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Altered dendritic cell subset distribution in patients with Parkinson's disease: Impact of CMV serostatus

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ABSTRACT

Parkinson's disease (PD) is characterised by low-level systemic inflammation, which may be at least partly due to pathophysiological activation of immunity. Here, the frequencies of different types of circulating dendritic cells (DCs) with and without a pro-inflammatory phenotype were determined in PD patients and controls. A high proportion of older people is infected with cytomegalovirus (CMV), which acts as a chronic antigenic stressor that could also contribute to increased inflammation. Following this idea, we found higher frequencies of myeloid DCs with a pro-inflammatory CD16+ILT2^{high} phenotype in CMV-positive PD patients than controls, suggesting the potential involvement of CMV in exacerbating PD.

1. Introduction

Parkinson's disease (PD) is characterised by an age-associated progressive degeneration of dopaminergic neurons in the *substantia nigra* (Qin et al., 2013). Systemic effects have also been identified, characterised by activation of peripheral immune cells which may infiltrate the brain through a compromised blood–brain barrier (Ferrari and Tarelli, 2011) and contribute to neuroinflammation (Monahan et al., 2008) and neurodegeneration (Brochard et al., 2009). CD4+ T-cells were shown to be activated and to differentiate into Th17 cells with a pro-inflammatory phenotype and a capacity to infiltrate the CNS in mice — these effects were dependent on dopamine receptor 5 expressed on dendritic cells, and the release of IL-6 and IL-23 (Prado et al., 2012). Unbalanced neurotransmitter levels in PD might have similar effects. Potential immunogens presented by DCs could be derived from the pigment neuromelanin from dopaminergic neurons; this is suggested to be a driving force for a DC-dependent auto-immune response against neuromelanin in PD (Koutsilieri et al., 2013). Moreover, DCs can also directly contribute to brain inflammation, as reviewed in the context of multiple sclerosis (Koutsilieri et al., 2013). Also in Alzheimer's disease, another brain disease with systemic inflammation, DCs are suggested to play an important role. Similar to alpha-synuclein in PD, in AD a prion-like protein accumulates (amyloid beta) and dendritic cells regulate brain infiltration of amyloid beta-specific T-cells (Fisher et al., 2011). It remains to be seen whether the interplay between DCs and alpha-synuclein specific T-cells is relevant in PD. It may therefore be useful to investigate the status of the peripheral immune system in detail in PD patients (Collins et al., 2012), because a specific therapy targeting single leukocyte subsets could pave the way for a well-balanced reduction of destructive brain inflammation and production of inflammation-triggered neuroprotective molecules without compromising immunity per se. One example of successful immune modulation is given by Lore et al. who targeted specific DC subsets with toll-like receptor (TLR) ligands promoting T-cell responses against cytomegalovirus (CMV) and HIV (Lore et al., 2003). Different TLR expression patterns by different DCs show that they respond to different stimuli. To investigate which DCs may be important in PD, we analysed different common DC subsets: IFN-α-producing and TLR7- and TLR9-expressing plasmacytoid DCs (pDCs), as well as TLR3-, 4- and 7-expressing myeloid DCs (mDCs) (Lore et al., 2003) that can be further divided into three subsets characterised as CD141+ (BDCA-3), CD1c+ (BDCA-1) and CD16+ mDCs (Kassianos et al., 2012). Upon TLR activation, CD16+ mDCs are able to produce much higher levels of cytokines, mainly TNF, compared to CD1c+ mDCs, identifying the former as the main pro-inflammatory type while the latter are the main chemokine-expressing DCs (Piccioli et al., 2007). CD141+ mDCs have been reported to present antigens from necrotic cells after viral infection (Jongbloed et al., 2010) and might also present PD-relevant

Abbreviations: α-syn, alpha-synuclein; CMV, cytomegalovirus; ILT, Ig-like transcript; mDC, myeloid dendritic cells; pDC, plasmacytoid dendritic cells; UPDRS, unified Parkinson disease rating scale; TLR, toll-like receptor.

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antigens from dying neurons; they do not express the negative signalling receptor Ig-like transcript 2 (ILT2, CD85j) whereas CD1c+ mDCs express it at low and CD16+ mDCs at high levels.

CMV infection is very common in the elderly population and has been repeatedly shown to influence immune reactivity (Derhovanessian et al., 2009; Pawelec et al., 2009). CMV can also infect mDCs but not pDCs (Kvale et al., 2006), although it may indirectly affect the latter, probably via TLR7 and/or TLR9 pathways, including partial maturation with MHC class II upregulation and secretion of IFN-α, TNF and IL-6 (Varani et al., 2007; Sinclair, 2008). CMV was shown to induce CD1c+ mDC maturation along with expression of CD83 and secretion of IFN-α, IL-6 and IL-12 (Sinclair, 2008). By activating DCs and promoting the development of certain phenotypes, CMV could deregulate cytokine homeostasis and contribute to the systemic inflammation present in PD.

2. Patients and methods

2.1. Study participants

Thirty four non-demented PD patients and their partners — all without history of neurological disease (n = 22) — as controls from the same environment and background were recruited from the outpatient clinic at the Neurodegenerative Department of the University of Tübingen, Germany (Table 1) (Maetzler et al., 2014, 2015). PD patients fulfilled the UKPDS Brain Bank criteria (Gibb and Lees, 1988). Participants scored a median of 29 points on the Mini Mental State Examination (MMSE, Folstein et al., 1975). Severity of disease-associated symptoms was assessed with the Hoehn and Yahr (H&Y) score (Hoehn and Yahr, 1967) and the unified Parkinson's disease rating scale (UPDRS) (Goetz et al., 2008) (Table 1). The Ethics Committee of the Medical Faculty of the University of Tübingen approved the study, and all participants gave their written informed consent.

2.2. Sample collection and processing

Plasma was obtained and CMV serostatus determined via a recombinant CMV IgG immunoblot (Mikrogen, Neuried, Germany) using six different target epitopes (IE1, p150, CM2, p65, gB1 and gB2). In parallel, blood was taken and PBMCs isolated using Ficoll–Hypaque (Linaris) gradient centrifugation. PBMCs were frozen at −80 °C and subsequently stored in liquid nitrogen until the day of the experiment, when they were thawed and thoroughly washed. After blocking of nonspecific antibody binding with 1% Gamunex (human IgG from Talecris), surface marker staining was performed with the antibodies listed in Table 2. Ethidium monoazide bromide (Biotium) was used to label dead cells. Measurements were performed on a BD LSRII flow cytometer using DIVA 6.1 software. To ensure performance quality control, BD CS&T tracking beads and BD 8 peak rainbow beads were applied, and each sample run included the same standard normal control donor as reference.

Table 1
Clinical data of the study participants.

Characteristic	Partners (CMV−; CMV+)	PD patients (CMV−; CMV+)
Number	22 (9; 13)	34 (17; 17)
Median age	65 (68; 61)	68 (68; 68)
Age range	53–77 (58–74; 53–77)	44–74 (55–74; 44–72)
Gender	9F/13M (3F/6M; 6F/7M)	12F/22M (7F/10M; 5F/12M)
Median MMSE	29 (29; 29)	29 (29; 29)
Median UPDRS I	1 (2; 0)	7 (7; 6)
Median UPDRS II	0 (0; 0)	8 (10; 6)
Median UPDRS III	1 (1; 1)	29 (29; 28)
Median UPDRS IV	0 (0; 0)	0 (0; 0)
Median total UPDRS	2 (2; 2)	44 (45; 42)
Total UPDRS range	0–13 (0–13; 0–11)	14–112 (24–112; 14–68)
H&Y score range	0 (0; 0)	1–4 (1–4; 1–3)
Median disease duration		6 years (9 years; 5 years)
Disease duration range		1–13 (2–13 years; 1–10 years)

2.3. Data analysis

For data evaluation, FlowJo software version 7.2.5 (Treestar) was used; the gating strategy is shown in the Supplemental figure. For statistical analysis, Mann–Whitney U tests and Bonferroni correction or linear regression analyses were performed with GraphPad Prism. To analyse ILT expression levels, a mean fluorescence index (MFI) was calculated, dividing the mean fluorescence of mDCs for each specific marker by the mean fluorescence of the highest visible peak of BD rainbow beads. These beads were added to each sample prior to measurement as a reference control, to compensate for potential LSRII performance variation between samples.

3. Results

3.1. Frequencies of dendritic cells

Frequencies of pDCs did not differ significantly between CMV− and CMV+ controls, or between CMV− and CMV+ PD patients (Fig. 1A). Frequencies did not significantly correlate with age. The frequency of ILT7-positive pDCs was also not significantly different between groups (Fig. 1B).

Within the mDC subsets, differences were observed between patients and controls, but only in CMV+ individuals. Three different analyses were performed. First, CD141+, CD1c+ and CD16+ mDCs were gated within the population of CD123−CD11c+ cells. Positive and negative populations were clearly visible, but to exclude potential false-positives, only those negative for the other markers (CD141+CD1c−CD16−, CD1c+cD141−CD16− and CD16+CD141−CD1c−) were selected. To confirm these results, the ILT2 pattern was included, as each subset displays a characteristic expression. In this way, CD141+ILT2−, CD1c+ILT2^{low} and CD16+ILT2^{high} mDCs were identified. All three analyses showed similar results, so that only the latter are presented in Fig. 1. Frequencies of mDCs were lower in controls than PD patients. The difference was significant in the CMV+ but not in the CMV− groups (CMV+ controls versus CMV+ patients, $p = 0.015$). Frequencies of CD141+ILT2− mDCs in relation to the total mDCs were not significantly different between groups, except in CMV+ PD patients who had significantly lower frequencies than either CMV+ controls or CMV− PD patients (Fig. 1D). Frequencies of CD1c+ILT2^{low} mDCs were higher in CMV+ controls than in the other groups (Fig. 1E). Frequencies of CD16+ILT2^{high} mDCs were significantly lower in CMV+ controls than in CMV+ PD patients ($p = 0.049$, Fig. 1F). All these differences disappeared when the total CD45+ cell population was taken as a reference population (Fig. 2).

3.2. Correlations with total UPDRS values

We investigated whether disease severity as defined by the total UPDRS score or disease duration influenced these results (Figs. 3 and 4). No significant correlations between total mDCs and pDCs and these parameters were observed. Next, ILT7 expression levels on pDCs and ILT2 expression on mDCs — assessed by mean fluorescence intensity — were compared with the above clinical measures. While ILT7 expression on pDCs did not significantly correlate with UPDRS total score, ILT2 expression on mDCs correlated positively with total UPDRS levels ($p = 0.014$) in CMV+ PD patients (Fig. 4). No correlations with disease duration were found (data not shown).