Critical Requirement for Aspartic Acid at Position 82 of Myelin Basic Protein 73–86 for Recruitment of Vβ8.2+ T Cells and Encephalitogenicity in the Lewis Rat

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Critical Requirement for Aspartic Acid at Position 82 of Myelin Basic Protein 73–86 for Recruitment of Vβ8.2+ T Cells and Encephalitogenicity in the Lewis Rat

Ronald B. Smeltz,* Marca H. M. Wauben,† Norbert A. Wolf,* and Robert H. Swanborg*

We synthesized single amino acid-substituted peptide analogues of guinea pig myelin basic protein (MBP) 73–86 to study the importance of aspartic acid at residue 82 (QKSQRSQDENPV), which previous reports have suggested is a critical TCR contact residue. Whereas the wild-type 73–86 peptide elicited severe experimental autoimmune encephalomyelitis (EAE) in the Lewis rat, none of the peptide analogues with substitutions at position 82 were capable of inducing EAE. The inability to cause EAE was not due to a failure to bind MHC or to elicit T cell proliferation and cytokine secretion. T cells specific for MBP73–86 did not cross-react with any of the analogues tested, further indicating the importance of this residue in T cell responses to 73–86. Analysis by flow cytometry showed that only the wild-type 73–86 peptide was capable of recruiting Vβ8.2+ T cells, which have been shown previously to be important for disease induction. Reduced expression of the Vβ8.2 TCR was also seen in Lewis rats protected from EAE by coimmunization of MBP73–86 with 73–86(82D→A), despite an increase in cytokine production when both peptides were present during in vitro culture. The data indicate that aspartic acid 82 is a critical TCR contact residue and is required for the recruitment of Vβ8.2+ T cells and the encephalitogenic activity of MBP73–86. The Journal of Immunology, 1999, 162: 829–836.

Materials and Methods

Animals and immunization

Female LEW rats were purchased from Charles River (Raleigh, NC), maintained in our American Association for the Accreditation of Laboratory Animal Care-accredited facility, and used at 8–10 wk of age. Rats were immunized s.c. with 25 µg of peptide emulsified in IFA supplemented with 200 µg of Mycobacterium butyricum (Difco, Detroit, MI) as described previously (8), and EAE was scored based on the following criteria: 0, no paralysis; 1, flaccid tail; 2, partial hind limb paralysis; and 3, complete hind limb paralysis with incontinence. Hematoxylin and eosin-stained spinal cord sections from representative rats were examined microscopically for the characteristic mononuclear cell infiltration without knowledge of experimental treatment and scored on a scale of 0–4 based on the intensity of inflammation.

Peptides

Peptides were synthesized using fluorenylmethoxycarbonyl chemistry with an automated Applied Biosystems Synergy model 432A peptide synthesizer (Perkin-Elmer, Foster City, CA). The m.w. and purity of each peptide was confirmed by mass spectrometry. All peptide analogues were synthesized based upon the dominant LEW rat encephalitogenic MBP epitope, residues 73–86 (QKSQRSQDENPV) of gpMBP.

Cell lines

Draining lymph nodes were removed from rats that had been immunized 9–10 days earlier, and single-cell suspensions were prepared as described previously (7). The primary culture consisted of lymph node cells (LNCs) cultured at 2 × 10⁶/ml in RPMI 1640 supplemented with 2-µM, l-glutamine, antibiotics, 1% normal rat serum (Life Technologies, Grand Island, NY). Cells were cultured at 2,000 cells per well in 96-well plates and stimulated with 73–86(82D→A) peptide.

Abbreviations used in this paper: gp, guinea pig; MBP, myelin basic protein; LEW, Lewis; EAE, experimental autoimmune encephalomyelitis; wt, wild type; LNC, lymph node cell; TCGF, T cell growth factor.
NY), and 20 μg/ml of the immunizing peptide as described previously (8). After 3 days of incubation at 37°C, lymphoblasts were isolated by Ficoll-Isoopaque (Pharmacia, Uppsala, Sweden) centrifugation. Viable blast cells were cultured at a density of $2 \times 10^5$ ml in RPMI 1640 supplemented as described above but with 10% FCS and with Con A supernatant containing 20 U/ml of IL-2 in place of Ag. After 4 days of expansion, T cells were collected and prepared for flow cytometry.

Flow cytometry

Cell lines were prepared for flow cytometry by first incubating $1 \times 10^6$ cells with PBS containing 0.02% sodium azide, 1% BSA (PBS-A-BSA), and 1% normal rat serum for 30 min as a blocking step (9). Cells were subsequently washed in PBS-A-BSA and incubated with the appropriate chromogen-labeled Ab for 20 min. The Abs used were: FITC-labeled anti-rat TCR (R73), FITC-labeled anti-rat Vβ8.2 (R78), phycoerythrin-labeled anti-rat CD25 (OX39), and FITC anti-rat DNP as an isotype control (all purchased from PharMingen, San Diego, CA); FITC anti-α4 HP2/1, which reacts with the α4-chain of very late Ag-4 (purchased from AMAC, Westbrooke, ME) was also used. Cells were analyzed in a FACScan flow cytometer using Lysis software (Becton Dickinson, Mountain View, CA).

Proliferation assay

T cell proliferation assays were performed as described previously (9, 10). Briefly, plastic nonadherent LNCs were applied to a T cell column (Biotex, Edmonton, Canada) and collected by washes with PBS supplemented with 2% FCS. Each well received $5 \times 10^5$ T cells and $5 \times 10^5$ syngeneic, irradiated thymocytes (2000 rad) as APCs. Cells were cultured in RPMI 1640 containing 5% FCS. Wells receiving Ag contained a final concentration of either 5 or 20 μM of the appropriate peptide, which was determined to be optimal in previous studies (9). Cultures were incubated for 96 h and subsequently pulsed with 0.5 μCi of [3H]thymidine for the last 18 h of the assay. Plates were harvested using a Tomtec Harvester 96

Table I. Altered peptide ligands of MBP73–86 are not encephalitogenic in the LEW rat

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>Dose</th>
<th>Clinical EAE</th>
<th>Histologic</th>
</tr>
</thead>
<tbody>
<tr>
<td>73–86</td>
<td>QKSORQDENPV</td>
<td>25 μg</td>
<td>3, 3, 3, 3</td>
<td>4, 4</td>
</tr>
<tr>
<td>(82D→A)</td>
<td>QKSORQDENPV</td>
<td>25 μg</td>
<td>0, 0, 0, 0</td>
<td>0, 0</td>
</tr>
<tr>
<td>(82D→E)</td>
<td>QKSORQDENPV</td>
<td>25 μg</td>
<td>0, 0, 0, 0</td>
<td>0, 0</td>
</tr>
<tr>
<td>(82D→N)</td>
<td>QKSORQDENPV</td>
<td>25 μg</td>
<td>0, 0, 0, 0</td>
<td>0, 0</td>
</tr>
<tr>
<td>(82D→Y)</td>
<td>QKSORQDENPV</td>
<td>25 μg</td>
<td>0, 0, 0, 0</td>
<td>0, 0</td>
</tr>
</tbody>
</table>

*LEW rats were immunized with 25 μg of the respective analog of MBP73–86 and observed for clinical signs of EAE over a period of 25 days.

Sections of rat spinal cord from immunized rats were stained and observed microscopically for the characteristic mononuclear cell infiltration. Severity ranged from 0 (no inflammation) to 4 (inflammatory lesions in every low power field).

FIGURE 1. Representative spinal cord sections from LEW rats immunized with either 73–86 (encephalitogenic) (A); analogue 73–86(82D→E) (B); analogue 73–86(82D→N) (C); analogue 73–86(82D→K) (D), showing numerous motor neurons; and analogue 73–86(82D→Y) (E). Rats were sacrificed around the time of appearance of clinical symptoms of EAE (days 11–14). Original magnification was ×100.
FIGURE 2. Competitive binding of MBP analogues to purified RT1.B1. Inhibition of the binding of 100 nM of biotinylated MBP73–86 to 3 μM of affinity-purified RT1.B1 is shown. Different dose ranges (0–256 μM) of competitor peptide were tested.

**Table:**

<table>
<thead>
<tr>
<th>Competitor peptide</th>
<th>Concentration of competitor peptide (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP73-86</td>
<td></td>
</tr>
<tr>
<td>MBP73-86 82D&gt;A</td>
<td></td>
</tr>
<tr>
<td>MBP73-86 82D&gt;E</td>
<td></td>
</tr>
<tr>
<td>MBP73-86 82D&gt;K</td>
<td></td>
</tr>
<tr>
<td>MBP73-86 82D&gt;N</td>
<td></td>
</tr>
<tr>
<td>MBP73-86 82D&gt;N</td>
<td></td>
</tr>
</tbody>
</table>

(Orange, CT) and counted with the 1450 Microbeta Plus liquid scintillation counter (Wallac, Gaithersburg, MD). Results are presented as cpm.

**MHC binding studies**

To determine whether peptide analogues bind to RT1.B1 (the rat equivalent of MHC class II I-A), each analogue was tested for its ability to compete with biotinylated 73–86 for binding to detergent-solubilized RT1.B1 molecules, as described previously (11). Briefly, biotinylated 73–86 peptide (100 nM) was incubated with MHC class II molecules (RT1.B1, 3 μM) that had been affinity-purified from the MBP-specific encephalitogenic Z1A T cell line. Various concentrations of competitor peptide (ranging from 0 to 256 μM) were subsequently added. The MHC-peptide mixtures were analyzed by SDS-PAGE followed by blotting onto nitrocellulose. The ability of each analogue to compete with biotinylated 73–86 was determined by measuring the reduction in signal from the biotinylated peptide by enhanced chemiluminescence. As a control, competition for binding to RT1.D1 (the rat equivalent of I-E) was determined in a similar fashion; biotinylated MBP 87–99, which is the minor encephalitogenic RT1.D1-restricted MBP epitope for LEW rats, was used as a marker peptide (12).

**Cytokine analysis**

The 72 h culture supernatants from peptide-stimulated T cells were evaluated for IFN-γ, TNF-α, and IL-10 using rat-specific commercial ELISA kits (Biosource International, Camarillo, CA; Life Technologies, Gaithersburg, MD) according to the manufacturers’ instructions.

**Results**

**Substitution of aspartic acid at position 82 of MBP73–86 abrogates encephalitogenic activity**

Immunizing LEW rats with native gpMBP epitope 73–86 (QKSQRSQDENPV) induces severe clinical EAE (Table I). Extensive mononuclear infiltration was present in the spinal cords (Fig. 1A). Demyelination is not a prominent finding in LEW rats with acute EAE (8). Although tested in this experiment at 25 μg, the same peptide also induced paralytic EAE at 2.5 μg (9). Aspartic acid at position 82 has been proposed to be a TCR contact residue (1). To test the hypothesis that this aspartic acid residue is important for encephalitogenicity, peptide analogues were synthesized with substitutions at position 82. The analogues we prepared included substitution of the native aspartic acid residue by one additional methyl group. LEW rats were immunized with 25 μg of an individual analogue in CFA and observed for clinical signs of EAE. None of the analogues tested induced clinical or histologic EAE (Table I, Fig. 1, B, C, D, and E). Interestingly, not even the conservative exchange of aspartic acid for glutamic acid at position 82 could maintain encephalitogenicity.

**Altered peptide ligands of MBP73–86 bind to MHC**

One possible explanation for the failure of the various peptide analogues to cause EAE is that MHC binding had been affected. This possibility was tested by determining whether any of the analogues could inhibit the binding of biotinylated 73–86 peptide to detergent-solubilized MHC class II RT1.B1 molecules (the homologue of murine I-A). The data confirm that each of the analogues binds to RT1.B1 at least as well as peptide 73–86 (Fig. 2). As expected, there was no binding to RT1.D1, the homologue of I-E (Fig. 3).

**Immunogenicity and cross-reactivity of analogue-primed T cells**

To determine whether the analogues were capable of inducing an immune response, lymph node T cells from analogue-primed rats were tested in vitro for proliferation to the immunizing peptide. As shown in Fig. 4, each analogue was immunogenic as reflected by T cell proliferation in vitro. T cells from rats immunized with 73–86 responded to the priming peptide, but did not proliferate when stimulated in vitro with any of the analogues (Fig. 4A). Similarly, T cells from rats immunized with 82D→A, 82D→K, or 82D→Y responded to the priming peptide but failed to cross-react with either the native peptide or the other analogues (Fig. 4B, C, D, and E). T cells from LEW rats immunized with 82D→E cross-reacted weakly with 82D→A (Fig. 4D), whereas T cells primed to 82D→N cross-reacted weakly with 82D→K and exhibited a heteroclitic response to 82D→A (Fig. 4C). Proliferative responses to a higher concentration of peptide were similar to those seen at the 5-μM concentration (Fig. 5).

Because the 82D→A analogue has been shown to have protective effects in vivo when coimmunized with 73–86, it was important to more closely examine cross-reactivity between 82D→A T cells and MBP73–86, 82D→A T cells, when stimulated in vitro with 5 μM of either gp73–86 or rat 73–86, did not show any
significant proliferation when compared with the proliferation induced by the 82D→A peptide (Table II). Proliferation using 20 μM of peptide gave similar results (data not shown).

To independently confirm the finding that all analogues induced an immune response, culture supernatants were also tested for the production of IFN-γ and IL-10 by ELISA. As shown in Fig. 6, LNCs from immunized rats secrete significant levels of IFN-γ and measurable, albeit lower quantities of IL-10, when stimulated with the immunizing peptide. Consistent with the observed lack of cross-reactivity at the level of proliferation, MBP73–86 T cells did not secrete IL-10 when stimulated with the different analogues (Fig. 7). Although IFN-γ was secreted at levels comparable with altered peptide ligand-immunized rats as shown in Fig. 6, a direct comparison cannot be made, because column-enriched 73–86-specific T cells were used in Fig. 7.

Only MBP73–86 elicits Vβ8.2+ T cells

Previous reports have shown that LEW rat T cells that bear the Vβ8.2 TCR predominate in both short-term T cell lines and in T cell clones specific for gpMBP73–86 (6, 7). TCR peptides consisting of complementarity-determining region-2 (13, 14) or complementarity-determining region-3 (15) of the Vβ8.2 TCR have been used successfully to treat EAE in LEW rats. To determine what effect, if any, the various substitutions at position 82 would have on the recruitment of T cells bearing the Vβ8.2 TCR, short-term T cell lines were generated from LEW rats immunized with each analogue and evaluated by flow cytometry. To confirm the dominance of the Vβ8.2 TCR in T cell responses to 73–86, T cells specific for the native 73–86 peptide were also stained with FITC anti-rat Vβ8.2. Fig. 8 shows that >50% of the 73–86-specific T cells express Vβ8.2. A majority of these T cells are also α-4+ and CD25+, demonstrating the activation status of the Vβ8.2+ T cells (Fig. 9). In contrast, when T cells specific for 82D→A, 82D→N, or 82D→E were analyzed, only 2–4% expressed the Vβ8.2 TCR (Fig. 8, B–D). Surprisingly, not even 82D→E, which differs from the wild-type (wt) peptide only by the addition of one methyl group, expanded the Vβ8.2+ T cell population (Fig. 8C). Because of the dramatic effect observed with such a conservative substitution, Vβ8.2 expression was not determined for the D→K and D→Y analogues. T cells specific for each analogue expressed αβ-TCR, α-4, and CD25 at levels equivalent to the 73–86 cells. The results of 82D→A are shown as a representative experiment with the analogue peptides (Fig. 9), and indicate that non-Vβ8.2+ T cells are preferentially expanded by the

![FIGURE 3. Competitive binding of MBP analogues to purified RT1.D1. Inhibition of the binding of 100 nM of biotinylated MBP87–99 to 3 μM of affinity-purified RT1.D1 is shown. Different dose ranges (0–256 μM) of competitor peptide were tested.](http://www.jimmunol.org/)

![FIGURE 4. T cell proliferation to various peptide analogues of MBP73–86. T cells were isolated from pooled lymph nodes of MBP73–86 (A), 73–86(82D→A) (B), 73–86(82D→N) (C), 73–86(82D→E) (D), 73–86(82D→K) (E), and 73–86(82D→Y) (F) peptide-immunized rats (three rats per group) and were cultured with irradiated APCs and peptide (5 μM) for 96 h. Proliferation is measured as incorporation of [3H]thymidine and is presented as cpm.](http://www.jimmunol.org/)
73–86 analogues. In support of this, 73–86-specific T cells that are cross-stimulated in vitro with the 82D→A analogue and then expanded in T cell growth factor (TCGF)-containing medium show a high degree of activation by virtue of CD25 expression but exhibit low Vβ8.2 expression (Figs. 11 and 12).

Previously it has been shown that when 73–86(82D→A) is coimmunized with encephalitogenic 73–86, LEW rats are protected from EAE (1, 2). To confirm this finding, we coimmunized 10 LEW rats with 73–86(82D→A) and 73–86 as described previously (1). Consistent with previous findings, 70% (7 of 10) of the rats were completely protected from EAE (data not shown). To determine Vβ8.2 expression in the coimmunized group, LNCs were harvested from protected rats and stimulated in vitro with either 73–86 alone showed a dominance of the Vβ8.2 TCR (Fig. 10H, Table III). However, when both peptides were included during the stimulation, there was a significant decrease in the percentage of T cells expressing the Vβ8.2 TCR (Fig. 10F, Table III). Both cell populations were predominantly αβ-TCR+ (Fig. 10, B and D) and were activated (CD25+; Fig. 10, E and G). However, the decrease in Vβ8.2 expression was not accompanied by a decrease in cytokine production, as supernatants from cultures in which both peptides were present during the stimulation period showed an additive effect.

### Table II. Cross-reactivity of 82D→A-specific T cells with gp and rat MBP73–86

<table>
<thead>
<tr>
<th>T Cells Activated</th>
<th>In Vitro with (5 µM)</th>
<th>cpm</th>
<th>Stimulation Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>No peptide</td>
<td></td>
<td>752</td>
<td>1</td>
</tr>
<tr>
<td>gpMBP73–86(82D→A)</td>
<td></td>
<td>5467</td>
<td>7.3</td>
</tr>
<tr>
<td>gpMBP73–86</td>
<td></td>
<td>776</td>
<td>1</td>
</tr>
<tr>
<td>rat MBP73–86</td>
<td></td>
<td>795</td>
<td>1</td>
</tr>
</tbody>
</table>

* Draining lymph nodes and spleen from LEW rats (n = 2) immunized with gpMBP73–86(82D→A) were pooled and T cells were isolated as described previously. A total of 5 × 10⁶ T cells were incubated with an equal number of irradiated thymocytes and peptide at 5 µM. Data are presented as cpm; the stimulation index was calculated by dividing the mean cpm of wells containing peptide by the mean cpm of wells containing no peptide.

### References


### Figures

**Figure 5.** T cell proliferation to various peptide analogues of MBP73–86. T cells were isolated from pooled lymph nodes of MBP73–86 (A), 73–86(82D→A) (B), 73–86(82D→N) (C), 73–86(82D→E) (D), 73–86(82D→K) (E), and 73–86(82D→Y) (F) peptide-immunized rats (three rats per group) and were cultured with irradiated APCs and peptide (20 µM) for 96 h.

**Figure 6.** Cytokine secretion by LNCs from 73–86- or analogue-primed rats. LNCs pooled from immunized rats (three rats per group) were stimulated in vitro with the priming peptide (vertical axis) for 72 h; IFN-γ and IL-10 production was measured by rat-specific ELISA.

**Figure 7.** Cytokine secretion by T cells from 73–86-primed rats. Column-enriched 73–86-specific T cells isolated from pooled LNCs of 73–86-immunized rats (n = 3) were stimulated in vitro for 72 h with either wt 73–86 or one of the peptide analogues (vertical axis); IFN-γ and IL-10 production was measured by rat-specific ELISA.
with a significant increase in IL-10 production in the coimmunized group (Table III).

Discussion

Wauben et al. (1) first studied single alanine-substituted analogues of MBP73–86 and determined that inhibition of EAE in LEW rats coimmunized with MBP73–86 plus 82D→A was due to a mechanism(s) other than MHC blockade alone. Their results suggested that TCR antagonism could play a role, although the precise mechanism was not delineated. The present study reveals that the aspartic acid residue at MBP residue 82 is essential for encephalitogenic activity in LEW rats, because no structural modification is tolerated at this position. Thus, even the conservative change from aspartic to glutamic acid (the addition of one methyl group), aspartic acid to alanine (the loss of the carboxyl group), or aspartic acid to asparagine (the change of a carboxyl to an amino group) rendered the analogues nonencephalitogenic.

Each of the analogues tested was an effective competitor of the wt MBP73–86 peptide in binding to detergent-solubilized RT1.B1 molecules, indicating that the aspartic acid residue at position 82 does not play a crucial role in MHC class II binding.

The failure of these analogues to induce EAE was not due to lack of immunogenicity, because proliferative responses were elicited in vitro by lymph node T cells stimulated with the priming peptide. The analogue-primed T cells did not cross-react in vitro with 73–86, the encephalitogenic epitope, as determined by proliferation. When 82D→A T cells were further tested for cross-reactivity by measuring proliferation to rat 73–86, there was also no significant cross-reactivity. This is an important point to address, because the 82D→A analogue has protective effects in vivo.

MBP73–86-primed cells also did not proliferate or secrete IL-10 in response to the analogues. Despite their immunogenicity, the analogues failed to stimulate the clonal expansion of T cells expressing the EAE-associated Vα8.2 TCR, suggesting that aspartic acid at MBP position 82 is a critical TCR contact residue for Vβ8.2+ T cells. Consistent with this possibility is the observation that a small population of 73–86-specific T cells cross-reacts with the 82D→A analogue, and

![FIGURE 8](http://www.jimmunol.org/)

**FIGURE 8.** Expression of the Vβ8.2 TCR by T cells specific for MBP73–86 or analogues of 73–86 LNCs pooled from rats (n = 3) immunized with MBP73–86 were cultured with 20 μg/ml of 73–86 peptide for 72 h. After 3 days of in vitro stimulation followed by 4 days of expansion in IL-2-containing medium, T cells were stained with FITC anti-rat-Vβ8.2 to determine the expression of Vβ8.2 TCR by flow cytometry. FITC anti-DNP was used as an isotype control. 73–86 (A), 73–86(82D→A) (B), 73–86(82D→E) (C), and 73–86(82D→N) (D).

![FIGURE 9](http://www.jimmunol.org/)

**FIGURE 9.** Expression of TCR and activation markers by activated T cells. 73–86-specific T cells express αβ-TCR, α-4, and CD25 (left panels). 82D→A-specific T cells also express αβ-TCR, α-4, and CD25 (right panels).
that these T cells exhibit high CD25 expression but low Vb8.2 expression. This finding helps to explain why 73–86-specific, encephalitogenic T cells cannot be primed with 82D3A in vitro to transfer disease (2). However, this cross-reactivity is not required for protection from EAE, because the 82D3A analogue will protect LEW rats from EAE induced with MBP87–99. Weissert et al. (16) recently compared Vb8.2 T cell activation by gp and rat MBP63–88, which contains the encephalitogenic 73–86 sequence. However, the rat sequence differs at position 78 (our numbering system), where threonine is substituted for serine in rat MBP. Although both peptides were encephalitogenic for LEW rats, only the gp peptide preferentially recruited Vb8.2 T cells (16). Thus, two residues influence Vb8.2 T cell activation, serine at position 78 (16) and aspartic acid at position 82 (this report), although only the latter residue is essential for encephalitogenic activity.

Because the 82D→A analogue has been shown to protect LEW rats from EAE when coimmunized with 73–86, it was of interest to determine Vb8.2 expression in these protected rats. There was a significant decrease in Vb8.2 expression when both the 73–86 and 82D→A peptides were included in culture, despite an additive increase in cytokine production, especially IL-10. Although the levels of IL-10 are relatively low, we cannot exclude a possible contribution of IL-10 in the protection. Additional experiments will determine whether the decrease in Vb8.2 expression is responsible for protection from EAE. Preliminary studies have shown that LEW rats are protected against EAE induced with MBP73–86 by adoptive transfer of LNCs from donors immunized with the nonencephalitogenic 82D→A (data not shown). This explanation would be consistent with the findings of Nicholson et al. (17), who reported that an analogue of proteolipid protein peptide 139–151, in which glutamine was substituted for the native tryptophan TCR contact residue at position 144, was no longer encephalitogenic but protected SJL mice against EAE. The W→Q modification elicited T cells that could transfer protection upon adoptive transfer into naive recipients (17). The results presented here suggest that the mechanism underlying the inhibition of EAE by 82D→A cannot solely be explained by MHC blockade (1). In support of this possibility, we have observed that analogue...
Table III. Cytokine and Vβ8.2 profile of T cells from MBP73–86(82D→A) coimmunized LEW rats activated in vitro

<table>
<thead>
<tr>
<th>T Cells Activated In Vitro with</th>
<th>IFN-γ</th>
<th>IL-10</th>
<th>Vβ8.2 TCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP73–86</td>
<td>4745*</td>
<td>&lt;40</td>
<td>45%</td>
</tr>
<tr>
<td>MBP73–86 + 82D→A (1:1)</td>
<td>8427</td>
<td>410</td>
<td>25%</td>
</tr>
</tbody>
</table>

* Draining lymph nodes from LEW rats (n = 4) coimmunized with 73–86 and 73–86(82D→A) were pooled and prepared as a single-cell suspension; cells were stimulated in vitro for 72 h with either 73–86 peptide or both 73–86 and 73–86(82D→A) peptides at equimolar concentrations. Supernatants were subsequently tested for IFN-γ and IL-10 using cytokine-specific ELISA kits. pg/ml.

References