# Ten Unsolved Questions About Neuroinflammation in Parkinson's Disease

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**Ten unsolved questions about neuroinflammation in Parkinson disease**

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**Abstract**

Parkinson disease is a progressive and debilitating disorder which has so far eluded attempts to develop disease modifying treatment. Both epidemiological and genetic studies support a role of neuroinflammation in the pathophysiology of Parkinson disease. Post mortem studies and experimental analyses suggest the involvement of both innate as well as adaptive immunity in the degenerative process. There is also some circumstantial evidence for effects of immune therapies on the disease. In the present article, we review ten unanswered questions related to neuroinflammatory processes in Parkinson disease with the goal of stimulating research in the field and accelerating the clinical development of neuroprotective therapies based on anti-inflammatory strategies.

## Introduction

Parkinson disease (PD) is a neurodegenenerative disorder classically characterized by a triad of motor symptoms: bradykinesia, rigidity and rest tremor. Recently, it has been recognized that the disease phenotype is in fact much broader. There are manifestations which precede these cardinal features and constitute the “premotor” symptoms of PD: constipation, bladder disorders, hyposmia, autonomic dysfunction, depression and sleep disorders. At the other extreme, in advanced disease patients suffer from poor balance, falls, cognitive impairment and dementia. (1)

Pathologically, the symptoms of PD are accompanied by degenerative changes involving the brain, spinal cord, and peripheral nerves. There is profound depletion of dopaminergic neurons in the substantia nigra pars compacta (SNc), but also extensive degeneration of other structures including the locus ceruleus, hippocampus, and cortical structures. These changes are accompanied by the appearance of Lewy bodies, proteinaceous aggregates found in the SNc and other areas, and degenerating Lewy neurites which are often abundant in the hippocampus and cortex.

A major advance in the field was the discovery of the role of the protein α-synuclein. Originally linked to the disease through genetic studies, it is now recognized as the core component of Lewy bodies and neurites. The use of immunohistochemical stains for α-synuclein has revealed the extensive pathology present in PD. Early stages are associated with abnormal accumulation of α-synuclein in enteric neurons of the gut, the vagus nerve, and other peripheral nerves. In the brain, the earliest changes are associated with α-synuclein aggregates in the brainstem and olfactory bulb, with more advanced pathology appearing in the midbrain and eventually involving much of the cerebral cortex. (2)

Over the last forty years a wide variety of hypotheses have been advanced to explain the cause of neuronal degeneration in PD. In the nineteen-eighties there were many studies of the role of oxidative stress, examining in particular dopamine auto-oxidation, neuromelanin formation, iron accumulation, reduced defense mechanisms against free radicals and mitochondrial dysfunction. (3) Excitotoxic mechanisms have also been explored, and there has been interest in environmental factors as well. At the beginning of the 21$^{th}$ century, influenced by the discovery of α-synuclein and its properties, the focus shifted to studies of protein misfolding and clearance. In this regard, reduced proteasome activity, deficient protein ubiquitination, mitophagy and intracellular organelle deficiencies have attracted much interest. (4) During the last ten years, the emphasis on protein misfolding has been further fueled by evidence that α-synuclein has prion-like properties, and that misfolded α-synuclein can seed further aggregation of endogenous protein and propagate from one cell to another, at least in experimental systems. (5)

This research on the mechanisms of PD degeneration (for review see 6) has allowed the identification of a myriad of potential targets for neuroprotection. However, all clinical trials of neuroprotection have failed so far. The lack of success with these strategies calls for a critical re-assessment of the underlying ideas and assumptions. While there many possible approaches to this, the authors believe that one of the core problems with current paradigms may be a misplaced emphasis on cell-autonomous “neuronal” mechanisms in PD. Despite long-standing evidence for glial reaction in PD, the role of non-cell autonomous mechanisms and neuroinflammation has only recently gained acceptance. (7) In this article, we summarize the data supporting a role of neuroinflammation in PD and highlight 10 unsolved questions regarding the role of immune responses in the pathogenesis of PD.

**Evidence for neuroinflammation in PD**

In their seminal studies of post-mortem PD brain, McGeer and co-workers were the first to describe the presence of activated microglial cells and infiltrating lymphocytes in the substantia nigra. (8) As a whole, the field was slow to recognize the significance of this work. Nearly 20 years elapsed before there was a more detailed study demonstrating and quantifying the infiltration of T-lymphocytes (CD4 and CD8) but not of B-lymphocytes in the affected brain regions in PD. (9) Subsequent studies have confirmed that neuroinflammation is associated with the PD pathological process, with increased concentrations of pro-inflammatory cytokines in blood, CSF and even the brain postmortem of PD patients. (10) There are also changes in cellular immunity in the blood, with modifications in blood monocytes and T cells, including changes in Treg/Teff ratios and appearance of memory T cells with reactivity to specific epitopes of α-synuclein. Evidence for brain inflammation in PD has also been obtained using neuroimaging approaches, primarily with ligands binding to TSPO. (11-13)

There have also been a number of studies which have demonstrated the importance of immune activation in animal models of PD. Neuroinflammation is observed across a broad range of PD model systems, including models based on neurotoxins such as 6-OHDA and MPTP and a variety of α-synculein based models including transgenic animals, models based on viral delivery of α-synculein, and models induced by administration of misfolded α-synuclein fibrils (for review see 7). Animal model systems allow studies of the causality of immune factors which is often difficult to assess directly in human PD, but of course these models need to be considered in the context of the authenticity with which they reproduce the disease mechanisms.

Studies of neuroinflammation in PD are quickly gaining speed in the field, and promise to open the door to entirely new approaches to neuroprotective therapies. An important aspect of these approaches is that it is possible that a therapy for PD might be based on existing immunomodulatory treatments such as those which have been developed for multiple sclerosis and other inflammatory disorders, perhaps speeding delivery to clinical application. There are, however, a number of key issues which must be addressed in order to make progress towards immune-based neuroprotection in PD. Here, we review ten unanswered questions related to neuroinflammatory processes in PD with the goal of stimulating research in the field and promoting clinical development of neuroprotective treatments based on anti-inflammatory strategies.

**Question 1: Does genetic variation in inflammatory genes contribute to the risk and progression of PD?**

Genome-wide association studies (GWAS's) suggest that neuroinflammation represents a risk factor for PD. Hamza and coworkers (14) first reported that common genetic variation in the HLA region is associated with risk for late-onset sporadic PD, a finding confirmed in an independent study. (15) Furthermore, Wissemann et al. (16) suggested that PD is associated with both structural and regulatory elements in the HLA region. In addition, they showed that noncoding SNPs in the HLA region can be associated with disease irrespective of HLA alleles, and that observed associations with HLA alleles can sometimes be secondary to a noncoding variant. Kannarkat et al. (17) extended this, showing that the non-coding SNP *rs3129882* is associated with functional changes in MHCII, including heightened baseline expression and inducibility of MHC class II molecules in B cells and monocytes.

GWAS is a potent tool for identifying single-gene associations with disease, but pathway analysis reveals a much deeper and stronger genetic connection between PD and immune function. Holmans et al. analyzed in detail two large, independent GWAS data sets and found that the strongest associations were with two biological groups: the regulation of leukocyte/lymphocyte activity and cytokine-mediated signaling. (18) Zhang et al. used data from 3 independent GWAS studies and similarly found a strong association with variation in immune loci and PD risk, including the key immune regulatory HLA complex: HLA-DRA, HLA-DRB9, HLA-DRB1, HLA-DQA1, HLA-DRB9, and HLA-DPB2. (19) Gagliano et al. compared gene-set enrichment in PD with MS, a classic immune-mediated disorder. (20) They found that both diseases exhibited enrichment of genes related to immune function. Interestingly, the sets of genes linked to PD were different from those in MS, suggesting different immunological processes at work in PD versus MS and thus the need for further study. Taking another track, Raj et al. studied expression quantitative trait loci (eQTL's) in monocytes from healthy control subjects, and found a marked over-representation of PD-related genes in monocyte populations. (21) Together, these data point to a strong link between the genetics of PD and modulation of immune function.

Nearly all of the genetic evidence linking inflammation and genetic variation is descriptive and based on associations. These type of studies cannot prove that neuroinflammation is causing PD. Further studies testing the role of each allele in animal models of the disease, and addressing the difficult challenges of modeling human immunity in another organism, are needed to determine their real implications in the pathological process. Another potential approach is examing the impact of genes which are unequivocally linked to forms of human PD, such as LRRK2, Parkin

and PINK1, on immune functions (for review see 22). Studies addressing both approaches are needed to help understand how neuroinflammation represents a risk factor for the development and progression of PD.

**Question 2: Is it possible to analyze the evolution of neuroinflammation during the course of PD?**

So far, the strongest evidence for neuroinflammation in PD has been obtained from studies of risk factors or in postmortem studies. A key unmet need is a reliable approach to studing neuroinflammatory processes in living patients during the evolution of the disease. The main approaches which have been taken so far are studies of blood and CSF cytokines and chemokines, examination of cellular immunity in the blood, and brain imaging. While there has been some success with each of these approaches, an overall strategy for monitoring inflammation in living patients with PD is still lacking.

Qin et al. have recently reported a detailed review and meta-analysis of blood cytokines in PD. (23) Based on a systematic review of the literature, they pooled data from a total of 25 studies encompassing 2654 unique study participants, including 1547 patients with PD and 1107 healthy controls. They found clear evidence for elevation of several cytokines and chemokines in PD blood, including IL-6, TNF, IL-1β, CRP, IL-2 and RANTES. The main limitation identified, however, was that the studies included in general examined a mixed group of patients of varying disease durations and severity, and the published reports did not contain sufficient information about clinical state, disease duration, or concomitant therapy to disentangle these variables. The only large cohort study of blood cytokines and chemokines in early, incident PD is the recently reported ICICLE study. (24) In this

series of 230 early PD cases and 90 controls, the authors found evidence for elevation of TNF, IL1-b, IL-2, and IL-10 in blood, and noted that pro-inflammatory phenotypes were associated with faster progression of clinical symptoms. CSF data were not available in this cohort. Indeed, in general data on CSF cytokines and chemokines are more limited, with most studies enrolling a broad range of disease durations and severities. (25-28) There is also the important question of whether blood and CSF inflammatory markers are correlated with each other, an area where there is little data.

Studies of changes in cellular immunity in PD are more limited. In a small patient cohort, Gardai et al. observed defects in phagocytosis in monocytes from PD patients (29), while Chahine et al found that PD patients with mutations in glucocerebrosidase, a strong genetic risk factor for PD, have elevated levels of monocyte-associated inflammatory markers in blood (30) More recently, Grozdanov et al. have described dysregulation of peripheral monocytes in PD, using cultured cells, flow cytometry and gene expression (31) They also found evidence of inflammatory activation, although the underlying patient population was very heterogeneous, with PD disease durations ranging from new onset to up to 20 years. Changes in circulating T cells have also been reported, including alterations in the ratios of Teff and Treg cells, a key regulator of inflammatory signaling. (32) Sulzer et al. have described the appearance of T cells with memory for α-synculein epitopes, and have recently reported that these may appear even before the onset of motor symptoms, but whether these can used as a measure of disease activity is unknown. (33, 34)

Positron emission tomography (PET) imaging analysis is another technique to analyze neuroinflammation in the brain. One of the first PET markers used to monitor

neuroinflammation in vivo in the brain was [11C](R)-PK11195. (12) This tracer binds to a site originally identified as a “peripheral benzodiazepine receptor” and now recognized to be the Translocator Protein 18KD (TSPO), expressed in mitochondria and abundant in microglia and other macrophages. These authors reported an increased mean levels of [11C](R)-PK11195 binding in the pons, basal ganglia and frontal and temporal cortical regions in PD patients. Furthermore, the binding level remained stable two years apart. Yet, a few years later, Bartels et al. (35) performed a similar study and they concluded that “In current practice, [(11)C]-PK11195 seems an unsuitable tracer for accurate or reliable quantification of neuroinflammation”.

A new generation of TSPO tracers have recently been developed which offer greater sensitivity and reduced background. These include $^{18}$F-DPA-714, and the related compound $^{11}$C-DPA-713. Terada et al. (13) recently reported imaging with $^{11}$C-DPA-713 in a cohort of 11 early PD patients. These were mostly, although not exclusively, non-medicated early PD; some had durations of disease as long as 8 years. In this somewhat heterogeneous group, they found elevated uptake of $^{11}$C-DPA-713 in both cortical and subcortical regions, and further increases in uptake with repeat scanning one year later. Ghadery et al. (36) noted that they could identify differences among genetic medium- and high-affinity TSPO binders, but not different between PD and control using $^{18}$F-DPA-714 uptake in a small cohort with a wider range of disease durations.

Recently, Horti et al. (37) have developed $^{11}$C-CPPC [5-cyano-N-(4-(4-$^{11}$C-methylpiperazin-1-yl)-2-(piperidin-1-yl)phenyl)furan-2-carboxamide], a positron-emitting, high-affinity ligand that is specific for the macrophage colony-stimulating factor 1 receptor (CSF1R), the expression of which is essentially restricted to microglia within brain. It is highly and specifically taken up in the rodent and non-

human primate brain indicating potential interest for human PET imaging of CSF1R and the microglial component of neuroinflammation. Increased binding to CSF1R has been described in Alzheimer disease, but so far has not been examined in PD. On the whole, none of these methods has so far yielded a reliable approach to monitoring PD-related inflammation in a living patient.This ability is critical for understanding the causal link between the progression of inflammation, neurodegeneration and α-synuclein spreading (38) and is a key challenge for the field. Furthermore, in the future, it will be important to develop PET ligands for microglia/macrophages that can discriminate between the pro-inflammatory and the regulatory forms of these cells, as they have vastly different functions (see below). This is particularly important considering the possibility of treatments to modulate the phenotypes of these cells .

**Question 3: Is neuroinflammation in experimental models and the real disease different?**

One of the difficulties in linking the results obtained in human studies of PD and in experimental models of the disease arises from the differing character of immune response in different species. Differences likely arise from both the fundamental biological differences between species and the environment in which they are living. Striking differences in the responses of humans and mice to inflammation and injury are well documented. (39) In addition, most of the animal studies are performed in specific-pathogen-free (SFP) animal facilities while we are all in contact with pathogens during our whole life and even before birth. This might have a major consequence on the inflammatory status as immunogens might prime our immune system. Indeed, Carvey et al. (40) showed that prenatal exposure to the bacteriotoxin lipopolysaccharide (LPS) leads to long-term losses of dopamine neurons in offspring.

Furthermore, this group also show that prenatal exposure to LPS exacerbates dopamine neuron degeneration induced by parkinsonian toxins. (30-42) One possible explanation for this increased susceptibility is that peripheral inflammation influences neuroinflammation. (43)

Another major difference between the clinical and the preclinical studies is related to gender, age and genetic background. Experimental studies are generally performed in males but inflammation is different in males and females; (44) studies are mostly performed in young animals and do not take into account immunosenescence which is different in PD and normal aging; (45) and studies are performed on inbred animals but genetic background influences the sensitivity to PD.

Behavioral factors might also affect the level and duration of neuroinflammation. Indeed, we have showed that chronic stress influences neuroinflammation via cortisol and glucorticoid receptors (GR) in the MPTP-mouse model of PD. (46) In this model, GR is important in curtailing microglial reactivity, and its deregulation in PD could lead to sustained inflammation-mediated DN injury.

Finally, most of the preclinical studies are performed in a single laboratory while clinical trials are multicentric. This calls for multicentric preclinical studies or replication studies.

Clearly, to reproduce the clinical situation, preclinical studies should be performed on males and females animals, grown in a stressful non-sterile environment and with mixed genetic background. Implementing such a strategy would require many more animals in each study, changes in the rules governing the animal facilities and much higher grant funding.

**Question 4: What triggers neuroinflammation in PD?**

If it is now well established that neurodegeneration is associated with neuroinflammation in PD but the trigger of neuroinflammation is still unknown. Many suspects are on the list but none of them are unambiguously guilty. Debris of degenerating neurons might represent an activating agent for microglial cells. As far as dopamine neurons are concerned, human neuromelanin induces neuroinflammation and neurodegeneration in the rat substantia nigra. (47) Yet, even before neurons disintegrate, affected neurons might activate microglial cells. Indeed, both in vitro and in vivo models of PD, degenerating neurons release heat shock protein (HSP 60) which acts throught TOLL like receptor 4 (TLR4) at the surface of the microglial cells to activate them. (48-49) Several groups of investigators have also shown that α-synuclein activates microglia in experimental models of PD, (50, 51) but the precise structure of the immunogenic protein is still unclear. Post-translational modifications of α-synuclein may be important; indeed, nitrated α-synuclein has been shown to be a potent stimulus and the response to this antigen is regulated by CD4+ T cell subsets. (52, 53) This concept is further supported by the fact that T cells from PD patients recognize specific α-synuclein epitopes. (33) Activation of alpha synuclein reactive T cells might occur in the immune system at peripheral level because alpha synuclein has been detected in the cervical lymph nodes in an animal model of the disease. (54) Finally, the trigger for neuroinflammation in PD might come from outside of the nervous system. Supporting this hypothesis, Sampson et al., (55) using a highly controlled gnotobiotic system, showed that gut microbiota modify motor deficits and neuroinflammation in an experimental model of the disease, but one has to keep in mind that microbiome in human is highly variable in PD and has not yet been shown to be a disease trigger. (56)

What causes neuroinflammation in PD remains to be identified. Whether there is a single mechanism of immune activation or a combination of factors and whether it is a consequence of neuronal degeneration or even solely neuronal suffering remains the subject of great interest. What perpetuates neuroinflammation remains also a matter of debate. Misfolded alpha synuclein transiting from one neuron to another in a prion like mechanism might be at the origin of the self pertuation of neuroinflammation. This phenomenon might be exacerbated by the age-dependent defects of alpha-synuclein oligomer uptake in microglia and monocytes. (57) Alternately, a chronic elevated level of chemokines or their receptors located on lymphocytes, astrocytes or microglial cells might account for the chronicity of neuroinflammation in PD (58), perhaps driven by “inflammaging.” (59)

**Question 5: What kind of cells are responsible for the innate immune response in PD? Angel or devil?**

The immune system is composed of two branches. Broadly speaking, innate immunity is driven by tissue resident macrophages, and provides the “first response” to antigen, while adaptive immunity is responsible for long-term memory responses. In the brain, microglial cells are the resident macrophages responsible for innate immunity. These are complex cells which have many different functions including neuromodulation, surveillance, sensing and phagocytosis. Whether these functions are shared by all microglial cells or are associated with specialized populations is not certain. (60) Furthermore, microglial cells can undergo activation and differentiation, classically described as “M1” pro-inflammatory and “M2” anti-inflammatory states, but recent work has revealed that there is in fact a spectrum of different activation states rather than a dichotomy. Futher complicating the analysis of microglia, it has been

recognized in recent years that in addition to the brain microglia, there may also be macrophages present which have arisen from blood monocytes that can infiltrate the brain. Morphologically, these blood-derived macrophages can appear similar to the resident microglia, but they have different embryological origins and different properties. (60) In addition, border-associated macrophages have been described in the CNS but their roles (if any) has yet to be analyzed in PD. (61)

Several recent studies have attempted to better characterize the innate inflammatory response to PD. Parrilaud et al. analyzed the expression of the chemokines and their receptors in the substantia nigra pars compacta in an MPTP-treated mouse model. (58) They found that some of the chemokines are upregulated rapidly during the degenerative process, some of them late, and some of them even down regulated. This suggest that the inflammatory reaction is likely to change during the degenerative process. Using the chemokine receptor CCR2 as a marker, they observed blood monocytes which had infiltrated the brain during dopaminergic neurodegeneration. One must, however, acknowledge that the MPTP model of PD in rodents does not fully reproduces the chronicity of degeneration observed in humans or non-human primates. Indeed, while MPTP-induced microglial activation is transient in rodents (62, 63), it is chronic in non-human primates (64) and humans (65). Similarly, Harms et al. (66) used an α-synuclein based model and showed not only infiltration of monocytes, but also that blocking this infiltration protected against the toxic effect of excess α-synuclein. These observations suggest a cross talk between infiltrating monocytes and resident microglial cells, an example of “brain-body communication” in neurodegenerative disorders (discussed further below). This concept is further supported by the fact that when a systemic inflammatory event is combined with chronic neurodegeneration, primed microglia are further activated and

damage endangered neurons, accelerating the pathology. (43) Blood-derived macrophages have not yet been directly observed in postmortem human PD brain, likely because current histological methods are not adequate to the task. Altogether, these studies suggest that resident microglia and monocyte-derived macrophages may play multiple roles in PD. They may have neuroprotective roles in clearing misfolded α-synuclein, the debris of degenerating cells and secreting anti-inflammatory chemokines. Alternately, by secreting pro-inflammatory cytokines or activating the adaptive immunity via the chemokine network, they might exacerbate neuronal degeneration. Their functions might evolve during the course of the degenerative process depending of the cellular environment. Elucidating these roles remains a major challenge for understanding their role in neurodegenerative disorders.

**Question 6: Do lymphocytes play a role in PD pathogenesis?**

Lymphocytes also enter the brain parenchyma in PD and experimental models of the disease, indicating adaptive immunity is also important. Most studies of human brain have reported T but not B lymphocytes in PD; both CD4 and CD8 cells are present (for review see 7). The role of different types of T cells in the disease process remains to be fully established. In the MPTP model, both CD4 and CD8 lymphocytes enter the brain but it appears that only CD4 cells participate in the degenerative process. (9) More recently, Sommer et al (67) have suggested that Th17 cells plays a specific role in the degenerative process. They observed a higher frequency of Th17 cells in the blood of PD patients and increased numbers of Th17 lymphocytes in postmortem PD brain tissues. Furthermore, using autologous co-cultures of activated T lymphocytes and iPSC-derived mid-brain neurons (MBNs) they showed that PD

iPSC-derived MBNs underwent increased neuronal death in presence of T lymphocytes or the addition of IL-17. Altogether, this suggest a critical role for IL-17-producing T lymphocytes in sporadic PD. Whether this is specific for Th17 lymphocytes or other lymphocytes also play a deleterious role remains to be established.

MHCII, a protein genetically linked to risk for PD, has the critical function of presenting peptide antigens on the surface of microglia for recognition by T cells. Harms et al (51) have shown that microglial MHCII is required for the activation of both the innate and adaptive immune responses to α-syn in PD and inducing of CD4 proliferation. This suggests a specific role of an α-synuclein related antigen in the engagement of adaptive immunity in PD. This hypothesis received recently a strong support with the discovery that T cells from the blood of patients with PD recognize α-synuclein peptides. (33, 34)

It is now clear that adaptive immunity also plays a role in neurodegeneration in PD. Yet, whether this is a consequence of other processes such a modification of α-synuclein or other cellular components such as neurofilaments also detected in Lewy bodies remains to be determined. Whether subpopulations of lymphocytes participate in the clearing of α-synuclein or exacerbate neuronal degeneration remains to be established. An answer to this question is crucial to determine how to counteract the deleterious effects of lymphocytes sub-populations in PD.

**Question 7: Are the brain-periphery interactions modified in the context of neuroinflammation in PD?**

The brain has been considered since the mid-20$^{th}$ century as a site of “immune privilege.” This idea is now being challenged by new data on brain-body interactions

under physiological conditions and in pathological states. In 2012, a new fluid-clearance pathway known as the glymphatic (glial-lymphatic) pathway was identified in the rodent brain, and the key elements of this pathway also appear to be present in human brain (for review see 68). This system might be involved in PD on several levels as it allows for immune cells to enter the brain to conduct surveillance, and might allow misfolded proteins of brain origin to gain access to the periphery and stimulate neuroinflammatory processes. (69)

There are some clear examples of brain-periphery interaction in PD, including the evidence for lymphocyte (and likely monocyte) infiltration into the brain parenchyma (described above). The status of the blood brain barrier in PD remains to be determined. Kortekaas et al. (70) reported an opening of and/or transport changes across the BBB in PD but this has never been confirmed. More generally, this has raised the question of the status of brain microvascularization in PD. Two decades ago, increased neovascularization in PD patients and non-human primates models (but not rodents) was reported. (71, 72) This confirmed more recently (73) but it appears that this is influences by both the degeneration of dopaminergic neurons as well as dopaminergic treatment (74). There may also be other pathways of brain-body communication in PD, including retrograde (and potentially anterograde) transport of proteins and signaling molecules through nerves such as the vagus, which provide for communication between the brain and peripheral organs. Elucidating these cellular, fluid and neuronal mechanisms of communication will be crucial to understanding the relationship of the brain to the periphery in PD.

**Question 8: When does inflammation begin in PD?**

It is well recognized that the onset of the motor symptoms of PD, such as tremor, occurs long after the onset of the disease process. While the details of the “pre-motor” phase of PD are still uncertain, most evidence suggests that this occupies a period of 10 years or more, during which there is progressive loss of dopaminergic function and appearance of non-motor symptoms such as autonomic impairment, hyposmia, and sleep disorders. This raises the corollary question of when inflammation begins in the course of PD. There is very little existing data on this point. Some investigators have begun to assemble cohorts of prodromal PD by screening high-risk populations. In line with this, Inflammatory biomarkers were recently examined in studies of RBD patients (75, 76) There is some circumstantial evidence from studies of blood cytokines which suggest that these are higher in early disease and decline with time, (77) as well as some evidence that blood lymphocytes recognizing α-synculein antigens can be detected early. Stokholm et al. (78) have observed increased PK 11195 binding in subjects with RBD. On the whole, however, this is a critical question which requires further exploration.

**Question 9: Is there any existing evidence for a disease-modifying effect of immunotherapies in PD?**

Numerous epidemiological studies have used retrospective approaches to determine whether the use of anti-inflammatory drugs is associated with reduced risk of developing PD. Most of these studies have examined the commonly used non-steroidal anti-inflammatory drugs (NSAID’s). Gao and co-workers first reported that the intake of non-aspirin NSAID’s reduced the risk of developing PD and later that this effect was largely explain by ibuprofen. (79) These results have proved controversial, with replication in some larger meta-analyses, but not in others. (80-82)

Similarly, minocycline, an inhibitor of microglial activation, did not appear promising in a phase 2 “futility” clinical study in PD, (83) but this does not exclude a positive effect in a more thorough study. A more convincing case can be made for treatments that target tumor necrosis factor alpha (TNFα). Considerable preclinical work has shown that this inflammatory mediator is important in PD model systems. In a large retrospective study using the Medicare database, Peter et al. (84) found that inflammatory bowel disease (IBD) increased risk for the development of PD, a result expected because PD and IBD share genetic links, including LRRK2. Surprisingly, however, patients treated for IBD using anti-TNFα medications had a drastic reduction in PD risk, with nearly a 90% reduction in PD in those who had received treatment.

All of these data are of course retrospective and associational; they cannot establish causality between immune treatments and reduced risk of PD. The next steps are likely prospective studies, and ultimately randomized, double-blind studies will be needed to fully assess the effects of immunomodulation in PD.

**Question 10: What is the future of anti-inflammatory interventions in PD?**

Interest in the role of the immune system in PD is growing rapidly. In 2002 there were less than 50 papers published on PD and inflammation, while in 2019 there were more than 700. Studies of neuro-inflammation are likely to help the field bring together disparate ideas about the cause of PD: there may be multiple factors which trigger PD, both genetic and environment, but inflammation may represent a common pathway by which the disease progresses. If this idea is correct, immunomodulatory treatments may prove valuable across the whole spectrum of PD, from pre-motor to more advanced stages.

A critical issue for therapeutics is identifying the most appropriate targets. It should be clear at this point is that there is a range of possibilities encompassing a variety of cellular and molecular mechanisms. There is also the question of therapeutic delivery; given the evidence for participation of circulating immune mechanisms, it is conceivable that a treatment targeting peripheral cells or their ability to enter the CNS might be effective (85).

Through these questions, we have tried to define some of the key open questions in the field and to stimulate further study. It is our hope that these ideas will accelerate progress towards a vision expressed by James Parkinson himself in 1817: "*There appears to be sufficient reason for hoping that some remedial process may ere long be discovered, by which, at least, the progress of the disease may be stopped."* (86)

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