Introduction

Multiple sclerosis (MS) is an inflammatory disease of the CNS affecting 0.1% of the population and is associated in northern European caucasoid MS patients with the HLA-DR2 (DRB1*1501) haplotype (1–3). The animal model of MS, experimental autoimmune encephalomyelitis (EAE), is a T cell–mediated autoimmune disease that can be induced by subcutaneous injection of peptides derived from myelin components such as myelin basic protein (MBP) (4–6), proteolipid protein (PLP) (7, 8), or myelin oligodendrocyte glycoprotein (MOG) (9). In the course of EAE, autoreactive CD4+ T cells recognize self-antigens presented by murine class II MHC molecules (e.g., H-2s), ultimately leading to pathological changes that can be monitored as clinical signs of disease. EAE provides a well-studied system for testing the efficacy of therapeutic compounds that suppress the disease. These have included treatment with cytokines (10, 11), peptide antigens that induce anergy (12), oral tolerance (13–15), or altered peptide ligands (16–19).

Copolymer 1 (Cop 1, Copaxone [Teva Marion Partners, Kansas City, Missouri, USA]), a random amino acid copolymer of tyrosine (Y), glutamic acid (E), alanine (A), and lysine (K), reduces the frequency of relapses by 30% in relapsing-remitting multiple sclerosis (MS) patients. In the present study, novel random four–amino acid copolymers, whose design was based on the nature of the anchor residues of the immunodominant epitope of myelin basic protein (MBP) 85-99 and of the binding pockets of MS-associated HLA-DR2 (DRB1*1501), have been synthesized by solid-phase chemistry. Poly (Y, F, A, K) (YFAK) inhibited binding of the biotinylated MBP 86-100 epitope to HLA-DR2 molecules more efficiently than did either unlabeled MBP 85-99 or any other copolymer including Cop 1. Moreover, YFAK and poly (F, A, K) (FAK) were much more effective than Cop 1 in inhibition of MBP 85-99–specific HLA-DR2–restricted T cell clones. Most importantly, these novel copolymers suppressed experimental autoimmune encephalomyelitis, induced in the susceptible SJL/J (H-2^s) strain of mice with the encephalitogenic epitope PLP 139-151, more efficiently than did Cop 1. Thus, random synthetic copolymers designed according to the binding motif of the human immunodominant epitope MBP 85-99 and the binding pockets of HLA-DR2 might be more beneficial than Cop 1 in treatment of MS.


Novel synthetic amino acid copolymers that inhibit autoantigen-specific T cell responses and suppress experimental autoimmune encephalomyelitis

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Thus, further investigation of the mechanisms involved, as well as examination of additional copolymers of this type, could potentially result in improved therapeutic reagents. Virtually all of the large variety of copolymers found in the random mixture of poly (Y, E, A, K) (YEAK) bound to purified human HLA-DR1, -DR2, and -DR4 molecules, showing that Cop 1 did indeed bind to purified class II MHC proteins (35). It also competed for binding of MBP 85-99 to HLA-DR2 (DRB1*1501) and inhibited responses of DR2-restricted T cells to MBP 85-99 (34, 35). The binding and inhibition of T cell clones occurred in spite of the fact that the P1 pocket in DR2 is too small to accommodate the hydrophobic residue Y (due to the presence of β86Val rather than β86Gly). However, at high concentration Y may be "forced" into this pocket (36).

Thus, the combination of amino acids Y, E, A, and K as in Cop 1 might not be the best for the potent inhibitory activity of this compound. Therefore, copolymers that took account of the binding motifs of the autoantigenic peptide derived from MBP (MBP 85-99) might be better therapeutic agents than Cop 1. In the present study, several random four–amino acid copolymers — 14-, 35-, and 50-mers with different ratios of amino acids — have been synthesized by the solid-phase method with particular reference to the anchor residues of MBP 85-99 bound to HLA-DR2 (DRB1*1501) (37, 38) in an attempt to improve the effectiveness of the copolymers. Biochemical analysis and effects of the new copolymers on autoantigen-specific T cell responses in MS and on disease progression in the animal model of MS (EAE) are presented here.

Methods

Copolymers, peptides, and proteins. YEAK, VEAK, and FEAK in molar ratios approximating those found in Cop 1 (1:1.5:5:3) were synthesized by the solid-phase method as 14-, 35-, and 50-mers (Mimotopes, Clayton, Australia), by using Fmoc amino acids mixed in the desired ratios at each cycle. YFAK, molar ratio 0.2:0.8:5:3; YFAK, molar ratio 0.8:0.2:5:3; YFAK, molar ratio 0.5:0.5:5:3; and FAK, molar ratio 1:5:3, were synthesized similarly. Three different preparations of the novel copolymers were employed with identical results. Cop 1 (glatiramer acetate, Copaxone [Teva Marion Partners, Kansas City, Missouri, USA]) was obtained from the pharmacy and kindly provided by H.L. Weiner (Center for Neurologic Diseases, Brigham and Women’s Hospital, Boston, Massachusetts, USA). Peptides were synthesized on an Applied Biosystems Peptide Synthesizer (Framingham, Massachusetts, USA) and purified by reverse-phase HPLC. Peptide sequences were: MBP 85-99, ENPVHVFKNIVTPR, molecular weight 1795; PLP 40-60, TGTEKLIYFTSKNYQDYEYI, molecular weight 2603; and PLP 139-151, HSLKGWLGHPDKF, molecular weight 1520; either unlabeled or with biotin linked to the N-terminus by the spacer SGSG and free acid at the C-terminus. MBP 86-100, rather than MBP 85-99, was used for biotinylation because of ease of synthesis. Soluble HLA-DR2 molecules were purified from Drosophila S2 cells as described (39).

Peptide binding to class II MHC proteins. Microtiter immunoassay 96-well plates (FALCON Pro-Bind; Becton Dickinson and Co., Lincoln Park, New Jersey, USA) were coated with 1 µg/well affinity-purified LB3.1 mAb’s in 100 µl PBS for 18 hours at 4°C, as previously described (35). The wells were then blocked with TBS (137 mM sodium chloride, 25 mM Tris [pH 8.0], 2.7 mM potassium chloride)/3% BSA for 1 hour at 37°C and washed three times with TTBS (TBS plus 0.05% Tween-20). Biotinylated MBP 86-100 (final concentration 0.13 µM) in 50 µl of PBS was co-incubated with unlabeled inhibitors (random copolymers or MBP 85-99), and with HLA-DR2 molecules, for 40 hours at 37°C and subsequently transferred to the 96-well plates treated as described above. Following 1 hour of incubation at 37°C, plates were washed three times with TTBS and incubated with 100 µl of streptavidin-conjugated alkaline phosphatase (diluted 1:3,000 in TTBS; Bio-Rad Laboratories Inc., Richmond, California, USA) for 1 hour at 37°C, followed by addition of 100 µl p-nitrophenyl phosphate in triethanolamine buffer (Bio-Rad Laboratories Inc.). The absorbance at 410 nm was monitored by a microplate reader (model MR4000; Dynatech Laboratories, Chantilly, Virginia, USA).

Antigen presentation assays. HLA-DR2–restricted T cells were MBP 84-102–specific (or, more exactly, MBP 85-99–specific) transfectants using T cell receptors (TCRs) obtained from patients with relapsing-remitting MS carrying DR2 [clone 8073, from patient Ob (DRB1*1501), and clone Hy1B, from patient Hy (DRB1*1602)], transfected into murine BW 58 TCR α/β cells. MBP 84-102–specific (2E12) and PLP 40-60–specific (106A) hybridomas from HLA-DR2 transgenic mice (6) were also used. Mouse T cell hybridomas were PLP 139-151–specific H-2k–restricted (hPLP/1 and hPLP/c4; ref. 40). APCs were L466 [L cells transfected with HLA-DR2 (DRB1*1501), L416 [L cells transfected with HLA-DR2a (DRB5*0101)], MGAR (Epstein-Barr virus–transformed B cells homozygous for DRB1*1501), and splenocytes from SJL/J (H-2s) mice. T cell stimulation experiments were performed in a total volume of 200 µl in 96-well microtiter plates. Irradiated (30 Gy) APCs (2.5 × 104 per well) were co-incubated with MBP 85-99, PLP 40-60, or PLP 139-151 and the random copolymers at concentrations indicated in Results, for 2 hours at 37°C; then T cells (5 × 104 per well) were added and incubated for 24 hours at 37°C. Supernatants (30 µl) were taken and incubated with 1450 MicroBeta Plus liquid scintillation counter (Perkin Elmer Wallac Inc., Gaithersburg, Maryland, USA).

Mice. SJL/J (H-2s) female mice (8–12 weeks of age) were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA) and maintained in the animal environment.
facility at Harvard University according to the Guidelines of the Committee on Animals of Harvard University and the Committee on Care and Use of Laboratory Animal Resources, National Research Council (Department of Health and Human Services Publication 85-23, revised 1987).

Induction and suppression of EAE. Mice were injected subcutaneously in both the base of the tail and the nape of the neck with either mouse whole spinal cord homogenate (WSCH, 500 μg/mouse) or PLP 139-151 peptide (50 μg/mouse) together with 400 μg Mycobacterium tuberculosis H37Ra (BD Diagnostic Systems, Sparks, Maryland, USA) in an emulsion containing equal parts of PBS and CFA (Sigma-Aldrich). Pertussis toxin (List Biological Laboratories Inc., Campbell, California, USA; 200 ng) was injected intravenously into the tail a day after immunization. Mice were scored daily for clinical signs of EAE on a scale from 1 to 5 according to the severity of the disease as previously described (40, 41). For suppression of EAE, different copolymers (500 μg/mouse) were injected together with the encephalitogenic emulsion as described above. Mice were evaluated in a blinded fashion. Maximum clinical score and mean day of onset were calculated as described in ref. 40.

Proliferation of lymph node primary cultures. Mice were immunized subcutaneously together with different copolymers emulsified in CFA, as described above, except that no pertussis toxin was administered intravenously. Lymph nodes and spleens were taken 13–16 days after immunization. Irradiated splenocytes (12 Gy, 5 × 10⁵ cells per well) were incubated in 96-well round-bottom plates together with PLP 139-151 or copolymers at concentrations indicated in Results, for 2 hours at 37°C, followed by addition of lymph node cells (5 × 10⁴ cells per well) in DMEM/10% FCS. For T cell proliferation, ³H-thymidine (1 μCi/well) was added to the duplicate set of cultures after 72 hours, and the plates were harvested and radioactivity monitored using a 1450 MicroBeta Plus liquid scintillation counter (Perkin Elmer Wallac Inc.) after 96 hours.

Neuropathology. For assessment of inflammation and demyelination, mice were perfused under anesthesia through the ascending aorta with 40 ml of Trump’s fixative (4% paraformaldehyde, 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4). Slices of the brain and spinal cord were postfixed in cold 1% osmium tetroxide for 1 hour, dehydrated through a graded series of ethanol, and embedded in epoxy resin. One-micrometer sections were stained with toluidine blue and examined by light microscopy.

Results
Preliminary synthesis and evaluation of novel copolymers VEAK and FEAK. Initially, two new amino acid copolymers were synthesized to provide a better residue for binding in the P1 pocket of HLA-DR2 than is available in Cop 1. Since this P1 pocket is small in HLA-DR2 (DRB1*1501), V, the residue of MBP 85-99 found at P1, and F, the largest hydrophobic amino acid that should fit in the P1 pocket of this HLA protein, were used instead of the Y present in Cop 1; i.e., VEAK and FEAK were synthesized. In addition, YEAK itself was synthesized by the solid-phase method to establish that this material has the same effectiveness as Cop 1 synthesized by solution chemistry. Various sizes of each copolymer, 14-mers, 35-mers and 50-mers, were synthesized. These copolymers were examined in three ways: (a) binding to “empty” HLA-DR2 (DRB1*1501) synthesized in a baculovirus system, (b) inhibition of the four MBP 85-99– or PLP 40-60–specific HLA-DR2–restricted T cell clones, as well as the two PLP 139-151–specific H-2s–restricted T cell hybridomas, and finally (c) ability to suppress EAE induced by either PLP 139-151 or WSCH in H-2s mice. The following results were obtained:
(a) In the binding experiment, VEAK and FEAK were less effective than Cop 1 or YEAK in competing for binding of biotinylated MBP 86-100 (data not shown); (b) When tested as inhibitors of MBP 85-99– or PLP 40-60–induced stimulation of the four HLA-DR2–restricted T cell clones or hybridomas, VEAK was substantially less effective than Cop 1 or YEAK in inhibition of proliferation, while FEAK was equivalent to Cop 1 using two of these clones and slightly less effective in the other two cases. Using the two PLP 139-151–specific H-2s–restricted T cell hybridomas, again FEAK was approximately equivalent to Cop 1 or YEAK, while VEAK was half as effective. In both the binding and the inhibition of T cell proliferation experiments, the 50-mers of all of the copolymers used were much more effective than the 35-mers or the 14-mers (data not shown).

<table>
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<tr>
<th>Copolymer</th>
<th>Incidence</th>
<th>Percent disease</th>
<th>Percent mortality</th>
<th>Maximal mean score</th>
<th>Mean day of onset</th>
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</table>

See Methods for details. Three identical experiments with Cop 1 and the 50-mers were combined. The 35-mers were used in only two of these.

Table 1
Suppression of EAE induced by WSCH in SJL/J mice by novel random copolymers VEAK and FEAK.
VEAK and Cop 1 were equally effective in partially reducing the severity of EAE induced by PLP 139-151 in H-2s mice, while FEAK completely prevented the appearance of disease, except for a few mice who developed a transient +1 score (limp tail). In immunohistology, brain sections of control animals and VEAK-treated animals showed substantial demyelination, while animals treated with Cop 1, YEAK, or FEAK were completely normal (data not shown). WSCH was also used to induce the disease, in which case a milder disease was produced, perhaps more comparable to MS. The mild disease persisted in some animals treated with either Cop 1 or YEAK and in an even larger number of animals treated with VEAK. However, no disease was detected in any animals treated with FEAK (Table 1).

Characterization of the additional novel random copolymers YFAK and FAK and their binding to HLA-DR2 molecules. Based on the initial results above, random four- and three- amino acid copolymers YFAK (at the molar Y/F ratios of 0.2:0.8, 0.5:0.5, and 0.8:0.2) and FAK were synthesized as 50-mers by the solid-phase method. F was substituted for E because: (a) E seemed unnecessary. Moreover, the P1 pocket of DRB1*1501 includes β86Val, resulting in a small pocket that can accommodate F but for which Y is too large to be accommodated (except at high peptide concentration; ref. 36). (b) The residue occurring at P4 in MBP 85-99 is F, although Y would provide a better fit (see Discussion). Amino acid analysis, reverse-phase HPLC separation, and microsequencing of the novel compounds showed that the solid-phase method yielded substances similar in amino acid composition, distribution, hydrophobicity, and size to Cop 1 that had been generated previously by solution chemistry (data not shown). To determine whether novel copolymers synthesized by the solid-phase method competed with the autoantigenic MS-associated epitope MBP 85-99 for binding to HLA-DR2, competitive binding assays were carried out with biotinylated MBP 86-100 and unlabeled random copolymers (see Methods for details). All of the YFAK and FAK 50-mers were equally effective and equivalent to or better than Cop 1 in binding to HLA-DR2 (Figure 1).

**Figure 1**
Inhibition of binding of biotinylated MBP 86-100 to HLA-DR2 molecules by different competitors. Recombinant water-soluble HLA-DR2 molecules were incubated with biotinylated MBP 86-100 (0.13 μM) and the unlabeled random copolymers at a range of concentrations. All incubations were carried out in duplicate at pH 7.0 for 40 hours at 37°C. Results represent one of three independent experiments. Specific binding is expressed as percentage of inhibition using the formula: percentage of inhibition = 100% - [(absorbance at 410 nm with competitor - background)/absorbance without competitor - background] × 100]. The signals at 410 nm without competitor were 0.8–1.0, and the background was 0.1.

(c) VEAK and Cop 1 were equally effective in partially reducing the severity of EAE induced by PLP 139-151 in H-2s mice, while FEAK completely prevented the appearance of disease, except for a few mice who developed a transient +1 score (limp tail). In immunohistology, brain sections of control animals and VEAK-treated animals showed substantial demyelination, while animals treated with Cop 1, YEAK, or FEAK were completely normal (data not shown). WSCH was also used to induce the disease, in which case a milder disease was produced, perhaps more comparable to MS. The mild disease persisted in some animals treated with either Cop 1 or YEAK and in an even larger number of animals treated with VEAK. However, no disease was detected in any animals treated with FEAK (Table 1).

**Figure 2**
Inhibition of HLA-DR2-restricted MBP 84-102-specific T cells 2E12, 8073, and Hy1B in the presence of the random copolymers. Irradiated MGAR cells were co-incubated in duplicate with the MBP 85-99 at the final concentration of 12.5 μM and different concentrations of the random copolymers for 2 hours at 37°C; then T cells were added and incubated for 24 hours at 37°C. Supernatants (30 μl) were incubated with IL-2–dependent cytotoxic T lymphoma line cells, followed by labeling with 3H-thymidine (1 μCi/well) for 12 hours. Other details were as described in Methods.
[L cell transfectant that expresses DR2a (DRB5*0101)] was used, no response was detected, confirming that all the T cell clones were restricted to the DR2b (DRB1*1501) allele (data not shown). Therefore, MGAR or L466 cells were subsequently used in the antigen presentation assays described below. The inhibition of proliferation of three different MBP 85-99–specific, DR2b-restricted T cell clones was examined in the presence of different copolymers. Two independent experiments are presented in Figure 2. For each MBP-specific HLA-DR2–restricted clone, YFAK 0.2:0.8, YFAK 0.5:0.5, and FAK were better inhibitors than YFAK 0.8:0.2 at lower concentrations of the inhibitors, with YFAK 0.2:0.8 being most effective. In any case, all these copolymers inhibited the MBP-specific HLA-DR2–restricted T cell response more efficiently than did Cop 1.

Treatment of EAE induced by PLP 139-151 epitope. To find out whether the novel random copolymers affected chronic-relapsing EAE, in vivo experiments were carried out by immunizing SJL/J (H-2s) mice subcutaneously with 50 µg of PLP 139-151 (the encephalitogenic epitope in the SJL/J strain) and 500 µg copolymer. Following disease induction, mice were observed daily for the appearance of typical signs of EAE during a period of 70 days. Immunization with the PLP 139-151 epitope alone in CFA resulted in EAE with far more severe clinical signs compared with WSCH-induced EAE. Thus, all 13 mice developed severe EAE, with a mortality of 77%. The first signs appeared around day 11, followed by subsequent fluctuation in disease attacks, with a maximal mean score of 4.6 (Figure 3; Table 2). Co-injection with the random copolymers differentially reduced the clinical signs of EAE. In the YFAK 0.2:0.8–treated group, only 2 out of 16 mice showed clinical signs of EAE (mortality, 6%), with a delay in the first attack to around day 37 (maximal mean score, 0.6; Figure 3; Table 2). Similarly, in the YFAK 0.5:0.5–treated group, there was one sick mouse out of 16 (mortality, 0%), with the first attack developing on day 33. In contrast, 8 out of 17 mice treated with YFAK 0.8:0.2 developed EAE with no mortality. In this group, the maximal mean score (1.5) was higher than with the other YFAKs and the onset was earlier (day 27). Treatment with FAK resulted in 3 sick mice out of 17, with 12% mortality, maximal mean score of 0.9, and the mean onset at day 25 (Figure 3; Table 2). However, when Cop 1 was co-injected with PLP 139-151, 12 out of 16 mice developed EAE, with the mean onset around day 22 and maximal mean score of 2.6. Figure 4 is a representative experiment.

### Table 2

<table>
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<tr>
<th>Copolymer</th>
<th>Incidence</th>
<th>Percent disease</th>
<th>Percent mortality</th>
<th>Maximal mean score</th>
<th>Mean day of onset</th>
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<td>33.0 ± 0</td>
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<tr>
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<td>22.7 ± 7.1</td>
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</table>

See Methods for details. Three identical experiments were combined.
showing progression of the disease in each individual mouse out of the group treated with a specific copolymer during the 67-day period. In summary, random copolymers YFAK 0.2:0.8 or 0.5:0.5 and FAK were much more effective than Cop 1 in suppression of EAE. None of the mice treated with YFAK 0.2:0.8 or 0.5:0.5 developed significant disease.

Discussion

A major goal in the treatment of autoimmune diseases has been the development of antigen-specific immunomodulating therapies that interfere with the trimolecular interaction of the autoreactive TCR with the autoantigenic peptides presented by self MHC receptors at the surface of APCs. These immunotherapies of T cell–mediated autoimmune diseases have been successful in animal models with known target antigens (10–19, 42–45). One of the strategies, namely the use of altered peptide ligands, has been used both to treat EAE (12, 17, 19, 46) and recently to treat MS (47, 48), although the results have been contradictory. Cop 1, which was approved as a therapy for relapsing-remitting MS, was proposed to act as a promiscuous binder to class II MHC molecules (29, 35), as an antagonist of the TCR (49), and/or as an inducer of suppressor cells (25, 32, 33). Although Cop 1 is currently in wide use, and a recent clinical study demonstrated its sustained efficacy in MS patients over a period of 6 years (50), it has only a modest effect on the course of disease, similar to other immunomodulatory drugs such as steroids or IFN-β. Development of novel compounds based on accumulated knowledge could thus result in improved
I-A\(s\) (the homologue of human HLA-DQ protein) has also been employed in the present study. The structure of these copolymers was mainly based on the residues of the immunodominant T cell epitope MBP 85-99 interacting with the MS-associated HLA-DR2 (DRB1*1501) molecule. Interestingly, the size of the copolymers was crucial for activity, with the 50-mers being most efficient. In line with these data, previous study showed that 14-mer fragments of Cop 1, obtained by chymotrypsin digestion, bound poorly to the APC of different haplotypes (51), suggesting that longer polypeptides or multiple promiscuous epitopes are required for the higher affinity binding of this compound. Indeed, aggregation of class II MHC molecules on the surface of the APC upon Cop 1 binding was previously detected (52), suggesting that random polypeptides with an average of about 50–70 amino acids may be able to link adjacent class II molecules. Oligomerized T cell epitopes may cross-link HLA-DR molecules and thus enhance T cell responses (53).

The novel copolymers employed in the present study were optimized for binding to HLA-DR2. However, the murine model used for the in vivo studies was the SJL/J mouse. This strain lacks expression of I-E (the murine homologue of human HLA-DQ) and expresses only the I-As class II MHC molecule. However, this mouse model has previously been used to evaluate efficacy of Cop 1 prior to its introduction for the treatment of MS (31). That study demonstrated that data from this murine model were relevant to the treatment of the human disease, and it was therefore employed in the present study. The structure of I-A\(s\) (the homologue of human HLA-DQ protein) has never been resolved, and it remains for future work to determine whether Cop 1 and the novel copolymers described here bind to I-A\(s\) in a manner similar to that of their binding to HLA-DR2. Earlier studies had indeed shown that Cop 1 binds promiscuously to living APCs of mouse origin (29), including SJL/J strain, suggesting that it interacts with the I-A\(s\) molecules.

Among different random copolymers synthesized and examined in this study, YFAK and FEAK, but not VEAK, were most effective. Tissue samples from the lumbar cord of diseased mice injected with WSCH only or with WSCH and the 50-mer of the ineffective copolymer VEAK showed extensive submeningeal, perivascular, and parenchymal infiltration, as well as demyelination. In contrast, no infiltration or demyelination was detected in samples from mice who developed no signs of disease after treatment with the other copolymers (data not shown).

Importantly, the combination of V, E, A, and K, which appeared in some of the synthetic peptide 15-mers designed according to binding motifs of the MBP 85-99 epitope, resulted in a low-affinity binding to HLA-DR2 molecules and low levels of inhibition of HLA-DR2-restricted MBP 85-99-specific T cells (34). This observation is in contrast to the finding that, in the MBP 85-99/HLA-DR2 complex, V is the anchor residue at position 89 of the peptide interacting with [B8]Val in the P1 pocket of the HLA-DR2 protein (38). However, the substitution of F for V89 in MBP 85-99 resulted in a peptide that could bind to HLA-DR2 molecules (37) and may explain the higher inhibition by FEAK than by VEAK of the HLA-DR2-restricted MBP 85-99-specific T cells. F can fit in the P1 pocket, but it also fits in the P4 pocket, as found at F92 in MBP 85-99. Thus, because FEAK binds in both pockets, it is a better binding copolymer. Although Y would make a tighter fit in the P4 pocket, it is too large for the P1 pocket (and hence the lower efficacy of Cop 1, VEAK). The P4 pocket of DRB1*1501 is very large and can also accommodate the largest amino acid, W (37). The large size and hydrophobicity of this pocket are due to the unusual presence of the small B71 Ala, while virtually all other DR proteins have large charged residues at this position (e.g., K in DRB1*0401) (38). Thus, the combination of F at P1 and Y at P4 would stabilize the complex with DR2 (DRB1*1501) and may account for the enhanced efficacy of YFAKs, among which YFAK with Y/F ratios of 0.5:0.5 and 0.2:0.8 appeared to be the most efficacious. It should be noted that YFAK 0.8:0.2 was the best inhibitor of the MBP 85-99 binding to purified HLA-DR2 molecules, and yet, in the antigen presentation assays, it was less effective than YFAK 0.2:0.8. While binding to purified proteins involves interactions of amino acids in the groove of HLA-DR and the anchor residues of the peptide with no contribution from other components, in the whole-cell assay the binding of the antigen to the MHC molecules on the surface of APCs and the interaction with the TCR might be influenced by the actual mode of presentation of the processed epitope to the T cell. Thus, the lower binding capacity of YFAK 0.2:0.8 might have generated a better T cell epitope.

The K residues in both YFAK and FEAK are certainly important for the interaction with the TCR, as is the K at position 93 of MBP 85-99 (37, 38). On the other hand, K located near the N-terminus of the copolymer in the binding site may contribute to stable interactions with the HLA-DR molecules and the TCR, similarly to residue K307 at P-1 of HA 306-318 bound to HLA-DR1, which can interact with the side chains of G1 helix residues at Stg53 or Eg55 (54). The A residue in these copolymers functions simply as a spacer for Y, F, and K.

Of particular importance is the observation that 50-mers of YFAK suppressed EAE induced by the PLP 139-151 peptide more efficiently than did Cop 1. Previously, Cop 1 was shown to inhibit EAE induced by either WSCH or the synthetic PLP peptides and to interfere with PLP-specific T cell responses only when mice were coimmunized with both antigens (31), suggesting that copolymers compete for binding to class II MHC molecules. The mechanism of activity of the novel random copolymers might be similar to that of Cop 1. One possibility is that they lead to the inhibition of binding of the
potential autoantigenic peptides to class II MHC proteins and subsequent T cell suppression, although alternatives have also been suggested (32, 33, 49). Detailed studies of their mechanism(s) — whether by blockade, by induction of T cell anergy, by induction of regulatory T cells, or by a combination of these — have been initiated.

Thus, in the present work, several novel random amino acid copolymers were shown to be more potent inhibitors of the autoantigen-specific immune response than Cop 1 both in vitro and in vivo. They appear to be candidates for therapeutic trial in MS.

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Note added in proof

Cop 1 has recently been shown to be more effective in HLA-DR2 (DRB1*1501)–positive than in HLA-DR2–negative MS patients (55).


