Selective loss of a subset of T helper cells in active multiple sclerosis

(Received for publication, July 1, 1985; accepted, July 29, 1985)

LYNN MASSMAN ROSE†‡, ARTHUR H. Ginsberg§, TED L. ROTHSTEIN§, JEFFREY A. LEBETTER*, and EDWARD A. CLARK†‡

*Immunology Group, Genetic Systems Corporation, †Northwest Hospital, and the ‡Department of Microbiology and Immunology, University of Washington, Seattle, WA 98195

Communicated by Leonard A. Herzenberg, July 1, 1985

ABSTRACT Patients with active multiple sclerosis (MS) have a selective loss of a subset of T helper cells (T<sub>H</sub>), detectable by two-color fluorescence-activated cell sorter analysis of peripheral blood lymphocytes. By using pairs of monoclonal antibodies to the T-cell subset markers CD4<sup>+</sup> and CD8<sup>-</sup> (T suppressor/cytotoxic cell (T<sub>S</sub>)) and the common leukocyte markers Lp220<sup>+</sup> and Lp95-150<sup>+</sup>, five phenotypically distinct T-cell subsets have been identified in peripheral blood: two CD4<sup>+</sup> T<sub>H</sub> cell subsets and three CD8<sup>-</sup> T<sub>S</sub> cell subsets. The frequencies and absolute numbers of these five populations were measured in patients with active and inactive MS and were compared with those in healthy age-matched controls and in patients with other neurologic diseases. A high frequency of patients with active MS (80%) had a selective reduction of one T<sub>H</sub> subset (CD4<sup>+</sup> Lp220<sup>+</sup>) compared with normal controls (P < 0.001) or patients with inactive MS (P < 0.001). Three patients examined sequentially had a further loss of the Lp220<sup>+</sup> T<sub>H</sub> subset as disease activity progressed. The proportion of two T<sub>S</sub> subsets was also abnormal in patients with active MS, but this defect was not restricted to that group. Total T<sub>H</sub> and T<sub>S</sub> cell frequencies and T<sub>H</sub>/T<sub>S</sub> ratios were not significantly different between patient and normal control groups. Thus, two-color analysis of T-cell subsets may be a more sensitive indicator than conventional single-marker assays of abnormal immune status in MS patients.

Recent studies have suggested that multiple sclerosis (MS) may be a disorder of immune regulation in which effector and regulatory T-cell populations are altered (1-5). Several studies have found a reduction in both the number and activity of T suppressor/cytotoxic (T<sub>S</sub>) cells in MS patients that may correlate with disease activity (6, 7). The ratio of T helper/inducer (T<sub>H</sub>) cells to T<sub>S</sub> cells (T<sub>H</sub>/T<sub>S</sub> ratio) has been used extensively to assess abnormalities in MS patients, with varying results. In several studies, MS patients with active disease were found to have a greater frequency of abnormal T<sub>H</sub>/T<sub>S</sub> ratios (6, 7), but this is not a consistent finding (8, 9). No clear consensus has been reached on the significance of changes in lymphocyte subsets associated with MS.

Improvements in multiparameter fluorescence-activated cell sorter (FACS) methods have made it possible to define lymphocyte subsets more readily (10-12). Subpopulations of B cells (13, 14), natural killer (NK) cells (15), and regulatory T cells (16) can be quantitated based on the relative expression of two or more cell-surface markers. This approach has been used to define abnormal lymphocyte subsets in immunodeficient and autoimmune mice (14, 17). Using pairs of monoclonal antibodies (mAb) to the CD4 and CD8 T-cell subset markers and to the common leukocyte antigens Lp220 and Lp95-150, we were able to define five distinct T-cell subsets in this study. A comparison between MS patients and controls revealed that patients with active MS have a selective loss of one T<sub>H</sub> subset (CD4<sup>+</sup> Lp220<sup>+</sup>). This CD4<sup>+</sup> T<sub>H</sub> subset did not possess the majority of helper function for B-cell antibody production. The frequency of this subset decreased in three patients as disease activity increased, suggesting that loss of Lp220<sup>+</sup> CD4<sup>+</sup> cells may relate to a worsening clinical status. One T<sub>S</sub> subset was depleted in active MS patients, but this defect was also seen in other neurologic diseases. No differences in conventional single-parameter T<sub>H</sub>/T<sub>S</sub> ratios were evident, suggesting that two-color FACS analysis may be a more sensitive method for monitoring abnormal immune status.

METHODS

Patients. All patients with MS satisfied the Schumacher criteria (18) for clinically definite disease. Those with inactive MS had been clinically stable for 6 months or longer, with an overall disability rating of <5 on the Kurtzke disability status scale (19). Those with active MS had acute exacerbations, defined as the report of new symptoms persisting >24 hr, that correlated with an objective change in the standard neurologic examination. Evanescent symptoms involving sensory or motor modalities that could not be objectively verified were not considered to represent a true exacerbation. Blood was obtained within 5 days of the onset of new symptoms. Normal control subjects consisted of hospital and laboratory personnel. Neurologic disease controls consisted of the following patient groups: Parkinson disease (n = 8), amyotrophic lateral sclerosis (n = 3), myasthenia gravis (n = 2), Alzheimer disease (n = 2), cerebral vascular accident (n = 2), alcoholic or diabetic neuropathy (n = 3), transient ischemic attack (n = 2), and essential tremors (n = 6). The MS groups consisted of 15 patients with active disease and 32 stable patients. Three patients with active MS were tested serially.

The control populations consisted of 58 age-matched healthy donors and 28 patients with other neurologic diseases. Patients and healthy donors were not treated with steroids for at least 1 month before analysis of lymphocytes and no patient had been on immunosuppressive drugs within 1 year.

Cell Preparations. Human peripheral blood lymphocytes (PBL) were isolated from heparinized venous blood by means of Ficoll-Hypaque density gradient centrifugation (Litton

Abbreviations: FACS, fluorescence-activated cell sorter; FITC, fluorescein 5-isothiocyanate; mAb, monoclonal antibody(ies); MS, multiple sclerosis; PE, R-phycoerythrin; T<sub>H</sub>, T helper/inducer; T<sub>S</sub>, T suppressor/cytotoxic cell; PBL, peripheral blood lymphocytes; EAE, experimental allergic encephalomyelitis.

To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.
Bionetics). In some experiments, unfractionated PBL were adhered to plastic for 2 hr at 37°C to remove monocytes and were then separated into E rosette-positive (E⁺) and E rosette-negative (E⁻) populations with 10% AET-treated sheep erythrocytes as described (20). The E⁻ fraction was used without further manipulation as a source of enriched B cells.

mAb. The following mAb were used in this study: the 60.3 mAb (IgG2a/k) to a common leukocyte-cell-surface antigen family (Lp95-150) related to the LFA-1 complex and involved in a cell activation pathway (21); the 3ACS mAb (IgG2a/k), which precipitates a 220,000 Da peptide (Lp220) that is part of the T200 complex (22); the G17-2 mAb (IgG1/k) to the CD4 (T4) Tp55 helper T-cell-associated antigen (23); the G10-1 mAb (IgG2a/k) to the CD8 (Tp32) T-cell-associated antigen (23); the G3-7 mAb (IgG1/k) to the LD7 (3A1) Tp41 pan T-cell antigen (23); and Leu8 (Becton-Dickinson), a marker expressed on leukocyte subsets (12).

Conjugation of mAb with Fluorescein 5-Isothiocyanate (FITC) and R-Phycocerythrin (PE). mAb were conjugated with fluorescein by using FITC (24) or with the phycobiliprotein PE derived from the red algae Porphyra yezoensis by using the heterobifunctional cross-linker SPDP as described (25, 26). Conjugated antibodies were used at two doubling dilutions above their titration end point on lymphocytes as measured by FACS IV analysis.

Two-Color FACS Analysis of Lymphocyte Subsets. FACS analysis with a modified FACS IV cell sorter (Becton-Dickinson) and quantitative two-color analyses were performed as described (26). Forward and right-angle scatter gates were set on lymphocytes and to exclude monocytes and other leukocytes. In all samples tested, 4% of the cells evaluated expressed the monocyte marker MO-1. Data were plotted as cell number versus logarithm of green fluorescence versus logarithm of red fluorescence (Fig. 1A). Every four to five dots represent a doubling of fluorescence intensity. Because only directly conjugated antibodies of high affinity were used, fluorescence intensity is a good indicator of density of the markers on cells (22, 26). Nonspecific staining by irrelevant isotype-matched antibodies was <1%. Furthermore, nonspecific two-color staining was not observed in control combinations where overlap would not be expected (e.g., PE-anti-CD3 versus FITC-anti-Bp35 or PE-anti-CD4 versus FITC-anti-CD8).

In experiments to separate Lp220⁺ CD4⁺ cells from Lp220⁻ CD4⁺ cells, PBL were stained with PE-anti-CD4 and FITC-anti-Lp220 mAb. Stained cells (2 x 10⁷) were sorted with the FACS IV. Viability after sorting was >95% by trypan blue exclusion. Purity of FACS-separated T-cell subsets was >98%.

Analysis of Data. All cell sorter analyses were performed without knowledge of patients' clinical status. Forty thousand cells per sample were analyzed and recorded on floppy discs. Five T-cell subsets were quantitated: two CD4⁺ subsets and three CD8⁺ subsets. Comparison of mean percent and mean absolute lymphocyte levels was done by a two-tailed t test. The functions of the CD8 and CD4 molecules are more closely related to class I and class II recognition, respectively, than to suppressor or helper activity (27). However, for the sake of clarity we have operationally defined Tsk cells as any cell expressing the CD4 (T4, Leu3a) marker and Tc cells as any cell expressing the CD8 (8, Leu2a) marker. Three ratios were calculated: a standard Tsk/Tc ratio ( percentage CD4⁺ cells/percentage CD8⁺ cells); Tsk subset ratio (% Lp220⁻ CD4⁺ cells/% Lp220⁺ CD4⁺ cells); and Tc subset ratio (% Lp95-150hi Lp220⁺ CD8⁺ cells/% Lp95-150lo Lp220⁺ CD8⁺ cells). The mean ± SD of the ratios for each group was calculated. Individuals with ratios >2 SD were designated abnormal. Comparisons of the frequency of 100 individuals with abnormal and normal ratios in each group were assessed by χ² analysis.

Detection of in Vitro Secretion of IgG. To determine the effect of subpopulations of the CD4⁺ subsets on the secretion of IgG by stimulated B cells, 5 x 10⁴ sorted CD4⁺ Lp220⁺ and CD4⁺ Lp220⁻ cell subsets were added to 5 x 10⁴ E⁻ mononuclear cells in a vol of 0.5 ml of pokeweed mitogen (Difco) at a final dilution of 1:100. Quadruplicate cultures were set up for each group. On day 7, supernatants were harvested, and IgG secretion into supernatants was determined by solid-phase immunoassay in which purified goat anti-human IgG (Qualex) was used as described (28).

RESULTS
To determine which pairs of mAb could best divide T-cell subsets, we tested >50 two-color combinations on normal blood lymphocytes before screening MS patients for T-cell abnormalities. When bIc lymphocytes were stained with a PE (red)-conjugated anti-CD4 (T4) antibody (Fig. 1B), >60% of total lymphocytes were stained. mAb to the Lp220 common leukocyte antigen clearly divided CD4⁺ Tsk cells into two subpopulations (Fig. 1D): an Lp220⁺ subset and an Lp220⁻ subset. The cutoff point for Lp220⁻ cells was determined by staining peripheral cells with anti-CD4 mAb alone (Fig. 1F). Most, but not all, of the CD4⁺ cells were also Lp220⁺ (Fig. 1D). Approximately 50% of CD4⁺ cells expressed the Lp220 molecule, which is distinct from Leu8⁺ (12) or CD7⁺ (26) subsets, which represent only 15–20% of the Tsk cells (data not shown).

We were able to subdivide CD8⁺ (T8) Tc cells into three subsets (Fig. 1C). Cells stained with PE-anti-CD8 antibody and a FITC-conjugated antibody to the common leukocyte marker Lp95-150 (60.3) divided Tc cells into three subsets: one CD8⁺ 0.1 Lp95-150 0.1 subset and two CD8⁺ 0.1 populations, Lp95-150 0.1 cells and Lp95-150 0.0 cells. The CD8⁺ 0.0 subset

Fig. 1. Characterization of two Tsk (CD4⁺) and three Tc (CD8⁺) T-cell subsets by using quantitative two-color FACS analysis. (A) Data plotted as cell number (vertical) vs. logarithm of green fluorescence vs. logarithm of red fluorescence. (B) Histogram of 40,000 blood lymphocytes stained with PE-anti-CD4 mAb only; note autofluorescence in both populations. (C) Three CD8⁺ Tc subsets are evident in normal PBL: CD8⁺ 0.0 Lp95-150 0.0 cells; CD8⁺ 0.1 Lp95-150 0.1 cells and CD8⁺ 0.0 Lp95-150 0.0 cells. (D) Two CD4⁺ Tsk cell subsets are present in normal lymphocytes, Lp220⁺ and Lp220⁻. Comparison of D with B enables determination of positive and negative cells. (E and F) Same as C and D, respectively, using blood lymphocytes from a patient with active MS. Tsk subsets appear normal (E) but Lp220⁻ Tsk cells (F) are depleted.

Immunology: Rose et al.

consisted of some cells expressing the IgG1 Fc receptor and having NK cell activity (29).

We compared the frequency of these five subsets in patients with active and inactive MS with the frequency in healthy age-matched controls and in patients with other neurologic diseases (Table 1). The frequency of total CD4+ Tc cells did not differ between groups. However, as illustrated in Fig. 1, a comparison between patients with active MS (Fig. 1F) and healthy individuals (Fig. 1D) revealed that active MS patients had a low frequency of the Lp220+ Thc subset. The mean percentage of Lp220+ Thc cells in patients with active MS (14.4%) was significantly less than that of patients with inactive MS (28.2%) or that of healthy controls (29%) (P < 0.001).

When ratios of Lp220+ Thc to Lp220+ Tc cells (Thc/Tc ratio) were calculated, the differences between active MS and control groups were even more evident. As shown in Fig. 2 and summarized in Table 1, increased Thc ratios in active MS were significantly different from those observed in healthy controls (P < 0.001), in patients with inactive MS (P < 0.001), or in those with other neurologic diseases (P < 0.001). Serial samples were obtained from three patients with active MS. Patient 1 had a Thc ratio of 2.0 five days before an exacerbation and a Thc ratio of 3.9 the second day of the exacerbation episode. Patient 2, who has active chronic progressive disease, had a Thc ratio of 1.3 when first tested, which rose to 4.7 during a superimposed acute exacerbation 10 days later. Patient 3, who had severe chronic progressive disease, had a Thc ratio of 3.4 at the time of the first test and 4.4 four weeks later when the patient was clinically worse. Little or no change in the Thc ratio was seen in a group of healthy controls tested repeatedly over a 3-month period (time 0 mean = 0.9 ± 0.1 vs. 2-month mean = 0.8 ± 0.1). In spite of these dramatic differences in Thc ratios between active MS patients and control groups, no difference in Thc/Tc ratios between groups was evident (Table 1). Thus, the Thc ratio was a more sensitive indicator of abnormal immune status than was the Thc/Tc ratio.

The decrease in the frequency of Lp220+ Thc cells in MS patients was due to an absolute decrease in Lp220+ Tc cells and not simply due to a conversion of Lp220+ Tc cells into Lp220+ Thc cells. As shown in Fig. 3 (Upper), the absolute number of p220+ CD4+ cells is similar in patients with active MS and normal healthy controls. However, Lp220+ CD4+ cells are significantly depleted, particularly in active MS patients with acute exacerbations (Lower).

Table 1. T-cell subset abnormalities in patients with MS

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>n</th>
<th>Median age, yr (range)</th>
<th>Sex</th>
<th>% Thc cells</th>
<th>% Tc cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>Total CD4+</td>
<td>CD4* Lp220+</td>
</tr>
<tr>
<td>Active MS</td>
<td></td>
<td>15</td>
<td>(23–70)</td>
<td>9</td>
<td>47 ± 3</td>
<td>14 ± 2*</td>
</tr>
<tr>
<td>Inactive MS</td>
<td></td>
<td>32</td>
<td>(16–72)</td>
<td>27</td>
<td>52 ± 1</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>Other neurological disease</td>
<td></td>
<td>28</td>
<td>(57–92)</td>
<td>18</td>
<td>52 ± 2</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>Total</td>
<td>58</td>
<td>(20–66)</td>
<td>38</td>
<td>51 ± 1</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>Ages 20–45</td>
<td></td>
<td>36</td>
<td>(20–66)</td>
<td>19</td>
<td>51 ± 2</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>Ages 46–66</td>
<td></td>
<td>22</td>
<td>(20–66)</td>
<td>19</td>
<td>51 ± 2</td>
<td>27 ± 2</td>
</tr>
</tbody>
</table>

Total Thc cells = mean percent CD4* cells ± SEM; total Tc cells = mean percent CD8* cells ± SEM. Thc ratio = % Lp220+ CD4* cells/ % Lp220+ CD4* cells (± SEM); Tc ratio = % CD8* Lp95-150bn cells/ % CD8* Lp95-150bn cells (± SEM). Thc/Tc ratio is expressed as mean ± SEM.

*p < 0.001 versus healthy controls, inactive MS, and other neurologic diseases.

In subsequent studies, we attempted to define the function of the Lp220 molecule and the Lp220+ Thc cell subset depleted during acute exacerbations. The CD4+ population is known to contain Thc cells that augment B-cell immunoglobulin production (30), so it was important to determine whether T-cell help for antibody production was restricted to Lp220+ CD4+ or Lp220+ CD4+ subpopulations. Unfractionated Tc cells or Lp220+ CD4+ Tc cells or Lp220− CD4+ Tc cells isolated by cell sorting were mixed with autologous B cells and cultured in vitro with pokeweed mitogen; after 7 days, total IgG production was measured (Table 2). Neither B cells, unfractionated Tc cells, nor the cell-sorter fractionated subsets alone produced significant IgG. However, in a series of experiments the helper effect of Lp220+ CD4+ cells was consistently 2 to 3 times greater than that of the Lp220− CD4+ subset. When equal numbers of Lp220+ and Lp220− were mixed together and cultured with autologous B cells, the helper activity was less than with Lp220− CD4+ Tc cells alone. The presence of anti-CD4 and anti-Lp220 mAb in cultures of unfractionated Tc cells did not inhibit Thc activity, ruling out...
the possibility that the lack of helper activity in the sorted Lp220+ CD4+ subset was due to a blocking effect of the anti-p220 mAb.

A second series of experiments suggested that the Lp220 molecule may play a role in T-cell activation. First, when T cells were fractionated into buoyant and dense fractions by using Percoll gradients, the proportion of CD4+ cells that were Lp220+ was highest in dense fractions (46% of total CD4+ cells) and lowest in buoyant fractions (12.5% of total CD4 cells). Second, when T cells were stimulated to proliferate, the expression of Lp220 molecule decreased as interleukin 2 receptor expression increased (31). The role the Lp220 molecule plays in promoting interleukin 2-dependent T-cell activation is described in detail elsewhere (31). Thus, the Th cells depleted in active MS express the Lp220 molecule that is found principally on dense Th cells and is involved in T-cell activation.

Several previous studies have reported that CD8+ Th cells are selectively depleted in patients with active MS (6). In this series of MS patients, we detected a depletion of a subset of CD8+ cells, but this depletion was also seen in other control groups (Table 1). Of the three Th subsets that were measured (Fig. 1C), the CD8+bull cells, thought to contain the NK cell subset (29), were not statistically different between groups.

Table 2. Quantitative comparison of helper function provided by p220+ CD4+ and p220+ CD4+ T cells for B-cell IgG production

<table>
<thead>
<tr>
<th>Lymphocyte population</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>p220+ CD4+ and p220+ CD4+ and B cells</td>
<td>1800</td>
<td>400</td>
<td>1050</td>
</tr>
<tr>
<td>p220+ CD4+ and B cells</td>
<td>1350</td>
<td>190</td>
<td>540</td>
</tr>
<tr>
<td>p220+ CD4+ and B cells</td>
<td>2500</td>
<td>400</td>
<td>2600</td>
</tr>
<tr>
<td>B cells alone</td>
<td>125</td>
<td>65</td>
<td>170</td>
</tr>
<tr>
<td>p220+ CD4+ and p220- CD4+</td>
<td>52</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>p220+ CD4+ alone</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>p220- CD4+ alone</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
</tbody>
</table>

(data not shown). However, the proportion of the two CD8+bull subsets was clearly altered in many MS patients. The frequency of Lp95-150bull CD8+bull cells was lower in many patients with active MS. The ratio of Lp95-150bri CD8+bri cells to Lp95-150bri CD8+bri Th cells (Tc cell ratio) was significantly higher in patients with active MS than in age-matched healthy controls (P < 0.001), minimally different from age-matched patients with inactive MS (P < 0.05), and not different from the other neurologic disease group (P < 0.1). The Tc cell ratios in patients with inactive MS (P < 0.01) and other neurologic diseases (P < 0.001) were also clearly different from normal controls, suggesting that the alterations in the Tc ratio may reflect an abnormal immune status. Thus, abnormal Tc ratios, unlike abnormal Th ratios, were not restricted principally to active MS. Further testing revealed that there was a clear difference between the Tc ratios of younger and older healthy individuals (P < 0.05). The Tc ratios of older healthy individuals were not statistically different from those of the other neurologic disease group (median age, 69 years).

Like the Lp220 marker, the Lp95-150 common leukocyte marker used for subdividing CD8+bull cells changes in expression after T-cell activation. Dense T-cell fractions have fewer Lp95-150bri CD8+bri cells (25% of CD8+bri cells); buoyant fractions have 50% of Lp95-150bri CD8+bri cells. Furthermore, after stimulation with mitogens such as phytohemagglutinin or pokeweed mitogen, virtually all CD8+bri cells become Lp95-150bri (unpublished data). Thus, the Lp95-150 marker on Tc cells may also distinguish cells at different stages of activation.

**DISCUSSION**

Using two-color FACS analysis of PBL, we have identified two Th and three Tc cell subsets. These Tc subsets were measured in patients with MS during clinically active and inactive stages of disease. We have found that a high frequency of patients with active MS have a selective depletion of one Th subset (Lp220+ CD4+). Both the frequency and absolute levels of this subset were greatly depleted in patients with active disease. In addition, when three patients with active MS were tested serially, the levels of Lp220- Th cells in peripheral blood decreased as disease activity increased. Our observation that MS patients with acute exacerbations have the lowest levels of Lp220+ Th cells (Fig. 3) supports this possibility. A longitudinal study will help determine more precisely the kinetic relation of Lp220+ Tc cell depletion to the onset of exacerbation.

The difference between patients with active MS and control groups was also clearly evident when the proportion of Lp220- to Lp220+ Tc cells was expressed as a "Tc subset" ratio. The depletion of Lp220+ Tc cells in patients with active MS was not apparent when we used standard single-marker analysis of CD4+ cells nor when we calculated conventional Th/Tc ratios (Table 1). Our results revive the possibility that monitoring of appropriate T-cell subsets may be an important indicator of immune status.

The loss of Lp220+ CD4+ cells from the peripheral blood of MS patients could be due to selective migration of these cells out of the blood stream or to selective destruction of this subset. Lesion progression in MS is associated with large numbers of CD4+ cells at the lesion margin with extension into the adjacent normal appearing white matter (32). Similarly, a selective migration of Lyt-1- cells to the central nervous system from the peripheral blood has been observed.
in mice induced to develop experimental allergic encephalomyelitis (EAE) (33). In addition, a selective depletion of Lp220+CD4+ cells has been observed during acute EAE in macaques (unpublished observations). The implication is that CD4+ cells may be leaving the blood and entering the central nervous system with sequestration or trapping of these cells in the brain. EAE can be induced by adoptively transferring myelin-basic protein-specific T-cell clones into mice (34); recently, Waldor and coworkers have shown that a rat mAb L3T4, specific for the CD4 homologue in mice, when inoculated in vivo cannot only prevent the development of EAE, but can also cure mice that already have EAE (35). These results strongly suggest that Th cells play an active role in this neurologic disease and presumably in MS as well.

Alternatively, the depletion of Lp220+CD4+ cells could be due to the selective lysis of this T-cell subset. The absolute decrease of Th cells seen in MS is reminiscent of the Th cell depletion observed in patients with acquired immunodeficiency syndrome (AIDS) (36) and chronic lymphadenopathies (37). In patients with chronic lymphadenopathy, a subset of CD4+ cells (Leu3+38) are depleted (38), and, as with MS patients, the depleted subset lacks Th cell activity.

The precise function of the Lp220-Th cell is not yet known. It is clear that the Lp220-Th cells, and not the Lp220+Th cells possess the majority of helper activity for B-cell IgG production (Table 2). The most likely possibility is that Lp220+ and Lp220-Th cells differ in their state of activation. Expression of the Lp220 molecule decreases as T cells are activated, and the Lp220 molecule itself apparently plays a role in lymphocyte activation (31). Antibody to the Lp220 molecule rapidly increases the expression of interleukin 2 receptors on activated T cells and promotes T-cell proliferation in interleukin 2-dependent T-cell proliferation assays (36). The loss of a Lp220-CD4+ T-cell subset in active MS could explain why some MS patients have defective T-cell proliferative responses (39).

The common leukocyte markers used to subdivide Th and Ts cells both change in expression after activation by mitogens: the Lp220 molecule decreases after T-cell activation (31) and the Lp95-150 molecule recognized by mAb 60.3 increases (unpublished data). The 60.3 mAb reacts with an epitope common to all members of the Lp 95-150 family of molecules, some of which are part of the LFA-1 complex. Thus, Lp220-Th cells and Lp95-150null CD8+bri T cells have surface phenotypes expected of resting T cells. In patients with active MS, the Th and Ts cell subsets that are depleted both have resting cell phenotypes. This pattern contrasts sharply with that observed in patients with juvenile rheumatoid arthritis (data not published); many of these patients have decreased numbers of T-cell subsets with an activated cell phenotype: Lp220-Th cells and Lp95-150null CD8+bri T cells. The significance of these differences between juvenile rheumatoid arthritis and MS is not yet known.

We thank Mr. Derek Hewgill for his help in preparing the sorted cell populations used in this study. This work was supported in part by Grants CA59935 and RR00166 from the National Institute of Health and by Genetic Systems Corporation.