complexes on T-cell activation in vitro, a clone derived from MBP 91–103-reactive T-cell line was generated from SJL mice (18). HS1 cells were depleted of antigen-presenting cells and incubated with I-A\textsuperscript{\(\mathbf{b}\)} protein plus MBP 91–103 complex at a concentration of 10 \(\mu\)g/ml for 24 hr. Fig. 1 shows that treatment of MBP 91–103-reactive HS1 clone with soluble I-A\textsuperscript{\(\mathbf{b}\)} protein plus MBP 91–103 complexes resulted in the unresponsiveness of HS1 cells to a subsequent challenge to MBP 91–103 peptide. Lack of proliferative response was not the result of cell death or loss of viability because HS1 cells responded adequately to interleukin 2 and HS1 cells treated with I-A\textsuperscript{\(\mathbf{b}\)} protein plus PLP 139–151 showed no loss of the proliferative response to peptide MBP 91–103. Incubation of HS1 cells with the 100-fold excess of peptide required to induce optimum proliferation or MHC alone had no effect on responsiveness or on the level of T-cell receptor expression (data not shown). Lack of T-cell receptor modulation in nonresponsive T cells agrees with observations reported for murine T-cell clones (7) but differs from the results on an influenza peptide-specific human T-cell clone (19, 20).

To determine whether treatment with I-A\textsuperscript{\(\mathbf{b}\)} protein plus MBP 91–103 complex will prevent EAE development after T-cell activation, SJL mice were injected with 1.3 \(\times\) 10\(^7\) MBP 91–103-reactive T-cell blasts in vivo. Mice that received encephalitogenic MBP 91–103-reactive T cells then received either 100 \(\mu\)g of soluble I-A\textsuperscript{\(\mathbf{b}\)} protein plus MBP 91–103 complexes, 100 \(\mu\)g of I-A\textsuperscript{\(\mathbf{b}\)} protein plus MBP 1–14 (a peptide

![Histology of cerebellum and periventricular areas of animals treated with I-A\textsuperscript{\(\mathbf{b}\)} protein plus MBP 1–14. (A) Single large perivascular cuff in cerebellum. (B) Two lymphocytic cuffs in periventricular area (\(\times\)100.)](https://example.com/histology.png)
Mice were treated with PBS, 15 μg of I-A<sup>a</sup>-alone, 15 μg of I-A<sup>a</sup> plus PLP 139–151, or 100 μg of PLP on day 1, 4, and 7 after immunization.

Table 1. Prevention of EAE in SJL mice after treatment with I-A<sup>a</sup> plus PLP complexes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animals paralyzed, no.</th>
<th>n</th>
<th>Mean severity</th>
<th>Mortality, %</th>
<th>Onset of paralysis, day</th>
</tr>
</thead>
<tbody>
<tr>
<td>None or saline</td>
<td>15</td>
<td>15</td>
<td>4.7</td>
<td>87</td>
<td>8</td>
</tr>
<tr>
<td>I-A&lt;sup&gt;a&lt;/sup&gt; alone</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>PLP 139–151</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td>I-A&lt;sup&gt;a&lt;/sup&gt; + PLP 139–151</td>
<td>6</td>
<td>6</td>
<td>2.2</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
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Histological analysis was done from four animals in the I-A<sup>a</sup> protein plus PLP 139–14-treated group and four animals in the I-A<sup>a</sup> protein plus MBP 91–103-treated groups. Overall 14 perivascular cuffs were identified in the I-A<sup>a</sup> protein plus MBP 1–14 group, whereas the I-A<sup>a</sup> protein plus MBP 91–103 group showed only 3 cuffs. Most of the inflammation was seen mainly in the periventricular area and in the white matter of the cerebellum (Fig. 3).

We next addressed the question of whether inhibition of disease induction was possible with other autoantigens presented by the I-A<sup>a</sup> allele. SJL mice were immunized with peptide 139–151 of PLP in complete Freund's adjuvant to induce EAE. Table 1 shows that animals that received the appropriate I-A<sup>a</sup> protein plus PLP 139–151 peptide complex were protected from the severe fulminant paralytic disease induced by the immunization with peptide in adjuvant. There was no mortality in the I-A<sup>a</sup> peptide plus PLP 139–151 peptide-treated group. Although all six animals did develop paralysis, the mean severity of animals paralyzed was 2.2, and the mean day of onset was 10.6. In contrast, all six animals that received I-A<sup>a</sup> protein alone had a mean day of onset of 8.2. Five animals died by day 11, and one animal died on day 21. Animals that received saline or no treatment had a mortality of 87%, and the average day of onset was 9.2 (P < 0.0001 I-A<sup>a</sup> protein plus PLP 139–151 versus no treatment; P < 0.0007 I-A<sup>a</sup> protein plus PLP 139–151 versus I-A<sup>a</sup> protein alone).

Our observations indicate that in vivo therapy with I-A<sup>a</sup> protein plus MBP 91–103 complexes (300 μg) results in the prevention of EAE induced by adoptive transfer of encephalitogenic T-cell lines. In addition, therapy with 45 μg of I-A<sup>a</sup> protein plus PLP 139–151 significantly lowered the mortality and morbidity in animals that received this therapy. Fluorescein-activated cell sorter analysis of T-cell subset distribution on spleen and lymph nodes failed to show any significant differences in their numbers, and on day 20 or at the time of sacrifice, there was no difference in the proliferative response to mitogens and alloantigens between the three treatment groups (data not shown).

Our current experiments were designed to determine whether treatment with I-A<sup>a</sup> protein plus peptide complexes could alter the course of disease after activation of encephalitogenic T cells in vivo. Although the in vitro studies with MBP-specific T cells have led us to conclude that induction of anergy occurs after incubation of antigen-specific T cells with MHC–peptide complexes in vitro, the mechanism of action of MHC–peptide complexes in the prevention of EAE in vivo remains to be elucidated. Because EAE is a T-cell-mediated disease, many strategies have been attempted to either eliminate or inactivate encephalitogenic T-cell clones as a means of both preventing and treating established disease. At the T-cell level, therapies such as T-cell vaccination (22), immunotherapy with anti-T-cell receptor antibody (23), and immunization with peptides that span the hypervariable regions of the T-cell receptor as immunogens have been successful in prevention of EAE in rats (24, 25). Although this form of therapy is attractive, it assumes that the encephalitogenic T-cell receptor repertoire is very limited. This assumption appears not to be the case, at least in the SJL strain of mice, in which at least three different variable region β-chain genes are known to be used in the T-cell response to MBP 91–101 (26). Furthermore, in patients with multiple sclerosis the T-cell response to MBP 86–102, a region over-represented in multiple sclerosis patients, was not restricted to a particular variable region gene (27, 28).

At the MHC level, the use of anti-I-A antibody (29), soluble antigens (30), MHC-blocking peptides (31), and therapy with antigen-presenting cells coupled to peptides through a chemical cross linker (21, 32–35) have been used either to block putative MHC-binding sites or to alter the activation signals propagated in T cells. The drawbacks of these strategies are the inherent problems with in vivo antibody therapy and the lack of evidence of the efficacy of MHC-blocking peptides after T-cell activation. Although use of antigen-coupled spleen cells to prevent EAE is described (21, 32–35), it suffers from the impracticality of use. The findings reported here lead us to conclude that the use of MHC–peptide complexes represents a very viable and promising method of treating autoimmune disease when the peptide determinants of the autoantigen are known. This treatment also offers the important advantage of inhibiting only those T cells with specificity for a self-peptide bound to the MHC class II-presenting element implicated by the genetics of disease association as being the restricting element. In addition, success of this approach does not depend on a limited repertoire of T-cell receptors specific for the MHC–peptide complex. Preliminary experiments suggest that treatment of rats with MHC antigens complexed to appropriate peptides prevents adjuvant arthritis in rats and established autoimmune myasthenia gravis; similarly, initial results indicate that this form of therapy has been found useful in treating established EAE (Hansha Bhayani, S.D.S., E.S., and S.S., unpublished observations). The antigen MHC specificity and the lack of global immunosuppression makes this an additional form of immunotherapy. Such a therapy has broad applications for a number of autoimmune diseases in which the HLA-D restriction and the putative antigens are known.