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Immune system responses in Parkinson's Disease: early and dynamic

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Abstract

The neuropathological hallmarks of Parkinson's disease (PD) are the degeneration and death of dopamine-producing neurons in the ventral midbrain, the widespread intraneuronal aggregation of alpha-synuclein (a-syn) in Lewy bodies and neurites, neuroinflammation, and gliosis. Signs of microglia activation in the PD brain postmortem as well as during disease development revealed by neuroimaging, implicate immune responses in the pathophysiology of the disease. Intensive research during the last two decades has advanced our understanding of the role of these responses in the disease process, yet many questions remain unanswered. A transformative finding in the field has been the confirmation that *in vivo* microglia are able to respond directly to pathological a-syn aggregates but also to neuronal dysfunction due to intraneuronal a-syn toxicity well in advance of neuronal death. In addition, clinical research and disease models have revealed the involvement of both the innate and adaptive immune systems. Indeed, the data suggest that PD leads not only to a microglia response, but also to a cellular and humoral peripheral immune response. Together, these findings compel us to consider a more holistic view of the immunological processes associated with the disease. Central and peripheral immune responses aimed at maintaining neuronal health will ultimately have consequences on neuronal survival. We will review here the most significant findings that have contributed to the current understanding of the immune response in PD, which is proposed to occur early, involve peripheral and brain immune cells, evolve as neuronal dysfunction progresses, and is likely to influence disease progression.

GRAPHICAL ABSTRACT

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CONTRIBUTIONS & CONFLICTS

MR-R drafted the first version of the manuscript. MGT & MR-R conceived, designed, and wrote jointly the final version of the manuscript. Both authors approved the final version of the manuscript. The authors declare no conflict of interest.

Clinical and basic research have revealed the involvement of both the innate and adaptive immune systems in Parkinson's disease (PD). PD leads not only to a microglia response in brain, but also to a cellular and humoral peripheral immune response. This has resulted into the current understanding of the immune response in PD, which is proposed to occur early, involve peripheral and brain immune cells, evolve as neuronal dysfunction progresses, and is likely to influence disease progression.

Keywords

alpha-synuclein; neuroinflammation; monocytes; microglia; T-cells; cytokines; toll-like-receptors; immunomodulation; neurodegeneration; MHC-II; auto-antibodies

INTRODUCTION

Parkinson's Disease (PD) is a neurodegenerative disorder characterized by the progressive dysfunction and loss of neurons in the brain, in particular the dopaminergic population in ventral tier of the substantia nigra (SN) pars compacta in the midbrain; as well as by the presence of intraneuronal fibrillar forms of α -synuclein (α -syn) in Lewy bodies and neurites. Besides the neuronal loss, and the Lewy body pathology, PD is characterized by significant gliosis. Although this overt gliosis was first reported in the beginning of the 20th century by Charles de Foix (Foix & Nicolesco, 1925), the contemporary description of microglia activation in human PD brains was reported by McGeer and colleagues in 1988 (McGeer *et al.*, 1988) when they described increased human leukocyte antigen DR (HLA-DR) expression in the SN of PD patients. Their study in human brains together with consecutive reports from other laboratories, showing Tumor Necrosis Factor (TNF) expression in SN from patients (Boka *et al.*, 1994), and the presence of elevated pro-inflammatory cytokines in brain and cerebrospinal fluid (CSF) (Mogi *et al.*, 1994a; Mogi *et al.*, 1994b; Mogi *et al.*, 1995) prompted the "inflammation hypothesis of neurodegeneration" in PD. Recent epidemiological studies in humans have reported that chronic consumption of certain non-steroidal anti-inflammatory drugs (NSAIDs) in mid-life was associated with decreased incidence of PD (Gagne & Power, 2010; Gao *et al.*, 2011b), suggesting an active role for inflammatory responses in PD pathogenesis.

Although the majority of diagnosed PD does not arise from genetic mutations, there is growing appreciation that specific genetic variants in various genes expressed in immune cells can modify risk for development of PD. In particular, mutations in the gene encoding Leucine-rich repeat kinase-2 (LRRK2) are the most common cause of familial PD and makes up about 1% of sporadic PD (Hernandez *et al.*, 2016). Although its function in cells is not completely understood, it is highly expressed in immune cells (Hakimi *et al.*, 2011) and we found it to be increased in immune cells in the peripheral blood of PD patients compared to age-matched healthy controls (Cook *et al.*, 2017); raising the possibility that it may play a role in PD pathogenesis due to its proposed function in the immune system (Dzamko *et al.*, 2015; Cook *et al.*, 2017). Genetic variants in the LRRK2 gene have not only been associated with increased risk for PD, but also for Crohn's disease (Hui *et al.*, 2018). Highlighting the relevance of the immune system, GWAS analyses have suggested common genetic pathways between PD and autoimmune disease (Witoelar *et al.*, 2017). Furthermore, specific single nucleotide polymorphisms (SNPs) in the TNF gene have been associated with increased risk of PD (Chu *et al.*, 2012), as have SNPs in the HLA genetic loci (Hamza *et al.*, 2010), which encodes part of the major histocompatibility complex (MHC) class II, and that functionally translate to augmented antigen presentation capacity in peripheral immune cells of PD patients who carry the high-risk SNP (Kannarkat *et al.*, 2015). In summary, multiple genetic studies support a role for the immune system in PD pathogenesis.

The resident immune cells of the brain, the microglia, are cells of myeloid lineage but they are distinctly different from bone marrow derived peripheral macrophages (Li & Barres, 2018). The microglia are influenced by brain signals, but also respond to peripheral signals during development and during adult life (Matcovitch-Natan *et al.*, 2016). They are essential for neuronal health and they are involved in neuronal plasticity (Weinhard *et al.*, 2018). Based on postmortem analysis of PD brains by the McGeers, the extensive microgliosis was believed to be the consequence of neuronal death that could potentially have a secondary role in neurodegeneration. Thus, most early studies of inflammation and immune responses in PD were focused on the nigrostriatal system in models where neuronal death was present and immune responses prior to degeneration were largely ignored. However, several important findings during the last two decades have modified our understanding of the immune response associated with PD. First, the acknowledgement that a-syn aggregation occurs in multiple brain areas (Braak *et al.*, 2003), and that this aggregation can lead to neuronal dysfunction without cell death (Phan *et al.*, 2017) led researchers to investigate areas where a-syn pathology is present beyond the nigrostriatal pathway. Second, the realization that a-syn neuronal pathology is associated with microglia activation even in the absence of neuronal loss (Sanchez-Guajardo *et al.*, 2010; Barkholt *et al.*, 2012; Watson *et al.*, 2012) suggests that microgliosis is not a mere response to neuronal death. Accordingly, microglia activation in PD patients has been described not only in SN where cell death is significant (Ouchi *et al.*, 2005), but also in other areas of the CNS, such as hippocampus, and cortex (Imamura *et al.*, 2003; Ouchi *et al.*, 2005; Iannaccone *et al.*, 2013; Doorn *et al.*, 2014), areas associated with a-syn pathology and symptoms of the disease, thereby suggesting a relationship between microgliosis, a-syn and neuronal dysfunction that does not require neuronal death.

Another significant change in the field stems from the novel Braak hypothesis, which proposes that a-syn pathology starts somewhere outside the CNS, in the peripheral nervous system (gut or nasal epithelia) (Braak *et al.*, 2003); therefore, advocating for a more holistic view of the disease and suggesting that the peripheral immune cells and not microglia might be the first ones to encounter misfolded a-syn. In that regard, mounting evidence now supports the involvement of peripheral immune cells in PD (reviewed herein). Moreover, it has been suggested that peripheral inflammation via the gastrointestinal system may contribute to disease pathogenesis or that infections can contribute somehow to the risk of developing PD (Deleidi & Isacson, 2012; Houser & Tansey, 2017).

Finally, the discovery of three additional important events related to a-syn have further contributed to this paradigm shift in the field: first, the active release of a-syn by neurons in culture (Emmanouilidou *et al.*, 2010); secondly, that immune cells, primarily microglia and macrophages, are the main cell types clearing extracellular a-syn (Lee *et al.*, 2008); and lastly, the description and acceptance of a-syn as an inflammagen capable of initiating a sterile immune response (reviewed below). Altogether these findings have contributed to the current understanding of the immune response in PD that is now proposed to i) occur early, ii) involve peripheral and brain immune cells, iii) evolve as neuronal dysfunction progresses, and iv) is likely to contribute to disease progression.

The microglia response happens early due to changes in neuronal health and correlates with a-synuclein pathology

Several labs have confirmed the initial observations by the McGeers regarding increased MHCII expression on cells in the brain of PD patients post mortem (McGeer *et al.*, 1988; Croisier *et al.*, 2005; Orr *et al.*, 2005; Miklossy *et al.*, 2006). In addition, multiple animal models of PD have corroborated this microgliosis seen in patients (see (Sanchez-Guajardo *et al.*, 2013b). As mentioned, this microgliosis is not a mere consequence of neuronal death, and we and others have shown that microglia respond early to the effects of a-syn toxicity in neurons, well in advance of cell death while still being modulated by it (Theodore *et al.*, 2008; Sanchez-Guajardo *et al.*, 2010; Barkholt *et al.*, 2012). Moreover, microgliosis in the absence of or prior to cell death has been reported in multiple a-syn based PD models, corroborating that cell death is not necessary to elicit a microglial response (Gomez-Isla *et al.*, 2003; Tofaris *et al.*, 2006; Su *et al.*, 2008; Emmer *et al.*, 2011; Watson *et al.*, 2012). Taken together, these data strongly suggest that cell death is not required for an adaptive microglial response.

A significant observation in PD *post-mortem* brains was the correlation of MHCII expression in microglia to a-syn pathology (Croisier *et al.*, 2005), but not necessarily to disease duration or motor symptoms (Croisier *et al.*, 2005; Orr *et al.*, 2005); while the expression of the lysosomal protein CD68 was (indirectly) correlated to disease duration (Croisier *et al.*, 2005). These findings argue for an early, long lasting but dynamic response of microglia that changes progressively as disease advances, as a-syn accumulates pathologically and cell death occurs. Indeed, we have shown that in the viral-vector-a-syn rat PD model the microglia early (but steady) response is associated with MHCII expression and that in contrast CD68 is associated with the microglia response during or after cell death

(Sanchez-Guajardo *et al.*, 2010; Barkholt *et al.*, 2012). Since the early microglia activation response is related to α -syn aggregation and degeneration and not cell death *per se*, this would be expected to occur in multiple CNS areas, consistent with *post-mortem* analyses of PD patient brains (Hunot *et al.*, 1996; Knott *et al.*, 2000; Imamura *et al.*, 2003; Croisier *et al.*, 2005; Halliday & Stevens, 2011; Doorn *et al.*, 2014). The microglia response should be expected to change and evolve with time as the disease progresses and this response will include changes in microglia number (proliferation), cytoskeletal changes (morphological changes), and protein expression (Sanchez-Guajardo *et al.*, 2010; Watson *et al.*, 2012).

The early and progressive microglia activation described above is supported by *in vivo* imaging human studies; PET binding of the peripheral benzodiazepine receptor (PBR/TSPO) ligand [11C]-(R) PK11195, suggest microglial activation in midbrain of patients with idiopathic REM sleep behavioral disorders (RBD, considered a prodromal form of PD) (Stokholm *et al.*, 2017). The microglia response seems therefore to occur early although is not only restricted to midbrain. Indeed, in fully diagnosed PD patients the microgliosis is not only found in midbrain, but also in other areas such as striatum, pons, cortex and hippocampus (Gerhard *et al.*, 2006; Iannaccone *et al.*, 2013). This widespread microglia activation has also been confirmed using another TSPO ligand, [11C]DPA713, (Terada *et al.*, 2016). Interestingly, this prevalent microglia activation was found at early stages, (PD patients Hoehn & Yahr stages 1–2), but it increased with time in particular in cortical areas, suggesting a progressive increase of the immune response as the disease develops and the α -syn pathology and neurodegeneration evolves (Terada *et al.*, 2016). Accordingly, levels of microglia activation (i.e. binding of PK11195 PET) correlates with disease severity as evaluated by the degree of motor defects using the UPRDS assessment (Ouchi *et al.*, 2005). Furthermore, the PK11195 PET signal, increased as the [11C]-CFT (a dopamine transporter ligand, i.e. a marker of dopaminergic terminals) decreased in the striatum in the patients (Ouchi *et al.*, 2009).

This microgliosis, seems to have consequences on neuronal function as cortical microgliosis, PK11195 PET signal, correlates with decreased glucose metabolism and inversely correlates to cognitive scores (MMSE) in PD patients with dementia (Edison *et al.*, 2013). This microglia response, can eventually contribute to neuronal death if a pro-inflammatory event is sustained. Indeed, increased oxidative enzymes COX1 and iNOS (Hunot *et al.*, 1996; Knott *et al.*, 2000) and pro-inflammatory cytokines have been reported to be increased in human PD SN (Boka *et al.*, 1994) and striatum (Nagatsu *et al.*, 2000) and if persistent, this pro-inflammatory response may hasten cell death. Accordingly, in rodents a robust immune activation via intracerebral injection of the bacterial membrane molecule: lipopolysaccharide (LPS) results in nigral dopaminergic cell death (Machado *et al.*, 2011; Hoban *et al.*, 2013).

Peripheral monocytes are modified in Parkinson's disease

It is suggested that during neurodegeneration peripheral monocytes/macrophages (as mentioned, different myeloid cells than microglia) infiltrate the CNS. Accordingly, proteins that are highly expressed in monocytes/macrophages but not in brain-resident microglia, such as the scavenger receptor CD163 (Polfliet *et al.*, 2006); or the chemokine receptor CCR2 (Mizutani *et al.*, 2012), are present in cells that infiltrate the brain of animal models of

PD-like degeneration (Tentillier *et al.*, 2016; Harms *et al.*, 2017; Harms *et al.*, 2018) and in PD and Alzheimer's patients' brains post mortem (Pey *et al.*, 2014). Furthermore, analyses of blood cells in patients revealed changes in the immune compartment during active disease. PD patients display increased monocyte precursors in blood and enriched classical monocytes, while the alternatively activated (CD16+, including intermediate and non-classical) monocytes were decreased (Grozdanov *et al.*, 2014). Another study suggested that classical monocytes showed increased levels of CCR2 expression, although the total number of CCR2+ cells were reduced (Funk *et al.*, 2013). The receptor CCR2 has been shown to be critical for recruitment of macrophages and controls the migration of peripheral cells into the CNS (Gliem *et al.*, 2016) where mainly astrocytes produce the respective ligand: the chemokine CCL2 (Farina *et al.*, 2007). The relevance of the infiltrating peripheral monocytes in the degenerative event in PD has been confirmed in rodent PD models of dopaminergic degeneration. The anti-inflammatory targeted modification of the CD163+ monocytes in the 6-OHDA rat model using dexamethasone (Tentillier *et al.*, 2016) or the deletion of the CCR2 (thus avoiding macrophage recruitment) in the viral-vector-a-syn PD mouse model (Harms *et al.*, 2018), both resulted in dopaminergic neuronal protection in SN. CCR2 deletion was however not protective for the SN neurons in the MPTP mouse model (Kalkonde *et al.*, 2007; Parillaud *et al.*, 2017), suggesting that the relevance and number of infiltrating monocytes may be timing and/or insult-dependent. The toxic models, 6-OHDA and MPTP lead to neuronal loss, but not to relevant a-syn pathology (only certain chronic MPTP protocol (not widely used) has been associated to a-syn pathology in mice (Fornai *et al.*, 2005)). While 6-OHDA is intracerebrally administered, MPTP is injected repeatedly in the periphery, and that could account for the differences observed. It should also be noted that MPTP has been reported to directly affect blood lymphocytes and spleen T-cells (Chi *et al.*, 1992), which could also account for the different immune responses. However, an excess of CCL2 production in brain leads to increased infiltration of macrophages that could enhance MPTP-induced dopaminergic death in mice, further suggesting a deleterious role for the infiltrating cells (Parillaud *et al.*, 2017). The authors suggest that it is the microglia that exert a tight regulation on the CCL2-CCR2 axis, and that if microglia are dysfunctional (for example by knocking out CX3CR1), this could lead to neurodegeneration (Parillaud *et al.*, 2017). Thus, healthy microglia may be critical to limit the extent of infiltration of peripheral monocytes into the CNS.

Indeed, microglia health and homeostasis and the CX3CR1-CX3CL1 axis have been shown to be essential for protection of neurons against inflammatory stress and toxicity, both local or peripheral (Cardona *et al.*, 2006). While the CX3CR1 is expressed in microglia and monocytes/macrophages, in brain, neurons produce a membrane-anchored CX3CL1 form that can be cleaved into a soluble circulating form. Several studies have demonstrated that soluble fractalkine (CX3CL1) can protect dopaminergic neurons in PD rodent models. Specifically, treatment with CX3CL1 was neuroprotective in the 6-OHDA rat model (Pabon *et al.*, 2011) and also in the MPTP mouse model (Morganti *et al.*, 2012). Accordingly, the lack of CX3CR1 expression in microglia lead to increase of cell death in the MPTP model (Cardona *et al.*, 2006). However, data from a-syn-based models are thus far contradictory. While one study showed that viral vector-mediated a-syn overexpression in the SN of CX3CR1 knock-out mice resulted in a reduced immune response (compared to wildtype

mice) and decreased dopaminergic neuron death (Thome *et al.*, 2015); another lab using the same a-syn model reported that overexpression of soluble fractalkine protected dopaminergic nigral neurons against the a-syn induced toxicity (Nash *et al.*, 2015).

Another important regulatory axis between immune cells and brain is the CD200-CD200R pair. CD200 is expressed in multiple cells including neurons, while the receptor is expressed only in myeloid cells including monocytes/macrophages and microglia, where it serves to limit inflammatory activation (Wright *et al.*, 2000; Wright *et al.*, 2003). Monocyte-derived macrophages from PD patients showed a decreased ability to upregulate CD200R expression upon inflammatory signals, and even when increased, this CD200R was unable to dampen the pro-inflammatory response (Luo *et al.*, 2010). These findings suggest a possible failure in the signal that controls macrophage activation in subjects with PD. In the MPTP model, CD200 has been shown to modulate microglia activation (Ren *et al.*, 2016); and blocking of the CD200R using an antibody leads to increased dopaminergic toxicity by rotenone and iron, further supporting the importance of the CD200-CD200R axis in PD pathophysiology (Wang *et al.*, 2011). Interestingly, in postmortem analysis of dementia with Lewy bodies (DLB), microglia activation and CD200 expression were associated with tau and amyloid pathology rather than with a-syn pathology (Walker *et al.*, 2017). These findings suggest a regional specificity of the immune response in human brain undergoing neurodegeneration.

Monocytes in CSF from PD patients display higher HLA-DR (MHCII) expression compared to those in blood (Fiszer *et al.*, 1994) and interestingly, monocytes infiltrating the brain in a rodent viral-vector-a-syn PD model also express high levels of MHCII (Harms *et al.*, 2017). PD patient monocytes also display elevated TLR2 and TLR4 expression (Drouin-Ouellet *et al.*, 2015), two receptors reported to interact with a-syn (Fellner *et al.*, 2013; Kim *et al.*, 2013). In addition, the monocytes in PD patients seems to have an abnormal response to defined immune stimuli that some have described as exaggerated (Reale *et al.*, 2009; Grozdanov *et al.*, 2014), while others describe them as impaired (Bessler *et al.*, 1999; Hasegawa *et al.*, 2000). Additionally, phagocytic capacities in PD patient monocytes are impaired (Grozdanov *et al.*, 2014; Bliederauser *et al.*, 2016) and they may have reduced capacity to clear a-syn (Gardai *et al.*, 2013). Thus, the peripheral monocyte population in PD is anomalous, it infiltrates brain and could eventually contribute to disease progression by virtue of being dysfunctional and hyper-inflamed.

The Adaptive immune system is also involved in Parkinson's disease

The expression of MHCII, a protein involved in antigen presentation, by microglia found in the vicinity of a-syn neuronal deposition (Croisier *et al.*, 2005) infers the involvement of the adaptive immune system, with the microglia acting as antigen presenting cells to T-cells. Indeed T-cell infiltration has been observed in the brain of PD patients (Brochard *et al.*, 2009) and in animal models of PD-like degeneration (Theodore *et al.*, 2008; Brochard *et al.*, 2009; Sanchez-Guajardo *et al.*, 2010; Harms *et al.*, 2017). The presence and infiltration of T-cells may be related to a decrease in the phagocytic activity of microglia and an increase in the pro-inflammatory phenotype of the myeloid compartment in the brain (Sommer *et al.*, 2016). The specific relevance of the CD4 T-cell population has been confirmed by the dopaminergic neuroprotection observed when: deleting CD4 cells using the MPTP mouse

model (Brochard *et al.*, 2009), or when using a MHC-II global knockout mouse (that also has arrested CD4 population) (Harms *et al.*, 2013) or by enhancing CD4 regulatory T-cells through a-syn vaccination in rats (Sanchez-Guajardo *et al.*, 2013a). Notably, neuronal MHC-I expression, and thus signaling to CD8 T-cells (T-cytotoxic, Tc), has also been implicated in PD-like neurodegeneration and specifically to the selective vulnerability of catecholaminergic neurons in rodents (Cebrian *et al.*, 2014).

Analysis of the lymphocytes in peripheral blood of PD patients suggests an impairment in the CD4 T-helper (Th) cells (Bas *et al.*, 2001; Gruden *et al.*, 2011; Stevens *et al.*, 2012) and alterations in the CD4:CD8 ratio (Bas *et al.*, 2001; Hisanaga *et al.*, 2001; Baba *et al.*, 2005). Moreover, examination of the CD4-Th subpopulations suggests an abnormal distribution with the effector memory (antigen-experienced) fraction overrepresented in detriment to the naïve T-cells (Fiszer *et al.*, 1994; Bas *et al.*, 2001; Saunders *et al.*, 2012; Stevens *et al.*, 2012). A recent study reported that the reduction in CD4-Th cells in PD patients is due to a decrease in Th2, Th17 and regulatory T-cell populations; furthermore the authors reported that CD4-Th cells from PD patients show a Th1-biased immune response with increased IFN-gamma and TNF production, strongly suggesting involvement of CD4 cells in the pathophysiology of the disease (Kustrimovic *et al.*, 2018).

With regards to which antigens are being processed by immune cells and presented via MHC-II to the T-cells, a-syn constitutes an obvious candidate. Indeed, a recent study reported that PD patient-derived T-cells respond to a-syn-derived peptides in ways that T-cells from healthy controls do not (Sulzer *et al.*, 2017). The authors implicate mainly a CD4-Th2 (IL5) response, normally associated with a protective response that could lead to B-cell activation and a protective humoral response (see below). We could speculate that the decrease in the Th2 population observed in patients (Kustrimovic *et al.*, 2018), leads to a loss of this putative protective immune response (for further reading see (Mosley & Gendelman, 2017)). Accordingly, a humoral response in PD patients has been described, which seems to decrease with time, as antibodies recognizing monomeric and fibrillar a-syn are found early in PD but decrease with time (5 vs. 10 years into disease duration) (Gruden *et al.*, 2011; Yanamandra *et al.*, 2011). These findings suggest an early protective role for auto-IgG against a-syn that is lost with time. Indeed, anti-a-syn antibodies were decreased in PD serum compared to controls in a cohort where disease duration was 10 years (Besong-Agbo *et al.*, 2013). A recent study highlights the importance of the affinity of the auto-anti-a-syn antibody and reports decreased levels of high-affinity autoantibodies against a-syn in PD patients compared to healthy controls (Brudek *et al.*, 2017). The protective role of the auto-IgG could be related to their ability to enhance clearance of a-syn, which seems more efficient via IgG through FcGamma receptors (FcγR) (see below). The reason for the decrease in IgG is unclear, but PD patients show a decrease in the number of B cells (Gruden *et al.*, 2011; Stevens *et al.*, 2012), and they actually produce less IgG (Marttila *et al.*, 1985). But as mentioned above, it could be related to the decrease in the Th2 CD4 cells that might result in decreased B cell activation (Kustrimovic *et al.*, 2018). Interestingly, the IgG deposition in neurons correlated negatively with the degree of cell death in PD brains, thereby suggesting a time-dependent response or possible a positive role in neuron survival (Orr *et al.*, 2005). More in-depth investigations are needed to clarify the role of B-cells in PD pathogenesis and pathophysiology. In summary, the immune response in PD involves both

the innate and adaptive immune systems and not only microglia but also peripheral immune cells. Moreover, the data suggest that while some aspects of the immune response seem to drive pathogenesis, others may be aimed at neuroprotection.

Soluble immune biomarkers may help stage disease and correlate with clinical symptoms in Parkinson's disease

Besides the mentioned changes in autoantibodies, multiple labs have reported abnormal cytokine profiles in blood and CSF from patients. A recent metanalysis reports that pro-inflammatory cytokines such as IL-1b, IL-6 and TNF, but also others such as IL-10 (known for its anti-inflammatory actions) and RANTES (known for its chemotactic properties) are increased in PD, suggesting a complex ongoing regulation of the immune response (Qin *et al.*, 2016). Interestingly, these soluble markers were correlated to non-motor symptoms, which are proposed to precede motor symptomatology (Pessoa Rocha *et al.*, 2014; Kohler *et al.*, 2016). In particular, depression in PD patients has been associated with increased of several cytokines, although there is no clean consensus of which factors are up or down in PD patients relative to healthy controls. Correlations have been reported for: IL-2 (Katsarou *et al.*, 2007; Lindqvist *et al.*, 2012), TNF (Menza *et al.*, 2010; Lindqvist *et al.*, 2012) and IL-6 (Selikhova *et al.*, 2002). However, IL-6 has also been correlated to other markers of disease such as UPDRS and activities of daily living (Muller *et al.*, 1998; Hofmann *et al.*, 2009; Delgado-Alvarado *et al.*, 2017). In summary, the immune system appears to be activated very early in PD and various aspects of the immune response evolve as the disease progresses. However, changes in cytokine levels are not specific to PD as they also found in other neurodegenerative or psychiatric diseases, suggesting common pathogenic inflammatory mechanisms across neurological disease, and supporting the relevance of the immune system in neuronal health and normal brain function. Therefore, no single inflammatory marker represents an ideal PD biomarker, but rather combinations of them or combinations with neuronal-related biomarkers will most likely give a better opportunity to help in future patient selection, diagnosis and prognosis. Specifically, biomarker panels using cytokines/chemokines (Brockmann *et al.*, 2017) or combining neuronal and inflammatory markers (Delgado-Alvarado *et al.*, 2017; Eidson *et al.*, 2017) are being proposed by us and others, as valuable tools to stage disease and potentially to distinguish clinical subtypes, and to monitor disease progression. For example chemokines IL-8, CCL2 (MCP-1) and CCL-4 (MIP-1beta) and the brain derived neurotrophic factor (BDNF) seem to be good markers to discriminate subtypes in PD-LRRK2 patients because these markers are highest in patients with diffuse/malignant PD as compared to those with mainly pure motor disease. (Brockmann *et al.*, 2017). These immune markers, IL-8, CCL2, CCL4, were also correlated to more severe motor defects PD (Reale *et al.*, 2009), and they were also higher in glucocerebrosidase (GBA)-PD (Chahine *et al.*, 2013). Interestingly, in GBD-PD patients IL-8 was correlated with higher cognitive deficits (Chahine *et al.*, 2013), suggesting that an immune component is more readily manifested in cases where the course of PD progression is aggressive and associated with cognitive impairment.

The role of a-synuclein in the microglia response in Parkinson's disease

As mentioned, one of the key factors influencing the immune response is a-syn pathology. The description of a-syn as a danger associated molecular pattern (DAMP) able of inducing

sterile inflammation has deeply influenced the study of the immune response in the disease. The pro-inflammagen property of α -syn in microglia and macrophages has been corroborated multiple times (Kannarkat *et al.*, 2013; Sanchez-Guajardo *et al.*, 2013b; Allen Reish & Standaert, 2015). It was Zhang *et al.* who first reported the ability of oligomeric α -syn to induce microglia activation that could lead to oxidative stress and neuronal death (Zhang *et al.*, 2005). Moreover, this microglia-driven oxidative stress will lead to oxidation of α -syn in nearby cells which will further feed the neuro-toxic process (Shavali *et al.*, 2006). The α -syn-induced oxidative stress has been related to CD11b and resultant PHOX NADPH oxidase activation (Zhang *et al.*, 2005; Jin *et al.*, 2007; Zhang *et al.*, 2007; Hou *et al.*, 2018). Multiple labs have shown now that α -syn can initiate a pro-inflammatory response in microglia inducing production of IL-6, IL-1 β and TNF (Klegeris *et al.*, 2008; Su *et al.*, 2008; Lee *et al.*, 2009; Lee *et al.*, 2010; Roodveldt *et al.*, 2010; Couch *et al.*, 2011). This induced inflammatory profile changes dependent on solubility and α -syn modification (such as truncation) (Fellner *et al.*, 2013). It has been suggested that the pro-inflammatory capacity increases as solubility of α -syn decreases, thus it is likely to be the fibrillar form of α -syn that induces the highest production of TNF and IL-1 β (Hoffmann *et al.*, 2016). Therefore, as disease progresses and α -syn aggregates into toxic forms, the proinflammatory effect would also be expected to escalate. In addition, α -syn-induced microglia activation results in release of soluble secondary molecules that can act as chemoattractants (Kim *et al.*, 2009; Wang *et al.*, 2015), thereby leading to the recruitment of immune cells in the vicinity of areas with high levels of pathogenic α -syn. This could therefore explain the described correlation between activated microglia and neurons with α -syn pathology (Croisier *et al.*, 2005).

We should note that microglia have different roles with respect to α -syn and PD: while their pro-inflammatory response can contribute to pathogenesis, other effector functions of microglia such as phagocytosis can contribute to the clearance of extracellular α -syn, which would be predicted to slow disease progression. The activation of cytokine secretion and clearance by phagocytosis are therefore processes that could occur in parallel, although most believe they do not and microglia that are highly secretory are not simultaneously highly phagocytic. Specifically, when in culture, microglia are the cells that most efficiently take up and clear α -syn, and this is dependent on the activation stage of microglia decreasing when a pro-inflammatory profile is acquired (Lee *et al.*, 2008). Accordingly, this phagocytic clearance might be compromised when microglia are exposed to aggregated α -syn due to its pro-inflammagen properties (Park *et al.*, 2008; Choi *et al.*, 2015; York *et al.*, 2017). The solubility of α -syn also influences the uptake of α -syn, increasing with fibrilization (Hoffmann *et al.*, 2016). For example the microglia TLR2 uptake of oligomeric α -syn seems to be conformation specific (Kim *et al.*, 2013), while, the TLR-4 uptake includes all types of α -syn, independent of its solubility (Stefanova *et al.*, 2011). Also Fc γ R seem to mediate α -syn internalization and clearance in microglia, as well as their activation (Cao *et al.*, 2012). Fc γ R are IgG receptors that are involved in processing and uptake of opsonized IgG-antigen complexes (Guilliams *et al.*, 2014). Fc γ R can be inhibitory or activating and it is highly expressed in monocytes, macrophages and dendritic cells (Guilliams *et al.*, 2014). In brain, microglia is the main cell that can express all the different Fc γ Rs, but they are also expressed in neurons and astrocytes (Fuller *et al.*, 2014). Interestingly, the uptake of α -syn by

glia Fc γ R is more efficient if a-syn is complexed with IgG (Bae *et al.*, 2012; Gustafsson *et al.*, 2017). Therefore, as disease progresses the type or amount of a-syn found and/or released by neurons might differ, thus these diverse a-syn forms would be taken up or presented to microglia differently, resulting in distinctive immune response that will evolve as the disease advances.

Besides being responsible for a-syn uptake, TLR 2 and 4 are also able to induce an immune response to a-syn (Fellner *et al.*, 2013; Kim *et al.*, 2013). This interaction signals through the adaptor protein Myeloid Differentiation primary response gene-88 (Myd-88), which leads to NF- κ B nuclear translocation (Daniele *et al.*, 2015). The NF- κ B transcription factor is consistently associated with a-syn microglia activation (Klegeris *et al.*, 2008; Reynolds *et al.*, 2008; Wilms *et al.*, 2009; Lee *et al.*, 2010; Couch *et al.*, 2011; Hoenen *et al.*, 2016). As mentioned TLR4 not only mediates a-syn inflammatory activation, but also phagocytosis (Fellner *et al.*, 2013). In fact, the absence of TLR4 increases a-syn-induced neurotoxicity and results in elevated a-syn levels and pro-inflammatory markers in an a-syn multiple system atrophy (MSA) mouse model (Stefanova *et al.*, 2011). This paradoxical observation suggests that the decrease of a-syn uptake by the absence of TLR4 results in elevated extracellular a-syn that can further promote a pro-inflammatory response possibly through other receptors (such as CD11b or TLR2). On the other hand, treatment with a selective TLR4 agonist that promoted a-syn clearance without inducing pro-inflammatory activation, resulted in neuroprotection *in vivo* in the a-syn MSA mouse model (Venezia *et al.*, 2017). In parallel, as mentioned TLR2 has been associated with microglia activation induced by a specific a-syn oligomeric confirmation associated with neuron-released a-syn (Kim *et al.*, 2013). Distinct from TLR4, the absence of TLR2 reduced microglia activation and dopaminergic neuronal cell death associated with a-syn *in vivo*, possibly suggesting a less relevant role of this receptor in the clearance process (Kim *et al.*, 2013). Additional studies are needed to explore the role of TLR4 in PD. Nevertheless, taken together these data suggest that not one, but several proteins can mediate the effect of a-syn on microglia, thus resulting in a complex event of parallel signaling (for a review please see (Ferreira & Romero-Ramos, 2018)).

Finally, it should be obvious that if Braak's hypothesis regarding the periphery (gut) being the initiation site of a-syn pathology is correct, the immune system would be expected to play a critical role in limiting the propagation of aggregated a-syn between cells. Specifically, the role of microglia and gut macrophages would be essential, as they should be the cells clearing the misfolded protein and thus avoiding spreading to other healthy neurons. Interestingly, membrane-associated proteins initially linked to the immune system have been proposed as putative a-syn receptors responsible for the spread and propagation of a-syn between neurons. For example, interaction of aggregated a-syn with Fc γ RIIB (an inhibitory Fc γ R) in neurons via endocytosis leads to the phosphorylation of the Fc γ RIIB immunoreceptor tyrosine-based inhibitory motif. This phosphorylation through a downstream mechanism involving SH-containing tyrosine phosphatase 1/2, (SHP 1/2), resulted in enhancement of a-syn uptake (Choi *et al.*, 2018). One study reported that the immune receptor lymphocyte activation gene 3 (LAG3) (Mao *et al.*, 2016) is necessary to mediate the uptake of a-syn in neurons, although expression of LAG3 in neurons has not been confirmed by other studies and LAG3 may be more relevant for uptake of a-syn by

immune cells. Finally, TLR2 has been implicated in a-syn uptake not only by microglia, as discussed above, but also by neurons (Dzamko *et al.*, 2017). Therefore, interactions of a-syn with specific membrane proteins on two distinct cell populations, microglia and neurons, has been shown to initiate a cascade of events in neuronal and/or immune cells that could contribute to disease progression.

Is Parkinson's disease a consequence of changes in the immune system function?

A-syn is expressed ubiquitously in the brain, but the protein is also found in immune cells: microglia, monocytes, lymphocytes and others (Shin *et al.*, 2000). Few labs have addressed the role of a-syn in immune cells, and most work has been done using an a-syn knockout mice. This line showed a defective B cell development and IgG production (Xiao *et al.*, 2014), and a reduction in T cells as well as a defective Th2 phenotype in CD4+ cells (Shameli *et al.*, 2016), which is consistent with the observations in PD patients discussed above. *In vitro*, the absence of a-syn in microglia lead to a hyper-responsive microglia phenotype with defective phagocytosis (Austin *et al.*, 2006). On the other hand, expression of a-syn in neurons, has also been associated with an immune response to viruses in the brain, in particular to neuron-to-neuron viral transmission (Beatman *et al.*, 2015). Furthermore downregulation of a-syn in dopaminergic neurons leads to a robust immune response, via MHC-I that results in cell death (Benskey *et al.*, 2018), underscoring the relevance of the MHC-I signaling in this neuronal population (Cebrian *et al.*, 2014).

Other PD-associated gene products, such as LRRK2 and GBA are expressed in immune cells and mutations in these genes will most likely result in functional alterations in immune cells. We will shortly highlight recent findings regarding LRRK2, however, a detailed discussion of these proteins is beyond the scope of this review and we refer the reader elsewhere (Dzamko *et al.*, 2015; Cook & Tansey, 2017). Studies regarding the function of LRRK2 on immune cells are numerous. Data from labs using knockouts or knock-down of LRRK2 or the LRRK2 G2019S mutation support a role for LRRK2 in microglia and macrophage motility and their inflammatory response (see review (Lee *et al.*, 2017)); but the extent to which *in vivo* inflammatory phenotypes are a result of cell autonomous effects of LRRK2 in neurons versus immune cells has yet to be clearly delineated. For example, transgenic LRRK2 G2019S mice injected with LPS display altered peripheral immune responses (rather than alterations in microglia activation) that promoted dopaminergic neurodegeneration via soluble mediators (Kozina *et al.*, 2018). LRRK2 has been associated with phagocytic activity of macrophages and microglia via phosphorylation of Scar (orthologous to human Wiskott-Aldrich syndrome protein-family verproline homologous 2 (Wave2)) that when compromised leads to dopaminergic cell death (Kim *et al.*, 2018). Moreover, the LRRK2 (G2019S) mutation has been associated with expansion of myeloid cells with a bigger suppressive activity on CD4-T cells that could not support a Th17 response (Park *et al.*, 2017). Nevertheless, as mentioned above we have shown that in sporadic PD, LRRK2 protein levels were increased in peripheral blood immune cells in patients compared to age-matched healthy controls (Cook *et al.*, 2017). Together, these studies support a plausible role for LRRK2 in the central and peripheral immune system response in PD that needs to be further investigated at the mechanism level.

PINK1 and parkin have also been associated with immune responses and neuronal dysfunction and death (review in (Dzamko *et al.*, 2015). Lack of parkin was shown by our group to result in nigral dopaminergic degeneration after chronic peripheral inflammation induced by intraperitoneal injections of LPS (Frank-Cannon *et al.*, 2008). We further showed that lack of parkin results in a NFkB-dependent increased production of TNF and IL-6 upon stimulation of macrophages as compared to wildtype cells (Tran *et al.*, 2011). More recent studies demonstrate that PINK and parkin suppress mitochondria antigen presentation through mitochondrial-derived vesicles (Matheoud *et al.*, 2016); specifically, a loss of function of these proteins leads to enhancement of immune signaling through MHCI. Moreover, another paper suggests that failure of PINK and or parkin function can lead to defective mitophagy during mitochondrial damage, which results in stimulator of interferon genes (STING)-mediated inflammatory activation, followed by dopaminergic neurodegeneration in the SN of mice (Sliter *et al.*, 2018). Mitochondrial damage and failure have long been proposed as central pathological events not only in genetic PD but also in idiopathic PD and in relation to a-syn pathology (Grunewald *et al.*, 2018). Together, all of these studies support a role for parkin in immunomodulation and additional investigations into this area is merited.

Infections as putative players in the risk of developing Parkinson's disease

The possibility that immune cells, in the brain or in the periphery, might play a role in the disease pathogenesis and/or progression, also raise the interesting possibility that early and unrelated inflammatory events in a person's life, like those induced by infections, might also be associated to the etiology of PD. The importance of peripheral bacterial-related inflammation is supported by the synergistic effect observed in studies using peripheral LPS injections in a-syn based rodent models. A single peripheral injection of LPS in a-syn transgenic mice resulted in persistent inflammation, increase oxidative stress, which induced nitration and aggregation of a-syn resulting in dopaminergic neurodegeneration (Gao *et al.*, 2011a). A key player in this synergy is microglia NOX2 activation (Zhang *et al.*, 2018). Therefore, this raises the possibility that diseases with peripheral inflammatory components could promote a-syn pathology (Houser & Tansey, 2017).

With regards to peripheral organs, several groups have reported alterations in the gut microbiome of PD patients (Hasegawa *et al.*, 2015; Keshavarzian *et al.*, 2015; Scheperjans *et al.*, 2015; Unger *et al.*, 2016; Hill-Burns *et al.*, 2017; Petrov *et al.*, 2017), which may be related to the effects of diets, infections, or genetic variants in immune-related genes that have not been investigated in depth. In association with dysbiosis in one PD cohort (Hill-Burns *et al.*, 2017), our group has been able to detect increased levels of NFkB-regulated inflammatory factors in the stool of patients with sporadic PD relative to household controls (Houser *et al.*, 2018), raising the possibility that gut inflammation may create a permissive environment for non-motor gastrointestinal symptoms and perhaps exacerbation of motor symptoms. For instance, a recent meta-analysis suggest that prevalence of infections with *Helicobacter pylori* is higher among PD patients; and that those PD patients infected with *H. pylori* showed more severe motor symptoms, suggesting that the infections can aggravate the disease (Dardiotis *et al.*, 2018). For further consideration of these hypotheses, we refer the reader to a review on how intestinal inflammation could potentially contribute to a-syn

upregulation and aggregation in peripheral organs such as the gut that are connected anatomically to the gut in various ways and therefore potentially involved in any propagation of a-syn from the periphery to the CNS (Houser & Tansey, 2017). Finally, although the effects of the gut microbiome on the immune environment of the human brain is likely to be much more complex than in rodents, the relevance of the gut microbiome has also been demonstrated in mouse studies. Gut microbiota has been shown to influence a-syn-induced neurodegeneration, and in fact gut microbiota from PD patients has been reported to enhance the a-syn toxicity, while antibiotics decrease it (Sampson *et al.*, 2016).

Regarding viral infection, several suggestions have been put forward in the literature, related to viruses with certain brain tropism (see review (Olsen *et al.*, 2018). Virus-induced models of PD have been reported following infection with influenza A virus (Takahashi *et al.*, 1995) and Japanese encephalitis virus (Ogata *et al.*, 1997). Influenza virus H5N1 leads to microgliosis and a-syn pathology (phosphorylation) in mouse (Jang *et al.*, 2009). A recent report suggests that inflammation-associated serine-threonine kinase, PKR (EIF2AK2), which is involved in protection against viral infection, leads to phosphorylation of Ser129 in a-syn, which is suggested to play a role in the disease phenotype (Reimer *et al.*, 2018). Therefore, certain viral infections that lead to a-syn pathology could contribute to the onset or progression of neurodegenerative disease. Accordingly, an increase in the incidence of PD has been associated with herpes simplex virus (HSV) and influenza virus infections (Harris *et al.*, 2012; Vlajinac *et al.*, 2013). Interestingly, cross-reactivity between a-syn and an HSV-1 epitope has been detected in PD patients, suggesting that HSV-1 could promote an auto-immune response against a-syn (Caggiu *et al.*, 2016). The same group also reported that homologous HSV1 and a-syn peptide can both stimulate T-cell responses (with TNF production) in PD patients, further suggesting that HSV-1 can contribute to disease phenotype (Caggiu *et al.*, 2017). Additional research is needed to clarify the role of viral infections, if any, in PD pathogenesis and progression.

How is knowledge of the role of the immune system and inflammation changing Parkinson's disease therapeutic approaches?

The data suggesting a putative neuroprotective role of IgG and the relevance of T-cells have aided in the design of immunotherapy approaches and both active and passive vaccination strategies have been used in PD models and are currently in human trials by Prothena (Jankovic *et al.*, 2018) Biogen (Brys *et al.*, 2018) and Affiris (<http://www.affiris.com/news/affiris-announces-encouraging-long-term-data-from-a-series-of-first-in-human-studies-using-affitope-pd01a-targeting-oligomeric-alpha-synuclein-in-early-parkinsons-disease-patients/>), among others. The overarching hypothesis underlying the passive immunization approach is that the administration of IgG will enhance a-syn clearance via FcγR. This approach has now been shown to be neuroprotective *in vivo* in multiple studies (Bae *et al.*, 2012; Games *et al.*, 2014; Mandler *et al.*, 2015; Spencer *et al.*, 2017). The antibodies prevented the spread of a-syn between neurons and astrocytes in a mouse a-syn transgenic, improved motor behavior, and decreased neurodegeneration (Bae *et al.*, 2012). Similarly, passive immunization also decreased axonal a-syn pathology and memory deficits induced by a-syn pathology in the hippocampus (Spencer *et al.*, 2017). In an a-syn MSA mouse model, active a-syn immunization lead to decreased a-syn-induced demyelination and

neurodegeneration, and to reduce a-syn spreading to astrocytes (Mandler *et al.*, 2015). These findings suggest that this approach could potentially exert neuroprotection in different brain areas, cell types and a-syn-related diseases, and result in diminished disease phenotypes.

The passive approach requires carefully selected high-affinity antibodies, as suggested by the findings in PD patients, and the epitope recognized seems to be critically important. Indeed, it has been suggested that anti-a-syn antibodies targeting the N-terminal of human a-syn might be the most efficient in limiting a-syn pathology (Shahaduzzaman *et al.*, 2015). However, other studies report C-terminal-targeted antibodies may be superior (Games *et al.*, 2014). Epitope selection and deletion of the binding domain for Fc γ R are also required to avoid maladaptive T-cell activation, and other modifications can be also done to enhance brain penetrance (Spencer *et al.*, 2016).

Active immunization with a-syn epitopes however, involves both IgG and cellular responses, and this will also require a careful selection of the epitope as well as of the adjuvant. For example, nitrated a-syn enhanced the effector CD4 T-cell response and induced neurotoxicity in the mouse MPTP model (Reynolds *et al.*, 2010), underscoring the relevance of the protein/peptide chosen. Consistent with this, we have demonstrated that after a-syn immunization the T-cell compartment recognizes and responds to different a-syn modifications (fibrillar and nitrated) *in vivo* (Olesen *et al.*, 2018). Such responses are essential for the fate of neurons, and the increase or adoptive transfer of regulatory T-cells (CD4⁺CD25⁺Foxp3) has been shown to be neuroprotective (Reynolds *et al.*, 2007; Reynolds *et al.*, 2010). Our group has also shown that active a-syn vaccination that leads to an increase in CD4 T-cells and regulatory T-cells in the brain and the heightened humoral response, reduces a-syn pathology in nigrostriatal neurons (Sanchez-Guajardo *et al.*, 2013a). This vaccination strategy was associated with modifications of the peripheral T-cell compartment, hence supporting the role of the peripheral immune system in PD-like degeneration (Christiansen *et al.*, 2016). Therefore, a careful combination of cellular and humoral responses seems like a valid and promising approach, which has been recently corroborated in an a-syn transgenic mouse (Rockenstein *et al.*, 2018). Another novel active vaccination strategy combines a-syn with the chaperone Glucose-related protein-94 (Grp94), which has been shown to result in specific cellular immune responses (Labrador-Garrido *et al.*, 2016) that could ameliorate the microglia activation response in the MPTP mouse model of dopaminergic degeneration, both directly and also by adoptive transfer of the resulting splenocytes (Villadiego *et al.*, 2018). Therefore multiple novel therapeutic approaches are being developed with the intent of modulating the immune system to achieve a neuroprotective profile rather than silencing or suppressing the immune system.

CONCLUSIONS

A wealth of epidemiological, immunohistological, biofluid, and genetic evidence supports a role for the innate and adaptive immune system in the pathophysiology of PD. Although microglia activation was initially thought to be present in post-mortem PD brains as a result of neuronal death, it is now clear that microglia activation occurs early in the disease likely in response to neuronal dysfunction caused by a-syn aggregation and toxicity (Fig. 1). While microglia are likely to be critical in clearing aggregated a-syn, other effector functions such

as production of reactive oxygen and nitrogen species and cytokines may bombard neurons with apoptotic signals that compromise their survival and contribute to their demise. Production of chemokines by microglia may also have critical roles in triggering infiltration of peripheral innate and adaptive immune cells into the CNS and importantly the consequences of this infiltration are still being delineated. However, growing evidences support an active role for these peripheral immune cells in the disease progression and the neuronal health. A more in-depth understanding of the immune response and how it evolves as the disease progresses is likely to lead to development of novel and more effective immunomodulatory therapies that can restore the immune response to homeostatic levels, rather than immunosuppress the elderly individual.

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ABBREVIATIONS

a-syn	alpha-synuclein
BDNF	brain derived neurotrophic factor
CCR2	C-C chemokine receptor type 2
Th	CD4 T-helper
CSF	cerebrospinal fluid
CCL2	chemokine (C-C motif) ligand 2 = monocyte chemoattractant protein 1 (MCP1)
CCL4	chemokine (C-C motif) ligand 4 = macrophage inflammatory protein 1 beta (MIP-1B)
CX3CL1	chemokine (C-X3-C motif) ligand 1 = fractalkine
Cx3CR1	Cx3C chemokine receptor 1
DAMP	damage-associated molecular pattern
FcγR	FcGamma receptors
GBA	glucocerebrosidase
GWAS	genome-wide association study
HSV	herpes simplex virus
MHC	histocompatibility complex
HLA-DR	human leukocyte antigen DR

6-OHDA	6-hydroxydopamine
IL	Interleukin
LRRK2	Leucine-rich repeat kinase-2
LPS	lipopolysaccharide
LAG-3	lymphocyte activation gene 3
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSA	multiple system atrophy
Myd-88	Myeloid Differentiation primary response gene-88
NSAIDs	non-steroidal anti-inflammatory drugs
PD	Parkinson's disease
PRRs	pattern recognition-receptors
PBR/TSPO	peripheral benzodiazepine receptor
PET	Positron emission tomography
RBD	REM sleep behavioral disorders
SNPs	single nucleotide polymorphisms
SN	substantia nigra
TLR	Toll like receptor

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Figure 1. Immune response to alpha-synuclein induced neurodegeneration.

In Parkinson's disease, α-synuclein (α-syn) undergoes modifications (phosphorylation, nitration, truncation...) and forms toxic oligomers that progressively form aggregates in the nervous system. The neurons, through exocytosis, will release all types of α-syn (monomeric, oligomeric or fibrillar) to the extracellular media, where it will be taken up and cleared by microglia and/or macrophages, but also by astrocytes. If this process fails, the α-syn can be taken up by neighboring neurons, promoting the spread of pathological forms of α-syn. Microglia will also encounter α-syn when phagocytosing (trogocytosis) synapses (not shown). α-Syn can act as a damage-associated molecular pattern (DAMP) and via diverse immune receptors, pattern recognition-receptors (PRRs), will initiate a sterile inflammatory response, which will result in a pro-inflammatory immune milieu that will contribute to neuronal dysfunction and neurodegeneration. The α-syn-induced microglia activation also leads to proliferation and migration of microglia and to the infiltration of peripheral monocytes/macrophages, likely through a CCL2-CCR2 process. In parallel, α-syn peptides will be presented by neurons, through MHC I, and by microglia (or by infiltrating monocytes/macrophages from the periphery) through MHC II. This will initiate an adaptive immune response through CD8 (T-cytotoxic cells, Tc) or CD4 (T-helper cells, Th),

respectively. Depending on the cytokines produced the CD4-Th cells will undergo differentiation/maturation to various helper subsets, including differentiation into Th1 or Th17 T-cells, which typically potentiate pro-inflammatory events; whereas differentiation into Th2 or regulatory (Treg) T-cells typically resolve the inflammation and lead to B-cell activation (in the periphery) with production of antibodies that may cross the BBB into the CNS. The clearance of α -syn will be aided by the presence of IgG, to mitigate the pro-inflammatory process and the spread of pathologic α -syn to other healthy neurons. In parallel to this event, the α -syn-induced neurodegenerative process will change the expression of proteins involved in neuron-glia interactions, such as CD200 and CX3CL1 (not shown), which will also trigger a microglia response (Image partially constructed using Servier Medical Art).