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Original Research

Frequencies of Circulating Immune Cells in Patients with Parkinson's Disease: Correlation with MDS-UPDRS Scores

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Abstract

Background: Parkinson's Disease (PD) is associated with dysregulated/chronic inflammation. The immune system has multiple roles including beneficial effects such as clearing alpha synuclein aggregates. However, peripheral immune cells entering the brain may also contribute to inflammation and neurodegeneration. To identify which cells might have a negative impact and could be potential therapeutic targets, we compared immune signatures of patients and healthy controls. **Methods**: Multicolor flow cytometry was used to determine the frequencies of major immune cell subsets in peripheral blood mononuclear cells (PBMCs) of PD patients and controls. Because of the major impact of Cytomegalovirus (CMV) infection on the distribution of immune cell subsets, particularly cluster of differentiation (CD)8+ T-cells, all participants were tested for CMV seropositivity. **Results**: Although the cohort of 35 PD patients exhibited the well-established T-cell differentiation signature driven by CMV infection, there were no differences in the frequencies of differentiated or pro-inflammatory T-cells, B-cells or natural killer cells (NK-cells) attributable to the disease. However, percentages of myeloid-derived suppressor cells (MDSCs) were higher in PD patients than controls. Moreover, percentages of CD14+CD16+ (intermediate) monocytes expressing the C-C chemokine receptor type 5 (CCR5) correlated with disease severity assessed by the Movement Disorder Society's revised version of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) score and disease duration. **Conclusions**: A comprehensive evaluation of the major subsets of circulating immune cells in PD patients revealed differences in myeloid cells between PD and healthy controls and some correlation of monocyte abundance with disease severity.

Keywords: Parkinson's disease; CMV; T-cells; B-cells; NK-cells; MDSC; CCR5; monocytes; inflammation; neurodegeneration; peripheral immune cells

1. Introduction

Parkinson's Disease (PD) is characterised by an age-associated progressive degeneration of dopaminergic neurons in the *substantia nigra* [1] and the accumulation of alpha-synuclein (α-syn) protein in brain and periphery which is one of the main components of characteristic inclusions termed Lewy bodies [2,3]. α-syn released by neurons activates microglia to secrete pro-inflammatory cytokines such as interleukin-1 beta (IL-1β) and to produce reactive oxygen and nitrogen species promoting neurodegeneration [2,4]. It is hypothesised that microglia are first "primed" and then activated by a secondary pro-inflammatory stimulus from the periphery enhancing neurotoxic responses (reviewed in [5]). Brain-periphery interactions are facilitated as the integrity of the blood-brain barrier (BBB) is affected in PD [6]. Cytokines are emerging as biomarkers of PD [7] and anti-inflammatory treatment has shown some beneficial effects, but this can act as a double-edged sword because inflammation also has a beneficial role by inducing the expression of molecules which help in repair and regeneration of damaged tissue [5]. A more specific therapy targeting single leukocyte subsets could keep the balance between reducing unwanted brain inflammation while retaining or increasing desired inflammation-triggered neuroprotection [8,9].

To be able to address the most promising candidates for immunotherapy, a comprehensive overview of immune signatures in PD compared to healthy controls is needed, especially as there is clear evidence in the literature that subsets of inflammatory cells are associated with

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PD pathology. For example, the levels of peripheral dendritic cells, especially those secreting pro-inflammatory cytokines, were found to be altered in PD patients compared to controls and an impact on disease severity was proposed [10,11]. A neuroprotective role of natural killer cells (NK-cells) (by supporting α-syn clearance [12]) explains increased PD neuropathology in mice with deletion of these cells [13]. In mice, B-cell haematopoiesis was reported to be regulated by α-syn [14] and this might influence the frequencies of B-cells and their phenotype. In mouse PD models, the balance of immune cell types is shifted towards inflammation due to deficits in regulatory T-cells [15]. These and other examples suggest the importance of peripheral immunity in PD.

Infection with the persistent herpesvirus cytomegalovirus (CMV) and high anti-CMV Immunoglobulin G (IgG) antibody titers are related to an inverted cluster of differentiation (CD)4:CD8 T-cell ratio andmay be associated with age-independent morbidity, mortality and cognitive and functional decline [16]. Another point to consider: T-helper 17 (Th17) cells affect BBB integrity [17] which can promote enhanced migration of peripheral cells into the brain and mediation of dopaminergic neurodegeneration as shown in a mouse model [18]. Therefore we investigated the capacity of T-cells to produce cytokines and determined T-helper cell profiles in the context of CMV-serostatus and PD. Taken together, functional and immunophentyping analysis leads to a better understanding of the complexity of PD immunity. Our study revealed an example of the immune network in PD with similar T-cell profiles between PD patients and controls and on the other hand a correlation of the frequencies of C-C chemokine receptor type 5 (CCR5)-expressing CD14+CD16+ monocytes with disease severity and disease duration.

2. Materials and Methods

PD patients without signs of dementia and their partners as controls free from any neurological disease and living in the same environment were recruited from the outpatient clinic at the Neurodegenerative Department of the University of Tübingen, Germany. Clinical data were collected (Table 1) with patients' written consent and approval by the Ethics Committee of the Medical Faculty of the University of Tübingen (project number 480/2015BO2) [19,20]. PD patients fulfilled the United Kingdom Parkinson's Disease Society (UKPDS) Brain Bank Diagnostic criteria [21]. The severity of motor malfunctions and disease progression was assessed with the Hoehn and Yahr [22] score and the Movement Disorders Society-revised version of the Unified Parkinson's Disease Rating scale (MDS-UPDRS) [23].

Plasma was collected and CMV serostatus determined via a recombinant CMV IgG immunoblot (Mikrogen, Neuried, Germany) using six different targets (IE1, p150, CM2, p65, gB1 and gB2). Peripheral blood mononuclear cells (PBMCs) were isolated from whole ethylenediaminetetraacetic acid (EDTA) blood using Fico-Lite FicoLite-H(human) (Cat.No.: GTF1511YK, Linaris, Dossenheim, Germany) gradient centrifugation. PBMCs in 10% dimethylsulfoxide (DMSO) (research grade, Cat.No.: 20385.01, Serva, Heidelberg, Germany) and 20% fetal-bovineSerum (FBS) (Cat.No.: F0804, Sigma, Darmstadt, Germany) were frozen at –80 °C and subsequently stored in liquid nitrogen. Cells were thawed and after blocking non-specific antibody binding with 1% Gamunex (human IgG from Talecris, Research Triangle Park, NC, USA) dead cells were stained with ethidium monoazide bromide (Cat.No.: 40015, Biotium, Fremont, CA, USA). Different surface marker stainings in phosphatebufferedsaline (PBS) (Cat.No.: H15-002, PAA, Paching, Austria) with 2% (v/v) FBS (Cat.No.: F0804, Sigma, Darmstadt, Germany), 2 mM EDTA (Cat.No.: 11278, Serva, Heidelberg, Germany) and 0.01% (w/v) sodiumazide (NaN_3) (Ca.No.: S-8032, Sigma, Darmstadt, Germany) were performed using the antibodies shown in **Supplementary Table 1**. To assess T-cell function 0.5×10^6 PBMCs were incubated for 16 h in XVIVO-15 (Cat.No.: 02-060Q, Lonza, Basel, Switzerland) culture medium either without stimuli or in the presence of phorbol 12-myristate 13-acetate (PMA) (Cat.No.: P-8139, Sigma, Darmstadt, Germany) plus ionomycin (Ionomycin Calcium Salt, Cat.No.: 407952, Calbiochem, Darmstadt, Germany) and the protein transport inhibitor Brefeldin A (GolgiPlug, Cat.No.: 555029, BD, Heidelberg, Germany). After incubation, cells were fixed and permeabilized (BD CytofixCytoperm, Cat.No.: 554714, BD, Heidelberg, Germany) and stained for intracellular cytokines. Measurements were conducted on a BD LSRII flow cytometer and DIVA 6.1 software (Becton Dickinson, Franklin Lakes, NJ, USA). To confirmn constant cytometer performance, control BD Cytometer setup&tracking beads (CS&T) (Cat.No.: 642412, BD, Heidelberg, Germany) tracking beads and BD 8 peak rainbow beads (Cat.No.: 559123, BD, Heidelberg, Germany) were applied. For data evaluation FlowJo software version 7.2.5 (Treestar, Ashland, OR, USA) was used. Gating strategies for the individual panels are displayed in the **Supplementary Figures**. For statistical analysis Mann-Whitney U test or linear regression analysis was performed using GraphPad Prism 5 (GraphPad Software, Inc., Boston, MA, USA).

3. Results

3.1 T-Cell Phenotypes

To investigate possible interactions between the systemic inflammation in PD described in the literature [5] and T-cell differentiation, the expression of different surface markers was analysed. To exclude the confounding effects of CMV infection, patients and controls were dichotomized into seropositives and seronegatives. First, the overall frequencies of CD4+ and CD8+ T-cells were quantified. Higher CD8+ T-cell frequencies in CMV+ individuals were

Table 1. Clinical data of the study participants.

	Controls	Controls	PD patients	PD patients
CMV serostatus	CMV-	CMV+	CMV-	CMV+
Number	9	13	17	18
Median age	68	60	68	68
Age range	58–74	53–77	55–74	43–73
Gender	3F/6M	6F/7M	7F/10M	6F/12M
Median MDS-UPDRS I	2	0	7	6
Median MDS-UPDRS II	0	0	10	6
Median MDS-UPDRS III	1	1	29	28
Median MDS-UPDRS IV	0	0	0	0
Median total MDS-UPDRS	2	2	45	42
Total MDS-UPDRS range	0–13	0–11	24–112	14–68
H&Y score range	0–2	0–1	1–4	1–4
Median disease duration			9 years	5 years
Disease duration range			2–13 years	1–10 years

CMV, Cytomegalovirus; PD, Parkinson's Disease; MDS-UPDRS, Movement Disorder Society-revised version of the Unified Parkinson's Disease Rating scale; H&Y, Hoehn and Yahr Scale; M, male; F, female.

found while patients and controls did not display different patterns except a slightly higher frequency of CD4+CD8+ T-cells (T-cells associated with chronic inflammatory disorders [24]) in PD patients. All differences between groups are summarized in Table 2. Also regarding the specific T-cell phenotypes the differences were limited to CMV- compared to CMV+ study participants, but not affected by disease. T-cells from patients and controls showed a similar differentiation profile. Naïve T-cells were characterised as CD45RO-CD45RA^{hi}CD27+CD28+ and late-differentiated T-cells as CD45RO+CD45RA-CD27-CD28- or CD95+ or CD57+. Applying the main published models [25] we found a shift from early- to late-differentiated T-cells in CMV+ compared to CMV- individuals, although not all marker combinations led to statistically significant results and CD8+CD45RO+ T-cells showed contrary results with slightly lower frequencies in CMV+ than CMV- PD patients. The differences between CMV- and CMV+ individuals were more pronounced for CD4+ T-cells than CD8+ T-cells and in patients than controls.

3.2 T-Cell Cytokine Production

To detect key players possibly contributing to systemic inflammation in PD, PBMCs were mitogenically stimulated and intracellular cytokines analysed by flow-cytometry for a panel of pro- and anti-inflammatory cytokines. CMV drives T-cell differentiation, as demonstrated by phenotypic analysis and also by functionality. However, the only statistically significant differences between groups in this analysis were limited to a higher frequency of unstimulated (basal *ex-vivo*) CD4+ T-cells producing pro-inflammatory interferon gamma (IFNγ) in CMV+ than CMV- controls and a lower frequency of CD8+ T-cells producing anti-inflammatory IL-4 in CMV+ than in CMV- PD patients (**Supplementary Table 2**). To assess the overall capacity to produce cytokines, PBMCs were stimulated with mitogenic PMA and ionomycin and frequencies of cytokine-producing CD4+ and CD8+ T-cells evaluated. Here, there were no significant differences between any groups. Hence, this analysis failed to reveal any important differences between patients and controls in either basal or stimulated T-cell cytokine production.

3.3 B-Cell Analysis

First, the frequencies of all B-cells (CD19+) and plasmablasts (CD19+CD20–) within the total leukocyte population (CD45+) were analysed. Then CD27+CD43+ B1 cell frequencies were investigated within CD19+ and CD20+ cells. Next, CD24 and CD38 expression was plotted against each other and double-positive cells gated to define transitional B-cells (CD38^{hi}CD24+). Further, CD38–CD24–, CD38^{hi}CD27+ and CD40^{hi} B-cells were gated. To determine differentiation states, IgD expression was plotted against CD27. IgD+CD27– were considered early- and double-negative B-cells late-differentiated. No significant differences between any of the populations analysed were found. These results are summarized in **Supplementary Table 3.**

3.4 Other Leukocyte Subsets

We next analysed the frequencies of the different major innate leukocytes but again found no differences regarding immature NK-cells (CD56^{hi}CD3–), mature NK-cells (CD56^{lo}CD3–) or NKT-like cells (CD14–CD3+CD16+). NKT-cell (CD56+CD3+) frequencies were higher in CMV+ than in CMV– controls and also in PD patients but decreased over the disease duration. No differences were observed when comparing patients and controls. Monocyte subsets (CD3–CD20–CD56–CD14+CD16–, CD3–CD20–CD56–CD14+CD16+, CD3–

Table 2. Comparisons of differences in T-cell phenotypes between groups.

Population	Parental	C– vs. C+	PD– vs. PD+	C– vs. PD–	C+ vs. PD+
N		7 vs. 11	14 vs. 14	7 vs. 14	11 vs. 14
CD4+	CD3+	–	↓	–	–
CD8+	CD3+	↑↑	↑↑	–	–
CD4+:CD8+	CD3+	–	↓↓	–	–
CD4–CD8–	CD3+	–	–	–	–
CD4+CD8+	CD3+	↑	–	↑	–
CD27+CD28+	CD4+	↓↓	↓↓	–	–
CD27–CD28–	CD4+	↑↑	↑↑↑	–	–
CD45RO–CD45RA^{hi}	CD4+	–	–	–	–
CD45RO+CD45RA–	CD4+	–	–	–	–
CD45RO–CD45RA^{hi}CD27+CD28+	CD4+	–	–	–	–
CD45RO+CD45RA–CD27–CD28–	CD4+	↑↑	↑↑↑	–	–
CD57+	CD4+	↑↑	↑↑	–	–
CD95+	CD4+	–	–	–	–
CD27+CD28+	CD8+	–	↓↓	–	–
CD27–CD28–	CD8+	↑	↑↑↑	–	–
CD27+CD28–	CD8+	–	–	–	–
CD27–CD28+	CD8+	–	–	–	–
CD45RO–CD45RA^{hi}	CD8+	–	–	–	–
CD45RO+CD45RA–	CD8+	–	↓	–	–
CD45RO+CD45RA+	CD8+	–	↓	–	–
CD45RO–CD45RA^{hi}CD27+CD28+	CD8+	–	–	–	–
CD45RO+CD45RA–CD27–CD28–	CD8+	–	–	–	–
CD57+	CD8+	–	↑	–	–
CD57+	CD4–CD8–	–	–	–	–

Footnotes: − $p \geq 0.05$, ↕ $p < 0.05$, ↕↕ $p < 0.01$, ↑↑↑ $p < 0.001$ after Mann-Whitney analysis; C, control; PD, PD patient; – , CMV-seronegativ; +, CMV-seropositive; CD, cluster of differentiation. See **Supplementary Fig. 1** for representative flow cytometry data.

CD20–CD56–CD14^{lo}CD16+) within CD45+ PBMCs also revealed no differences between groups. However percentages of CD14–CD3–CD16–CD20+ cells negatively correlated with total MDS-UPDRS values and disease duration in CMV- PD patients. CCR5 expression was not different on B-cells, but frequencies of CCR5+CD14+CD16+ monocytes positively correlated with disease duration and total MDS-UPDRS values. Several different myeloid-derived suppressor cells (MDSCs) were also different between the groups as follows: CD14+CD15–CD11b^{lo} human leucocyte antigen(HLA)-DR- higher in CMV+ vs. CMV– PD patients, CD14–HLA-DR–CD33–CD11b^{lo} lower in CMV+ vs. CMV– controls and higher in CMV– PD patients vs. controls. Notably, frequencies of CD14+CD15+HLA-DR–CD11b+CD33+ MDSCs correlated positively with total MDS-UPDRS values in CMV+ PD patients (Table 3).

4. Discussion

Systemic inflammation has been documented in several neurodegenerative diseases. Peripheral immune profiles differ between patients and controls in several published studies, mostly in Alzheimer's Disease (AD) where a shift from early- to late-differentiated T-cells is observed [25–27]. In this disease also higher frequencies of activated T-cells have been reported [25] as well as T-cells expressing chemokine receptors, especially CCR6 [27], which is a key player for brain-homing [28]. To the best of our knowledge, for PD the situation is less clear, especially as previous studies generally failed to account for the effects of CMV infection [15,29–31]. Therefore we performed comprehensive phenotyping of the main T-cell subsets as well as other immune cell types in peripheral blood of PD patients and a balanced control cohort. Because CMV has been shown to have a great impact on T-cell differentiation profiles [32], we analysed CMV– and CMV+ study participants separately. The previously reported decrease in the frequency of naïve T-cells and the accumulation of late-stage differentiated T-cells in CMV+ compared to CMV- individuals [32] could be confirmed in the present study. In PD patients the difference between CMV-seronegative and seropositive individuals was slighter greater, but this does not necessarily mean that CMV can have a greater impact due to an exhausted immune system in PD. While CMV– and CMV+ PD patients were age-matched, in the controls the CMV– participants were slightly older than the CMV-seropositives. It is known that chronological age is

Table 3. Frequencies of leukocyte subsets in relation to CMV, PD, disease duration and MDS-UPDRS scores.

Population	C– vs. C+	PD– vs. PD+	C– vs. PD–	C+ vs. PD+	PD– dis.duration	PD+ dis.duration.	PD– MDS-UPDRS	PD+ MDS-UPDRS
n	9 vs. 13	17 vs. 17	9 vs. 17	13 vs. 17	17	17	17	17
CD56+CD3^{hi}	–	–	–	–	–	–	–	–
CD56+CD3+	↑↑	↑	–	–	–	↓	–	–
CD56^{hi}CD3–	–	–	–	–	–	–	–	–
CD56^{lo}CD3–	–	–	–	–	–	–	–	–
CD14–CD3–CD16+	–	–	–	–	–	–	–	–
CD14–CD3–CD16+HLA-DR–	–	–	–	–	–	–	–	–
CD14–CD3–CD16+HLA-DR+	–	–	–	–	–	–	–	–
CD14–CD3+CD16+	–	–	–	–	–	–	–	–
CD14–CD3–CD16–	–	–	–	–	–	–	↓	–
CD14–CD3+CD16–	–	–	–	–	–	–	–	–
CD14–CD3+CD16–CCR5+	–	–	–	–	–	–	–	↓
CD14–CD3–CD16–CD20+	–	–	–	–	↓	–	↓	–
CD20+CCR5+	–	–	–	–	–	–	–	–
lin–CD14+CD16+	–	–	–	–	–	–	–	–
lin–CD14+CD16–	–	–	–	–	–	–	–	–
lin–CD14^{lo}CD16+	–	–	–	–	–	–	–	–
lin–CD14–CD16–	–	–	–	–	–	–	–	↓
CD14+CD16–CCR5+	–	–	–	–	–	–	–	–
CD14+CD16+CCR5+	–	–	–	–	↑↑	–	↑↑	–
CD14–HLA-DR–	–	–	–	–	–	–	–	–
CD14–HLA-DR–CD33–CD11b^{lo}	–	↓↓	↑	–	–	–	–	–
CD15^{hi}	–	–	–	–	–	–	–	–
CD14–CD15+	–	–	–	–	–	–	–	–
CD14–CD15+CD11b^{lo}	–	–	–	–	–	–	–	–
CD14+CD15+	–	–	–	–	–	–	–	–
CD14+CD15+HLA-DR–	–	–	–	–	–	–	–	–
CD14+CD15+HLA-DR–CD11b+CD33+	–	–	–	–	–	–	–	↑
CD14–CD15–	–	–	–	–	–	–	–	–
CD14+CD15–	–	–	–	↑	–	–	–	–
CD14+CD15-CD11b^{lo}HLA-DR–	–	–	–	↑	–	↓	–	–
CD11b+CD33–	–	–	–	–	–	–	–	–
CD11b+CD33+	–	–	–	–	–	–	–	↑
CD11b–CD33–	–	–	–	–	–	–	–	–
CD11b–CD33+	–	–	–	–	–	–	–	–

Footnotes: – $p \geq 0.05$, ↕ $p < 0.05$, ↕↕ $p < 0.01$; dis.duration, disease duration; MDS-UPDRS = total MDS-UPDRS I–IV, parental = CD45+, lin = CD3, CD20, CD56. HLA, human leucocyte antigen; CCR5, C-C chemokine receptor type 5. Columns 2–5 show results of Mann Whitney analysis and columns 6-9 results of linear regression after plotting frequencies against disease duration or total MDS-UPDRS values.

also independently associated with greater T-cell differentiation status [33,34] and it could be that this counteracted the effect of CMV in our study, such that fewer differences between CMV– and CMV+ individuals were seen. That the disease does not play a main role can be definitely concluded, because comparing patients and controls revealed no significant differences regarding the phenotypes of either CD4+ or CD8+ T-cell subsets. Other studies have also reported no relevant differences in CD4+ phenotypes, but a shift to more early differentiated CD8+ T-cells, even in the context of CMV [35–37].

In PD, a pro-inflammatory environment was reported, so we asked whether this might be associated with higher frequencies of cytokine-producing T-cells as the source in patients. However, the frequencies of anti-inflammatory cytokine-positive T-cells were not lower in PD patients and neither were the levels of pro-inflammatory cytokine-producing T-cells higher. A possible reason why a shift from early- to late-differentiated T-cells was observed in AD [25–27], but not here in PD patients might be the different stimulatory environment; cerebrospinal fluid (CSF) of PD patients was reported to differ from CSF of AD patients and healthy controls in respect to selected proteins [38], which could act as immune stimulatory antigens or induce cell death. This could lead to immune dysfunction as reported for Tregs in PD patients [39]. Calopa *et al.* [40] detected an increase in apoptosis of peripheral CD4+ T-cells in PD patients. As a consequence the level of CD4+ T-cells was lower in patients than controls. This was mainly related to cells which were considered as naïve (CD4+CD45RA+), while the frequencies of CD25+ activated CD4+ T-cells were higher in PD patients [40]. In the present study, lower frequencies of CD4+ T-cells within the total CD3+ T-cells were observed only in CMV+ patients relative to CMV+ controls. A reason for the difference between our results and those of Calopa *et al.* [40] could be that they referred to the lymphocyte count (decreased in PD) and we only analysed frequencies. Further they revealed higher CD95 (FAS) expression on CD4+ T-cells of PD patients than controls [40] and another study found an association between CD45RO+CD95+CD4+ effector memory T-cells and the motor part of the MDS-UPDRS III score, independent of age and disease duration [39]. We also failed to find correlations with disease duration, but also with MDS-UPDRS values, although we used total MDS-UPDRS values and not only MDS-UPDRS III.

In parallel to T-cell phenotypes, we determined frequencies of Th17 cells *in vitro* as well as other CD4+ and CD8+ T-cells producing pro- or anti-inflammatory cytokines, but again found no significant differences between patients and controls or between CMV- and CMV+ individuals as reported for Th17 cells in other studies [41]. Tregs did counteract this effect in mice [18], so possibly regulatory cells play an important role in PD. In our study frequencies of CD14+CD15–and CD14+CD15–CD11b^{lo}HLA-DR– cells were higher in PD patients compared to controls, but only in CMV+ patients. In addition, it is noteworthy that other MDSC subsets, CD14+CD15+HLA-DR–CD11b+CD33+ and CD11b+CD33+ cells, correlated positively with total MDS-UPDRS values, but only in CMV+ donors. This could be a sign that CMV activates the immune system which as a consequence can react more readily to other targets, such as PD-related molecules. Other leucocyte subsets with suppressive function such as CD4+ CD25+ regulatory T-cells can reduce neuroinflammation and production of reactive oxygen species via IL-10 and neurotrophic factors (reviewed in [5]). Frequencies of Forkhead-Box-Protein P3 (FoxP3)+CD4+ regulatory T-cells were reported to be higher in older than in younger people, but not different in AD or PD patients. On the other hand, the suppressive activity of these regulatory T-cells was stronger in a previous patient cohort [42].

In PD patients and animal models increased neurodegeneration has been associated with systemic inflammation originating in the periphery [5,43]. In this context, peripheral immune cells have been shown to be activated, infiltrate the brain and contribute to neuroinflammation [6,44] and neurodegeneration, as shown in a mouse model of PD [45]. The hypothesis in this latter model was that peripheral inflammation starts the cascade and promotes activation of microglia, which induces oxidative stress in dopaminergic neurons. As a consequence, α-syn is nitrated and in this form damages neurons. Together with cytokine-mediated apoptosis this leads to PD pathology [6,46]. In parallel, activated microglia secrete chemokines and recruit immune cells into the brain, thus perpetuating a vicious circle. In the present study, analysis of CCR5+ monocytes of the CD14+CD16+ subset did indeed reveal a positive correlation with disease duration and total MDS-UPDRS values. Of note, this association was limited to CMV-seronegative patients, suggesting that CMV infection overrides this effect, which would therefore remain unrecognized unless patients were stratified for CMV infection as done here. Because CCR5 plays an important role for cells crossing the blood-brain barrier [47], higher frequencies of CCR5+ cells may indicate greater brain infiltration; different chemokine levels were reported in CSF of PD patients, which might attract peripheral cells.

Monocytic cells might reduce disease burden by phagocytosis of α-synuclein or suppress inflammatory immune reactions. Therefore we speculated that PD might be characterized by an altered monocyte subset distribution with different expression of the scavenger receptor CD36, whose levels were reported to be lower in AD patients where amyloid beta could be the target for phagocytosis. According to their expression of surface markers CD14 and CD16, several monocyte subsets were identified, but frequencies were not different in the different groups. A previous study indicated that frequencies of classical (mostly anti-inflammatory) monocytes might be elevated in early

PD and then decreased in late PD while the percentages of CD14+CD16+ (potentially inflammatory) monocytes were clearly higher in PD patients of a later stage [48].

To comprehensively assess immune signatures, we also investigated the frequencies and phenotypes of other leukocytes including B-cells and NK-cells, but found no significant differences between either CMV- and CMV+ individuals or between PD patients and controls. However, this does not necessarily imply that they play no role in PD pathology. For example, higher NK-cell activation as reflected by natural killer cell 2D (NKG2D) expression was reported in early PD [31], but we did not test this marker. For the B-cell phenotypes too few samples for a correlation with disease severity were available, but the frequencies of total CD20+ cells within the CD45+ correlated negatively with disease duration and total MDS-UPDRS values. This was also only seen in CMV-seronegative patients. In another study age-related decrease of B-cell numbers was reported, but only for the very old over 85 years of age [49]. As reported for T-cells, age is also a driver of B-cell differentiation and higher frequencies of late-differentiated IgG+IgD–CD27– B-cells, with a reduced capacity to respond to new antigens (maybe also PD-related antigens) were found in older adults [50]. This finding was related to CD19+CD38–CD24– B-cells, which show low expression of immunosenescence-associated marker CD180, produce pro-inflammatory TNF, and whose frequencies were higher in older individuals [51]. Because PD pathology might cause B-cell stress, we investigated different subsets and phenotypes, but found no impact of the disease.

5. Conclusions

From a comprehensive analysis of immune signatures in CMV-seronegative and CMV-seropositive PD patients, significantly higher frequencies of certain MDSCs were found in PD patients than controls, and CCR5 expression correlated with total MDS-UPDRS values hinting at higher cell migration with increased disease severity. No significant differences in frequencies of other immune cells emerged from this study, overall implying that the interaction between the (circulating) immune system and brain pathology/function in PD is very specific and potentially best visible in CMV-negative PD patients. This study was considered a pilot study to identify PD immune signatures covering a broad overview of immune cells in a limited number of patients. Further studies are needed to confirm these findings in larger cohorts. Thereby a focus on MDSC populations, chemokine receptors and regulatory T-cells (previously found relevant) could be considered.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

DG, TF, DB, WM and GP designed the research study. DG, LO performed the research. LO, CS and WM managed the biobank. CS supported the study as biobank manager and recruiter of study participants and organising clinical data. KH performed CMV-serostatus testing. DG and LO analyzed the data. DG and GP wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Study participants were recruited by the outpatient clinic at the Neurodegenerative Department of the University of Tübingen, Germany and clinical data and blood samples collected and biobanked with patients' written consent. The study was carried out in accordance with the guidelines of the Declaration of Helsinki. The Ethics Committee of the Medical Faculty of the University of Tübingen approved this study under project number 480/2015BO2.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/JIN26393.

References

[1] Qin L, Liu Y, Hong JS, Crews FT. NADPH oxidase and aging drive microglial activation, oxidative stress, and dopaminergic neurodegeneration following systemic LPS administration. Glia. 2013; 61: 855–868. https://doi.org/10.1002/glia.22479.

[2] Earls RH, Lee JK. The role of natural killer cells in Parkinson's disease. Experimental & Molecular Medicine. 2020; 52: 1517–1525. https://doi.org/10.1038/s12276-020-00505-7.

[3] Surguchov A, Surguchev A. Synucleins: New Data on Misfolding, Aggregation and Role in Diseases. Biomedicines. 2022; 10: 3241. https://doi.org/10.3390/biomedicines10123241.

[4] Tansey MG, Romero-Ramos M. Immune system responses in Parkinson's disease: Early and dynamic. The European Journal of Neuroscience. 2019; 49: 364–383. https://doi.org/10.1111/ejn.14290.

[5] Ferrari CC, Tarelli R. Parkinson's disease and systemic inflammation. Parkinson's Disease. 2011; 2011: 436813. https://doi.org/10.4061/2011/436813.

[6] Abdi IY, Ghanem SS, El-Agnaf OM. Immune-related biomark-

ers for Parkinson's disease. Neurobiology of Disease. 2022; 170: 105771. https://doi.org/10.1016/j.nbd.2022.105771.
[7] Marogianni C, Sokratous M, Dardiotis E, Hadjigeorgiou GM, Bogdanos D, Xiromerisiou G. Neurodegeneration and Inflammation-An Interesting Interplay in Parkinson's Disease. International Journal of Molecular Sciences. 2020; 21: 8421. https://doi.org/10.3390/ijms21228421.
[8] Lee SYH, Yates NJ, Tye SJ. Inflammatory Mechanisms in Parkinson's Disease: From Pathogenesis to Targeted Therapies. The Neuroscientist: a Review Journal Bringing Neurobiology, Neurology and Psychiatry. 2022; 28: 485–506. https://doi.org/10.1177/1073858421992265.
[9] Su R, Zhou T. Alpha-Synuclein Induced Immune Cells Activation and Associated Therapy in Parkinson's Disease. Frontiers in Aging Neuroscience. 2021; 13: 769506. https://doi.org/10.3389/fnagi.2021.769506.
[10] Ciaramella A, Salani F, Bizzoni F, Pontieri FE, Stefani A, Pierantozzi M, *et al*. Blood dendritic cell frequency declines in idiopathic Parkinson's disease and is associated with motor symptom severity. PloS One. 2013; 8: e65352. https://doi.org/10.1371/journal.pone.0065352.
[11] Goldeck D, Maetzler W, Berg D, Oettinger L, Pawelec G. Altered dendritic cell subset distribution in patients with Parkinson's disease: Impact of CMV serostatus. Journal of Neuroimmunology. 2016; 290: 60–65. https://doi.org/10.1016/j.jneuroim.2015.11.008.
[12] Menees KB, Lee JK. New Insights and Implications of Natural Killer Cells in Parkinson's Disease. Journal of Parkinson's Disease. 2022; 12: S83–S92. https://doi.org/10.3233/JPD-223212.
[13] Fleming SM, Davis A, Simons E. Targeting alpha-synuclein via the immune system in Parkinson's disease: Current vaccine therapies. Neuropharmacology. 2022; 202: 108870. https://doi.org/10.1016/j.neuropharm.2021.108870.
[14] Xiao W, Shameli A, Harding CV, Meyerson HJ, Maitta RW. Late stages of hematopoiesis and B cell lymphopoiesis are regulated by α-synuclein, a key player in Parkinson's disease. Immunobiology. 2014; 219: 836–844. https://doi.org/10.1016/j.imbio.2014.07.014.
[15] Huang Y, Liu Z, Wang XQ, Qiu YH, Peng YP. A dysfunction of CD4+ T lymphocytes in peripheral immune system of Parkinson's disease model mice. Chinese Journal of Applied Physiology. 2014; 30: 567–576.
[16] Luz Correa B, Ornaghi AP, Cerutti Muller G, Engroff P, Pestana Lopes R, Gomes da Silva Filho I, *et al*. The inverted CD4:CD8 ratio is associated with cytomegalovirus, poor cognitive and functional states in older adults. Neuroimmunomodulation. 2014; 21: 206–212. https://doi.org/10.1159/000356827.
[17] Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, *et al*. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nature Medicine. 2007; 13: 1173–1175. https://doi.org/10.1038/nm1651.
[18] Reynolds AD, Stone DK, Hutter JAL, Benner EJ, Mosley RL, Gendelman HE. Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. Journal of Immunology. 2010; 184: 2261–2271. https://doi.org/10.4049/jimmunol.0901852.
[19] Maetzler W, Karam M, Berger MF, Heger T, Maetzler C, Ruediger H, *et al*. Time- and frequency-domain parameters of heart rate variability and sympathetic skin response in Parkinson's disease. Journal of Neural Transmission. 2015; 122: 419–425. https://doi.org/10.1007/s00702-014-1276-1.
[20] Maetzler W, Ellerbrock M, Heger T, Sass C, Berg D, Reilmann R. Digitomotography in Parkinson's disease: a cross-sectional and longitudinal study. PLoS ONE. 2015; 10: e0123914. https://doi.org/10.1371/journal.pone.0123914.
[21] Gibb WR, Lees AJ. A comparison of clinical and pathological features of young- and old-onset Parkinson's disease. Neurology. 1988; 38: 1402–1406. https://doi.org/10.1212/wnl.38.9.1402.
[22] Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology. 1967; 17: 427–442. https://doi.org/10.1212/wnl.17.5.427.
[23] Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, *et al*. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. Movement Disorders. 2008; 23: 2129–2170. https://doi.org/10.1002/mds.22340.
[24] Parel Y, Chizzolini C. CD4+ CD8+ double positive (DP) T cells in health and disease. Autoimmunity Reviews. 2004; 3: 215–220. https://doi.org/10.1016/j.autrev.2003.09.001.
[25] Pellicanò M, Larbi A, Goldeck D, Colonna-Romano G, Buffa S, Bulati M, *et al*. Immune profiling of Alzheimer patients. Journal of Neuroimmunology. 2012; 242: 52–59. https://doi.org/10.1016/j.jneuroim.2011.11.005.
[26] Larbi A, Pawelec G, Witkowski JM, Schipper HM, Derhovanessian E, Goldeck D, *et al*. Dramatic shifts in circulating CD4 but not CD8 T cell subsets in mild Alzheimer's disease. Journal of Alzheimer's Disease. 2009; 17: 91–103. https://doi.org/10.3233/JAD-2009-1015.
[27] Goldeck D, Larbi A, Pellicanó M, Alam I, Zerr I, Schmidt C, *et al*. Enhanced Chemokine Receptor Expression on Leukocytes of Patients with Alzheimer's Disease. PLoS ONE. 2013; 8: e66664. https://doi.org/10.1371/journal.pone.0066664.
[28] Sallusto F, Impellizzieri D, Basso C, Laroni A, Uccelli A, Lanzavecchia A, *et al*. T-cell trafficking in the central nervous system. Immunological Reviews. 2012; 248: 216–227. https://doi.org/10.1111/j.1600-065X.2012.01140.x.
[29] Cen L, Yang C, Huang S, Zhou M, Tang X, Li K, *et al*. Peripheral Lymphocyte Subsets as a Marker of Parkinson's Disease in a Chinese Population. Neuroscience Bulletin. 2017; 33: 493–500. https://doi.org/10.1007/s12264-017-0163-9.
[30] Zhang Z, Xie X, Cai Y, Liu P, Liu S, Chen R, *et al*. Abnormal immune function of B lymphocyte in peripheral blood of Parkinson's disease. Parkinsonism & Related Disorders. 2023; 116: 105890. https://doi.org/10.1016/j.parkreldis.2023.105890.
[31] Holbrook J, Patel B, Camacho M, Kahanawita L, Greenland J, Williams-Gray CH. Natural killer cells have an activated profile in early Parkinson's disease. Journal of Neuroimmunology. 2023; 382: 578154. https://doi.org/10.1016/j.jneuroim.2023.578154.
[32] Pourgheysari B, Khan N, Best D, Bruton R, Nayak L, Moss PAH. The cytomegalovirus-specific CD4+ T-cell response expands with age and markedly alters the CD4+ T-cell repertoire. Journal of Virology. 2007; 81: 7759–7765. https://doi.org/10.1128/JVI.01262-06.
[33] Derhovanessian E, Maier AB, Hähnel K, Beck R, de Craen AJM, Slagboom EP, *et al*. Infection with cytomegalovirus but not herpes simplex virus induces the accumulation of late-differentiated CD4+ and CD8+ T-cells in humans. The Journal of General Virology. 2011; 92: 2746–2756. https://doi.org/10.1099/vir.0.036004-0.
[34] Pawelec G, Bronikowski A, Cunnane SC, Ferrucci L, Franceschi C, Fülöp T, *et al*. The conundrum of human immune system "senescence". Mechanisms of Ageing and Development. 2020; 192: 111357. https://doi.org/10.1016/j.mad.2020.111357.
[35] Williams-Gray CH, Wijeyekoon RS, Scott KM, Hayat S, Barker RA, Jones JL. Abnormalities of age-related T cell senescence in Parkinson's disease. Journal of Neuroinflammation. 2018; 15: 166. https://doi.org/10.1186/s12974-018-1206-5.
[36] Kouli A, Jensen M, Papastavrou V, Scott KM, Kolenda C, Parker

C, *et al*. T lymphocyte senescence is attenuated in Parkinson's disease. Journal of Neuroinflammation. 2021; 18: 228. https://doi.org/10.1186/s12974-021-02287-9.

[37] Vavilova JD, Boyko AA, Ponomareva NV, Fokin VF, Fedotova EY, Streltsova MA, *et al*. Reduced Immunosenescence of Peripheral Blood T Cells in Parkinson's Disease with CMV Infection Background. International Journal of Molecular Sciences. 2021; 22: 13119. https://doi.org/10.3390/ijms222313119.

[38] Zhang J, Sokal I, Peskind ER, Quinn JF, Jankovic J, Kenney C, *et al*. CSF multianalyte profile distinguishes Alzheimer and Parkinson diseases. American Journal of Clinical Pathology. 2008; 129: 526–529. https://doi.org/10.1309/W01Y0B808EMEH12L.

[39] Saunders JAH, Estes KA, Kosloski LM, Allen HE, Dempsey KM, Torres-Russotto DR, *et al*. CD4+ regulatory and effector/memory T cell subsets profile motor dysfunction in Parkinson's disease. Journal of Neuroimmune Pharmacology. 2012; 7: 927–938. https://doi.org/10.1007/s11481-012-9402-z.

[40] Calopa M, Bas J, Callén A, Mestre M. Apoptosis of peripheral blood lymphocytes in Parkinson patients. Neurobiology of Disease. 2010; 38: 1–7. https://doi.org/10.1016/j.nbd.2009.12.017.

[41] Fu J, Huang Y, Bao T, Liu C, Liu X, Chen X. The role of Th17 cells/IL-17A in AD, PD, ALS and the strategic therapy targeting on IL-17A. Journal of Neuroinflammation. 2022; 19: 98. https://doi.org/10.1186/s12974-022-02446-6.

[42] Rosenkranz D, Weyer S, Tolosa E, Gaenslen A, Berg D, Leyhe T, *et al*. Higher frequency of regulatory T cells in the elderly and increased suppressive activity in neurodegeneration. Journal of Neuroimmunology. 2007; 188: 117–127. https://doi.org/10.1016/j.jneuroim.2007.05.011.

[43] Jurcau A, Andronie-Cioara FL, Nistor-Cseppento DC, Pascalau N, Rus M, Vasca E, *et al*. The Involvement of Neuroinflammation in the Onset and Progression of Parkinson's Disease. International Journal of Molecular Sciences. 2023; 24: 14582. https://doi.org/10.3390/ijms241914582.

[44] Monahan AJ, Warren M, Carvey PM. Neuroinflammation and peripheral immune infiltration in Parkinson's disease: an autoimmune hypothesis. Cell Transplantation. 2008; 17: 363–372.

[45] Brochard V, Combadière B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, *et al*. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. The Journal of Clinical Investigation. 2009; 119: 182–192. https://doi.org/10.1172/JCI36470.

[46] Du XY, Xie XX, Liu RT. The Role of α-Synuclein Oligomers in Parkinson's Disease. International Journal of Molecular Sciences. 2020; 21: 8645. https://doi.org/10.3390/ijms21228645.

[47] Ubogu EE, Callahan MK, Tucky BH, Ransohoff RM. CCR5 expression on monocytes and T cells: modulation by transmigration across the blood-brain barrier in vitro. Cellular Immunology. 2006; 243: 19–29. https://doi.org/10.1016/j.cellimm.2006.12.001.

[48] Konstantin Nissen S, Farmen K, Carstensen M, Schulte C, Goldeck D, Brockmann K, *et al*. Changes in CD163+, CD11b+, and CCR2+ peripheral monocytes relate to Parkinson's disease and cognition. Brain, Behavior, and Immunity. 2022; 101: 182–193. https://doi.org/10.1016/j.bbi.2022.01.005.

[49] Potestio M, Pawelec G, Di Lorenzo G, Candore G, D'Anna C, Gervasi F, *et al*. Age-related changes in the expression of CD95 (APO1/FAS) on blood lymphocytes. Experimental Gerontology. 1999; 34: 659–673. https://doi.org/10.1016/s0531-5565(99)00041-8.

[50] Buffa S, Bulati M, Pellicano M, Dunn-Walters DK, Wu YC, Candore G, *et al*. B cell immunosenescence: different features of naive and memory B cells in elderly. Biogerontology. 2011; 12: 473–483.

[51] Buffa S, Pellicanò M, Bulati M, Martorana A, Goldeck D, Caruso C, *et al*. A novel B cell population revealed by a CD38/CD24 gating strategy: CD38(-)CD24 (-) B cells in centenarian offspring and elderly people. Age. 2013; 35: 2009–2024. https://doi.org/10.1007/s11357-012-9488-5.