Lymphocyte populations in Parkinson’s disease and in rat models of parkinsonism

Jordi Bas\textsuperscript{a,}\textsuperscript{*}, Mátíl Calopa\textsuperscript{b}, Mariona Mestre\textsuperscript{c}, David G. Molleví\textsuperscript{a}, Blanca Cutillas\textsuperscript{c}, Santiago Ambrosio\textsuperscript{c}, Enric Buendia\textsuperscript{a}

\textsuperscript{a}Immunology Service, Hospital Duran i Reynals, CSU de Bellvitge, Autovía de Castelldefels Km 2.7, L’Hospitalet de Llobregat, 08907 Barcelona, Catalonia, Spain

\textsuperscript{b}Neurology Service, Ciatat Sanitaria Universitària de Bellvitge, Barcelona, Catalonia, Spain

\textsuperscript{c}Biochemistry Department, Campus de Bellvitge, Universitat de Barcelona, Barcelona, Catalonia, Spain

Received 16 May 2000; received in revised form 28 September 2000; accepted 10 October 2000

Abstract

To assess the involvement of the immune system in Parkinson’s disease we studied the phenotype of circulating lymphocytes in 30 untreated and 34 treated patients. We found a numeric decrease in helper T cells (higher in CD4\textsuperscript{+}CD45RA\textsuperscript{+} than in CD4\textsuperscript{+}CD29\textsuperscript{+}) and B cells, and a rise in activated, CD4\textsuperscript{+}CD25\textsuperscript{+} lymphocytes that was correlated with lymphocyte depletion. All these alterations were independent of levodopa treatment. In addition, we performed striatal dopamine depletion in rats with either MPP\textsuperscript{7} or 6-OHDA, showing that MPP\textsuperscript{7} but not 6-OHDA can increase CD4\textsuperscript{+}CD25\textsuperscript{+} lymphocytes. Thus, mechanisms other than dopamine deficit may explain the immune activation in Parkinson’s disease. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Parkinson’s disease; Lymphocytes; Dopamine; Rat; Human; Activation

1. Introduction

Idiopathic Parkinson’s disease (PD) is a degenerative neurological disorder of unknown etiology. Several pathogenic mechanisms have been proposed to cause neuronal death such as oxidative stress, mitochondrial dysfunction or metabolic impairment. The major neurochemical feature is the loss of dopamine in nigrostriatal pathway but perturbations in other neurotransmitter systems also occur, including noradrenergic and cholinergic, which are related with the regulation of the immune system. Actually, it is not known whether immune mechanisms have a role in neurodegenerative diseases. However, in the last years the implication of the immune system and chronic inflammation in the neuronal death has been suggested (McGeer and McGeer, 1995). In PD, some indications of a certain degree of intracerebral immune activation have been described. These include activated microglia in the substantia nigra (McGeer et al., 1988; McGeer and McGeer, 1997a), proinflammatory cytokines (Mogi et al., 1994; McGeer and McGeer, 1997b), and enhanced expression of HLA-DR antigens on CSF monocytes (Fiszer et al., 1994). In addition, several authors have described immunological alterations such as the presence of antibodies against dopaminergic neurons in serum (Defazio et al., 1994) and CSF (Carvey et al., 1991) of PD patients, and higher ADCC activity (Bokor et al., 1993). Less data are available on phenotypic alterations of circulating lymphocytes, but changes in the percentage of CD8\textsuperscript{+} cells and CD4\textsuperscript{+} subsets (Fiszer, 1989; Fiszer et al., 1994) have been reported. All these findings are suggestive of an ongoing immune response that could play a role in the pathogenesis of the disease; however, the existence of immune activation in peripheral blood remains controversial. Indirect signs of lymphocyte activation, such as increased adenosine deaminase activity, have been observed by some authors (Chiba et al., 1995), but defective production of interleukin-2 by peripheral blood lymphocytes has also been demonstrated (Kluter et al., 1995).

In this context, the present study was aimed to assess the
composition of peripheral blood lymphocyte subsets and the presence of activated T cells in peripheral blood of PD patients. Furthermore, since the neuroendocrine regulation of the immune system could be impaired, we also assessed the role of dopamine in the described alterations of lymphocyte subsets. This was accomplished by comparing treated with untreated patients and by testing the effect of experimentally induced dopamine depletion in rats on the ‘in vivo’ activation of lymphocytes.

2. Material and methods

2.1. Patients

Sixty-four patients controlled in the Movement Disorders Unit of the Neurology Department of our Center and meeting strict inclusion criteria were selected for the study. These criteria included to fulfill diagnostic criteria of idiopathic Parkinson’s disease (Calne et al., 1992) and the absence of central nervous system lesions by a CT scan. The exclusion criteria were the presence of diseases that could affect significantly the immunological parameters assessed, such as inflammatory processes, autoimmune diseases and neoplasia. Moreover, any patient should be under immunosuppressive treatment. Concomitant illnesses at the time of the study are summarized in Table 1.

The clinical status was assessed by the Unified Parkinson’s Disease Rating Scale (UPDRS) (Lang and Fann, 1989) and Hoehn and Yahr stage (Hoehn and Yahr, 1967). Thirty patients (mean age 66±11 S.D. years old) were of ‘de novo’ diagnosis (1.8±2.2 years after presentation of symptoms) and the immunological study was carried out before treatment initiation. All of them showed a good response to antiparkinsonian treatment. Thirty-four patients (mean age 65±9 S.D. years old) were under antiparkinsonian treatment (levodopa in all cases, plus dopaminergic agonists in 19 cases, seven of which were also treated with selegiline) for a mean time of 5±3.7 years (from 1 to 13) at the time of study.

Thirty-eight healthy blood donors (mean age 63±4 S.D. years old) were included as a control group.

2.2. Analysis of human lymphocyte subsets

Blood extraction was always performed from 08:00 to 10:00 h. Blood was drawn in tubes containing K$_2$EDTA. Sample processing for flow cytometry was carried out directly from whole blood as described elsewhere (Mestre et al., 1992), since density gradient enrichment of mononuclear cells before analysis results in significant alterations of several lymphocyte subset percentages (Romieu et al., 1992). The cells were stained with the following fluochrome-conjugated monoclonal antibodies, alone or in double combinations: FITC-Leu4 (CD3), FITC-Leu3a (CD4), FITC-Leu2a (CD8), PE-IL2R (CD25) and PE-Leu12 (CD19) all from Becton-Dickinson (San Jose, CA) and with FITC-T4 (CD4), TRD1-4B4 (CD29), TRD1-2H4 (CD45RA), from Coulter (Hialeah, FL). A negative control stained with an irrelevant monoclonal antibody was always included. The samples were analyzed in direct double immunofluorescence by a FACScan flow cytometer (Becton-Dickinson). Four thousand events were acquired per sample and the percentage of lymphocyte subsets was calculated by using the CELLQuest™ software.

2.3. Soluble IL2 receptor quantitation

Measurement of soluble IL-2 receptor was carried out in 19 PD patients and 21 healthy subjects. Blood was allowed to clot and centrifuged at 4000×g. Serum was stored at −80°C until assay. The concentration of soluble IL-2 receptor was determined by a commercially available enzyme immunoassay (Immunotech, France) following the manufacturer’s directions. The assay uses monoclonal antibodies directed against two different epitopes of the IL-2 receptor and reports a sensitivity of 5 pM.

2.4. Induction of Parkinsonism in rats

Two different models of dopamine neuronal degeneration have been used: nigrostriatal degeneration by 6-OHDA injected into the substantia nigra and striatonigral degeneration by MPP+ injected into the striatum. The time after the lesion corresponds for each treatment to the maximal effect described on the striatal dopamine content and turnover (Espino et al., 1995). The parkinsonism models employed have been previously described (Espino et al., 1994b, 1995). Male Sprague–Dawley rats were bilaterally lesioned with 6-hydroxydopamine (6-OHDA) (24 μg in 3 μl) into the substantia nigra (coordinates: A, +4.8; L, ±1.6; H, −7.8) (n=6) or with MPP+ (12 μg in 4 μl) into the striatum (coordinates: A, +0.7; L, ±3.2; H, −5) (n=6). Sham-operated rats (n=12) received an equal

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Arterial hypertension</td>
</tr>
<tr>
<td>12</td>
<td>Dyslipemia</td>
</tr>
<tr>
<td>10</td>
<td>Depression</td>
</tr>
<tr>
<td>7</td>
<td>Diabetes mellitus type II</td>
</tr>
<tr>
<td>5</td>
<td>Ischemic cardiomyopathy</td>
</tr>
<tr>
<td>4</td>
<td>Benign hyperplasia of prostate</td>
</tr>
<tr>
<td>3</td>
<td>Arrhythmia</td>
</tr>
<tr>
<td>1</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>1</td>
<td>Chronic hepatitis C</td>
</tr>
<tr>
<td>9</td>
<td>Other (arthrosis, hyperuricemia, ulcus)</td>
</tr>
</tbody>
</table>
of the cytometer were adjusted for rat lymphocytes and the patients showed lower lymphocyte counts in Parkinson's disease patients. Comparison with healthy subjects (Student's t-test). Two-tailed P values: (a) P<0.001; (b) P=0.003; (c) P=0.047; (d) P=0.020.

3. Results

3.1. Peripheral blood T cell subsets in Parkinson’s disease

The analysis of lymphocyte subsets including all 64 patients showed lower lymphocyte counts in Parkinson patients than in control subjects (1839±577 vs. 2227±541; P=0.001) due to the decrease in the number of both T (CD3⁺) and B (CD19⁺) cells. As is shown in Table 2, the changes in T cells were caused by a decrease in the number of helper T cells (CD4⁺ lymphocytes) while its counterpart, the cytotoxic/suppressor CD8⁺ lymphocytes remained unchanged. When the results were expressed as percentages (Table 3) the values of T cells (CD3⁺) were maintained in comparison with the group of healthy subjects and the percentage of B cells (CD19⁺ lymphocytes) was slightly decreased. The observed decrease in absolute counts of CD4⁺ cells was also reflected in a decrease in the percentage of CD4⁺ lymphocytes with a concomitant increase in the relative values of CD8⁺ cells. This yielded a lower CD4/CD8 ratio (1.26±0.52 vs. 1.79±0.65; P<0.001). The fall in the absolute values of CD4⁺ lymphocytes was caused mainly by the ‘naive’
subset (CD4⁺CD45RA⁺ cells). However, the number of ‘memory’ helper T cells (CD4⁺CD29⁺) was also significantly decreased, but at a lesser extent. As it is shown in Table 3, there were no differences in the percentage of ‘memory’ cells but a decrease in the percentage of the ‘naive’ subset that caused the increase in the CD29/CD45RA ratio (3.16±2.89 vs. 1.70±0.87; \( P < 0.001 \)).

In addition, there was a high increase in the number and percentage of activated helper T cells (CD4⁺CD25⁺). The rise in activated CD4⁺ cells above the reference range established in our laboratory was observed in 24 treated and 26 untreated patients but only in six control individuals. Moreover, the percentage of CD4⁺CD25⁺ T cells showed a negative correlation with the number of total lymphocytes (Pearson coefficient: \(-0.3183; \ P = 0.012\) (Fig. 1) and with the absolute number of CD4⁺CD45RA⁺ T cells (Pearson coefficient: \(-0.2542; \ P = 0.048\)). These correlations were not found in the control group of healthy subjects. Parkinson patients did not show increased concentration of soluble IL-2 receptor with respect to the control group of healthy subjects (58.5 ± 46 vs. 57 ± 14 pM).

In order to assess the influence of the standard antiparkinsonian therapy on the lymphocyte populations treated and untreated patients were compared separately with the same control group (Tables 2 and 3). The behavior of the lymphocyte subsets in both groups of patients was essentially identical, maintaining the differences that were observed with respect to control individuals in the global analysis. Both untreated and treated PD patients showed a significant decrease in the ‘naive’ subset (CD4⁺CD45RA⁺) and a marked increase in activated, CD4⁺CD25⁺ T cells. However, the decrease in the ‘memory’ CD4⁺CD29⁺ cells lost its statistical significance in the treated group. Finally, to assess the possible role of dopamine agonists, patients under agonist treatment (19 cases) were compared with those not receiving agonists (15 cases), but no significant differences were observed, although a tendency (\( P = 0.0925 \)) towards an increase in lymphocyte count was observed in the cases under agonist therapy.

Finally, the absolute number and percentage of lymphoid subsets did not correlate with either the severity of the disease, expressed as the UPDRS, or the duration of the disease. As expected, a negative correlation was observed between the CD4⁺CD45RA⁺ lymphocytes and the age of both, patients and control individuals (not shown).

3.2. Lymphocyte activation in parkinsonian rats

We tested whether the activation of the CD4⁺ lymphocyte subset observed in PD patients could be secondary to the dopaminergic deficit. In this way, rats were depleted of endogenous dopamine by bilateral striatal injection of MPP⁺ or by bilateral injection of 6-OHDA into substantia nigra nuclei. Peripheral blood samples were drawn by cardiac puncture but their cytometric analysis revealed too low numbers of activated CD4⁺ lymphocytes expressing the α chain of the interleukin-2 receptor (CD25) to allow a reliable analysis. Thus, we studied lymphocytes extracted from mesenteric lymph nodes instead. Control rats showed very similar values and were grouped for comparisons with treated rats. Animals with bilateral lesions in the substantia nigra may be considered to have lost almost all the dopamine striatal content, and MPP⁺-treated animals have not such an extensive dopamine striatal depletion but they have damage to other neurotransmitter systems (i.e., GABA) and the blood–brain barrier integrity (Espino et al., 1994a, 1995). 6-OHDA treatment failed to increase significantly the percentage of activated lymphocytes. Only the group treated with MPP⁺ showed a slight but significant increase in CD4⁺CD25⁺ lymphocytes (Fig. 2).

4. Discussion

In recent years, a number of studies have suggested the involvement of immunological mechanisms in some neurodegenerative diseases, and this represents a conceptual challenge. The alterations in lymphocyte subsets have been widely described in autoimmune diseases, but data in neurodegenerative diseases such as PD are scarce. Our results are very interesting because they showed the depletion of some lymphocyte subsets in peripheral blood of PD patients and a certain degree of immunological activation. It is the first study in which cytometry data are
expressed in absolute counts, in addition to relative values. This approach allows a more accurate description of the variations in every lymphocyte population studied.

Our data on relative values of lymphocyte subsets supports part of the observations of Fiszer, who reported an increase in the proportion of CD8\(^{+}\) suppressor lymphocytes that lowered the ratio helper/suppressor cells (Fiszer, 1989). However, the expression of our results as absolute cell counts allowed us to extend their findings by showing that the percent increase in CD8\(^{+}\) T cells was actually caused by a selective decrease in the number of CD4\(^{+}\) T cells without changes in the number of CD8\(^{+}\) lymphocytes.

We also found that the decrease in CD4\(^{+}\) T cell number in Parkinson patients involved mainly the ‘naive’ CD4\(^{+}\)CD45RA\(^{+}\) T cells. However, the ‘memory’ helper T cells (which can be defined by either CD29 or CD45RO expression) also showed a significant reduction. The only previous report of these subpopulations in PD observed a percentual decrease in CD4\(^{+}\)CD45RA\(^{+}\) T cells with an increase in the CD4\(^{+}\)CD45RO\(^{+}\) T cells (Fiszer et al., 1994). Since CD45RA\(^{+}\) and CD29\(^{+}\)/CD45RO\(^{+}\) subsets of CD4\(^{+}\) T cells are mutually exclusive this apparent increase may be the result of a more marked depletion of CD45RA\(^{+}\) than of CD45RO\(^{+}\) helper T cells as we have observed in the present study.

The CD4\(^{+}\)CD45RA\(^{+}\) subset mainly consists of ‘naive’ T cells that are believed to exert a suppressor-inducer function. The decrease of this subset has been described in several autoimmune diseases such as systemic lupus (Morimoto et al., 1987), rheumatoid arthritis (Emery et al., 1987) and diabetes mellitus (Al-Kassab and Raziuddin, 1990). It has also been observed in multiple sclerosis, where the reduction of suppressor-inducer T cells during clinical exacerbation has been related to a concomitant decrease in suppressor function (Chofflon et al., 1988; Calopa et al., 1995). However, the finding of a decrease in CD4\(^{+}\)CD45RA\(^{+}\) T cells in a different pathological context such as PD, suggests that there are mechanisms linked to the neurodegeneration that lead to qualitative and quantitative variations in circulating lymphocytes similar to those observed in autoimmune diseases. Changes in functional differentiation or recirculation by alterations in the neuroendocrine regulation of the immune system, even selective apoptosis of susceptible lymphocyte populations may have a role.

Interleukin-2 has an essential role in activation, growth and differentiation of lymphocytes. It exerts its effects via a three-chain surface receptor where the \(\alpha\)-chain is the CD25 molecule. Since it is not expressed on resting T cells, the detection of CD25 on the cell membrane or its soluble form in plasma indicates lymphocyte activation. The increase in CD4\(^{+}\)CD25\(^{+}\) T cells is a very consistent finding in our study and clearly indicates that systemic immune activation is associated with the degeneration of neural structures in PD. Our findings are in agreement with a report of a slight increase in circulating cells positive for CD25 and HLA-DR in PD patients (Chiba et al., 1995). However, this study used a single labeling technique and the mean percentages of CD25\(^{+}\) cells found in both healthy controls and PD patients were much lower than those from our study. Our results also support more indirect indications of T lymphocyte activation such as an increase in adenosine deaminase activities (Chiba et al., 1995) and the finding of decreased lymphocyte receptors for IFN-\(\gamma\) and TNF-\(\alpha\), in PD (Bongioanni et al., 1997a,b). Interestingly, this is in contrast to some inflammatory neurological diseases, including multiple sclerosis, where the presence of activated T cells in peripheral blood is not a consistent observation (Bellamy et al., 1985; Hafler et al., 1985; Selmaj et al., 1986; Calopa et al., 1995). Again, the alterations of the immune system in neurological diseases seem to depend on the particular pathogenic mechanisms involved in each process. Furthermore, the degree of T cell activation in PD is not comparable to those found in systemic autoimmune diseases such as active lupus or during allograft rejection, in view of the low levels of soluble form of CD25 detected in our patients.

In the search for a link between the neurodegenerative processes and the peripheral immune activation observed in Parkinson patients it was of interest to assess the role of the depletion of endogenous dopamine in the rise of CD4\(^{+}\)CD25\(^{+}\) T cells because this is the neurotransmitter most severely affected in PD. Dopamine receptors have been described in lymphocytes (Faraj et al., 1991; Nagai et al., 1996; Ricci et al., 1997; Barbanti et al., 1999) and experimental lesions of dopaminergic pathways induced changes in T lymphocyte proliferation in mice (Deleplanque et al., 1994). Surprisingly, the depletion of dopamine...
by bilateral 6-OHDA injection into substantia nigra in rats failed to reproduce the increase in CD4^+CD25^+ T cells observed in our patients.

This fact, together with the finding that the alterations in phenotype and activation of lymphocytes in our Parkinson patients are not reversed by levodopa treatment, suggests that the loss of nigrostriatal dopamine by itself is not involved in the immune activation observed in PD. On the other hand, the fact that the bilateral lesion of striatum by MPP^+ yielded moderate increases in lymph node CD4^+CD25^+ cells suggests that activation may be induced by changes in other striatal factors (Espino et al., 1995), since MPP^+-striatal lesions cause not only dopamine loss but also affects gabaergic and other cells. However, since we have found that MPP^+ treatment also causes a partial disruption of the blood–brain barrier with macrophage and lymphocyte invasion of the striatum (Espino et al., 1994a), it cannot be ruled out that this inflammatory response could contribute to the activation of T helper cells in this model.

The finding of a significant decrease in the number of lymphocytes and of CD4^+CD45RA^+ T cells in our patients that was inversely correlated with the percentage of CD4^+CD25^+ T cells, suggests other mechanism in Parkinson patients as an alternative explanation for the increase in lymphocyte activation. It is known that the interaction between Fas ligand (CD95L) and Fas (CD95) on activated lymphocytes, is an important pathway in the regulation of cell death and this plays a significant role in the maintenance of immunological homeostasis. Lymphocytes dying of apoptosis do express activation molecules such as CD25 (Kishimoto et al., 1995), even Fas-mediated apoptosis is controlled by signals generated by the IL-2 receptor (Fournel et al., 1996). At present, there is an emerging body of evidence of the participation of apoptosis as a mechanism of cell death in neurodegenerative diseases such as Parkinson (Ziv and Melamed, 1998). Moreover, an increase in both lymphocyte activation in peripheral blood and 'in vitro' apoptosis (Fas antigen expression) has been found recently in some cases of Alzheimer’s disease (Lombardi et al., 1999). In view of these data, we suggest that the observed increase in CD4^+CD25^+ T cells may be related with the presence of increased lymphocyte apoptosis, which could cause lower lymphocyte numbers in PD.

In conclusion, the data presented here indicate the existence of a significant decrease in circulating T CD4^+ cells, particularly with 'naïve' phenotype, and B lymphocytes. In addition, there are signs of immune activation in peripheral blood that we failed to relate with the depletion of endogenous dopamine that occurs in PD. Other neurotransmitters with immunosuppressive function such as norepinephrine may be involved, but, since the activation is correlated with the decrease in circulating lymphocytes, we also suggest that apoptosis may explain the observed alterations. Therefore, further studies are currently in progress in our laboratory to test this hypothesis and its implications for the pathogenesis of the disease.

Acknowledgements

The authors thank Mr. Jordi Bonet and Mrs. Carme Salarich for his skillful technical assistance. We also thank Dr. A. Rubió for providing blood donor samples. Financial support from the 'Fondo de Investigaciones Sanitarias de la Seguridad Social', grant 94/1100.

References


Espino, A., Tortosa, A., Bendahan, G., Bartrons, R., Calopa, M., Ferrer, I., Ambrosio, S., 1994a. Stereotaxic administration of 1-methyl-4-


