Changes in Activated T Cells in the Blood Correlate With Disease Activity in Multiple Sclerosis

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Objective: To determine whether changes in activation markers on peripheral blood T cells correlate with disease activity in patients with multiple sclerosis.

Design: In a prospective longitudinal study during 1 year, we analyzed the change in percentage of activated T lymphocytes in the peripheral blood of 40 patients with multiple sclerosis in relation to clinical findings and changes on brain magnetic resonance imaging (MRI) scans. The patients underwent repeated imaging of the brain (mean number of MRIs for each patient, 22) at the time blood samples were obtained as well as at monthly neurologic examinations, and at the time of scoring on the Kurtzke Expanded Disability Status Scale (EDSS) and ambulation index scale.

Results: A change in the percentage of cells expressing the activation markers interleukin 2 receptor (CD25), class II major histocompatibility complex (MHC) (I) or surface dipeptidyl peptidase (CD26) correlated significantly with a change in lesion volume or a change in number of gadolinium-enhancing lesions as detected on MRI. Changes in CD25+ cells and in CD4+ cells expressing class II MHC also correlated with changes in disability as measured by EDSS in patients with relapsing-remitting disease, and changes in CD4+CD25+ cells correlated with the occurrence of attacks in patients with relapsing-remitting disease. These correlations are dependent on measurement of changes between time points sampled at 1- or 2-week intervals.

Conclusion: There is a linkage between peripheral T lymphocyte activation as measured by cell surface markers and disease activity in patients with multiple sclerosis.

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MULTIPLE sclerosis (MS) is thought to result from aberrant immune responses to myelin antigens, and abnormalities of immune functions have been described. Lymphocytes express a large number of cell surface structures that define the functional diversity of the lymphocyte populations identified by monoclonal antibodies. Lymphocyte subsets in the peripheral blood of patients with MS have been the subject of numerous reports. However, a comprehensive study of specific changes in cell surface markers with disease activity as measured clinically and by magnetic resonance imaging (MRI) has not been done. We studied untreated patients with MS in various categories of disease with serial measurements of T-cell activation markers performed at the time of MRI. The primary hypothesis we tested was whether changes in activated T cells in the peripheral blood were linked to clinical or radiologic measures of disease activity.

RESULTS

CORRELATIONS BETWEEN CHANGES IN LYMPHOCYTE SUBSETS AND CLINICAL MEASURES

The data from normal individuals were used to calculate the SD for each measurement in the absence of disease. The data from MS patients were then weighted according to the SD in normal individuals. Using these weighted data, changes in percentage of activated T cells were correlated with changes in EDSS and AI and with the occurrence of attacks.

We found that an increase in percentage of CD4+CD25+ cells by 6% over 2 consecutive measurements separated by 1 week increased the odds of having an attack (odds ratio, 1.28; 95% confidence interval [CI], 1.19-1.38; P<.001; r = 0.13) in patients with RR disease. An increase in percentage of CD25+ cells was significantly correlated with an increase in EDSS in patients with RR MS (P = .001). In ad-
PATIENTS AND METHODS

PATIENTS

Forty patients with definite MS were recruited from our clinic. The study was approved by the institutional review board at the Brigham and Women’s Hospital, Boston, Mass, and all patients gave informed consent. The patients were between the ages of 20 and 55 years, were ambulatory with Kurtzke Expanded Disability Status Scale (EDSS) scores between 0 and 6.5, and had an MRI of the brain consistent with MS. There were 12 patients with relapsing-remitting (RR) MS with a minimum of 2 exacerbations in the 2 years before study entry; 14 with relapsing-remitting progressive (RR-P) MS who experienced clear-cut attacks but did not return to their pretreatment functional status within 6 months and displayed a stepwise increase in disability in the 6 months prior to entry; and 14 with chronic progressive (CP) MS (7 were classified as primary progressive and 7 as secondary progressive) who had increasing disability without stabilization in the 12 months prior to study entry, as shown by at least a 1-point decline in EDSS or ambulation index (AI) scores. Enrollment in the study was started prior to the introduction of the new clinical classification of MS. The patients’ initial characteristics are shown in Table 1. Patients previously treated with immunosuppressive, cytotoxic, or immunomodulatory drugs were excluded. Patients could have corticosteroids in the past but not within 2 months of study entry. Patients with significant medical or psychiatric illness; patients who were pacemakers, aneurysm clips, other metal implants, or shrapnel fragments present; and patients with claustrophobia were excluded.

STUDY DESIGN

Patients were evaluated at the Brigham and Women’s Hospital MS clinic and had a complete neurologic examination with EDSS and AI ratings. Baseline testing included complete blood cell count and SMA-20 blood chemistry tests. Magnetic resonance imaging examinations of the brain were performed weekly for 8 weeks, biweekly for 16 weeks, and then monthly for 6 months (mean number of MRIs per patient, 22). Clinical examinations were performed monthly and at the time of exacerbations. Patients with a clinical exacerbation were treated with steroids at the discretion of the examining physician. Exacerbations were defined as episodes of clinical worsening associated with a measurable change in EDSS or AI occurring in a patient who was otherwise stable or who had not taken steroids for more than 30 days.

PHENOTYPING OF PERIPHERAL BLOOD MONONUCLEAR CELLS

Blood samples were obtained at the time of each MRI. Staining of peripheral lymphocyte surface antigens was performed on fresh whole blood samples using a Q-prep machine from Coulter Immunology, Hialeah, Fla (Table 2). The samples were analyzed on a Coulter flow cytometer within 24 hours of staining and fixation and the results stored in list mode for reanalysis. Table 3 shows the results of the mean ± SD percentage of cells in each clinical group. Analysis was performed with the same gating parameters for each individual for all time points. Voltages on the flow cytometer were kept constant and the data were acquired on a clinical flow cytometer in which quality controls were run daily. Day-to-day variability in the assay and normal variations in T-lymphocyte subsets were accounted for by weekly phenotyping of peripheral blood mononuclear cells in a group of 10 normal individuals for 8 consecutive weeks. Information regarding intercurrent infections and medications was obtained at each visit.

MRI SCANS

Imaging was performed on a General Electric Signa 1.5-T unit (General Electric, Milwaukee, Wis). Proton density and T2-weighted images were obtained using 2 interleaved dual-echo (echo time, 30 and 80 milliseconds) and long repetition time (3000 milliseconds) sequences. Contiguous 3-mm-thick slices covered the whole brain from the foramen magnum to the higher convexity with an in-plane voxel size of 1.25 × 1.25 mm.

Table 1. Characteristics of the Patient Population With Multiple Sclerosis by Disease Category

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RR</th>
<th>RR-P</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, MF</td>
<td>4/10</td>
<td>2/10</td>
<td>7/7</td>
</tr>
<tr>
<td>Age, y</td>
<td>35.4 ± 1.7</td>
<td>36.7 ± 2.0</td>
<td>43.5 ± 1.5</td>
</tr>
<tr>
<td>Kurtzke EDSS</td>
<td>2.1 ± 0.3</td>
<td>4.6 ± 0.5</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>Ambulation index</td>
<td>1.1 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>No. of relapses</td>
<td>17</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>No. of patients with relapses</td>
<td>9</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Lesion volume, cm²</td>
<td>5.0 ± 1.1</td>
<td>10.0 ± 2.8</td>
<td>5.9 ± 1.6</td>
</tr>
</tbody>
</table>

*RR indicates relapsing remitting; RR-P, relapsing-remitting progressive; CP, chronic progressive; and EDSS, Experimental Disability Status Scale.
†Values are mean ± SE.
peptidyl peptidase, a marker of activation (CD4+CD26+ cells), and an increase in number of new Gd+ lesions (P = .004) in the patients with RR disease. In patients with RR-P disease, an increase in 13’ cells (cells expressing class...
of 0.94 x 0.94 mm (24-cm field of view with a 256 x 192 acquisition matrix). The scan duration was approximately 12 minutes (using the 1/2 Fourier technique). Images were also obtained by applying a T1-weighted spin-echo pulse sequence after administration of an intravenous bolus of 10 mL of 0.5-mol/L gadolinium-diethyltriaminepentaacetic acid (Gd-DTPA) (Magnevir; Berlex Laboratories, Wayne, NJ). Postcontrast T1-weighted gadolinium positive (Gd+) images in the axial plane resulted from a 600/19/1 (repetition time/echo time/excitations) spin-echo sequence. Slice thickness was 4 mm with a 1-mm gap.

**IMAGE EVALUATION**

Proton density and T2-weighted images were analyzed using an automated computerized procedure. An outline of the intracranial cavity (ICC) was obtained for all studies. This ICC mask was used to define the region of interest containing brain parenchyma and cerebrospinal fluid (CSF). The ICC model was also used to automatically reposition the images from all time points to the reference frame of one of the time points. Each point (pixel) in the images was then assigned to 1 of 4 tissues: white matter, gray matter, CSF, or lesion. This was performed by combining the expectation-maximization algorithm described by Wells and coworkers with an automatic correction step for partial-volume artifacts arising from admixtures of brain parenchyma and CSF in boundary pixels. Lesion volumes were calculated by summing all pixels classified as lesions by this pipeline and by multiplying by 0.00064, corresponding to the volume in cubic centimeters of an individual voxel (smallest volume element resolved in the image). Only lesions that attained a minimum size of 20 pixels during the course of the study were included in this measurement. Individual lesions were defined by spatial-temporal (4-dimensional) connectivity. Six radiologists participated in rating the Gd-enhanced images. The total number of Gd-enhancing lesions and the number of new Gd-enhancing lesions at each time point were determined independently by 2 radiologists for each patient data set and the mean was used in the analysis.

**STATISTICAL METHODS**

The data from the clinical, radiologic, and immunologic databases were combined, and correlation analysis was performed on those dates when simultaneous EDSS and immunologic data were recorded and when simultaneous MRI and immunologic data were recorded. From this clinical/immunological data set, each EDSS value was compared with the previous value to calculate the change in EDSS for each patient. The analogous simultaneous change was calculated for each immunologic measure. A repeated-measures linear regression was then performed to determine if the change in EDSS was associated with a change in any of the immunologic measures. To avoid colinearity, each immunologic measure was entered into a separate regression, although each regression was adjusted for the administration of steroids, cyclophosphamide (in 1 patient), and cold medications. The adjustment was performed as follows: the regression analyses included a binary marker for steroid (or other medication) administration, which is coded as 1 for any time point when steroids (or other medication) were administered and coded as 0 for other time points. The program would then take this marker into account when generating a correlation.

The repeated-measures linear regressions were calculated again using the change in the AI, the change in MRI-measured lesion volume, the change in the total number of Gd-enhancing lesions, and the number of new Gd-enhancing lesions as alternative outcomes. In addition, a repeated-measures logistic regression was used to assess the relationship between the occurrence of an attack and the change in each immunologic measure. All of the repeated-measures regressions were implemented using generalized estimating equations, allowing correlation between successive changes in clinical or MRI parameters to decrease over time (i.e., a first-order autoregressive correlation structure). Such models do not require a normally distributed outcome, only an additive effect of new lesions on change in EDSS, and variability that is unrelated to the mean.

Because of the large number of outcomes and predictors considered, we report only on relationships that are significant with 2-sided P ≤ .01 in the correlations between predictors and multiple outcomes.

**Table 2. Antibodies to Cell Surface Molecules**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen Recognized</th>
<th>Cells Expressing the Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>Associated with the T-cell receptor</td>
<td>Mature T cells</td>
</tr>
<tr>
<td>CD4</td>
<td>Interacts with class II MHC</td>
<td>Subset of CD3 cells</td>
</tr>
<tr>
<td>CD8</td>
<td>Interacts with class I MHC</td>
<td>Subset of CD3 cells</td>
</tr>
<tr>
<td>CD68</td>
<td>Transmembrane glycoprotein/peptidase</td>
<td>Activates T cells</td>
</tr>
<tr>
<td>CD4/CD26</td>
<td>Transmembrane glycoprotein/peptidase</td>
<td>Subset of activated CD4 cells</td>
</tr>
<tr>
<td>CD25</td>
<td>Low-affinity IL-2R (Tac antigen)</td>
<td>Activated peripheral T cells</td>
</tr>
<tr>
<td>CD4/CD25</td>
<td>Low-affinity IL-2R (Tac antigen)</td>
<td>Subset of activated CD4 cells</td>
</tr>
<tr>
<td>I3</td>
<td>Nonpolymorphic class II antigens</td>
<td>Monocytes, macrophages, B cells, and activated T cells</td>
</tr>
<tr>
<td>CD34/13</td>
<td>Nonpolymorphic class II antigens</td>
<td>Subset of activated CD4 cells</td>
</tr>
</tbody>
</table>

*MHC indicates major histocompatibility complex; IL-2R, interleukin 2 receptor.*

II major histocompatibility complex (MHC) correlated with an increase in T2-weighted lesion volume on MRI. In patients with CP disease, an increase in percentage of CD4+ cells expressing class II MHC (CD4+13+) correlated with the appearance of new enhancing lesions on MRI (P = .002). When patients with CP disease were separated into primary (n = 7) and secondary (n = 7) progressive categories, this correlation was found only in the patients with secondary progressive disease (P < .001).

**Correlations Between Changes in Cell Surface Marker Expression and Delayed Change in MRI Parameters**

This analysis was performed to test the hypothesis that immunologic changes in the periphery may precede MRI changes. Thus, we analyzed the data comparing changes in percentage of activated T cells measured at 2 time points separated by 7 days with changes in MRI parameters measured at 2 overlapping time points separated by 14 days (Table 5). With this analysis, we found that increases in...
Table 3. Activated T Cells in Patients With Multiple Sclerosis and Normal Individuals by Disease Category*

<table>
<thead>
<tr>
<th>T Cells</th>
<th>Normal</th>
<th>RR</th>
<th>RR-P</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD25+</td>
<td>30 ± 5.9</td>
<td>25 ± 7.3</td>
<td>31 ± 7.7</td>
<td>30 ± 7.5</td>
</tr>
<tr>
<td>CD4+CD25+</td>
<td>14 ± 2.8</td>
<td>10 ± 4.3†</td>
<td>15 ± 4.7</td>
<td>11 ± 0.4</td>
</tr>
<tr>
<td>CD4+</td>
<td>72 ± 7.3</td>
<td>64 ± 11.5†</td>
<td>68 ± 7.9</td>
<td>61 ± 10.26</td>
</tr>
<tr>
<td>CD4-CD26+</td>
<td>37 ± 3.7</td>
<td>41 ± 7.0</td>
<td>40 ± 5.7</td>
<td>35 ± 5.9†</td>
</tr>
<tr>
<td>I3+</td>
<td>24 ± 3.4</td>
<td>24 ± 3.9</td>
<td>24 ± 3.9</td>
<td>29 ± 2.0</td>
</tr>
<tr>
<td>CD4+I3-</td>
<td>44 ± 9.9</td>
<td>43 ± 2.4</td>
<td>52 ± 3.2</td>
<td>46 ± 0.5</td>
</tr>
</tbody>
</table>

*Values are mean ± SD percentages. RR indicates relapsing remitting; RR-P, relapsing remitting progressive; and CP, chronic progressive.
†P < .04 for RR vs normal; P < .05 for RR vs RR-P.
‡P < .002 for RR vs normal; P < .05 for RR vs RR-P.
§P < .001 for CP vs normal; P < .05 for CP vs RR-P.
¶P < .001 for CP vs RR-P.
#P < .05 RR vs CP.

Table 4. Correlations Between Changes in Percentage of Activated T Cells and Simultaneous MRI Changes*

<table>
<thead>
<tr>
<th>T Cells</th>
<th>MS Category</th>
<th>Volume (cm³)†</th>
<th>No. of Gd+ Lesions‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+CD26+ RR</td>
<td>...</td>
<td>...</td>
<td>.23 ± .08 (P = .004; r = .16)</td>
</tr>
<tr>
<td>I3+ RR-P</td>
<td>...</td>
<td>...</td>
<td>.32 ± .11 (P = .004; r = .19)</td>
</tr>
<tr>
<td>CD4+I3- CP</td>
<td>...</td>
<td>...</td>
<td>.12 ± .04 (P = .002; r = .19)</td>
</tr>
</tbody>
</table>

*MRI indicates magnetic resonance imaging; MS, multiple sclerosis; Gd+, presence of gadolinium-enhancing lesions; RR, relapsing remitting; RR-P, relapsing remitting progressive; and CP, chronic progressive.
†The number of Gd+ lesions that correlated with a change in the percentage of I3+ cells of 3.1% and a change in the percentage of CD4+CD26+ cells of .89% (corresponding to 1 SD of variation in normal individuals).
‡The number of Gd+ lesions that correlated with a change in the percentage of CD4+CD26+ cells of 3.1% and a change in the percentage of CD4+I3- cells of 0.89% (corresponding to 1 SD of variation in normal individuals).

Table 5. Correlations Between Changes in Percentage of Activated T Cells and Lagged MRI Changes*

<table>
<thead>
<tr>
<th>T Cells</th>
<th>MS Category</th>
<th>Volume (cm³)†</th>
<th>No. of Gd+ Lesions‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD25+ RR</td>
<td>...</td>
<td>...</td>
<td>.36 ± .13 (P = .006; r = .33)</td>
</tr>
<tr>
<td>CD26+ CP</td>
<td>...</td>
<td>...</td>
<td>.22 ± .06 (P = .01; r = .22)</td>
</tr>
<tr>
<td>CD4+CD26+ CP</td>
<td>...</td>
<td>...</td>
<td>.27 ± .10 (P = .01; r = .27)</td>
</tr>
<tr>
<td>CD4+I3- CP</td>
<td>...</td>
<td>...</td>
<td>-0.44 ± 0.11 (P = .001; r = .33)</td>
</tr>
</tbody>
</table>

*Lagged changes were obtained by comparing changes in percentages of activated T cells measured at 2 time points separated by 7 days with changes in the percentage of CD25+ cells of .5%, or a change in the percentage of CD4+CD26+ cells of 3.1%, or a change in the percentage of CD4+I3- cells of 0.89% (corresponding to 1 SD of variation in normal individuals).
†The number of Gd+ lesions that correlated with a change in the percentage of CD4+I3- cells of 0.89% (corresponding to 1 SD of variation in normal individuals).

Multiple sclerosis is a chronic inflammatory disease of the central nervous system. There is increasing evidence that the condition is related to the immune system, with studies showing that activated T cells and changes in MRI can be correlated. The table above shows the correlations between changes in activated T cells and MRI changes, suggesting a possible link between immune activity and disease progression. It is important to note that while these correlations are significant, further research is needed to understand the exact mechanisms involved and to develop effective treatments.
evidence suggesting that MS is a T-cell-mediated autoimmune disease associated with a variety of immune abnormalities. A number of antigen-nonspecific abnormalities have been described. Studies have demonstrated selective changes in certain lymphocyte subpopulations in patients with MS. However, cross-sectional studies cannot give information on changes that are linked to disease activity if those in the population have the same mean percentage as normal individuals.

In the present study, we investigated the hypothesis that systemic activation of the immune system is associated with radiologic or clinical evidence of disease activity. In the analysis of the results, care was taken to account for changes that may be secondary to intercurrent infections or the intake of medications. Furthermore, we believe that analyzing the data by looking at changes that are weighted for the normal SD of a measurement is essential to ensure that we are investigating biologically significant changes and not fluctuations that are inherent to the method. Using the same approach, we have recently reported correlations between change in serum levels of intracellular adhesion molecule 1 and tumor necrosis factor receptors and the change in Gd-enhancing lesions on MRI. We analyzed each clinical subgroup separately because evidence from our laboratory and others showed that clinical subgroups were immunologically different and different by their MRI characteristics. In this study, we found no correlations between activation markers and clinical or radiologic markers when the patients were grouped together.

The major finding in this study was that changes in activated T-lymphocyte populations correlated with clinical and radiologic measures of disease activity. These data support the hypothesis that the immune dysregulation in MS is found not only in the central nervous system but also in the peripheral blood. On T-cell activation, CD25 (interleukin 2 receptor [IL-2R]), CD26, and I3 (class II MHC) are up-regulated. As a control for the activation markers studied, we measured changes in percentage of total T cells (CD3+) and CD4+ cells. Change in these populations did not correlate with clinical or MRI measures of disease activity.

Activated T cells produce IL-2, which stimulates other cells of the immune system and up-regulates the expression of its own receptor on T cells. The human IL-2R is composed of 2 different polypeptide chains, α and β, each capable of binding IL-2 with different affinities.
finding raises 2 possibilities: The first is that the percentage of T3*CD4+ cells decreases 1 week prior to MRI activity and peaks at the time of appearance of Gd-enhancing lesions. The second possibility is that a decrease in percentage of CD4+T3* cells in the periphery reflects the migration of these cells to lymphoid organs or to the central nervous system just prior to the appearance of new lesions on MRI.

The membrane-bound dipeptidyl peptidase CD26 serves as an accessory molecule for T-cell activation.43 It is a surface protease that has the ability to inactivate cytokines and is closely associated with adenosine deaminase.44,45 The number of CD26+ cells is increased in patients with human T-lymphotropic virus type I–associated myelopathy.46 Patients with active rheumatoid arthritis have increased CD4+CD26+ cells in the blood.47 In MS, an increase in Tα1+ cells (Tα1 recognizes an epitope of CD2648) in the CSF has been reported.49 In this study, we found correlations between changes in the CD26+expressing T lymphocytes and lesion activity on MRI in patients with RR and CP disease, consistent with the presumed function of CD26 in T-cell activation.

A high sampling frequency appears to be crucial for measuring these correlations. Thus, the change in percentage of activated T cells occurs rapidly and must be measured at frequent intervals.

In conclusion, we have demonstrated that changes in activated T cells in the peripheral blood reflect disease activity as determined clinically and radiologically. These measurements may be useful for understanding the pathogenesis of MS and for immune monitoring in phase 1 and 2 clinical trials.

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Antibody pairs labeled with fluorescein isothiocyanate (FITC) and phycocyanin conjugate (PE) (mouse IgG-FITC/mouse IgG-PE, CD3/CD4, CD4/CD26, CD4/CD25, and CD4/ I3) were a kind gift from Coulter Immunology, Hialeah, Fla.

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REFERENCES


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Call for Papers

Theme Issues

The ARCHIVES is planning a theme approach for several issues in 2000 and 2001. The topics for these special issues will include cerebrovascular diseases and stroke, demyelinating diseases and multiple sclerosis, the epilepsies, neuromuscular diseases, aging of the nervous system and the dementia, molecular and genetic neurology, and ethical issues in neurology. Papers are requested for consideration that represent important information related to these areas. Both clinical and basic science papers are requested. We look forward to receiving your contributions to these important issues.

Roger N. Rosenberg, MD
Editor