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Increased CD8⁺ Cytotoxic T Cell Responses to Myelin Basic Protein in Multiple Sclerosis¹

Ying C. Q. Zang,²* Sufang Li,* Victor M. Rivera,* Jian Hong,* Rachel R. Robinson,* Wini T. Breitbach,* James Killian,* and Jingwu Z. Zhang* †‡

Autoreactive T cells of CD4 and CD8 subsets recognizing myelin basic protein (MBP), a candidate myelin autoantigen, are thought to contribute to and play distinct roles in the pathogenesis of multiple sclerosis (MS). In this study we identified four MBP-derived peptides that had high binding affinity to HLA-A2 and HLA-A24 and characterized the CD8⁺ T cell responses to these peptides and their functional properties in patients with MS. There were significantly increased CD8⁺ T cell responses to 9-mer MBP peptides, in particular MBP₁₁₁⁻₁₁₉ and MBP₈₇⁻₉₅ peptides that had high binding affinity to HLA-A2, in patients with MS compared with healthy individuals. The resulting CD8⁺ T cell lines were of the Th1 phenotype, producing TNF-α and IFN-γ and belonged to a CD45RA⁻/CD45RO⁺ memory T cell subset. Further characterization indicated that the CD8⁺ T cell lines obtained were stained with MHC class I tetramer (HLA-A₂/MBP₁₁₁⁻₁₁₉) and exhibited specific cytotoxicity toward autologous target cells pulsed with MBP-derived peptides in the context of MHC class I molecules. These cytotoxic CD8⁺ T cell lines derived from MS patients recognized endogenously processed MBP and lysed COS cells transfected with genes encoding MBP and HLA-A2. These findings support the potential role of CD8⁺ CTLs recognizing MBP in the injury of oligodendrocytes expressing both MHC class I molecules and MBP. These findings have raised new questions about whether CD8⁺ cytotoxic MBP-reactive T cells play a similar role in MS. In MS, however, the role of MBP-reactive T cells is not clear. There is some evidence indicating that the CD4⁺ T cell responses to MBP and other candidate myelin Ags may play an important role in the disease processes (6–16). The results accumulated to date suggest that CD4⁺ MBP-reactive T cells undergo in vivo activation and clonal expansion in MS patients compared with healthy controls (15, 17–20). Compared with their CD4⁺ MBP-reactive T cell counterparts, the potential involvement of CD8⁺ cytotoxic MBP-reactive T cells in the pathogenesis of MS is unknown. Tsuchida and coworkers (21) reported the identification of CD8⁺ CTLs in the blood of MS patients as well as in healthy individuals. Some of these CD8⁺ T cells appeared to recognize endogenously processed myelin peptides, suggesting their potential role in the injury of oligodendrocytes constitutively express MHC class I molecules (but not class II molecules) and the myelin Ags. A subsequent study confirmed that HLA-A₂-restricted CD8⁺ T cell lines recognized the 110–118 peptide of MBP could mediate lysis of human oligodendrocytes (22). However, it remains unknown whether CD8⁺ T cells recognizing MBP are sensitized in vivo to undergo activation and expansion in MS patients compared with healthy controls and whether there are additional epitopes associated with other MHC class I molecules.

The present study was undertaken to identify CD8⁺ CTLs recognizing MBP-derived peptides in MS patients and healthy individuals and to characterize the precursor frequency and the functional properties of these CD8⁺ T cells in relation to their potential role in the injury of oligodendrocytes. Four sequence regions of human MBP were identified for HLA-A₂ and -A₂₄ by TEPITOPE, a software program that allows the identification of HLA class I ligand binding epitopes. CD8⁺ T cell responses to synthetic 9-mer peptides corresponding to the predicted HLA-A₂ and -A₂₄ binding sequences were analyzed using an experimental approach that allows efficient expansion of CD8⁺ T cells by predepleting CD4⁺

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Abbreviations used in this paper: MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; LDH, lactate dehydrogenase; MBP, myelin basic protein.
T cells in PBMC. The findings described in this study are important for understanding the role of CD8\(^+\) CTLs recognizing self myelin peptides in the injury of oligodendrocytes and their potential relevance to the disease process in MS.

Materials and Methods

Subjects

Fifteen patients with relapsing-remitting or secondary progressive MS, as determined by the Poser criteria, were included. Patients had not been treated with immunosuppressive or immunomodulatory drugs (azathioprine, cyclophosphamide, IFN-\(\beta\), or glatiramer acetate) at least 3 mo before entering the study. The protocol was approved by the institutional review board at Baylor College of Medicine. A group of 15 healthy subjects matched for age and sex with the MS group were included as controls.

Table 1. Clinical characteristics and HLA haplotypes of MS patients and normal subjects (NS)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Sex</th>
<th>RR/SP</th>
<th>EDSS</th>
<th>Duration (years)</th>
<th>HLA Haplotypes</th>
</tr>
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<tr>
<td>MS-1</td>
<td>34</td>
<td>F</td>
<td>RR</td>
<td>6.0</td>
<td>6</td>
<td>A<em>02,24; B</em>40(60)(w6),57(w4); Cw*03(10),06</td>
</tr>
<tr>
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<td>33</td>
<td>F</td>
<td>RR</td>
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<td>9</td>
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<td>RR</td>
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<td>M</td>
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<td>8</td>
<td>A<em>0201 positive, A</em>2402 negative by PCR</td>
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<td>RR</td>
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<td>13</td>
<td>A<em>01,02; B</em>08(6),15(6); Cw*03,07</td>
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<td>SP</td>
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<tr>
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<td>SP</td>
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<td>46</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A<em>02,04; B</em>08(6),44(6); Cw*05,07</td>
</tr>
<tr>
<td>NS-2</td>
<td>34</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A<em>11,24; B</em>08(6),44(6); Cw*05,07</td>
</tr>
<tr>
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<td>42</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A<em>03,24; B</em>13(4),40(60)(w6); Cw*03,06</td>
</tr>
<tr>
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<td>42</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A<em>02,24; B</em>15(62)(w6),36(6); Cw*03,07</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
<td>A<em>02,30; B</em>40(61); Cw*01,03</td>
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<tr>
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<td>22</td>
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<td>A<em>02,34; B</em>14(65)(w6),35(6); Cw*06,08</td>
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<tr>
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<td>43</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A<em>0201 negative, A</em>2402 negative by PCR</td>
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<tr>
<td>NS-8</td>
<td>40</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A<em>01,02; B</em>15(62)(w6),37(4); Cw*03,06</td>
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<tr>
<td>NS-9</td>
<td>26</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A<em>02,24; B</em>07(6),15(63)(w4); Cw*04,06</td>
</tr>
<tr>
<td>NS-10</td>
<td>45</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A<em>02,24; B</em>44(4),57(4); Cw*05,06</td>
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<tr>
<td>NS-11</td>
<td>30</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>A<em>02,11, B</em>15(62)(w6),35(6); Cw*03(10),04</td>
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<tr>
<td>NS-12</td>
<td>46</td>
<td>F</td>
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<td>-</td>
<td>-</td>
<td>A<em>02,30; B</em>39(6),44(6); Cw*02,07</td>
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<tr>
<td>NS-13</td>
<td>37</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A<em>02,24; B</em>15(35)(6),44(6); Cw*01,05</td>
</tr>
<tr>
<td>NS-14</td>
<td>35</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A<em>24,33; B</em>15(7)(w6),44(4); Cw*07</td>
</tr>
<tr>
<td>NS-15</td>
<td>35</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A<em>03,24; B</em>27(4),44(4); Cw*01,05</td>
</tr>
</tbody>
</table>

Reagents and peptides

TEPITOPE is a Windows application that allows identification of HLA class I and binding epitopes (15-25). Four peptides corresponding to the predicted HLA-A2 and -A24 binding sequences (1% threshold) were synthesized using the Mayfield method and were purified using HPLC (M. D. Anderson Cancer Center Peptide Core, Houston, TX). The purity of the peptides was >90%. The amino acid sequences of the peptides were VVH–FFKNIV (MBP\(^{111-119}\)) and SLSRFSWGA (MBP\(^{111-119}\)) for HLA-A2 and DYKSAHKGF (MBP\(^{142-149}\)) and KYLATASTM (MBP\(^{142-149}\)) for HLA-A24. A synthetic peptide corresponding to an immunodominant sequence of tetanus toxoid (residues 830–838) was included in the frequency analysis. A reference peptide of unrelated TCR CDR3 sequence (STROGQPQET) was used as a control. HLA-A2-MBP\(^{111-119}\) tetramer was obtained from Immunomics (San Diego, CA).

Precursor frequency analysis of CD8\(^+\) T cells recognizing MBP-derived peptides and the generation of short term CD8\(^+\) T cell lines and clones

PBMC were isolated from the peripheral blood of MS patients and healthy individuals by Ficoll separation. CD4\(^+\) T cells were predepleted using magnetic beads coupled with an anti-CD4 Ab (Dynal Biotech, Oslo, Norway). Briefly, PBMC were incubated with magnetic beads coated with the Ab at a bead to cell ratio of 10 for 30 min with gentle shaking. Unbound PBMC fractions were collected by magnetic separation. The depletion rate for CD4\(^+\) T cells was >98% in all cases. The resulting CD4-depleted fractions typically contained 72 ± 8% CD8\(^+\) T cells as determined by flow cytometric analysis. A representative experiment is shown in Fig. 1. Cells were then seeded in 96-well, U-bottom microtiter plates at a density of 50,000 cells/well together with 10\(^3\) of autologous unfraccionated PBMC that were irradiated to provide helper function of CD4\(^+\) T cells, whereas they themselves were unable to proliferate in response to the Ags. Peptides were added at a final concentration of 20 \(\mu\)g/ml to cultures. A total of 24 wells were set for each peptide. Cells were cultured at 37°C in a 5% CO\(_2\) atmosphere. After 7 days, all cultures were tested for specific proliferation to the corresponding peptides by titrated thymidine incorporation. In brief, each well was split into four aliquots (~10\(^4\) cells/aliquot) and cultured in duplicate with 10\(^3\) autologous PBMC in the presence and the absence of the corresponding MBP-derived peptides at 20 \(\mu\)g/ml. Cultures were kept...
Subjects          Stimulating peptides       Total positive wells / 360 w tested /group # of positive wells in subjects (24 w tested /subject)
MS (n=15)            MBP$_{87-95}$             51
                      MBP$_{111-119}$            78
                      MBP$_{14-22}$             62
                      MBP$_{134-142}$          49
                      TT$_{830-838}$           214
NS (n=15)             MBP$_{87-95}$             16
                      MBP$_{111-119}$          26
                      MBP$_{14-22}$            25
                      MBP$_{134-142}$         29
                      TT$_{830-838}$           232

FIGURE 2. Direct comparison of the number of positive wells/cultures of CD8$^+$ T cells reactive to MBP-derived peptides in patients with MS and normal subjects (NS). The analyses were performed by the split-well method in which responder PBMC fractions predepleted for CD4$^+$ T cells were cultured with irradiated autologous PBMC in the presence of the indicated MBP-derived peptides. A synthetic peptide corresponding to an immunodominant epitope of tetanus toxoid (TT; residues 830–838) was used as a control. The results are expressed as the total number of wells/cultures scored positive in CD4-depleted fractions of PBMC (see Materials and Methods) in each group (360 wells for each group) and as the number of positive wells in each subject (24 wells for each subject). ●, Number of positive wells in each subject; †, mean of positive wells in each group. * Statistically significant differences ($p < 0.05$) between the two groups.

for 3 days and pulsed with $[^{3}H]$thymidine (Nycomed, Amersham Pharmacia Biotech, Arlington Heights, IL) at 1 μCi/well during the last 16 h of culture. Cells were then harvested using an automated cell harvester and $[^{3}H]$thymidine incorporation was measured in a Betaplate counter (Wallac, Turku, Finland). A well/culture was defined as specific for the peptide when there were >1500 cpm and exceeded the reference value (in the absence of the peptide) by at least 3 times. Results were expressed as the number of wells scored positive.

A panel of 39 resulting CD8$^+$ T cell lines was characterized for phenotypic expression, cytokine profile, and specific cytotoxic activity toward autologous target cells. Four such representative T cell lines (E11, D10, B9, and F12) were selected for their recognition of the four MBP peptides. Four such representative T cell lines (E11, D10, B9, and F12) were selected for their recognition of the four MBP peptides. The T cell clones obtained were further expanded by alternate stimulation with the corresponding MBP peptides and PHA-protein in the presence of autologous APC.

Phenotypic analysis of CD8$^+$ T cell lines

To analyze the phenotypic expression, 10$^5$ cells of each T cell line were washed in PBS containing 1% FBS and 0.1% sodium azide (FBS-PBS) and resuspended in 100 μl FBS-PBS containing a 1/100 dilution of fluorochrome-labeled Ab (Simultest CD4/CD8, CD45RA/CD45RO, and TCRβ/TCRγδ; BD Immunocytometry Systems, San Jose, CA) or appropriate Ig isotype controls (γ2a-FITC/γ1-PE; BD Immunocytometry Systems). After incubation for 30 min on ice, the cells were washed three times in FBS-PBS and fixed in 1% formaldehyde for flow cytometric analysis. The same procedure was used for the analysis of CD8$^+$ T cell lines using MHC class I tetramer.

Cytokine production of CD8$^+$ T cell lines

The selected CD8$^+$ T cell lines were first challenged with autologous APC pulsed with the corresponding MBP peptides. The cytokine profile was determined quantitatively using ELISA kits (BD PharMingen, San Diego, CA). Microtiter plates (96-well; Maxisorp; Nunc, Naperville, IL) were coated overnight at 4°C with 1 μg/well of a purified mouse capturing mAb to human cytokine (IL-4, IL-10, TNF-α, and IFN-γ; BD PharMingen). Plates were washed, and nonspecific binding sites were saturated with 10% (w/v) FBS for 1 h and subsequently washed. Supernatants and cytokine standards were diluted with PBS and added in duplicate wells. Plates were incubated at 37°C for 2 h and subsequently washed with PBS-T. Matched biotinylated detecting Ab was added to each well and incubated at room temperature for 2 h. After washing, avidin-conjugated HRP was added, and plates were incubated for 1 h. 3,3′,5,5′-Tetramethylbenzidine (Sigma-Aldrich, St. Louis, MO) was used as a substrate for color development. OD was measured at 450 nm using an ELISA reader (Bio-Rad, Hercules, CA), and cytokine concentrations were quantitated by Microplate computer software (Bio-Rad) using a double eight-point standard curve.

COS cell transfectants with human MBP and HLA-A2

To prepare transfectants expressing both human MBP and HLA-A2 as target cells, CDNA encoding human MBP and human HLA-A2 were constructed into pBud CE4.1 vector that contained two promoters (P<sub>CMV</sub> promoter and P<sub>EF-α1</sub> promoter). The recombinant DNA was transfected into COS-7 cells using Lipofectamine 2000 (Invitrogen, San Diego, CA). The
stable transfectants were selected using selective medium containing zeocin at 400 μg/ml (Invitrogen). Stable expression of MBP and HLA-A2 was evaluated by incubating the cells with conjugated mAbs to MBP (Sigma-Aldrich) or HLA-A2 (BD PharMingen) and analyzed subsequently by flow cytometry.

Cytotoxicity assay

In experiments with autologous target cells, EBV-transformed B cell lines were generated from each individual included in this study using a procedure described previously (26, 27). B cells were pulsed with corresponding peptides and used as autologous target cells. Pulsing of B cells was conducted by incubating cells with MBP-derived peptides or a control TCR peptide (40 μg/ml), respectively, for 2 h, followed by washing to remove free peptides. In experiments with COS cells, stable transfectants and non-transfectants were washed and used as target cells. Cytotoxicity was performed using a lactate dehydrogenase (LDH) release assay (Promega, Madison, WI). LDH release was measured in an enzymatic assay according to the manufacturer’s instructions. Briefly, CD8+ T cells (50,000 effector cells/well) were incubated with target cells (autologous B cells or COS cells) at an E:T cell ratio of 10 and centrifuged once at 250 x g. Unpulsed autologous B cells and nontransfectants were used as controls. RPMI 1640 without phenol red was used throughout the assay to avoid the background absorbance. After incubation at 37°C and 5% CO2 for 4 h, the plates were centrifuged again. Fifty microliters of supernatant was transferred to another plate and mixed with the substrate mix provided in the test kits. The reaction was stopped after 30 min and read at 490 nm absorbance. Specific cytotoxicity was calculated as: % cytotoxicity = (experimental release – spontaneous release)/(maximum release – spontaneous release) x 100. For MHC restriction experiments, purified mAbs to MHC class I (W6/32) or MHC class II (HB55) were added at (20 μg/ml) during incubation of effector cells with target cells in cytotoxicity assays described above.

Results

Estimated precursor frequency of CD8+ T cells recognizing MBP-derived peptides in MS patients and healthy controls

The clinical characteristics/demographic data and HLA class I haplotypes of MS patients and control subjects are shown in Table I. Four sequence regions of human MBP were identified for predicted binding motifs to HLA-A2 (residues 87–95 and 111–119) and HLA-A24 (residues 134–142 and 14–22). A panel of four 9-mer peptides was synthesized corresponding to the identified sequence regions. The CD8+ T cell responses to the selected peptides of MBP were examined in MS patients and controls using a split-well method previously described (6, 15). The initial attempts to detect CD8+ T cell responses to the MBP peptides in unfractonated PBMC yielded low frequencies of specific T cell isolates of mixed CD4 and CD8 phenotypes.

The approach to detecting CD8+ T cells reactive to MBP-derived peptides was subsequently improved by predepleting CD4+ T cells. The resulting PBMC fractions, depleted for CD4+ T cells, were cultured with selected MBP peptides in the presence of irradiated autologous PBMC as a source of inactivated CD4+ T cells that could provide helper function, but were unable to proliferate in responses to the peptides. CD8+ T cell wells/cultures scored positive for reactivity to the peptides of MBP were compared directly between the two groups as all individual experiments were
performed under the same conditions. The experiments were repeated ~12 mo later, with new samples obtained from the same MS patients and control subjects. When compared directly as the number of wells scored positive for each peptide, the results were consistent, as the differences between the two analyses were <15% for all peptides of MBP tested, and the differences between the patient group and the control group in the two analyses were similar. As shown in a representative analysis (Fig. 2), the total number of wells/cultures scored positive for the peptides of MBP was considerably increased in MS patients compared with that in control subjects, especially for MBP<sub>111–119</sub> and MBP<sub>87–95</sub> (p < 0.05). In contrast, the number of positive wells for tetanus toxoid, a recall Ag, did not differ significantly between the two groups. The findings indicate that CD8<sup>+</sup> T cells recognizing the selected peptides of MBP occur at an increased frequency in MS patients.

**Phenotypic characterization of resulting CD8<sup>+</sup> T cell lines isolated from MS patients**

A panel of 39 resulting CD8<sup>+</sup> T cell lines recognizing various MBP-derived peptides was selected to include 25 T cell lines from MS patients and 14 T cell lines from healthy controls and was representative of reactivity to all four MBP-derived peptides (Table II). They were first characterized for phenotypic expression and were found to express TCRαβ/CD8 (∼95%, on the average), but not CD4 (<5%), and CD45RO, but not CD45RA, regardless of their reactivity to the various MBP-derived peptides. The findings indicate that they belonged to the CD8<sup>+</sup> memory T cell subset. Four CD8<sup>+</sup> T cell lines were successfully cloned from three MS patients and were examined in detail. Two such cloned CD8<sup>+</sup> T cell lines recognized MBP<sub>111–119</sub> peptide (E11) and MBP<sub>87–95</sub> peptide (D10), respectively, in the context of HLA-A2, whereas two other CD8<sup>+</sup> T cell lines were A24 restricted and reacted with MBP<sub>134–142</sub> peptide (B9) and MBP<sub>14–22</sub> peptide (F12). Fig. 3 illustrates the representative phenotypic expression of the four cloned T cell lines described above. We then examined specific binding of an HLA-A2 tetramer to selected CD8<sup>+</sup> T cell lines. Only one tetramer (HLA-A2/MBP<sub>111–119</sub>) could be made successfully by a commercial source. As shown in Fig. 4, the HLA-A2/MBP<sub>111–119</sub> tetramer exhibited >90% specific binding to a CD8<sup>+</sup> T cell line (E11) recognizing peptide MBP<sub>111–119</sub>, but not to an A2<sup>+</sup> CD8<sup>+</sup> T cell line (D10) recognizing peptide MBP<sub>87–95</sub>

![FIGURE 4](image)

**Analysis of MHC class I tetramer for binding to cloned CD8<sup>+</sup> T cell lines by flow cytometry.** Two A2-restricted CD8<sup>+</sup> T cell lines that recognized MBP<sub>111–119</sub> peptide (E11) and MBP<sub>87–95</sub> peptide (D10) were analyzed by flow cytometry using an HLA-A2-PE-MBP<sub>111–119</sub> tetramer. The open profiles represent staining of T cells with a PE-conjugated control antibody. The solid profiles indicate staining of T cells with the tetramer in the same representative experiment.

![FIGURE 5](image)

**FIGURE 5.** The cytokine profile of CD8<sup>+</sup> T cells recognizing MBP-derived peptides. Cytokine production of the resulting CD8<sup>+</sup> MBP-reactive T cell lines derived from patients with MS (n = 25) and from normal subjects (NS; n = 14) was measured by ELISA. The T cell lines were challenged with the corresponding peptide, and the supernatants were tested after 48 h for concentrations of the indicated cytokines. The bars indicate the mean concentration (picograms per milliliter) ± SEM. The detection limit of the assays for all cytokines was <25 pg/ml.
lected CD8 T cell lines reactive to MBP-derived peptides against autologous target cells. Four representative CD8+ T cell lines reactive to MBP-derived peptides, E11 for MBP111–130, D10 for MBP85–95, B9 for MBP134–142, and F12 for MBP119–122, were examined for cytotoxicity in LDH release assays. A, CD8+ T cell lines were tested for cytotoxic activity against autologous target cells pulsed with corresponding peptides at the indicated effector (CD8+ T cells) to target (autologous EBV-transformed B cells) ratio. A synthetic 9-mer peptide corresponding to a unrelated TCR CDR3 sequence (STRQPQET) was used as a control. B, The same CD8+ T cell lines were analyzed for cytotoxicity against autologous target cells pulsed with different peptides of MBP. The same autologous target cells pulsed with the irrelevant TCR peptide served as a control peptide. The E:T cell ratio was 10.

FIGURE 6. Cytotoxic activity of CD8+ T cell lines recognizing MBP-derived peptides against autologous target cells. The same CD8+ T cell lines were used as a control. The selected CD8+ cytotoxic T cell lines were tested for cytotoxic activity in LDH release assays using COS cells transfected with human MBP and HLA-A2 genes. The selected CD8+ cytotoxic T cell lines were tested for cytotoxic activity in LDH release assays using COS cells transfected with human MBP and HLA-A2 genes. The E:T cell ratio was 10. Nontransfected COS cells were used as a control.

FIGURE 7. MHC restriction of CD8+ cytotoxic T cell lines. The selected CD8+ cytotoxic T cell lines recognizing MBP-derived peptides were tested for specific cytotoxicity against autologous target cells in the presence and the absence of mAbs to MHC class I (W6/32) and class II (HB55), used at a concentration of 20 μg/ml. The E:T cell ratio was 10 for all experiments. Data are expressed as the percent specific cytotoxicity. The procedure used is the same as that described in Fig. 6.

FIGURE 8. Cytotoxic activity of CD8+ T cell lines reactive to MBP-derived peptides against COS cells transfected with human MBP and HLA-A2 genes. The selected CD8+ cytotoxic T cell lines were tested for cytotoxic activity in LDH release assays using COS cells transfected with human MBP and HLA-A2 genes. The E:T cell ratio was 10. Nontransfected COS cells were used as a control.

Discussion
Unlike CD4+ MBP-reactive T cells that induce extensive CNS inflammation and mild demyelination in EAE, CD8+ CTLs recognizing MBP-derived peptides directly contribute to severe CNS demyelination in EAE, presumably through induction of injury of oligodendrocytes (4). The distinct role of these CD8+ CTLs is of particular relevance to MS where demyelination represents the most significant CNS pathology associated with neurologic deficits. There is evidence in the literature on the CD4+ T cell responses to candidate myelin Ags in MS and the preliminary therapeutic attempts to suppress or eliminate CD4+ myelin-reactive T cells (28, 29). In contrast, the frequency, the functional properties, and the potential role of CD8+ T cells recognizing myelin Ags in MS are virtually unknown. Part of the reasons for lack of advances in this area is related to technical difficulties in the detection and generation of CD8+ T cells reactive to myelin Ags, as we initially experienced in this study. In this study we developed an approach that allows effective generation of CD8+ T cells by predepleting CD4+ T cells in responder PBMC fractions and culturing them with irradiated unfractionated PBMC. The study has provided new insights into the role of CD8+ CTLs recognizing MBP-derived peptides in the pathogenesis of MS.

First, the study has demonstrated that, similar to CD4+ MBP-reactive T cells, CD8+ cytotoxic MBP-reactive T cells can also be detected in healthy individuals (6, 7, 15, 16). However, the CD8+ T cell responses to the identified MHC class I peptides of MBP are considerably elevated in MS patients compared with those in control subjects, indicating in vivo activation of CD8+ T cells recognizing the MBP peptides in MS patients. The observed differences appear to be more significant than those for CD4+ MBP-reactive T cells seen in MS patients and controls (6, 7, 16). It should also be noted that unlike CD4+ MBP-reactive T cells that are naïve T cells expressing both CD45RA and CD45RO (30), the CD8+ CTLs identified in this study belong to an Ag-experienced memory T cell subset expressing the CD45RO, but not the CD45RA, phenotype. The findings described in this study also suggest that these CD8+ CTLs recognizing MBP-derived peptides may undergo in vivo activation in MS patients. In this regard, there is increasing evidence indicating that MBP-reactive T cells can be activated by...
a variety of microbial Ags through the mechanism known as molecular mimicry (31–34). Recently, we identified sequence homology between HHV-6, a suspected etiologic agent for MS, and MBP and demonstrated that CD4+ T cells cross-reactive with both Ags are sensitized in MS patients as opposed to healthy individuals (34). Although the regions of MBP identified in this study do not share complete sequence homology with myelin proteins, TCR degeneracy seen in MBP-reactive T cells may render them cross-reactive with microbial antigenic peptides of incomplete sequence match as long as the TCR contact residues required for T cell recognition are preserved (35–37).

Furthermore, although the cell culture-based, split-well method used in this study has been proven useful in comparing the frequency of CD4+ myelin-reactive T cells between individual samples when used consistently (6, 15, 16, 28, 38), it is problematic to calculate the actual precursor frequency of CD8+ CTLs in this study. Consequently, direct comparison of specific CD8+ T cell wells/cultures was made between MS patients and controls to discern differences in the CD8+ T cell responses to the selected peptides of MBP. The split-well method may have considerably different sensitivity from other existing methods for the analysis of T cell recognition, as shown by Huseby and colleagues (22) who reported the specificity of human T lymphocyte lines of different HLA restriction molecules.

In this study further characterization has confirmed that these CD8+ T cells recognizing MBP-derived peptides are cytotoxic in nature. They recognize and are cytotoxic toward both autologous target cells pulsed with the MBP peptides and endogenously processed MBP in the context of MHC class I molecules, as evidenced in a series of experiments involving COS cells doubly transfected with HLA-A2 and human MBP genes. This finding is of particular importance in view of a potential role of CD8+ CTLs in the injury of oligodendrocytes that express both class I molecules and MBP. The findings are in agreement with an earlier study by Jurewicz and colleagues (22) who reported the specific cytotoxicity of CD8+ MBP110–118-reactive T cells toward A2+ human oligodendrocytes. The CD8+ CTLs reactive to MBP-derived peptides as described in this study are reminiscent of CD8+ T cells of similar functional properties in EAE, which are able to induce extensive CNS demyelination potentially through specific recognition and cytotoxic activity toward oligodendrocytes (4). Further investigation is warranted to define the mechanism underlying in vivo activation and the functional role of CD8+ cytotoxic myelin-reactive T cells in the disease process of MS.

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References


