Increase in Peripheral CD4 Bright+ CD8 Dull+ T Cells in Parkinson Disease

Kinya Hisanaga, MD; Misa Asagi, MA; Yasuto Itoyama, MD; Yuzo Iwasaki, MD

Background: Immune abnormalities are known to be involved in the pathogenesis of sporadic Parkinson disease.

Objective: To examine whether abnormalities in peripheral lymphocytes exist in Parkinson disease.

Methods: Immune mediators, including CD1a, CD3, CD4, CD8, CD45RO, and Fas (CD95), were examined in peripheral lymphocytes of patients by 3-color flow cytometry.

Results: Patients with Parkinson disease displayed a significantly greater population of circulating CD3+ CD4 bright+ CD8 dull+ lymphocytes than age-matched control subjects (P = .005) and patients with cerebrovascular disease (P = .002). The increase in these cells appeared to continue for at least 17 months. These T cells also expressed CD45RO and Fas, markers for activated T cells, while CD1a, a marker for thymic T cells, was negative, suggesting that these cells are mature T cells with immune activities.

Conclusions: As CD4+ CD8+ T cells are known to increase after some specific viral infections, the continuous increase in CD4 bright+ CD8 dull+ T cells shown here may indicate postinfectious immune abnormalities that are possibly associated with the pathogenesis of this slowly progressive, multifactorial neurodegenerative disease.

Arch Neurol. 2001;58:1580-1583

PARKINSON DISEASE (PD), characterized by various neurologic symptoms including resting tremor, bradykinesia, rigidity, and pulsion, has been extensively studied, but the cause is still unclear. Many factors, such as neurotoxins, excitatory amino acids, oxidative stress, mitochondrial dysfunction, and genetic abnormalities, appear to be involved.

Immune abnormalities have also been observed in PD, such as the occurrence of antineuronal antibodies, increases in HLA-DR+–activated microglia in the substantia nigra, increases in HLA-DR expression on cerebrospinal fluid monocytes, decreases in CD4+CD45RA− (naive) T cells and increases in CD4+CD45RO+ (memory) T cells and TCRγδ cells, and CD38+ cells (activated T) and interleukin 2 receptor (CD25)+ cells (activated T, B, and macrophage) in peripheral blood.

In the present study, we found that patients with PD displayed a significantly high population of circulating CD4 bright+ CD8 dull+ lymphocytes, which are usually seen in the thymus, as compared with control subjects. These cells appeared to be mature T cells with immune activities.

Table 1 shows proportions of peripheral lymphocyte subsets in PD and the other groups. Peripheral lymphocytes were slightly lower in patients with PD than in control subjects. The percentages of CD4+CD8−, CD4+CD8−, and CD4+CD8+ lymphocytes in peripheral blood remained unchanged in patients with PD. On the other hand, we found significantly higher proportions of the CD4+CD8− lymphocytes in patients with PD compared with control subjects or patients with CVD. The CD4+CD8− lymphocytes could be clearly separated into CD4 bright+ CD8 dull+ lymphocytes and CD4 dull+ CD8 bright+ lymphocytes according to the density of CD4 and CD8 immunoreactivity (Figure 1). The expression of CD4 in CD4 bright+ CD8 dull+ cells was identical to that of CD4+ cells in the same sample. The expression of CD8 in these cells was lower than that of CD8+ cells. The situation in CD4 dull+ CD8 bright+ cells was the...
SUBJECTS AND METHODS

We studied 40 patients with idiopathic PD (20 men and 20 women) without immune disorders and/or other neurologic disorders. For each patient, the clinical diagnosis had been established on the basis of the medical history and physical examinations. The age of patients was 68.9±6.6 years (mean±SD). The patients were treated with levodopa, dopamine receptor agonists (bromocriptine mesylate or pergolide mesylate), an anticholinergic agent (trihexyphenidyl hydrochloride), amantadine hydrochloride, and/or droxidopa. The clinical stage of each patient during his or her “on” period was estimated as I to V according to the criteria described by Hoehn and Yahr.10

The other groups studied were 22 control subjects without any neurologic disorders (8 men and 14 women, 60.4±6.5 years old) and 33 patients with mild cerebrovascular disease (CVD; 19 men and 14 women, 67.7±8.4 years old).

Venous blood samples of patients were collected with informed consent between 6 AM and 10 AM to minimize the influence of diurnal fluctuations of lymphocyte subsets, and mixed with citrate. Mononuclear cells were isolated from the blood by density gradient centrifugation on Ficoll-sodium diatrizoate solution (Ficoll-Paque Plus; Amersham Pharmacia Biotech, Uppsala, Sweden) at 300g for 30 minutes at room temperature, and diluted with phosphate-buffered saline (pH 7.4) containing 2% calf serum. The cells were stained with fluorescein isothiocyanate (FITC)−, phycoerythrin (PE)−, or phycoerythrin-cyanin 5 (PC5)−conjugated antibodies to CD1a (PE), CD3 (PC3), CD4 (FITC), CD8 (PE or PC5), CD25 (PE), CD28 (PE), CD38 (PE), CD45RO (PE), CD54 (PE), CD95 (PE), and CD126 (PE) (Beckman-Coulter, Fullerton, Calif). The CD3, CD4, and CD8 were studied in all the patients and control subjects as described above, and the other markers were used for the 3-color analysis of CD4 bright CD8 dull lymphocytes. Control staining was performed with FITC-IgG1, PE-IgG1, PE-IgG2a, or PC5-IgG1. All monoclonal antibodies were applied at saturating concentrations. The population of stained cells was calculated by flow cytometric analysis with the use of a fluorescence-activated cell sorter (FACScalibur; Becton Dickinson, San Jose, Calif). A sample gate that contained lymphocytes but excluded granulocytes and monocytes was used to acquire data. At least 10000 mononuclear cells were analyzed. The populations of stained cells in patients with PD were compared with those in control subjects and patients with CVD by statistical analysis using the Mann-Whitney test.

Table 1. Lymphocytic Subpopulations in Peripheral Blood of Control Subjects and Patients With Cerebrovascular Disease (CVD) or Parkinson Disease (PD)*

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n = 22)</th>
<th>Patients With CVD (n = 33)</th>
<th>Patients With PD (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes, 10⁹/L</td>
<td>6.158 ± 1.540</td>
<td>5.754 ± 1.514</td>
<td>5.782 ± 1.555</td>
</tr>
<tr>
<td>Lymphocytes, per µL</td>
<td>2218 ± 665</td>
<td>1790 ± 599</td>
<td>1748 ± 602†</td>
</tr>
<tr>
<td>CD2+, %</td>
<td>61.4 ± 12.8</td>
<td>59.3 ± 14.5</td>
<td>59.3 ± 14.0</td>
</tr>
<tr>
<td>CD4+CD8−, %</td>
<td>0.98 ± 0.57</td>
<td>1.09 ± 1.05</td>
<td>1.63 ± 1.02‡</td>
</tr>
<tr>
<td>CD4+CD8+, %</td>
<td>0.29 ± 0.32</td>
<td>0.25 ± 0.33</td>
<td>0.26 ± 0.29</td>
</tr>
<tr>
<td>CD4b+CD8d+, %</td>
<td>0.69 ± 0.37</td>
<td>0.85 ± 1.01</td>
<td>1.36 ± 0.98§</td>
</tr>
<tr>
<td>CD4d+CD8b−, %</td>
<td>38.3 ± 9.1</td>
<td>35.7 ± 10.1</td>
<td>35.8 ± 11.4</td>
</tr>
<tr>
<td>CD4d+CD8d+, %</td>
<td>32.5 ± 7.3</td>
<td>35.7 ± 10.4</td>
<td>38.1 ± 11.6</td>
</tr>
<tr>
<td>CD4b+CD8d+, %</td>
<td>38.3 ± 9.1</td>
<td>35.7 ± 10.1</td>
<td>35.8 ± 11.4</td>
</tr>
</tbody>
</table>

*Data are mean ± SD. P values greater than .05 are not shown.
CD4b+CD8d+ indicates CD4 bright CD8 dull+ cells; CD4d+CD8b+, CD4 dull+CD8 bright+ cells.
P= .02 vs control subjects.
P= .002 vs patients with CVD.
P= .004 vs patients with CVD.
P= .005 vs control subjects, P= .002 vs patients with CVD.

Figure 1. Flow cytometry of lymphocytes (A, control subject; B, patient with Parkinson disease). Cells in the square above were counted as CD4 bright CD8 dull+ lymphocytes. Cells in the square above were counted as CD4 dull+ CD8 bright+ lymphocytes. PE indicates phycoerythrin; FITC, fluorescein isothiocyanate.

however, failed to achieve significance (Table 2). The results were not significantly correlated with any medications (not shown). No significant differences were observed in CD4 dull+ CD8 bright+ lymphocytes (Table 1). Three-color flow cytometry showed that these CD4 bright+ CD8 dull+ lymphocytes were also CD3+, indicating that these cells were T cells (not shown). These T cells demonstrated CD54 (intercellular adhesion mol-
ecule 1)* (not shown), CD45RO dull*, and CD95 (Fas) dull* (Figure 3). On the other hand, CD1a, CD25 (interleukin 2 receptor α), CD28, and CD126 (interleukin 6 receptor α) were negative (not shown).

Seven patients with PD who demonstrated greater populations of peripheral CD4 bright* CD8 dull* T cells were reexamined 17 to 25 months later. All of these patients showed similar percentages of this cell subpopulation (not shown).

**COMMENT**

In this study, we found further evidence of the presence of abnormalities of immune function in PD; that is, patients with PD demonstrated increased CD4 bright* CD8 dull* T cells in peripheral blood as compared with control subjects and patients with CVD.

T cells differentiate from CD4−CD8− cells to CD4+CD8+ cells during maturation in the thymus. After selection, the surviving T cells lose the expression of 1 of these 2 molecules and become either CD4+ or CD8+ T cells, and then normally enter the circulation. Therefore, the concomitant expression of CD4 and CD8 on the cell surface of the T lymphocytes has been regarded as representing immu-

<table>
<thead>
<tr>
<th>No. of Cases</th>
<th>CD4 Bright*, %</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>22</td>
<td>0.69 ± 0.37</td>
</tr>
<tr>
<td>Parkinson disease†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yahr I, II</td>
<td>10</td>
<td>1.50 ± 1.25</td>
</tr>
<tr>
<td>Yahr III</td>
<td>15</td>
<td>1.50 ± 0.99</td>
</tr>
<tr>
<td>Yahr IV, V</td>
<td>15</td>
<td>1.14 ± 0.78</td>
</tr>
</tbody>
</table>

*There was a trend for patients with Parkinson disease in Yahr stages I to III (mild to moderate) to demonstrate a higher proportion of CD4 bright* CD8 dull* T cells than those in Yahr stages IV and V (more advanced). Data are mean ± SD; P values are compared with control subjects.
†Yahr stages are described in Hoehn and Yahr.10

---

![Figure 2](image-url)  
**Figure 2.** Percentages of CD4 bright* CD8 dull* lymphocytes in control subjects, patients with cerebrovascular disease (CVD), and patients with Parkinson disease (PD). Note that the cell population in patients with PD is significantly greater than that in control subjects and patients with CVD.

![Figure 3](image-url)  
**Figure 3.** Three-color flow cytometry for CD4, CD8, and CD45RO (A-C) or CD95 (Fas) (D-F). Left, center, and right graphs show CD8 bright*, CD8 dull*, and CD8−, respectively. Arrows indicate CD4 bright* CD8 dull* lymphocytes. Note that these cells are CD45RO+ dull and CD95 (Fas) dull*. PE indicates phycoerythrin; FITC, fluorescein isothiocyanate.
turity.\textsuperscript{11} However, the increased peripheral CD4 bright$^+$ CD8 dull$^-$ T cells in the present results were not immature thymocytes, since they lacked CD1a expression, a characteristic of later developmental stages of thymocytes.\textsuperscript{11} CD4$^+$ CD45RO$^+$ T cells and CD8$^+$ CD45RO$^+$ T cells are known to be memory T cells and cytotoxic T cells, respectively. CD95 (Fas) has been shown to be increased in activated T cells. Therefore, these CD4 bright$^+$ CD8 dull$^-$ lymphocytes may be mature T cells with immune activities.

CD4 dull$^-$ CD8 bright$^+$ T cells were found for less than a month in association with Epstein-Barr virus.\textsuperscript{12} Cytomegalovirus, human herpesvirus 6, human T-cell leukemia virus type 1, and human immunodeficiency virus are also known to induce this phenotype.\textsuperscript{13} On the other hand, CD4 bright$^+$ CD8 dull$^-$ T cells were observed in patients with neoplasms as well as in small populations of healthy adults and were continuously present in similar percentages for a long period (about 3 years).\textsuperscript{15} An increase in CD4$^+$CD8$^+$ lymphocytes in peripheral blood also has been demonstrated during the rejection of renal transplants and in patients with multiple sclerosis or myasthenia gravis. However, the origin and functional characteristics of this immunophenotype are unknown.\textsuperscript{14,15} A previous report showed that interleukin 4 can induce the expression of CD8 on CD4$^+$ lymphocytes, resulting in the induction of the cytotoxic activity to cells in culture.\textsuperscript{16}

Why CD4 bright$^+$ CD8 dull$^-$ T cells increase in PD and whether these circulating lymphocytes contribute to the pathogenesis of neuronal death in PD remain to be investigated. Wekerle et al\textsuperscript{17} demonstrated that the nervous system is constantly patrolled by small numbers of T lymphocytes, which penetrate the blood-brain barrier nonspecifically and play a major role in the initiation and subsequent regulation of the intracellular immune response. Therefore, CD4 bright$^+$ CD8 dull$^-$ T cells may contact central nervous system cells. As CD4$^+$ CD8$^+$ T cells are known to increase after some specific viral infections as described above, the continuous increase of CD4 bright$^+$ CD8 dull$^-$ T cells shown herein may indicate postinfectious immune abnormalities that are possibly associated with the pathogenesis of PD, which is a slowly progressive, multifactorial neurodegenerative disease.

Accepted for publication June 12, 2001.

This study was supported in part by a grant from the Research Committee on Neuroimmunological Diseases, Ministry of Health and Welfare, Tokyo, Japan.

We thank Brent Bell, MA, for reading the manuscript.

Corresponding author and reprints: Kinya Hisanaga, MD, Department of Neurology, Miyagi National Hospital, 100 Kassenhara, Takase, Yamamoto, Watari, Miyagi 989-2202, Japan.

REFERENCES