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Increased Levels of Pro-Inflammatory and Anti-Inflammatory Cellular Responses in Parkinson's Disease Patients: Search for a Disease Indicator

Authors' Contribution:
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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder and it arises when most of the dopaminergic neurons of substantia nigra region die. Several mechanisms have been postulated as the causative event in PD pathology, and neuroinflammation is most crucial among them.





Material/Methods: We analyzed T-helper 17 (Th17) cells and myeloid-derived suppressor cells (MDSCs) from 80 PD patients to assess inflammatory processes and to find a cost-effective means to evaluate PD prognosis.

Results: We found significantly increased numbers of Th17 cells and MDSCs count in peripheral circulation in PD patients compared with controls ($p < 0.001$). A positive correlation was found between Th17 cells and MDSCs in PD patients ($r = 0.421$, $p < 0.05$).

Conclusions: Our results show the effector role of Th17 cells and MDSCs in PD pathology and shows their utility as effective biomarkers for PD diagnosis.

MeSH Keywords: **Biological Markers • Myeloid Cells • Neurogenic Inflammation • Parkinsonian Disorders • T-Lymphocytes, Helper-Induc**

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Background

The pathophysiology of Parkinson's disease (PD) arises when progressive neurodegeneration in the substantia nigra region occurs, which is the confirmatory clinical diagnostic feature of PD [1]. As the disease expresses phenotypically only when 80–90% neuronal loss has already occurred, the early diagnosis of PD is a difficult challenge. Moreover, the global scenario of a growing trend of PD makes it a serious concern [2]. Over the last 100 years, studies have shown that several mechanisms influence the pathogenesis of PD, including oxidative stress, inflammation, apoptosis, and endogenous and exogenous neurotoxins [3–5]. Among them, inflammation holds a crucial role as most brain cells are glia, which have an important role in quickly spreading inflammation [6,7]. More specifically, clustered differentiation of 4+ (CD4+) T lymphocytes produces a subset of cells called T-helper 17 (Th17) cells [8], which act as a pro-inflammatory factor in inflammation [9–11]. Reports have shown that Th17 cells are active participants in PD pathogenesis [8]. On the other hand, CD14-/CD11b+/CD33+ cells in humans are one of the subtypes of myeloid-derived suppressor cells (MDSCs), which have the potency to inhibit the ongoing inflammatory process by acting on Th17 cells [12]. Reports have shown that the growing numbers of MDSCs are traceable in various inflammatory diseases [13–15], but the actual mechanistic pathway underlying MDSCs induced inhibition of Th17 cells in PD is largely unknown [16]. However, it has been reported that the occurrence of PD is highly correlated with the occurrence of inflammation [17]. Particularly in PD, the role of TH17 has been documented as a critical determinant for neurodegeneration through the inflammatory pathway [18], and MDSCs are also found to be highly active in neurodegeneration [19]. Hence, the estimation of Th17 cells as well as MDSCs is important in the diagnosis of PD. Therefore, in the present study, we have determined the quantity of Th17 and MDSCs to get an insight into the PD pathology and to introduce the measure as a novel biomarker in PD diagnosis.

Material and Methods

Subject and experimental design

Subjects were chosen randomly and matched for age and sex. At the end of the selection procedure, a total of 80 patients in the initial stages of PD were enrolled from the No. 101 Hospital of Chinese PLA (from January 2012 to December 2016). Hoehn and Yahr classification (H&Y) and Unified Parkinson's Disease Rating Scale (UPDRS) were used to access the PD pathology. Inclusion criteria also included the following: willingness to undergo neurological assessment, data on patient nutritional status, and willingness to provide blood samples (blood, glucose, liver function, lipid profile) at checkup. As the study was

focused on the early diagnostic feature of PD, we excluded PD patients with tremors, PD+, secondary parkinsonian symptoms, immunity-related complications, and patients on anti-PD drug treatment. The study was approved by the Medical Ethics Committee of Affiliated No. 101 Hospital of Chinese PLA. We also enrolled and obtained informed consent from 80 volunteers who served as the control group.

Flow cytometry analysis

Detection of Th17 in peripheral blood

Blood was collected from the peripheral circulation from both PD and control subjects in heparinized tube with utmost care and security. Collected blood cells were incubated in the medium containing RPMI 1640 with Ionomycin 500 ng/mL, PMA 50 ng/mL (Sigma-Aldrich, St Louis, MO). In the initial step, GolgiPlug™ 1 µg/mL (Becton Dickinson Biosciences, San Jose, CA) was added in the culture. Ficoll-Paque density gradient separation was performed prior to storage of lymphocytes in culture medium. Culture media was stored at 37°C in a 5% CO₂ atmosphere for 4–6 h. Activated cells were washed with PBS and stained with APC-CD3, FITC-CD8, and PE-IL-17A antibodies (BD PharMingen, San Diego, CA), then fixed and permeabilized with the Cell Fixation and Permeabilization Kit (BD PharMingen, San Diego, CA). The re-suspended cells were analyzed by FACSCalibur (Becton Dickinson Biosciences, Shanghai, China) equipped with FlowJo software.

Detection of MDSC in peripheral blood

For the detection of MDSCs, EDTA was used as anticoagulant and whole blood was processed without any time lag. MDSCs cells were separated by Ficoll-Hypaque density gradient centrifugation and then stained with FITC-CD33 antibodies (BD PharMingen, San Diego, CA, USA). MDSCs cells were visualized and analyzed using FACS Calibur (Becton Dickinson Biosciences, Shanghai, China) equipped with FlowJo software.

Statistical analyses

The experimental data were mean ± standard deviation for the statistical description. Differences between groups were determined by nonparametric statistical analysis. Correlations between 2 variables were quantified by determining the Spearman's rank correlation coefficients. $p < 0.05$ was considered to indicate a statistically significant difference. SPSS 24.0 statistical software was used for statistical analysis. Graph Pad Prism 7 and Microsoft Excel 2007 were used for drawing statistical figures.

Table 1. Analysis of different serological and psychometrics.

| Characteristics or stages | PD group | Control group | p value |
|---------------------------|--------------|---------------|--------------------|
| Gender male/female | 40/40 | 40/40 | 1.00* |
| Age (range) | 66.23±6.54 | 66.74±5.34 | 0.261 [#] |
| HB | 123.32±12.33 | 124.55±13.54 | 0.553 [#] |
| WBC | 6.45±1.04 | 5.35±2.48 | 0.537 [#] |
| NEU% | 58.51±6.40 | 56.36±4.61 | 0.455 [#] |
| LYMPH% | 32.47±3.50 | 31.02±4.54 | 0.054 [#] |
| MONO | 6.54±2.59 | 6.50±1.87 | 0.432 [#] |
| H&Y | 1.23±0.17 | N/A | |
| UPDRS-I | 2.98±1.21 | N/A | |
| UPDRS-II | 8.85±3.47 | N/A | |
| UPDRS-III | 13.45±5.29 | N/A | |
| UPDRS-IV | 6.93±1.30 | N/A | |
| UPDRS-Total | 32.11±12.02 | N/A | |

PD – Parkinson's disease; Data presented as n or mean ± standard deviation. HB – Hemoglobin; WBC – the counts of the white blood cells; NEU% – percentage of Neutrophils; LYMPH% – the percentage of lymphocyte; MONO – monocytes; H&Y – Hoehn and Yahr classification; UPDRS I, II, III, IV and Total – Unified Parkinson's Disease Rating Scale I, II, III, IV and Total; N/A – not applicable. Tests performed in the table were: * chi-square and [#] student's test.

Results

Characteristics of subjects

No significant difference was observed ($p > 0.05$) between the 2 groups in age, sex, hemoglobin, white blood cell counts, monocytes, and the percentage of neutrophils and lymphocytes. On the basis of PD severity and symptoms (H&Y and UPDRS scores), patients had mild-to-moderate PD. Data are presented as mean ± standard deviation in Table 1.

Detection of Th17 cells and MDSCs

The percentages of Th17 cells and MDSCs in peripheral blood of PD patients were significantly higher than those of the control group ($1.56 \pm 1.38\%$ vs. $0.13 \pm 0.08\%$; $11.26 \pm 2.38\%$ vs. $1.26 \pm 1.36\%$, $p < 0.001$) (Figures 1, 2, and Table 2).

Correlation of the percentages between Th17 cells and MDSCs

The result showed that the percentage of Th17 cells and MDSCs in peripheral blood in the PD group was positively correlated ($r = 0.421$, $p < 0.05$) (Figure 3A); however, 2 indexes had no correlation in the control group ($r = 0.116$, $p = 0.5$) (Figure 3B).

Discussion

It has been reported that in PD pathogenesis, inflammatory responses play crucial roles, which has been evaluated by the increased expression of interferon gamma (IFN- γ), interleukin 6 (IL-6), and interleukin 1 beta (IL-1 β) in the brain [20]. Such overexpression leads to neuroinflammation and becomes the crucial event in the neurodegeneration in the dopaminergic center of the substantia nigra region of the midbrain. Exclusive work of Brochard and colleagues has documented that Th17 cells actively participate in nigral neurodegeneration by infiltrating the region, which results in excessive activation of microglial cells [21]. It is well known that brain parenchyma is separated by the presence of the blood brain barrier (BBB), which restricts the entry of inflammatory substances. However, physical damage to the BBB has been reported in chronic inflammatory spectrum, which is also evident in the scenario of PD [22]. Damage in the BBB allows inflammatory cells and various cytotoxic entities into the brain parenchyma of people with PD, which not only initiates the detrimental pathways of neuroinflammation, but also influences other mechanistic pathways associated with neurodegeneration, such as oxidative stress and mitochondrial dysfunction [23]. Infiltration of T lymphocytes is quite common in individuals with a damaged BBB [24,25]. Such infiltration has been reported several times in different disease profiles, where the infiltrated Th17 plays a crucial detrimental role [26,27]. It has been reported that Th17

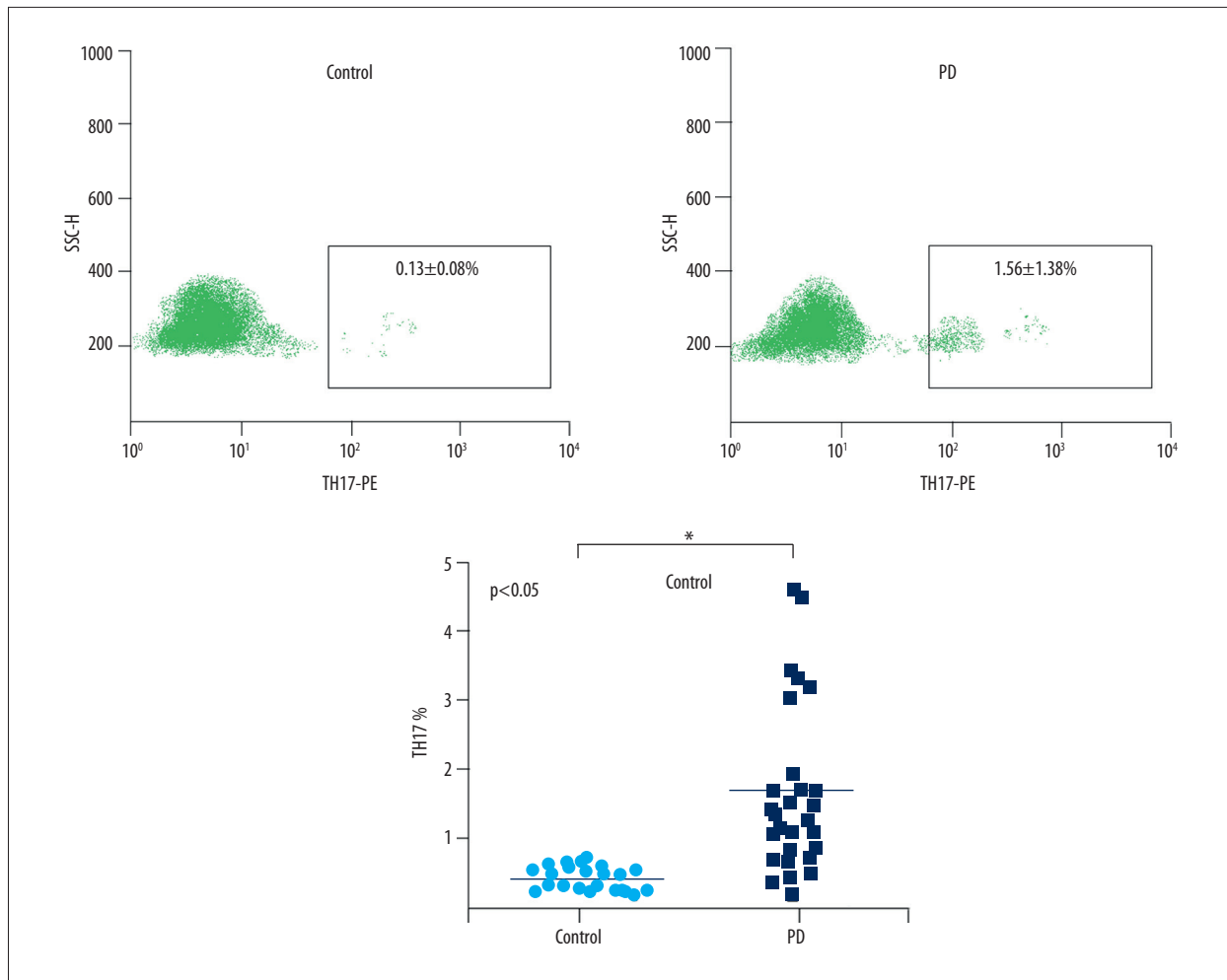


Figure 1. Detection of Th17 cells from PD and control group: Blood collected from peripheral circulation of PD patients contained significantly more Th17 cells than in the control subjects ($1.56 \pm 1.38\%$ in PD group, $0.13 \pm 0.08\%$ in control group, $p < 0.05$). The line represents the mean of the individual values.

increases release of IL-17, which is an important inflammatory factor, and is also associated with activation of other detrimental inflammatory factors like tumor necrosis factor alpha (TNF- α) and interleukin-1 (IL-1). These inflammatory factors have been shown to be released from brain microglial cells, which are the most numerous type of brain cell; therefore, inflammatory responses quickly spread throughout the brain [28]. MDSCs are immature bone marrow cells, which are assumed to have a crucial role in inhibition of inflammation [29]. It is interesting that differentiation of initial CD4+ T cells or Th17 cells are greatly influenced by different subsets of MDSCs [30]. Induction of CD14 with HLA-DR has been reported to induce Th17 cell differentiation, which promotes brain inflammation [30]. However, a similar combination with low CD14 has been shown to have a different mechanism of action that includes production of T_{reg} cells, which are a type of CD4+ T lymphocyte responsible for proper immune regulation. This process also promotes negative regulation of neuroinflammation in

brains affected by PD [29,30]. It was also reported that MDSCs are responsible for the maturation and transformation of Th17 and T_{reg} cells, and this transformation is highly controlled by cytokines. These findings show that MDSCs can regulate the balance between Th17 and T_{reg} cells to regulate immune balance in the brain [31]. Because administration of an anti-PD drug could alter the expression of Th17 cells [32], the present study assessed newly-diagnosed PD patients without anti-PD drug treatment. We demonstrated an increased percentage of Th17 and MDSCs in the peripheral circulation of PD patients, which was significantly higher than in the control subjects. Such a scenario confers the assurance of neuroinflammation and also depicts the involvement of these cell types in brain inflammation. Earlier reports have documented that, in the PD brain, the quantity of inflammatory markers, such as IL-6, transforming growth factor beta (TGF- β), and IL-1, increases significantly [33,34]. Moreover, MDSCs are reported to induce the CD4+ T cells to transform into Th17 cell types

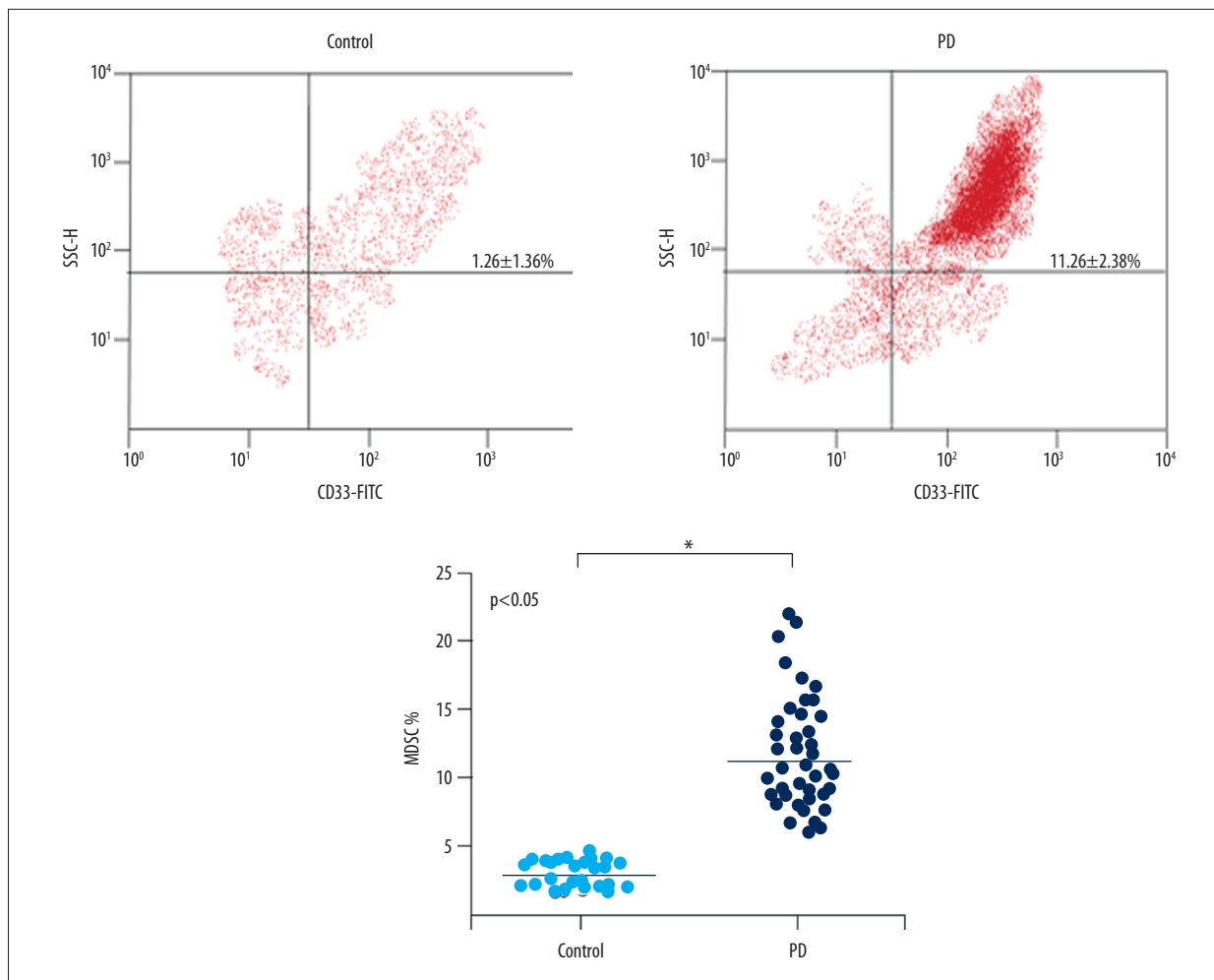


Figure 2. Detection of CD33 MDSC from PD and control groups: The results show the percentage of MDSCs in peripheral blood, which was significantly higher in PD patients than in control subjects (11.26±2.38% in PD group, 1.26±1.36% in control group, $p < 0.05$). The line represents the mean of the individual values.

Table 2. Comparison of the percentage of Th17 and MDSC of peripheral blood in different groups.

| Group | n | Th17% | MDSC% |
|---------|----|------------|-------------|
| PD | 80 | 1.56±1.38* | 11.26±2.38* |
| Control | 80 | 0.13±0.08 | 1.26±1.36 |

Data were presented as mean ± standard deviation; * $p < 0.001$, compared with the control group.

under certain condition [18, 35]. The prime function of MDSCs is to suppress the immune responses, but transforming CD4+ T lymphocytes into Th17 cells could increase the inflammation for a limited time, which promotes PD progression. The occurrence and development of Th17 and MDSCs are involved in PD because they showed positive correlation in our study, but the detailed mechanism of action behind Th17-MDSCs interaction in PD needs further study. The present study is the first to show that Th17 and MDSCs are involved in the early stages of PD, and might be useful as biomarkers in the diagnosis of

PD. Because reducing the percentage of Th17 could help delay the progression of PD, the study also shows the promise of a new therapeutic intervention for PD and associated neuroinflammatory complications.

Conclusions

The present study is the first to report the possibility of early diagnosis of PD through quantification of TH17 and CD33

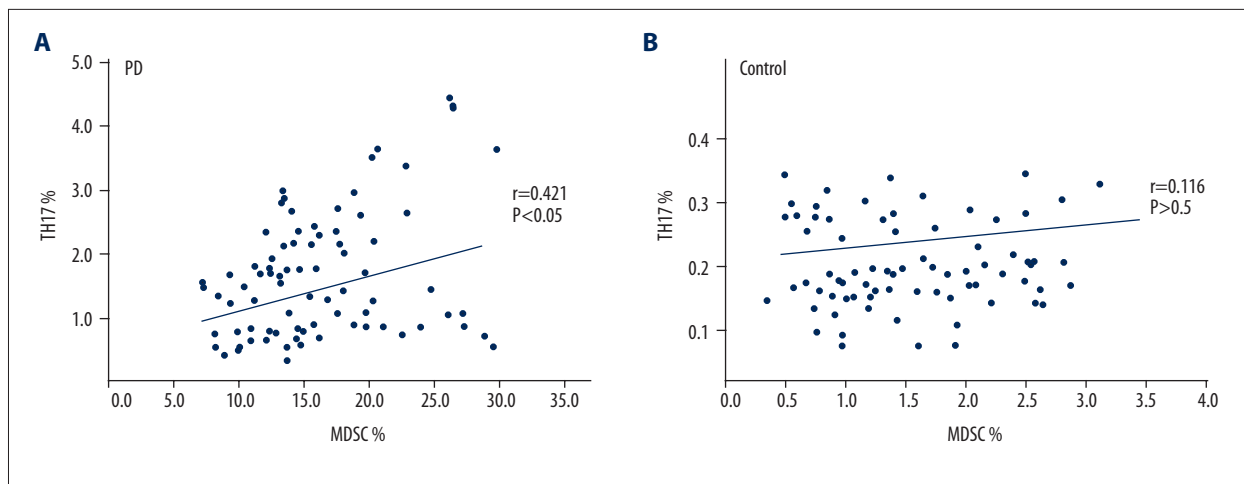


Figure 3. The ratio between Th17 cells and MDSCs in peripheral blood of the PD group was positively correlated ($r=0.421$, $p<0.05$) (A). However, no correlation was seen between Th17 cells and MDSCs ratio in the control group ($r=0.116$, $p>0.5$) (B).

MDSC from peripheral circulation. Experimental evidence showed that high levels of TH17 are associated with neuroinflammation and play the determining role in PD disease progression. Similar quantification from cerebrospinal fluid could give better and more accurate results. However, for the time being, clinical diagnosis of PD from peripheral circulation could be the most cost-effective approach.

Conflicts of interest

None.

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