Peptides Based on MHC-TCR Binding Motifs

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Immunomodulation of Experimental Autoimmune Encephalomyelitis with Ordered Peptides Based on MHC-TCR Binding Motifs


T cell-mediated destruction of the myelin sheath causes inflammatory damage of the CNS in multiple sclerosis (MS). The major T and B cell responses in MS patients who are HLA-DR2 (about two-thirds of MS patients) react to a region between residues 84 and 103 of myelin basic protein (MBP). The crystal structure of HLA-DR2 complexed with myelin basic protein (MBP) confirmed that Lys91 is the major TCR contact site, whereas Phe90 is a major anchor to MHC and binds the hydrophobic P4 pocket. We have identified a repetitive tetrapeptide sequence, EYYKEYYKEYYK, that can reverse paralysis and reduce proinflammatory cytokine production in the Lewis rat. One such sequence, EYYKEYYKEYYK, ameliorates experimental autoimmune encephalomyelitis in Lewis rats, an animal model of MS. This suggests that repetitive tetrapeptide sequences may have therapeutic potential in the treatment of autoimmune diseases.

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Materials and Methods

Animals
Female Lewis rats (6–8 wk old) were purchased from Harlan Sprague Dawley (Indianapolis, IN).

Peptides
For immunization and disease reversal, peptides were synthesized on a peptide synthesizer (model 9050; MilliGen, Burlington, MA) by standard 9-fluorenylmethoxycarbonyl chemistry. Peptides were purified by HPLC. Structure was confirmed by amino acid analysis and mass spectrometry. Peptides used for the experiments were: ENPVVHHFKNIYTPR (MBPp85-99), ENPVVHHFKNIYTPR (MBPp85-99 K→A), EYYKEYYKEYYK (EYYK), KYYKYYKYYKYYK (KYY), and AYEYAKEYAYEK (AYEY).

Peptide-MHC binding assay
Peptide binding to class II molecules was measured as described before (8) with some modifications. Briefly, APCs were purified from spleen cells by negative selection using magnetic beads (Dynal, Oslo, Norway) conjugated with Abs specific for T cells (CD32), macrophages (HIS36), and NK cells (NKR-P1A) (BD PharMingen, San Diego, CA). After selection, cells were plated at a concentration of 0.5 × 10^6 cells/well in flat-bottom 96-well microtiter plates (Corning Costar, Corning, NY). EYYK and KYY peptides were added to the wells at different concentrations (ranging from 0.01 to 0.80 mM). Additional data points were not included because the reaction saturated at concentrations above 80 μM.

Computer modeling
The structural modeling of the peptide-MHC complexes was performed using Insight II (Accelrys, San Diego, CA). The crystal structure of HLA-DR2 complexed with a peptide from human MBP was used as a template for all studies. Substitutions in the native MBP sequence were done using the residue replace function of InsightII. The backbone and side chain torsion angles of the original residues were retained. Steric clashes were avoided. A RasMol-readable script was then exported from InsightII and imported into RasMol for rapid imaging.

Alignment of human and rat MHC class II
Homology analysis was done using ClustalW with a Blosum matrix (12). The protein sequences are identified in GenBank as: 60497 for RT1.Dl and 1BX2B for HLA-DR2. The ClustalW output was formatted in BoxShade for presentation.

EAE induction
Guinea pig spinal cord homogenate (gSCH) or synthetic MBPp85-99 was dissolved in 1 M PBS to a final concentration of 5 mg/ml or 2 mg/ml, respectively, and emulsified with an equal volume of IFA supplemented with 4 mg/ml heat-killed Mycobacterium tuberculosis H37Ra (Difco, Detroit, MI). Rats were injected s.c. with 0.1 ml of the peptide emulsion. Experimental animals were assessed for signs of clinical disease as follows: 0, no clinical disease; 1, tail weakness or paralysis; 2, hind limb weakness; 3, hind limb paralysis; 4, forelimb weakness or paralysis; and 5, moribund or dead animal.

Peptide treatment
For disease prevention, peptides were emulsified in IFA to a final concentration of 1 mg/ml. A total of 0.1 ml was injected intradermally, once on day −20, and again on day −10. Animals were challenged for EAE with gSCH at day 0. For treatment of ongoing disease, EAE was induced with a 0.1-mg emulsion of MBPp85-99 in CFA. On the first day of acute clinical disease, rats were injected i.p. with a dose of 0.5 mg peptide (EYYK or KYY) in 1 ml 1 M PBS.

Results
The ordered peptide EYYKEYYKEYYK inhibits MBPp85-99 binding to rat MHC class II molecules
A competition assay was performed to test whether the ordered peptide EYYK inhibits the binding of MBPp85-99 to the MHC class II molecule. The ordered peptides EYYK and KYY were incubated with APCs and allowed to bind the MHC cleft. After 1 h of incubation, biotinylated MBPp85-99 was added and incubated for 4 h more. The APCs were analyzed by flow cytometry to determine the amount of biotinylated MBPp85-99 bound to MHC class II. As shown in Fig. 1, the ordered peptide EYYK is capable of blocking MBPp85-99 from the Ag-binding cleft of MHC class II. The IC_{50} value of EYYK calculated from a linear regression curve was 83.0 μM.

Molecular modeling of binding interactions
Because EYYK was capable of displacing MBPp85-99, we created a hypothetical model of the bound structure of EYYK in the HLA-DR2 peptide-binding groove. The previously published crystal structure of HLA-DR2 complexed with MBPp85-99 was used as a template. The sequence of MBP residues His^90, Phe^91, and Phe^92 were changed to Gla^90, Tyr^91, and Tyr^92; Lys^93 remained the same (Fig. 2). Although energy minimization or interatomic contacts were not calculated, obvious steric clashes and chemical incompatibilities were avoided.

In our model, residues 90, 91, and 93 project out of the cleft and are implicated in TCR recognition. The glutamate that replaces His^90 carries a negative charge and may block TCR signaling by electrostatic repulsion. The addition of a polar hydroxyl group onto a hydrophobic TCR contact residue (Phe^92→Tyr) could also be deleterious to TCR engagement. In our model, the Phe^92→Tyr change results in the submergence of the aromatic ring into the cleft (Fig. 3). In the native peptide, Phe^92 is important in anchoring the peptide to the P4 hydrophobic pocket of the MHC class II molecule. However, the P4 pocket is not entirely hydrophobic, and several residues could form hydrophilic interactions with the polar tyrosine hydroxyl of EYYK. Hydrophilic residues that could potentially form hydrogen bonds with tyrosine in the EYYK peptide are shown in Fig. 4. These residues may stabilize the binding of EYYK in the MHC cleft, helping it block native MBPp85-99.

The residues derived from the crystal structure are based on a human HLA-DR molecule (2). Because we conducted our experiments in Lewis rats and the residues are not identical, we tested the homology of the two sequences. The rat (RT1-D^I) and human MHC class II (DRB1*1501) molecules were aligned and analyzed to determine whether peptide-MHC interactions are conserved. The result of this alignment is shown in Fig. 5. In humans, the peptide KYY which is in the DRB1*1501 molecule, is replaced by the ordered peptide EYYK. Because of the similarity between the two sequences, the peptide configuration in human MHC class II is predicted to be similar to that observed in our model.
residues lining the P4 pocket are Arg^{13}, Phe^{26}, Asp^{28}, Gln^{70}, Ala^{71}, and Tyr^{78}. In rats, these residues are Gly, Leu, Ala, Gln, Leu, and Ala, respectively. The presence of the large aromatic residues in the human molecule may decrease the size of the P4 pocket. However, the HLA-DR2 P4 pocket can accommodate a phenylalanine residue, so size is most likely not a factor. Based on our modeling, the hydrophilic residues in the predominantly hydrophobic P4 binding pocket may anchor EYYK to the MHC.

Hydrophilic residues that could stabilize EYYK in the MHC cleft are found in both molecules. The crystal structure of HLA-DR2 with MBP\textsubscript{85-99} showed that Gln\textsuperscript{70} is positioned over the P4 pocket (2). Gln\textsuperscript{70} is conserved in RT1.D\textsuperscript{l}, making it capable of hydrophilic interactions with EYYK.

The EYYK ordered peptide inhibits the development of EAE

We tested the potential of the predicted peptide sequences to prevent or to revert the development of EAE in Lewis rats. Induction of EAE in Lewis rats can be achieved by immunization with SCH, whole MBP, or MBPps. Responses to MBP\textsubscript{88-88} are restricted by the RT1.B MHC class II allele, and responses to MBP\textsubscript{85-99} are restricted by the RT1.D allele (13). It has been demonstrated that autoimmune responses to Ags containing more than one pathogenic epitope can be controlled by the previous exposure of the immune system to an APL derived from one of those epitopes (14, 15). To measure the degree of immunomodulation by the ordered peptide EYYK on EAE induced by gpSCH known to contain the dominant epitope MBP\textsubscript{72-88}, the experimental animals were injected on days 0 and 10 intradermally with a 0.1-mg emulsion of 0.1 mg ordered peptide in IFA. Ten days after the last injection, rats were immunized with an emulsion of 0.5 mg gpSCH in CFA. As seen in Fig. 6, injection of EYYK in IFA has a protective effect when EAE is induced with an emulsion of gpSCH and CFA, as compared with animals immunized with KYY peptide or IFA alone ($p = 0.052$).

![FIGURE 2](http://www.jimmunol.org/images/figure2.png)

**FIGURE 2.** Stick models of the major contact residues of (A) MBP\textsubscript{85-99} and (B) EYYK with HLA-DR2. The MBP\textsubscript{85-99} contains a positively charged amino acid at the 90th position (histidine), whereas EYYK consists of a negative charge (glutamic acid). Both phenylalanines have been modified to tyrosines. The lysine residue remains unchanged.

![FIGURE 3](http://www.jimmunol.org/images/figure3.png)

**FIGURE 3.** A molecular model of the binding region of (A) MBP\textsubscript{85-99} in the cleft of HLA-DR2 and (B) the modified EYYK peptide with HLA-DR2. The peptides are shown as stick models; the MHC class II molecule is a rendered surface. The charged residues, His\textsuperscript{90} in MBP\textsubscript{85-99} and Glu in EYYK, stick out of the cleft and are implicated in involvement with the TCR. The second tyrosine of EYYK is embedded in the cleft, potentially interacting with hydrophilic residues of HLA-DR2 and RT1.D\textsuperscript{l}. Gln\textsuperscript{70} of the MHC $\beta$-chain is positioned over the embedded tyrosine and may contribute to binding via hydrophilic interactions.
We then tested whether this inhibition was specific for a blockade of anti-MBPp85–99 responses. In MBPp85–99-immunized Lewis rats, we delivered a single dose of EYYK (a solution of 0.5 mg peptide in 1 ml 1 M PBS i.p.) on the day when the first signs of clinical disease became apparent. As seen in Fig. 7, administration of EYYK suppresses the development of clinical disease in experimental animals when compared with KYY- or PBS-treated animals.

Discussion

In this report, we used MHC class II binding motifs of MBP to design optimized analogs containing repetitive peptide sequences that prevent and suppress EAE in Lewis rats.

One currently approved drug for MS therapy is a random synthetic amino acid copolymer, GA (poly Tyr, Glu, Ala, and Lys) (16). Glatiramer suppresses EAE in rodents, slows the progression of disability, and reduces the relapse rate in MS. The mechanism of action is likely MHC blocking and TCR antagonism of MBPp82–100-specific T cells (16). Binding-motif analyses of glatiramer after elution from HLA-DR molecules showed tyrosine at the first anchor position followed by alanine in the subsequent pockets (17). Although glatiramer is a random sequence of amino acids and each batch has considerable variability, Fridkis-Hareli et al. (17) have suggested that substitutions of valine or phenylalanine for tyrosine may improve glatiramer’s efficacy for binding to the P1 pocket of HLA molecules. APLs, in contrast to glatiramer, have a defined sequence and change autoimmune responses in part by altering cytokine production in autoreactive T cells (18–20). Previously, an APL of MBPp85–99 was designed based on TCR-MHC contact residues (21). Administration of MBPp87–99 (Lys91→Ala), an APL in which the primary TCR contact residue is changed, reversed EAE induced paralysis in the Lewis rat and reduced production of the proinflammatory cytokine TNF-α (8). In a recent report, the minimal structural requirements for a peptide to tolerize animals with ongoing EAE were determined (22). Using a panel of truncated variants of the MBPp87–99, a 7-aa peptide, FKNIVPT, was found to induce remission of EAE in the Lewis rat model. These results indicate that only one MHC anchor and one TCR binding site are essential for initiation of Ag-specific T cell tolerance (22). However, these peptides closely resemble the original self peptide and have the potential to induce cross-reactive adverse side effects when used for therapeutic purposes.

The peptides we designed contain repetitive sequences of three (KYY) or four (EYYK and AYEK) amino acids and resemble the

![FIGURE 4](image-url). Modified crystal structure of EYYK in the binding cleft of HLA-DR2. All residues are shown as stick models. The binding residues of EYYK are yellow, and those of HLA-DR2 are blue. Residues colored red represent hydrophilic residues of HLA-DR2 in close proximity with the tyrosine of EYYK. These residues could stabilize the polar head group of tyrosine through hydrophilic interactions and/or hydrogen bonding. Glu76 is conserved in both rat RT1.D1 and human HLA-DR2.

![FIGURE 5](image-url). Sequence alignment of HLA-DR2 (DRB1*1501) β-chain and rat RT1.D1 β-chain. Identical residues are shaded black; similar residues are shaded gray. Residues indicated by an asterisk comprise the P4 binding pocket of HLA-DR2. The human P4 pocket consists of amino acids with larger R groups, but is capable of accommodating a phenylalanine inside of it.

![FIGURE 6](image-url). Ordered peptide EYYKEYYKEYYK prevents the induction of EAE in Lewis rats. Animals were injected with a 0.1-mg emulsion of EYYKEYYKEYYK (●), KYYKYYKYYKYY (●), or PBS (○) in IFA at day −20 and again at day −10. At day 0, the animals were challenged with gpSCH in CFA. Results are expressed as mean disease score of a group of six rats.
MBPp85–99 core motif. The ordered peptide EYYKEYYKEYYK is effective in preventing and reverting the development of EAE in the Lewis rat. Although prevention of disease may be related to immune deviation, the abrogation of disease progression by the ordered peptide is correlated in vitro with competitive binding of the peptide to the rat class II MHC. Computer modeling reveals that the substitution of His90 by Glu may be an important alteration from the original HFFK TCR-MHC motif. The presence of the glutamate residue may affect the stability of TCR binding, whereas the two tyrosines and the lysine may resemble the original FFK structure.

Previously, MBPp96–100 was oligomerized into a 16-mer. This oligomerized peptide treated ongoing disease and prevented disease when administered before EAE induction. Interestingly, the peptide also induced EAE in traditionally resistant strains (23). In this study, we have minimized the number of amino acids required to treat and prevent disease. In addition, we have created a peptide that is not homologous to MBP and does not induce EAE or cause B or T cell cross-reactivity to MBP (data not shown). EYYK and AYEK are tetrad repeats, similar to the native HFFK structure. AYEK, a similar tetrad structure, resulted in partial abrogation of EAE (data not shown). Unlike AYEK or EYYK, KYY is a triplet repeat and contains fewer HFFK-analogous binding regions (Fig. 8). KYY did not bind MHC or prevent disease. This observation suggests that there is a minimum of four residues per repeat required for efficient APL binding.

The oligomerized MBPp96–100 improved the response of low avidity T cell clones (23). The repetitive structure may effectively raise the local concentration of peptide. If the tyrosine residues of EYYK are structurally predisposed to bind in pockets P3 and P4 of the rat MHC class II, then EYYK contains three potential binding sites. When one block of EYYK releases, two others are immediately adjacent to bind MHC. This may raise the avidity of the interaction and result in stronger, sustained binding. In contrast, the KYY peptide has only two binding sites.

Further studies should be conducted to determine the importance of the first amino acid of the repeat in abrogating EAE. In the native HFFK sequence, histidine is first and carries a positive charge (His90). KYY, which starts with another positively charged amino acid, resulted in a slight increase in EAE scores. Electrostatic interactions between His90 and TCR residues may trigger a nontolerogenic signal. Altering the charge of this residue can affect the stimulatory capacity; when converted to a nonpolar alanine (AYEK), partial abrogation of EAE is observed. Furthermore, when changed to the negatively charged amino acid glutamate (EYYK), there is a significant reduction in EAE scores.

The P4 pocket is a major anchor for the peptide to the MHC cleft. Although the residues that make up the cleft differ between human and rat MHC, the crystal structure of human HLA-DR2 suggests that tyrosine can act as an anchor residue in the P4 pocket (2). The polar end group of tyrosine potentially interacts with Gln71, a residue positioned over the P4 pocket in human and rat MHC. The EYYK peptide was designed with the structure of rat RTI.D in mind, where the P4 pocket is composed of mainly nonpolar residues that could interact with the aromatic portion of tyrosine. In humans, the P4 pocket consists of multiple polar and charged residues. Despite structural studies suggesting that tyrosine will fit in the human P4 pocket, peptides designed for human use could be engineered with a polar residue. MBP analogs that contain histidine, arginine, lysine, glutamine, or asparagine instead of Phe92 bind the P4 pocket (2, 4).

The possibility of using crystal structures of HLA molecules to design TCR-MHC antagonists should improve the pace of discovery for new therapies in autoimmune diseases. As the interactions between MHC, peptide, and TCR are defined, disease- and genotype-specific APLs will become possible.

References


