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Immunology

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

(two-color cell sorter analysis/T helper subpopulations/neuroimmunologic disease)

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ABSTRACT **Patients with active multiple sclerosis (MS) have a selective loss of a subset of T helper cells (T_h), 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 (T_h) and CD8 [T suppressor/cytotoxic cell (T_s)] and the common leukocyte markers Lp220 and Lp95-150, five phenotypically distinct T-cell subsets have been identified in peripheral blood: two $CD4^+$ T_h cell subsets and three $CD8^+$ T_s 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_h subset ($CD4^+$ $Lp220^+$) 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 $Lp220^+$ T_h subset as disease activity progressed. The proportion of two T_s subsets was also abnormal in patients with active MS, but this defect was not restricted to that group. Total T_h and T_s cell frequencies and T_h/T_s 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_s) cells in MS patients that may correlate with disease activity (6, 7). The ratio of T helper/inducer (T_h) cells to T_s cells (T_h/T_s 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_h/T_s 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 multiparametric 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_h subset ($CD4^+$ $Lp220^+$). This $CD4^+$ T_h 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 $Lp220^+$ $CD4^+$ cells may relate to a worsening clinical status. One T_s subset was depleted in active MS patients, but this defect was also seen in other neurologic diseases. No differences in conventional single-parameter T_h/T_s 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 ($n = 8$), amyotropic 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

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

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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 3AC5 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 (T8) Tp32 suppressor T-cell-associated antigen (23); the G3-7 mAb (IgG1/k) to the CD7 (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-Phycoerythrin (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. 1*A*). 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 (2×10^7) 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 percentages 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 T_h cells as any cell expressing the CD4 (T4, Leu3a) marker and T_s cells as any cell expressing the CD8 (8, Leu2a) marker. Three ratios were calculated: a standard T_h/T_s ratio (% $CD4^+$ cells/% $CD8^+$ cells); T_h subset ratio (% $Lp220^-$ $CD4^+$ cells/% $Lp220^+$ $CD4^+$ cells); and T_s subset ratio (% $Lp95\text{-}150^{bri}$ $CD8^{bri}$ cells/% $Lp95\text{-}150^{dull}$ $CD8^{bri}$ 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 χ^2 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, 5×10^4 sorted $CD4^+$ $Lp220^+$ and $CD4^+$ $Lp220^-$ cell subsets were added to 5×10^4 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 blc lymphocytes were stained with a PE (red)-conjugated anti-CD4 (T4) antibody (Fig. 1*B*), ≈60% of total lymphocytes were stained. mAb to the Lp220 common leukocyte antigen clearly divided $CD4^+$ T_h cells into two subpopulations (Fig. 1*D*): 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. 1*B*). Most, but not all, of the $CD4^-$ cells were also $Lp220^+$ (Fig. 1*D*). 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 T_h cells (data not shown).

We were able to subdivide $CD8^+$(T8) T_s cells into three subsets (Fig. 1*C*). Cells stained with PE–anti-CD8 antibody and a FITC-conjugated antibody to the common leukocyte marker Lp95-150 (60.3) divided T_s cells into three subsets: one $CD8^{dull}$ $Lp95\text{-}150^{bri}$ subset and two $CD8^{bri}$ populations, $Lp95\text{-}150^{bri}$ cells and $Lp95\text{-}150^{dull}$ cells. The $CD8^{dull}$ subset

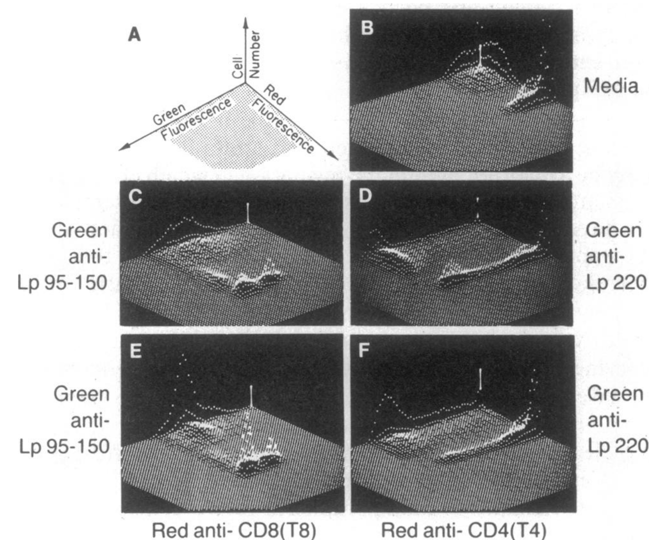

FIG. 1. Characterization of two T_h ($CD4^+$) and three T_s ($CD8^+$) T-cell subsets by using quantitative two-color FACS analysis. (*A*) Data plotted as cell number (vertical) vs. logarithm of green fluorescence vs. logarithm of red fluorescence. (*B*) Histogram of 40,000 blood lymphocytes stained with PE–anti-CD4 mAb only; note autofluorescence in both populations. (*C*) Three $CD8^+$ T_s subsets are evident in normal PBL: $CD8^{dull}$ $Lp95\text{-}150^{bri}$ cells; $CD8^{bri}$ $Lp95\text{-}150^{dull}$ cells and $CD8^{bri}$ $Lp95\text{-}150^{bri}$ cells. (*D*) Two $CD4^+$ T_h 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. T_s subsets appear normal (*E*) but $Lp220^+$ T_h cells (*F*) are depleted.

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 $CD4^+$ T_h cells did not differ between groups. However, as illustrated in Fig. 1, a comparison between patients with active MS (Fig. 1*F*) and healthy individuals (Fig. 1*D*) revealed that active MS patients had a low frequency of the $Lp220^+$ T_h subset. The mean percentage of $Lp220^+$ T_h 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 $Lp220^-$ T_h cells to $Lp220^+$ T_h cells (T_h 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 T_h 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 T_h ratio of 2.0 five days before an exacerbation and a T_h ratio of 3.9 the second day of the exacerbation episode. Patient 2, who has active chronic progressive disease, had a T_h 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 T_h 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 T_h 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 T_h ratios between active MS patients and control groups, no difference in T_h/T_s ratios between groups was evident (Table 1). Thus, the T_h ratio was a more sensitive indicator of abnormal immune status than was the T_h/T_s ratio.

The decrease in the frequency of $Lp220^+$ T_h cells in MS patients was due to an absolute decrease in $Lp220^+$ cells and not simply due to a conversion of $Lp220^+$ T_h cells into $Lp220^-$ T_h 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*).

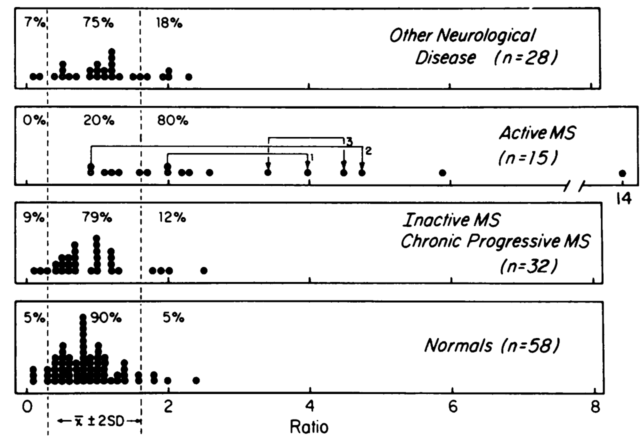

FIG. 2. Comparison of T_h cell subset ratios in patients with active and inactive MS with ratios in healthy controls and patients with other neurologic diseases. The percentages of $Lp220^-$ $CD4^+$ cells and $Lp220^+$ $CD4^+$ cells were calculated for each of four sample groups: active MS, inactive MS, other neurologic diseases, and normal controls. The T_h cell ratio was determined by dividing the percentage of $Lp220^-$ $CD4^+$ cells by the percentage of $Lp220^+$ $CD4^+$ cells. The mean T_h ratios for each group are shown in Table 2. Lines indicate changes where repeat samples were tested.

In subsequent studies, we attempted to define the function of the Lp220 molecule and the $Lp220^+$ T_h cell subset depleted during acute exacerbations. The $CD4^+$ population is known to contain T_h 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 T cells or $Lp220^+$ $CD4^+$ T cells or $Lp220^-$ $CD4^+$ T 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 T 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 $Lp220^-$ $CD4^+$ T cells alone. The presence of anti-CD4 and anti-Lp220 mAb in cultures of unfractionated T cells did not inhibit T_h activity, ruling out

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

Group		Median age, yr	Sex		% T_h cells			% T_s cells			
Category	n	(range)	F	M	Total	$CD4^+$ $Lp220^+$	T_h ratio	Total	$CD8^{bri}$ $Lp95\text{-}150^{dull}$	T_s ratio	T_h/T_s ratio
Active MS	15	38 (23–70)	9	6	47 ± 3	14 ± 2*	3.3 ± 0.9*	29 ± 2	8 ± 2	5.9 ± 2.1†	1.6 ± 0.1
Inactive MS	32	38 (16–72)	27	5	52 ± 1	28 ± 1	0.9 ± 0.1	29 ± 2	13 ± 1	1.3 ± 0.2	2.0 ± 0.1
Other neurological disease	28	69 (37–92)	10	18	52 ± 2	26 ± 2	1.1 ± 0.1	26 ± 1	7 ± 1	3.4 ± 0.7	2.2 ± 0.2
Healthy controls											
Total	58	34 (20–66)	38	20	51 ± 1	29 ± 1	0.9 ± 0.1	31 ± 1	12 ± 1	1.4 ± 0.2	1.9 ± 0.1
Ages 20–45	36	30	19	17	51 ± 2	30 ± 2	0.8 ± 0.1	30 ± 1	15 ± 1	0.8 ± 0.1	1.9 ± 0.1
Ages 46–66	22	53	19	3	51 ± 2	27 ± 2	1.0 ± 0.1	32 ± 2	8 ± 1	2.6 ± 0.5	1.9 ± 0.2

Total T_h cells = mean percent $CD4^+$ cells ± SEM; total T_s cells = mean percent $CD8^+$ cells ± SEM. T_h ratio = % $Lp220^-$ $CD4^+$ cells/% $Lp220^+$ $CD4^+$ cells (± SEM); T_s ratio = % $CD8^{bri}$ $Lp95\text{-}150^{bri}$ cells/% $CD8^{bri}$ $Lp95\text{-}150^{dull}$ cells (± SEM). T_h/T_s ratio is expressed as mean ± SEM.

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

†$P < 0.001$ versus healthy controls.

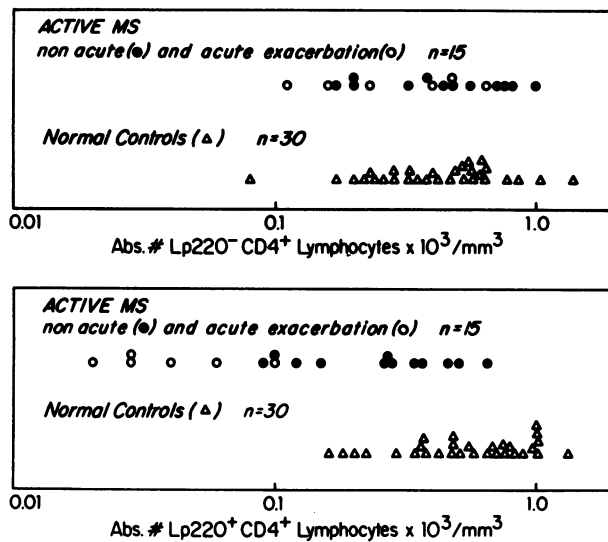

FIG. 3. Absolute numbers of $CD4^+$ cell subsets in patients with active MS and in normal controls. The total leukocytes and cell differential were obtained for each individual tested. The absolute numbers for $Lp220^-$ and $Lp220^+$ $CD4^+$ subsets were calculated by multiplying the percentage of each subset by the absolute lymphocyte number per mm^3 of whole blood. The distinction between nonacute and acute active MS was made on the basis of the presence or absence of a clinical exacerbation, defined as neurological changes occurring over a period of 1–5 days. $Lp220^+$ cells were clearly depleted in acute MS patients.

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 T_h cells depleted in active MS express the Lp220 molecule that is found principally on dense T_h cells and is involved in T-cell activation.

Several previous studies have reported that $CD8^+$ T_s 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 T_s subsets that were measured (Fig. 1*C*), the $CD8^{dull}$ 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

Lymphocyte population	IgG, ng/ml Exp. 1	Exp. 2	Exp. 3
$p220^+$ $CD4^+$ and $p220^-$ $CD4^+$ and B cells	1800	400	1050
$p220^+$ $CD4^+$ and B cells	1350	190	540
$p220^-$ $CD4^+$ and B cells	2500	400	2600
B cells alone	125	65	170
$p220^+$ $CD4^+$ and $p220^-$ $CD4^+$	52	25	50
$p220^+$ $CD4^+$ alone	25	25	50
$p220^-$ $CD4^+$ alone	25	25	50

(data not shown). However, the proportion of the two $CD8^{bri}$ subsets was clearly altered in many MS patients. The frequency of Lp95-150^{dull} $CD8^{bri}$ cells was lower in many patients with active MS. The ratio of Lp95-150^{bri} $CD8^{bri}$ cells to Lp95-150^{dull} $CD8^{bri}$ T_s cells (T_s 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 T_s 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 T_s ratio may reflect an abnormal immune status. Thus, abnormal T_s ratios, unlike abnormal T_h ratios, were not restricted principally to active MS. Further testing revealed that there was a clear difference between the T_s ratios of younger and older healthy individuals ($P < 0.05$). The T_s ratios of older healthy individuals were not statistically different from those of the other neurologic disease group (median age, 69 years). Therefore, the high proportion of "abnormal" T_s ratios found in the other neurologic disease group may be related to age as well as disease status. The median age for both MS groups was 38 years, similar to the control group (34 years), indicating that the difference in T_s ratios between these groups was not age related.

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

DISCUSSION

Using two-color FACS analysis of PBL, we have identified two T_h and three T_s cell subsets. These T-cell 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 T_h 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 $Lp220^+$ T_h cells in peripheral blood decreased as disease activity increased. Our observation that MS patients with acute exacerbations have the lowest levels of $Lp220^+$ T_h cells (Fig. 3) supports this possibility. A longitudinal study will help determine more precisely the kinetic relation of $Lp220^+$ T_h 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 $Lp220^+$ T_h cells was expressed as a "T_h subset" ratio. The depletion of $Lp220^+$ T_h cells in patients with active MS was not apparent when we used standard single-marker analysis of $CD4^+$ cells nor when we calculated conventional T_h/T_s 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 T_h 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 T_h cells seen in MS is reminiscent of the T_h 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 ($Leu8^+$) are depleted (38), and, as with MS patients, the depleted subset lacks T_h activity.

The precise function of the $Lp220^+$ T_h cells is not yet known. It is clear that the $Lp220^-$ T_h cells, and not the $Lp220^+$ T_h cells possess the majority of helper activity for B-cell IgG production (Table 2). The most likely possibility is that $Lp220^+$ and $Lp220^-$ T_h 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 T_h and T_s 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, $Lp220^+$ T_h cells and $Lp95\text{-}150^{dull}$ $CD8^{bri}$ T_s cells have surface phenotypes expected of resting T cells. In patients with active MS, the T_h and T_s 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: $Lp220^-$ T_h cells and $Lp95\text{-}150^{bri}$ T_s cells. The significance of these differences between juvenile rheumatoid arthritis and MS is not yet known.

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