Aberrant T cell responses to myelin antigens during clinical exacerbation in patients with multiple sclerosis

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Keywords: myelin, multiple sclerosis, T cells

Abstract

Multiple sclerosis (MS) is a demyelinating disease of presumed T cell autoimmunity against self myelin. We hypothesized that if myelin-reactive T cells are associated with the disease processes, they may undergo activation and expansion during acute exacerbation. In this study, we examined the precursor frequency, epitope recognition and cytokine profile of myelin-reactive T cells in 14 relapsing/remitting MS patients during exacerbation and remission. The study revealed that T cells recognizing the immunodominant peptides of candidate myelin antigens, including myelin basic protein (MBP), proteolipid protein and myelin oligodendrocyte glycoprotein, occurred at increased precursor frequency during acute exacerbation. The T cell responses to MBP focused on the immunodominant regions (residues 83–99 and 151–170) during exacerbation and shifted toward other epitopes of MBP at the time of remission. Furthermore, there was a marked increase in the production of Th1 cytokines among T cell lines obtained during exacerbation compared to those obtained during remission. The study demonstrated that myelin-reactive T cells underwent selective activation and expansion during acute MS exacerbation. In contrast, myelin-reactive T cells found during remission in the same patients generally resembled those identified in healthy controls with some discrepancies. The findings suggest potential association of aberrant myelin-reactive T cell responses with acute exacerbation in MS, which may reflect transient activation of myelin-reactive T cell populations of pathogenic potential.

Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system characterized pathologically by focal infiltration of inflammatory cells in the white matter (1). There are two basic clinical forms of MS, relapsing/remitting and chronic progressive MS, which exhibit distinct features (2–4). The relapsing-remitting form of MS is characterized clinically by episodic worsening (exacerbation) and recovery of neurologic functions (remission). Although the etiology and pathogenesis of MS is unknown, there is increasing evidence suggesting that the T cell responses to myelin antigens may play an important role in the disease processes (5–9). There are at least three candidate myelin antigens, i.e. myelin basic protein (MBP), proteolipid protein (PLP) (10) and myelin oligodendrocyte glycoprotein (MOG) (11,12), that have been implicated in the pathogenesis of MS for their ability to induce experimental autoimmune encephalomyelitis (EAE), an animal model for MS (13,14). It has been demonstrated that only a limited number of epitopes on these myelin antigens are encephalitogenic and associated with the induction of EAE (14). T cells recognizing a variety of epitopes, including the encephalitogenic epitopes, are present as part of the normal T cell repertoire, exhibiting a diverse clonal distribution in naive animals (15). It has been demonstrated that the diversity in clonal distribution of MBP-reactive T cells is lost in EAE.
Immunization with the whole MBP induces selective activation and expansion of encephalitogenic T cell populations, resulting in a skewed pattern of clonal distribution toward the encephalitogenic epitopes (16). This clonal distribution pattern characteristic of EAE returns to its pre-clinical diversity when animals recover from the disease.

Substantial T cell responses to all three myelin antigens have been reported in patients with MS (7,17–23). In a recent study, the frequency of T cells recognizing an immunodominant epitope was found to be 1/300–1/1000 in the blood of patients with MS (24). There is preliminary evidence that the TCR-CDR3 sequence of MBP-reactive T cells derived from MS patients was present in post-mortem MS lesions (25). However, these myelin-reactive T cells can also be isolated from normal individuals (7,9,17,18,26,27), suggesting that myelin-reactive T cells are part of the normal T cell repertoire. Furthermore, the T cell responses to these candidate myelin antigens are considerably heterogeneous in the epitope recognition among different individuals with MS (7–9,28–31). Nevertheless, some regions of MBP and PLP, including residues 83–99 and 151–170 for MBP and residues 30–49 and 181–199 for PLP, are preferentially recognized in patients with MS (6,7,10,19,32), and the immunodominant properties of residues 83–99 and 151–170 of MBP are associated with their high binding affinity to HLA-DR2 (33). One of the important discrepancies between myelin-reactive T cells in MS patients and those in normal individuals is the activation state of the T cells. MBP-reactive T cells are found to undergo in vivo activation and clonal expansion in patients with MS, as opposed to healthy individuals (6,34,35).

It was hypothesized that if myelin-reactive T cells were associated with acute exacerbation, they would exhibit characteristic changes in the precursor frequency and the functional properties during exacerbation as a result of selective in vivo activation and expansion. This study was undertaken to address these issues by analyzing the precursor frequency, epitope recognition and cytokine profile of myelin-reactive T cells during acute exacerbation, and comparing them with those occurring during remission in the same patients. Seven healthy individuals were examined in parallel. The study revealed that aberrant T cell responses to the candidate myelin antigens correlated with acute exacerbation in MS patients. The myelin-reactive T cells occurred at increased precursor frequency in MS patients during acute exacerbation, and exhibited distinct functional properties and epitope recognition as compared to those obtained from the same patients during remission. The findings described in this study provide new insights into the potential role of myelin-reactive T cells in the pathogenesis of MS and encourage further investigation to identify myelin-reactive T cells associated with disease activity in MS.

Methods

Reagents and peptides

Human MBP was purified from the white matter of the human brain by the method previously described by Deibler et al. (36). Media used for cell culture were AIM-V serum-free medium (Gibco/BRL, Grand Island, NY), and RPMI 1640 supplemented with l-glutamine, sodium pyruvate, non-essential amino acids, 10 mM HEPES buffer (Gibco), 10% (v/v) FBS (Gibco) and 1% penicillin/streptomycin (Gibco). Recombinant human IL-2 (rIL-2) was purchased from Boehringer Mannheim (Indianapolis, IN).

A panel of overlapping peptides of human MBP (residues 83–99 and 151–170) was synthesized by the Merrifield solid-phase method and purified by HPLC (courtesy of Dr Stefan Boheme, Neurocrine Biosciences). Two peptides of human PLP (residues 30–49 and 180–199) and a MOG peptide (residues 41–60) were synthesized by Chiron (San Diego, CA). The purity of all peptides used in this study was >95%.

Patient selection and clinical criteria

Fourteen patients with clinically definite relapsing-remitting MS (six males and eight females) were recruited in the study. The diagnosis was made based on standard clinical manifestations and laboratory studies confirmed by magnetic resonance imaging (3,4). All patients were characterized as having relapsing-remitting MS for >2 years. The patients were not treated with immunosuppressive or immunomodulatory agents for at least 2 months prior to entry. Patients were recruited at the time of an acute exacerbation according to the clinical criteria routinely used in MS studies (37,38). Briefly, an acute exacerbation was defined as the appearance of new symptoms or worsening of old symptoms consistent with MS with objective signs sufficiently severe to produce a deterioration of 1 point on expanded disability scale score (EDSS). The symptoms lasted for at least 24 h, which were not attributable to other medical conditions of the patients. Informed consent was obtained from the patients after explaining the experimental procedures. The protocol was approved by the Institutional Human Subjects Committee at Baylor College of Medicine. Seven asymptomatic healthy individuals were recruited as control subjects.

Blood specimens were obtained from patients at the time of acute exacerbation within 2–4 days following the onset of new or worsened neurologic deficits and before any treatment was initiated. Sixty percent of the patients received a 3-day course of methylprednisolone (1 g, i.v. daily) and the remaining patients opted to defer the treatment in fear of side effects. No other immunosuppressive agents were used. Blood specimens corresponding to remission were obtained 2–3 months following the onset of acute exacerbation when all patients returned to their baseline neurologic functions as determined clinically by a neurologist.

HLA-DR2 typing

Total cellular RNA was extracted from peripheral blood mononuclear cell (PBMC) specimens using the RNeasy mini kit (Qiagen, Santa Clarita, CA). RNA was reverse transcribed to first-strand complementary DNA (cDNA) using an oligo(dT) primer and the superscript pre-amplification system (Gibco, Gaithersburg, MD). cDNA was amplified by PCR using oligonucleotide primers specific for DRB1*1501 as described elsewhere (39). Briefly, 1 µl cDNA was added into the following amplification mixture: 5 µl of 10×PCR buffer II (100 mM Tris–HCl, pH 8.3, 500 mM KCl), 3 µl of 25 mM magnesium chloride, 1 µl of 10 mM dNTP mix, 0.3 µl of Taq polymerase (5 U/µl) (AmpliTaq Gold; Perkin-Elmer, Norwalk, CT), 10 pmol of a
specific primer as the forward primer and 10 pmol of a specific primer as the reverse primer. The amplification profile used was 1 min at 95°C for denaturation, 20 s at 65°C for annealing and 40 s at 72°C for extension in a total of 30 cycles. The amplified PCR products were separated on a 1% agarose gel by electrophoresis and stained with ethidium bromide for visualization.

Estimation of the precursor frequency and generation of myelin-reactive T cell lines from PBMC

PBMC were isolated from heparinized venous blood by Ficoll density gradient separation and washed 3 times with sterile HBSS (Gibco). PBMC were seeded at 2×10⁵ cells/well in a 96-well, U-bottomed plates (Costar, Cambridge, MA) in the presence of various myelin antigens respectively. The myelin antigens used in this study included the whole human MBP (40 µg/ml) and five myelin peptides, including MBP83–99, MBP151–199, PLP30–49, PLP180–199 and MOG40–61. All peptides were used at a concentration of 10 µg/ml. Hen egg lysozyme (HEL) was used as a control antigen (40 µg/ml). The total number of wells for each peptide varied from 24 to 32. Seven days later, the cultures were re-stimulated with corresponding peptides in the presence of 10⁵ irradiated (6000 rad) autologous PBMC as a source of antigen-presenting cells. IL-2 (50 IU/ml) was added 48 h later to supplement T cell growth. Two weeks later, each culture was examined for specific proliferation in response to the corresponding peptide in a proliferation assay. Briefly, each well was split into four aliquots and cultured in duplicate with 10⁵ irradiated autologous PBMC in the presence and the absence of the corresponding peptide. Culture supernatants were collected after 48 h for cytokine ELISA. The remaining cultures were maintained for an additional 24 h and pulsed subsequently with [³H]thymidine (Amersham, Arlington Heights, IL) at 1 µCi/well during the last 16 h of culture. Cells were then harvested using an automated cell harvester (Tomtec, Orange, CT) and [³H]thymidine incorporation was measured in a β-counter.

A T cell line was defined as specific for MBP or a peptide when c.p.m. > 1000 and exceeded the reference c.p.m. (in the absence of the antigen) by at least 3 times. The frequency of peptide-reactive T cells was then estimated by dividing the number of positive wells by the total number of PBMC seeded in the initial culture (6,40). The same calculation was used consistently in all experiments.

Cytokine quantification by ELISA

Cytokines were determined quantitatively in culture supernatants using ELISA kits obtained from PharMingen (San Diego, CA). The kits were used according to the manufacturer’s instructions. Microtiter plates (96-wells, Maxisorp; Nunc, Roskilde, Denmark) were coated overnight at 4°C with 2 µg/well of a purified mouse capturing mAb to human cytokine [IL-4, IL-10, tumor necrosis factor (TNF)-α and IFN-γ] (PharMingen, San Diego, CA) in 100 µl of a carbonate buffer (100 mM, pH 9.5). Plates were washed with PBS (pH 7.0) containing 0.05% Tween 20 (PBS/T). Non-specific binding sites were saturated with 10 % (w/v) FBS in PBS (FBS/PBS) for 1 h and washed subsequently with PBS/T. Supernatants and cytokine standards were diluted with PBS and added in duplicate wells. Plates were incubated at 4°C overnight and subsequently washed 5 times with PBS/T. Then 100 µl of the matched biotinylated detecting antibody (0.5 µg/ml for IL-4 and IL-10 and 1 µg/ml for IFN-γ and TNF-α; PharMingen) were added to each well and incubated at room temperature for 2 h. After washing, avidin-conjugated horseradish peroxidase (1:5000 dilution) was added and plates were incubated for 1 h. Plates were then washed and 3,3′,5,5′-tetramethylbenzidine/1.2 mM H₂O₂ in citrate buffer (pH 5.0) was used as a substrate for color development. The reaction was stopped by adding 1N HCl. Optical density was measured at 450 nm by using an ELISA reader (BioRad, Hercules, CA) and cytokine concentrations were quantitated by Microplate computer software (BioRad) using a double eight-point standard curve. The detection limit of the assays was <30 pg/ml for all cytokines.

Statistical analysis

A Student’s t-test (for normally distributed variables) and the Mann-Whitney rank-sum test (for non-normally distributed variables) was used for data analysis. P < 0.05 was considered statistically significant.

Results

The estimated frequency of myelin-reactive T cells in the blood of MS patients and control subjects during acute exacerbation and remission

Fourteen patients with definite relapsing-remitting MS (age range 33–66) were included in this study. The clinical
Characteristics and the HLA-DR2 (DRB1*1501) status of the patients are shown in Table 1. The mean EDSS of the patient group was 4.5 (range 2.5–6.0). To address whether acute exacerbation is associated with activation and expansion of myelin-reactive T cells, the precursor frequency of myelin-reactive T cells was analyzed at two time points, corresponding to acute exacerbation (2–4 days following acute onset) and remission (2–3 months after acute exacerbation). A group of seven healthy volunteers (age range 26–56) was examined in the same experimental setting at two time points. The myelin antigens used in this study included the whole human MBP, two peptides of MBP (residues 83–99 and 151–170), two peptides of human PLP (residues 31–49 and 180–199) and a MOG peptide (residues 41–60). These myelin peptides were previously shown to represent immunodominant regions of the candidate myelin antigens in patients with MS (6,7,10,19,22,23). Some of these peptides are encephalitogenic in rodents (14,41).

As shown in Fig. 1(A), T cells recognizing MBP and the immunodominant myelin peptides occurred at an increased frequency at the time of acute exacerbation, and the T cell frequency declined substantially during remission in patients with relapsing-remitting MS ($P < 0.05$). The mean frequency of T cells was $1.55 \times 10^{-6}$ for the whole MBP molecule, $1.80 \times 10^{-6}$ for MBP83–99 and $2.74 \times 10^{-6}$ for MBP151–170, during exacerbation. The estimated frequency of MBP-reactive T cells reduced to $0.93 \times 10^{-6}$ for MBP, $0.34 \times 10^{-6}$ for MBP83–99 and $0.38 \times 10^{-6}$ for MBP151–170, during remission.

Similarly, as illustrated in Fig. 1(B and C), the frequency of T cells recognizing the PLP and MOG peptides was also elevated during acute exacerbation ($1.78 \times 10^{-6}$ for PLP30–49, $1.97 \times 10^{-6}$ for PLP180–199 and $1.99 \times 10^{-6}$ for MOG41–60) and decreased significantly during remission ($0.45 \times 10^{-6}$ for PLP30–49, $0.43 \times 10^{-6}$ for PLP180–199 and $0.53 \times 10^{-6}$ for MOG41–60) in the same patients. In contrast, the frequency of T cells specific for HEL (a control antigen) did not differ significantly between acute exacerbation and remission (mean frequency $0.9 \times 10^{-6}$ versus $0.8 \times 10^{-6}$) in eight patients examined. There was no significant correlation between the expression of DRB1*1501 and the frequency of T cells recognizing any of the myelin antigens in this group of patients.

We then examined whether myelin-reactive T cells would undergo a similar change in the precursor frequency in healthy individuals. As shown in Fig. 2, the frequency of T cells reactive to the myelin antigens was generally lower ($<1.0 \times 10^{-6}$) than that in MS patients during acute exacerbation and was highly comparable to that seen in the MS patients during remission. No statistical difference in the precursor frequency of myelin-reactive T cells was detected between the two time points (2–3 months apart), even though...
Myelin-reactive T cell responses during exacerbation in MS

Fig. 2. Estimated frequency of myelin-reactive T cells in healthy individuals. The precursor frequency of myelin-reactive T cells was estimated in a group of seven healthy individuals at two time points (2–3 months apart). As in Fig. 1, the bars in the upper panels represent the mean frequency ± SEM during entry (solid) and 2–3 months later (open bars) respectively. The lower panels indicate the changes in individual subjects between the first (filled circles) and second time point (open circles).

The T cell frequency varied slightly in some healthy individuals examined. Taken together, the results suggest correlation of transient activation and expansion of myelin-reactive T cells with acute exacerbation in patients with relapsing-remitting MS.

Shifts in dominant epitope recognition among MBP-reactive T cells obtained during acute exacerbation and remission

It is of considerable interest to address whether myelin-reactive T cells undergo shifts in epitope recognition during acute exacerbation and remission, and whether such shifts are associated with certain myelin antigen(s) or peptide(s). The combined use of the whole MBP molecule containing all potential epitopes with the two immunodominant MBP peptides allowed us to discern whether shifts occurred between the immunodominant epitopes and potential epitopes other than those within the 83–99 and 151–170 regions of MBP. As illustrated in Fig. 3(a), during acute exacerbation, the T cell reactivity to MBP was predominantly directed at the immunodominant regions, with the 151–170 region being the most dominant in the majority of the patients, while the reactivity to the MBP molecule was less dominant. This pattern of epitope recognition appeared to shift toward the whole MBP molecule among MBP-reactive T cells occurring during remission (Fig. 3a). The results suggest that in the case of MBP, the T cell reactivity was focused predominantly on the immunodominant regions during acute exacerbation and shifted toward other epitope(s) of MBP. Furthermore, as shown in Fig. 3(b), in healthy individuals, MBP-reactive T cells were found to react predominantly with the whole MBP molecule, generally resembling those derived from MS patients during remission. The pattern of epitope recognition of myelin-reactive T cells seen in healthy individuals remained essentially the same between the two time points (2–3 months apart).

Cytokine profile of myelin-reactive T cell lines obtained during acute exacerbation and remission

Next, we examined whether myelin-reactive T cells occurring during acute exacerbation would exhibit a different cytokine profile from those obtained from the same patients during remission. To this end, a panel of 650 short-term myelin-reactive T cell lines, including 402 lines obtained during acute exacerbation and 248 lines obtained during remission, were assayed for the production of both Th1 (IFN-γ, TNF-α) and Th2 (IL-4, IL-10) cytokines. The culture supernatants were collected 48 h after the T cell lines were challenged with corresponding antigens and measured for the absolute concentration of the cytokines in ELISA. The distribution of the epitope specificity of the two panels of myelin-reactive T cell lines examined for the cytokine profile was representative of those shown in Fig. 1, with the T cell lines specific for the immunodominant myelin peptides being the dominant population in acute exacerbation. The majority of the MBP-
Fig. 3. The recognition pattern of myelin-reactive T cell lines. The T cell reactivity to MBP and the myelin peptides is expressed as a percentage by dividing the estimated precursor frequency of T cells specific for the indicated antigen (a myelin peptide or MBP) by the total precursor frequency of myelin-reactive T cells found in a given MS patient during exacerbation and remission (a), and healthy individuals between two time points (b).

reactive T cell lines obtained during remission were predominantly directed at the whole MBP molecule.

As a group, the myelin-reactive T cell lines obtained during acute exacerbation produced significantly increased amounts of IFN-γ and TNF-α as compared to these derived from the same patients during remission as well as healthy individuals. The production of IL-4 in MBP-reactive T cells recognizing the whole MBP and the 150–170 region was slightly higher during exacerbation than that during remission in MS patients (Table 2). The IL-4 production of MS-derived MBP-reactive T cells was lower than that derived from healthy individuals. The production of IL-10 was not changed in any of the panels of myelin-reactive T cells. Furthermore, a panel of 85 HEL-specific T cell lines derived from MS patients during acute
Myelin-reactive T cell responses during exacerbation in MS

Table 2. Average cytokine concentrations (pg/ml) produced by myelin-reactive T cell lines

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Antigen No. T cell lines</th>
<th>IL-10</th>
<th>IL-4</th>
<th>TNF</th>
<th>IFN</th>
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</thead>
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<tr>
<td>MS-RR exacerbation</td>
<td>whole MBP 66</td>
<td>38 ± 5</td>
<td>49 ± 8</td>
<td>491 ± 12</td>
<td>1051 ± 217 a</td>
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<td></td>
<td>MBP38-99 91</td>
<td>38 ± 4</td>
<td>68 ± 14</td>
<td>908 ± 18 a</td>
<td>956 ± 163 a</td>
</tr>
<tr>
<td></td>
<td>MBP150-170 92</td>
<td>185 ± 37</td>
<td>53 ± 10 b</td>
<td>984 ± 26 a</td>
<td>1646 ± 227 a,b</td>
</tr>
<tr>
<td></td>
<td>PLP30-49 53</td>
<td>66 ± 10</td>
<td>35 ± 6</td>
<td>610 ± 15 a,b</td>
<td>909 ± 153 a,b</td>
</tr>
<tr>
<td></td>
<td>PLP180-199 47</td>
<td>64 ± 11</td>
<td>53 ± 11</td>
<td>1154 ± 58 a,b</td>
<td>1690 ± 176 a,b</td>
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<tr>
<td></td>
<td>MOG41-60 53</td>
<td>67 ± 8</td>
<td>111 ± 28</td>
<td>392 ± 74</td>
<td>956 ± 181 a,b</td>
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<td>MS-RR remission</td>
<td>whole MBP 71</td>
<td>38 ± 4</td>
<td>28 ± 9 b</td>
<td>231 ± 50</td>
<td>84 ± 18 b</td>
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<tr>
<td></td>
<td>MBP38-99 31</td>
<td>38 ± 12</td>
<td>31 ± 5</td>
<td>113 ± 25 b</td>
<td>85 ± 20 b</td>
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<tr>
<td></td>
<td>MBP150-170 34</td>
<td>31 ± 4</td>
<td>26 ± 7 b</td>
<td>164 ± 30</td>
<td>91 ± 28 b</td>
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<td>MOG41-60 44</td>
<td>38 ± 6</td>
<td>29 ± 9 b</td>
<td>220 ± 30</td>
<td>118 ± 40</td>
</tr>
<tr>
<td>Healthy subjects</td>
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<td>143 ± 14</td>
<td>276 ± 43</td>
<td>500 ± 75</td>
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<td>65 ± 20</td>
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<td></td>
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<tr>
<td></td>
<td>MOG41-60 19</td>
<td>26 ± 9</td>
<td>196 ± 5</td>
<td>181 ± 40</td>
<td>189 ± 6</td>
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aStatistically significant (P < 0.05) MS-RR exacerbation versus MS-RR remission.
bStatistically significant (P < 0.05) MS versus healthy subjects

Exacerbation and remission were found to display a Th0-like cytokine profile. The average cytokine concentrations were 110 ± 26 versus 155 ± 15 pg/ml for IL-10, 97 ± 7 versus 107 ± 13 pg/ml for IL-4, 332 ± 37 versus 398 ± 45 pg/ml for TNF-α and 111 ± 6 versus 108 ± 14 pg/ml for IFN-γ respectively in myelin-reactive T cell lines derived during acute exacerbation and remission. However, the changes in the cytokine concentrations between the two time points were not statistically significant (P > 0.05).

Discussion

The role of myelin-reactive T cells in the pathogenesis of MS remains speculative despite extensive research effort in this area (5–8, 22, 23). One of the approaches to defining the potential role of myelin-reactive T cells is to examine the precursor frequency in patients with MS, which provides an indication for selective in vivo activation and expansion of some subsets of myelin-reactive T cells. To date, the results accumulated from studies involving randomly recruited patients with chronic progressive and relapsing-remitting MS indicate that T cells recognizing MBP and PLP occur at a slightly higher or similar precursor frequency in MS patients as compared to control subjects (7,18,27). In this study, we took a different approach by analyzing the precursor frequency and functional properties of myelin-reactive T cells occurring during acute exacerbation and remission. We demonstrated for the first time that a high precursor frequency of myelin-reactive T cells correlated with acute exacerbation in relapsing-remitting MS. The precursor frequency of myelin-reactive T cells detected during acute exacerbation is also higher than that reported previously in randomly recruited MS patients (6–8). The increased precursor frequency of T cells correlated with reactivity to the immunodominant peptides of the candidate myelin antigens, most noticeably the 150–170 peptide, as well as Th1 cytokine profile at the time of exacerbation.

The observed change in the precursor frequency of myelin-reactive T cells is unlikely attributable to the inhibitory effect of the steroids given to some of the patients as a standard treatment for acute exacerbation since the treatment was very brief (a 3-day course) and the subsequent frequency analysis was 2–3 months following the treatment. In this study, the wash-out period for steroids treatment was significantly longer than that routinely used in the MS trials (30 days) (38, 42, 37). Furthermore, in patients who deferred the treatment for various reasons, the precursor frequency of myelin-reactive T cells was similar to that seen in the treated patients. Selective activation and expansion of myelin-reactive T cells during acute exacerbation is contrasted by the observation that T cells recognizing a control antigen (HEL) did not undergo a similar change in the same patients between two time points. It is likely that episodic activation and expansion of myelin-reactive T cells may be related to triggering events, such as clinically overt or subtle viral infection (31), correlating with the relapsing-remitting features of the disease. For example, several infectious agents, including human herpes virus-6 (HHV-6), have been found to correlate with clinical exacerbation in relapsing-remitting MS (43). Activation and expansion of myelin-reactive T cells may in turn trigger immune regulation through various peripheral regulatory mechanisms (e.g. apoptosis and clonal anergy), which down-regulate activated myelin-reactive T cells shortly after exacerbation (10, 44, 45). However, in the absence of the TCR V gene analysis, it is not clear whether the increased precursor frequency reflects the expansion of the existing myelin-reactive T cell populations or whether it is attributable to in vivo activation of distinct myelin-reactive T cell subsets. In addition to the increased T cell precursor frequency during acute exacerbation, the T cell responses to the myelin
antigens undergo characteristic shift in epitope recognition. It is of interest to note that the C-terminal MBP151–170 peptide represents the most dominant epitope of MBP in the majority of patients examined during acute exacerbation. The results suggest that there may be selective activation and expansion of T cells recognizing the immunodominant myelin peptides at the time of exacerbation. During remission, the pattern of epitope recognition shifted largely toward MBP, resembling that found in the normal T cell repertoire in healthy individuals. There are several related issues that need further investigation. First, although the combined use of the whole MBP (as a substitute for all potential epitopes) and the two immunodominant peptides of MBP helped to discern shift in the recognition of the immunodominant regions during acute exacerbation and other epitopes outside these regions during remission, it did not distinguish whether the T cell recognition of the whole MBP during remission was directed at one or few epitope(s) outside the 83–99 and 151–170 regions or whether it represented diverse responses to a variety of potential epitopes. Further study is needed to clarify the issue using overlapping peptides of the myelin antigens. Second, it remains to be determined in future investigations whether activation and expansion of myelin-reactive T cells is characteristic of relapsing-remitting MS and whether chronic progressive MS patients exhibit a distinct pattern of shift in the precursor frequency of myelin-reactive T cells and the epitope recognition. It is conceivable that epitope shift described here during acute exacerbation and remission in MS patients may be different from the phenomenon of epitope spreading originally demonstrated in EAE as the majority of MS patients had already undergone a lengthy clinical course prior to the study. However, epitope spreading among candidate myelin antigens may be detectable in MS if patients are monitored at the early stage of the disease as suggested in recent studies by Tuohy et al. (46,47).

In addition to the changes in the precursor frequency, myelin-reactive T cells occurring during exacerbation and remission exhibited a distinct cytokine profile. Myelin-reactive T cells derived during acute exacerbation produce increased amounts of pro-inflammatory cytokines (IFN-γ and TNF-α) resembling a Th1-like profile as compared to those during remission and in healthy individuals, while the production of IL-4 was decreased in MBP-reactive T cells during acute exacerbation. The cytokine profile of myelin-reactive T cells associated with acute exacerbation is highly relevant to the inflammatory potential of the T cells. There is evidence suggesting that Th1 cytokines (e.g. IFN-γ and TNF-α) are associated with the worsening of the disease (48–50). For example, administration of IFN-γ was shown to induce clinical exacerbation in patients with MS (51). On the other hand, the Th2 cytokines, including IL-4 and IL-10, are thought to be beneficial for MS. The therapeutic benefit of IFN-β in MS is at least partially attributable to the enhanced production of IL-4 and IL-10 (52,53). It is conceivable that these Th1-like myelin-reactive T cells occurring during acute exacerbation have the migratory advantage and may release pro-inflammatory cytokines locally within the central nervous system.

Taken together, the study described herein provides new evidence suggesting that activation of myelin-reactive T cells is associated with acute exacerbation in patients with relapsing-remitting MS and these cells exhibit a distinct cytokine profile from those occurring during remission. The findings consistently suggest that myelin-reactive T cells detected in MS patients during remission are similar to those identified in the normal T cell repertoire in healthy individuals. The study provides critical information for further investigating the potential role of myelin-reactive T cells in MS. For example, it is important to further delineate whether myelin-reactive T cells associated with recurrent exacerbation would express identifiable markers (e.g. TCR V gene usage and CD83 sequence motifs). Furthermore, the findings described here have practical implications in the development of effective immunotherapy for MS, reinforcing the importance in selecting pathologically relevant myelin-reactive T cells for specific immunotherapy, such as T cell vaccination and TCR peptide vaccination. In this regard, specific depletion or suppression of the myelin-reactive T cells associated with clinical exacerbation is potentially more efficacious. Conversely, elimination of the ‘house-keeping’ T cell population representing the normal T cell repertoire is not only ineffective and may be harmful because of their potential regulatory functions (e.g. IL-4 and IL-10) that are not well understood at the present time.

Acknowledgements
This work was supported by the National Multiple Sclerosis Society (RG 2871) and in part by the National Institutes of Health (NS 36140). We thank Dr Dennis Mosier for critical review of the manuscript. Y. C. Q. Z. is the recipient of an advanced postdoctoral fellowship award from the National Multiple Sclerosis Society (FA 1282).

Abbreviations
EAE experimental autoimmune encephalomyelitis
EDSS expanded disability scale score
HEL hen egg lysozyme
MBP myelin basic protein
MOG myelin oligodendrocyte glycoprotein
MS multiple sclerosis
PBMC peripheral blood mononuclear cells
PLP proteolipid protein
TNF tumor necrosis factor

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