T Cell Epitopes of Human Myelin Oligodendrocyte Glycoprotein Identified in HLA-DR4 (DRB1*0401) Transgenic Mice Are Encephalitogenic and Are Presented by Human B Cells


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T Cell Epitopes of Human Myelin Oligodendrocyte Glycoprotein Identified in HLA-DR4 (DRB1*0401) Transgenic Mice Are Encephalitogenic and Are Presented by Human B Cells


Myelin oligodendrocyte glycoprotein (MOG) is an Ag present in the myelin sheath of the CNS thought to be targeted by the autoimmune T cell response in multiple sclerosis (MS). In this study, we have for the first time characterized the T cell epitopes of human MOG restricted by HLA-DR4 (DRB1*0401), an MHC class II allele associated with MS in a subpopulation of patients. Using MHC binding algorithms, we have predicted MOG peptide binding to HLA-DR4 (DRB1*0401) and subsequently defined the in vivo T cell reactivity to overlapping MOG peptides by testing HLA-DR4 (DRB1*0401) transgenic mice immunized with recombinant human (rh)MOG. The data indicated that MOG peptide 97–108 (core 99–107, FFRDHSYQE) was the immunodominant HLA-DR4-restricted T cell epitope in vivo. This peptide has a high in vitro binding affinity for HLA-DR4 (DRB1*0401) and upon immunization induced severe experimental autoimmune encephalomyelitis in the HLA-DR4 transgenic mice. Interestingly, the same peptide was presented by human B cells expressing HLA-DR4 (DRB1*0401), suggesting a role for the identified MOG epitopes in the pathogenesis of human MS. The Journal of Immunology, 2001, 167: 7119–7125.

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the CNS believed to be mediated by an autoimmune T cell response directed at proteins of the myelin sheath, such as myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG) (1–5). Although considerable research has focused on MBP, observations of a predominant MS patient T cell response to MOG, and MS-like lesions in marmosets immunized with MOG have heightened interest in this Ag (6–8). Knowledge of the MOG epitopes targeted by the T cell response in patients would be important for the understanding of the pathogenesis of MS, and to identify possible candidate peptides for immune intervention. However, the analysis of Ag-specific T cell responses in genetically diverse MS patients has remained technically challenging. Frequencies of Ag-specific T cells in freshly isolated human tissues, such as peripheral blood, are frequently at or below the detection limit of current technologies (9). Therefore, the exact nature of the T cell epitopes of MOG presented by MHC molecules in MS patients has remained unresolved. The human MHC molecules most frequently associated with MS are HLA-DR2 (DRB1*1501, DRB5*0101, and DQB1*0602) (10, 11) and HLA-DR4 (DRB1*0401) in a subpopulation of patients with Mediterranean background (10–13).

MHC molecules are highly polymorphic and have unique binding motifs. Therefore, results on determinant usage obtained in conventional animal models cannot necessarily be extrapolated to Ags presented in the context of human MHC molecules (14). To overcome these limitations, we have applied MHC binding algorithms to predict MOG peptide binding to HLA-DR4 (DRB1*0401), and subsequently probed HLA-DR4 (DRB1*0401) transgenic mice immunized with rhMOG for T cell reactivity to overlapping MOG peptides by computer-assisted cytokine ELISPOT assays at single cell resolution. The data showed that MOG peptide (p)97–108 (core 99–107, FFRDHSYQE) was the immunodominant HLA-DRB1*0401-restricted T cell epitope. This region had a high in vitro binding affinity for HLA-DR4, showed complete sequence homology among mice, rats, and humans, and induced severe experimental autoimmune encephalomyelitis (EAE) in the HLA-DR4 transgenic mice. Interestingly, the same peptide was presented by human HLA-DR4 homozygous B cells, suggesting a role for this epitope in the pathogenesis of MS.

Materials and Methods

Mice, Ags, and injections

HLA-DR4 (DRB1*0401) transgenic mice were generated as described previously (15) and bred at Case Western Reserve University (Cleveland, OH).
under special pathogen-free conditions. Female transgenic mice were injected at 6–10 wk of age with the Ag in CFA. Pertussis toxin (List Biological Laboratories, Campbell, CA) was added to the immunization regimen, as described previously. Recombinant MOG was prepared as described and subsequently. Overlapping MOG peptides were obtained from Princeton Biomolecules (Langhorne, PA). CFA was prepared by mixing IFA (Life Technologies, Grand Island, NY) with Mycobacterium tuberculosis H37RA at 5 mg/ml (Difco, Detroit, Michigan). Ags were mixed with the adjuvant to yield a 2-μg/ml emulsion, of which 50 μl was injected i.c.s. as specified.

Cell separations

Single cell suspensions were prepared from HLA-DR4 lymph node cells. CD4+ or CD8+ T cells were obtained by passing the cells through a murine CD8+ or CD4+ T cell enrichment column (R&D Systems, Minneapolis, MN) following the manufacturer’s suggested protocol. FACS analysis showed >95% enrichment for either CD4+ or CD8+ T cells. Irradiated syngeneic spleen cells or EBV-transfected HLA-DR4 (DRB1*0401) homozygous B cells (16) were added at 1 × 10^6 cells per well as indicated in Fig. 5.

Cytokine measurements by ELISPOT and computer-assisted ELISPOT image analysis

Cytokine ELISPOT assays were performed as described previously (17). Briefly, ELISPOT plates (ImmuNoSpot; Cellular Technology, Cleveland, OH) were coated overnight with IFN-γ (R46A2, 4 μg/ml) or IL-5 (TRFK5, 5 μg/ml)-specific capture Ab diluted in 1× PBS. The plates were blocked with 1% BSA in PBS for 1 h at room temperature, then washed four times with PBS/Tween. Wells from draining lymph nodes were placed in assays at 1 × 10^6 cells/well alone or with MOG peptides (7 μM) in HL-1 medium supplemented with 1% l-glutamine and cultured for 24 h for IFN-γ and 48 h for IL-5. Subsequently, the cells were removed by washing four times with PBS then four times with PBS/Tween, and the biotinylated detection Ab was added to wells. The plate-bound second Ab was then visualized by adding streptavidin–alkaline phosphatase (DAKO, Carpenta- ria, CA) and nitroblue tetrazolium (Bio-Rad, Hercules, CA)/5-bromo-4-chloro-3-indolyl phosphate substrate (Sigma-Aldrich, St. Louis, MO). Image analysis of ELISPOT assays was performed on a Series 1 ImmunoSpot Image Analyzer (Cellular Technology) as described previously (17, 18). In brief, digitized images of individual wells of the ELISPOT plates were analyzed for cytokine spots based on the comparison of experimental wells and FACS analysis showing >95% enrichment for either CD4+ or CD8+ T cells. Images of Ab-labeled cytokine spots were analyzed for cytokine spots based on the comparison of experimental wells and FACS analysis showing >95% enrichment for either CD4+ or CD8+ T cells. T cells were obtained by passing the cells through a murine CD8+ or CD4+ T cell enrichment column (R&D Systems, Minneapolis, MN) following the manufacturer’s suggested protocol. FACS analysis showed >95% enrichment for either CD4+ or CD8+ T cells. Irradiated syngeneic spleen cells or EBV-transfected HLA-DR4 (DRB1*0401) homozygous B cells (16) were added at 1 × 10^6 cells per well as indicated in Fig. 5.

Evaluation of clinical disease

Mice were monitored daily for 30 days and on alternate days thereafter. A mean clinical score was assigned for each group using the following scale: 0, no clinical symptoms; 1, limp tail; 2, moderate hind limb weakness; 3, complete hind limb paralysis; 4, quadriplegia or paraparesis; 5, death.

Histopathology

At the time of the experiment the brain and spinal cord of the mice were removed and either preserved in 10% formalin or snap-frozen in 2-methylbutane. Five-micrometer slices of the CNS tissue were prepared and stained with H&E. The tissue was then examined by light microscopy in a blinded fashion by a neuropathologist and evaluated for the extent of in- flammation and graded as follows: −, no inflammation; +, -, a few mono- nuclear cells; +, organization of inflammatory infiltrates around positive vessels; ++, extensive perivascular cuffing with extension into the sub- arachnoid space; and +++, extensive perivascular cuffing with increasing subarachnoid inflammation (19, 20). Immunofluorescence staining of the brain tissue was performed as described above and analyzed with FITC- or PE-conjugated anti-mouse CD3, CD4, CD8, CD19, MAC-1, and F4/80 Abs (BD PharMingen, San Diego, CA). Images of Ab-labeled tissue sections were captured using a Leica fluorescence microscope equipped with a charge-coupled device camera and image analysis software. Sections were analyzed in a blinded fashion.

Prediction of MOG sequences with high affinity for HLA-DRB1*0401

To identify potential core peptides with predicted high binding affinity for HLA-DRB1*0401, the MOG amino acid sequence was analyzed with two published HLA-DR4 binding algorithms (22, 23). Briefly, a computer program was written that parsed the MOG sequence into successive 9-mer, each beginning one amino acid after the start of the previous 9-mer. The contribution toward binding of each amino acid was summed based on the matrix published by Hammer et al. (22), yielding a peptide score. Higher peptide scores indicate relatively higher affinity binding to HLA-DRB1*0401. Several immunodominant peptides predicted by this algorithm yield relative affinity scores in the range of 4–22 (22). This hierarchy of predicted high affinity peptides was compared with predictions based on the published algorithm of Marshall et al. (23), which calculates an IC50 concentration rather than a relative score. The Marshall et al. (23) algorithm was based on the effects of single amino acid substitutions within the 11-residue core of a 13-mer polyalanine peptide (AAYAAAAGAAAA) that binds promiscuously to most HLA-DR alleles. The contributions of each of the 11 amino acid residues were multiplied consecutively, and the sum was multiplied by the IC50 of the parent polyalanine peptide (14.7 nM) to derive a predicted IC50 for the sequence. Lower IC50 values indicate higher predicted affinity of a peptide containing the sequence. To compare the affinity of an amino acid sequence predicted by the two algorithms, 9-mer sequences with the anchor residue (F, I, L, M, V, W, or Y) at position 1 were first evaluated by the Hammer et al. (22) algorithm. Each 9-mer, along with the N- and C- flanking amino acids, were then evaluated by the Marshall et al. (23) algorithm, providing an 11-mer peptide with the anchor residue (F, I, L, M, V, W, or Y) at the second position as required by this algorithm.

In vitro peptide binding studies

In vitro binding competition assays were performed as described previously (17). In brief, biotinylated peptides were immobilized on ELISA plates to establish optimal binding conditions for the purified HLA-DR4 (DRB1*0401) molecules. Relative affinities of MOG peptides for the HLA-DR4 molecules were assessed by an inhibition ELISA based on a dissociation-enhanced lanthanide fluoro-immunnoassay (DELFIA; Wallac, Turku, Finland). In the inhibition assay, HLA-DR4 molecules (10 nM) were incubated with fixed amounts of the tracer peptide (class II-associated invariant chain peptide (CLIP)120) in the presence of various concen- trations (10-fold dilutions between 1 nM and 100 μM) of the unlabeled MOG peptides. The concentration yielding 50% inhibition of binding of the tracer peptide (IC50) was plotted by the percentage of inhibition vs the concentration of MOG peptide. Peptides were tested in three independent experiments.

Cloning and bacterial expression of human MOG

The cDNA encoding amino acids 23–247 of human MOG was obtained by reverse transcription of total RNA from the human glioma cell line 45/8. RNA was prepared with TRizol Reagent (Life Technologies) and first- strand cDNA was synthesized using Moloney murine leukemia virus re- verse transcriptase, RNase H Minus (Promega, Madison, Wisconsin), and the gene-specific primer R785. The amplified PCR product was separated by agarose gel electrophoresis on a 1.2% gel and purified with a QIAEX II kit (Qiagen). The amplified PCR product was subcloned into the Bluescript II KS+ vector (Stratagene, La Jolla, California), which was previously treated with Smal and alkaline phosphatase according to conven- tional protocols. The nucleotide sequences of the various clones were determined for both strands by using the PRISM Ready Reaction Dye Deoxy Terminator cycle sequencing kit in conjunction with an Applied Biosystems model 310 DNA Sequencer (both from Applied Biosystems, Foster City, CA). After the purification of the resulting PCR product by phenol/chloroform extraction and ethanol precipitation, the fragment was digested with Ncol and BamHI and ligated into the Ncol/BamHI linearized pQE60 vector (Qiagen). To improve the bacterial expression level, it was necessary to replace some of the original, N-terminal MOG codons by PCR mutation. The nucleotide sequences of the resulting clones were confirmed by sequence analysis, and clone mog-1His was used for expression in Escherichia coli. The His-tagged MOG fusion peptides documented in the liter- ature domain of MOG (amino acids 1–125) was purified under denaturing conditions by metal chelate affinity chromatography on Ni-NTA Agarose columns (Qiagen) according to the manufacturer’s guidelines.
Results

Predicting the binding of MOG epitopes to HLA-DR4 (DRB1∗0401) molecules

To characterize the complement of MOG peptides that could bind to the human HLA-DR4 molecule and could therefore play a role in MS, we screened the MOG protein sequence with peptide binding algorithms which had been reported previously to predict the binding of peptides to HLA-DRB1∗0401 (22, 23). These algorithms take into account the properties of the Ag binding site and of the amino acid side chains of a peptide to predict its binding to MHC molecules (25). According to the method developed by Hammer et al. (22), a numeric value is generated for all 9-mer amino acid sequences of a given protein, and only peptides with binding scores greater than 2 are expected to bind to HLA-DRB1∗0401. The results for the screening of the extracellular region of the MOG protein are summarized in Table I. Of these, the sequence MOGp99–107 (FFRDHSYQE) was the peptide with the highest binding score (3.9). This peptide has an aromatic amino acid (phenylalanine) at the anchor position P1, and an amino acid with a hydroxyl group (serine) at position P6, consistent with optimal binding to HLA-DRB1∗0401 (26–28). Several other peptides achieved predicted binding scores above 2, such as MOGp83–91 (LRIRNVRFs), which has an aliphatic anchor at P1 (leucine) and a valine at position P6 of the sequence, and was scored at 2.8. These results were confirmed overall when compared with a second binding algorithm developed by Marshall et al. (23). However, the predictions conflicted for some of the peptides. For example, MOGp83–91 had a good binding score of 2.8 with the Hammer method (22) but had an IC50 of 17.83 with the Marshall algorithm (23), indicative of low-affinity binding. Furthermore, the Hammer algorithm (22) predicted several peptides to be nonbinders for HLA-DRB1∗0401, which were, however, estimated to be high-affinity binders by the Marshall method (Ref. 23 and data not shown). Hence, the binding algorithms alone were not sufficient to unambiguously determine the complement of MOG sequences that could be targeted by T cells in HLA-DR4∗ MS patients.

Mapping of MOG epitopes in HLA-DR4 (DRB1∗0401) transgenic mice

To test whether or not the predicted and/or any of the other MOG epitopes were generated in vivo and induced T cell immunity, we used a transgenic mouse line that expressed HLA-DR4 (DRA1-E0/HLA-DRB1∗0401-Eβ) molecules under the mouse MHC class II promoter (15). These transgenic mice are deficient for endogenous mouse MHC class II molecules, have no significant deletions in their T cell repertoire, and have previously been shown to mount strong T cell responses to various self and foreign Ags (15, 29).

As shown in Fig. 1, HLA-DR4 transgenic mice were immunized with the extracellular portion of MOG (rhMOG, amino acids 1–125), and T cell responses were measured in draining lymph node cells after recall with overlapping 20-mer MOG peptides by cytokine ELISPOT assay. Vigorous IFN-γ responses were detected after recall with the peptides MOGp91–110 and MOGp81–100, and occasionally with MOGp21–40. Furthermore, the mice mounted a vigorous Ag-specific IL-2, but not IL-5, recall response to these peptides (data not shown), consistent with the induction of Th1 immunity. None of the other MOG peptides induced significant cytokine production. The same MOG determinant hierarchy was seen when the mice were tested in parallel in proliferation assays for recall responses to the overlapping MOG peptides (data not shown). Mice not immunized, or immunized with irrelevant control Ags (e.g., hen egg white lysozyme, MBP), showed no responses to any of the MOG peptides (data not shown), demonstrating the specificity of the T cell response.

The minimal core epitope of the immunodominant region 91–110 of MOG peptide was comprised of amino acids 99–107, as determined by recall with overlapping MOG peptides shifted by one amino acid covering the region amino acids 90–110 (Fig. 1B). Cell separation for CD4 and CD8 T cells demonstrated that the observed T cell responses were mediated by CD4+ T cells, but not by CD8+, T cells (data not shown).

Taken together, the data showed that MOGp99–107 was the immunodominant MOG peptide 91–110 of MOG peptide was comprised of amino acids 99–107, as determined by recall with overlapping MOG peptides shifted by one amino acid covering the region amino acids 90–110 (Fig. 1B). Cell separation for CD4 and CD8 T cells demonstrated that the observed T cell responses were mediated by CD4+, but not by CD8+, T cells (data not shown).

In vitro binding of the region MOGp99–107 (FFRDHSYQE) to HLA-DR4 (DRB1∗0401)

The data on the predicted binding and ex vivo T cell reactivity suggested that the region MOGp99–107 had a high affinity for the HLA-DR4 molecule. However, to formally determine the binding affinity of this region, purified HLA-DRB1∗0401 molecules were incubated with a biotinylated reference peptide (CLIP, 250 nM), and serial dilutions of the respective overlapping 9-mer peptides (100–0.001 μM) covering amino acids 91–108 of the MOG sequence were added. As shown in Fig. 2, this region of the MOG sequence indeed bound with a high affinity to HLA-DR4, as demonstrated by IC50 values of 4, 0.07, and 7.4 μM for the peptides MOGp97–105 (TCFRDHSY), MOGp98–106 (CFRDSHYQ), and MOGp99–107 (FRFDSHYQE) respectively. Thus, the in vitro binding data confirmed the algorithm prediction.

Induction of EAE with MOG peptides in HLA-DR4 transgenic mice

Several HLA-DR4 (DRB1∗0401)-restricted MOG epitopes, including the region MOGp91–110 (core 99–107, FFRDSHYQE), induced T cell immunity in HLA-DR4 (DRB1∗0401) transgenic mice. To establish whether or not these MOG peptides played a role in the induction of EAE, we immunized the HLA-DR4 transgenic mice with MOGp97–108 (TCFRRDHSYQE) and observed the mice for clinical and histological signs of EAE (Fig. 3A.

### Table I. Side chain scanning of MOG for HLA-DR4 (DRB1∗0401) binding sequences

<table>
<thead>
<tr>
<th>Amino Acid Position</th>
<th>MOG Peptide Sequence</th>
<th>Binding Score</th>
<th>Predicted IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>99–107</td>
<td>FFRDHSYQE</td>
<td>3.9</td>
<td>0.8</td>
</tr>
<tr>
<td>15–23</td>
<td>LVGDEVELP</td>
<td>3.0</td>
<td>1.18</td>
</tr>
<tr>
<td>83–91</td>
<td>LRIRNVRFs</td>
<td>2.8</td>
<td>17.83</td>
</tr>
<tr>
<td>120–128</td>
<td>YWVSPGVLV</td>
<td>2.5</td>
<td>0.09</td>
</tr>
<tr>
<td>40–48</td>
<td>YRPFRSRRV</td>
<td>2.1</td>
<td>14.5</td>
</tr>
</tbody>
</table>

* Scanning of the MOG sequence and calculation of peptide scores and estimated IC50 were performed as outlined in Materials and Methods. Shown are the results for the extramembraneous region of MOG (amino acids 1–125). Sequences with combined peptide binding scores above 2 were arranged according to their binding score (third column). Estimated IC50 values for the corresponding 13-mer MOG amino acid sequences are shown in the last column. Lower estimated IC50 indicate higher-affinity HLA-DRB1∗0401 binding and are inversely proportional to peptide binding scores.
Interestingly, immunization of the transgenic mice with this peptide resulted in severe EAE at 8–14 days after immunization. Brain sections of mice with EAE stained by H&E showed perivascular and periventricular inflammatory infiltrates consisting of lymphocytes, macrophages, and occasional neutrophils (Fig. 4, A and B). Immunofluorescence staining of brain sections with mAbs demonstrated that the infiltrates consisted of macrophages/microglia (MAC-1<sup>+</sup>, F4/80<sup>+</sup>) and CD4<sup>+</sup> T cells (Fig. 4, C and D). No B cells or CD8<sup>+</sup> T cells were detected (data not shown). Furthermore, immunization of the transgenic mice with the subdominant MOG epitopes MOGp21–40 or MOGp80–91 also induced EAE in the HLA-DR4 transgenic mice (Fig. 3B, □ and △, respectively), demonstrating that these determinants were encephalitogenic.

Taken together, the results show that EAE could be induced in the transgenic mice by immunization with HLA-DR4-restricted MOG epitopes.

**FIGURE 1.** Mapping of HLA-DR4 (DRB1*0401)-restricted MOG epitopes. Six- to 10-wk old HLA-DR4 (DRB1*0401) transgenic mice were immunized with rhMOG (amino acids 1–125) in CFA s.c. Ten days later, frequencies of Ag-specific IFN-γ-producing T cells were measured by cytokine ELISPOT assay in single cell suspensions of draining lymph node cells. A, Shown are the mean and SE of the frequencies of MOG peptide-specific IFN-γ spots per 5 × 10<sup>5</sup> cells after recall with overlapping 20-mer MOG peptides spanning the whole MOG sequence (n = 12 mice). B, Frequencies of MOG peptide-specific IFN-γ spots per 5 × 10<sup>5</sup> cells after recall with overlapping 9-mer peptides shifted by one amino acid covering the region MOG amino acids 90–110 (n = 6 mice). The means were calculated from the results of triplicate wells, with the background subtracted (usually fewer than five spots), obtained in three independent experiments. Stimulation index of greater than 3 are considered positive as outlined in Materials and Methods.

**FIGURE 2.** In vitro binding affinity of MOGp91–108 peptides to HLA-DR4 (DRB1*0401) molecules. Overlapping 9-mer peptides of the region MOGp91–108 were probed for their binding to purified HLA-DR4 (DRB1*0401) molecules, and IC<sub>50</sub> values for each of the peptides were derived from the inhibition curves obtained by ELISA as described in Materials and Methods. A, Inhibition of the binding of the biotinylated tracer peptide CLIP by MOG peptides. B, Inverse IC<sub>50</sub> of the respective MOG peptides.
Human B cells process and present the immunodominant MOG epitope

To test whether or not human APCs could present the encephalitogenic MOG peptides, we purified CD4^+ T cells from MOGp97–108-immunized transgenic mice and stimulated them with Ag and a human HLA-DR4 homozygous B cell line as APCs (16). As shown in Fig. 5, the human B cells, pulsed either with the MOGp97–108 peptide or with the whole rhMOG protein, induced vigorous cytokine production by the MOGp97–108-specific T cells (filled bars). No T cell response was induced with the irrelevant control peptide, human collagen type II p261–273. In contrast, hCIIp261–273-specific CD4^+ T cells (gray bars) responded to the hCII peptide, but not to MOG or the MOG peptide. Furthermore, fixing of the HLA-DR4^+ human B cells in 1% paraformaldehyde completely abrogated the induction of T cell responses when pulsed with the MOG protein, whereas the fixed cells induced strong T cell responses to the MOGp97–108 (data not shown). Similarly, HLA-DR4^+ B cells failed to induce specific T cell responses to either the MOG protein or the MOG peptide (data not shown).

Taken together, the data show that human APCs processed and presented the immunodominant HLA-DR4-restricted MOG peptide that we have identified in the transgenic mice. Therefore, the data suggest that this MOG peptide could also play a role in the pathogenesis of human MS.

Discussion

We have characterized for the first time the human MOG epitopes presented in the context of HLA-DR4 (DRB1*0401). The observation that the T cell response was directed against MOG peptides with high binding affinity for HLA-DR4 may reflect the notion that this self-Ag is not expressed in the thymus (30, 31). Thus, MOG-specific T cells are not negatively selected and express high-avidity TCRs for the MHC:peptide complex. How profoundly thymic negative selection is affected by the expression (or lack thereof) of myelin Ags in the thymus has recently been demonstrated for MBP

**FIGURE 3.** MOGp97–108 induces EAE in HLA-DR4 (DRB1*0401) transgenic mice. Transgenic mice were immunized with MOG peptides in CFA and pertussis toxin. Mice were observed daily for clinical signs of EAE and scored accordingly (see Materials and Methods). Shown is a representative experiment of six experiments performed. A, HLA-DR4 transgenic mice immunized with MOGp97–108 (●, n = 10). B, HLA-DR4 transgenic mice immunized with MOGp21–40 (▲, n = 6) or MOGp80–91 (▲, n = 5) respectively.

**FIGURE 4.** MOGp97–108-induced EAE is characterized by CD4^+ T cell infiltrates in the CNS. Histologic analysis of brain sections of representative H&E-stained (A and B) HLA-DR4 (DRB1*0401) transgenic mice was performed as outlined in Materials and Methods. A, Unimmunized HLA-DR4 transgenic control mouse without EAE. No inflammation is present. B, Representative MOGp97–108-immunized HLA-DR4 mouse with EAE shows extensive perivascular inflammatory infiltrates. C and D, Immunofluorescence microscopy of the same animals was performed as outlined in Materials and Methods. Shown in D is a representative section of anti-CD3-PE/CD4-FITC-stained brain from HLA-DR4 transgenic mice with MOGp97–108-induced EAE. Extensive infiltrates consisting of CD3^-CD4^- T cells are present. MAC-1^- cells were also present, but no CD8^- or CD19^- cells were detected (not shown).
and PLP (32–35). However, there are alternative mechanisms by which myelin-specific T cells could escape negative selection. For example, the immunodominant MBP epitope Ac1–11 is expressed in the thymus of B10.PL mice. However, MBP Ac1–11 is an extremely weak H-2d binder and forms unstable MHC:peptide complexes that fail to negatively select T cells (36, 37). This may explain why a documented immunodominant MBP peptide in HLA-DR4+ MS patients binds HLA-DR4 (DRB1*0401) with relatively low affinity (13). In contrast, the immunodominant MOG region 97–108 is a high-affinity binder to HLA-DR4 (Fig. 2). Hence, our results suggest that it is not sufficient to identify myelin epitopes as targets for the treatment of autoimmune diseases based on peptide binding predictions or in vitro binding assays. Confirmation of the relevance of the autoantigenic epitopes by in vivo studies is required, such as we have demonstrated in the HLA-DR4 transgenic mouse.

Theoretically, myelin epitopes need to be presented in the CNS to activate encephalitogenic T cells. That this actually occurs in vivo has recently been shown for MBPp84–102 in the context of HLA-DR2 (38). However, MBP is an abundant Ag in the CNS, whereas MOG comprises only 0.01–0.05% of the myelin mass (39). Therefore, it is not known whether it is processed and presented in the CNS, and by which type of APCs. It is particularly interesting that in our studies, MOGp97–108-reactive T cells induced EAE, suggesting that this peptide was presented in the CNS. Furthermore, the complete sequence homology of this region with human MOG (and >90% homology for the other MOG peptides identified) suggested that this peptide could similarly be presented in the CNS of MS patients and play a role in the pathogenesis of this disease. The significance of MOG for the autoimmune process is supported by the observation that MOG-reactive T cells are readily detectable in MS patients (40, 41), and MOG immunization induced severe EAE in a non-human primate model of MS (42).

The presented data confirm reports by other investigators that Ag processing and presentation by human and mouse APCs are sufficiently similar to permit the identification of antigenic epitopes within foreign- and self-Ags based on the binding properties of the MHC molecule (43, 44). Furthermore, by using in our studies transgenic mice that expressed HLA-DRA1-E01/HLA-DRB1*0401-I-E01 molecules under the control of the mouse MHC class II promoter (15), it was ensured that T cell maturation and Ag recognition in these mice were unaffected. Thus, the TCR repertoire of the transgenic mice had no significant deletions, and hence there was enough plasticity of the T cell repertoire to detect the MOG epitopes presented in the context of HLA-DRB1*0401 (45). Most importantly, human B cells pulsed with the MOG protein activated MOGp97–108-reactive T cells (Fig. 5), demonstrating that this peptide could be naturally processed and presented to high-avidity T cells in HLA-DR4-expressing MS patients.

If MOG-reactive T cells have a high avidity for their Ag (experiments are under way in our laboratory to formally test for this), then it may be easier to activate the naive T cell precursors, by molecular mimicry, for example. Furthermore, the frequency of MOG-reactive T cells necessary to induce MS may be very low, perhaps even beyond the detection limit of current assays. Subsequently, T cells specific for other myelin Ags (MBP, PLP) or bystander T cells (specific for unrelated Ags) may cross the blood-brain barrier and perpetuate the MOG-induced disease process.

In summary, we have defined for the first time the nature of the MOG T cell epitopes restricted by human HLA-DR4. Our results show that the HLA-DR4 molecule selected for T cell responses to a high-affinity MHC binding peptide. This peptide was encephalitogenic and was also processed and presented by human APCs. Thus, the identified MOG epitopes are potential targets for Ag-specific immunotherapy.

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References


