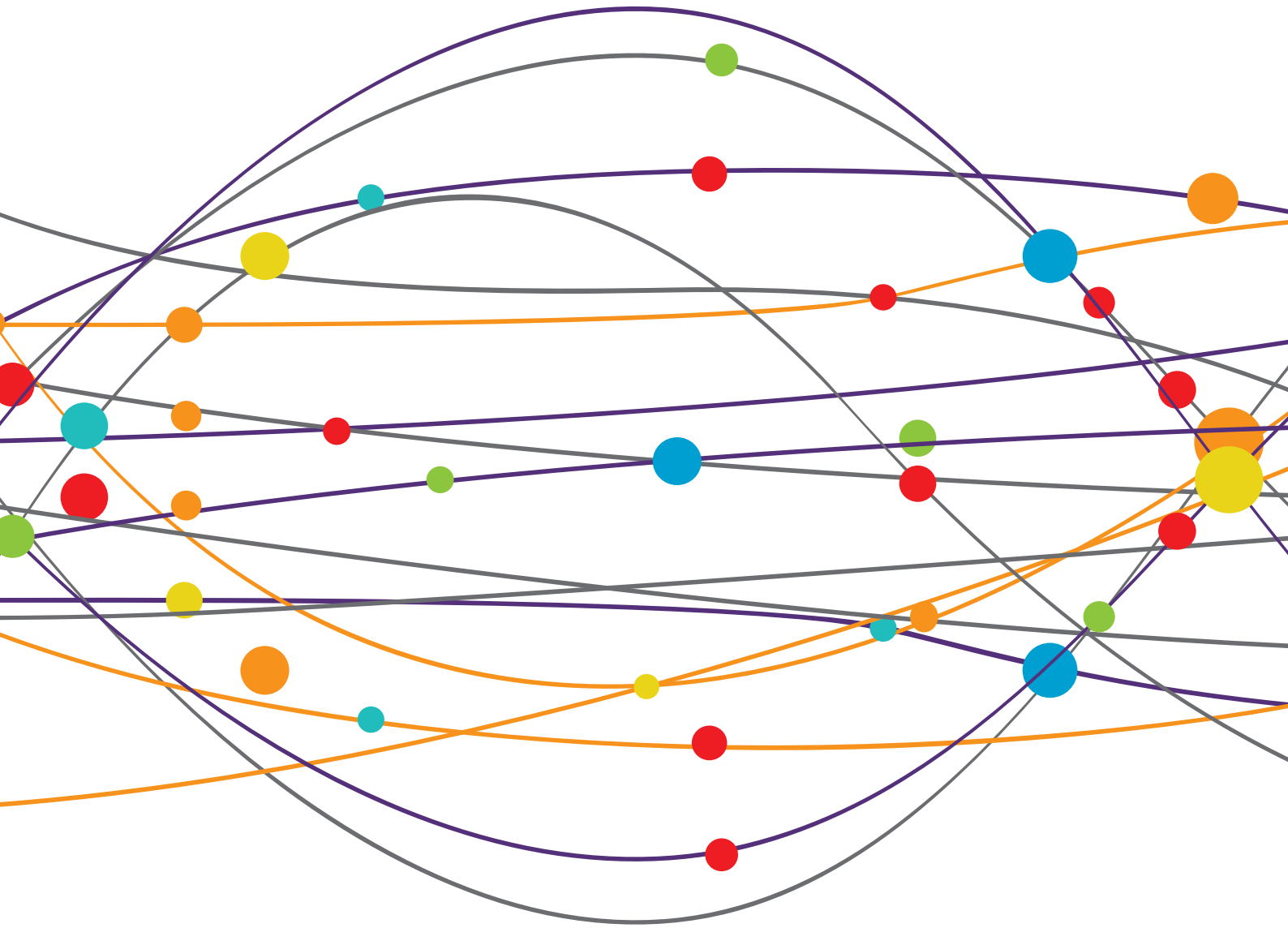
PERIPHERAL IMMUNITY IN PARKINSON'S DISEASE: EMERGING ROLE AND NOVEL TARGET FOR THERAPEUTICS

EDITED BY: Cristoforo Comi, Marco Cosentino and Rodrigo Pacheco
PUBLISHED IN: Frontiers in Neurology and Frontiers in Immunology



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-285-5
DOI 10.3389/978-2-88963-285-5

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

PERIPHERAL IMMUNITY IN PARKINSON'S DISEASE: EMERGING ROLE AND NOVEL TARGET FOR THERAPEUTICS

Topic Editors:
Cristoforo Comi, University of Eastern Piedmont, Italy
Marco Cosentino, University of Insubria, Italy
Rodrigo Pacheco, Fundación Ciencia and Vida, Chile

Citation: Comi, C., Cosentino, M., Pacheco, R., eds. (2019). Peripheral Immunity in Parkinson's Disease: Emerging Role and Novel Target for Therapeutics. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-285-5

Table of Contents

frontiers in Neurology

EDITORIAL
published: 15 October 2019
doi: 10.3389/fneur.2019.01080

Editorial: Peripheral Immunity in Parkinson's Disease: Emerging Role and Novel Target for Therapeutics

Cristoforo Comi[1]*, *Marco Cosentino*[2] *and Rodrigo Pacheco*[3]

[1] Neurology Unit, Department of Translational Medicine, Interdisciplinary Research Center of Autoimmune Diseases, Movement Disorders Center, University of Piemonte Orientale, Novara, Italy, [2] Center of Research in Medical Pharmacology, University of Insubria, Varese, Italy, [3] Laboratory of Neuroimmunology, Fundación Ciencia and Vida, Ñuñoa, Departamento de Ciencias Biológicas, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile

Keywords: immunity, alpha-synucein, gut-brain axis, T cell, probiotics

Editorial on the Research Topic

Peripheral Immunity in Parkinson's Disease: Emerging Role and Novel Target for Therapeutics

Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting up to 10 million people worldwide. PD has no cure yet, and patients rely only on symptomatic treatments. The hallmarks of PD are progressive loss of dopaminergic neurons in the substantia nigra, appearance of intracellular inclusions of aggregated α-synuclein (α-syn), called Lewy bodies (LB), and neuroinflammation resulting from microglia activation (1, 2). Understanding the causes of neurodegeneration in PD remains, so far, a challenging goal. Nevertheless, the recent identification of the involvement of peripheral immunity is raising increasing interest, as it may provide unprecedented opportunities to better understand PD pathogenesis, to identify clinically meaningful biomarkers and hopefully also novel therapeutic strategies (3, 4).

Besides a strong genetic association between the major histocompatibility class II locus and PD risk (5, 6), evidence supporting the role of peripheral immunity in PD include more rapid PD progression in the presence of a pro-inflammatory cytokine profile in the blood (7), a Th1-biased CD4+ T cell profile (8, 9), as well as an altered CD8+ T cell profile, with increased activation and reduced senescence markers (10). Remarkably, α-syn may trigger infiltration into the brain of T cells which in turn contribute to exacerbation of α-syn pathology, neurotoxicity and neurodegeneration (11–13), and the reported ability of T cells from PD patients to generate an autoimmune response to α-syn (14, 15) is even leading to reconsider PD as an autoimmune disorder (16).

Despite all this knowledge, however, the nature of immune dysregulation in PD, its relationship with neuroinflammation and neurodegeneration in the brain, the changes in peripheral immunity during disease progression, and whether targeting peripheral immunity may be beneficial to PD patients, all remain to be established. This Research Topic has been launched with the aim to collect high-quality and state-of-the-art articles covering all the aspects relevant to the relationship between immunity and neurodegeneration in PD, and bringing together research teams from different but synergistic scientific fields.

The 12 articles, seven review and five original articles, that were finally accepted for publication, offer the opportunity to explore the most relevant aspects of this emerging Research Topic. Of course, review articles discuss the contribution of immunity and inflammation to PD pathogenesis in a more general framework. This is the case of Caggiu et al., who focus on evidence linking infections and abnormal protein accumulation to immune system activation and critically discuss the possibility that autoimmunity may take part in PD pathogenesis. Fuzzati-Armentero et al. make an interesting parallel between PD patients and toxin-induced animal models, discussing differences and similarities in the context of neuroinflammation and immune responses,

Edited and reviewed by:
Hans-Peter Hartung, Heinrich Heine University of Düsseldorf, Germany

***Correspondence:**
Cristoforo Comi
cristoforo.comi@med.uniupo.it

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *21 June 2019*
Accepted: *24 September 2019*
Published: *15 October 2019*

Citation:
Comi C, Cosentino M and Pacheco R (2019) Editorial: Peripheral Immunity in Parkinson's Disease: Emerging Role and Novel Target for Therapeutics. Front. Neurol. 10:1080. doi: 10.3389/fneur.2019.01080

including the potential to guide novel therapeutic strategies. Troncoso Escudero et al. provide an updated perspective on the complex dynamics which orchestrate immune responses bothinside the central nervous system (CNS) and in the periphery. In this context, authors also discuss the potential targeting of astrocytes and microglia, as well as gut microbiome, with their therapeutic implications in PD.

García-González et al. review the involvement of stress signaling by the endoplasmic reticulum, which is crucially involved in protein aggregation and proteostasis dysfunction. Authors discuss how such signaling impacts on brain-associated immune cells and the possible implications to neuroinflammation and development of neurodegenerative diseases.

Scott et al. focus on the role of α-syn autoantibodies in PD, showing that there is weak evidence for an increase in α-syn auto-antibodies in PD patients particularly in early disease phase, but also underlining that more evidence is needed to support a robust relationship.

Finally, two reviews point to the role of T cells. Campos-Acuña et al. critically discuss the possibility that T cell driven inflammation, which has a crucial role in dopaminergic degeneration in PD, is triggered in the gut mucosa. Accordingly, they show how structural components of commensal bacteria and/or mediators produced by gut-microbiota, including short-chain fatty acids and dopamine, may affect the behavior of T cells, triggering the development of T cell responses against LB, initially confined to the gut mucosa but later extended to the brain. Storelli et al. summarize the current knowledge on the contribution of Th17 cells and IL-17 in PD, also assessing their therapeutic relevance. They underline that both animal and clinical studies are limited. Only a few studies provide mechanistic evidence and none of them investigates the eventual relationship between Th17/IL-17 and clinically relevant endpoints.

As regards original articles, Peralta Ramos et al. investigated the role of peripheral immune cells in the spreading of α-syn strains to the CNS. Authors provide evidence that α-syn administration in mice can induce microglia activation and leukocytes recruitment toward the CNS. Monocytes primed by intraperitoneal LPS administration internalize α-syn, with subsequent CNS dissemination. In addition, the α-syn ribbons strain determines differential recruitment of CD4+ and CD8+ T cells.

Elgueta et al. explore the role of dopamine receptor D3 (DRD3) signaling in peripheral blood CD4+ T cells from PD patients. They find that immune phenotypes favored by DRD3 signaling, namely Th1 and Th17 cells, are increased in the peripheral blood cells of PD patients compared to controls.

Further, they support their findings by selective DRD3-antagonism in this subset of lymphocytes in parkinsonian mice, obtaining a therapeutic effect on motor impairment.

Magistrelli et al. studied the effects of an *in vitro* challenge with probiotic bacterial strains to peripheral blood mononuclear cells (PBMCs) of PD patients and controls. All strains inhibited inflammatory cytokines and ROS production in both patients and controls, but most strikingly *Lactobacillus salivarius* and *acidophilus*. Furthermore, most strains restored the integrity of an artificial membrane model integrity and inhibited *Escherichia coli* and *Klebsiella pneumoniae* overgrowth. Finally, authors showed that the studied strains did not express tyrosine decarboxylase genes, which are known to decrease levodopa bioavailability.

Wijeyekoon et al. assessed monocyte functions in early-moderate PD compared to age and gender-matched controls. They found that PD monocytes display enhanced phagocytosis, but no significant differences in migration or cytokine secretion compared to controls.

White et al. investigated cell-extrinsic factors in systemic immune activation by using α-syn monomers and fibrils, as well as bacterial toxins, to stimulate PBMCs from PD patients and controls. They found no differences in cytokine production, nor in mRNA expression in patients vs. controls. By contrast, α-syn monomers increased production of IL-1β and IL-18 to levels significantly increased compared to those induced by low-level endotoxin.

In conclusion, this Research Topic provides a comprehensive overview of our current understanding of how adaptive and innate immune systems in the periphery are affected by infectious agents, commensal bacteria and pathogenic forms of α-syn, triggering an immune response in the central nervous system, possibly targeted at endogenous neoantigens such as α-syn itself, which eventually feeds neuroinflammation and neurodegeneration. Despite all this knowledge, however, much research is still required to establish the nature of immune dysregulation occurring in PD, how the immune system is involved in the prodromal phases of PD, and whether targeting peripheral immunity may favorably affect disease progression. We strongly hope that this collection of articles, providing a new insight of the physiopathology of PD, will encourage more laboratory and clinical research leading to the development of novel immunotherapeutics and probiotics as treatments of this disorder.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

The authors gratefully acknowledge the support provided by: Fondazione Cariplo (Progetto 2011-0504); Fondazione Unione Banche Italiane per Varese onlus (grant 18/07/2017); the AGING Project, Department of Excellence, Università degli Studi del Piemonte Orientale; Programa de Apoyo a Centros con Financiamiento Basal AFB-170004 from Comisión Nacional de Investigación Científica y Tecnológica de Chile (CONICYT); grant FONDECYT-1170093 from Fondo Nacional de Desarrollo Científico y Tecnológico de Chile; grant MJFF-10332.01 and MJFF-15076 (to RP) from Michael J. Fox Foundation for Parkinson Research. All these grant programs contributed to the development of the ideas and of the experimental research reviewed in the text.

REFERENCES

1. Eriksen JL, Wszolek Z, Petrucelli L. Molecular pathogenesis of Parkinson disease. *Arch Neurol*. (2005) 62:353–7. doi: 10.1001/archneur.62.3.353
2. Dehay B, Bourdenx M, Gorry P, Przedborski S, Vila M, Hunot S, et al. Targeting α-synuclein for treatment of Parkinson's disease: mechanistic and therapeutic considerations. *Lancet Neurol*. (2015) 14:855–66. doi: 10.1016/S1474-4422(15)00006-X
3. Mosley RL, Hutter-Saunders JA, Stone DK, Gendelman HE. Inflammation and adaptive immunity in Parkinson's disease. *Cold Spring Harb Perspect Med*. (2012) 2:a009381. doi: 10.1101/cshperspect.a009381
4. Sommer A, Winner B, Prots I. The Trojan horse - neuroinflammatory impact of T cells in neurodegenerative diseases. *Mol Neurodegener*. (2017) 12:78. doi: 10.1186/s13024-017-0222-8
5. Saiki M, Baker A, Williams-Gray CH, Foltynie T, Goodman RS, Taylor CJ, et al. Association of the human leucocyte antigen region with susceptibility to Parkinson's disease. *J Neurol Neurosurg Psychiatry*. (2010) 81:890–1. doi: 10.1136/jnnp.2008.162883
6. International Parkinson Disease Genomics Consortium, Nalls MA, Plagnol V, Hernandez DG, Sharma M, Sheerin UM, et al. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet*. (2011) 377:641–9. doi: 10.1016/S0140-6736(10)62345-8
7. Williams-Gray CH, Wijeyekoon R, Yarnall AJ, Lawson RA, Breen DP, Evans JR, et al. Serum immune markers and disease progression in an incident Parkinson's disease cohort (ICICLE-PD). *Mov Disord*. (2016) 31:995–1003. doi: 10.1002/mds.26563
8. Kustrimovic N, Rasini E, Legnaro M, Bombelli R, Aleksic I, Blandini F, et al. Dopaminergic receptors on CD4+ T naive and memory lymphocytes correlate with motor impairment in patients with Parkinson's Disease. *Sci Rep*. (2016) 6:33738. doi: 10.1038/srep33738
9. Kustrimovic N, Comi C, Magistrelli L, Rasini E, Legnaro M, Bombelli R, et al. Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/T17 and Treg in drug-naïve and drug-treated patients. *J Neuroinflammation*. (2018) 15:205. doi: 10.1186/s12974-018-1248-8
10. Williams-Gray CH, Wijeyekoon RS, Scott KM, Hayat S, Barker RA, Jones JL. Abnormalities of age-related T cell senescence in Parkinson's disease. *J Neuroinflammation*. (2018) 15:166. doi: 10.1186/s12974-018-1206-5
11. Ferreira SA, Romero-Ramos M. Microglia response during Parkinson's Disease: alpha-synuclein intervention. *Front Cell Neurosci*. (2018) 12:247. doi: 10.3389/fncel.2018.00247
12. Sommer A, Fadler T, Dorfmeister E, Hoffmann AC, Xiang W, Winner B, et al. Infiltrating T lymphocytes reduce myeloid phagocytosis activity in synucleinopathy model. *J Neuroinflammation*. (2016) 13:174. doi: 10.1186/s12974-016-0632-5
13. Sommer A, Maxreiter F, Krach F, Fadler T, Grosch J, Maroni M, et al. Th17 lymphocytes induce neuronal cell death in a human iPSC-based model of Parkinson's Disease. *Cell Stem Cell*. (2018) 23:123–31.e6. doi: 10.1016/j.stem.2018.06.015
14. Garretti F, Agalliu D, Lindestam Arlehamn CS, Sette A, Sulzer D. Autoimmunity in Parkinson's Disease: the role of α-Synuclein-specific T cells. *Front Immunol*. (2019) 10:303. doi: 10.3389/fimmu.2019.00303
15. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize alpha-synuclein peptides. *Nature*. (2017) 546:656–61. doi: 10.1038/nature22815
16. Bandres-Ciga S, Cookson MR. Alpha-synuclein triggers T-cell response. Is Parkinson's disease an autoimmune disorder? *Mov Disord*. (2017) 32:1327.doi: 10.1002/mds.27116

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

SYSTEMATIC REVIEW
published: 01 October 2018
doi: 10.3389/fneur.2018.00815

A Systematic Review and Meta-Analysis of Alpha Synuclein Auto-Antibodies in Parkinson's Disease

Kirsten M. Scott[1], Antonina Kouli[1], Su L. Yeoh[2], Menna R. Clatworthy[3†] and Caroline H. Williams-Gray[1†]*

[1] Department of Clinical Neurosciences, John van Geest Centre for Brain Repair, University of Cambridge, Cambridge, United Kingdom, [2] University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom, [3] MRC Laboratory of Molecular Biology, Molecular Immunity Unit, Department of Medicine, University of Cambridge, Cambridge, United Kingdom

OPEN ACCESS

Edited by:
Cristoforo Comi, Università degli Studi del Piemonte Orientale, Italy

Reviewed by:
Tomasz Brudek, Bispebjerg Hospital, Denmark
Natalia P. Rocha, University of Texas Health Science Center at Houston, United States

***Correspondence:**
Kirsten M. Scott
kms61@cam.ac.uk

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *10 July 2018*
Accepted: *10 September 2018*
Published: *01 October 2018*

Citation:
Scott KM, Kouli A, Yeoh SL, Clatworthy MR and Williams-Gray CH (2018) A Systematic Review and Meta-Analysis of Alpha Synuclein Auto-Antibodies in Parkinson's Disease. Front. Neurol. 9:815. doi: 10.3389/fneur.2018.00815

Immune dysfunction has been associated with Parkinson's disease (PD) and its progression. Antibodies play an important role in both innate and adaptive responses, acting as powerful effector molecules that can propagate inflammation by activating innate immune cells. Alpha synuclein binding antibodies have been described in PD patients with conflicting associations. In this article, we consider the potential mechanistic basis of alpha synuclein auto-antibody development and function in PD. We present a systematic review and meta-analysis of antibody studies in PD cohorts showing that there is weak evidence for an increase in alpha synuclein auto-antibodies in PD patients particularly in early disease. The confidence with which this conclusion can be drawn is limited by the heterogeneity of the clinical cohorts used, inclusion of unmatched controls, inadequate power and assay related variability. We have therefore made some recommendations for the design of future studies.

Keywords: antibodies (Abs), alpha synuclein (α syn), auto-antibodies, Parkinson's disease (PD), peripheral inflammation, Fcγ receptor

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra resulting in a movement disorder and many non-motor symptoms, including dementia, postural hypotension and gut dysfunction (1). Whilst dopaminergic treatments may alleviate the motor symptoms, there are currently no disease-modifying therapies that slow clinical progression.

Immune dysfunction has been associated with PD and its progression (2–5) and represents a tractable target for disease modification. However, the cellular and molecular mechanisms underpinning this association have yet to be elucidated. Potential pathways include the activation of adaptive immunity via antigen-specific recognition of alpha-synuclein or non-specific innate immune activation due to cell damage and death.

The accumulation of aggregated alpha-synuclein within CNS neurons is the pathological hallmark of PD (6). There is also evidence that misfolded alpha synuclein accumulates in the periphery, for example in the gut, in early stages of disease (7), providing a route for the exposure

of the peripheral immune system to a central nervous system (CNS) antigen. Monomeric alpha synuclein is abundant in the CNS in pre-synaptic terminals of neurons and is also produced by platelets and red blood cells peripherally. Pathological forms of the protein range from soluble oligomers to mature insoluble fibrillar forms (8). Multiple studies have sought to measure alpha synuclein in either the blood or serum [reviewed in (9)] for use as a biomarker. Substantial variation in levels may be a confounding factor in studies measuring alpha synuclein antibodies as these may be undetectable if already bound.

A recent study demonstrated the presence of alpha-synuclein specific CD4 and CD8 T cells in PD patients, implicating an alpha-synuclein specific adaptive immune response in disease pathogenesis (10). CD4 T cells orchestrate adaptive immunity, including humoral responses which result in the production of antibodies. Antibodies are powerful immune effector molecules produced by plasma cells, terminally differentiated B lymphocytes. The most common circulating antibody isotype is IgG, that can readily initiate and propagate inflammation by activating complement and engaging cell surface antibody receptors [Fcγ receptors (FcγR)] that are expressed by most innate immune cells.

Alpha synuclein-specific IgG antibodies have been described in PD, but their role is unclear with many conflicting studies. Publication bias favoring positive findings in this field may also further complicate attempts to unmask a true effect. The presence of alpha synuclein specific antibodies in early disease could potentially contribute to pathology by exacerbating local inflammation in the brain, promoting neuronal damage and causing disease progression. Consistent with this hypothesis, IgG isolated from PD patients and injected into rat substantia nigra causes selective dopaminergic cell death that was absent in animals receiving control IgG (11). There is also attenuation of disease in Fcγ receptor knockout mice receiving PD IgG, confirming that activation of microglia by PD IgG is pathogenic (12). Approximately 30% of dopaminergic cells in the substantia nigra of post-mortem PD brains were bound by IgG highlighting that immunoglobulins do cross the blood brain barrier in PD and may play a role in disease (13).

Alternatively, alpha synuclein auto-antibodies may play a protective role, facilitating the clearance of toxic protein species by opsonizing alpha-synuclein for FcγR-mediated uptake by phagocytes. Consistent with this hypothesis, the passive peripheral transfer of alpha-synuclein specific antibodies in some mouse models of PD improved disease outcomes (14). Trials of both passive and active immunization therapies targeting alpha synuclein are underway (15, 16).

Clearly, it is critical to have a better understanding of how alpha synuclein autoantibodies relate to PD and its progression. In particular, there is a need to discern whether they constitute a useful diagnostic or prognostic biomarker or may have potential therapeutic relevance. In this article, we will consider the potential mechanistic basis of their role in PD, present a systemic review of antibody studies in PD cohorts, critically discuss the value and limitations of existing data and make recommendations for future studies.

POTENTIAL MECHANISMS UNDERLYING ANTIBODY GENERATION IN PD

B lymphocytes can produce antibodies via T cell-independent (TI) and T cell-dependent pathways (TD) (see **Figure 1**). TI pathways involve the recognition of multimeric carbohydrate and lipid antigens by the B cell receptor (BCR) or by toll like receptors (TLR) on the cell surface of "B1" cells (or marginal zone B cells in the spleen). This leads to the production of polyreactive IgM that binds with low affinity and can facilitate the removal of blood borne encapsulated organisms (17). Antibodies produced in this context are called "natural antibodies." Most of the literature on alpha synuclein antibodies suggests that these are natural antibodies (18–21). Natural antibodies are part of innate immune surveillance against pathogens or cell damage and are present from an early point in development (22). They are predominantly IgM but IgG and IgA natural antibodies have also been described (22). Antibodies to alpha synuclein epitopes could be generated via this process.

The recent description of alpha synuclein specific T cells in patients with PD (10) supports the thesis that alpha synuclein antibodies may be generated by a TD response. These antibodies recognize protein antigens and their production requires a cognate interaction between "B2" cells and CD4 T cells. This facilitates iterative rounds of somatic hypermutation and clonal selection within a germinal center reaction to generate class-switched long lived plasma cells or memory B cells capable of initiating a secondary response upon further encounter of the antigen (22). The plasma cells that arise from this process are able to produce large quantities of specific, high affinity class-switched antibodies (17). Humoral responses to self-antigen are limited by negative selection of self-reactive clones during B cell development. However, if the self-antigen is modified sufficiently, as in the case of alpha synuclein toxic species, and is present in an immunogenic context, such as cell death, some B cell clones may be activated to produce alpha synuclein antibodies. Such an antibody response might change over time; firstly IgM may dominate, but with progression of the germinal center reaction, there is class switching to IgG or IgE. Secondly, with persistent exposure to neo-antigen, clones with higher levels of somatic hypermutation and higher affinity antibodies would be selected. Thirdly, the overall level of alpha synuclein antibody might change with age, as older age is associated with decreasing antibody response to antigens (e.g., vaccines) and immunosenescence of the B cell compartment (23).

B cell activation to generate plasma cell-producing antibodies generally occurs within secondary lymphoid organs (lymph nodes and spleen) but may also occur in tertiary lymphoid follicles that develop in inflamed tissues. The site of B cell activation to generate alpha synuclein-specific responses is unclear and may be peripheral or within the CNS. Follicles have been described in the meninges of patients with multiple sclerosis (24), with the potential to generate CNS localized antibodies, but whether such structures exist in PD is unknown.

Antibodies bind non-specifically to Fcγ receptors on other immune cells (e.g., phagocytes, monocytes, dendritic cells) or via engagement of their Fc region with complement components

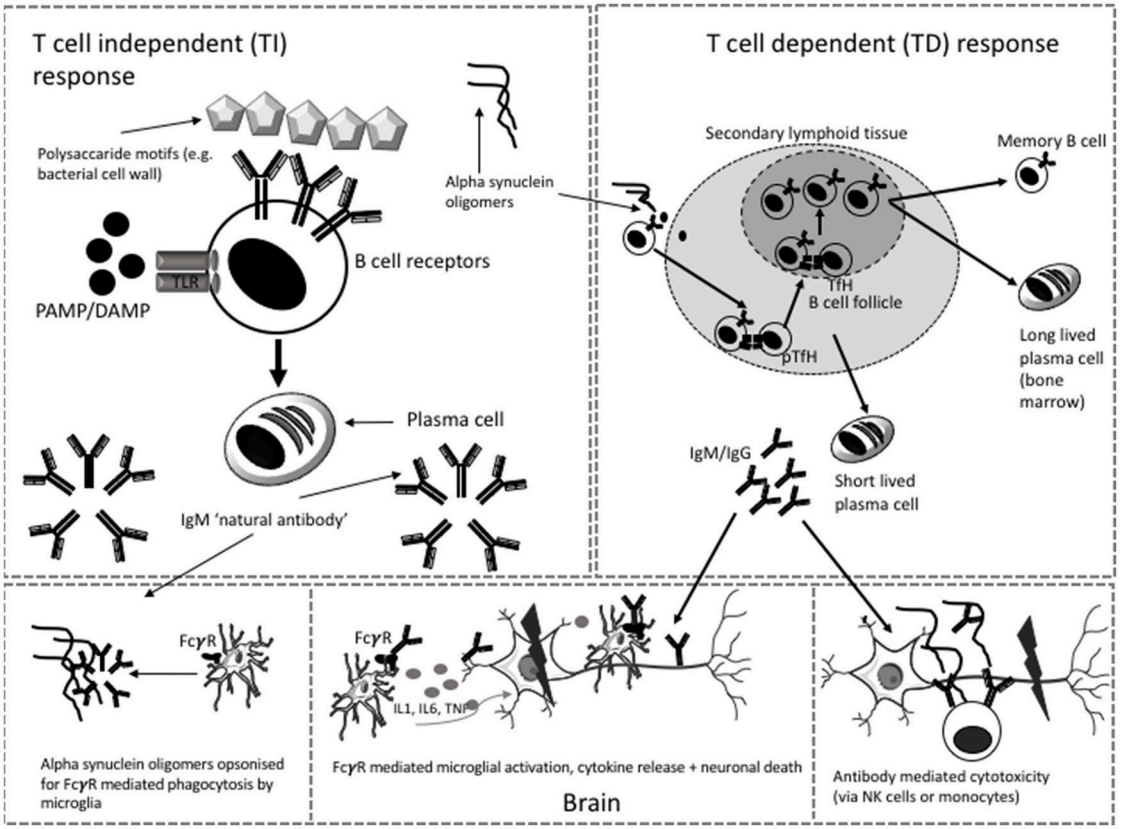


FIGURE 1 | Possible T independent and T dependent mechanisms of antibody generation in PD. Microglia and neuron images modified from templates obtained https://smart.servier.com/smart_image/microglia-3/ under Creative Commons Attribution 3.0 Unported License. TfH, T follicular helper cell; pTfH, peripheral T follicular helper cell; NK, Natural killer; PAMP, pathogen associated molecular motif; DAMP, damage associated molecular motif, TLR, toll like receptor.

(25). Different subclasses of IgG (IgG1, IgG2, IgG3 and IgG4) have different affinities for the FcγR on cells which can be either activating or inhibitory [see (25)]. There is evidence that FcγRI and FcγRIIB/C are required for uptake of alpha synuclein by CNS derived cells in culture and that this is mediated by the presence of alpha synuclein specific antibodies (26). One recent paper suggested that FcγRIIB (a low affinity inhibitory Fc receptor) is not only responsible for the inhibition of phagocytosis of alpha synuclein fibrils (via low affinity binding with the fibrils themselves) but also mediates cell to cell transmission of alpha synuclein (27). In addition, the glycosylation status of immunoglobulin also affects downstream binding and effector function (28). A study investigating the IgG glycome in PD showed significant differences between patients and controls, with the authors concluding that the changes observed in PD may result in enhanced Fcγ RIIIa-mediated antibody-dependent cellular cytotoxity (with the potential to contribute to chronic inflammation) (29).

SYSTEMATIC REVIEW OF ALPHA SYNUCLEIN ANTIBODY STUDIES IN PD

We [AK, SLY and KS] searched the literature for studies published prior to 1st June 2018 using Pubmed, Medline, Cochrane database, Embase, Google scholar and Keele Web of Science. We used the following search terms: "Antibody and Parkinson's Disease," "Auto-antibody and Parkinson's disease," "Alpha synuclein antibody," "Alpha synuclein auto-antibody." To ensure complete study capture we also searched using "Auto-antibody dementia" "Antibody dementia." Reference lists of the selected papers were also manually searched to identify additional studies. Papers were excluded if they did not involve PD patients, if they did not measure alpha synuclein antibodies and if there was no control group. Otherwise all papers measuring antibodies to alpha synuclein or its epitopes in Parkinson's disease patients were included in the systematic review. The literature searches were done between 1 May 2018 and 6 June 2018. Summary information from each study was compiled into a table (**Table 1**).

In order to assess whether studies were adequately powered, mean alpha synuclein antibody titres (or optical density) in each group and standard deviations were recorded and used to calculate required sample size to detect a difference of the magnitude reported. The following formula was used to calculate sample size [modified from (43)].

$$nA = \kappa nB \; and \; nB = (1 + \frac{1}{\kappa})(Swithin \frac{z1 - \frac{a}{2} + z1 - \beta}{\mu A - \mu B})^2$$

TABLE 1 | Summary of studies measuring alpha synuclein antibodies in Parkinson's disease.

	Paper	Method	Fluid	N (HC = healthy controls)	Matched	Mean age of PD (SD)	Disease duration years (SD)	H and Y (SD)	Finding (in PD vs. controls)	Required N^^
<=5 years DD	Xu et al. (30)***	Electrochemical impedance spectroscopy	Serum	60 PD, 29 HC	Yes	69.4 (SD10.8)	1.4 (1.44)	20 HandY1, 20 HandY2, 20 HandY3	↑ in PD, more in HandY 1 and 2 than controls, no diff betweeen stages	382
	Horvath et al. (31)***	indirect ELISA	Plasma, CSF	20 PD, 20 HC	Yes	Mild: 65.5 (38–79*) Moderate: 67.2 (56–77*)	2.8 (1–8*) months (<1 year)	1.5 to 2	↑ in PD vs. HC in CSF and plasma Decreased in moderate vs. mild disease	N/A
	Smith et al. (9)	ELISA	Serum	14 PD, 11 PD syndrome, RBD 10, 9 HC	Yes	RBD 58 (SD 9), PD 63 (9)	Median 3.5 (1–12*)	1.3 (range 1–3.5)	No difference	N/A
	Gruden et al. (32)***	ELISA	Serum	32 PD, 26 HC	Yes	60.8 (2)**	8.6 (3.4)** Subgroup <5	2.1 (0.6)	↑ in PD vs. HC, greater difference with monomers than oligomers	23
	Shalash et al. (33)	ELISA	Serum	46 PD, 20 HC	Yes	56.26 (SD12.26)	5.2 (3.36)	3 (1.5–3.5 range)	↑ in PD vs. HC	N/A
7-10 years DD	Akhtar et al. (34)	ELISA	Serum, CSF	Serum: 53 PD, 16 HC CSF: 93 PD, 52 HC Both CSF and serum for 24 participants	No	Serum 70.9 (7) CSF 67.1 (9.4)	7.9 (5)	3 (1–4) (median + range)	CSF ↑, serum →	77
	Brudek et al. (19)	ELISA, MSD	Plasma	46 iPD, 46 HC	No	62.4 (6.7)	7.9 (5)	2 (median)	↓ in PD vs. HC	126
	Papachroni et al. (35)	Immunoblot	Serum	31 iPD, 20 FPD, 26 HC	Yes	Idiopathic: 65.1 (11.6), Familiial: 66.1 (12.7)	Calculated 7.2 iPD, 9.4 FPD	2.4 (FPD), 2.5 iPD	↑ in FPD vs. PD or controls	N/A
	Yanamandra et al. (36)***	Elisa, western blot, biocore surface plasmon resonance	Serum	39 PD, 23 HC	Yes	55.7 (10)	7.7 (5.6)	HandY1-2 27, HandY 2.5–4 12	↑ PD vs. HC	N/A
	Caggiu et al. (37)	ELISA	Serum	40 PD, 40 HC	Yes	69.8 (7.95)	8.42(4.29)	3.01 (0.88)	↑ in PD vs. controls to three peptides (similar to HSV)	N/A
	Maetzler et al., (38)	ELISA	Serum	93 PD (demented subgroup 31), 194 controls	No	68.5(SD9) PDND, 76.7 (SD8) PDD	9.5 (1–26*)	2 (1–4)	No difference	N/A
10-12 years DD	Alvarez-Castelao et al. (39)	ELISA immunoblots	Plasma	55 iPD, 104 LRRK2 carriers, 85 HC	No	67.8 (9.9) iPD 68.37 (10.2) LRRK2	12 (8.7) iPD 13 (11) LRRK2	2.44 (0.8) iPD, 2.55 (0.88) LRRK2	Controls and iPD no difference Using stringent criteria ↑ antibodies in LRKK2 pre-manifest	N/A

(Continued)

TABLE 1 | Continued

	Paper	Method	Fluid	N (HC = healthy controls)	Matched	Mean age of PD (SD)	Disease duration years (SD)	H and Y (SD)	Finding (in PD vs. controls)	Required N^^
	Besong-Agbo et al. (18)	ELISA	Serum	62 iPD, 46 HC	Yes	68.6 (9)	10.2(6)	>3	↓ in PD vs. HC	60
Unknown DD	Bryan et al. (40)	Electrochemical impedance spectroscopy	Serum	30 PD, 14 HC	No	Not reported	Not reported	1 to 3	↑ in PD vs. HC, increasing up to HandY 2 then decreasing for HandY 3.	N/A
	Heinzel et al. (20)	ELISA	Serum, CSF	66 PD, 69 HC (CSF 59 PD and 46 controls)	Yes	No ages reported	Not reported	2	No difference	214
	Woulfe et al. (41)	ELISA	Serum	Serum: 28 PD, 19 HC CSF: 4 PD, five controls	Not reported	Not reported	Not reported	Not reported	No difference	N/A

*Numbers refer to mean and standard deviation unless otherwise specified. The table is ordered according to disease duration. Patient and control groups were considered "matched" if there were no significant between-group differences in age and gender distributions. One paper was removed due to cohort overlap (42). N/A was recorded in the sample size column if there was not sufficient data to do the power calculation. *Range, **SEM, ***author overlap, ^^ in each group, DD, Disease duration.*

Where:

K = nA/nB (matching ratio between groups—nA = PD patients, nB = controls)

Swithin = pooled standard deviation across groups

α = Type I error (set at 0.05)

β = Type II error (1-β = power, set at 0.8)

The pooled within sample standard deviation was calculated to overcome differences in variation between the groups [from (44)]:

$$Swithin = \sqrt{\frac{(n1-1)\,S1^2 + (n2-1)\,S2^2}{n1+n2-2}}$$

n1 = sample size (SS) in patients, n2 = SS in controls

S1 = SD in patients, S2 = SD in controls.

META-ANALYSIS OF ALPHA SYNUCLEIN ANTIBODY STUDIES IN PD

We undertook a meta-analysis, stratified by disease duration given the suggestion in the literature that this is a relevant factor [e.g., (36)]. Studies with mean disease durations of 5.9 years and less were included in an "early disease" meta-analysis and those with disease durations of 7 years or more were included in a "later disease" meta-analysis given the trends noted in the review above and in **Table 1**.

More stringent data quality criteria were adopted for the meta-analysis than for the systematic review described above.

Inclusion criteria:

i) The study measured antibodies to full length alpha synuclein
ii) The antibodies were measured using titres (either relative or absolute) as a continuous measure
iii) The study included both idiopathic PD patients and controls
iv) The study stipulated a measure of disease duration for the cohort
v) The controls were age and gender matched to the patients
vi) Antibodies were measured in either serum or plasma

If a study had not published appropriate statistical tests to determine whether the controls were matched appropriately to the patients this was performed (independent samples *t*-test for age; chi-squared test for gender). The study estimates were extracted from the included papers according to the protocol below;

Study estimate extraction:

i) Means and standard deviations were used as the basis for the study estimates, if reported.
ii) If these were not reported, then the median and interquartile ranges were extracted and converted into means and standard deviations using the methodology described in (45) and an online calculator (http://www.comp.hkbu.edu.hk/~xwan/median2mean.html).
iii) If the above estimates were not described in the text then they were estimated from the boxplots or graphs published in the text.

As all studies used different assays and units of measurement, it was not possible to do a direct comparison using the raw unstandardised mean difference. The study estimates were therefore used to calculate the standardized difference and the associated variance (yi and vi, respectively) using the metafor package for R in R studio (version 1.0.153), and the following formulas (44):

$$yi = \frac{\overline{X}1 - \overline{X}2}{Swithin}$$

Where yi = standardized mean difference (d)

$\overline{X}1$ = sample mean in PD patients

$\overline{X}2$ = sample mean in controls

Swithin = within groups standard deviation, pooled across groups (as used above for the power calculation)

$$Swithin = \sqrt{\frac{(n1-1)\,S1^2 + (n2-1)\,S2^2}{n1+n2-2}}$$

S1 = standard deviation in PD group

S2 = standard deviation in controls

A random effects model was used to assess the overall difference between patients and controls. Forest plots were generated to show the results graphically. Funnel plots were generated to plot standardized mean difference (x axis) against standard error (y axis) to assess the impact of publication bias and heterogeneity.

The variance of d (referred to as vi) is given by the following formula (see (44) page 27):

$$vi = \frac{n1+n2}{n1n2} + \frac{d^2}{2(n1+n2)}$$

RESULTS

A total of 17 papers met the inclusion and exclusion criteria for the systematic review (**Table 1**). Eight studies found a statistically significant increase in alpha synuclein antibodies in idiopathic PD patients compared to controls (30–33, 36, 37, 40, 42). These studies included a total of 305 patients and 198 controls but two of the papers appear to use overlapping patient samples with identical demographic tables and results figures and so the second of these was excluded (32, 42).

Three papers found raised alpha synuclein antibodies in subgroups of PD patients, either in familial PD (35), pre-manifest LRRK2 carriers (39) or only in CSF and not serum (34). Four studies reported no difference in peripheral anti-alpha synuclein antibodies (9, 20, 38, 41) and two studies found that alpha synuclein antibodies were decreased in patients vs. controls (18, 19). Importantly the Brudek et al. paper focused on high affinity antibodies only which may underlie the difference in findings.

Three studies investigated antibodies in CSF as well as in plasma or serum (20, 31, 34) with two of these finding raised alpha synuclein antibodies in the CSF (31, 34).

All studies investigated the antibody response to full length alpha synuclein apart from the Caggiu et al study that assessed the response to specific epitopes deemed to be relevant due to their similarity to EBV (37).

Clinical Heterogeneity

There is wide variation in disease stage and duration across studies (see **Table 1**). Previous studies have noted an increase in early disease e.g., (42). Of the five papers reporting a mean disease duration of 5 years or less (see **Table 1**), four report an increase in alpha synuclein antibodies in patients compared to controls (representing a total of 196 patients and 121 controls excluding the first Gruden et al paper as described above) (9, 30, 31, 33, 36, 42). Only the smallest of the studies in early PD showed no PD-control difference (N = 14 PD patients and nine controls) (9). Even taking a conservative interpretation of these results, the larger studies are consistent in reporting an increase in alpha synuclein antibodies in early disease. An additional study for which disease duration was unavailable reported an association with HY disease stage with increasing titres from HY stage 1 to 2, decreasing at stage 3 (40). Alvarez-Castelao et al. found increased alpha synuclein antibodies in LRRK2 carriers vs. controls but not in patients with longer disease durations (>10 years) (39). Other studies have also reported a similar association with HY staging (33, 46). Of six studies with mean disease durations between 7 and 10 years, two studies report a clear increase in patients vs. controls (36, 37). Two further studies show an increase in a subgroup, in familial PD vs. controls (but not idiopathic PD) (35) in one study and in CSF only and not serum in another (34). The two studies that showed either no difference (38) or a difference in the opposite direction (19) did not have age and gender matched control groups. In the two studies with disease duration beyond 10 years there was either no difference (39) or a decrease in patients compared to controls (18).

Patient age also varies between study cohorts, ranging from a mean of 55.7 (36) to 69.8 [(37); **Table 1**]. Antibody responses vary with age and gender (47). It is therefore also critical to ensure that patient and control groups are well-matched. Of the 17 studies reviewed, seven either did not report appropriate demographic information or the control group was not matched to the patients.

Assay Variability

Most studies have made use of custom ELISAs with one study using a commercial ELISA for serum anti-alpha synuclein antibodies (33). Two positive studies by the same group in different patient cohorts used electroimpedence spectroscopy (30, 40). Several others used immunoblots or western blots (35, 36, 39). ELISAs are limited by many factors including the requirement for two independent binding events and problems with non-specific binding (30). There is also variation in conditions between studies, such as buffers used, protein coating concentration and temperature of the assay which are particularly relevant for an intrinsically disordered protein, such as alpha-synuclein.

Most of the alpha synuclein for the use in ELISAs was generated in *E. coli* in-house, and therefore may not include post-translational modifications present in mammalian cells (30, 31, 34–36, 38, 40) (with other papers obtaining commercially generated protein). Alvarez-Castelao et al. attempted to replicate

their ELISA findings using immunoblots and identified that some of the ELISA positive samples were recognizing something other than alpha synuclein (39). This effect disappeared when they introduced an additional purification step suggesting the possibility that at least some of the findings in the literature may be due to interfering antibodies to bacterial toxins rather than to alpha synuclein itself. Antibodies present in serum may also be bound to serum protein (either specifically or non-specifically) which may interfere with antibody detection (38). Most of the papers investigated antibody responses to

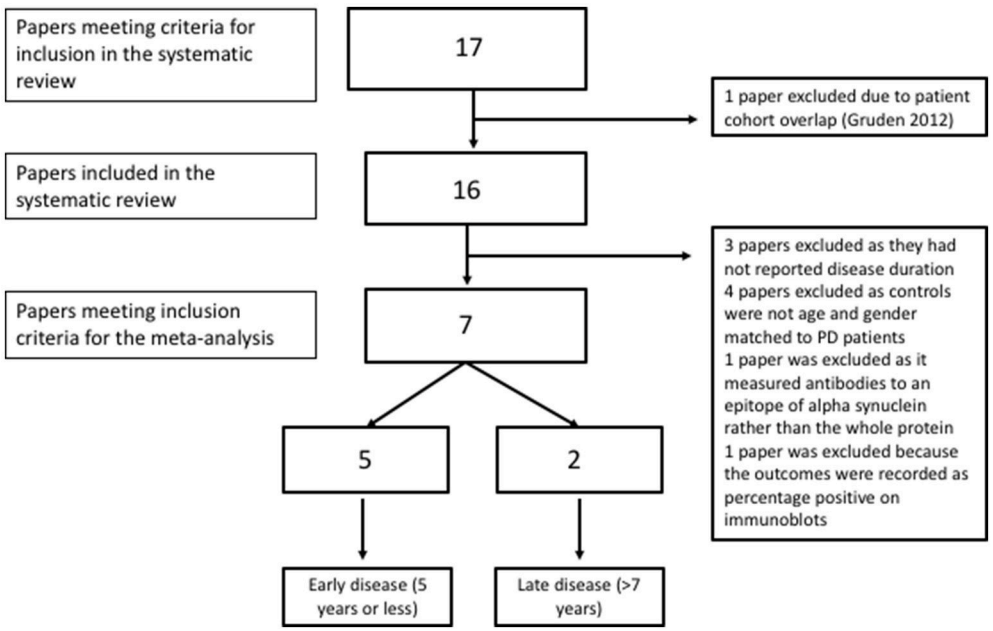


FIGURE 2 | Flow diagram showing inclusion and exclusion of studies in the meta-analysis.

TABLE 2 | Study estimates, standardized effect sizes (yi) and variance (vi) ("early disease" <5.9 years disease duration).

	Year	Controls			PD			yi	vi
		Mean	SD	N	Mean	SD	N		
Gruden	2011	25.00	50.99	26	310.00	452.55	32	0.83	0.08
Xu	2012	1.24	1.44	29	1.62	2.04	60	0.20	0.05
Smith	2012	0.83	1.13	9	1.06	1.81	14	0.14	0.18
Horvath	2017	5.00	0.67	20	6.50	2.72	20	0.74	0.11
Shalash	2017	0.49	0.69	20	4.39	1.78	46	2.50	0.12

Model results: Estimate = 0.88 (95% CI 0.005–1.17), SE = 0.42, Z = 2.09, p = 0.036.
I^2 = 89.32%.
Q(df = 4) = 33.71, p = 0.0001.

TABLE 3 | Study estimates, standardized effect sizes (yi) and variance (vi) ("later disease," >7 years disease duration).

Study	Year	Controls			PD			yi	vi
		Mean	SD	N	Mean	SD	N		
Yanamandra 6.7 years	2011	108.67	126.43	23	696.44	821.82	27	0.95	0.09
Yanamandra 9.7 years	2011	108.67	126.43	23	313.11	490.58	12	0.66	0.13
Besong-Agbo	2013	153.5	103.77	46	105.40	85.83	62	−0.51	0.04

Model results: Estimate 0.34 (95% CI −0.57–1.25), SE 0.46, Z = 0.73, p = 0.47.
I^2 = 87.6%.
Q(df = 2) = 19.80, p = 0.0001.

monomeric alpha synuclein (which is not necessarily the disease relevant species) with only a minority assessing responses to fibrils, mutated alpha synuclein (36, 39, 42), oligomers or other pathological forms [e.g., phosphorylated alpha synuclein (19) or specific peptides (37)]. The Brudek et al. paper focused on high affinity antibodies finding that these were decreased in patients compared to controls which is consistent with them having a role in alpha synuclein clearance. As other studies have investigated the overall antibody response it is not useful to directly compare these.

Lastly, some of the variation between studies may be due to the use of either serum or plasma (although only two studies used plasma rather than serum, see **Table 1**). It is possible that factors present in plasma but not in serum (e.g., alpha synuclein produced by platelets) may affect subsequent results and therefore it would be wise to standardize the use of serum across studies.

Power

Lack of adequate power may be an important factor leading to false negative findings in a number of studies. The largest study included 93 PD patients and 194 controls (38) but unfortunately the controls were not age and gender matched to the patients (see **Table 1**). Of the 17 studies, seven included appropriate information to calculate power. Of those with incomplete information, this was usually because the data were presented as graphs or as medians and IQ range. The estimated sample sizes required to detect the differences reported ranged from 23 to 382, with a mean of 147 per group (see **Table 1**). The only study that was adequately powered was that by Gruden et al. that reported much larger difference between controls and patients than other studies and is therefore an outlier. Excluding this study, the estimated required sample size per group is between 60 and 382.

Meta-Analysis

All of the "early disease" papers shown in **Table 1** met the inclusion criteria (see also flow plot in **Figure 2**) Means and standard deviations were available from two of the studies (30, 32). The means and standard deviations from Horvath et al. (31) were estimated based on the reported medians and interquartile ranges. The medians and interquartile ranges from the other two papers were estimated from boxplots and subsequently converted to means and standard deviations as described in the methods (9, 33). Study effect size estimates and model results are shown in **Table 2**. Overall, there is a significant increase in antibodies in patients vs. controls across studies (see forest plot in **Figure 3**) but the effect size is modest (0.88, 95% CI 0.05–1.71, p-value = 0.036). There was significant heterogeneity across studies (I^2 = 89.32%).

Only two of the "later disease" studies (mean disease duration >7 years) met inclusion criteria (18, 36). Means and standard deviations were published in the Besong-Agbo study and therefore used to calculate study estimates. Medians and interquartile ranges were estimated from boxplots in the Yanamandra study which was divided into two subgroups (mean disease duration 6.7 years and mean disease duration 9.7 years as there was no available data for the patient group overall). Means and standard deviations were then derived from this data.

Three studies were excluded due to a lack of reported disease duration (20, 40, 41); four studies were excluded due to a lack of age and gender matching between patients and controls (19, 34, 38, 39). The Alvarez-Castelao paper did not include published significance testing of the age difference which was therefore done as part of this review. There was

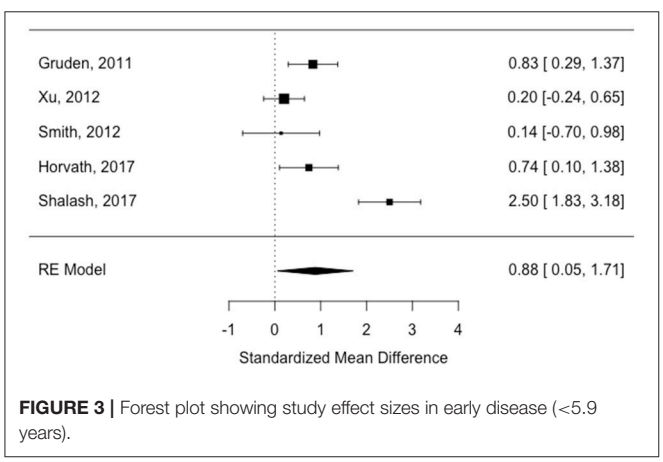


FIGURE 3 | Forest plot showing study effect sizes in early disease (<5.9 years).

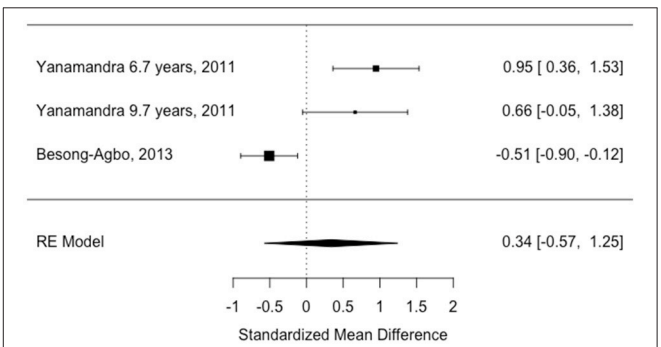


FIGURE 4 | Forest plot showing study effect sizes in later disease (>7 years).

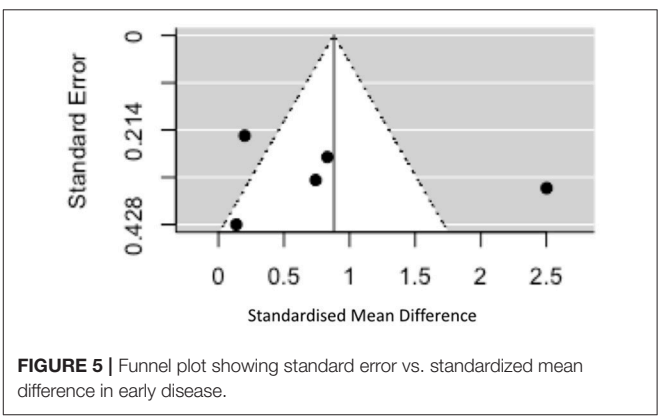


FIGURE 5 | Funnel plot showing standard error vs. standardized mean difference in early disease.

a significant difference in age between patients and controls according to a independent samples *t*-test (idiopathic PD mean 67.81, SD 9.98 and controls mean 61.4, SD 14.7), $t[136] = 2.83$, $p = 0.005$). One study was excluded as it only measured antibodies to specific epitopes of alpha synuclein rather than the entire protein (37) and one other study was excluded because outcomes were recorded as percentage positive on immunoblots (35).

The study estimates are shown in **Table 3** and the overall random effects model is shown in the forest plot in **Figure 4**. There was no overall difference between groups in this small sample (estimate = 0.34, 95% CI = −0.57–1.24, $p = 0.46$) and there was also significant heterogeneity ($I^2 = 87.67\%$).

Given the significant heterogeneity, funnel plots were generated plotting standardized mean difference on the *x* axis against standard error on the y axis for studies in the "early disease" group (there were too few in the later disease group to make interpretation of these plots meaningful). The plot is symmetrical around the effect size of 0.88 ($z = 0.20$, $p = 0.84$) but shows that two of the studies fall outside of the 95% CI of an assumed true effect (see **Figure 5**). One of the many explanations for the shape of this plot is the presence of true heterogeneity between studies (both clinical and assay related factors discussed above). If we were able to include more studies in the analysis one would expect, assuming the same true effect, that effect estimates from smaller studies would spread widely along the bottom with those from larger, more powerful studies appearing at the top (see **Figure 5**). One cannot fully discount the role played by publication bias in this context as positive findings in this field will be more likely to be written up and published than negative results particularly in the context of smaller studies.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

Whilst the available data does not suggest elevation of alpha synuclein antibodies universally across all stages of PD, it is consistent with the hypothesis that there is an increased antibody response in early disease that wanes during disease progression, which is biologically plausible. According to our meta-analysis the effect size is modest in early disease but the analysis is limited by significant study heterogeneity.

There are many caveats to this conclusion based on both the systematic review and the meta-analysis, including the limitations of the assays used, clinical heterogeneity of cohorts, the lack of any longitudinal data and poor matching of controls to patient groups, meaning that the overall quality of evidence is poor (for example, seven of nine studies in later disease did not meet the inclusion criteria for the meta-analysis making it difficult to draw any firm conclusions from this aspect of the study). Hence the value of alpha synuclein auto-antibodies as a diagnostic or prognostic biomarker remains uncertain. Further studies are needed to demonstrate a consistent, reproducible effect in early PD cases vs. controls (or indeed between different groups of PD patients), to investigate the specificity of raised antibody titres in PD vs. other alpha-synucleinopathies, and to track longitudinal changes in antibody titres and their relationship to disease onset and clinical disease progression. The possible utility of using antibody based biomarkers for identifying patients who would potentially benefit from either immune modulating or antibody based therapies is also unknown.

There is a clear need for further studies in this field and we recommend that future studies should focus on the following points:

1. Appropriate sample size with an absolute minimum of 60 in each group (based on approximate power calculations from existing studies)
2. Well-characterized clinical cohorts with appropriately matched controls using both serum and CSF if possible
3. Longitudinal assessment to measure changes in antibody levels over the course of the disease and relationship with clinical disease progression
4. Study of prodromal PD cohorts to establish whether the antibody response is truly an early feature of the disease
5. Using a robustly validated method (ideally with validation using a second method in the same samples) to measure antibodies including standardization and testing of different coating concentrations, buffers and assay temperature.
6. Study of epitope-specific antibodies and Ig subclasses to allow a fuller understanding of the adaptive immune response to PD.

AUTHOR CONTRIBUTIONS

KS designed the study, reviewed the literature, performed the meta-analysis and wrote the first draft of the manuscript. SY and AK reviewed the literature, made summary tables and critically reviewed the manuscript. MC and CW-G contributed significantly to the design of the study and critically reviewed the final manuscript.

FUNDING

KS is supported by a Ph.D. fellowship from the Wellcome Trust Grant (106565/Z/14/Z). CW-G is supported by a Clinician Scientist fellowship from the Medical Research Council. MC is supported by a Medical Research Council New Investigator Research Grant (MR/N024907/1) and an Arthritis Research UK Cure Challenge Research Grant (21777). This work was also supported by the NIHR Cambridge Biomedical Research Centre.

REFERENCES

1. Schapira AHV, Chaudhuri KR, Jenner P. Non-motor features of Parkinson disease. *Nat Rev Neurosci.* (2017) 18:435–50. doi: 10.1038/nrn.2017.62
2. Gagne JJ, Power MC. Anti-inflammatory drugs and risk of Parkinson disease: a meta-analysis. *Neurology* (2010) 74:995–1002. doi: 10.1212/WNL.0b013e3181d5a4a3
3. Racette BA., Gross A, Vouri SM, Camacho-Soto A, Willis AW, Searles Nielsen S. Immunosuppressants and risk of Parkinson disease. *Ann Clin Transl Neurol.* (2018) 5:870–5. doi: 10.1002/acn3.580
4. Williams-Gray CH, Wijeyekoon R, Yarnall AJ, Lawson RA, Breen DP, Evans JR, et al. Serum immune markers and disease progression in an incident Parkinson's disease cohort (ICICLE-PD). *Mov Disord.* (2016) 31:995–1003. doi: 10.1002/mds.26563
5. Williams-Gray CH, Wijeyekoon RS, Scott KM, Hayat S, Barker RA, Jones JL. Abnormalities of age-related T cell senescence in Parkinson's disease. *J Neuroinflammation* (2018) 15:166. doi: 10.1186/s12974-018-1206-5
6. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature* (1997) 388:839–40. doi: 10.1038/42166
7. Braak H, Müller CM, Rüb U, Ackermann H, Bratzke H, de Vos RAI, et al. Pathology associated with sporadic Parkinson's disease–where does it end? *J Neural Transm Suppl.* (2006) 89–97. doi: 10.1007/978-3-211-45295-0_15
8. Stefanis L. α-Synuclein in Parkinson's disease. *Cold Spring Harb Perspect Med.* (2012) 2:a009399. doi: 10.1101/cshperspect.a009399
9. Smith LM, Schiess MC, Coffey MP, Klaver AC, Loeffler DA. α-Synuclein and anti-α-synuclein antibodies in Parkinson's disease, atypical Parkinson syndromes, REM sleep behavior disorder, and healthy controls. *PLoS ONE* (2012) 7:e52285. doi: 10.1371/journal.pone.0052285
10. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize α-synuclein peptides. *Nature* (2017) 546:656–61. doi: 10.1038/nature22815
11. Chen S, Le WD, Xie WJ, Alexianu ME, Engelhardt JI, Siklós L, et al. Experimental destruction of substantia nigra initiated by Parkinson disease immunoglobulins. *Arch Neurol.* (1998) 55:1075–80. doi: 10.1001/archneur.55.8.1075
12. He Y, Le W-D, Appel SH. Role of Fcgamma receptors in nigral cell injury induced by Parkinson disease immunoglobulin injection into mouse substantia nigra. *Exp Neurol.* (2002) 176:322–7. doi: 10.1006/exnr.2002.7946
13. Orr CF, Rowe DB, Mizuno Y, Mori H, Halliday GM. A possible role for humoral immunity in the pathogenesis of Parkinson's disease. *Brain* (2005) 128:2665–74. doi: 10.1093/brain/awh625
14. Masliah E, Rockenstein E, Adame A, Alford M, Crews L, Hashimoto M, et al. Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. *Neuron* (2005) 46:857–68. doi: 10.1016/j.neuron.2005.05.010
15. Takamatsu Y, Ho G, Koike W, Sugama S, Takenouchi T, Waragai M, et al. Combined immunotherapy with "anti-insulin resistance" therapy as a novel therapeutic strategy against neurodegenerative diseases. *NPJ Park Dis.* (2017) 3:4. doi: 10.1038/s41531-016-0001-1
16. Dolhun R. Alpha-Synuclein: A Therapeutic Target and Potential Biomarker in Parkinson's Disease. *Pract Neurol.* (2016) 1–5.
17. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. *Blood* (2008) 112:1570–80. doi: 10.1182/blood-2008-02-078071
18. Besong-Agbo D, Wolf E, Jessen F, Oechsner M, Hametner E, Poewe W, et al. Naturally occurring α-synuclein autoantibody levels are lower in patients with Parkinson disease. *Neurology* (2013) 80:169–75. doi: 10.1212/WNL.0b013e31827b90d1
19. Brudek T, Winge K, Folke J, Christensen S, Fog K, Pakkenberg B, et al. Autoimmune antibody decline in Parkinson's disease and multiple system atrophy; a step towards immunotherapeutic strategies. *Mol Neurodegener.* (2017) 12:44. doi: 10.1186/s13024-017-0187-7
20. Heinzel S, Gold M, Deuschle C, Bernhard F, Maetzler W, Berg D, et al. Naturally occurring alpha-synuclein autoantibodies in Parkinson's disease: sources of (error) variance in biomarker assays. *PLoS ONE* (2014) 9:e114566. doi: 10.1371/journal.pone.0114566
21. Kronimus Y, Albus A, Balzer-Geldsetzer M, Straub S, Semler E, Otto M, et al. Naturally occurring autoantibodies against tau protein are reduced in Parkinson's disease dementia. *PLoS ONE* (2016) 11:e0164953. doi: 10.1371/journal.pone.0164953
22. Panda S, Ding JL. Natural antibodies bridge innate and adaptive immunity. *J Immunol.* (2015) 194:13–20. doi: 10.4049/jimmunol.1400844
23. Aw D, Silva AB, Palmer DB. Immunosenescence: emerging challenges for an ageing population. *Immunology* (2007) 120:435–46. doi: 10.1111/j.1365-2567.2007.02555.x
24. Magliozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain* (2006) 130:1089–104. doi: 10.1093/brain/awm038
25. Willcocks LC, Smith KGC, Clatworthy MR. Low-affinity Fcγ receptors, autoimmunity and infection. *Expert Rev Mol Med.* (2009) 11:1–29. doi: 10.1017/S1462399409001161
26. Gustafsson G, Eriksson F, Möller C, da Fonseca TL, Outeiro TF, Lannfelt L, et al. Cellular uptake of α-synuclein oligomer-selective antibodies is enhanced by the extracellular presence of α-synuclein and mediated via Fcγ receptors. *Cell Mol Neurobiol.* (2017) 37:121–31. doi: 10.1007/s10571-016-0352-5
27. Choi YR, Cha SH, Kang SJ, Kim JB, Jou I, Park SM. Prion-like propagation of α-synuclein is regulated by the FcγRIIB-SHP-1/2 signaling pathway in neurons. *Cell Rep.* (2018) 22:136–48. doi: 10.1016/j.celrep.2017.12.009
28. Jennewein MF, Alter G. The immunoregulatory roles of antibody glycosylation. *Trends Immunol.* (2017) 38:358–72. doi: 10.1016/j.it.2017.02.004
29. Russell AC, Šimurina M, Garcia MT, Novokmet M, Wang Y, Rudan I, et al. The N-glycosylation of immunoglobulin G as a novel biomarker of Parkinson's disease. *Glycobiology* (2017) 1–10. doi: 10.1093/glycob/cwx022
30. Xu Q, Evetts S, Hu M, Talbot K, Wade-Martins R, Davis JJ. An impedimetric assay of α-synuclein autoantibodies in early stage Parkinson's disease. *RSC Adv.* (2014) 4:58773–7. doi: 10.1039/C4RA10100F
31. Horvath I, Iashchishyn IA, Forsgren L, Morozova-Roche LA. Immunochemical detection of alpha-synuclein autoantibodies in Parkinson's disease: correlation between plasma and cerebrospinal fluid levels. *ACS Chem Neurosci.* (2017) 8:1170–6. doi: 10.1021/acschemneuro.7b00063
32. Gruden MA, Sewell RDE, Yanamandra K, Davidova TV, Kucheryanu VG, Bocharov EV, et al. Immunoprotection against toxic biomarkers is retained during Parkinson's disease progression. *J Neuroimmunol.* (2011) 233:221–7. doi: 10.1016/j.jneuroim.2010.12.001
33. Shalash A, Salama M, Makar M, Roushdy T, Elrassas HH, Mohamed W, et al. Elevated serum alpha-synuclein autoantibodies in patients with Parkinson's disease relative to Alzheimer's disease and controls. *Front Neurol.* (2017) 8:720. doi: 10.3389/fneur.2017.00720
34. Akhtar RS, Licata JP, Luk KC, Shaw LM, Trojanowski JQ, and Lee VM. Measurements of auto-antibodies to α-synuclein in the serum and cerebral spinal fluids of patients with Parkinson's disease. *J Neurochem.* (2018) 145:489–503. doi: 10.1111/jnc.14330
35. Papachroni KK, Ninkina N, Papapanagiotou A, Hadjigeorgiou GM, Xiromerisiou G, Papadimitriou A, et al. Autoantibodies to alpha-synuclein in inherited Parkinson's disease. *J Neurochem.* (2007) 101:749–56. doi: 10.1111/j.1471-4159.2006.04365
36. Yanamandra K, Gruden MA, Casaite V, Meskys R, Forsgren L, Morozova-Roche LA. α-Synuclein reactive antibodies as diagnostic biomarkers in blood sera of Parkinson's disease patients. *PLoS ONE* (2011) 6:e18513. doi: 10.1371/journal.pone.0018513
37. Caggiu E, Paulus K, Arru G, Piredda R, Sechi GP, Sechi LA. Humoral cross reactivity between α-synuclein and herpes simplex-1 epitope in Parkinson's disease, a triggering role in the disease? *J Neuroimmunol.* (2016) 291:110–4. doi: 10.1016/j.jneuroim.2016.01.007
38. Maetzler W, Apel A, Langkamp M, Deuschle C, Dilger SS, Stirnkorb JG, et al. Comparable autoantibody serum levels against amyloid- and inflammation-associated proteins in Parkinson's disease patients and controls. *PLoS ONE* (2014) 9:e88604. doi: 10.1371/journal.pone.0088604
39. Alvarez-Castelao B, Gorostidi A, Ruíz-Martínez J, López de Munain A, Castaño JG. Epitope mapping of antibodies to alpha-synuclein in LRRK2 mutation carriers, idiopathic Parkinson disease patients, and healthy controls. *Front Aging Neurosci.* (2014) 6:169. doi: 10.3389/fnagi.2014.00169

40. Bryan T, Luo X, Forsgren L, Morozova-Roche LA, Davis JJ. The robust electrochemical detection of a Parkinson's disease marker in whole blood sera. *Chem Sci.* (2012) 3:3468–73. doi: 10.1039/C2SC21221H
41. Woulfe JM, Duke R, Middeldorp JM, Stevens S, Vervoort M, Hashimoto M, et al. Absence of elevated anti-synuclein and anti-EBV latent membrane protein antibodies in PD. *Neurology* (2002) 58:1435. doi: 10.1212/WNL.58.9.1435
42. Gruden MA, Yanamandra K, Kucheryanu VG, Bocharova OR, Sherstnev VV, Morozova-Roche LA, et al. Correlation between protective immunity to α-synuclein aggregates, oxidative stress and inflammation. *Neuroimmunomodulation* (2012) 19:334–42. doi: 10.1159/000341400
43. LLC HC. *Power and Sample Size*. Hylown consulting. Available online at: http://powerandsamplesize.com/ (Accessed Jun 1, 2018).
44. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. *Introduction to Meta-Analysis*. Chichester: John Wiley & Sons, Ltd (2009).
45. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol.* (2014) 14:135. doi: 10.1186/1471-2288-14-135
46. Xu Y, Yan J, Zhou P, Li J, Gao H, Xia Y, et al. Neurotransmitter receptors and cognitive dysfunction in Alzheimer's disease and Parkinson's disease. *Prog Neurobiol.* (2012) 97:1–13. doi: 10.1016/j.pneurobio.2012.02.002
47. Candore G, Di Lorenzo G, Mansueto P, Melluso M, Fradà G, Li Vecchi M, et al. Prevalence of organ-specific and non organ-specific autoantibodies in healthy centenarians. *Mech Ageing Dev.* (1997) 94:183–90. doi: 10.1016/S0047-6374(96)01845-3

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 15 October 2018
doi: 10.3389/fneur.2018.00860

Outside in: Unraveling the Role of Neuroinflammation in the Progression of Parkinson's Disease

*Paulina Troncoso-Escudero [1,2,3,4], Alejandra Parra [1,2,3,4], Melissa Nassif [1] and Rene L. Vidal [1,2,4,5]**

[1] Faculty of Sciences, Center for Integrative Biology, Universidad Mayor, Santiago, Chile, [2] Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile, Santiago, Chile, [3] Program of Cellular and Molecular Biology, Institute of Biomedical Sciences, University of Chile, Santiago, Chile, [4] Geroscience Center for Brain Health and Metabolism (GERO), Santiago, Chile, [5] Neurounion Biomedical Foundation, Santiago, Chile

Neuroinflammation is one of the most important processes involved in the pathogenesis of Parkinson's disease (PD). The current concept of neuroinflammation comprises an inflammation process, which occurs in the central nervous system due to molecules released from brain-resident and/or blood-derived immune cells. Furthermore, the evidence of the contribution of systemic delivered molecules to the disease pathogenesis, such as the gut microbiota composition, has been increasing during the last years. Under physiological conditions, microglia and astrocytes support the well-being and well-function of the brain through diverse functions, including neurotrophic factor secretion in both intact and injured brain. On the other hand, genes that cause PD are expressed in astrocytes and microglia, shifting their neuroprotective role to a pathogenic one, contributing to disease onset and progression. In addition, growth factors are a subset of molecules that promote cellular survival, differentiation and maturation, which are critical signaling factors promoting the communication between cells, including neurons and blood-derived immune cells. We summarize the potential targeting of astrocytes and microglia and the systemic contribution of the gut microbiota in neuroinflammation process archived in PD.

Keywords: Parkinson's disease, neuroinflammation, gut microbiota, neurotrophic factor, neurodegenerative disease

OPEN ACCESS

Edited by:
Rodrigo Pacheco, Fundación Ciencia and Vida, Chile

Reviewed by:
Jiawei Zhou, Shanghai Institutes for Biological Sciences (CAS), China
Hideyuki Takeuchi, Yokohama City University, Japan

***Correspondence:**
Rene L. Vidal
rene.vidal@umayor.cl;
rene.vidal@neurounion.com

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *13 July 2018*
Accepted: *24 September 2018*
Published: *15 October 2018*

Citation:
Troncoso-Escudero P, Parra A, Nassif M and Vidal RL (2018) Outside in: Unraveling the Role of Neuroinflammation in the Progression of Parkinson's Disease. Front. Neurol. 9:860. doi: 10.3389/fneur.2018.00860

INTRODUCTION

Parkinson's disease (PD) is the second most frequent neurodegenerative disease worldwide, affecting approximately 1% of adults whose age exceeds 50 years. PD is characterized by classical symptoms including bradykinesia, rigidity, tremor and later postural instability (1). Some non-motor symptoms such as depression, dementia, anxiety olfactory dysfunction and sleep disorders are also associated with PD and may precede motor symptoms by more than a decade, involving several neurotransmitter pathways beyond dopaminergic projections (2–5).

PD is caused by dysfunctions of the nigrostriatal pathway, which involve the loss of dopaminergic neurons in the Substancia Nigra pars compacta (SNpc) and the following loss of the dopamine circuit in the striatum (6). The onset of the cellular neuropathology of PD appears decades before the onset of the motor symptoms. Around 30% of the dopaminergic neurons are lost when the first symptoms of PD occur (5, 7). However, the cause of PD remains unknown. In

less than 10% of PD cases, the disease is associated to genetic mutations (familiar Parkinson's), such as the mutation of the alpha-synuclein (*SCNA*) gene that encodes for the alpha-synuclein (α-syn) protein (8, 9). In the other 90% of the cases, the causes of the disease are unknown (idiopathic Parkinson's). Although mutations in the *SCNA* gene are not the most frequent mutations that cause familial PD, idiopathic cases of PD also show overexpression of the wild-type α-syn (10–13). The misfolded protein α-syn is present in presynaptic cells as cytoplasmic inclusions named Lewy bodies, which are a biological hallmark of PD (14). Also, vast evidence shows a toxic effect of misfolded α-syn, particularly in dopaminergic neurons (15–18).

Despite the advance in our understanding about PD pathogenesis in the last decades, several details are still missing, hampering the rational development of therapies interfering with the processes underlying neuronal degeneration. Current therapeutic approaches provide symptomatic relief but fail to stop or slow down the course of the disease. In addition, the diagnosis of PD relies primarily on the clinical assessment of motor symptoms that become detectable only when a large part of the nigral dopaminergic neurons have already degenerated. Thus, unravel novel effective therapies that can slow down or reverse disease progression, specifically dopaminergic neurodegeneration, are urgently required.

In the ceaseless search for new therapies for PD, neurotrophic factors (NFs) have demonstrated to exert neuroprotection in animal models of PD, and are also under different phases of clinical trials as a treatment for PD patients (19–23). NFs are molecules produced mainly by neurons, which mediate synaptic plasticity, neuroprotection, neurorestoration and maintenance of neuronal functions. Moreover, after neural injury, some NFs facilitate tissue regeneration via their anti-inflammatory, anti-apoptotic, re-myelination and axon regeneration properties as well as by promoting adult stem cells to contribute to tissue repair (24). These molecules are also secreted by glial cells like microglia and astrocytes, which activate survival signaling pathways in neurons. Bidirectional communication between glial cells and neurons is critical to maintaining brain homeostasis. A loss in this communication occurs in the brain of PD patients, which cause the development of neuroinflammation observed in PD. In the following sections, we summarize the role of neuroinflammation in PD progression, as well as the implication of NFs from the central nervous system to this process.

INFLAMMATION IN PD: CAUSE OR CONSEQUENCE OF THE DISEASE?

The central nervous system (CNS) has long been considered a privileged immune tissue due to (a) the absence of dendritic cells, (b) the presence of an immunosuppressant microenvironment in the brain parenchyma under physiological conditions and (c) the presence of the blood-brain barrier (BBB) that separates the brain parenchyma and the peripheral immune system (25). Despite this, the CNS can initiate an immune response against insults such as pathogens or endogenous danger signals. This response is initiated by microglia, the resident tissue macrophages of the CNS, which can be activated by various stimuli (26). All the inflammatory reaction must be terminated to maintain the tissue structure and homeostasis, including the elimination of pathogens, dead cells or other cellular debris, and tissue restoration. If the insult persists or the mechanisms involved in the termination of the inflammation are inadequate, chronic inflammation can arise (27). Furthermore, inflammation can also occur in response to secreted molecules from neurons under degeneration, a condition called neuroinflammation, a crucial player in neurodegenerative diseases (28). If neuroinflammation is a cause or consequence of neurodegenerative diseases, it remains unknown.

Neuroinflammation: Hallmarks in Parkinson's Disease

In PD, as well as in other neurodegenerative diseases, there is dysfunction and loss of specific neurons in a specific region of the brain. Several mechanisms have been described as triggers of neurodegeneration that are common among neurodegenerative diseases, such as protein aggregation due to protein misfolding or no degradation, formation of reactive oxygen species (ROS) and reactive nitrogen species, which causes oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, dysfunction of neurotrophic factor, chronic neuroinflammation, among others (29). Inflammation is a highly regulated self-defensive mechanism against pathogenic stimuli or injury, generated by an activated immune system that seeks to protect the host organism and get rid of the pathogenic stimuli to promote a healing process (30). The immune system can be classified as innate or adaptive. The innate immune system is the first line of defense against insults, creating a rapid but short-term response. Mononuclear phagocytes (dendritic cells, macrophages, microglia, and monocytes), natural killer cells and neutrophils are responsible for triggering this response. On the other hand, the adaptive immune system generates a pathogen-specific, non-rapid and long-lasting response, in which T- and B-lymphocytes are responsible (30).

Under physiological conditions, inflammatory molecules are not expressed, given that the expression of their genes is suppressed. However, under a stress condition such an infection or necrosis signals, non-self-molecules are recognized by pattern recognition receptors such as Toll-like receptors (TLRs) and nucleotide oligomerization domain receptors (NLRs) (31). Stranger molecules that are specific to bacteria or virus are collectively known as pathogen-associated molecular patterns (PAMPs), whereas endogenous molecules that came from the host cells are known as damage-associated molecular patterns (DAMPs), and include chromatin, adenosine, ATP, heat-shock proteins, β-amyloid, tau, α-syn, among many others (32, 33). Upon the presence of PAMPs and/or DAMPs, TLRs and NLRs are activated in microglia and astrocytes, which secrete NFs that promote tissue repair and regrowth (32).

Studies in experimental animal models of PD have shown that neuroinflammation plays a key role in disease progression (34). It has been demonstrated the interplay between neuroinflammation

and other proposed pathogenic mechanisms of PD, such as mitochondrial dysfunction and oxidative stress (35), the participation of protein products of parkinsonian genes, such as α-syn, Parkin and DJ-1 in innate immune responses (36–39). An immunoregulatory role of dopamine has also been described during neuroinflammation (40). Inflammatory responses may also contribute to the intrinsic vulnerability of nigral dopaminergic neurons, explained by several factors such as dopaminergic metabolism, high iron content, differential transcriptional profile, different calcium channel expression and a low antioxidant defense system (6).

Since many studies have demonstrated the complex neuroimmune interactions occurring both at homeostatic and pathological conditions in the CNS, the notion of the CNS as a tissue immune privilege has been refined (25). For example, in PD the integrity of the BBB is compromised, and the components of the innate immune system are activated, allowing the recruitment and activation of the adaptive arm of the immune system [(25); **Figure 1**]. While the role of the immune system is not clear and has not been extensively studied in the etiology of PD, it is well known that the immune system is critical for the progression of the disease (41–43). The initial activation of the innate immune system may have protective roles, but when these innate defense mechanisms become dysregulated and maladaptive, it leads to disease progression.

Role of Microglia in Neuroinflammation

Microglia are the immune cells of the CNS, constituting 5-10% of total brain cells and the 20% of the glial cell population of the brain (26). Microglia are in a quiescent state in the absence of any stimulus, which is achieved by the immunosuppressant microenvironment present in the CNS, where immunoregulatory molecules are expressed and/or released by healthy neurons (44). Among these immunoregulatory molecules are CX3CL1, CD200, CD22, CD47, CD95 and neuronal cell adhesion molecule (NCAM), and the receptor for these molecules are almost exclusively expressed in microglia, evidencing the important role of neuron-microglia interactions in the regulation of neuroinflammation (45). This communication between neurons and glia are of special importance because microglia have the potential to damage brain tissue, which has limited capacity for regeneration and repair. Microglia are the responsible cells for mediating the innate immune response in the brain through antigen-presenting and effector functions such as phagocytosis (26). Besides its immunological functions, microglia play other roles that are beneficial for neurons, such as NFs release, removal of toxic substances, neuronal repair, synaptic remodeling and synaptic pruning (26). Microglia localize in specific structures in the human brain, including the medulla oblongata, pons, basal ganglia, and SNpc (46). In addition to microglia, which are in the brain parenchyma, the CNS contains other types of mononuclear phagocytes, which are meningeal macrophages, choroid plexus macrophages, epipexus cells and perivascular macrophages (26).

Microglial activation can be triggered in response to a variety of environmental challenges, a process that involves morphological changes and upregulation of a spectrum of intracellular molecules and surface antigens. Microglia can be activated by bacterial and viral molecules, as well as with disease-related proteins (amyloid β and α-syn) and soluble molecules released by dying neurons (47). When microglia are activated, transformation and proliferative events take place to form reactive microglia (48), which are distinguished classically by two distinct phenotypes: M1 phenotype (pro-inflammatory) and the M2 phenotype (anti-inflammatory) (**Figure 1**). During transformation, the resting ramified phenotype of microglia changes into an intermediate hyper-ramified morphology, which consists of a large soma and an amoeboid morphology to initiate phagocytosis (49). Together with this changes, microglia upregulate cell surface markers of inflammation, including MHC class I and II, and cytokine and chemokine receptors (48). Acute or chronic activation of microglia can occur, depending on the type and duration of the external stimuli or activated factor (50). Short-term activation of microglia is generally believed to be neuroprotective, while chronic activation has been implicated as a potential mechanism in neurodegenerative diseases (51). The mechanism that underlies the change from a neuroprotective to an autoaggressive effector microglia, which causes neurodegeneration, has long been elusive, but recent findings are shedding light on the mechanisms involved in this change (52). In their work, Du et al. describe that the deficiency of Kir6.1-containing ATP-sensitive potassium (Kir6.1/K-ATP) channel favors the M1 phenotype which exacerbates the inflammatory response and dopaminergic neuronal loss in a MPTP model through the activation of p38 MAPK–NF-kβ pathway and the increasing the ratio of M1/M2 markers in SNpc (52).

Several studies have shown that dying neurons release soluble mediators, such as α-syn, matrix metalloproteinase-3 (MMP-3), neuromelanin, ATP and m-calpain, which cause the secretion of toxic mediators by microglia that are lethal to neighboring cells and stressed neurons (53). Pro-inflammatory mediators released by activated astrocytes act on their cognate receptors expressed in microglia and further increase the microglial activation by rendering them to an overactivated state (**Figure 1**). Moreover, misfolded proteins induce the activation of microglia toward an M1 phenotype in *in vitro* and *in vivo* models of PD (54–56). For example, chronic administration of MPTP leads to a reduction of CD206, a molecular marker of M2 microglia, suggesting downregulation of this phenotype activation in this model of PD (57). Cell culture experiments have demonstrated that dopaminergic neurons incubated with conditioned medium (CM) from M1 microglia increase the death of these neurons, whereas a mixture of CM from both M1 and M2 microglia reverses the neurotoxicity elicited by the M1-CM (58).

Role of Astrocytes in Neuroinflammation

Astrocytes are the most abundant glial cells in the CNS and are five times the number of neurons (59). Astrocytes have numerous extensions that connect directly with neurons and blood vessels of the BBB to form a functional network via gap junctions, which is called the neurovascular unit (NVU) (60). Given this phenomenon, astrocytes participate in the maintenance and permeability of the BBB and are key regulators of neuronal activity and cerebral blood flow (**Figure 1**).

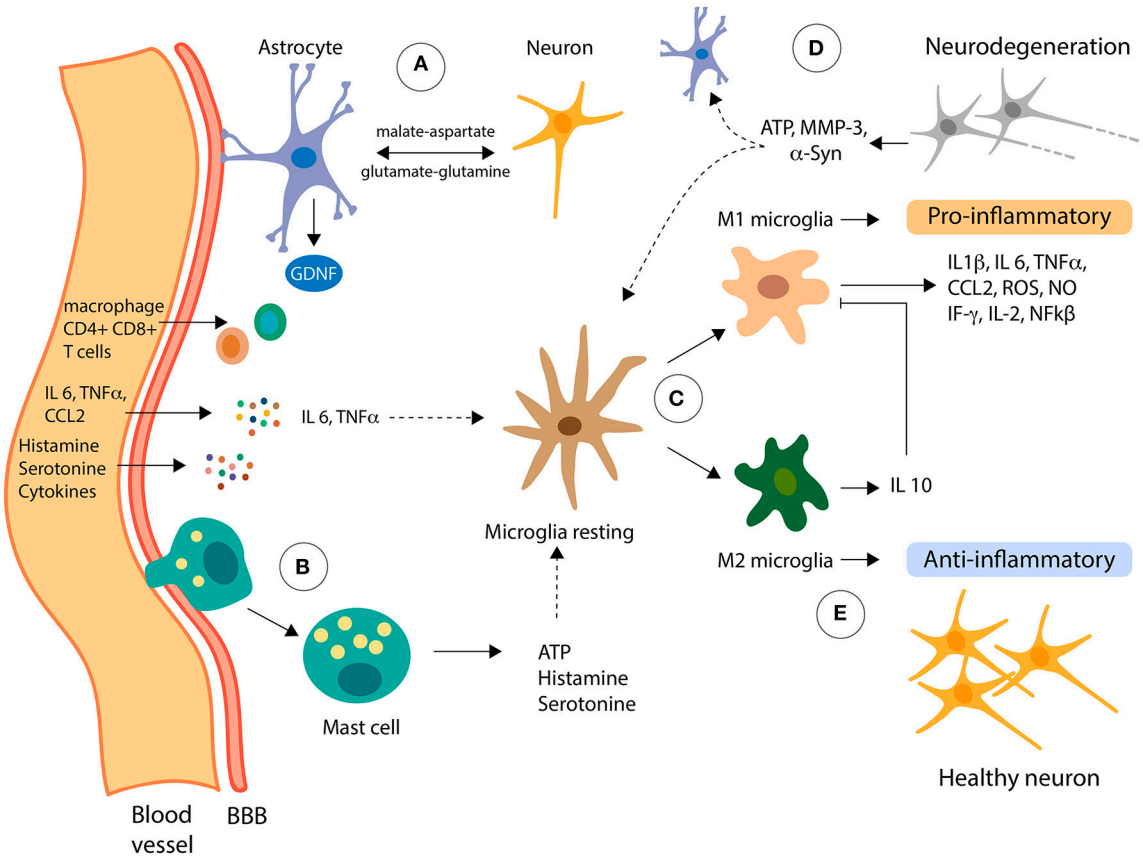


FIGURE 1 | Role of non-neuronal cells in neuroinflammation. Pro-inflammatory molecules can reach the Central Nervous System (CNS) from the periphery going across the blood-brain barrier (BBB). **(A)** Astrocytes, the most abundant cells in the CNS, are functionally connected with the BBB, receiving signals from the periphery and from inside the CNS. Also, astrocytes metabolically support neurons via the shuttle systems malate-aspartate and glutamate-glutamine. **(B)** Mast cells can infiltrate the CNS, inducing changes in the microglia by the delivery of proinflammatory effectors, including ATP, which stimulates transcription of proinflammatory cytokines through PKC. **(C)** Resting microglia can be activated in two classical phenotypes, M1 and M2, depending on the effector signals from its microenvironment. **(D)** In presence of LPS and IFNγ, microglia cells polarize to M1 phenotype and secrete the proinflammatory cytokines which contribute to the dysfunction of dopaminergic neurons (neurodegeneration). Moreover, neuron failed can release α-Syn, ATP, MMP-3, among other molecules, in a cross-talk signaling with astrocytes and microglia, increasing the toxic-loop of neuroinflammation. **(E)** Contrary, IL 4 and IL 13 induce activation of microglia to M2 phenotype that downregulates M1 functions by release of IL 10 cytokines contributing to anti-inflammatory of CNS.

Metabotropic glutamate receptors in the membrane of astrocytes release arachidonic acid metabolites, which causes an increase of calcium levels as a result of activating the inositol triphosphate (IP3) pathway at the astrocyte end feet. If this activation occurs near a blood vessel, it results in the dilation of blood vessels (61). Moreover, astrocytes give biochemical and nutritional support to neurons, extracellular ion balance, and repair of scarring of the brain and spinal cord tissues (60). Astrocytes also produce and secrete NFs, including the glial-derived neurotrophic factor (GDNF) (**Figure 1**), which is especially important for the development and survival of dopaminergic neurons (62). Also, via specific shuttle systems, such as the malate-aspartate and glutamate-glutamine shuttle systems, astrocytes transport nutrients and metabolites to neurons (63, 64) (**Figure 1**).

In vitro and *in vivo* studies show the vital role that astrocytes play in the neuroinflammatory processes in PD. Astrocytes, like microglia, respond to inflammatory stimulations such as IL-1β, LPS, and TNF-α, producing more proinflammatory cytokines (65, 66). Reactive astrogliosis has been reported in different PD animal models, and importantly, in the affected brain regions of PD patients, indicating a possible involvement of astrocytes in the immune response in PD. Treatment of astrocytes primary culture with α-Syn increase the expression levels of IL-6 and TNF-α (67) and overexpression of mutant α-Syn in astrocytes causes astrogliosis, microglial activation and degeneration of dopaminergic neurons and motor neurons in mice (68).

As a consequence of brain diseases such as infection and neurodegeneration, and brain injuries like trauma and ischemia, astrocytes become reactive, a process known as reactive astrogliosis. Reactive astrocytes experience changes in gene expression (69) and in morphology (70), which leads to the formation of a glial scar in the site of injury. For a long time, it has been debated if reactive astrogliosis is

beneficial or detrimental for the recovery of the injured CNS. Several studies have demonstrated that reactive astrocytes can play both roles (71–74), which raises the question of whether there are different populations of reactive astrocytes activated by different stimulus, and which can in turn, have different functions. This question was addressed by Zamanian group who elegantly and meticulously demonstrated that reactive astrocytes gene expression differs depending on the brain injury model used: focal ischemic stroke produced by transient occlusion of the cerebral middle artery (MCAO) or neuroinflammation induced by systemic LPS injection (75). The authors obtained pure reactive astrocytes from both models, and identified a total of 263 reactive glial genes, 150 of which were preferentially expressed by MCAO reactive astrocytes, 57 were preferentially expressed by LPS reactive astrocytes, and 56 genes were shared, including GFAP and vimentin, classical markers of reactive astrocytes (75). Importantly, the authors identify a new set of genes induced under both brain injury studies, which can now be used as new markers for reactive astrogliosis. Finally, the authors hypothesized, based on their results and the findings by Sofroniew group (69), that MCAO reactive astrocytes are protective, given the expression of high levels of neurotrophic factors and cytokines, which may help repair and rebuild damaged synapses (76). On the contrary, the authors postulated that LPS reactive astrocytes may be harmful, due to the upregulation of genes for the classical complement cascade, which is thought to cause synapse loss and neuronal loss in neurodegenerative diseases (77). Therefore, reactive astrocytes induced by neuroinflammation are termed A1 reactive astrocytes, and those induced by ischemia are termed A2 reactive astrocytes, in analogy to the M1/M2 macroglia nomenclature.

Recent findings have uncovered how A1 reactive astrocytes are activated under LPS stimulation. Starting with the premise that A1 reactive astrocytes are induced by LPS and that LPS is an activator of microglia through TLR4. Liddelow group demonstrated that LPS-activated microglia secreted IL-1a, TNF and complement component 1, subcomponent q (C1q) (78) which changes astrocytes toward an A1 phenotype nearly identical to the phenotype found by LPS induction *in vivo* (75). Furthermore, astrocytes activation is completely dependent on the presence of microglia, because LPS treatment of animals lacking microglia, or pure astrocytes *in vitro* cultures, fails to achieve the activation of astrocytes. Also, the authors showed that LPS treatment of a triple knockout mouse for IL-1a, TNF and C1q causes no A1 reactive astrocytes, further confirming that these molecules are necessary and sufficient to activate astrocytes toward an A1 phenotype. Another important finding is that A1 reactive astrocytes show loss of normal astrocytes function and a gain of toxic functions. A1 reactive astrocytes lose the ability to form functional synapses *in vitro* and loose phagocytosis activity both *in vitro* and *in vivo*. On the other hand, neurons cultured with A1 astrocytes show increased percentage of cell death, which account for a loss of the protective role of astrocytes on neuronal survival. The finding that IL-1a and TNF secreted by activated microglia is able to activate astrocytes toward an A1 phenotype, which causes neuronal loss and possibly contributes to neurodegeneration seen in different brain diseases, opens the possibility for the study using antibodies against both molecules, which are already approved by the FDA for the treatment of others maladies.

Several studies have demonstrated that LPS injection is sufficient to cause dopaminergic neurons degeneration that simulates PD (79). Human dopaminergic neurons cultured together with A1 astrocytes show a 25% increase in cell death, which is attributed to the activation of apoptosis in these neurons (78). Furthermore, analysis of human post-mortem tissues shows an important amount of A1 reactive astrocytes in the brain areas affected in different neurodegenerative diseases, such Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and multiple sclerosis. The presence of these cells, together with activated microglia, could be accounting for the selective neurodegeneration seen in these diseases, which is accompanied with neuroinflammation, which furthers enhances neuronal loss.

Increasing evidence suggests that disruption of astrocyte biology is involved in dopaminergic neuron degeneration in PD. As mentioned before, monogenic mutations in 17 genes have been identified in the development of the disease, and many of these genes are expressed in astrocytes at levels comparable to, and even higher than, in neurons (80). Recently, proteins encoded by eight of these genes have been shown to have a role in astrocyte biology [reviewed in (81)].

Overall, it is clear that microglia and astrocytes play important roles in the maintenance of the CNS homeostasis, and these neuroprotective roles are lost under brain injury. Also, it is clear that both glial cells are constantly interacting, where activated microglia can activate astrocytes toward a neurotoxic phenotype (78). For many years, therapies aimed to slow down or stop CNS diseases have neurons as the principal objective. With these new findings, it becomes increasingly interesting to target microglia and astrocytes, given that the prevention of glia activation has a positive outcome in neuronal survival. Recently, it has been demonstrated that the GLP1R agonist NLY01 protects against dopaminergic neurons loss in two models of PD (82). Interestingly, this protection was due to the prevention of astrocytes A1 activation by activated microglia, not by a direct effect of NLY01 on neurons. Targeting glial cells may be the next step in the development of therapies for the treatment of different CNS maladies.

In vivo Evidence of Neuroinflammation in PD

The most widely used preclinical model of PD is based in the administration of 6-hydroxydopamine (6-OHDA), a selective catecholaminergic neurotoxin that upon injection into the striatum, causes retrograde degeneration of the nigrostriatal dopaminergic circuit (83). Because 6-OHDA cannot cross the BBB, it has to be injected into the brain by stereotaxic surgery. The neurotoxic effect of 6-OHDA is due to the oxidative stress triggered by ROS production (83). Cellular

and molecular evidence of inflammation is observed in the 6-OHDA-induced animal model of PD. Intranigral 6-OHDA injection in mice generates acute astrogliosis and microgliosis in the nigrostriatal system, which is accompanied by degeneration of nigral dopaminergic cell bodies (84). Reactive microglia precede astrogliosis, demonstrated using GFAP immunohistochemistry, and these active microglia upregulates the expression of the gene coding for TNF-α, a pro-inflammatory molecule known to drive the progression of neurodegeneration (85). A recent study demonstrated that unilateral injection of 6-OHDA in the striatum of mice induces an increase in the levels of the pro-inflammatory cytokines TNF-α, IF-γ, IL-1β, IL-2, IL-6, and NF-kβ, in parallel with a decrease in the levels of the anti-inflammatory cytokine IL-10 in the striatum of these mice (86). Importantly, this was reversed when mice were treated with Chrysin, a natural flavonoid known to have neuroprotective effects (87, 88).

Another potent neurotoxin used to mimic PD in a wide range of organisms including non-human primates, guinea pigs, mice, dogs and cats is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (83). Because MPTP is a lipophilic molecule, it rapidly crosses the BBB and is converted by astrocytes into the toxic metabolite MPP^+ (89). MPP^+ is released by striatal and nigral astrocytes and is taken up by dopaminergic neurons through the dopamine receptor. Inside neurons, MPP^+ induces neurotoxicity by inhibiting the mitochondrial electron transport chain complex I, resulting in ATP depletion and increased oxidative stress (90). Inflammation markers have also been evaluated in the MPTP model. Mice treated with MPTP have a significant increase in the pro-inflammatory cytokines IL-1, TNF-α, and IL-6 mRNA levels both in the SNpc and striatum (91). Additionally, both mRNA and protein levels of the receptors for these three cytokines were increased in the SNpc of MPTP-treated mice, although this increase was not observed in the striatum of these mice (91). Rai et al. have demonstrated an increase of the inflammation-related molecules GFAP, iNOS, ICAM, and TNF-α in the SNpc of mice treated with MPTP (92). This increase was reverted when MPTP-treated mice were administered with *Macuna pruriens* (Mp) seed, which have been previously demonstrated to be neuroprotective in a mouse model of MPTP (93). In MPTP-treated mice, Mp also inhibited the activation of NF-kβ and promoted the activation of pAkt1, preventing the apoptosis of dopaminergic neurons (92). A decrease in the mRNA levels of the pro-inflammatory cytokines IL-1β and TNF-α was also seen in mice treated with MPTP and naringenin, another natural product (94). The use of plant-derived natural products to treat PD have been extensively studied, but its effects in neuroinflammation have to be further investigated. Blocking microglial activation by minocycline also protects the nigrostriatal dopaminergic pathway against MPTP model, suggesting that microglial activation plays a crucial role in the pathogenesis of PD (95). The treatment with paeonol decreased MPTP/p-induced oxidative stress and neuroinflammation through the increase of the brain-derived neurotrophic factor (BDNF), one of the most critical NFs in the physiology of CNS (96).

THE ANTI-INFLAMMATORY CONTRIBUTION OF NEUROTROPHIC FACTORS IN PRECLINICAL MODELS OF PD

As described above, an inflammatory response in the CNS mediated by activated microglia and astrocytes contributes to neuronal degeneration in PD. For this reason, the incessant search for therapeutic alternatives with neuroprotective and anti-inflammatory effects is a relevant consideration in research. Several NFs have been considered as an alternative for the treatment of neurodegenerative pathologies such as PD. However, only subsets of NFs have shown to be neuroprotective and neurorestorative in pre-clinical animal models of PD, highlighting among them the glial cell line-derived neurotrophic factor (GDNF), cerebral dopamine neurotrophic factor (CDNF) and vascular endothelial growth factor (VEGF).

GDNF belongs to the TGF-β superfamily of NFs (97), which was first purified from a rat glioma cell line (B49) medium and identified to promote the survival of embryonic dopaminergic neurons in culture and increase dopamine uptake (62). GDNF is considered the most potent neuroprotective agent tested in cellular and animal models of PD (62, 98). The neuroprotective and neurorestorative effect of GDNF has been shown in numerous neurotoxic PD models including mouse, rat (99–101) and no-human primates (102). In a recent study, Chen et al. demonstrated that GDNF derived from macrophages diminished the loss of dopaminergic neurons and improved motor symptoms in a mouse model of PD (103). In this study, bone marrow hematopoietic stem cells were transduced with a lentiviral vector expressing macrophage promoter-driven GDNF and transplanted into the MitoPark mice (103, 104). These genetically modified macrophages were able to infiltrate the midbrain of MitoPark mice, but not control littermates. This was accompanied with GDNF secretion, an improvement in motor and non-motor symptoms and a reduction of dopaminergic neurons loss in the SNpc and its axonal terminals in the striatum (103). The mechanisms that are involved in the neuroprotective effect of GDNF are still unknown. *In vitro* and *in vivo* assays suggest that GDNF can protect from neurodegeneration thought the inhibition of neuroinflammation. Using an inflammatory model of PD based on LPS treatment, it has been demonstrated that GDNF delivery by mesenchymal stem cells provides localized neuroprotection of dopaminergic neurons (105). Additionally, in a neurotoxic model of PD, the intracerebral administration of GDNF by microspheres reduced the TNF-α levels, an important pro-inflammatory cytokine involved in neuronal death (98, 106). Using an *in vitro* assay, it was demonstrated that astrocyte-derived GDNF is an inhibitor of the activation of microglia. In this experiment, midbrain microglia cultures were incubated with astrocytes conditioned media that reduced microglial activation, however, when the medium was neutralized with GDNF antibody the effect was abrogated (107). However, in genetic PD models generated by overexpression of mutant or wild-type α-syn into the midbrain, it has been reported that

GDNF fails to exert robust neuroprotection (21, 108). According to the later, overexpression of α-syn would cause an alteration in GDNF signaling and a decrease in its neurotrophic effects in dopaminergic neurons (108). Nevertheless, it has been recently demonstrated that α-syn accumulation does not block the expression of GDNF in patients and preclinical models of PD (109). More recently, clinical application of GDNF (clinical trial phase 2) has failed to demonstrate a significant positive effect (110). New approaches for GDNF administration are being tested in animals models (103). For example, the intrastriatal infusion of a variant of GDNF, which was designed to promote a better tissue distribution and to enhance its chemical stability, increased the dopamine turnover and protected midbrain dopaminergic neurons function in 6-OHDA-lesioned rats (110). In mice model of PD, intravenous-injected GDNF-transfected macrophages can cross the blood-brain barrier, reduce microglial activation and the loss of dopaminergic neurons in the SNpc, to improve the motor dysfunction observed in 6-OHDA-lesioned mice (111).

CDNF is an unconventional neurotrophic factor that presents a robust effect in reducing dopaminergic neurons loss (112, 113), along with the ability to promote neurorestoration in a neurotoxic model of PD (114). Recently, it has been reported the neurorestorative effect of CDNF by its single administration or as co-treatment with subthalamic nucleus deep brain stimulation (STN-DBS), which might be explained by the interaction of electric stimulation and NFs (115). CDNF can regulate ER stress and exhibit anti-inflammatory properties that promote neuronal survival (24, 116). In *in vitro* assay, the overexpression of CDNF reduces the cytokine secretion by astrocytes under ER-stress (117). Moreover, overexpression of human CDNF into the rat SNpc reduces de levels of glial markers and IL-6 in pharmacological model of PD (118). In LPS-treated microglial cultures it has been showed that CDNF treatment has anti-inflammatory effects, attenuating the production of pro-inflammatory cytokines and cytotoxicity by inhibition of JNK signaling (119). Additionally, *in vitro* assays revealed that CDNF protects against toxicity induced by α-syn oligomers in primary cultures of mesencephalic neurons (120).

The members of the VEGF family are key regulators of vascular biology, modulating angiogenesis, vasculogenesis, and maintaining vasculature during embryogenesis and in adults (121, 122). However, the neuroprotective role of this growth factor family for the treatment of neurodegenerative diseases has also been studied (122). VEGF-A is the most studied of the VEGF family, highlighting its angiogenic role (123). Additionally, it has been reported it neuroprotective effect both *in vitro* and *in vivo* in PD models (124–126). However, an increase in the levels of VEGF-A contributes to the development of L-DOPA-induced dyskinesia (LID), which has been associated with its angiogenic effect (127, 128). On the other hand, VEGF-B has emerged as an alternative for the treatment of neurodegenerative diseases, given its anti-apoptotic effects in different cell types, by suppressing the expression of genes related to apoptosis and its angiogenic effect (129, 130). *In vitro* assays have shown that exogenous administration of VEGF-B reduces neuronal loss in a PD model generated by the addition of Rotenone (131), a toxin used as a pesticide that reproduces the pathological characteristics of PD in cellular and animal models (132, 133). VEGF-B has also shown a neuroprotective effect in an animal model of PD, which is accompanied by an improvement in motor symptoms, but with no changes regarding dopaminergic neuronal loss (101, 134). However, its use in combination with other neurotrophic factors such as GDNF in nanoparticles, has shown a synergistic effect, favoring neuroprotection and neurorestoration processes (135). Although the use of VEGF has shown clear neuroprotective effects in PD pharmacological models, clinical trials using this therapeutic target have not yet been carried out.

Neurotrophic Factors as Therapeutic Targets for PD

Currently, the success of the NFs application in clinical trials has been modest. This could be explained considering that PD preclinical models present a partial lesion (early stage PD) unlike the patients condition, which receive this alternative treatment after the onset of motor symptoms (late-stage PD), with a 80% decrease in dopamine content in the striatum, 50–80% loss of striatal dopaminergic innervations and a 30% loss of dopaminergic neuron in SNpc (136–138). Another challenge in the clinical use of NFs for the treatment of neurodegenerative diseases is their inability cross the BBB. Direct needle or catheter delivery has a limited clinical use. Non-invasive drug delivery for early-stage patients throughout the diseased regions may be critical to improve patient response.

EXTRACEREBRAL ORIGIN OF PARKINSON'S DISEASE: DOES ALPHA-SYNUCLEIN REACH THE BRAIN FROM PERIPHERAL ORGANS?

Role of the Peripheral System in the Pathogenesis of Parkinson's Disease

As mentioned before, the CNS was considered for many years to be immune-privileged, being excluded from the effects of immune-molecules released by inflammatory cells from systemic reactions. However, the process of "neuroinflammation" is a consequence of the complex signaling between systemic and CNS cells. The players are the inflammatory substances that arrive to the CNS from the periphery, the infiltrated mast cells or T-lymphocytes after the delivery of chemoattraction substances or rupture of the BBB and the sustained activation of glial cells in the CNS (microglia, astrocytes and even oligodendrocytes) [reviewed in (139, 140)]. Astrocytes, the cells that are functionally connected with the BBB by surrounding the endothelial cells in the brain, can be activated directly by molecules from the circulation, and secrete pro-inflammatory molecules and NFs (**Figure 1**). In PD, for instance, the loss of dopaminergic neurons was shown to be accompanied by activated microglia and T-cells infiltration (141). Indeed, it was recently shown a pathway in which activated microglia release cytokines that stimulated MHC-I expression in dopaminergic neurons, which finally are attacked by T-CD8$^+$ cells (142). Another player in the connection between the peripheral system and the CNS is the gut microbiota, with surprising incidence in the development of

neurodegenerative diseases. In the next section, we will review the recent findings about the gut microbiome alteration on PD and its implication en the progression of this disease.

Impact of the Enteric Nervous System in PD

Although studies of neurodegenerative diseases have historically been performed in brain tissue, the influence of peripheral organs emerges as an important niche to study and understand the origin and/or progression of diseases affecting the CNS, giving the direct contact between gut neurons and the CNS (143). Recently, there has been an increase in the number of studies describing close bidirectional communication between the gut and the brain in neuropsychiatric disorders such as anxiety, depression, autism, among others (144–146). Additionally, gastrointestinal physiology is influenced by signals generated both locally in the intestine and from the brain. Neurotransmitters, immune signaling, hormones, growth factors (GFs) and neuropeptides produced in the intestine can, in turn, affect the brain (147, 148).

From clinical studies, it has been described that PD patients present intestinal inflammation (149) and gastrointestinal anomalies, such as constipation, which often precedes for many years the motor deficits characteristic of this disease (150, 151). The Braak hypothesis suggests that the aberrant accumulation of the α-syn protein starts in the intestine and spreads through the vagus nerve to the brain like a prion disease (151). This idea is supported by surprising physiopathological evidence that describes the presence of protein inclusions of α-syn in the Enteric Nervous System (ENS) and in the glossopharyngeal and vagal nerves in early stages of PD (152). Additionally, vagotomized individuals have a lower risk of developing PD (153).

The concept regards to the extracerebral origin of PD is becoming increasingly relevant. For example, the injection of α-syn fibrils into the intestinal tissue of healthy rodents is sufficient to induce pathology in the vagus nerve and brainstem (154). Due to the immediate proximity of the ENS to feces, the gut microbiota and the metabolic products of the microbiota, are presented as potential candidates that could initiate a process that eventually results in the formation of α-syn protein aggregates in the ENS and that this spreads to the brain.

Alterations of the Intestinal Microbiota in Parkinson's Disease

Microorganisms permanently colonize the human body in virtually all environmentally exposed surfaces, where the most significant percentage of these reside within the gastrointestinal tract (155). Intestinal bacteria control the differentiation and function of immune cells in the intestine and the brain (156–158). The impact of gut microbiota on neurological development and neurodegenerative diseases emerges as an innovative alternative to understand the molecular processes that govern these complex biological processes (146).

Similarly, perturbations of the bidirectional network known as the "intestinal microbiota-brain axis" can affect brain physiology (159) and have been linked to numerous diseases (160). Alterations in the gut microbiota can affect both brain neurochemistry (altered levels of neurotransmitters, their receptors and various neurotrophic factors), as well as behavior (161–164). Recently, evidence has described the role of gut microbiota in the regulation of the expression levels of synaptic components, such as the 5-hydroxytryptamine (5-HT1A, serotonin) receptor, BDNF and the subunit 2 of the NMDA receptor (NR2A) (161, 162, 165). In addition, it can alter the enteric and circulating production of serotonin in mice (166), which in turn generates anxiety, hyperactivity and cognitive alterations (147, 162, 167, 168). These alterations of the gut microbiota, known as dysbiosis, have been observed in patients diagnosed with various neurological diseases (145).

In PD, it has been determined clear differences in the gut microbiome from PD patients and healthy people (169–171). Recent studies have described that alterations in the intestinal microbiota promote the pathology of α-syn, neuroinflammation and the motor symptoms of PD in a mouse model of this disease [**Figure 2**; (172)]. In this work, the authors performed fecal transplantation from PD patients to healthy mice, which generates a significant deterioration in the motor function of these animals (172). Surprisingly, they also identified specific metabolites of the microbiota present in the feces of patients that are sufficient to promote the PD symptoms. The gut microbiota is exclusively responsible for several metabolic functions, including the production of short chain fatty acids and vitamins (SCFAs), amino acid synthesis (AAs), biotransformation of bile acids, hydrolysis and fermentation of non-digestible substrates (173). In addition, the beneficial functions of gut microbiota include (i) homeostasis and development of immune system cells, (ii) homeostasis of epithelial cells, (iii) enteric nerves regulation and (iv) angiogenesis, food digestion and fat metabolism induction (160, 174). Interesting, mouse models of gut injury have shown that gut microbiota can penetrate injured areas and induce macrophages to migrate to the damaged sites, triggering the expression of specific GFs to recover tissue homeostasis (175).

The molecules that are produced by the microbiota can cross the epithelial barriers to cause systemic effects at distant sites of the organism. Moreover, the fermentation of dietary fiber by gut microbiota produce SCFAs, such as acetate, propionate and butyrate, which are absorbed by epithelial cells and used as an energy source (176). For instance, an association has been shown between the abundance of specific gut bacteria and PD development (177), where patients with PD have a decrease in the number of intestinal bacteria that are capable of producing SCFA. The SCFA can modulate the activity of the ENS and therefore increase gastrointestinal motility (178). Therefore, altered concentrations of SCFA could contribute to decreased gastrointestinal motility in patients with PD (171). The presence of metabolic biomarkers in the blood is especially useful for the diagnosis of diseases because they can reveal the physiological state of both the host and its microbiota (179, 180). Such biomarkers may correspond to the final products of the metabolism of microorganisms, providing mechanistic explanations for the association between changes in the microbiota and the development of the disease (171).

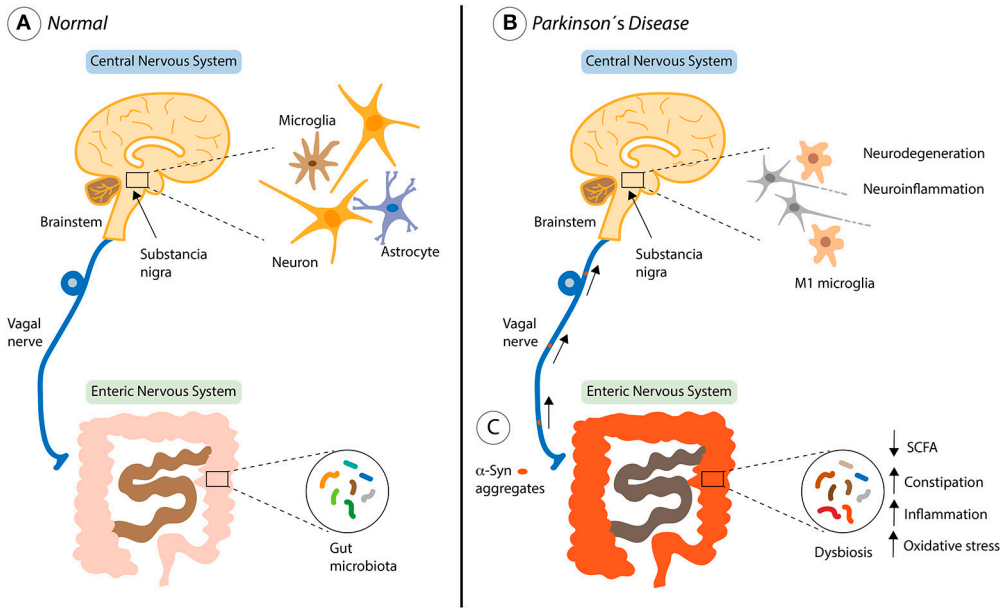


FIGURE 2 | Contribution of the gut microbiota in Parkinson's disease progression. **(A)** The healthy bi-directional communication between the brain and the gut, highlighting the involvement of the vagus nerve. **(B)** The brain-gut axis and non-motor symptoms of Parkinson's disease (PD) including both central and gastrointestinal dysfunction. **(C)** Environmental factors such as the gut microbiota, might begin a pathological process within enteric nerve cell plexus, causing mucosal inflammation and oxidative stress, thereby initiating alpha-synuclein (α-syn) accumulation. The vagal nerve might provide a footpath for the spread of α-Syn from the Enteric Nervous System to the brain through the brainstem, Substancia nigra, basal forebrain and finally the cortical areas where is activated the neurodegeneration and neuroinflammation process described in PD.

The appearance of gastrointestinal symptoms, the dysbiosis present in patients with PD, and the studies that show that the microbiota can affect brain functions, bring us to the conclusion that intestinal bacteria can regulate the progression of motor deficits and the pathophysiology observed in patients with PD.

The Olfactory Bulb as a Possible Initial Site of α-syn Spreading

Although the trigger of PD pathogenesis is unclear, several hypotheses were outlined in the last years. One of them proposes that the beginning of the neurodegeneration in PD occurs in the olfactory bulb, in a called olfactory vector hypothesis (150, 181–183). Around 90% of the PD patients present a loss of the sense of smell in early stages of PD (184, 185), and this olfactory dysfunction is one of the first symptoms during disease progression, years before motor symptoms appear. The olfactory sensory neurons are bipolar neurons, in which dendrites are exposed to the exterior environment, and the axons project directly to the brain. It means that our olfactory mucosa is exposed for decades to the air components, and might be the via of entrance to the CNS of environmental contaminants, such as xenobiotics, viruses and metals. As an example of environmental contaminants is paraquat, a herbicide known to cause parkinsonism. In a recent study, a group of researchers showed for the first time significant structural differences between the olfactory bulb from PD patients and age-matched controls. The total volume occupied by the functional units of the olfactory bulb (glomeruli) in PD is around half that in controls (186). Remarkable, the researchers establish an indirect relationship between the volume of the olfactory bulb and the phosphorylation of α-syn: smaller the olfactory bulb, increased phospho-α-syn was found (186). Since the modification of α-syn found in the olfactory bulb neurons might predict the brain α-syn pathology in the CNS (187), and the olfactory dysfunction is presented years before the first motor symptoms of PD, this study supports the olfactory vector hypothesis, including the modifications of α-syn and its prion-like spread to the CNS (183).

The olfactory bulb could be the start or the intermediate point before arriving at the brain of other responses as well, such as the inflammatory response. For instance, a study conducted in rats showed that intravenously delivered LPS provoked a robust inflammatory response in the olfactory bulb, with the presence of peripherical immune cells and increased levels of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-10 (188). However, if it is enough to follow to the Snigra reaction, is unknow.

CONCLUDING REMARKS

Researchers continuously revisit the role of inflammation in the progression of neurodegenerative diseases. It was already known that during aging there is a decline in physiologic protective processes, vital in maintaining the body homeostasis. However, some pathways are persistently activated, causing a chronic state of cellular stress, such as the chronic inflammation. This persistent inflammation state, or non-resolved inflammation,

can contribute directly or indirectly to the etiology of the most common of neurodegenerative diseases, including PD. In fact, immune cells are in general more reactive during aging, in a state called "primed," being more susceptive to secondary inflammatory stimulus. This is the case of aged-microglia, which behavior is overexcitable and resistant to regulation, causing an amplified immune reaction in the CNS (189, 190). Also, the activation of microglia is influenced by astrocytes and neurons, in a cell-to-cell interaction, direct or indirectly through cytokines and neurotransmitters. The initial activation of the innate immune system may have protective roles, but when these innate defense mechanisms become dysregulated and maladaptive, it leads to disease progression. A possible scenario could be that chronic circulating inflammatory cytokines derived from glial cells, from blood-derived immune cells and/or from an imbalanced microbiota in the progress of aging can result in a non-autonomous degeneration of dopaminergic neurons in PD. For instance, necroptosis, a different mechanism of cell death, is triggered by an excessive inflammatory response, especially due to TNF-α signaling. In a recent study, it was demonstrated that the dopaminergic cell death induced by treatment with 6-OHDA *in vitro* (191) or MPTP *in vivo* (192) was blocked by the pre-treatment with an inhibitor of necroptosis (necrostatin-1). Moreover, a recently published paper links the lack of *PINK1* in glial cells with enhanced inflammation-induced neuronal death in an *in vivo* model of PD (39). The understanding of the contribution of these cells in the etiology and/or progression of PD will support the design of more effective lines of treatment for this devasting pathology.

AUTHOR CONTRIBUTIONS

PT-E, AP, and MN drafted the contents of this review, and together with RV wrote the text. All authors contributed equally to the critical reading of the final manuscript, including text and figures.

ACKNOWLEDGMENTS

This work was funded by FONDECYT 1150608, Millennium Institute No. P09-015-F, and FONDAP 15150012 (RV), FONDECYT 11160288 and FDP-UM (MN), CONICYT Ph.D. fellowship 21160843 (PT-E) and CONICYT Ph.D. fellowship 21181114 (AP).

REFERENCES

1. Jankovic J. Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry* (2008) 79:368–76. doi: 10.1136/jnnp.2007.131045
2. Langston JW. The Parkinson's complex: parkinsonism is just the tip of the iceberg. *Ann Neurol* (2006) 59:591–6. doi: 10.1002/ana.20834
3. Muzerengi S, Contrafatto D, Chaudhuri KR. Non-motor symptoms: identification and management. *Parkinsonism Relat Disord* (2007) 13 (Suppl 3):S450–6. doi: 10.1016/S1353-8020(08)70048-8
4. Salat-Foix D, Suchowersky O. The management of gastrointestinal symptoms in Parkinson's disease. *Expert Rev Neurother* (2012) 12:239–48. doi: 10.1586/ern.11.192
5. Peall KJ, Kuiper A, de Koning TJ, Tijssen MA. Non-motor symptoms in genetically defined dystonia: Homogenous groups require systematic assessment. *Parkinsonism Relat Disord* (2015) 21:1031–40. doi: 10.1016/j.parkreldis.2015.07.003
6. Surmeier DJ, Obeso JA, Halliday GM. Selective neuronal vulnerability in Parkinson disease. *Nat Rev Neurosci.* (2017) 18:101–13. doi: 10.1038/nrn.2016.178
7. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain* (1991) 114 (Pt 5):2283–301.
8. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* (1997) 276:2045–7.
9. Corti O, Lesage S, Brice A. What genetics tells us about the causes and mechanisms of Parkinson's disease. *Physiol Rev* (2011) 91:1161–218. doi: 10.1152/physrev.00022.2010
10. Golbe LI, Di Iorio G, Bonavita V, Miller DC, Duvoisin RC. A large kindred with autosomal dominant Parkinson's disease. *Ann Neurol.* (1990) 27:276–82. doi: 10.1002/ana.410270309
11. Michell AW, Barker RA, Raha SK, Raha-Chowdhury R. A case of late onset sporadic Parkinson's disease with an A53T mutation in alpha-synuclein. *J Neurol Neurosurg Psychiatry* (2005) 76:596–7. doi: 10.1136/jnnp.2004.046425
12. Chelban V, Vichayanrat E, Schottlaende L, Iodice V, Houlden H. Autonomic dysfunction in genetic forms of synucleinopathies. *Mov Disord* (2018) 33:359–371. doi: 10.1002/mds.27343
13. Shprecher DR, Adler CH, Zhang N, Hentz JG, Serrano GE, Dugger BN, et al. Predicting alpha-synuclein pathology by REM sleep behavior disorder diagnosis. *Parkinsonism Relat Disord* (2018) doi: 10.1016/j.parkreldis.2018.05.020. [Epub ahead of print].
14. Schulz-Schaeffer WJ. The synaptic pathology of alpha-synuclein aggregation in dementia with Lewy bodies, Parkinson's disease and Parkinson's disease dementia. *Acta Neuropathol.* (2010) 120:131–43. doi: 10.1007/s00401-010-0711-0
15. Petrucelli L, O'Farrell C, Lockhart PJ, Baptista M, Kehoe K, Vink L, et al. Parkin protects against the toxicity associated with mutant alpha-synuclein: proteasome dysfunction selectively affects catecholaminergic neurons. *Neuron* (2002) 36:1007–19. doi: 10.1016/S0896-6273(02)01125-X
16. Maries E, Dass B, Collier TJ, Kordower JH, Steece-Collier K. The role of alpha-synuclein in Parkinson's disease: insights from animal models. *Nat Rev Neurosci.* (2003) 4:727–38. doi: 10.1038/nrn1199
17. Deng H, Yuan L. Genetic variants and animal models in SNCA and Parkinson disease. *Ageing Res Rev.* (2014) 15:161–76. doi: 10.1016/j.arr.2014.04.002
18. Volpicelli-Daley LA, Kirik D, Stoyka LE, Standaert DG, Harms AS. How can rAAV-alpha-synuclein and the fibril alpha-synuclein models advance our understanding of Parkinson's disease? *J Neurochem.* (2016) 139 (Suppl. 1):131–55. doi: 10.1111/jnc.13627
19. Kalia LV, Kalia SK, Lang AE. Disease-modifying strategies for Parkinson's disease. *Mov Disord* (2015) 30:1442–50. doi: 10.1002/mds.26354
20. Kordower JH, Bjorklund A. Trophic factor gene therapy for Parkinson's disease. *Mov Disord* (2013) 28:96–109. doi: 10.1002/mds.25344
21. Lo Bianco C, Schneider BL, Bauer M, Sajadi A, Brice A, Iwatsubo T, et al. Lentiviral vector delivery of parkin prevents dopaminergic degeneration in an alpha-synuclein rat model of Parkinson's disease. *Proc Natl Acad Sci USA.* (2004) 101:17510–5. doi: 10.1073/pnas.0405313101
22. Qudrat A, Unni N. Theoretical Approaches to Lentiviral Mediated Neurotrophin Delivery in Potential Treatments of Parkinson's Disease. *Yale J Biol Med.* (2016) 89:215–25.
23. Domanskyi A, Saarma M, Airavaara M. Prospects of Neurotrophic Factors for Parkinson's Disease: Comparison of Protein and Gene Therapy. *Hum Gene Ther.* (2015) 26:550–9. doi: 10.1089/hum.2015.065
24. Nasrolahi A, Mahmoudi J, Akbarzadeh A, Karimipour M, Sadigh-Eteghad S, Salehi R, et al. Neurotrophic factors hold promise for the future of

Parkinson's disease treatment: is there a light at the end of the tunnel? *Rev Neurosci.* (2018) 29:475–89. doi: 10.1515/revneuro-2017-0040
25. Louveau A, Harris TH, Kipnis J. Revisiting the Mechanisms of CNS Immune Privilege. *Trends Immunol.* (2015) 36:569–77. doi: 10.1016/j.it.2015.08.006
26. Li Q, Barres BA. Microglia and macrophages in brain homeostasis and disease. *Nat Rev Immunol.* (2018) 18:225–42. doi: 10.1038/nri.2017.125
27. Sochocka M, Diniz BS, Leszek J. Inflammatory Response in the CNS: Friend or Foe? *Mol Neurobiol.* (2017) 54:8071–89. doi: 10.1007/s12035-016-0297-1
28. DiSabato DJ, Quan N, Godbout JP. Neuroinflammation: the devil is in the details. *J Neurochem.* (2016) 139 (Suppl. 2):136–53. doi: 10.1111/jnc.13607
29. Jellinger KA. Basic mechanisms of neurodegeneration: a critical update. *J Cell Mol Med.* (2010) 14:457–87. doi: 10.1111/j.1582-4934.2010.01010.x
30. Kannarkat GT, Boss JM, Tansey MG. The role of innate and adaptive immunity in Parkinson's disease. *J Parkinsons Dis.* (2013) 3:493–514. doi: 10.3233/JPD-130250
31. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell* (2010) 140:918–34. doi: 10.1016/j.cell.2010.02.016
32. Thundyil J, Lim KL. DAMPs and neurodegeneration. *Ageing Res Rev.* (2015) 24:17–28. doi: 10.1016/j.arr.2014.11.003
33. Richards RI, Robertson SA, O'Keefe LV, Fornarino D, Scott A, Lardelli M, et al. The enemy within: innate surveillance-mediated cell death, the common mechanism of neurodegenerative disease. *Front Neurosci.* (2016) 10:193. doi: 10.3389/fnins.2016.00193
34. Cebrian C, Loike JD, Sulzer D. Neuroinflammation in Parkinson's disease animal models: a cell stress response or a step in neurodegeneration? *Curr Top Behav Neurosci.* (2015) 22:237–70. doi: 10.1007/7854_2014_356
35. Hunter RL, Dragicevic N, Seifert K, Choi DY, Liu M, Kim HC, et al. Inflammation induces mitochondrial dysfunction and dopaminergic neurodegeneration in the nigrostriatal system. *J Neurochem.* (2007) 100:1375–86. doi: 10.1111/j.1471-4159.2006.04327.x
36. Roodveldt C, Labrador-Garrido A, Gonzalez-Rey E, Fernandez-Montesinos R, Caro M, Lachaud CC, et al. Glial innate immunity generated by non-aggregated alpha-synuclein in mouse: differences between wild-type and Parkinson's disease-linked mutants. *PLoS ONE* (2010) 5:e13481. doi: 10.1371/journal.pone.0013481
37. Couch Y, Alvarez-Erviti L, Sibson NR, Wood MJ, Anthony DC. The acute inflammatory response to intranigral alpha-synuclein differs significantly from intranigral lipopolysaccharide and is exacerbated by peripheral inflammation. *J Neuroinflamm.* (2011) 8:166. doi: 10.1186/1742-2094-8-166
38. Chien CH, Lee MJ, Liou HC, Liou HH, Fu WM. Microglia-Derived Cytokines/Chemokines Are Involved in the Enhancement of LPS-Induced Loss of Nigrostriatal Dopaminergic Neurons in DJ-1 Knockout Mice. *PLoS ONE* (2016) 11:e0151569. doi: 10.1371/journal.pone.0151569
39. Sun L, Shen R, Agnihotri SK, Chen Y, Huang Z, Bueler H. Lack of PINK1 alters glia innate immune responses and enhances inflammation-induced, nitric oxide-mediated neuron death. *Sci Rep.* (2018) 8:383. doi: 10.1038/s41598-017-18786-w
40. Sarkar C, Basu B, Chakroborty D, Dasgupta PS, Basu S. The immunoregulatory role of dopamine: an update. *Brain Behav Immun.* (2010) 24:525–8. doi: 10.1016/j.bbi.2009.10.015
41. Chao Y, Wong SC, Tan EK. Evidence of inflammatory system involvement in Parkinson's disease. *Biomed Res Int.* (2014) 2014:308654. doi: 10.1155/2014/308654
42. Su X, Federoff HJ. Immune responses in Parkinson's disease: interplay between central and peripheral immune systems. *Biomed Res Int.* (2014) 2014:275178. doi: 10.1155/2014/275178
43. Moehle MS, West AB. M1 and M2 immune activation in Parkinson's Disease: Foe and ally? *Neuroscience* (2015) 302:59–73. doi: 10.1016/j.neuroscience.2014.11.018
44. Ransohoff RM. How neuroinflammation contributes to neurodegeneration. *Science* (2016) 353:777–83. doi: 10.1126/science.aag2590
45. Le W, Wu J, Tang Y. Protective Microglia and Their Regulation in Parkinson's Disease. *Front Mol Neurosci.* (2016) 9:89. doi: 10.3389/fnmol.2016.00089
46. Mittelbronn M, Dietz K, Schluesener HJ, Meyermann R. Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. *Acta Neuropathol.* (2001) 101:249–55. doi: 10.1007/s004010000284
47. von Bernhardi R, Eugenin-von Bernhardi L, Eugenin J. Microglial cell dysregulation in brain aging and neurodegeneration. *Front Aging Neurosci.* (2015) 7:124. doi: 10.3389/fnagi.2015.00124
48. Subramaniam SR, Federoff HJ. Targeting Microglial Activation States as a Therapeutic Avenue in Parkinson's Disease. *Front Aging Neurosci.* (2017) 9:176. doi: 10.3389/fnagi.2017.00176
49. Graeber MB, Streit WJ. Microglia: biology and pathology. *Acta Neuropathol.* (2010) 119:89–105. doi: 10.1007/s00401-009-0622-0
50. Cherry JD, Olschowka JA, O'Banion MK. Neuroinflammation and M2 microglia: the good, the bad, the inflamed. *J Neuroinflammation* (2014) 11:98. doi: 10.1186/1742-2094-11-98
51. Perry VH, Holmes C. Microglial priming in neurodegenerative disease. *Nat Rev Neurol.* (2014) 10:217–24. doi: 10.1038/nrneurol.2014.38
52. Du RH, Sun HB, Hu ZL, Lu M, Ding JH, Hu G. Kir6.1/K-ATP channel modulates microglia phenotypes: implication in Parkinson's disease. *Cell Death Dis.* (2018) 9:404. doi: 10.1038/s41419-018-0437-9
53. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci.* (2007) 8:57–69. doi: 10.1038/nrn2038
54. Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, et al. Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J.* (2005) 19:533–42. doi: 10.1096/fj.04-2751com
55. Austin SA, Floden AM, Murphy EJ, Combs CK. Alpha-synuclein expression modulates microglial activation phenotype. *J Neurosci.* (2006) 26:10558–63. doi: 10.1523/JNEUROSCI.1799-06.2006
56. Hoenen C, Gustin A, Birck C, Kirchmeyer M, Beaume N, Felten P, et al. Alpha-Synuclein Proteins Promote Pro-Inflammatory Cascades in Microglia: Stronger Effects of the A53T Mutant. *PLoS ONE* (2016) 11:e0162717. doi: 10.1371/journal.pone.0162717
57. Pisanu A, Lecca D, Mulas G, Wardas J, Simbula G, Spiga S, et al. Dynamic changes in pro- and anti-inflammatory cytokines in microglia after PPAR-gamma agonist neuroprotective treatment in the MPTPp mouse model of progressive Parkinson's disease. *Neurobiol Dis.* (2014) 71:280–91. doi: 10.1016/j.nbd.2014.08.011
58. Tang Y, Li T, Li J, Yang J, Liu H, Zhang XJ, et al. Jmjd3 is essential for the epigenetic modulation of microglia phenotypes in the immune pathogenesis of Parkinson's disease. *Cell Death Differ* (2014) 21:369–80. doi: 10.1038/cdd.2013.159
59. Freeman MR. Specification and morphogenesis of astrocytes. *Science* (2010) 330:774–8. doi: 10.1126/science.1190928
60. Sofroniew MV Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol.* (2010) 119:7–35. doi: 10.1007/s00401-009-0619-8
61. Takano T, Tian GF, Peng W, Lou N, Libionka W, Han X, et al. Astrocyte-mediated control of cerebral blood flow. *Nat Neurosci.* (2006) 9:260–7. doi: 10.1038/nn1623
62. Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* (1993) 260:1130–2.
63. Mangia S, Simpson IA, Vannucci SJ, Carruthers A. The *in vivo* neuron-to-astrocyte lactate shuttle in human brain: evidence from modeling of measured lactate levels during visual stimulation. *J Neurochem.* (2009) 109 (Suppl. 1):55–62. doi: 10.1111/j.1471-4159.2009.06003.x
64. Falkowska A, Gutowska I, Goschorska M, Nowacki P, Chlubek D, Baranowska-Bosiacka I. Energy metabolism of the brain, including the cooperation between astrocytes and neurons, especially in the context of glycogen metabolism. *Int J Mol Sci.* (2015) 16:25959–81. doi: 10.3390/ijms161125939
65. Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, et al. A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. *Cell* (2009) 137:47–59. doi: 10.1016/j.cell.2009.01.038
66. Tanaka T, Kai S, Matsuyama T, Adachi T, Fukuda K, Hirota K. General anesthetics inhibit LPS-induced IL-1beta expression in glial cells. *PLoS ONE* (2013) 8:e82930. doi: 10.1371/journal.pone.0082930

67. Fellner L, Irschick R, Schanda K, Reindl M, Klimaschewski L, Poewe W, et al. Toll-like receptor 4 is required for alpha-synuclein dependent activation of microglia and astroglia. *Glia* (2013) 61:349–60. doi: 10.1002/glia.22437
68. Gu XL, Long CX, Sun L, Xie C, Lin X, Cai H. Astrocytic expression of Parkinson's disease-related A53T alpha-synuclein causes neurodegeneration in mice. *Mol Brain* (2010) 3:12. doi: 10.1186/1756-6606-3-12
69. Sofroniew MV. Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci.* (2009) 32:638–47. doi: 10.1016/j.tins.2009.08.002
70. Wilhelmsson U, Bushong EA, Price DL, Smarr BL, Phung V, Terada M, et al. Redefining the concept of reactive astrocytes as cells that remain within their unique domains upon reaction to injury. *Proc Natl Acad Sci USA*. (2006) 103:17513–8. doi: 10.1073/pnas.0602841103
71. Karimi-Abdolrezaee S, Billakanti R. Reactive astrogliosis after spinal cord injury-beneficial and detrimental effects. *Mol Neurobiol.* (2012) 46:251–64. doi: 10.1007/s12035-012-8287-4
72. Li X, Yang B, Xiao Z, Zhao Y, Han S, Yin Y, et al. Comparison of subacute and chronic scar tissues after complete spinal cord transection. *Exp Neurol.* (2018) 306:132–137. doi: 10.1016/j.expneurol.2018.05.008
73. Teh DBL, Prasad A, Jiang W, Ariffin MZ, Khanna S, Belorkar A, et al. Transcriptome Analysis Reveals Neuroprotective aspects of Human Reactive Astrocytes induced by Interleukin 1beta. *Sci Rep*. (2017) 7:13988. doi: 10.1038/s41598-017-13174-w
74. Robel S, Buckingham SC, Boni JL, Campbell SL, Danbolt NC, Riedemann T, et al. Reactive astrogliosis causes the development of spontaneous seizures. *J Neurosci.* (2015) 35:3330–45. doi: 10.1523/JNEUROSCI.1574-14.2015
75. Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, et al., Genomic analysis of reactive astrogliosis. *J Neurosci.* (2012) 32:6391–410. doi: 10.1523/JNEUROSCI.6221-11.2012
76. Eroglu C, Allen NJ, Susman MW, O'Rourke NA, Park CY, Ozkan E, et al. Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell* (2009) 139:380–92. doi: 10.1016/j.cell.2009.09.025
77. Stephan AH, Barres BA, Stevens B. The complement system: an unexpected role in synaptic pruning during development and disease. *Annu Rev Neurosci.* (2012) 35:369–89. doi: 10.1146/annurev-neuro-061010-113810
78. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* (2017) 541:481–487. doi: 10.1038/nature21029
79. Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, et al. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia* (2007) 55:453–62. doi: 10.1002/glia.20467
80. Zhang Y, Sloan SA, Clarke LE, Caneda C, Plaza CA, Blumenthal PD, et al. Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. *Neuron* (2016) 89:37–53. doi: 10.1016/j.neuron.2015.11.013
81. Booth HDE, Hirst WD, Wade-Martins R. The Role of Astrocyte Dysfunction in Parkinson's Disease Pathogenesis. *Trends Neurosci.* (2017) 40:358–70. doi: 10.1016/j.tins.2017.04.001
82. Yun SP, Kam TI, Panicker N, Kim S, Oh Y, Park JS, et al. Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease. *Nat Med.* (2018) 24:931–8. doi: 10.1038/s41591-018-0051-5
83. Tieu K. A guide to neurotoxic animal models of Parkinson's disease. *Cold Spring Harb Perspect Med.* (2011) 1:a009316. doi: 10.1101/cshperspect.a009316
84. Fricke IB, Viel T, Worlitzer MM, Collmann FM, Vrachimis A, Faust A, et al. 6-hydroxydopamine-induced Parkinson's disease-like degeneration generates acute microgliosis and astrogliosis in the nigrostriatal system but no bioluminescence imaging-detectable alteration in adult neurogenesis. *Eur J Neurosci.* (2016) 43:1352–65. doi: 10.1111/ejn.13232
85. Haas SJ, Zhou X, Machado V, Wree A, Krieglstein K, Spittau B. Expression of Tgfbeta1 and Inflammatory Markers in the 6-hydroxydopamine Mouse Model of Parkinson's Disease. *Front Mol Neurosci*. (2016) 9:7. doi: 10.3389/fnmol.2016.00007
86. Goes, ATR, Jesse CR, Antunes MS, Lobo Ladd FV, Lobo Ladd ABA, Luchese C, et al. Protective role of chrysin on 6-hydroxydopamine-induced neurodegeneration a mouse model of Parkinson's disease: Involvement of neuroinflammation and neurotrophins. *Chem Biol Interact.* (2018) 279:111–20. doi: 10.1016/j.cbi.2017.10.019
87. He XL, Wang YH, Bi MG, Du GH. Chrysin improves cognitive deficits and brain damage induced by chronic cerebral hypoperfusion in rats. *Eur J Pharmacol.* (2012) 680:41–8. doi: 10.1016/j.ejphar.2012.01.025
88. Souza LC, Antunes MS, Filho CB, Del Fabbro L, de Gomes MG, Goes AT, et al. Flavonoid Chrysin prevents age-related cognitive decline via attenuation of oxidative stress and modulation of BDNF levels in aged mouse brain. *Pharmacol Biochem Behav.* (2015) 134:22–30. doi: 10.1016/j.pbb.2015.04.010
89. Ransom BR, Kunis DM, Irwin I, Langston JW. Astrocytes convert the parkinsonism inducing neurotoxin, MPTP, to its active metabolite, MPP+. *Neurosci Lett*. (1987) 75:323–8.
90. Nicklas WJ, Vyas I, Heikkila RE. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *Life Sci.* (1985) 36:2503–8.
91. Lofrumento DD, Saponaro C, Cianciulli A, De Nuccio F, Mitolo V, Nicolardi G, et al. MPTP-induced neuroinflammation increases the expression of pro-inflammatory cytokines and their receptors in mouse brain. *Neuroimmunomodulation* (2011) 18:79–88. doi: 10.1159/000320027
92. Rai SN, Birla H, Singh SS, Zahra W, Patil RR, Jadhav JP, et al. Mucuna pruriens Protects against MPTP Intoxicated Neuroinflammation in Parkinson's Disease through NF-kappaB/pAKT Signaling Pathways. *Front Aging Neurosci.* (2017) 9:421. doi: 10.3389/fnagi.2017.00421
93. Yadav SK, Prakash J, Chouhan S, Westfall S, Verma M, Singh TD, et al. Comparison of the neuroprotective potential of Mucuna pruriens seed extract with estrogen in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mice model. *Neurochem Int.* (2014) 65:1–13. doi: 10.1016/j.neuint.2013.12.001
94. Mani S, Sekar S, Barathidasan R, Manivasagam T, Thenmozhi AJ, Sevanan M, et al. Naringenin Decreases *alpha*-Synuclein Expression and Neuroinflammation in MPTP-Induced Parkinson's Disease Model in Mice. *Neurotox Res.* (2018) 33:656–70. doi: 10.1007/s12640-018-9869-3
95. Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, et al. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J Neurosci* (2002) 22:1763–71. doi: 10.1523/JNEUROSCI.22-05-01763.2002
96. Shi X, Chen YH, Liu H, Qu HD. Therapeutic effects of paeonol on methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid-induced Parkinson's disease in mice. *Mol Med Rep.* (2016) 14:2397–404. doi: 10.3892/mmr.2016.5573
97. Airaksinen MS, Saarma M. The GDNF family: signalling, biological functions and therapeutic value. *Nat Rev Neurosci.* (2002) 3:383–94. doi: 10.1038/nrn812
98. Tomac A, Lindqvist E, Lin LF, Ogren SO, Young D, Hoffer BJ, et al. Protection and repair of the nigrostriatal dopaminergic system by GDNF *in vivo*. *Nature* (1995) 373:335–9. doi: 10.1038/373335a0
99. Hoffer BJ, Hoffman A, Bowenkamp K, Huettl P, Hudson J, Martin D, et al. Glial cell line-derived neurotrophic factor reverses toxin-induced injury to midbrain dopaminergic neurons *in vivo*. *Neurosci Lett.* (1994) 182:107–11.
100. Rosenblad C, Kirik D, Devaux B, Moffat B, Phillips HS, Bjorklund A. Protection and regeneration of nigral dopaminergic neurons by neurturin or GDNF in a partial lesion model of Parkinson's disease after administration into the striatum or the lateral ventricle. *Eur J Neurosci.* (1999) 11:1554–66.
101. Yue X, Hariri DJ, Caballero B, Zhang S, Bartlett MJ, Kaut O, et al. Comparative study of the neurotrophic effects elicited by VEGF-B and GDNF in preclinical *in vivo* models of Parkinson's disease. *Neuroscience* (2014) 258:385–400. doi: 10.1016/j.neuroscience.2013.11.038
102. Garbayo E, Ansorena E, Lana H, Carmona-Abellan MD, Marcilla I, Lanciego JL, et al. Brain delivery of microencapsulated GDNF induces functional and structural recovery in parkinsonian monkeys. *Biomaterials* (2016) 110:11–23. doi: 10.1016/j.biomaterials.2016.09.015
103. Chen C, Li X, Ge G, Liu J, Biju KC, Laing SD, et al. GDNF-expressing macrophages mitigate loss of dopamine neurons and improve Parkinsonian symptoms in MitoPark mice. *Sci Rep.* (2018) 8:5460. doi: 10.1038/s41598-018-23795-4
104. Biju K, Zhou Q, Li G, Imam SZ, Roberts JL, Morgan WW, et al. Macrophage-mediated GDNF delivery protects against dopaminergic neurodegeneration:

a therapeutic strategy for Parkinson's disease. *Mol Ther.* (2010) 18:1536–44. doi: 10.1038/mt.2010.107
105. Hoban DB, Howard L, Dowd E. GDNF-secreting mesenchymal stem cells provide localized neuroprotection in an inflammation-driven rat model of Parkinson's disease. *Neuroscience* (2015) 303:402–11. doi: 10.1016/j.neuroscience.2015.07.014
106. Wang X, Cui G, Yang X, Zhang Z, Shi H, Zu J, et al. Intracerebral administration of ultrasound-induced dissolution of lipid-coated GDNF microbubbles provides neuroprotection in a rat model of Parkinson's disease. *Brain Res Bull.* (2014) 103:60–5. doi: 10.1016/j.brainresbull.2014.02.006
107. Rocha SM, Cristovao AC, Campos FL, Fonseca CP, Baltazar G. Astrocyte-derived GDNF is a potent inhibitor of microglial activation. *Neurobiol Dis.* (2012) 47:407–15. doi: 10.1016/j.nbd.2012.04.014
108. Decressac M, Ulusoy A, Mattsson B, Georgievska B, Romero-Ramos M, Kirik D, et al. GDNF fails to exert neuroprotection in a rat alpha-synuclein model of Parkinson's disease. *Brain* (2011) 134:2302–11. doi: 10.1093/brain/awr149
109. Su X, Fischer DL, Li X, Bankiewicz K, Sortwell CE, Federoff HJ. Alpha-Synuclein mRNA Is Not Increased in Sporadic PD and alpha-synuclein accumulation does not block GDNF Signaling in Parkinson's disease and disease models. *Mol Ther.* (2017) 25:2231–5. doi: 10.1016/j.ymthe.2017.04.018
110. Grondin R, Littrell OM, Zhang Z, Ai Y, Huettl P, Pomerleau F, et al. GDNF revisited: a novel mammalian cell-derived variant form of GDNF increases dopamine turnover and improves brain biodistribution. *Neuropharmacology* (2018) doi: 10.1016/j.neuropharm.2018.05.014
111. Zhao Y, Haney MJ, Gupta R, Bohnsack JP, He Z, Kabanov AV, et al. GDNF-transfected macrophages produce potent neuroprotective effects in Parkinson's disease mouse model. *PLoS ONE* (2014) 9:e106867. doi: 10.1371/journal.pone.0106867
112. Petrova P, Raibekas A, Pevsner J, Vigo N, Anafi M, Moore MK, et al. MANF: a new mesencephalic, astrocyte-derived neurotrophic factor with selectivity for dopaminergic neurons. *J Mol Neurosci* (2003) 20:173–88. doi: 10.1385/JMN:20:2:173
113. Lindholm P, Voutilainen MH, Lauren J, Peranen J, Leppanen VM, Andressoo JO, et al. Novel neurotrophic factor CDNF protects and rescues midbrain dopamine neurons *in vivo*. *Nature* (2007) 448:73–7. doi: 10.1038/nature05957
114. Airavaara M, Voutilainen MH, Wang Y, Hoffer B. Neurorestoration. *Parkinsonism Relat Disord.* (2012) 18 (Suppl. 1):S143–6. doi: 10.1016/S1353-8020(11)70045-1
115. Huotarinen A, Penttinen AM, Back S, Voutilainen MH, Julku U, Piepponen TP, et al. Combination of CDNF and deep brain stimulation decreases neurological deficits in late-stage model Parkinson's Disease. *Neuroscience* (2018) 374:250–63. doi: 10.1016/j.neuroscience.2018.01.052
116. Voutilainen MH, Arumae U, Airavaara M, Saarma M. Therapeutic potential of the endoplasmic reticulum located and secreted CDNF/MANF family of neurotrophic factors in Parkinson's disease. *FEBS Lett* (2015) 589:3739–48. doi: 10.1016/j.febslet.2015.09.031
117. Cheng L, Zhao H, Zhang W, Liu B, Liu Y, Guo Y, et al. Overexpression of conserved dopamine neurotrophic factor (CDNF) in astrocytes alleviates endoplasmic reticulum stress-induced cell damage and inflammatory cytokine secretion. *Biochem Biophys Res Commun.* (2013) 435:34–9. doi: 10.1016/j.bbrc.2013.04.029
118. Nadella R, Voutilainen MH, Saarma M, Gonzalez-Barrios JA, Leon-Chavez BA, Jimenez JM, et al. Transient transfection of human CDNF gene reduces the 6-hydroxydopamine-induced neuroinflammation in the rat substantia nigra. *J Neuroinflamm.* (2014) 11:209. doi: 10.1186/s12974-014-0209-0
119. Zhao H, Cheng L, Liu Y, Zhang W, Maharjan S, Cui Z, et al. Mechanisms of anti-inflammatory property of conserved dopamine neurotrophic factor: inhibition of JNK signaling in lipopolysaccharide-induced microglia. *J Mol Neurosci* (2014) 52:186–92. doi: 10.1007/s12031-013-0120-7
120. Latge C, Cabral KM, de Oliveira GA, Raymundo DP, Freitas JA, Johanson L, et al. The Solution Structure and Dynamics of Full-length Human Cerebral Dopamine Neurotrophic Factor and Its Neuroprotective Role against alpha-Synuclein Oligomers. *J Biol Chem.* (2015) 290:20527–40. doi: 10.1074/jbc.M115.662254
121. Roy H, Bhardwaj S, Yla-Herttuala S. Biology of vascular endothelial growth factors. *FEBS Lett.* (2006) 580:2879–87. doi: 10.1016/j.febslet.2006.03.087
122. Rosenstein JM, Krum JM. New roles for VEGF in nervous tissue–beyond blood vessels. *Exp Neurol.* (2004) 187:246–53. doi: 10.1016/j.expneurol.2004.01.022
123. Shibuya M. Vascular Endothelial Growth Factor (VEGF) and Its Receptor (VEGFR) Signaling in Angiogenesis: A Crucial Target for Anti- and Pro-Angiogenic Therapies. *Genes Cancer* (2011) 2:1097–105. doi: 10.1177/1947601911423031
124. Silverman WF, Krum JM, Mani N, Rosenstein JM. Vascular, glial and neuronal effects of vascular endothelial growth factor in mesencephalic explant cultures. *Neuroscience* (1999) 90:1529–41.
125. Yasuhara T, Shingo T, Kobayashi K, Takeuchi A, Yano A, Muraoka K, et al. Neuroprotective effects of vascular endothelial growth factor (VEGF) upon dopaminergic neurons in a rat model of Parkinson's disease. *Eur J Neurosci.* (2004) 19:1494–504. doi: 10.1111/j.1460-9568.2004.03254.x
126. Tian YY, Tang CJ, Wang JN, Feng Y, Chen XW, Wang L, et al. Favorable effects of VEGF gene transfer on a rat model of Parkinson disease using adeno-associated viral vectors. *Neurosci Lett.* (2007) 421:239–44. doi: 10.1016/j.neulet.2007.05.033
127. Ohlin KE, Francardo V, Lindgren HS, Sillivan SE, O'Sullivan SS, Luksik AS, et al. Vascular endothelial growth factor is upregulated by L-dopa in the parkinsonian brain: implications for the development of dyskinesia. *Brain* (2011) 134:2339–57. doi: 10.1093/brain/awr165
128. Munoz A, Garrido-Gil P, Dominguez-Meijide A, Labandeira-Garcia JL. Angiotensin type 1 receptor blockage reduces l-dopa-induced dyskinesia in the 6-OHDA model of Parkinson's disease. Involvement of vascular endothelial growth factor and interleukin-1beta. *Exp Neurol.* (2014) 261:720–32. doi: 10.1016/j.expneurol.2014.08.019
129. Li X, Lee C, Tang Z, Zhang F, Arjunan P, Li Y, et al. VEGF-B: a survival, or an angiogenic factor? *Cell Adh Migr* (2009) 3:322–7. doi: 10.4161/cam.3.4.9459
130. Caballero B, Sherman SJ, Falk T. Insights into the Mechanisms Involved in Protective Effects of VEGF-B in dopaminergic neurons. *Parkinsons Dis.* (2017) 2017:4263795. doi: 10.1155/2017/4263795
131. Falk T, Zhang S, Sherman SJ. Vascular endothelial growth factor B (VEGF-B) is up-regulated and exogenous VEGF-B is neuroprotective in a culture model of Parkinson's disease. *Mol Neurodegener.* (2009) 4:49. doi: 10.1186/1750-1326-4-49
132. Alam M, Schmidt WJ. Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. *Behav Brain Res.* (2002) 136:317–24. doi: 10.1016/S0166-4328(02)00180-8
133. Testa CM, Sherer TB, Greenamyre JT. Rotenone induces oxidative stress and dopaminergic neuron damage in organotypic substantia nigra cultures. *Brain Res Mol Brain Res.* (2005) 134:109–18. doi: 10.1016/j.molbrainres.2004.11.007
134. Falk T, Yue X, Zhang S, McCourt AD, Yee BJ, Gonzalez RT, et al. Vascular endothelial growth factor-B is neuroprotective in an in vivo rat model of Parkinson's disease. *Neurosci Lett.* (2011) 496:43–7. doi: 10.1016/j.neulet.2011.03.088
135. Herran E, Requejo C, Ruiz-Ortega JA, Aristieta A, Igartua M, Bengoetxea H, et al. Increased antiparkinson efficacy of the combined administration of VEGF- and GDNF-loaded nanospheres in a partial lesion model of Parkinson's disease. *Int J Nanomed.* (2014) 9:2677–87. doi: 10.2147/IJN.S61940
136. Leenders KL, Salmon EP, Tyrrell P, Perani D, Brooks DJ, Sager H, et al. The nigrostriatal dopaminergic system assessed *in vivo* by positron emission tomography in healthy volunteer subjects and patients with Parkinson's disease. *Arch Neurol.* (1990) 47:1290–8.
137. Nandhagopal R, McKeown MJ, Stoessl AJ. Functional imaging in Parkinson disease. *Neurology* (2008) 70:1478–88. doi: 10.1212/01.wnl.0000310432.92489.90
138. Cheng HC, Ulane CM, Burke RE. Clinical progression in Parkinson disease and the neurobiology of axons. *Ann Neurol.* (2010) 67:715–25. doi: 10.1002/ana.21995
139. Appel SH, Beers DR, Henkel JS. T cell-microglial dialogue in Parkinson's disease and amyotrophic lateral sclerosis: are we listening? *Trends Immunol.* (2010) 31:7–17. doi: 10.1016/j.it.2009.09.003

140. Skaper SD, Facci L, Zusso M, Giusti P. An Inflammation-centric view of neurological disease: beyond the neuron. *Front Cell Neurosci.* (2018) 12:72. doi: 10.3389/fncel.2018.00072
141. Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, et al. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest.* (2009) 119:182–92. doi: 10.1172/JCI36470
142. Cebrian C, Zucca FA, Mauri P, Steinbeck JA, Studer L, Scherzer CR, et al. MHC-I expression renders catecholaminergic neurons susceptible to T-cell-mediated degeneration. *Nat Commun.* (2014) 5:3633. doi: 10.1038/ncomms4633
143. Dinan TG, Cryan JF. The impact of gut microbiota on brain and behaviour: implications for psychiatry. *Curr Opin Clin Nutr Metab Care* (2015) 18:552–8. doi: 10.1097/MCO.0000000000000221
144. Mayer EA, Padua D, Tillisch K. Altered brain-gut axis in autism: comorbidity or causative mechanisms? *Bioessays* (2014) 36:933–9. doi: 10.1002/bies.201400075
145. Schroeder BO, Backhed F. Signals from the gut microbiota to distant organs in physiology and disease. *Nat Med.* (2016) 22:1079–1089. doi: 10.1038/nm.4185
146. Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The Central Nervous System and the Gut Microbiome. *Cell* (2016) 167:915–932. doi: 10.1016/j.cell.2016.10.027
147. Selkrig J, Wong P, Zhang X, Pettersson S. Metabolic tinkering by the gut microbiome: Implications for brain development and function. *Gut Microbes* (2014) 5:369–80. doi: 10.4161/gmic.28681
148. Wall R, Cryan JF, Ross RP, Fitzgerald GF, Dinan TG, Stanton C. Bacterial neuroactive compounds produced by psychobiotics. *Adv Exp Med Biol.* (2014) 817:221–39. doi: 10.1007/978-1-4939-0897-4_10
149. Devos D, Lebouvier T, Lardeux B, Biraud M, Rouaud T, Pouclet H, et al. Colonic inflammation in Parkinson's disease. *Neurobiol Dis.* (2013) 50:42–8. doi: 10.1016/j.nbd.2012.09.007
150. Braak H, Rub U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J Neural Transm (Vienna)* (2003) 110:517–36. doi: 10.1007/s00702-002-0808-2
151. Del Tredici K, Braak H. A not entirely benign procedure: progression of Parkinson's disease. *Acta Neuropathol.* (2008) 115:379–84. doi: 10.1007/s00401-008-0355-5
152. Shannon KM, Keshavarzian A, Dodiya HB, Jakate S, Kordower JH. Is alpha-synuclein in the colon a biomarker for premotor Parkinson's disease? Evidence from 3 cases. *Mov Disord.* (2012) 27:716–9. doi: 10.1002/mds.25020
153. Svensson E, Horvath-Puho E, Thomsen RW, Djurhuus JC, Pedersen L, Borghammer P, et al. Vagotomy and subsequent risk of Parkinson's disease. *Ann Neurol* (2015) 78:522–9. doi: 10.1002/ana.24448
154. Holmqvist S, Chutna O, Bousset L, Aldrin-Kirk P, Li W, Bjorklund T, et al. Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathol.* (2014) 128:805–20. doi: 10.1007/s00401-014-1343-6
155. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* (2006) 124:837–48. doi: 10.1016/j.cell.2006.02.017
156. Erny D, Hrabe de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci.* (2015) 18:965–77. doi: 10.1038/nn.4030
157. Matcovitch-Natan O, Winter DR, Giladi A, Vargas Aguilar S, Spinrad A, Sarrazin S, et al. Microglia development follows a stepwise program to regulate brain homeostasis. *Science* (2016) 353:aad8670. doi: 10.1126/science.aad8670
158. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol.* (2016) 16:341–52. doi: 10.1038/nri.2016.42
159. Mohle L, Mattei D, Heimesaat MM, Bereswill S, Fischer A, Alutis M, et al. Ly6C(hi) Monocytes Provide a Link between Antibiotic-Induced Changes in Gut Microbiota and Adult Hippocampal Neurogenesis. *Cell Rep.* (2016) 15:1945–56. doi: 10.1016/j.celrep.2016.04.074
160. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-gut microbiota metabolic interactions. *Science* (2012) 336:1262–7. doi: 10.1126/science.1223813
161. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology* (2011) 141:599–609, e1-3. doi: 10.1053/j.gastro.2011.04.052
162. Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci USA.* (2011) 108:3047–52. doi: 10.1073/pnas.1010529108
163. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci.* (2012) 13:701–12. doi: 10.1038/nrn3346
164. Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, Ebrat B, et al. Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology* (2013) 144:1394–401, e1–4. doi: 10.1053/j.gastro.2013.02.043
165. Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol.* (2004) 558:263–75. doi: 10.1113/jphysiol.2004.063388
166. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* (2015) 161:264–76. doi: 10.1016/j.cell.2015.02.047
167. Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney RD, Shanahan F, et al. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol Psychiatry* (2013) 18:666–73. doi: 10.1038/mp.2012.77
168. Neufeld KM, Kang N, Bienenstock J, Foster JA. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil.* (2011) 23:255–64, e119. doi: 10.1111/j.1365-2982.2010.01620.x
169. Hasegawa S, Goto S, Tsuji H, Okuno T, Asahara T, Nomoto K, et al. Intestinal Dysbiosis and Lowered Serum Lipopolysaccharide-Binding Protein in Parkinson's Disease. *PLoS ONE* (2015) 10:e0142164. doi: 10.1371/journal.pone.0142164
170. Keshavarzian A, Green SJ, Engen PA, Voigt RM, Naqib A, Forsyth CB, et al. Colonic bacterial composition in Parkinson's disease. *Mov Disord* (2015) 30:1351–60. doi: 10.1002/mds.26307
171. Unger MM, Spiegel J, Dillmann KU, Grundmann D, Philippeit H, Burmann J, et al. Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinsonism Relat Disord* (2016) 32:66–72. doi: 10.1016/j.parkreldis.2016.08.019
172. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell* (2016) 167:1469–1480, e12. doi: 10.1016/j.cell.2016.11.018
173. Putignani L, Del Chierico F, Vernocchi P, Cicala M, Cucchiara S, Dallapiccola B, et al. Gut Microbiota Dysbiosis as Risk and Premorbid Factors of IBD and IBS Along the Childhood-Adulthood Transition. *Inflamm Bowel Dis.* (2016) 22:487–504. doi: 10.1097/MIB.0000000000000602
174. Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK. Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. *Trends Microbiol.* (2011) 19:349–59. doi: 10.1016/j.tim.2011.05.006
175. Pull SL, Doherty JM, Mills JC, Gordon JI, Stappenbeck TS. Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. *Proc Natl Acad Sci USA.* (2005) 102:99–104. doi: 10.1073/pnas.0405979102
176. Gilbert JA, Quinn RA, Debelius J, Xu ZZ, Morton J, Garg N, et al. Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature* (2016) 535:94–103. doi: 10.1038/nature18850
177. Noyce AJ, Bestwick JP, Silveira-Moriyama L, Hawkes CH, Giovannoni G, Lees AJ, et al. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. *Ann Neurol.* (2012) 72:893–901. doi: 10.1002/ana.23687
178. Cersosimo MG, Benarroch EE. Pathological correlates of gastrointestinal dysfunction in Parkinson's disease. *Neurobiol Dis.* (2012) 46:559–64. doi: 10.1016/j.nbd.2011.10.014

179. Shahanavaj K, Gil-Bazo I, Castiglia M, Bronte G, Passiglia F, Carreca AP, et al. Cancer and the microbiome: potential applications as new tumor biomarker. *Expert Rev Anticancer Ther.* (2015) 15:317–30. doi: 10.1586/14737140.2015.992785
180. Griffin JL, Wang X, Stanley E. Does our gut microbiome predict cardiovascular risk? A review of the evidence from metabolomics. *Circ Cardiovasc Genet.* (2015) 8:187–91. doi: 10.1161/CIRCGENETICS.114.000219
181. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* (2003) 24:197–211.
182. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. *Neuropathol Appl Neurobiol.* (2007) 33:599–614. doi: 10.1111/j.1365-2990.2007.00874.x
183. Rey NL, Wesson DW, Brundin P. The olfactory bulb as the entry site for prion-like propagation in neurodegenerative diseases. *Neurobiol Dis.* (2018) 109:226–248. doi: 10.1016/j.nbd.2016.12.013
184. Braak H, Ghebremedhin E, Rub U, Bratzke H, Del Tredici K. Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res.* (2004) 318:121–34. doi: 10.1007/s00441-004-0956-9
185. Doty RL. Olfactory dysfunction in Parkinson disease. *Nat Rev Neurol.* (2012) 8:329–39. doi: 10.1038/nrneurol.2012.80
186. Zapiec B, Dieriks BV, Tan S, Faull RLM, Mombaerts P, Curtis MA. A ventral glomerular deficit in Parkinson's disease revealed by whole olfactory bulb reconstruction. *Brain* (2017) 140:2722–36. doi: 10.1093/brain/awx208
187. Beach TG, White CL, III, Hladik CL, Sabbagh MN, Connor DJ, Shill HA, et al. Olfactory bulb alpha-synucleinopathy has high specificity and sensitivity for Lewy body disorders. *Acta Neuropathol.* (2009) 117:169–74. doi: 10.1007/s00401-008-0450-7
188. Doursout MF, Schurdell MS, Young LM, Osuagwu U, Hook DM, Poindexter BJ, et al. Inflammatory cells and cytokines in the olfactory bulb of a rat model of neuroinflammation; insights into neurodegeneration? *J Interferon Cytokine Res.* (2013) 33:376–83. doi: 10.1089/jir.2012.0088
189. Eggen BJ, Raj D, Hanisch UK, Boddeke HW. Microglial phenotype and adaptation. *J Neuroimmune Pharmacol.* (2013) 8:807–23. doi: 10.1007/s11481-013-9490-4
190. Norden DM, Godbout JP. Review: microglia of the aged brain: primed to be activated and resistant to regulation. *Neuropathol Appl Neurobiol.* (2013) 39:19–34. doi: 10.1111/j.1365-2990.2012.01306.x
191. Wu JR, Wang J, Zhou SK, Yang L, Yin JL, Cao JP, et al. Necrostatin-1 protection of dopaminergic neurons. *Neural Regen Res.* (2015) 10:1120–4. doi: 10.4103/1673-5374.160108
192. Iannielli A, Bido S, Folladori L, Segnali A, Cancellieri C, Maresca A, et al. Pharmacological Inhibition of Necroptosis Protects from Dopaminergic Neuronal Cell Death in Parkinson's Disease Models. *Cell Rep.* (2018) 22:2066–2079. doi: 10.1016/j.celrep.2018.01.089

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 16 October 2018
doi: 10.3389/fneur.2018.00870

Monocyte Function in Parkinson's Disease and the Impact of Autologous Serum on Phagocytosis

Ruwani S. Wijeyekoon[1], Deborah Kronenberg-Versteeg[2], Kirsten M. Scott[1], Shaista Hayat[1], Joanne L. Jones[3], Menna R. Clatworthy[4], R. Andres Floto[4], Roger A. Barker[1,2] and Caroline H. Williams-Gray[1]*

[1] John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom, [2] Wellcome Trust-MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, United Kingdom, [3] Department of Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom, [4] Department of Medicine, University of Cambridge, Cambridge, United Kingdom

Background: Increasing evidence implicates involvement of the innate immune system in the initiation and progression of Parkinson's disease (PD). Monocytes and monocyte-derived cells perform a number of functions, such as phagocytosis, chemotaxis, and cytokine secretion, which may be particularly relevant to PD pathology. The behavior of these cells in early-moderate disease, in conditions more similar to the *in-vivo* environment has not been fully evaluated.

Research Question: Does monocyte function, including phagocytosis, chemotaxis and cytokine secretion, differ in early-moderate PD compared to age and gender-matched controls?

Methods: Participants included PD patients ($n = 41$) with early-moderate stage disease (Hoehn and Yahr ≤ 2) and age and gender matched controls ($n = 41$). Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood and monocytes were further separated using CD14 magnetic beads. Functional assays, including bead phagocytosis (in standard medium and autologous serum), Boyden chamber trans-well chemotaxis, and cytokine secretion on lipopolysaccharide stimulation were performed. Monocyte surface markers relating to chemotaxis were measured using immunohistochemistry and flow cytometry. Between-group analysis was performed using paired *t*-tests.

Results: An autologous serum environment significantly increased bead phagocytosis compared to standard medium as expected, in both patients and controls. When in autologous serum, PD monocytes demonstrated enhanced phagocytosis compared to control monocytes ($p = 0.029$). The level of serum-based phagocytosis was influenced by complement inactivation and the origin of the serum. There were no significant differences between PD and controls in terms of standard medium based monocyte migration or cytokine secretion in this cohort.

Conclusions: Autologous serum has a significant influence on monocyte phagocytosis and reveals increased phagocytic capacity in early-moderate PD compared to controls. These conditions may better reflect the function of monocytes *in-vivo* in PD patients than standard medium based phagocytosis assays. Further studies will be required to

OPEN ACCESS

Edited by:
Cristoforo Comi, Università degli Studi del Piemonte Orientale, Italy

Reviewed by:
Viviane Labrie, Van Andel Institute, United States
Johannes Schlachetzki, University of California, San Diego, United States

***Correspondence:**
Ruwani S. Wijeyekoon
rsw27@cam.ac.uk

Specialty section:
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

Received: *09 July 2018*
Accepted: *26 September 2018*
Published: *16 October 2018*

Citation:
Wijeyekoon RS, Kronenberg-Versteeg D, Scott KM, Hayat S, Jones JL, Clatworthy MR, Floto RA, Barker RA and Williams-Gray CH (2018) Monocyte Function in Parkinson's Disease and the Impact of Autologous Serum on Phagocytosis. Front. Neurol. 9:870. doi: 10.3389/fneur.2018.00870

replicate these results in larger cohorts, including earlier and later stages of disease, and to understand which serum factors are responsible for this observation and the potential mechanistic relevance to PD pathogenesis.

Keywords: Parkinson's, monocyte, phagocytosis, migration, cytokine, serum

INTRODUCTION

There is increasing evidence of an association between the innate immune system and Parkinson's disease (PD), with genetic, cellular and biomarker studies suggesting variations in innate immune genes and cells in this condition (1). A few studies have found peripheral innate immune changes in PD patients, including changes in peripheral monocyte phenotype and function (2–4), but results are inconsistent between studies and may be related to variations in participant characteristics and assay protocols.

Monocytes are a major component of the peripheral innate immune system and have a number of important functions, such as migration into tissues, phagocytosis of pathogens and cell debris, secretion of cytokines and other proteins, antigen presentation to cells of the adaptive immune system and differentiation into dendritic cells and macrophages which take on more specialized roles (5). The 3 principal subtypes of monocyte–classical, intermediate and non-classical-specialize in different combinations of functions. However, there is a considerable degree of overlap, and variability between study findings suggests that the functional abilities of monocytes may vary depending on a variety of intrinsic and extrinsic factors (5–8).

With relevance to PD pathology, monocytes and related cells, such as macrophages have been found to be capable of entering and interacting with the central nervous system via the meninges and choroid plexus (9, 10) and may be involved in the recognition and/or phagocytosis of protein aggregates (11–13), of debris from degenerating neurons and other cells, or of microbial organisms and their components. However, this may not be entirely beneficial, as they may also generate inflammatory cytokines, such as IL-1β in response to TLR and inflammasome stimulation by forms of alpha-synuclein or other pathogen or damage associated molecular patterns (PAMPs and DAMPs) (12), which in turn may then drive an ongoing inflammatory response. Monocyte derived cells may also conceivably play a role in the presentation of pathogenic alpha-synuclein and other antigens, to T lymphocytes, mediated by the PD-associated risk allele HLA-DR (14–16). These functions may occur within the intravascular compartment, or monocytes could also migrate into tissues to function as monocytes or differentiated cells, such as dendritic cells and macrophages (15).

Studies on peripheral monocyte function in PD to date have reported impaired phagocytosis, with higher monocyte alpha-synuclein levels associated with greater phagocytic impairment (2, 3). Uptake of alpha-synuclein fibrils by monocytes (12) has been shown to increase production of pro-inflammatory cytokines, such as IL-1β, while monocyte uptake of alpha-synuclein oligomers has been reported to decrease with age (11). Monocyte cytokine secretion in response to stimulation has been found to be increased (3, 17) or decreased (4) in PD compared to controls in separate studies. No studies have assessed monocyte migration in PD, but alpha-synuclein monomers and oligomers have been found to act as monocyte chemotactic factors (18).

While studies to date have not been entirely consistent, they have provided some indication that there may be differences in monocyte function between PD and controls. However, most studies have been performed on relatively late stage PD patients with average disease duration of at least 8–10 years, in whom secondary effects of the disease process on the innate immune system are likely to be predominant. Functional differences in monocytes of PD patients at an earlier disease duration and stage than in these previous studies, have not been fully assessed.

Functions, such as phagocytosis may also be affected by changes in cell surface marker expression and properties, which occur on prolonged incubation in different forms of artificial media (19). Thus, assessment of functional phagocytic differences in conditions which more closely replicate the *in-vivo* state, with less pre-incubation and an autologous serum environment, may also be important in order to obtain a better understanding of monocyte function in PD.

Therefore, this study investigated key functions of *ex-vivo* monocytes (phagocytosis, migration and cytokine secretion), in early-moderate PD patients [Hoehn and Yahr (HY) stage $\leq$2, mean disease duration 4.2 $\pm$ 1.1 years] and age and gender matched controls, using conditions more representative of the *in-vivo* state where possible. Monocyte migration-related markers [CCR2, C-X3-C motif chemokine receptor 1 (CX3CR1)] were also evaluated using immunocytochemistry and flow cytometry.

MATERIALS AND METHODS

Participant Recruitment and Sample Collection

The study was carried out and the protocol was approved in accordance with the recommendations of the Cambridgeshire Research Ethics Committee (03/303), with written, informed consent from all subjects in accordance with the Declaration of Helsinki. Patients were recruited from the PD Research Clinic at the John van Geest Centre for Brain Repair in Cambridge.

Inclusion criteria were fulfillment of UK PD Brain Bank Criteria for a diagnosis of PD, age 55–80 years and Hoehn and Yahr (HY) stage $\leq$2 as defined by the Movement Disorder Society, with absence of postural instability (20). Exclusion criteria were: other neurodegenerative disorders, chronic inflammatory or autoimmune disorders, current clinically significant infection, surgery within last month, vaccinations in the last 3 weeks, use of anti-inflammatory/immunomodulatory

medications [steroids (within 3 months), high dose aspirin >75 mg (2 weeks), ibuprofen and other nonsteroidal anti-inflammatory drugs (2 weeks) and other long-term immunosuppressant drugs e.g., azathioprine, mycophenolate, methotrexate, rituximab or other antibody therapy (1 year)].

Control participants were recruited from the NIHR Cambridge BioResource (http://www.cambridgebioresource.org.uk). They were age and gender matched to the patients and had no history of neurological disease, self-reported memory problems or depression. Exclusion criteria for controls were the same as for the patients.

50 ml venous blood was collected (45 ml lithium heparin and 5 ml serum in Sarstedt, S-Monovette® tubes) between 9 and 11 a.m. and patients were on their regular medication and had no dietary restrictions. Serum was extracted by centrifuging samples at 2,000 rpm for 15 min, following 15 min clotting time. Separated serum was stored at 4°C prior to subsequent processing. PBMCs were isolated for immunohistochemistry and flow cytometry. Functional assays were performed on fresh cells and serum, depending on cell availability. Patient and paired control samples were processed together on the same day.

Basic demographic and clinical data were obtained from the patients and included disease duration, medication history, Unified Parkinson's Disease Rating Scale (UPDRS) score and Addenbrooke's Cognitive Examination-Revised (ACE-R) score.

Separate data from this participant cohort contributed toward our previously published study investigating T cell senescence in PD (21).

PBMC Isolation, Immunocytochemistry and Flow Cytometry

PBMCs were extracted using the standard Ficoll gradient centrifugation method (Ficoll® Paque Plus, GE Healthcare). Cell suspensions were centrifuged, and cell pellets were blocked with fluorescence activated cell sorting (FACS) buffer with 2% mouse serum (Sigma) per 0.5–1 $\times$ 10^6 cells. Following blocking for 30 min, the PBMCs were stained with a panel of relevant conjugated antibodies including [CX3CR1-APC and CCR2-PE (Biolegend)] or appropriate isotype controls [Rat IgG2b κ-APC and Mouse IgG2a κ-PE (Biolegend)] and incubated at 4°C for 30 min. Following incubation, the PBMCs were washed and then fixed with 2% paraformaldehyde (PFA) and re-suspended in FACS buffer for flow cytometry. Flow cytometry was performed using the BD LSR Fortessa machine with BD FACS Diva software.

Monocytes were gated as described in the literature (22) (**Supplementary Figure 4**) and a minimum number of 10,000 monocyte events were collected per sample. PBMCs from healthy controls, labeled with single conjugated antibodies, were used to determine the appropriate compensation for spectral overlap of fluorophores.

Flow cytometry data was analyzed using Flow Jo software, version 10. The percentage of positive cells and marker expression levels were determined with reference to isotype control samples (Median Fluorescence Intensity (MFI) Test/Isotype ratio).

Monocyte Separation

$CD14^+$ cells were separated using MACS® magnetic $CD14^+$ beads (Miltenyi Biotec) and "LS" columns, according to the manufacturer's instructions. Patient and control pairs were separated using the same method. $CD14^+$ cell purity following magnetic bead separation was >97% (CD14-APC-H7, Biolegend; **Supplementary Figure 1A**). The monocytes obtained following $CD14^+$ magnetic bead separation undertaken in standard cooled conditions produced consistent patterns of CD14/CD16 staining, demonstrating the presence of all monocyte subtypes (Classical, Intermediate and Non-Classical; **Supplementary Figure 1B**).

Monocyte Bead Phagocytosis Assays

$CD14^+$ cells were centrifuged at 350 g for 5 min and re-suspended in either phenol-red free, clear RPMI (Life Technologies) + 10% heat inactivated FCS (Sigma), or in 100% of the participant's own serum (200 µl per 0.5 $\times$ 10^6 monocytes). The cells were placed in 96 well-plates at a density of 0.5 $\times$ 10^6 monocytes in 200 ul per well and equilibrated in the incubator (37°C, 5% CO_2; Test plates) or the fridge (4°C plates) for 45 min.

Latex beads [Fluorescent Carboxyl Polymer, Dragon green, 2–5 µm (Bangs Laboratories)] were added at a 1:1 ratio and mixed into the appropriate wells. The Test plates were placed in the incubator (37°C, 5% CO_2) to simulate *in-vivo* conditions. Reference plates were placed in the fridge (4°C) to inhibit monocyte phagocytosis and endocytosis and used as a reference to account for non-specific adherence of fluorescent beads to the cells for each condition. Cells were incubated for 60 min, then washed with ice cold PBS and then with FACS buffer. Cells were fixed in 2% PFA prior to flow cytometry (BD LSR Fortessa). The gating strategy is illustrated in **Figure 1**. The bead positive monocyte percentage and bead positive monocyte MFI ratio values for bead uptake were calculated with reference to the 4°C samples (Bead positive monocyte % = Test sample bead positive monocyte %−4C sample bead positive monocyte %; Bead positive monocyte MFI ratio = Test sample bead positive MFI/4C sample bead positive MFI).

The effect of heat mediated serum complement inactivation on these serum bead uptake assays was assessed by heating the serum to 56°C for 30 min prior to performing the assay.

Microscopy was performed on a subset of samples. The cells were re-suspended in PBS and smeared onto a glass slide and air dried with protection from light. A glass cover slip was applied onto the slide with FluorSave™ reagent solution. Light microscope images of latex bead uptake were taken on the Leica DM light microscope.

Monocyte Migration Assays

Monocyte migration assays were performed using Neuro Probe ChemoTx® chemotaxis system 96 well-plates with 5 µm pores (http://www.neuroprobe.com/product/chemo_tx/). 29 µl of medium (RPMI (Life Technologies) and 10% FCS) with or without the chemoattractant CCL2 (100 ng/ml; Peprotech) was added to the appropriate lower wells of the plate in triplicate. 50 µl of monocyte suspension (2 $\times$ 10^5 monocytes per 50 µl RPMI and 10% FCS) was added to the top of the filter above

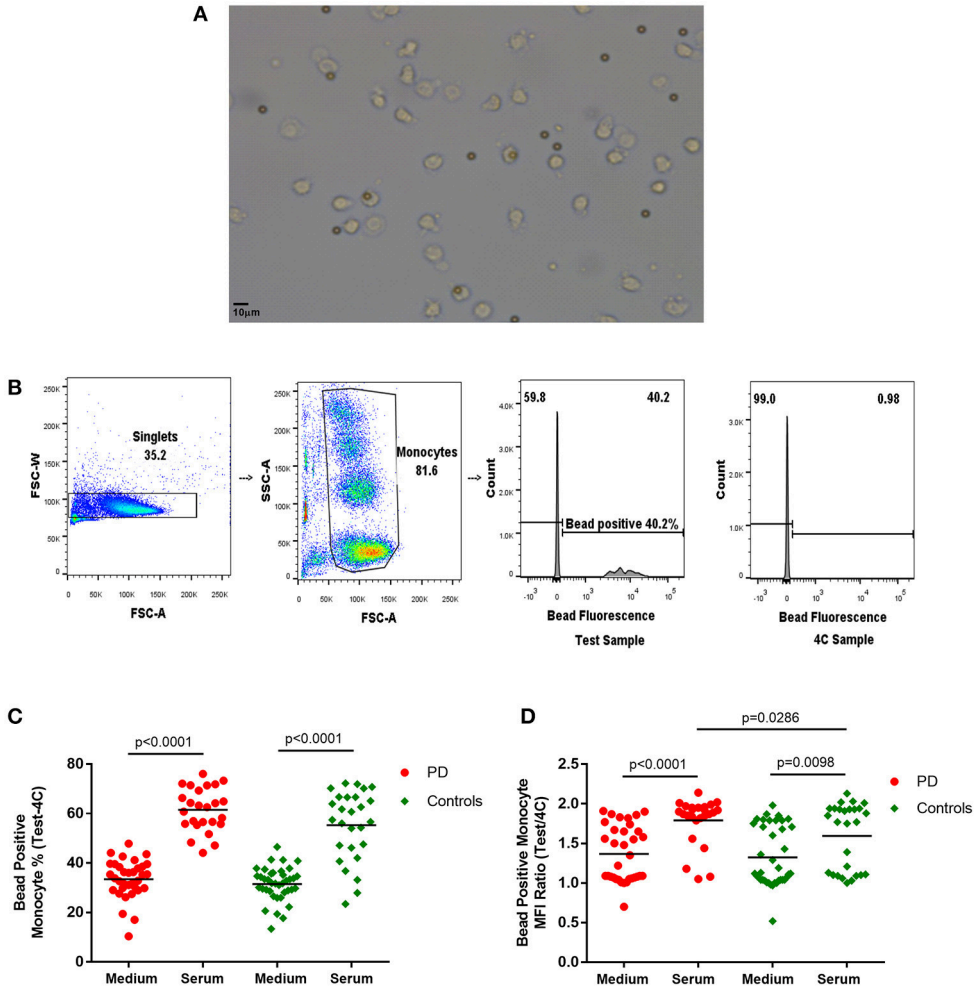


FIGURE 1 | (A) Light microscope image of monocytes with phagocytosed 2–5 μm latex beads (×20). **(B)** Flow cytometry gating strategy for monocyte latex bead phagocytosis (1:1 ratio) analysis. Monocyte gate extended upwards to include bead phagocytosed monocytes, which have increased side scatter. Dividing gate on histogram based on position of 4°C sample. FSC-A, forward scatter-area; FSC-W, forward scatter-width; SSC-A, side scatter-area. **(C,D)** Monocyte latex bead phagocytosis in standard medium and in autologous serum–**(C)** percentage bead positive monocytes (37–4°C); **(D)** bead positive monocyte median fluorescence intensity (MFI) ratio (37/4°C) [Medium-PD = 34, Controls = 39; Serum-PD = 25, Controls = 27; *p*-values relate to the significance of paired *t*-tests performed between matched PD and control pairs following the exclusion of experimental outliers >3 SD above or below mean (excluded pairs Medium = 1, Serum = 0)].

each well and plates were incubated at 37°C with 5% CO_2 for 2 h. Cell migration into the lower wells was counted using a haemocytometer. The percentages of monocytes that migrated with and without CCL2 and the percentage increase in monocyte migration with CCL2 were calculated.

Monocyte Cytokine Secretion Assays

$CD14^+$ monocytes were re-suspended in RPMI and 10% FCS at a concentration of 1×10^6 cells per ml. 1 ml cell suspension was added per well into a 24 well-culture plate with and without the potent monocyte stimulant bacterial lipopolysaccharide (LPS) from Escherichia Coli O111:B4 (1 ng/ml; Sigma). Cells were cultured for 24 h at 37°C and 5% CO_2. Post-culture supernatants were separated by centrifugation at 350 g for 5 min and stored at −80°C.

Cytokines were measured using a Mesoscale Discovery (MSD) platform V-Plex Pro-inflammatory panel 1 electrochemiluminescence assay (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α). The assays were run according to the manufacturer's instructions. Supernatant samples were diluted 1:10 in the appropriate buffer and assayed in duplicate.

Statistical Analysis

Between-group comparisons (PD vs. matched controls) were performed using paired *T*-tests (IBM SPSS statistics version 25). Experimental outliers with values >3 standard deviations (SD) above or below the mean were excluded prior to analysis. Values which were unpaired for any reason (e.g., outlier exclusion or cell unavailability for assay completion) were automatically excluded

TABLE 1 | Demographics of overall cohort.

Variable	Patients	Paired controls	*p*
Number (*n*)	41	41	
Age (years)	68.4 ± 6.3	68.1 ± 5.6	0.784
Gender (% male)	68.3	68.3	0.594
Disease duration (years)	4.2 ± 1.1		
MDS-UPDRS motor score	35.2 ± 12.3		
Equivalent Levodopa dose	591.5 ± 292.9		
ACE-R score	92.9 ± 8.2		

Values indicate Mean ± SD (Standard deviation); UPDRS, Unified Parkinson's Disease Rating Scale; ACE-R, Addenbrooke's Cognitive Examination (Revised).

during the statistical analysis of paired *T*-tests. As analyses were always performed as a comparison between paired PD and control pairs, the exclusion of unpaired values did not influence sample matching.

RESULTS

Participant Demographics

41 PD patients and 41 age and gender matched controls were recruited in total. Demographic and clinical characteristics of the cohort are shown in **Table 1**. Subsets of samples were used for different functional assays depending on cell availability.

Monocyte Bead Phagocytosis

The latex bead phagocytosis assay was performed using both standard medium (RPMI and 10% FCS) and freshly extracted autologous serum. While autologous serum was used to partially simulate the *in-vivo* intravascular environment, the standard medium represented assessment of cell-intrinsic phagocytic ability under standard culture conditions. A significantly higher percentage of monocytes phagocytosed latex beads in the presence of serum compared to standard medium (**Figure 1**), as would be expected due to the increased presence of opsonizing factors, such as antibodies and complement in autologous serum (23–25).

Paired analysis indicated no differences in measures of bead phagocytosis in standard medium between patients and controls (**Figure 1**). However, with autologous serum, the bead positive monocyte MFI ratio in patient monocytes was significantly higher than in controls ($p = 0.0286$; **Figure 1**), indicating increased uptake in PD bead positive monocytes compared to controls. Patients also tended to have a higher overall serum bead positive monocyte percentage, but this did not reach statistical significance (**Figure 1**). There were no statistically significant correlations between the uptake measures and clinical data, including the UPDRS motor score, in this cohort.

The influence of serum factors on uptake was subsequently examined in separate smaller *ad hoc* groups of patients and controls. These groups included patients of later disease stage compared to the original study, who otherwise fulfilled similar criteria. The effect of swapping PD and control serum with their paired monocytes was examined in 6 age and gender matched PD and control pairs [age- (mean ± standard deviation (SD)) PD 72.67 ± 2.34, controls 68.00 ± 6.89 ($p = 0.148$); gender 83.3% male (PD and controls); PD disease duration (mean ± SD) 7.35 ± 2.94]. The autologous serum bead positive monocyte percentage was significantly higher in PD patients compared to controls in this cohort ($p = 0.0002$; **Supplementary Figure 2**), but this difference was no longer apparent when phagocytosis was measured in the swapped, non-autologous serum condition ($p = 0.6541$). Thus, the origin of the serum appeared to account for the PD-control difference in monocyte phagocytosis (bead positive monocyte percentage) seen with autologous serum (**Supplementary Figure 2**).

Serum complement inactivation by heat treatment was examined in PD ($n = 6$) and controls ($n = 5$) [age (mean ± SD) PD 72.0 ± 2.44, controls 66.25 ± 8.18 ($p = 0.135$); gender PD 83.3% male, controls 80% male; disease duration PD 7.50 ± 2.69]. Serum heat inactivation resulted in significant decreases in autologous serum-based monocyte bead uptake in both PD and controls [bead positive monocyte percentage (PD $p = 0.0001$; controls $p = 0.006$); bead positive monocyte MFI ratio (PD $p = 0.0034$; controls $p = 0.0163$)], indicating that it had a significant effect on the overall level of phagocytosis (**Supplementary Figure 3**). Bead positive monocyte uptake was significantly higher in PD patients compared to paired controls ($p = 0.0087$) in this cohort, but this significant difference was lost post-heat inactivation ($p = 0.0558$; **Supplementary Figure 3**).

Monocyte Migration

There were no significant differences in monocyte migration between this PD cohort and controls overall with or without the presence of CCL2 (**Figure 2A**). Monocyte trans-well migration levels were low in the baseline condition, but increased significantly, as expected, in response to CCL2. However, there was no difference in the magnitude of this response between PD and controls (**Figure 2B**).

There were no significant differences between patients and controls in the surface expression of the migration-associated surface markers CCR2 and CX3CR1 (**Supplementary Figure 4**). Migration measures also did not demonstrate any statistically significant relationships with clinical data, including the UPDRS motor score.

Monocyte Cytokine Secretion

Cytokine secretion in the unstimulated condition was low for most cytokines and only IL-1β, IL-6, IL-8, IL-10, and TNF-α had measurable results in >75% of participants. There were no significant differences between paired patients and controls (**Table 2**).

With LPS stimulation for 24 h, measurable concentrations of all 10 cytokines were present in the supernatant in >75% of participants. However, there were no statistically significant differences between paired patients and controls overall in this cohort (**Table 2**). There were also no statistically significant relationships between cytokine secretion in either condition and clinical measures.

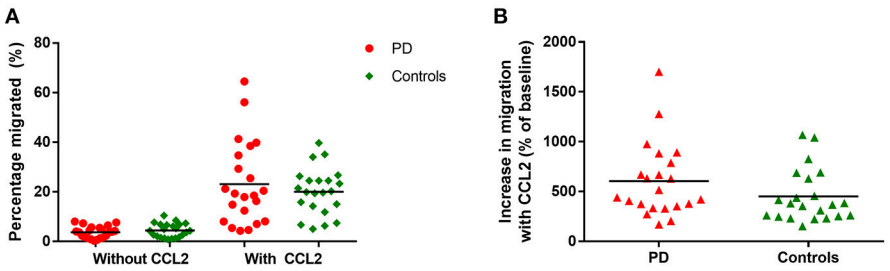


FIGURE 2 | (A) Graph showing percentage monocytes migrated in all PD patients and controls, with and without the presence of CCL2 as a chemoattractant. PD = 22, Controls = 22. **(B)** Graphs showing percentage increase in monocyte migration with CCL2 in all PD and controls.

TABLE 2 | Summary of unstimulated and LPS stimulated monocyte supernatant cytokine results.

Cytokine	Cytokine concentration (pg/ml) ± SD					
	Unstimulated			LPS Stimulated		
	Patients ($n = 20$)	Paired controls ($n = 22$)	p	Patients ($n = 21$)	Paired controls ($n = 23$)	p
IFN-γ	X	X		46.09 ± 33.06	41.08 ± 35.00	0.764
IL-1β	42.74 ± 72.76	115.18 ± 232.63	0.176	771.38 ± 469.57	819.50 ± 500.82	0.222
IL-2	X	X		17.28 ± 21.16	14.37 ± 15.05	0.604
IL-4	X	X		4.87 ± 3.18	12.08 ± 16.68	0.082
IL-6	691.64 ± 2,027.38	1,060.22 ± 2,606.12	0.329	9,566.62 ± 4,558.47	10,024.70 ± 5,594.38	0.825
IL-8	28,776.83 ± 37,982.80	27,404.75 ± 39,478.76	0.591	126,025.80 ± 129,595.00	146,257.96 ± 166,969.57	0.794
IL-10	42.52 ± 104.54	63.59 ± 127.92	0.379	336.90 ± 235.92	327.90 ± 175.87	0.836
IL-12p70	X	X		22.78 ± 13.19	19.01 ± 11.05	0.605
IL-13	X	X		62.14 ± 51.80	50.93 ± 37.86	0.286
TNF-α	55.22 ± 123.60	215.93 ± 457.47	0.160	971.69 ± 612.36	962.76 ± 393.42	0.754

All results represent mean cytokine concentration (pg/ml) ± standard deviation (SD). p-values indicate significance on paired t-tests between matched PD and control pairs. X indicates concentration above detection threshold in <75% of participants.

DISCUSSION

The findings demonstrate significantly increased monocyte phagocytic capacity in an autologous serum environment in PD, but no significant differences in standard medium-based monocyte phagocytosis, monocyte migration or cytokine secretion, compared to age and gender matched parallel processed controls, in this cohort.

The use of the participant's own serum as an environment for the uptake assays attempted to more faithfully replicate the intravascular *in-vivo* environment. The presence of serum increased bead phagocytosis overall, compared to standard medium in both PD and controls, consistent with the presence of opsonising factors, such as antibodies and complement in the serum (25, 26). Complement inactivation through heat treatment had a profound negative effect on bead uptake in both PD and controls, indicating an important contribution of complement to serum-based monocyte phagocytosis (**Supplementary Figure 2**). Interestingly, derivatives of complement factors, such as C3, have been found to be higher in PD serum (27) compared to controls and may contribute to the differences seen. We did not specifically investigate the effect of serum antibodies in this study, but they may also play an important role. Further studies including antibody depletion and Fc receptor blocking and measurement of serum complement and antibody levels will be required to fully determine the factors responsible for the serum assay differences observed.

Our data suggests that serum components in disease are an important factor in determining the differential phagocytosis observed between PD and controls. When the environmental conditions are equal (standard medium) there is no significant difference between PD and control monocyte phagocytosis, whereas an autologous serum environment brings out a difference. This indicates a strong influence from cell extrinsic factors rather than cell-intrinsic factors in bringing about the PD-control difference in phagocytosis observed in this study. The data also suggests that swapping patient and control serum with their paired monocytes removes this PD-control difference, further supporting the hypothesis that external disease related serum-based factors critically influence monocyte phagocytic behavior.

In contrast to our study, previous studies investigating monocyte phagocytosis in PD using standard medium conditions have found a relative impairment in PD compared to controls in smaller numbers of participants (2, 3). The standard medium bead phagocytosis assays in the current study, which

would be most similar to those previous studies (3) did not show a similar significant impairment. However, the assays used in the previous studies utilized different types of medium, different types and sizes of uptake particles (different concentrations of 1 μm beads, fluorescent red blood cells or zymosan particles) and longer assay periods (up to 4 h) and were also performed on relatively later stage PD patients (2, 3), which may have contributed to the differences in outcome. Shorter incubation periods used in the current study may have also resulted in persistence of elements of influence from the *in-vivo* environment, which may have affected the results.

Previous studies have also shown the presence of differences in monocyte surface markers and other cell-intrinsic factors in PD vs. controls (3, 28). It is possible that intrinsic monocyte differences may enhance or enable the effects of serum and the overall phagocytosis level is likely to be influenced by a combination and/or interaction of intrinsic and extrinsic factors. Further studies investigating these factors in larger numbers of PD and control pairs will be important to confirm and extend these findings.

This was also the first study to assess monocyte migration behavior in PD, and we found no significant overall differences in standard medium based migration when compared to controls in this cohort. One previous study has found increased expression of the chemokine receptor CCR2 in PD monocytes compared to controls (28), but our data did not replicate this finding. Alpha-synuclein monomers and oligomers have been shown to have monocyte and neutrophil chemoattractant properties (18), but this was not assessed in the current study. Considering the phagocytosis differences seen with autologous serum, it would be interesting to also assess monocyte migration in autologous serum, to investigate any differences which may be revealed in this more *in-vivo* relevant environment. However, these assays would need to be controlled for serum CCL2 and other chemokine levels, which may be potential confounding factors.

The current study showed no significant differences in standard medium based monocyte cytokine secretion between PD and controls. Previous studies using smaller sample sizes have reported inconsistent results, with increased (3) or partially decreased/unchanged (4) cytokine secretion by PD monocytes with LPS stimulation compared to controls. The largest of these (21 PD, 8 controls), reported no significant differences in monocyte IL-1β, IL-6, IL-8, and IL-10 production, in keeping with our study, but found a decrease in TNF-α secretion by PD monocytes (4). Elevated cytokine production by PBMCs has also been reported in PD compared to controls (29), suggesting that other immune cell types may also be involved in the mediation of an increased inflammatory response in PD patients.

As with migration, monocyte cytokine secretion could also be assessed in autologous serum, but measurement of supernatant cytokine levels would require controlling for serum intrinsic cytokine levels. However, measurement of monocyte intracellular cytokine levels using intracellular staining and flow cytometry and cytokine gene expression may be alternative methods of assessing cytokine secretion in the serum environment.

The functional assays were performed on positively selected $CD14^+$ monocytes and the overall functional status of total monocytes will be influenced by the relative proportions of the different monocyte subsets in PD and controls, with the predominant classical monocytes (∼60–80% of total monocytes), which have been found to be higher in PD, likely to have the most influence on functions, such as phagocytosis (3). However, this would not explain the presence of significant differences in phagocytosis in autologous serum, but not in standard medium. $CD14^+$ selection may also lead to the relative loss of CD14 low non-classical monocytes, which may subsequently influence the magnitude of any potential differences in non-classical predominant cytokine secretion. Nevertheless, a previous study demonstrating significant differences in monocyte secretion also used $CD14^+$ selection, suggesting that this may not be a major factor (3). In general, the goal of the study was to obtain a global overview of the status of total monocyte function in PD compared to controls, while accepting that a variety of factors will likely be contributing toward the overall picture.

It is possible that the use of CD14 positive magnetic bead selection may have affected monocyte behavior (30). However, both patient and control samples underwent the same process of monocyte extraction and previous studies have also used this method of monocyte isolation with differing results (3), hence it is unlikely to be a major factor influencing differences between PD and controls.

Most of the PD patients involved in this study were on levodopa or dopamine agonist medications, and dopamine has been reported to have effects on immune cells, including augmentation of T follicular helper cell-B lymphocyte interactions in germinal centers (31) and of monocyte functions, such as migration (32). However, we found no significant differences in monocyte migration behavior in PD when compared to controls and this coupled to the finding of no significant correlations between monocyte uptake measures or other functions and levodopa equivalent dose in this study, suggests that it is unlikely that these drugs could explain the differences in behavior that we report. Dietary, circadian and other medication factors may also potentially influence monocyte function and serum. In this study all patients and controls had samples taken within the same time period in the morning and would have been expected to have had breakfast and their regular medication prior to their visit. Further work will require additional assessment of these assays in dopamine medication naïve earlier stage PD patients and paired controls, prior to food and medication intake in the morning.

It is possible that clinical subgroups of patients, with differing levels of risk factors for cognitive and motor progression (33, 34) may have differential differences compared to paired controls and that potential differences are masked with the use of overall combined analysis. This study did not have sufficient numbers for each assay, to enable subgroup analysis. Future studies will need to repeat these assays in larger numbers of different clinical subgroups of patients to determine any underlying differential changes seen only within specific clinical subgroups. The uptake assays investigating serum factors provide some indication that serum-based uptake is higher in PD even at later stages of disease.

Thus, it will be important to assess monocyte function in larger cohorts at different stages of PD, as well as longitudinally, in order to identify any changes correlated with disease severity and duration, which may be useful as potential biomarkers.

In conclusion, we have investigated a range of monocyte functions in early-moderate PD compared to age and gender matched controls and demonstrated an increased capacity for bead phagocytosis in PD which appears to be mostly driven by cell-extrinsic factors in PD serum. This adds to existing evidence implicating changes in innate immune function in PD, but further work will be required to investigate what serum factors are important, as well as the physiological and clinical relevance of these findings, particularly in relation to the disease process and the alpha-synuclein pathology in PD.

AUTHOR CONTRIBUTIONS

RW, CW-G, and DK-V designed the study and planned the assays. RW, CW-G, KS, and SH carried out the study. MC, RAF, JJ, and DK-V advised on the assay protocols, interpretation and analysis. RW prepared the first draft of the manuscript. CW-G, DK-V, RB, KS, JJ, MC, RAF, and SH reviewed the manuscript.

FUNDING

Funding for this work was provided by Addenbrooke's Charitable Trust, the Rosetrees Trust and the NIHR Cambridge Biomedical Research Centre. RW was supported by a Fellowship from Addenbrooke's Charitable Trust. DK-V is supported by a Junior Research Fellowship from Homerton College, Cambridge. KS is supported by a Fellowship from the Wellcome Trust. CW-G is supported by a Clinician Scientist Fellowship from the Medical Research Council. RB is an NIHR Senior Investigator and is supported by the Wellcome Trust-MRC Cambridge Stem Cell Institute.

ACKNOWLEDGMENTS

We gratefully acknowledge the participation of all our patient and control volunteers and NIHR Cambridge BioResource volunteers and thank the NIHR Cambridge BioResource Centre and staff for their contribution. We thank the National Institute for Health Research and NHS Blood and Transplant. We also acknowledge the support of the Cambridge NIHR BRC Cell Phenotyping Hub and the Core Biochemical Assay Laboratory at Cambridge University Hospitals.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2018.00870/full#supplementary-material

REFERENCES

1. Kannarkat GT, Boss JM, Tansey MG. The role of innate and adaptive immunity in parkinson's disease. *J Parkinsons Dis.* (2013) 3:493–514. doi: 10.3233/JPD-130250
2. Gardai SJ, Mao W, Schüle B, Babcock M, Schoebel S, Lorenzana C, et al. Elevated alpha-synuclein impairs innate immune cell function and provides a potential peripheral biomarker for parkinson's disease. *PLoS ONE* (2013) 8:e71634. doi: 10.1371/journal.pone.0071634
3. Grozdanov V, Bliederhaeuser C, Ruf WP, Roth V, Fundel-Clemens K, Zondler L, et al. Inflammatory dysregulation of blood monocytes in Parkinson's disease patients. *Acta Neuropathol.* (2014) 128:651–63. doi: 10.1007/s00401-014-1345-4
4. Hasegawa Y, Inagaki T, Sawada M, Suzumura A. Impaired cytokine production by peripheral blood mononuclear cells and monocytes/macrophages in Parkinson's disease. *Acta Neurol Scand.* (2000) 101:159–64. doi: 10.1034/j.1600-0404.2000.101003159.x
5. Wong KL, Yeap WH, Tai JJY, Ong SM, Dang TM, Wong SC. The three human monocyte subsets: implications for health and disease. *Immunol Res.* (2012) 53:41–57. doi: 10.1007/s12026-012-8297-3
6. Boyette LB, Macedo C, Hadi K, Elinoff BD, Walters JT, Ramaswami B, et al. Phenotype, function, and differentiation potential of human monocyte subsets. *PLoS ONE* (2017) 12:e0176460. doi: 10.1371/journal.pone.0176460
7. Mukherjee R, Kanti Barman P, Kumar Thatoi P, Tripathy R, Kumar Das B, Ravindran B. Non-classical monocytes display inflammatory features: validation in sepsis and systemic lupus erythematous. *Sci Rep.* (2015) 5:13886. doi: 10.1038/srep13886
8. Lee J, Tam H, Adler L, Ilstad-Minnihan A, Macaubas C, Mellins ED. The MHC class II antigen presentation pathway in human monocytes differs by subset and is regulated by cytokines. *PLoS ONE* (2017) 12:e0183594. doi: 10.1371/journal.pone.0183594
9. Herz J, Filiano AJ, Smith A, Yogev N, Kipnis J. Myeloid cells in the central nervous system. *Immunity* (2017) 46:943–56. doi: 10.1016/j.immuni.2017.06.007
10. Harms AS, Thome AD, Yan Z, Schonhoff AM, Williams GP, Li X, et al. Peripheral monocyte entry is required for alpha-Synuclein induced inflammation and neurodegeneration in a model of parkinson disease. *Exp Neurol.* (2018) 300:179–87. doi: 10.1016/j.expneurol.2017.11.010
11. Bliederhaeuser C, Grozdanov V, Speidel A, Zondler L, Ruf WP, Bayer H, et al. Age-dependent defects of alpha-synuclein oligomer uptake in microglia and monocytes. *Acta Neuropathol.* (2016) 131:379–91. doi: 10.1007/s00401-015-1504-2
12. Codolo G, Plotegher N, Pozzobon T, Brucale M, Tessari I, Bubacco L, et al. Triggering of inflammasome by aggregated α-synuclein, an inflammatory response in synucleinopathies. *PLoS ONE* (2013) 8:e55375. doi: 10.1371/journal.pone.0055375
13. Weltzien RB, Pachter JS. Visualization of beta-amyloid peptide (Abeta) phagocytosis by human mononuclear phagocytes: dependency on Abeta aggregate size. *J Neurosci Res.* (2000) 59:522–7. doi: 10.1002/(SICI)1097-4547(20000215)59:4<522::AID-JNR7>3.0.CO;2-L
14. Kannarkat GT, Cook DA, Lee J-K, Chang J, Chung J, Sandy E, et al. Common genetic variant association with altered HLA expression, synergy with pyrethroid exposure, and risk for Parkinson's disease: an observational and case–control study. *NPJ Park Dis.* (2015) 1:15002. doi: 10.1038/npjparkd.2015.2
15. Jakubzick CV, Randolph GJ, Henson PM. Monocyte differentiation and antigen-presenting functions. *Nat Rev Immunol.* (2017) 17:349–62. doi: 10.1038/nri.2017.28
16. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize α-synuclein peptides. *Nature* (2017) 546:656–61. doi: 10.1038/nature22815

17. Cook DA, Kannarkat GT, Cintron AF, Butkovich LM, Fraser KB, Chang J, et al. LRRK2 levels in immune cells are increased in Parkinson's disease. *NPJ Park Dis.* (2017) 3:11. doi: 10.1038/s41531-017-0010-8
18. Stolzenberg E, Berry D, Yang D, Lee EY, Kroemer A, Kaufman S, et al. A role for neuronal alpha-synuclein in gastrointestinal immunity. *J Innate Immun.* (2017) 9:456–63. doi: 10.1159/000477990
19. Bernardo J, Billingslea AM, Ortiz MF, Seetoo KF, Macauley J, Simons ER. Adherence-dependent calcium signaling in monocytes: induction of a CD14-high phenotype, stimulus-responsive subpopulation. *J Immunol Methods* (1997) 209:165–75. doi: 10.1016/S0022-1759(97)00157-9
20. Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, et al. Movement disorder society-sponsored revision of the unified parkinson's disease rating scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov Disord.* (2008) 23:2129–70. doi: 10.1002/mds.22340
21. Williams-Gray CH, Wijeyekoon RS, Scott KM, Hayat S, Barker RA, Jones JL. Abnormalities of age-related T cell senescence in Parkinson's disease. *J Neuroinflamm.* (2018) 15:166. doi: 10.1186/s12974-018-1206-5
22. Ziegler-Heitbrock L, Hofer TPJ. Toward a refined definition of monocyte subsets. *Front Immunol.* (2013) 4:23. doi: 10.3389/fimmu.2013.00023
23. Swartz RP, Naai D, Vogel CW, Yeager H. Differences in uptake of mycobacteria by human monocytes: a role for complement. *Infect Immun.* (1988) 56:2223–7.
24. Tebo AE, Kremsner PG, Luty AJF. Fcgamma receptor-mediated phagocytosis of Plasmodium falciparum-infected erythrocytes *in vitro*. *Clin Exp Immunol.* (2002) 130:300–6. doi: 10.1046/j.1365-2249.2002.01972.x
25. Tanaka K, Usui Y, Kojo S. Role of serum components in the binding and phagocytosis of oxidatively damaged erythrocytes by autologous mouse macrophages. *Cell Mol Life Sci.* (2001) 58:1727–33. doi: 10.1007/PL00000811
26. Spycher MO, Spycher-Burger M, Späth PJ, Burckhardt JJ. Human serum induced opsonization of immunoglobulin G-coated polystyrene microspheres with complement components C3 and C4 as measured by flow cytometry. *J Immunol Methods* (1991) 145:83–92.
27. Goldknopf IL, Sheta EA, Bryson J, Folsom B, Wilson C, Duty J, et al. Complement C3c and related protein biomarkers in amyotrophic lateral sclerosis and Parkinson's disease. *Biochem Biophys Res Commun.* (2006) 342:1034–9. doi: 10.1016/j.bbrc.2006.02.051
28. Funk N, Wieghofer P, Grimm S, Schaefer R, Bühring H-J, Gasser T, et al. Characterization of peripheral hematopoietic stem cells and monocytes in Parkinson's disease. *Mov Disord.* (2013) 28:392–5. doi: 10.1002/mds.25300
29. Reale M, Iarlori C, Thomas A, Gambi D, Perfetti B, Di Nicola M, et al. Peripheral cytokines profile in Parkinson's disease. *Brain Behav Immun.* (2009) 23:55–63. doi: 10.1016/j.bbi.2008.07.003
30. Bhattacharjee J, Das B, Mishra A, Sahay P, Upadhyay P. Monocytes isolated by positive and negative magnetic sorting techniques show different molecular characteristics and immunophenotypic behaviour. *F1000Res.* (2018) 6:2045. doi: 10.12688/f1000research.12802.3
31. Papa I, Saliba D, Ponzoni M, Bustamante S, Canete PF, Gonzalez-Figueroa P, et al. TFH-derived dopamine accelerates productive synapses in germinal centres. *Nature* (2017) 547:318–23. doi: 10.1038/nature23013
32. Coley JS, Calderon TM, Gaskill PJ, Eugenin EA, Berman JW. Dopamine increases $CD14^{+}CD16^{+}$ monocyte migration and adhesion in the context of substance abuse and HIV neuropathogenesis. *PLoS ONE* (2015) 10:e0117450. doi: 10.1371/journal.pone.0117450
33. Williams-Gray CH, Foltynie T, Brayne CEG, Robbins TW, Barker RA. Evolution of cognitive dysfunction in an incident Parkinson's disease cohort. *Brain* (2007) 130:1787–98. doi: 10.1093/brain/awm111
34. Velseboer DC, de Bie RMA, Wieske L, Evans JR, Mason SL, Foltynie T, et al. Development and external validation of a prognostic model in newly diagnosed Parkinson disease. *Neurology* (2016) 86:986–93. doi: 10.1212/WNL.0000000000002437

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 02 November 2018
doi: 10.3389/fimmu.2018.02541

Interplay Between the Unfolded Protein Response and Immune Function in the Development of Neurodegenerative Diseases

Paulina García-González[1†], *Felipe Cabral-Miranda*[2,3,4,5†], *Claudio Hetz*[2,3,4,6,7*] *and Fabiola Osorio*[1*]

[1] *Laboratory of Immunology and Cellular Stress, Program of Immunology, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile,* [2] *Faculty of Medicine, Biomedical Neuroscience Institute, University of Chile, Santiago, Chile,* [3] *Brain Health and Metabolism, FONDAP Center for Geroscience, Santiago, Chile,* [4] *Program of Cellular and Molecular Biology, Institute of Biomedical Sciences, University of Chile, Santiago, Chile,* [5] *Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil,* [6] *Buck Institute for Research on Aging, Novato, CA, United States,* [7] *Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, United States*

Emerging evidence suggests that the immune and nervous systems are in close interaction in health and disease conditions. Protein aggregation and proteostasis dysfunction at the level of the endoplasmic reticulum (ER) are central contributors to neurodegenerative diseases. The unfolded protein response (UPR) is the main transduction pathway that maintains protein homeostasis under conditions of protein misfolding and aggregation. Brain inflammation often coexists with the degenerative process in different brain diseases. Interestingly, besides its well-described role in neuronal fitness, the UPR has also emerged as a key regulator of ontogeny and function of several immune cell types. Nevertheless, the contribution of the UPR to brain inflammation initiated by immune cells remains largely unexplored. In this review, we provide a perspective on the potential role of ER stress signaling in brain-associated immune cells and the possible implications to neuroinflammation and development of neurodegenerative diseases.

Keywords: UPR, neurodegeneration, immune system, inflammation, protein protein misfolding diseases, ER stress, immune cells, misfolded proteins

OPEN ACCESS

Edited by:
Marco Cosentino,
Università degli Studi dell'Insubria, Italy

Reviewed by:
Paola Perin,
University of Pavia, Italy
Tatsuro Misu,
Tohoku University, Japan

***Correspondence:**
Claudio Hetz
chetz@med.uchile.cl
Fabiola Osorio
fabiolaosorio@med.uchile.cl

[†] *These authors have contributed equally to this work*

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology

Received: *05 September 2018*
Accepted: *15 October 2018*
Published: *02 November 2018*

Citation:
García-González P, Cabral-Miranda F, Hetz C and Osorio F (2018) Interplay Between the Unfolded Protein Response and Immune Function in the Development of Neurodegenerative Diseases. Front. Immunol. 9:2541. doi: 10.3389/fimmu.2018.02541

INTRODUCTION

The Unfolded Protein Response (UPR)

Proteostasis encompasses the dynamic interrelation of processes governing generation and localization of functional proteins (1). Physiological and pathological factors can impair the balance between protein load and protein processing, resulting into accumulation of improperly folded proteins (2, 3). Abnormal protein aggregation is a key feature of several neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) and prion-related disorders amongst others, collectively classified as protein misfolding diseases (PMDs) (4, 5).

Protein misfolding is sensed by dedicated stress-response pathways that include the cytoplasmic heat shock response (HSR) and the unfolded protein response originated in the mitochondria and in the endoplasmic reticulum (ER) (3). Activation of these intracellular mechanisms by the presence of misfolded proteins leads to ameliorating the protein

folding load and resolving proteotoxic stress (1, 3). In this context, the ER is a central node of the proteostasis network controlling folding, processing and trafficking of up to a third of the protein load in the cell (6). The UPR originated in the ER (for now referred as "UPR") is a main intracellular mechanism responsible to safeguard the fidelity of the cellular proteome and for this reason, it will be the main focus of the current review (6, 7). The UPR is an adaptive reaction controlled by three ER-located signal transducers: inositol requiring enzyme 1 (IRE1) α and β, protein kinase R-like ER kinase (PERK) and activating transcription factor 6 (ATF6) alpha and beta (6) (**Figure 1**). Upon activation, these signal transducers activate gene expression programs through specific downstream transcription factors, restoring proteostasis and increasing ER and Golgi biogenesis (6, 8). IRE1α cleaves the mRNA encoding for the X-box binding protein (XBP1), removing a 26 nucleotide intron, which followed by RTCB (RNA 2′,3′-Cyclic Phosphate and 5′-OH ligase) ligation changes the coding reading frame, prompting the translation of a protein with transcription factor activity termed XBP1s (XBP1 spliced) (7). XBP1s controls the expression of genes involved in ER-associated degradation (ERAD), lipid biosynthesis, folding and quality control (9, 10). IRE1α RNase also directly degrades diverse mRNAs and microRNAs through a process termed "Regulated IRE1-Dependent Decay" (RIDD) (11), originally proposed to contribute to alleviating the detrimental effects of ER stress by reducing the protein folding load (12), in addition to regulating inflammation and apoptosis (13). Activation of PERK mediates protein translation shutdown via phosphorylation of eukaryotic initiation factor 2α (P-eIF2α), which also favors selective translation of certain mRNAs encoding proteins involved in cell survival, ER homeostasis and anti-oxidant responses, such as ATF4 and nuclear erythroid related factor 2 (NRF2) (6, 14). ATF6, translocates to the Golgi apparatus where it is cleaved by site-1 and site-2 proteases, releasing a transcription factor that directs the expression of genes encoding ERAD components, ER chaperones and molecules involved in lipid biogenesis (15, 16). XBP1s and ATF6 can also heterodimerize to control selective gene expression patterns (9). Moreover, the activity (signaling amplitude and kinetics) of the three UPR stress sensors is controlled by several cofactors through the assembling of distinct platforms termed the UPRosome (17). Binding of adapter proteins to the IRE1α UPRosome also mediates the crosstalk with other stress pathways including MAP kinases and NF-κB (6). Thus, the UPR integrates information regarding intensity and duration of the stress stimuli toward cell fate control in cells suffering from ER stress.

UPR in Brain Homeostasis And Protein Misfolding Diseases

ER stress signaling has a physiological as well as pathological role in brain function and development (18–20). In neurodegeneration, the UPR influences several aspects including cell survival, synaptic plasticity, axonal regeneration, protein aggregation and control of the secretory pathway (21–23). By mediating synthesis and secretion of the brain-derived neurotrophic factor (BDNF), XBP1s regulates neuronal plasticity at a structural, molecular and behavioral level (18, 24–27). Moreover, postmortem tissue analyses revealed that ER stress markers often co-localize with cells containing protein aggregates in brain of patients affected with PMDs (4, 5, 22, 28). In AD, the expression of Grp78/BiP, PDI and HRD1 is increased in the hippocampus and temporal cortex; and the phosphorylated forms of PERK, IRE1α and eIF2α are found in AD neurons and substantia nigra of PD patients (22, 29, 30). Phosphorylated IRE1α levels directly correlate with the degree of histopathological changes, where most cells showing neurofibrillary tangles exhibit signs of ER stress (31). Furthermore, ER stress signs are also observed in different brain areas in PD patients, a phenomenon also observed in incidental cases of subjects who died without PD symptoms but presented α-synuclein inclusions in the brain (32). Moreover, components of all UPR signaling branches are overexpressed in spinal cord samples of patients with familial and sporadic forms of ALS (33), as well as in striatum, parietal cortex and caudate putamen of HD and Prion disease patients (22, 34–39).

In support of a dual role of UPR in controlling cell fate in neurodegenerative diseases, genetic disruption and pharmacological intervention modulating ER stress signaling revealed that depending on disease type and the UPR component targeted, distinct and even opposite effects are observed [reviewed in (21, 40)]. Conditional deletion of XBP1 in the central nervous system (CNS) provides protective effects through upregulation of autophagy levels, improving motor performance in ALS, PD and Huntington's disease models (35, 37, 41), whereas XBP1 deficiency does not affect Prion pathogenesis *in vivo* (42). Ablation of IRE1α signaling in neurons decreases astrogliosis and amyloid β accumulation in an animal model of AD, correlating with improved neuronal function (31). Conversely, therapeutic gene delivery of active UPR components or ER chaperones to specific brain areas has shown outstanding effects in different animal models of PMDs (43). Different studies have shown that ectopic delivery of XBP1s into the hippocampus restored synaptic plasticity in an AD model (27), promoted axonal regeneration (44), reduced mutant huntingtin aggregation (45) and protected dopaminergic neurons against PD-inducing neurotoxins (41, 46).

Targeting the PERK pathway also provides contradicting results. PERK signaling supports oligodendrocyte survival in animal models of multiple sclerosis (MS) (47) and enhancement of eIF2α phosphorylation is protective in ALS and other models (32, 48), whilst ATF4 deficiency has a detrimental effect in spinal cord injury models, diminishing locomotor recovery following lesion, also impacting oligodendrocyte survival (49). Conditional deletion of PERK in the brain however, improved cognition in an AD model, correlating with decreased amyloidogenesis and restoration of normal expression of plasticity-related proteins (50, 51). Similarly, genetic targeting of CHOP has neuroprotective effects in a PD model, and ATF4 ablation protects against ALS (52, 53). Consistent with this, sustained PERK signaling has been shown to enhance neurodegeneration due to acute repression of synaptic proteins, resulting in

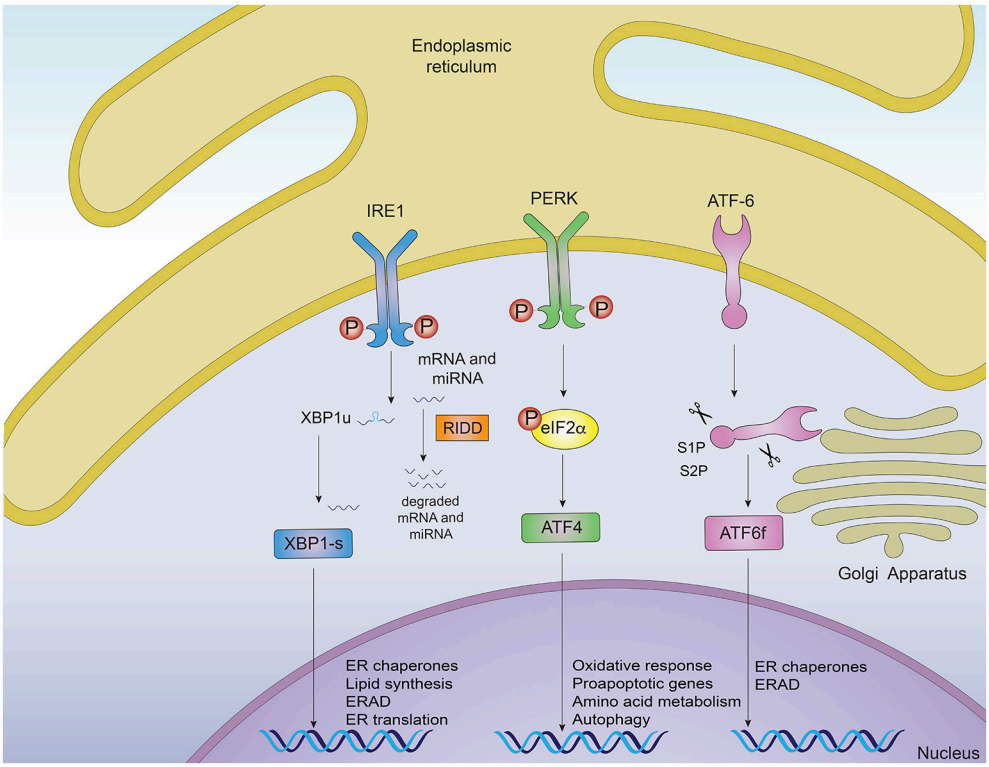


FIGURE 1 | Signaling pathways of the unfolded protein response. Noxious stimuli in cells may induce endoplasmic reticulum (ER) stress and trigger an adaptive response known as the unfolded protein response (UPR), which is controlled by three main ER-resident sensors: IRE1α, PERK and ATF6. Upon ER stress, IRE1α autophosphorylates, leading to the activation of its RNase domain and the processing of the mRNA encoding for XBP1s, a transcriptional factor that upregulates genes involved in protein folding and quality control, in addition to regulating ER/Golgi biogenesis and ER-mediated degradation (ERAD). Additionally, IRE1α RNase also degrades a subset of specific RNAs and microRNAs, a process termed Regulated IRE1α-Dependent Decay (RIDD). The second ER sensor, PERK, phosphorylates the translation of the eukaryotic initiation factor eIF2α, decreasing the synthesis of proteins and the overload of misfolded proteins at the ER. PERK phosphorylation also leads to the specific translation of ATF4, a transcription factor that promotes the expression of genes related to amino acid metabolism, anti-oxidant response, autophagy and apoptosis. The third UPR sensor, ATF6, is a type II ER transmembrane protein that encodes a bZIP transcriptional factor in its cytosolic domain. Following ER stress, ATF6 translocates to the Golgi apparatus where it is processed, releasing a transcription factor which directs the expression of genes encoding ER chaperones, ERAD components and molecules involved in lipid biogenesis.

abnormal neuronal function, as demonstrated through PERK inhibitors in Prion disease (54), frontotemporal dementia (48) and PD models (32). ATF6, on the other hand, protected dopaminergic neurons in another PD model, by upregulating ER chaperones and ERAD components (55, 56). Overall, UPR mediators have a pivotal role in the progression of various PMDs, nurturing the hypothesis that UPR components could be used as therapeutic targets in neurodegeneration (21, 22, 43).

UPR in Neuroinflammation

Immune surveillance is an active process in the brain. The mammalian CNS harbors several subtypes of leukocytes, which display physiological roles related to tissue homeostasis and regulation of the inflammatory response (57, 58). However, if unrestrained, inflammation can have detrimental effects in the CNS, contributing to the type of tissue malfunction that precedes pathological processes (59). During neuroinflammation, the immune response in the CNS is drastically altered, and it is typified by activation of resident microglia and invasion of peripheral immune cells into the parenchyma, including granulocytes, monocytes and, in pathologies like multiple sclerosis, lymphocytes (60–63). Interestingly, the UPR has shown to regulate inflammation in peripheral tissues, emerging as an interesting candidate for targeting CNS-associated inflammation in a field that remains largely unexplored. Thus, in addition to the well-described role of the UPR in neuronal fitness, it is also plausible that UPR activation in CNS-associated immune cells could contribute to modulating PMD development.

One hallmark of neuroinflammation is the presence of tumor necrosis factor (TNF), interleukin (IL)-1β, and IL-6 in brain, cerebrospinal fluid (CSF) and serum of patients with AD, PD and HD (63–65). Production of pro-inflammatory cytokines across tissues depends on activation of innate immune sensors (known as pattern recognition receptors, PRRs) specialized in the recognition of microbes and stress signals (63). In the brain, PRRs can promote pro-inflammatory cytokine production upon recognition of "neurodegeneration associated molecular

patterns" (NAMPs) that consists in CNS-specific danger signals such as extracellular protein aggregates, molecules exposed by dying neurons, lipid degradation byproducts and myelin debris, among others (66). The most relevant PRRs associated to the development of PMDs are TLRs (Toll-like Receptors) and NLR (Nucleotide-binding domain, leucine-rich repeat containing) inflammasomes (63). These receptors are broadly expressed in CNS-myeloid cells including microglia, macrophages and infiltrating cells such as monocytes and dendritic cells (DCs) (63, 67). Interestingly, PRR-signaling and the UPR converge on several levels for amplification of inflammatory responses via activation of NF-kB, IRF-3, JNK and JAK/STAT modules (68–71). Signaling via TLR2 and TLR4 induces ER stress in peripheral macrophages and activates IRE1α and XBP1s, which in turn is required to increase production of IL-6 and TNF, thus connecting activation of the IRE1α-XBP1s branch of the UPR with TLR-dependent pro-inflammatory programs (68). In the CNS, misfolded α-synuclein and Fibrillar Aβ, characteristic in patients with PD and AD, can be sensed by TLR1/2 and TLR4, further promoting inflammation (63) (**Figure 2**). Moreover, injection of lipopolysaccharide (LPS), an agonist of TLR4, into the *substantia nigra* induces dopaminergic neuronal death resembling animal PD models (73). LPS-induced neurotoxicity and LPS-derived inducible nitric oxide synthase (iNOS) expression was shown to be mediated by the UPR related chaperone BiP/Grp78 and NF-kB (74, 75). Correspondingly, *Tlr4* null mice are protected from PD in a mouse model induced with neurotoxins (63, 76). Overall, TLR pathways activating the IRE1α-XBP1s axis are relevant drivers of PMDs, although the precise contribution of this UPR branch to TLR-induced neuroinflammation remains to be formally demonstrated.

Another PRR relevant in neurodegeneration modulated by the UPR, is the NLRP3 (NLR Family Pyrin Domain-Containing-3) inflammasome, a multimeric protein complex composed of the NLRP3 sensor, the adaptor ASC and activated caspase 1, which mediates the proteolytic activation of IL-1β and IL-18 and promotes a type of inflammatory cell death referred to as pyroptosis (63). In the brain, the NLRP3 inflammasome is activated by amyloid β and α-synuclein aggregates (63). The relevance of this protein complex is underscored by studies with *Nlrp3* deficient mice carrying mutations associated with familiar AD, which are protected from the disease (77). On a mechanistic level, the interplay between the UPR and inflammasome activation has been connected to IRE1α signaling (78), where the RNase domain of IRE1α increases the expression of TXNIP, an activator of the NLRP3 inflammasome, through degradation of the TXNIP-destabilizing microRNA miR-17 (78) (**Figure 2**). Considering the relevance of the NLRP3 inflammasome in AD progression and its dependence on IRE1α endonuclease, it is tempting to speculate that IRE1α activation in CNS-resident myeloid cells may contribute to the development of AD (79–84). Additionally, the B-class scavenger receptor CD36, upon recognition of amyloid β fibrils, forms a complex with TLR4/6, which triggers activation of the NLRP3 inflammasome, promoting cytokine and ROS production (67, 85).

On the other hand, in models of peripheral nerve damage, XBP1 expression has been shown to enhance nerve regeneration after injury, involving increased expression of the chemokine MCP-1 and macrophage infiltration, essential to remove myelin debris and allow axonal regeneration (44). PERK expression correlates with astroglial activation and production of IL-6 and the chemokines CCL2 and CCL20, which promotes microglial activation (71, 86). In spinal cord injury, ATF4 deficiency reduced microglial activation, which is associated with altered levels of IL-6, TNFα, and IL-1β (44–49). Similarly, ATF6 deficiency in the context of PD induced by neurotoxins leads to suppression of astroglial activation and decreased production of BDNF and anti-oxidative genes, such as heme oxygenase-1 (HO-1) and xCT (56). To sum up, ER stress and inflammation are both prevalent in many neurodegenerative diseases and NAMPs can alter neuronal function as well as promote inflammation through the activation of innate defense mechanisms of immune cells in the CNS, which can be modulated by UPR activity and vice versa.

Immune Targets of the UPR in the Central Nervous System

Although it is clear that inflammation contributes to neurodegeneration (61), there has been limited knowledge about the homeostasis of immune cells residing in the CNS. Recent technological advances in single cell analysis have provided insights into the identification and characterization of the vast diversity of immune cell lineages present in the healthy and pathogenic brain (61, 62). The potential role of the UPR in immune cell lineages in the CNS is illustrated in **Figure 2**.

Microglia

Microglia is the CNS-resident macrophage and most prominent myeloid cell in the brain (87). Microglia fine-tunes the development of neuronal circuits, neurogenesis and synaptic plasticity through the production of neurotrophic factors (88, 89). Given that several PRRs that signal via IRE1α and XBP1s such as TLR1/2 and TLR4, the NLRP3 inflammasome and nucleic acid sensors are expressed in this cell lineage, it is plausible that microglial XBP1s activation may contribute to the initiation of neuroinflammation. The ATF6 branch has also been associated with microglial activation and production of inflammatory mediators via NF-kB (90). Furthermore, although long conceived as a homogeneous cell type that becomes destructive in neurodegeneration (62), comprehensive single cell RNA analysis has demonstrated that a subset known as "disease-associated microglia" plays an important role in several CNS diseases including AD, ALS, MS and also in aging (62, 91–93). Thus, it is vital to elucidate whether protective microglial populations engage the UPR upon innate recognition of NAMPs, and whether microglial UPR is an intrinsic mechanism of sensing danger in the CNS.

Border Associated Macrophages

Border associated macrophages (BAMs) are a recently characterized population distinct from microglia and from infiltrating monocyte-derived macrophages, which display high heterogeneity and are classified per phenotype, development and location in the CNS (62, 94). Single cell analysis, fate

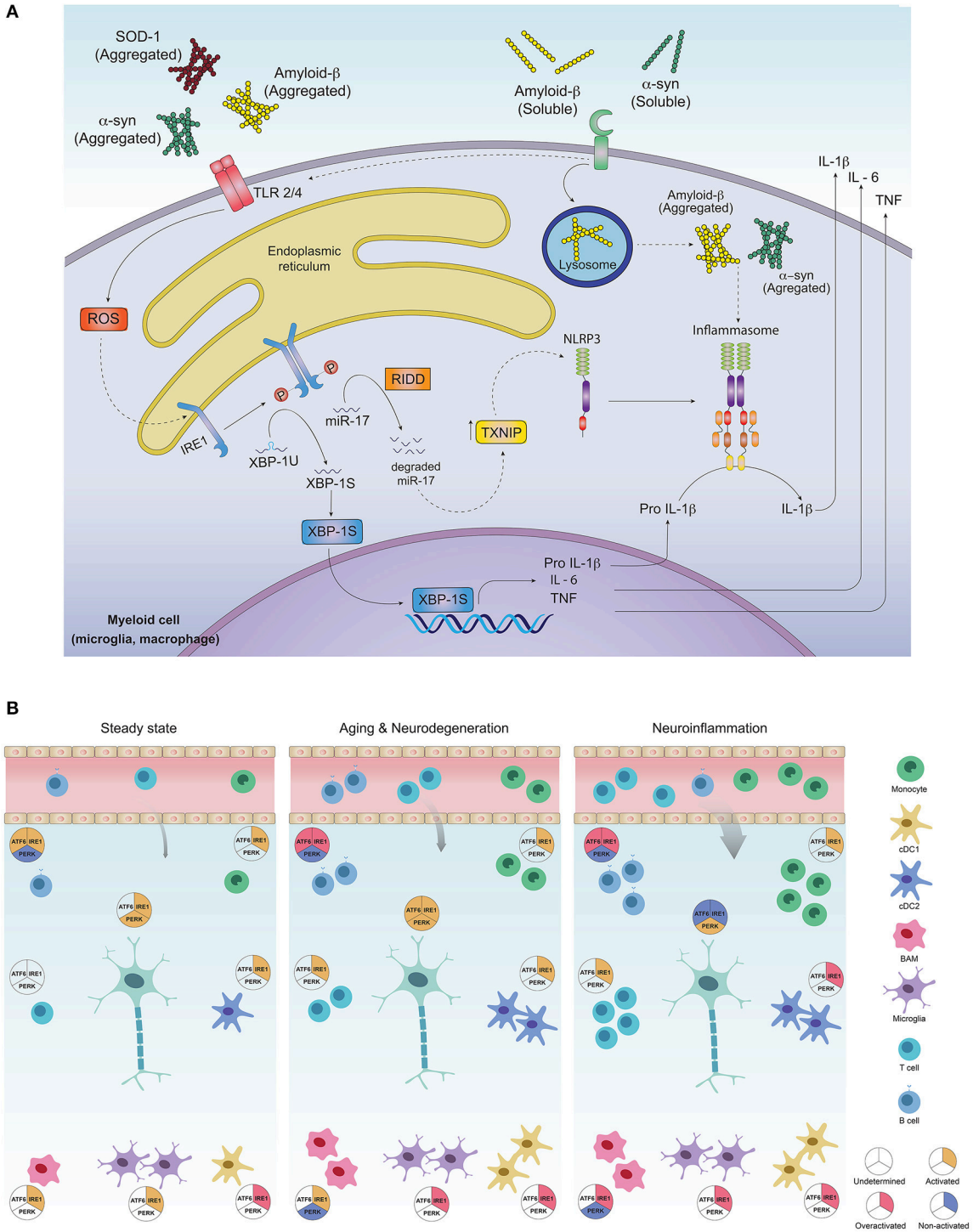


FIGURE 2 | Activation of the unfolded protein response in CNS-residing immune cells may contribute to neuroinflammation and PMDs development. **(A)** Protein aggregates can promote inflammation via triggering of innate receptors and activation of the UPR. Neurodegeneration associated molecular patterns (NAMPS) such as protein aggregates are recognized by pattern recognition receptors (TLRs and PRRs) present on immune cells and signal through ROS production, which in turn could activate the IRE1α/XBP1s axis for co-transcriptional activation of IL-6, TNF and IL-1β. On the other hand, through RIDD, IRE1α induces degradation of the TXNIP-destabilizing microRNA mir-17, allowing activation of the NLRP3 inflammasome and processing of IL-1β into its active form. **(B)** Most of the immune cell lineages residing in the healthy and pathogenic brain are known targets of the UPR in peripheral tissues. In **steady state**, the most abundant immune

(Continued)

FIGURE 2 | cells in the brain are microglia, which along with border associated macrophages ("BAMs") and dendritic cells act as sentinels, sampling the environment and clearing cell debris, maintaining CNS homeostasis. Except for dendritic cells and macrophages, which exhibit IRE1α/XBP1s activation, little is known about UPR activation in additional myeloid subsets, although microglia, macrophages and monocytes could potentially activate this axis downstream of PRR signaling. While very rare, B and T cells have been identified in the steady state brain, and activation of IRE1α/XBP1s has been proposed to be critical for their differentiation and activation. ATF6 axis is also necessary for B cell development and activation whilst absence of PERK contributes to plasma cell differentiation and immunoglobulin synthesis. Basal activation of UPR in neurons is still a matter of debate in literature as the function of IRE1α and PERK pathways has just begun to be understood in the context of normal neuronal physiology (72). In **aging and neurodegeneration,** the number of immune cells within the brain increases, due to higher cell activation as well as blood brain barrier infiltrates. Extracellular protein aggregation promotes activation of immune cells via PRRs, in addition to inducing ER stress and activation of the UPR, mainly the IRE1α/XBP1s axis. Microglia and dendritic cells become more activated, with higher production of pro-inflammatory and oxidative mediators and loss of their protein clearance function. This is further aggravated by antibodies against CNS-derived antigens by B cells accumulated in the CSF, mediated by the activation of IRE1α and ATF6 signaling. Activation of infiltrating T cells reactive to α-synuclein, amyloid-β and myelin constituents further amplify inflammation, resulting in more protein aggregation and neuronal loss. In neurons, UPR triggering may elicit both, adaptive or neurodegenerative responses, since all three UPR pathways are engaged in brain diseases and have been found to be altered during the normal aging process. Different inducers of **neuroinflammation**, have shown to engage the UPR in neurons and promote a greater inflammatory response due to immune cell infiltration, mainly B and T cells. The cDC1 subset of dendritic cells could activate IRE1α for cross presentation of antigens to infiltrating $CD8^+$ T cells, and cDC2 as well as monocyte-derived DCs may set an inflammatory environment through cytokine secretion and activation of infiltrating $CD4^+$ T cells. Macrophages and microglia also become highly activated and could tune IRE1α/XBP1s upon recognition of NAMPs. Inflammatory mediators such as cytokines prime axonal destruction and neuronal loss. It remains to be addressed weather UPR triggering in these cells corresponds to a homeostatic (adaptive) response, or a terminal (neurodegenerative) response due to sustained unresolved ER stress.

mapping and parabiosis experiments revealed that these cells express distinct surface markers and differentially populate the pia mater, perivascular space, choroid plexus and dura mater (62, 94). Most of these subsets sample the environment, clear apoptotic cells and amyloid β plaques, and help maintaining CNS homeostasis in steady state. Up to date, there is no evidence available on the extent of UPR activation in BAMs. However, it has been described that splenic F4/80 macrophages display basal levels of IRE1α RNase activity and upon bacterial infection, peripheral macrophages induce XBP1s for enhancing cytokine production in a mechanism mediated by TLRs and reactive oxygen species (68, 95). However, whether CNS macrophages show a functional analogy to peripheral macrophages and also engage the IRE1α-XBP1s branch upon recognition of NAMPs (68) remains undetermined.

Dendritic Cells

DCs are major APCs in the CNS, acting as sentinels between brain and periphery (87, 96–99). Steady-state CNS is populated by most DC subtypes, including plasmacytoid DCs (pDCs), and conventional DC type 1 (cDC1) and type 2 (cDC2) (62). These cells locate in the choroid plexus, pia mater and dura mater, but not in the perivascular space, suggesting that these compartments may serve as entry sites for MHC-dependent T cells (62, 96, 97). Importantly, DCs are key targets of the UPR. XBP1s is constitutively expressed by DCs and high XBP1s is a hallmark of cDC1s across tissues, although the CNS remains to be examined (95, 100, 101). Furthermore, cDC1s activate the IRE1α -XBP1 axis for development, survival in mucosal tissues and cross-presentation of antigens to $CD8^+$ T cells, which may be of relevance in infections with neurotropic viruses (2, 102). In addition, cDC1s are highly sensitive to perturbations in XBP1 signaling and counter activate RIDD upon XBP1 loss (95, 101). The implication of RIDD and XBP1s signaling in DC subtypes in the CNS has not been explored so far but relevant aspects downstream of XBP1s and RIDD may encompass cytokine production upon recognition of protein aggregates, cell survival and cross-presentation of antigens to $CD8^+$ T cells.

Lymphocytes

T and B cells survey the steady-state CNS exerting a neuroprotective role, but can become pathogenic under unresolved inflammation (57, 103–106). T cell numbers have been found to be increased in AD, PD, ALS and MS, and to contribute both to inflammation and neuronal dysfunction as well as to deferring inflammatory responses leading to nerodegeneration (107, 108). The immune response elicited by these cells in the CNS depends on their functional phenotype, although observations regarding cell number and T cell subset involved varies between different disease types and model of study (108–113). UPR activation in T cells is not completely elucidated, however the IRE1α-XBP1s branch has shown to regulate cell differentiation and cytokine production in $CD8^+$ and $CD4^+$ T cells under infection and chronic ER stress (114–118). During neuroinflammation and aging, B cells play a pathogenic role by producing pro-inflammatory cytokines, promoting effector T cells and activating macrophages via Fc receptors (62, 119–123). B cell development, activation and differentiation is critically regulated by IRE1α-XBP1s and ATF6, whilst absence of PERK favors plasma cell differentiation and immunoglobulin synthesis (124–128).

Overall, as proposed on **Figure 2**, activation of UPR components could occur in CNS-residing and infiltrating immune cells upon PRR recognition of protein aggregates, or due to noxious threats. The IRE1α-XBP1s axis has a key role in immune cell development from hematopoietic progenitors, cell survival and effector function, and it could be activated by NAMPs through PRR signaling in microglia, macrophages or dendritic cells, inducing cell maturation and activation (66, 68, 88, 97). The PERK pathway in contrast, is mostly deactivated to allow immune cells to fulfill their function under different inflammatory settings without going through apoptosis. In AD or PD however, sustained stimulation triggered by amyloid β or α-synuclein aggregates could lead to a dysfunctional activated phenotype associated to defective clearance and increased production of inflammatory mediators. This process could, in turn, attract more immune cells that

exert a neurotoxic effect, promoting the accumulation of more protein aggregates, axonal destruction and neuronal malfunction (129, 130). Under this chronic ER stress, UPR signaling would be expected to be highly activated in CNS-related immune cells, in line with observations in brain samples of patients. Nevertheless, it remains to be addressed whether the UPR output in CNS-associated immune cells proves to be beneficial or detrimental for the development of PMDs, as is the case of neurons and astrocytes (131, 132).

CONCLUDING REMARKS

The interplay between the UPR, the immune system and the CNS in neurodegenerative diseases remains in its early stages. Intensive research will be required to accurately understand the role of ER stress in the immune-related aspects of CNS pathology and to determine whether UPR signaling in immune cells answers to a homeostatic or a terminal fate. It is also important to keep in mind the potential differences between human and mice immune cell types, since most knowledge gained in this matter emerges from studies in murine models. Through our knowledge on the UPR role in peripheral immunity and neurodegeneration models, better access to human samples and the advent of novel analytic tools for identification of the diversity of cell lineages, the cell-specific contribution of the UPR to neural and CNS-associated immune cells will begin to be elucidated, generating valuable knowledge that may provide therapeutic opportunities.

AUTHOR CONTRIBUTIONS

All authors read and approved the final version of the manuscript. PG-G and FC-M contributed equally to the work. PG-G, FO, FC-M, and CH participated in manuscript conception and design.

FUNDING

This work was primary funded by FONDECYT 1161212 and an International Research Scholar grant from the Howard Hughes Medical Institute # 55008744 (FO); and by FONDECYT 1140549, FONDAP program 15150012, Millennium Institute P09-015-F, European Commission R&D MSCA-RISE 734749 (CH). We thank the support from Michael J Fox Foundation for Parkinson's Research–Target Validation grant 9277, FONDEF ID16I10223, FONDEF D11E1007, US Office of Naval Research-Global N62909-16-1-2003, U.S. Air Force Office of Scientific Research FA9550-16-1-0384, ALSRP Therapeutic Idea Award AL150111, Muscular Dystrophy Association 382453, and CONICYT-Brazil 441921/2016-7 (CH), We also thank FONDECYT for postdoctoral fellowships 3180195 and from FONDAP program 15150012 (FC-M).

ACKNOWLEDGMENTS

We thank members of the laboratory of immunology and cellular stress for helpful discussions.

REFERENCES

1. Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. *Science* (2008) 319:916–9. doi: 10.1126/science.1141448
2. Tavernier SJ, Lambrecht BN, Janssens S. The unfolded protein response in the immune cell development: putting the caretaker in the driving seat. *Curr Top Microbiol Immunol.* (2018) 414:45–72. doi: 10.1007/82_2017_1
3. Taylor RC, Berendzen KM, Dillin A. Systemic stress signalling: understanding the cell non-autonomous control of proteostasis. *Nat Rev Mol Cell Biol.*(2014) 15:211. doi: 10.1038/nrm3752
4. Cabral-Miranda F, Hetz C. ER Stress and neurodegenerative disease: a cause or effect relationship? *Curr Top Microbiol Immunol*. (2018) 414:131–57. doi: 10.1007/82_2017_52
5. Soto C. Unfolding the role of protein misfolding in neurodegenerative diseases. *Nat Rev Neurosci.* (2003) 4:49–60. doi: 10.1038/nrn1007
6. Hetz C, Papa FR. The unfolded protein response and cell fate control. *Mol. Cell* (2018) 69:169–81. doi: 10.1016/j.molcel.2017.06.017
7. Hetz C, Chevet E, Oakes SA. Proteostasis control by the unfolded protein response. *Nat Cell Biol*. (2015) 17:829–38. doi: 10.1038/ncb3221
8. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* (2011) 334:1081–6. doi: 10.1126/science.1209038
9. Shoulders MD, Ryno LM, Genereux JC, Moresco JJ, Tu PG, Wu C, et al. Stress-independent activation of XBP1s and/or ATF6 reveals three functionally diverse ER proteostasis environments. *Cell Rep*. (2013) 3:1279–92. doi: 10.1016/j.celrep.2013.03.024
10. Lee AH, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol*. (2003) 23:7448–59. doi: 10.1128/MCB.23.21.7448-7459.2003
11. Maurel M, Chevet E, Tavernier J, Gerlo S. Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem Sci.* (2014) 39:245–54. doi: 10.1016/j.tibs.2014.02.008
12. Coelho DS, Domingos PM. Physiological roles of regulated Ire1 dependent decay. *Front Genet.* (2014) 5:76. doi: 10.3389/fgene.2014.00076
13. Oakes SA, Papa FR. The role of endoplasmic reticulum stress in human pathology. *Annu Rev Pathol*. (2015) 10:173–94. doi: 10.1146/annurev-pathol-012513-104649
14. Kranz P, Neumann F, Wolf A, Classen F, Pompsch M, Ocklenburg T, et al. PDI is an essential redox-sensitive activator of PERK during the unfolded protein response (UPR). *Cell Death Dis*. (2017) 8:e2986. doi: 10.1038/cddis.2017.369
15. Yamamoto K, Sato T, Matsui T, Sato M, Okada T, Yoshida H, et al. Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6α and XBP1. *Dev Cell* (2007) 13:365–76. doi: 10.1016/j.devcel.2007.07.018
16. Hillary RF, FitzGerald U. A lifetime of stress: ATF6 in development and homeostasis. *J Biomed Sci.* (2018) 25:48. doi: 10.1186/s12929-018-0453-1
17. Hetz C, Glimcher LH. Fine-tuning of the unfolded protein response: assembling the IRE1alpha interactome. *Mol Cell* (2009) 35:551–61. doi: 10.1016/j.molcel.2009.08.021
18. Martínez G, Vidal RL, Mardones P, Serrano FG, Ardiles AO, Wirth C, et al. Regulation of memory formation by the transcription factor XBP1. *Cell Rep*. (2016) 14:1382–94. doi: 10.1016/j.celrep.2016.01.028
19. Costa-Mattioli M, Gobert D, Stern E, Gamache K, Colina R, Cuello C, et al. eIF2α phosphorylation bidirectionally regulates the switch from short- to long-term synaptic plasticity and memory. *Cell* (2007) 129:195–206. doi: 10.1016/j.cell.2007.01.050
20. Martínez G, Khatiwada S, Costa-Mattioli M, Hetz C. ER proteostasis control of neuronal physiology and synaptic function. *Trends Neurosci*. (2018) 41:610–24. doi: 10.1016/j.tins.2018.05.009

21. Hetz C, Saxena S. ER stress and the unfolded protein response in neurodegeneration. *Nat Rev Neurol.* (2017) 13:477–91. doi: 10.1038/nrneurol.2017.99
22. Scheper W, Hoozemans JJ. The unfolded protein response in neurodegenerative diseases: a neuropathological perspective. *Acta Neuropathol.* (2015) 130:315–31. doi: 10.1007/s00401-015-1462-8
23. Smith HL, Mallucci GR. The unfolded protein response: mechanisms and therapy of neurodegeneration. *Brain* (2016) 139(Pt 8):2113–21. doi: 10.1093/brain/aww101
24. Hayashi A, Kasahara T, Iwamoto K, Ishiwata M, Kametani M, Kakiuchi C, et al. The role of brain-derived neurotrophic factor (BDNF)-induced XBP1 splicing during brain development. *J Biol Chem.* (2007) 282:34525–34. doi: 10.1074/jbc.M704300200
25. Hayashi A, Kasahara T, Kametani M, Kato T. Attenuated BDNF-induced upregulation of GABAergic markers in neurons lacking Xbp1. *Biochem Biophys Res Commun.* (2008) 376:758–63. doi: 10.1016/j.bbrc.2008.09.059
26. Saito A, Cai L, Matsuhisa K, Ohtake Y, Kaneko M, Kanemoto S, et al. Neuronal activity-dependent local activation of dendritic unfolded protein response promotes expression of brain-derived neurotrophic factor in cell soma. *J Neurochem.* (2018) 144:35–49. doi: 10.1111/jnc.14221
27. Cissé M, Duplan E, Lorivel T, Dunys J, Bauer C, Meckler X, et al. The transcription factor XBP1s restores hippocampal synaptic plasticity and memory by control of the Kalirin-7 pathway in Alzheimer model. *Mol Psychiatry* (2017) 22:1562–75. doi: 10.1038/mp.2016.152
28. Hetz C, Mollereau B. Disturbance of endoplasmic reticulum proteostasis in neurodegenerative diseases. *Nat Rev Neurosci.* (2014) 15:233–49. doi: 10.1038/nrn3689
29. Hoozemans JJ, Veerhuis R, Van Haastert ES, Rozemuller JM, Baas F, Eikelenboom P, et al. The unfolded protein response is activated in Alzheimer's disease. *Acta Neuropathol.* (2005) 110:165–72. doi: 10.1007/s00401-005-1038-0
30. Hoozemans JJ, van Haastert ES, Eikelenboom P, de Vos RA, Rozemuller JM, Scheper W, et al. Activation of the unfolded protein response in Parkinson's disease. *Biochem Biophys Res Commun.* (2007) 354:707–11. doi: 10.1016/j.bbrc.2007.01.043
31. Duran-Aniotz C, Cornejo VH, Espinoza S, Ardiles ÁO, Medinas DB, Salazar C, et al. IRE1 signaling exacerbates Alzheimer's disease pathogenesis. *Acta Neuropathol.* (2017) 134:489–506. doi: 10.1007/s00401-017-1694-x
32. Mercado G, Castillo V, Soto P, López N, Axten JM, Sardi SP, et al. Targeting PERK signaling with the small molecule GSK2606414 prevents neurodegeneration in a model of Parkinson's disease. *Neurobiol Dis.* (2018) 112:136–48. doi: 10.1016/j.nbd.2018.01.004
33. Medinas DB, Valenzuela V, Hetz C. Proteostasis disturbance in amyotrophic lateral sclerosis. *Hum Mol Genet.* (2017) 26:R91–104. doi: 10.1093/hmg/ddx274
34. Ilieva EV, Ayala V, Jové M, Dalfó E, Cacabelos D, Povedano M, et al. Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. *Brain* (2007) 130(Pt 12):3111–23. doi: 10.1093/brain/awm190
35. Hetz C, Thielen P, Matus S, Nassif M, Court F, Kiffin R, et al. XBP-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy. *Genes Dev.* (2009) 23:2294–306. doi: 10.1101/gad.1830709
36. Atkin JD, Farg MA, Walker AK, McLean C, Tomas D, Horne MK. Endoplasmic reticulum stress and induction of the unfolded protein response in human sporadic amyotrophic lateral sclerosis. *Neurobiol Dis.* (2008) 30:400–7. doi: 10.1016/j.nbd.2008.02.009
37. Vidal RL, Figueroa A, Court FA, Thielen P, Molina C, Wirth C, et al. Targeting the UPR transcription factor XBP1 protects against Huntington's disease through the regulation of FoxO1 and autophagy. *Hum Mol Genet.* (2012) 21:2245–62. doi: 10.1093/hmg/dds040
38. Fernandez-Fernandez MR, Ferrer I, Lucas JJ. Impaired ATF6alpha processing, decreased Rheb and neuronal cell cycle re-entry in Huntington's disease. *Neurobiol Dis.* (2011) 41:23–32. doi: 10.1016/j.nbd.2010.08.014
39. Medinas DB, Rozas P, Martínez Traub F, Woehlbier U, Brown RH, Bosco DA, et al. Endoplasmic reticulum stress leads to accumulation of wild-type SOD1 aggregates associated with sporadic amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA.* (2018) 115:8209–14 doi: 10.1073/pnas.1801109115
40. Remondelli P, Renna M. The endoplasmic reticulum unfolded protein response in neurodegenerative disorders and its potential therapeutic significance. *Front Mol Neurosci.* (2017) 10:187. doi: 10.3389/fnmol.2017.00187
41. Valdés P, Mercado G, Vidal RL, Molina C, Parsons G, Court FA, et al. Control of dopaminergic neuron survival by the unfolded protein response transcription factor XBP1. *Proc Natl Acad Sci USA.* (2014) 111:6804–9. doi: 10.1073/pnas.1321845111
42. Hetz C, Lee AH, Gonzalez-Romero D, Thielen P, Castilla J, Soto C et al. Unfolded protein response transcription factor XBP-1 does not influence prion replication or pathogenesis. *Proc Natl Acad Sci USA.* (2008) 105:757–62. doi: 10.1073/pnas.0711094105
43. Valenzuela V, Jackson KL, Sardi SP, Hetz C. Gene therapy strategies to restore ER proteostasis in disease. *Mol Ther.*(2018) 26:1404–13 doi: 10.1016/j.ymthe.2018.04.004
44. Oñate M, Catenaccio A, Martínez G, Armentano D, Parsons G, Kerr B, et al. Activation of the unfolded protein response promotes axonal regeneration after peripheral nerve injury. *Sci Rep.* (2016) 6:21709. doi: 10.1038/srep21709
45. Zuleta A, Vidal RL, Armentano D, Parsons G, Hetz C. AAV-mediated delivery of the transcription factor XBP1s into the striatum reduces mutant Huntingtin aggregation in a mouse model of Huntington's disease. *Biochem Biophys Res Commun.* (2012) 420:558–63. doi: 10.1016/j.bbrc.2012.03.033
46. Sado M, Yamasaki Y, Iwanaga T, Onaka Y, Ibuki T, Nishihara S, et al. Protective effect against Parkinson's disease-related insults through the activation of XBP1. *Brain Res.* (2009) 1257:16–24. doi: 10.1016/j.brainres.2008.11.104
47. Clayton BLL, Popko B. Endoplasmic reticulum stress and the unfolded protein response in disorders of myelinating glia. *Brain Res.* (2016) 1648(Pt B):594–602. doi: 10.1016/j.brainres.2016.03.046.
48. Hughes D, Mallucci GR. The unfolded protein response in neurodegenerative disorders - therapeutic modulation of the PERK pathway. *Febs J.* (2018) doi: 10.1111/febs.14422. [Epub ahead of print].
49. Valenzuela V, Collyer E, Armentano D, Parsons GB, Court FA, Hetz C. Activation of the unfolded protein response enhances motor recovery after spinal cord injury. *Cell Death Dis.* (2012) 3:e272. doi: 10.1038/cddis.2012.8
50. Ma T, Trinh MA, Wexler AJ, Bourbon C, Gatti E, Pierre P, et al. Suppression of eIF2α kinases alleviates AD-related synaptic plasticity and spatial memory deficits. *Nat Neurosci.* (2013) 16:1299–305. doi: 10.1038/nn.3486
51. Halliday M, Hughes D, Mallucci GR. Fine-tuning PERK signaling for neuroprotection. *J Neurochem.* (2017) 142:812–26. doi: 10.1111/jnc.14112
52. Matus S, Lopez E, Valenzuela V, Nassif M, Hetz C. Functional contribution of the transcription factor ATF4 to the pathogenesis of amyotrophic lateral sclerosis. PLoS ONE (2013) 8:e66672. doi: 10.1371/journal.pone.0066672
53. Silva RM, Ries V, Oo TF, Yarygina O, Jackson-Lewis V, Ryu EJ, et al. CHOP/GADD153 is a mediator of apoptotic death in substantia nigra dopamine neurons in an *in vivo* neurotoxin model of parkinsonism. *J Neurochem.* (2005) 95:974–86. doi: 10.1111/j.1471-4159.2005.03428.x
54. Moreno JA, Halliday M, Molloy C, Radford H, Verity N, Axten JM, et al. Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. *Sci Transl Med* (2013) 5:206ra138. doi: 10.1126/scitranslmed.3006767
55. Egawa N, Yamamoto K, Inoue H, Hikawa R, Nishi K, Mori K, et al. The endoplasmic reticulum stress sensor, ATF6alpha, protects against neurotoxin-induced dopaminergic neuronal death. *J Biol Chem.* (2011) 286:7947–57. doi: 10.1074/jbc.M110.156430
56. Hashida K, Kitao Y, Sudo H, Awa Y, Maeda S, Mori K, et al. ATF6alpha promotes astroglial activation and neuronal survival in a chronic mouse model of Parkinson's Disease. PLOS ONE (2012) 7:e47950. doi: 10.1371/journal.pone.0047950
57. Tanabe S, Yamashita T. The role of immune cells in brain development and neurodevelopmental diseases. *Int Immunol.* (2018) 30:437–44 doi: 10.1093/intimm/dxy041
58. Schwartz M, Kipnis J, Rivest S, Prat A. How do immune cells support and shape the brain in health, disease, and aging? *J Neurosci.* (2013) 33:17587–96. doi: 10.1523/JNEUROSCI.3241-13.2013
59. Medzhitov R. Origin and physiological roles of inflammation. *Nature* (2008) 454:428–35. doi: 10.1038/nature07201

60. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol*. (2015) 15:545–58. doi: 10.1038/nri3871
61. Kempuraj D, Thangavel R, Selvakumar GP, Zaheer S, Ahmed ME, Raikwar SP, et al. Brain and peripheral atypical inflammatory mediators potentiate neuroinflammation and neurodegeneration. *Front Cell Neurosci*. (2017) 11:216. doi: 10.3389/fncel.2017.00216
62. Mrdjen D, Pavlovic A, Hartmann FJ, Schreiner B, Utz SG, Leung BP, et al. High-dimensional single-cell mapping of central nervous system immune cells reveals distinct myeloid subsets in health, aging, and disease. *Immunity* (2018) 48:599. doi: 10.1016/j.immuni.2018.02.014
63. Labzin LI, Heneka MT, Latz E. Innate immunity and neurodegeneration. *Annu Rev Med*. (2018) 69:437–49. doi: 10.1146/annurev-med-050715-104343
64. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. *Nat Rev Immunol*. (2014) 14:463–77. doi: 10.1038/nri3705
65. Becher B, Spath S, Goverman J. Cytokine networks in neuroinflammation. *Nat Rev Immunol*. (2017) 17:49–59. doi: 10.1038/nri.2016.123
66. Deczkowska A, Keren-Shaul H, Weiner A, Colonna M, Schwartz M, Amit I. Disease-associated microglia: a universal immune sensor of neurodegeneration. *Cell* (2018) 173:1073–81. doi: 10.1016/j.cell.2018.05.003
67. El Khoury JB, Moore KJ, Means TK, Leung J, Terada K, Toft M, et al. CD36 mediates the innate host response to β-Amyloid. *J Exp Med*. (2003) 197:1657–66. doi: 10.1084/jem.20021546
68. Martinon F, Chen X, Lee AH, Glimcher LH. TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. *Nat Immunol*. (2010) 11:411–8. doi: 10.1038/ni.1857
69. Moretti J, Roy S, Bozec D, Martinez J, Chapman JR, Ueberheide B, et al. STING Senses microbial viability to orchestrate stress-mediated autophagy of the endoplasmic reticulum. *Cell* (2017) 171:809–23.e13. doi: 10.1016/j.cell.2017.09.034
70. Beisel C, Ziegler S, Martrus Zapater G, Chapel A, Griesbeck M, Hildebrandt H, et al. TLR7-mediated activation of XBP1 correlates with the IFNalpha production in humans. *Cytokine* (2017) 94:55–8. doi: 10.1016/j.cyto.2017.04.006
71. Meares GP, Liu Y, Rajbhandari R, Qin H, Nozell SE, Mobley JA, et al. PERK-dependent activation of JAK1 and STAT3 contributes to endoplasmic reticulum stress-induced inflammation. *Mol Cell Biol*. (2014) 34:3911–25. doi: 10.1128/MCB.00980-14
72. Godin JD, Creppe C, Laguesse S, Nguyen L. emerging roles for the unfolded protein response in the developing nervous system. *Trends Neurosci*. (2016) 39:394–404. doi: 10.1016/j.tins.2016.04.002
73. Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, et al. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia* (2007) 55:453–62. doi: 10.1002/glia.20467
74. Bellezza I, Grottelli S, Mierla AL, Cacciatore I, Fornasari E, Roscini L, et al. Neuroinflammation and endoplasmic reticulum stress are coregulated by cyclo(His-Pro) to prevent LPS neurotoxicity. *Int J Biochem Cell Biol*. (2014) 51:159–69. doi: 10.1016/j.biocel.2014.03.023
75. Ho HJ, Huang DY, Ho FM, Lee LT, Lin WW. Inhibition of lipopolysaccharide-induced inducible nitric oxide synthase expression by endoplasmic reticulum stress. *Cell Signal*. (2012) 24:2166–78. doi: 10.1016/j.cellsig.2012.07.018
76. Noelker C, Morel L, Lescot T, Osterloh A, Alvarez-Fischer D, Breloer M, et al. Toll like receptor 4 mediates cell death in a mouse MPTP model of Parkinson disease. *Sci Rep*. (2013) 3:1393. doi: 10.1038/srep01393
77. Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A., et al. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature* (2013) 493:674–8. doi: 10.1038/nature11729
78. Lerner AG, Upton JP, Praveen PV, Ghosh R, Nakagawa Y, Igbaria A, et al. IRE1alpha induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. *Cell Metab*. (2012) 16:250–64. doi: 10.1016/j.cmet.2012.07.007
79. Kim S, Joe Y, Kim HJ, Kim YS, Jeong SO, Pae HO, et al. Endoplasmic reticulum stress-induced IRE1alpha activation mediates cross-talk of GSK-3beta and XBP-1 to regulate inflammatory cytokine production. *J Immunol*. (2015) 194:4498–506. doi: 10.4049/jimmunol.1401399
80. Tam AB, Mercado EL, Hoffmann A, Niwa M. ER stress activates NF-kappaB by integrating functions of basal IKK activity, IRE1 and PERK. PLoS ONE (2012) 7:e45078. doi: 10.1371/journal.pone.0045078
81. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, et al. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* (2000) 287:664–6. doi: 10.1126/science.287.5453.664
82. Zhang K, Shen X, Wu J, Sakaki K, Saunders T, Rutkowski DT, et al. Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell* (2006) 124:587–99. doi: 10.1016/j.cell.2005.11.040
83. Hosoi T, Honda M, Oba T, Ozawa K. ER stress upregulated PGE/IFNgamma-induced IL-6 expression and down-regulated iNOS expression in glial cells. *Sci Rep*. (2013) 3:3388. doi: 10.1038/srep03388
84. Lin W, Harding HP, Ron D, Popko B. Endoplasmic reticulum stress modulates the response of myelinating oligodendrocytes to the immune cytokine interferon-gamma. *J Cell Biol*. (2005) 169:603–12. doi: 10.1083/jcb.200502086
85. Stewart CR, Stuart LM, Wilkinson K, van Gils JM, Deng J, Halle A, et al. CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. *Nat Immunol*. (2010) 11:155–61. doi: 10.1038/ni.1836
86. Guthrie LN, Abiraman K, Plyler ES, Sprenkle NT, Gibson SA, McFarland BC, et al. Attenuation of PKR-like ER Kinase (PERK) signaling selectively controls endoplasmic reticulum stress-induced inflammation without compromising immunological responses. *J Biol Chem*. (2016) 291:15830–40. doi: 10.1074/jbc.M116.738021
87. Herz J, Filiano AJ, Smith A, Yogev N, Kipnis J. et al. Myeloid cells in the central nervous system. Immunity, (2017) 46:943–56. doi: 10.1016/j.immuni.2017.06.007
88. Colonna M, Butovsky O. Microglia function in the central nervous system during health and neurodegeneration. *Annu Rev Immunol*. (2017) 35:441–68. doi: 10.1146/annurev-immunol-051116-052358
89. Vezzani A, Viviani B. Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability. *Neuropharmacology* (2015) 96(Pt A):70–82. doi: 10.1016/j.neuropharm.2014.10.027
90. Ta HM, Le TM, Ishii H, Takarada-Iemata M, Hattori T, Hashida K, et al. Atf6alpha deficiency suppresses microglial activation and ameliorates pathology of experimental autoimmune encephalomyelitis. *J Neurochem*. (2016) 139:1124–37. doi: 10.1111/jnc.13714
91. Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, et al. A unique microglia type associated with restricting development of Alzheimer's Disease. *Cell* (2017) 169:1276–90 e17. doi: 10.1016/j.cell.2017.05.018
92. Ulland TK, Song WM, Huang SC, Ulrich JD, Sergushichev A, Beatty WL, et al. TREM2 maintains microglial metabolic fitness in Alzheimer's Disease. *Cell* (2017) 170:649–63.e13. doi: 10.1016/j.cell.2017.07.023
93. Krasemann S, Madore C, Cialic R, Baufeld C, Calcagno N, El Fatimy R, et al. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity* (2017) 47:566–81.e9. doi: 10.1016/j.immuni.2017.08.008
94. Goldmann T, Wieghofer P, Jordão MJ, Prutek F, Hagemeyer N, Frenzel K, et al. Origin, fate and dynamics of macrophages at central nervous system interfaces. *Nat Immunol*. (2016) 17:797–805. doi: 10.1038/ni.3423
95. Osorio F, Tavernier SJ, Hoffmann E, Saeys Y, Martens L, Vetters J, et al. The unfolded-protein-response sensor IRE-1alpha regulates the function of CD8alpha+ dendritic cells. *Nat Immunol*. (2014) 15:248–57. doi: 10.1038/ni.2808
96. Anandasabapathy N, Victora GD, Meredith M, Feder R, Dong B, Kluger C, et al. Flt3L controls the development of radiosensitive dendritic cells in the meninges and choroid plexus of the steady-state mouse brain. *J Exp Med*. (2011) 208:1695–705. doi: 10.1084/jem.20102657
97. Bossù P, Spalletta G, Caltagirone C, Ciaramella A. Myeloid dendritic cells are potential players in human neurodegenerative diseases. *Front Immunol*. (2015) 6:632. doi: 10.3389/fimmu.2015.00632
98. Fischer HG, Reichmann G. Brain dendritic cells and macrophages/microglia in central nervous system inflammation. *J Immunol*. (2001) 166:2717–26. doi: 10.4049/jimmunol.166.4.2717

99. Pashenkov M, Huang YM, Kostulas V, Haglund M, Söderström M, Link H. Two subsets of dendritic cells are present in human cerebrospinal fluid. *Brain* (2001) 124(Pt 3):480–92. doi: 10.1093/brain/124.3.480
100. Iwakoshi NN, Pypaert M, Glimcher LH. The transcription factor XBP-1 is essential for the development and survival of dendritic cells. *J Exp Med*. (2007) 204:2267–75. doi: 10.1084/jem.20070525
101. Tavernier SJ, Osorio F, Vandersarren L, Vetters J, Vanlangenakker N, Van Isterdael G, et al. Regulated IRE1-dependent mRNA decay sets the threshold for dendritic cell survival. *Nat Cell Biol*. (2017) 19:698–710. doi: 10.1038/ncb3518
102. Osorio F, Lambrecht BN, Janssens S. Antigen presentation unfolded: identifying convergence points between the UPR and antigen presentation pathways. *Curr Opin Immunol*. (2018) 52:100–7. doi: 10.1016/j.coi.2018.04.020
103. Ellwardt E, Walsh JT, Kipnis J, Zipp F. Understanding the role of T cells in CNS homeostasis. *Trends Immunol*. (2016) 37:154–65. doi: 10.1016/j.it.2015.12.008
104. Kurnellas MP, Ghosn EE, Schartner JM, Baker J, Rothbard JJ, Negrin RS, et al. Amyloid fibrils activate B-1a lymphocytes to ameliorate inflammatory brain disease. *Proc Natl Acad Sci USA*. (2015) 112:15016–23. doi: 10.1073/pnas.1521206112
105. Matsumoto M, Baba A, Yokota T, Nishikawa H, Ohkawa Y, Kayama H, et al. Interleukin-10-producing plasmablasts exert regulatory function in autoimmune inflammation. *Immunity* (2014) 41:1040–51. doi: 10.1016/j.immuni.2014.10.016
106. Korn T, Kallies A. T cell responses in the central nervous system. *Nat Rev Immunol*. (2017) 17:179–94. doi: 10.1038/nri.2016.144
107. Bryson KJ, Lynch MA. Linking T cells to Alzheimer's disease: from neurodegeneration to neurorepair. *Curr Opin Pharmacol*. (2016) 26:67–73. doi: 10.1016/j.coph.2015.10.003
108. González H, Pacheco R. T-cell-mediated regulation of neuroinflammation involved in neurodegenerative diseases. *J Neuroinflamm*. (2014) 11:201. doi: 10.1186/s12974-014-0201-8
109. Bailey-Bucktrout SL, Martinez-Llordella M, Zhou X, Anthony B, Rosenthal W, Luche H, et al. Self-antigen-driven activation induces instability of regulatory T cells during an inflammatory autoimmune response. *Immunity* (2013) 39:949–62. doi: 10.1016/j.immuni.2013.10.016
110. Brochard V, Combadière B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, et al. Infiltration of CD4(+) lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest*. (2009) 119:182–92. doi: 10.1172/JCI36470
111. Monsonego A, Zota V, Karni A, Krieger JI, Bar-Or A, Bitan G, et al. Increased T cell reactivity to amyloid beta protein in older humans and patients with Alzheimer disease. *J Clin Invest*. (2003) 112:415–22. doi: 10.1172/JCI200318104
112. Saresella M, Piancone F, Tortorella P, Marventano I, Gatti A, Caputo D, et al. T helper-17 activation dominates the immunologic milieu of both amyotrophic lateral sclerosis and progressive multiple sclerosis. *Clin Immunol*. (2013) 148:79–88. doi: 10.1016/j.clim.2013.04.010
113. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize alpha-synuclein peptides. *Nature* (2017) 546:656–61. doi: 10.1038/nature22815
114. Kamimura D, Bevan MJ. Endoplasmic reticulum stress regulator XBP-1 contributes to effector CD8+ T cell differentiation during acute infection. *J Immunol*. (2008) 181:5433–41. doi: 10.4049/jimmunol.181.8.5433
115. Kemp KL, Lin Z, Zhao F, Gao B, Song J, Zhang K, et al. The serine-threonine kinase inositol-requiring enzyme 1alpha (IRE1alpha) promotes IL-4 production in T helper cells. *J Biol Chem*. (2013) 288:33272–82. doi: 10.1074/jbc.M113.493171
116. Brunsing R, Omori SA, Weber F, Bicknell A, Friend L, Rickert R, et al. B- and T-cell development both involve activity of the unfolded protein response pathway. *J Biol Chem*. (2008) 283:17954–61. doi: 10.1074/jbc.M801395200
117. Omar I, Lapenna A, Cohen-Daniel L, Tirosh B, Berger M. Schlafen2 mutation unravels a role for chronic ER stress in the loss of T cell quiescence. *Oncotarget* (2016) 7:39396–407. doi: 10.18632/oncotarget.9818
118. Scheu S, Stetson DB, Reinhardt RL, Leber JH, Mohrs M, Locksley RM. Activation of the integrated stress response during T helper cell differentiation. *Nat Immunol*. (2006) 7:644–51. doi: 10.1038/ni1338
119. Cepok S, von Geldern G, Grummel V, Hochgesand S, Celik H, Hartung H, et al. Accumulation of class switched IgD-IgM- memory B cells in the cerebrospinal fluid during neuroinflammation. *J Neuroimmunol*. (2006) 180:33–9. doi: 10.1016/j.jneuroim.2006.06.031
120. Sudduth TL, Greenstein A, Wilcock DM. Intracranial injection of gammagard, a human IVIg, modulates the inflammatory response of the brain and lowers Aβ in APP/PS1 mice along a different time course than anti-Aβ antibodies. *J Neurosci*. (2013) 33:9684–92. doi: 10.1523/JNEUROSCI.1220-13.2013
121. Fuller JP, Stavenhagen JB, Teeling JL. New roles for Fc receptors in neurodegeneration-the impact on Immunotherapy for Alzheimer's Disease. *Front Neurosci*. (2014) 8:235. doi: 10.3389/fnins.2014.00235
122. Peress NS, Fleit HB, Perillo E, Kuljis R, Pezzullo C. Identification of Fc gamma RI, II and III on normal human brain ramified microglia and on microglia in senile plaques in Alzheimer's disease. *J Neuroimmunol*. (1993) 48:71–9. doi: 10.1016/0165-5728(93)90060-C
123. Biragyn A, Aliseychik M, Rogaev E. Potential importance of B cells in aging and aging-associated neurodegenerative diseases. *Semin Immunopathol*. (2017) 39:283–94. doi: 10.1007/s00281-016-0615-8
124. Kharabi Masouleh B, Geng H, Hurtz C, Chan LN, Logan AC, Chang MS, et al. Mechanistic rationale for targeting the unfolded protein response in pre-B acute lymphoblastic leukemia. *Proc Natl Acad Sci USA*. (2014) 111:E2219–28. doi: 10.1073/pnas.1400958111
125. Zhang K, Wong HN, Song B, Miller CN, Scheuner D, Kaufman RJ. The unfolded protein response sensor IRE1alpha is required at 2 distinct steps in B cell lymphopoiesis. *J Clin Invest*. (2005) 115:268–81. doi: 10.1172/JCI200521848
126. Gass JN, Jiang HY, Wek RC, Brewer JW. The unfolded protein response of B-lymphocytes: PERK-independent development of antibody-secreting cells. *Mol Immunol*. (2008) 45:1035–43. doi: 10.1016/j.molimm.2007.07.029
127. Hu CC, Dougan SK, McGehee AM, Love JC, Ploegh HL. XBP-1 regulates signal transduction, transcription factors and bone marrow colonization in B cells. *EMBO J*. (2009) 28:1624–36. doi: 10.1038/emboj.2009.117
128. Gass JN, Gifford NM, Brewer JW. Activation of an unfolded protein response during differentiation of antibody-secreting B cells. *J Biol Chem*. (2002) 277:49047–54. doi: 10.1074/jbc.M205011200
129. Aguzzi A, Barres BA, Bennett ML. Microglia: scapegoat, saboteur, or something else? *Science* (2013) 339:156–61. doi: 10.1126/science.1227901
130. Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer disease. *Nat Rev Neurosci*. (2015) 16:358–72. doi: 10.1038/nrn3880
131. Jiang P, Gan M, Ebrahim AS, Lin WL, Melrose HL, Yen SH. ER stress response plays an important role in aggregation of alpha-synuclein. *Mol Neurodegener*. (2010) 5:56. doi: 10.1186/1750-1326-5-56
132. Lee DY, Lee KS, Lee HJ, Kim DH, Noh YH, Yu K, et al. Activation of PERK signaling attenuates Abeta-mediated ER stress. PLoS ONE (2010) 5:e10489. doi: 10.1371/journal.pone.0010489

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

BRIEF RESEARCH REPORT
published: 20 November 2018
doi: 10.3389/fneur.2018.00946

The Peripheral Inflammatory Response to Alpha-Synuclein and Endotoxin in Parkinson's Disease

Alice J. White, Ruwani S. Wijeyekoon, Kirsten M. Scott, Nushan P. Gunawardana, Shaista Hayat, I. H. Solim, H. T. McMahon, Roger A. Barker and Caroline H. Williams-Gray*

John van Geest Center for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom

The immune system is activated in Parkinson's Disease (PD), as evidenced by neuroinflammatory changes within the brain as well as elevated immune markers in peripheral blood. Furthermore, inflammatory cytokine levels in the blood are associated with disease severity and rate of progression. However, the factors driving this immune response in PD are not well established. We investigated cell-extrinsic factors in systemic immune activation by using α-synuclein monomers and fibrils, as well as bacterial toxins, to stimulate peripheral blood mononuclear cells (PBMCs) derived from 31 patients and age/gender-matched controls. α-synuclein monomers or fibrils resulted in a robust cytokine response (as measured by supernatant cytokine concentrations and mRNA expression in cultured cells) in both PD and control PBMCs, similar to that induced by bacterial LPS. We found no PD vs. control differences in cytokine production, nor in mRNA expression. Levels of endotoxin within the recombinant α-synuclein used in these experiments were very low (0.2–1.3EU/mL), but nonetheless we found that comparable levels were sufficient to potentially confound our cytokine concentration measurements for a number of cytokines. However, α-synuclein monomers increased production of IL-1β and IL-18 to levels significantly in excess of those induced by low-level endotoxin. In conclusion, this study: (i) highlights the importance of accounting for low-level endotoxin in antigen-PBMC stimulation experiments; (ii) indicates that cell-extrinsic factors may be a major contributor to immune activation in PD; and (iii) suggests that α-synuclein may play a role in inflammasome-related cytokine production in the periphery.

Keywords: Parkinson's disease, immune system, alpha-synuclein, endotoxin, cytokines

OPEN ACCESS

Edited by:
Cristoforo Comi, Università degli Studi del Piemonte Orientale, Italy

Reviewed by:
Cintia Roodveldt, Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), Spain
Ali Keshavarzian, Rush University, United States

****Correspondence:***
Alice J. White
ajw283@cam.ac.uk

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *09 July 2018*
Accepted: *23 October 2018*
Published: *20 November 2018*

Citation:
White AJ, Wijeyekoon RS, Scott KM, Gunawardana NP, Hayat S, Solim IH, McMahon HT, Barker RA and Williams-Gray CH (2018) The Peripheral Inflammatory Response to Alpha-Synuclein and Endotoxin in Parkinson's Disease. Front. Neurol. 9:946. doi: 10.3389/fneur.2018.00946

INTRODUCTION

The immune system is known to be altered in Parkinson's disease (PD). Whilst some of these changes may be secondary phenomena, a growing body of evidence suggests that the immune system may play a contributory role in the primary progression of PD (1, 2). α-synuclein is the key protein implicated in the pathogenesis of PD, forming intracellular aggregates known as Lewy bodies (3). Fibrillar α-synuclein is the principal pathological form present in Lewy bodies (4), but the protein also exists in monomeric and oligomeric forms within the CNS, and all three may trigger a central immune response orchestrated by microglia (5–7). Mutations in α-synuclein are known to be associated with PD risk (8) and *in-vitro* studies of the behavior of monocytes

and microglia stimulated with mutant α-synuclein monomers demonstrate increased cytotoxic immune responses in comparison to wild-type α-synuclein-exposed cells (9, 10). Components of α-synuclein have also been shown to lead to activation of T-lymphocytes and monocytes (11, 12), all of which suggests that α-synuclein may drive both a neuronal pathology and an inflammatory process in PD. Overproduction of cytokines in PD perpetuates the inflammatory response centrally and systemically (13). Serum cytokines [for example, IL-1ß, IL-2, IL-10, IFNγ, and TNF-α (14, 15)] and peripheral blood mononuclear cell (PBMC) cytokine production has been correlated with PD symptom severity (16, 17) and rate of disease progression (2). The question therefore arises: could this be driven by α-synuclein in the periphery?

Aberrant α-synuclein is distributed throughout peripheral organs, blood, interstitial and extracellular fluids in PD (18–20) and may act as a catalyst for activation of the peripheral immune system (1). Indeed, selected α-synuclein peptides stimulate a specific T-cell response in 40% of patients, via presentation by MHC alleles which are known to be genetically associated with PD risk (11). Fibrillar α-synuclein has been shown to act via Toll-like receptor (TLR) and inflammasome pathways in monocytes leading to IL-1β production (12).

However, other factors such as infections or translocation of bacterial toxins from the gut may also contribute to inflammation in PD (21, 22). For example, lipopolysaccharides (LPS) stimulate PBMCs via the TLR and inflammasome pathways to produce an inflammatory cytokine response (12), and several studies have investigated this response in PD albeit with inconsistent results. LPS-stimulated cytokine production has been reported to be elevated in PD compared to controls, along with the basal production of some cytokines (IL-1ß, IFNγ, and TNF-α) (16). A second study showed that production of IL-1ß, IL-6, and TNF-α is enhanced in PD PBMCs, while IL-2 is reduced (23). However, in another study, production of IFNγ by LPS-stimulated PBMCs was lower in patients than controls, while IL-6, IL-1α, and IL-1ß levels were no different, but decreasing concentrations correlated worsened disease severity (24).

Given this ambiguity in the literature and the absence of any study investigating both α-synuclein and LPS stimulation of PBMCs in PD patients, we sought to understand how stimulation by α-synuclein monomers, fibrils, and LPS affects PBMC cytokine production in PD patients and matched controls.

METHODS

Early-stage PD patients (Hoehn and Yahr $\leq$2), fulfilling UK PD Brain Bank Criteria, aged 55–80, were recruited from the PD Research Clinic at the John van Geest Center for Brain Repair, Cambridge. A movement disorder accredited neurologist conducted clinical and neuropsychological assessments.

Age and gender matched control participants were recruited from the NIHR Cambridge Bioresource (http://www.cambridgebioresource.org.uk) and had no history of neurological disease, self-reported memory problems, or depression. Ethical approval was obtained from the East of England-Cambridge Central Research Ethics Committee (REC 03/303). Exclusion criteria were: other neurodegenerative disorders, chronic inflammatory or autoimmune disorders, current clinically significant infection or use of anti-inflammatory/immunomodulatory medications, surgery within the last month, or recent vaccinations. Data from this cohort also contributed to our previously published study (25).

PBMCs were extracted from venous blood by centrifugation over a Ficoll gradient, washed and cultured (37°C, 5% CO_2) for 24 h in RPMI (Life Technologies) and 10% fetal calf serum (FCS, Sigma) in aliquots of 1 million cells per mL per well, either unstimulated, or with LPS (1 ng/mL), α-synuclein monomers (2 nmol/mL) or fibrils (2 nmol/mL). Supernatant was collected and stored at −80°C, and cultured PBMCs were washed and stored in RNA protect (Qiagen) at −80°C. Matched samples were processed in parallel.

Recombinant α-synuclein was produced by expression in *E.coli* Rosetta using human α-synuclein cDNA, and aggregates were confirmed on SDS-PAGE gel (**Supplementary Methods** and **Supplementary Figure 1**). Endotoxin levels were determined using LAL assays (Lonza Verviers SPRL, Belgium).

Cytokines (IFNγ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α) were measured in culture supernatants using the Meso Scale Discovery (Rockville) platform V-Plex Pro-inflammatory panel 1 electrochemiluminescence assay. Secondary analyses were performed in a subset of samples/conditions to measure IL-18 (MSD U-PLEX Human IL-18 assay) and caspase-1 (Human caspase-1/ICE Quantikine ELISA kit, R&D Systems) as markers of inflammasome activation. Assays were run according to manufacturer's instructions. Supernatant samples were diluted 1:10 or 2:3 in the appropriate buffer and assayed in duplicate. Cytotoxicity post-culture was quantified with the Pierce LDH Cytotoxicity Assay Kit (ThermoFisher).

RNA was extracted from cultured PBMCs using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. RNA was reverse-transcribed using SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR (ThermoFisher Scientific). TaqMan Real-Time PCR was used for quantification of gene expression, and primers were IL-6 (HS00174131M1) and IL-1ß (HS001555410M1) (ThermoFisher Scientific). Assays were run in triplicate. Relative quantification was carried out on a QuantStudio 12K Flex Real-Time PCR machine and calculated using comparative cycle threshold (ΔΔCT method) relative to the housekeeping gene GAPDH, and a randomly selected endogenous control common to all plates.

Cytokine concentrations across antigens and PD status were compared using repeated-measures ANOVAs, and mRNA production using 2-way ANOVAs (GraphPad Prism version 7, SPSS version 25). Outliers were removed using Grubbs' tests ($p < 0.05$).

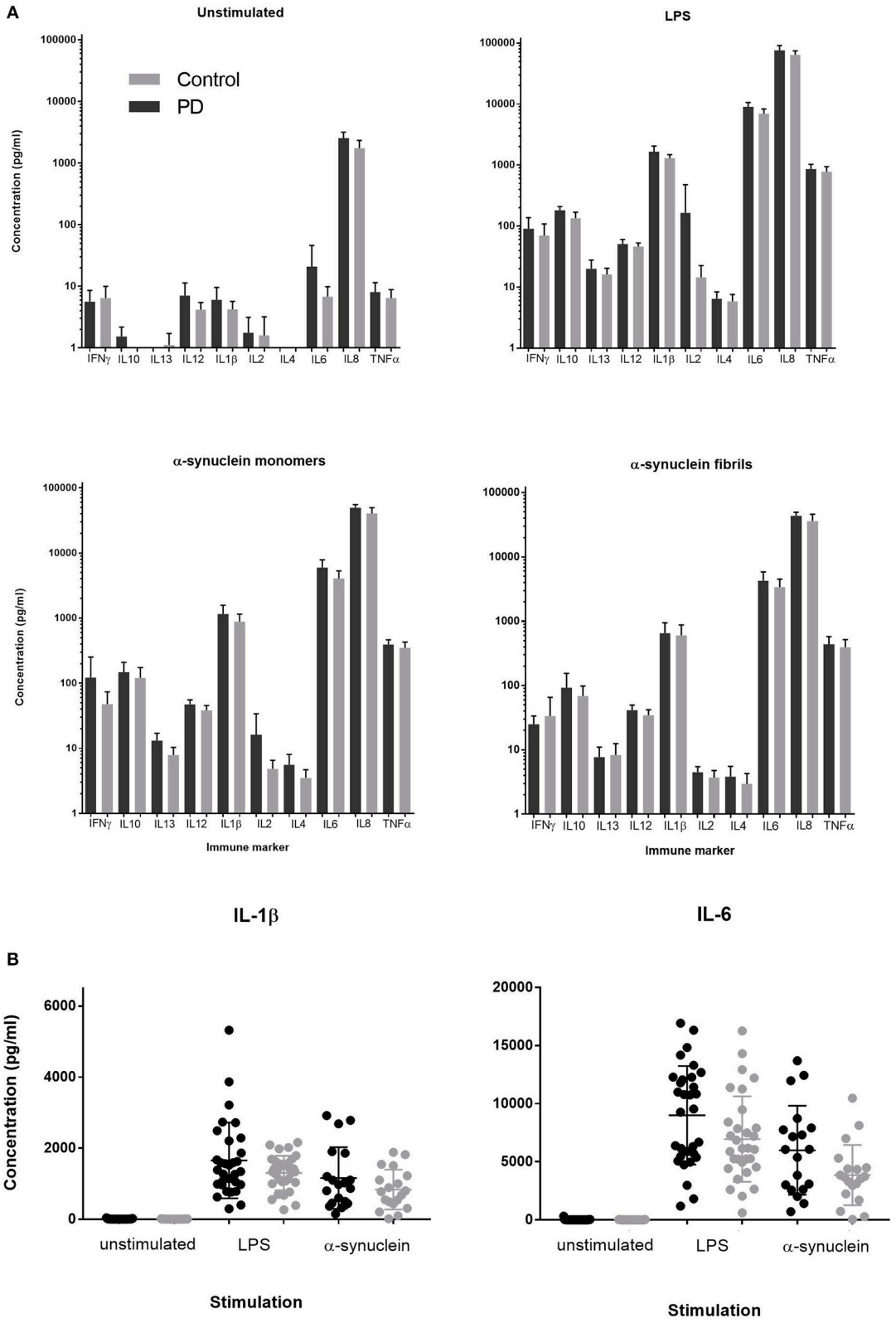


FIGURE 1 | (A) Supernatant cytokine concentration produced by PD and control PBMCs cultured for 24 h in media containing LPS (1ng/ml), α-synuclein fibrils, or α-synuclein monomers (1 nmol = 27.3 ng/ml). **(B)** Individual level data for IL-1β and IL-6 concentrations in post-culture supernatant in PD cases and matched controls for direct comparison between unstimulated, LPS and α-synuclein monomer stimulation for two key inflammatory cytokines. Error bars represent SEM.

RESULTS

31 PD patients [mean disease duration 4.3 (1.1) years] and 31 controls (matched for age and gender) were included (**Supplementary Table 1**).

PBMCs were cultured with LPS ($n = 31$ case-control pairs), and α-synuclein monomers and fibrils ($n = 19$ case-control pairs). A subset of α-synuclein-cultured PBMCs were used for the gene expression assays based on RNA availability.

Stimulation with either LPS, α-synuclein monomers or fibrils led to robust cytokine stimulation compared to the unstimulated condition ($p < 0.0001$, RMANOVA, main effect of antigen). There was no main effect of patient vs. control status on cytokine production for any antigen ($p > 0.05$, RMANOVA; **Figure 1**). PBMC supernatant LDH levels were not different between α-synuclein, LPS, or unstimulated cultures. Expression of IL-6 and IL-1β was quantified by qRT-PCR, given that these cytokines showed the greatest PD-control differences on LPS stimulation in previous studies (16, 24). IL-6 and IL-1β expression were elevated in response to stimulation by both LPS and α-synuclein (2-way ANOVA and *post-hoc* Tukey's test $p < 0.0001$; Gene expression, relative units mean ± standard deviation(SD), IL-6: unstimulated PD = 2.18 ± 4.28, Control = 1.67 ± 2.82; α-synuclein PD = 929.72 ± 485.93, Control = 845.05 ± 419.67; LPS PD = 989.05 ± 968.66, Control = 810.00 ± 731.10, IL-1β: unstimulated PD = 2.99 ± 3.72, Control = 2.54 ± 3.76; α-synuclein PD = 207.01 ± 95.13, Control = 171.39 ± 60.10; LPS PD = 155.59 ± 116.50, Control = 136.77 ± 86.64). There was no main effect of disease status between PD and control groups ($p > 0.05$, two-way ANOVA).

Given that α-synuclein produced a similar magnitude of cytokine response to LPS, we examined the α-synuclein for the presence of any associated endotoxin. Despite procedures to remove contaminating endotoxin as detailed in the methods, endotoxin concentrations in samples at 2 nmol/mL were 0.2–1.3EU/mL on testing multiple aliquots (Lonza). LPS (1 ng/mL) contained >10 EU/mL. To ascertain whether the levels of contaminating endotoxin were sufficient to confound cytokine measurements in the α-synuclein cultures, we used six endotoxin standard dilutions (0, 0.1, 0.26, 0.64, 1.6, and LPS>10EU/mL) to stimulate PBMCs using otherwise identical conditions (PD $n = 5$, Control $n = 4$, age = 68.9 (not different from previous cohort) and compared supernatant cytokine concentrations with data obtained in our initial experiments (**Figure 2**). An endotoxin concentration of 1.6EU/mL produced similar levels of cytokine to α-synuclein (monomers or fibrils) for most cytokines thus suggesting a possible confounding effect of endotoxin. However, for IL-1β and IL-18, monomeric α-synuclein had a significantly greater effect than 1.6EU/mL endotoxin ($p = 0.01$), an EU level in excess of the measured level of contaminating endotoxin. A similar pattern was seen for α-synuclein fibrils compared to endotoxin at 1.6EU/mL, but this did not reach statistical significance. Despite the excess production of inflammasome-related cytokines IL-1β and IL-18 by PBMCs stimulated with α-synuclein monomers, there was no corresponding increase in caspase-1 secretion ($p > 0.05$, **Figure 2D**).

DISCUSSION

We found that PBMCs collected from both PD patients and age/gender-matched controls stimulated by α-synuclein (both monomeric and fibrillary) produced a robust inflammatory cytokine response. The response was similar in magnitude to LPS stimulation as assessed by both cytokine concentrations in culture supernatant and mRNA expression. Whilst this response may have been confounded by low levels of endotoxin in the α-synuclein preparation, the response of the IL1-β and IL-18 is greater than this low level endotoxin effect, which suggests that α-synuclein may have a specific independent effect on inflammasome-related pathways. Interestingly, it has previously been shown that α-synuclein fibrils (produced from a strain of E. Coli with strongly reduced endotoxicity) stimulate the NLRP3 inflammasome pathway in monocytes to produce IL-1β, in addition to the TLR pathway that is activated by bacterial endotoxin (12). In our study, we found that α-synuclein monomers had a more pronounced effect on IL-1β production than fibrils, but comparison between studies is difficult given the likely variability in aggregate size according to the methodology used to prepare fibrils. We found no significant increase in the PBMC supernatant levels of the inflammasome pathway mediator caspase-1 with α-synuclein stimulation, suggesting that α-synuclein may be acting via caspase-1 independent inflammasome pathways in this setting (26).

In contrast to previous studies (16, 23, 24), there was no evidence of PD-control difference in cytokine production or mRNA expression. Notably, our case-control pairs were well-matched for age and gender and processed in parallel to eliminate variation that may have confounded previous studies. Hence, our data do not support a differential effect of PBMC stimulation in PD cases vs. controls, irrespective of the stimulating antigen. The lack of any patient vs. control differences in cytokine production in response to PBMC stimulation suggests raised levels of inflammatory markers in the serum in PD may relate more to levels of exogenous stimulating antigens or other cytokine sources, rather than to intrinsic properties of the peripheral mononuclear cells. Additionally, oligomeric α-synuclein species might contribute to inflammation in PD but this has not been specifically tested in this study.

The generation of α-synuclein for experimental use typically involves producing recombinant protein in *E.coli*, which invariably leads to endotoxin contamination of the protein product; contamination which can be removed to some extent by cleaning methods, but may remain at low levels and confound cellular processes with sensitivity to endotoxin (27). Our data confirms that even very low level endotoxin levels can have a significant confounding effect. A previous study found that α-synuclein-derived peptides drive specific T-cell responses in PD (11), but it is unclear whether the presence of associated endotoxin had been entirely excluded in these experiments. However, it may be relevant to further study co-stimulation with endotoxin and α-synuclein, given that endotoxin may act synergistically with α-synuclein in TLR stimulation (12), as has been shown in α-synuclein-primed murine microglia (28).

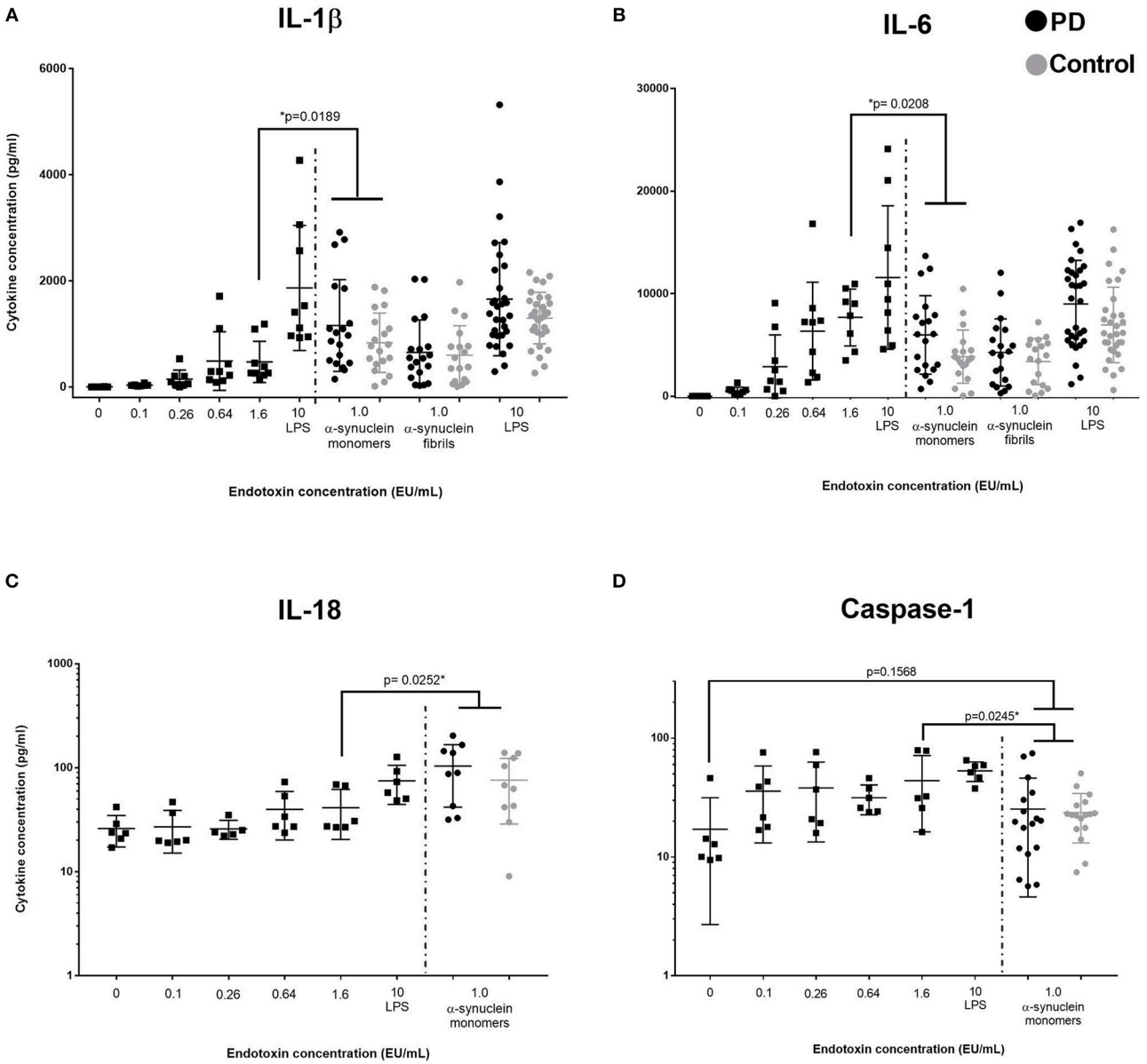


FIGURE 2 | Comparison of low concentration endotoxin and α-synuclein stimulation of PBMCs. Squares represent cytokine concentrations from PBMC cultures with varying concentrations of endotoxin (**A, B**: $n = 9$, 5 PD and 4 controls, **C,D**: $n = 6$, 3PD and 3 controls, assayed in duplicate; left of dashed line). Circles represents PD (black) and control (gray) concentrations in post-culture supernatants from the original assays cultured with α-synuclein monomers, fibrils, or LPS (α-synuclein conc: 1 nmol = 27.3 ng/ml, LPS conc: 1 ng/ml). *P*-values indicates comparison between 1.6 EU/ml (comparable to endoxin level in our α-synuclein preparation) and grouped PD/control cohort stimulated by α-synuclein monomers (No PD/control differences were observed in post-culture cytokine concentrations) Bars represent mean value and first and third quartiles. **(A)** Data suggest that IL-1β production is stimulated by α-synuclein in excess of stimulation by the equivalent value of endotoxin present as a contamiant. **(B)** IL-6 (and all other measured cytokines) do not show this increased production. Data indicates that endotoxin is the primary driver of elevated IL-6 concentration, rather tha α-synuclein. **(C)** IL-18 prodution is also significantly increased in response to α-synuclein stimulation, compared to stimulation with an endotoxin concentration comparable to contaminating levels. **(D)** Caspase-1 levels are not significantly increased by α-synuclein stimulation compared to the unstimulated condition.

Furthermore, endotoxin may influence the conformation of α-synuclein, with different LPS β-sheet content driving alterations in fibril density and changes in associated behavioral phenotypes in animal models (29). However, these mechanisms are not well understood in patients.

A limitation of this study is that the assessment of the PBMC response to varying endotoxin concentrations was undertaken in an independent sub-sample. However, subjects included were similar in age and disease status and the measured cytokine concentrations had minimal

between-subject variation suggesting that the responses were representative.

In conclusion, our data suggest that even low levels of endotoxin can confound the measurement of immune cell responses to α-synuclein *in-vitro* and future studies should consider endotoxin quantification. α-synuclein may have independent effects on production of inflammasome-related cytokines, which may perpetuate the immune response in PD. Furthermore, PD and control PBMCs behaved similarly in the face of stimulation in our study which suggests that cell-extrinsic factors may be an important contributor to the chronic inflammation which has been observed in PD. The nature of these agents remains to be fully determined but both α-synuclein and bacterial endotoxins may play a critical role.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Health Research Authority East of England-Cambridge Central Research Ethics Committee with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the East of England-Cambridge Central Research Ethics Committee (REC 03/303).

AUTHOR CONTRIBUTIONS

AW acquisition of data, analysis and interpretation of data, drafting of the manuscript. RW acquisition of data, critical revision of the manuscript. KS acquisition of data, critical revision of the manuscript. NG preparation of α-synuclein species, acquisition of data, critical revision of the manuscript. HM preparation of α-synuclein species, acquisition of data, critical revision of the manuscript. SH acquisition of data, critical revision of the manuscript. IS acquisition of data, critical revision of the manuscript. RB study supervision, critical revision of the manuscript. CW-G study design and supervision, critical revision of the manuscript.

FUNDING

Grant funding from the Academy of Medical Sciences UK, the Rosetrees Trust, the Stevenage Biosciences Catalyst and Addenbrooke's Charitable Trust contributed to this work. The research was also supported by the NIHR Cambridge Biomedical Research Center (Cambridge University Hospitals NHS Trust/University of Cambridge). CH Williams-Gray is supported by a fellowship from the Medical Research Council. AJ White was funded by Homerton College. RS Wijeyekoon was supported by a fellowship from Addenbrooke's Charitable Trust. KM Scott is supported by a fellowship from the Wellcome Trust. R A Barker is supported by the Wellcome-MRC Cambridge Stem Cell Institute and is an NIHR Senior Investigator.

The funding bodies had no role in the design of the study, the collection, analysis, and interpretation of data or the writing of the manuscript.

ACKNOWLEDGMENTS

We gratefully acknowledge the participation of all our patient volunteers, and NIHR Cambridge BioResource volunteers, and we thank the NIHR Cambridge BioResource center and staff for their contribution. We thank the National Institute for Health Research and NHS Blood and Transplant. We also acknowledge the support of the Core Biochemical Assay Laboratory at Cambridge University Hospitals.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2018.00946/full#supplementary-material

REFERENCES

1. Mosley RL, Hutter-Saunders JA, Stone DK, Gendelman HE. Inflammation and adaptive immunity in Parkinson's Disease. *Cold Spring Harb Perspect Med*. (2012) 2:a009381. doi: 10.1101/cshperspect.a009381
2. Williams-Gray CH, Wijeyekoon R, Yarnall AJ, Lawson RA, Breen DP, Evans JR, Cummins GA, et al. Serum immune markers and disease progression in an incident Parkinson's disease cohort (ICICLE-PD). *Mov Disord*. (2016) 31:995–1003. doi: 10.1002/mds.26563
3. Spillantini MG, Schmidt ML, Lee VM-Y, Trojanowski JQ, Jakes R, Goedert M. α-Synuclein in Lewy bodies. *Nature* (1997) 388:839–40. doi: 10.1038/42166
4. Wakabayashi K, Tanji K, Mori F, Takahashi H. The lewy body in Parkinson's disease: molecules implicated in the formation and degradation of alpha-synuclein aggregates. *Neuropathol Off J Jpn Soc Neuropathol*. (2007) 27:494–506. doi: 10.1111/j.1440-1789.2007.00803.x
5. Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, et al. Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J Off Publ Fed Am Soc Exp Biol*. (2005) 19:533–42. doi: 10.1096/fj.04-2751com
6. Lee S-J. Origins and effects of extracellular α-synuclein: implications in Parkinson's Disease. *J Mol Neurosci*. (2008) 34:17–22. doi: 10.1007/s12031-007-0012-9
7. Sanchez-Guajardo V, Barnum CJ, Tansey MG, Romero-Ramos M. Neuroimmunological processes in Parkinson's disease and their relation to α-synuclein: microglia as the referee between neuronal processes and peripheral immunity. *ASN NEURO* (2013) 5:113–39. doi: 10.1042/AN20120066
8. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* (1997) 276:2045–7.
9. Klegeris A, Pelech S, Giasson BI, Maguire J, Zhang H, McGeer EG, et al. Alpha-synuclein activates stress signaling protein kinases in THP-1 cells and microglia. *Neurobiol Aging* (2008) 29:739–52. doi: 10.1016/j.neurobiolaging.2006.11.013
10. Roodveldt C, Labrador-Garrido A, Gonzalez-Rey E, Fernandez-Montesinos R, Caro M, Lachaud CC, et al. Glial innate immunity generated by non-aggregated alpha-synuclein in mouse: differences between wild-type and Parkinson's disease-linked mutants. *PloS ONE* (2010) 5:e13481. doi: 10.1371/journal.pone.0013481

11. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize α-synuclein peptides. *Nature* (2017) 546:656–61. doi: 10.1038/nature22815
12. Codolo G, Plotegher N, Pozzobon T, Brucale M, Tessari I, Bubacco L, et al. Triggering of inflammasome by aggregated α-synuclein, an inflammatory response in synucleinopathies. *PloS ONE* (2013) 8:e55375. doi: 10.1371/journal.pone.0055375
13. Whitton PS. Inflammation as a causative factor in the aetiology of Parkinson's disease. *Br J Pharmacol*. (2007) 150:963–76. doi: 10.1038/sj.bjp.0707167
14. Gao H-M, Hong J-S. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. *Trends Immunol*. (2008) 29:357–65. doi: 10.1016/j.it.2008.05.002
15. Collins LM, Toulouse A, Connor TJ, Nolan YM. Contributions of central and systemic inflammation to the pathophysiology of Parkinson's disease. *Neuropharmacology* (2012) 62:2154–68. doi: 10.1016/j.neuropharm.2012.01.028
16. Reale M, Iarlori C, Thomas A, Gambi D, Perfetti B, Di Nicola M, et al. Peripheral cytokines profile in Parkinson's disease. *Brain Behav Immun*. (2009) 23:55–63. doi: 10.1016/j.bbi.2008.07.003
17. Banks WA, Erickson MA. The blood–brain barrier and immune function and dysfunction. *Neurobiol Dis*. (2010) 37:26–32. doi: 10.1016/j.nbd.2009.07.031
18. Mollenhauer B, Locascio JJ, Schulz-Schaeffer W, Sixel-Döring F, Trenkwalder C, Schlossmacher MG. α-Synuclein and tau concentrations in cerebrospinal fluid of patients presenting with parkinsonism: a cohort study. *Lancet Neurol*. (2011) 10:230–40. doi: 10.1016/S1474-4422(11)70014-X
19. Malek N, Swallow D, Grosset KA, Anichtchik O, Spillantini M, Grosset DG. Alpha-synuclein in peripheral tissues and body fluids as a biomarker for Parkinson's disease - a systematic review. *Acta Neurol Scand*. (2014) 130:59–72. doi: 10.1111/ane.12247
20. El-Agnaf OMA, Salem SA, Paleologou KE, Curran MD, Gibson MJ, Court JA, et al. Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. *FASEB J Off Publ Fed Am Soc Exp Biol*. (2006) 20:419–25. doi: 10.1096/fj.03-1449com
21. Houser MC, Tansey MG. The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *NPJ Park Dis*. (2017) 3:3. doi: 10.1038/s41531-016-0002-0
22. Fang F, Wirdefeldt K, Jacks A, Kamel F, Ye W, Chen H. CNS infections, sepsis and risk of Parkinson's disease. *Int J Epidemiol*. (2012) 41:1042–9. doi: 10.1093/ije/dys052
23. Bessler H, Djaldetti R, Salman H, Bergman M, Djaldetti M. IL-1 beta, IL-2, IL-6 and TNF-alpha production by peripheral blood mononuclear cells from patients with Parkinson's disease. *Biomed Pharmacother Biomedecine Pharmacother*. (1999) 53:141–5. doi: 10.1016/S0753-3322(99)80079-1
24. Hasegawa Y, Inagaki T, Sawada M, Suzumura A. Impaired cytokine production by peripheral blood mononuclear cells and monocytes/macrophages in Parkinson's disease. *Acta Neurol Scand*. (2000) 101:159–164. doi: 10.1034/j.1600-0404.2000.101003159.x
25. Williams-Gray CH, Wijeyekoon RS, Scott KM, Hayat S, Barker RA, Jones JL. Abnormalities of age-related T cell senescence in Parkinson's disease. *J Neuroinflam*. (2018) 15:166. doi: 10.1186/s12974-018-1206-5
26. Afonina IS, Müller C, Martin SJ, Beyaert R. Proteolytic processing of interleukin-1 family cytokines: variations on a common theme. *Immunity* (2015) 42:991–1004. doi: 10.1016/j.immuni.2015.06.003
27. Schwarz H, Schmittner M, Duschl A, Horejs-Hoeck J. Residual endotoxin contaminations in recombinant proteins are sufficient to activate human CD1c+ dendritic cells. *PloS ONE* (2014) 9:e113840. doi: 10.1371/journal.pone.0113840
28. Roodveldt C, Labrador-Garrido A, Gonzalez-Rey E, Lachaud CC, Guilliams T, Fernandez-Montesinos R, et al. Preconditioning of microglia by α-synuclein strongly affects the response induced by toll-like receptor (TLR) stimulation. *PloS ONE* (2013) 8:e79160. doi: 10.1371/journal.pone.0079160
29. Kim C, Lv G, Lee JS, Jung BC, Masuda-Suzukake M, Hong C-S, et al. Exposure to bacterial endotoxin generates a distinct strain of α-synuclein fibril. *Sci Rep*. (2016) 6:30891. doi: 10.1038/srep30891

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 24 January 2019
doi: 10.3389/fneur.2019.00013

Do Th17 Lymphocytes and IL-17 Contribute to Parkinson's Disease? A Systematic Review of Available Evidence

*Elisa Storelli, Niccolò Cassina, Emanuela Rasini, Franca Marino and Marco Cosentino**

Center of Research in Medical Pharmacology, University of Insubria, Varese, Italy

Parkinson's disease (PD) is a neurodegenerative disease characterized by progressive loss of dopaminergic neurons, appearance of Lewy bodies and presence of neuroinflammation. No treatments currently exist to prevent PD or delay its progression, and dopaminergic substitution treatments just relieve the consequences of dopaminergic neuron loss. Increasing evidence points to peripheral T lymphocytes as key players in PD, and recently there has been growing interest into the specific role of T helper (Th) 17 lymphocytes. Th17 are a proinflammatory CD4+ T cell lineage named after interleukin (IL)-17, the main cytokine produced by these cells. Th17 are involved in immune-related disease such as psoriasis, rheumatoid arthritis and inflammatory bowel disease, and drugs targeting Th17/IL-17 are currently approved for clinical use in such disease. In the present paper, we first summarized current knowledge about contribution of the peripheral immune system in PD, as well as about the physiopharmacology of Th17 and IL-17 together with its therapeutic relevance. Thereafter, we systematically retrieved and evaluated published evidence about Th17 and IL-17 in PD, to help assessing Th17/IL-17-targeting drugs as potentially novel antiparkinson agents. Critical appraisal of the evidence did not allow to reach definite conclusions: both animal as well as clinical studies are limited, just a few provide mechanistic evidence and none of them investigates the eventual relationship between Th17/IL-17 and clinically relevant endpoints such as disease progression, disability scores, intensity of dopaminergic substitution treatment. Careful assessment of Th17 in PD is anyway a priority, as Th17/IL-17-targeting therapeutics might represent a straightforward opportunity for the unmet needs of PD patients.

Keywords: Parkinson's disease, Th17 lymphocytes, interleukin-17, neuroinflammation, neurodegeneration, peripheral immunity

OPEN ACCESS

Edited by:
Salvatore Galati, Neurocenter of Southern Switzerland (NSI), Switzerland

Reviewed by:
Anindya Bhattacharya, Janssen Research and Development, United States
Graziella Madeo, National Institutes of Health (NIH), United States
Héctor Alberto González-Usigli, Instituto Mexicano del Seguro Social (IMSS), Mexico

****Correspondence:***
Marco Cosentino
marco.cosentino@uninsubria.it

Specialty section:
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

Received: *20 September 2018*
Accepted: *07 January 2019*
Published: *24 January 2019*

Citation:
Storelli E, Cassina N, Rasini E, Marino F and Cosentino M (2019) Do Th17 Lymphocytes and IL-17 Contribute to Parkinson's Disease? A Systematic Review of Available Evidence. Front. Neurol. 10:13. doi: 10.3389/fneur.2019.00013

PARKINSON'S DISEASE AND PERIPHERAL ADAPTIVE IMMUNITY

Parkinson's disease (PD) is a progressive neurodegenerative disease affecting 7 to 10 million people worldwide (1, 2) and is characterized by the progressive loss of dopaminergic neurons in the *substantia nigra pars compacta*, by the appearance of Lewy bodies, which are intracellular inclusions of aggregated α-synuclein, and by the presence of neuroinflammation (3–8). People with PD suffer from motor symptoms, such as bradykinesia, rigidity, resting tremor, and postural

instability, as well as from non-motor symptoms, such as autonomic disturbances, depression, and cognitive impairment (9–11). Available therapies are just symptomatic (12), resulting in improved patients' quality of life as disease progresses (13, 14), but unfortunately no treatments exist to prevent or delay PD progression, due to the limited comprehension of the events that lead to neurodegeneration.

Understanding the causes of neurodegeneration in PD remains so far a challenging goal, although novel clues are possibly coming from evidence concerning the role of peripheral adaptive immunity in the regulation of neuroinflammation and neurodegeneration (15–18). Preclinical and epidemiological data strongly suggest that chronic neuroinflammation may slowly bring to neuronal dysfunction during the asymptomatic stage of PD (7, 19). The activation of resident microglia seems to precede dopamine (DA) neuron loss and activators may include interferon (IFN)-γ and tumor necrosis factor (TNF)-α, inducing microglia to commit to a phagocytic activity (1, 20). Activated microglia secretes several neurotoxic substances such as superoxide anions, matrix metalloproteases, nitric oxide, chemokines, proinflammatory cytokines, and glutamate (1, 21). Microglia-derived pro-inflammatory mediators may favor blood-brain barrier (BBB) permeabilisation and subsequent infiltration of peripheral leukocytes into the CNS (22).

Indeed, presence of T lymphocytes has been reported in the *substantia nigra* of parkinsonian brains (20, 23), and both CD8+ and CD4+ T cell subtypes were found in post-mortem brain specimens from PD patients, as well as in animal models of PD (23). CD4+ T lymphocytes are pivotal in the orchestration of an effective immune response during host defense as well as in the pathogenesis of inflammatory diseases. CD4+ T cells may choose either pro-inflammatory phenotypes, such as T helper (Th) 1 and Th17, or anti-inflammatory phenotypes, such as Th2 and the T regulatory (Treg) (24, 25). Interestingly, evidence from both animal models of PD and from clinical studies, suggests that, on one hand, Th1 and Th17 may be detrimental to neurons, and on the other hand, Th2 and Treg may be protective (26, 27).

Understanding whether these cell subsets are imbalanced and how their functions are dysregulated in PD patients could possibly provide novel clues for the understanding of PD pathogenesis and progression as well as for the development of novel therapeutic approaches. Indeed it is now apparently established that in PD patients there is a decreased number of circulating CD4+ T lymphocytes (28), however the relative proportion of CD4+ T cell subsets and their functional profile is still a matter of debate. Our group recently reported that in peripheral blood of PD patients reduction of CD4+ T cells is mostly due to reduced Th2, Th17, Treg, and T naïve cells (29, 30). Consequently Th1 cells, which do not differ between PD patients and healthy subjects in terms of absolute count, are increased with respect to other subsets, leading to a putative Th1 bias, also confirmed by a preferential differentiation of naïve CD4+ T cells of PD patients toward the Th1 lineage and by increased production of IFN-γ and TNF-α (but not of other cytokines, including IL-17) (30). Altogether, such results may not support a role for Th17 in PD, however they are in possible conflict with other studies. For instance, a recent investigation reported increased frequency of Th17 cells in PD patients and a role for IL-17 in T cell-induced cell death of midbrain neurons (31). Since an increasing number of pharmacological agents are being developed targeting IL-17 and Th17 function, we felt mandatory to establish the role—if any—of Th17 cells and IL-17 in neuroinflammation and neurodegeneration occurring in PD, as this would also pave the way for repositioning Th17/IL-17 targeting drugs in PD.

OVERVIEW ABOUT TH17 CELLS AND IL-17

Physiology and General Pathology of Th17

Th17 have been recognized in 2005 as a distinct lineage and named after IL-17A, which they produce in high amounts (32). Th17 cells function prominently at mucosal surfaces where they trigger pro-inflammatory danger signals that promote clearance of extracellular bacteria and fungi by recruiting and activating neutrophil granulocytes and expressing antimicrobial factors (33, 34). They also directly stimulate the production of mucins (MUC5AC and MUC5) in primary human bronchial epithelial cells *in vitro* (35) as well as the expression of human beta defensin-2 (36) and CC-chemokine ligand 20 (CCL-20) in lung epithelial cells (37).

Th17 cell differentiation is regulated by several transcription factors, including signal transducer and activator of transcription 3 (STAT3), retinoic acid receptor-related orphan receptor-γt (RORγt) and aryl hydrocarbon receptor, and it is driven by transforming growth factor-β (TGF-β), IL-1 and IL-6. IL-23 and TGF-β are critical differentiation factor for Th17 cells. IL-23 (secreted by dendritic cells and tissue-resident macrophages) is required to expand and stabilize the cell population (38). Exposure to this cytokine, after priming with TGF-β and IL-6 (39), is fundamental for functional maturation and pathogenic function of Th17 (40–42). In the absence of inflammation, however, TGF-β alone induces Foxp3 leading to the production of Treg, this way maintaining immune tolerance (43).

Th17 produce several cytokines in addition to IL-17, such as IL-8, IL-21, IL-22, IL-26, TNF-α, granulocyte-monocyte colony stimulatory factor (GM-CSF), CCL20, and IL-10, that allow the recruitment of neutrophils in inflammatory sites (44, 45), even though some of them are not Th17 specific. IL-21 binding to its receptor leads to CD8+ T cell differentiation and proliferation (together with IL-17 and IL-15), B cell differentiation, IL-8 production from dendritic cells and natural killer (NK) cells differentiation (45). IL-22 induces antimicrobial agents and β-defensins in keratinocytes and promotes epidermal hyperplasia (46). Although GM-CSF is not specifically produced by Th17 cells, but also from Th1 cells, it is important in the pathogenicity of Th17 cells in experimental allergic encephalomyelitis (EAE), the animal model of multiple sclerosis (MS), where it seems to induce antigen presenting cells to produce pro-inflammatory cytokines (including IL-6 and IL-23), promoting generation, maturation and survival of Th-17 cells (47–49). Finally, Th17 cells and their secretes are mainly pro-inflammatory and have been linked to several autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), psoriasis, inflammatory bowel disease (IBD) (45). Evidence however also

exists that Th17 may be possibly skewed toward an immune-suppressive regulatory type in a micro-environmental-dependent manner (47, 50). In RA patients there is a high number of IL-17+ and IL-22+ CD4+ T cells in peripheral blood, and IL-17 is present at the sites of inflammatory arthritis, where it amplifies the inflammation induced by other cytokines (45, 51). In patients with SLE there are increased levels of IL-23, IL-21, and IL-17 as a result of the expansion of Th17 cells associated with the depletion of Treg population and increased Th17/Th1 ratio (45, 52). Th1 and Th17 cells infiltrate psoriatic skin lesions, and in particular Th17 determine increased local amounts of IL-17, IL-22, CCL-20, and TNF-α (45, 53). Finally, in IBD patients, high serum concentrations of IL-17 and IL-21 have been reported (45, 54).

Interleukin-17 Biology and Pharmacology

IL-17A is the founding member of the IL-17 family of cytokines and the major product of Th17 cells. IL-17 family includes six members, designated IL-17A-F. The IL-17 receptor (IL-17R) is expressed ubiquitously, therefore most cells can potentially respond to IL-17 (55). Different IL-17 cytokines have specific receptors (IL-17RA-E). Receptors belonging to the IL-17R family have unique structural features and mediate signaling events that differ from those triggered by other cytokine receptors. All IL-17R subunits are single transmembrane domain-containing proteins with common signaling regions used by at least four ligands (38). The IL-17R complex contains an undetermined number of IL-17RA and IL-17RC subunits, although studies so far indicate that it might be at least trimeric. Both IL-17A and IL-17F signal through these subunits, although IL-17A has far higher affinity for IL-17RA than for IL-17RC, whereas IL-17F has a greater affinity for IL-17RC than for IL-17RA in humans (56). Many cytokine-targeting strategies have been proposed to block signaling through IL-17R, antibodies specific for individual ligands or individual receptor subunits being the most straightforward approach. In addition, soluble IL-17R subunits have been evaluated in pre-clinical models (38).

Among all IL-17 family members, IL-17A and IL-17F are the best characterized. Besides Th17 cells, they are also produced by γδ T cells (57). IL-17 can be produced also by several other innate immune cell types, such as lymphoid tissue inducer cells, natural killer and natural killer T cells, macrophages, Paneth cells (58, 59) and type 3 innate lymphoid cells (ILC-3) (60).

IL-17 immunity has been shown to be essential for muco-cutaneous protection against *Candida albicans* in mice and humans (61), however its dysregulation may cause a variety of disturbances, including autoimmune diseases such as psoriasis and RA, and inflammation-associated cancers such as colorectal carcinoma (62), and blocking IL-17 activity with neutralizing antibodies has emerged as a highly effective therapy for psoriasis and psoriatic arthritis (63). On the other side, in murine models of SLE, deficiency of IL-17 was protective, supporting IL-17 blockade as a potential therapeutic approach in SLE (64).

Th17 in Autoimmune Disorders of the Nervous System

The contribution of Th17 cells is well established in **MS** (65, 66). In mice with EAE, Th17 cells infiltrate the brains (67) and T cell trafficking to the meninges is supported by ILC-3 cells which produce IL-17, eventually sustaining T cell-induced neuroinflammation and neurodegeneration (60). In MS patients, Th17 frequency, serum levels of IL-17, and IL-17 production by PBMC are higher during relapses (68, 69), and Th17 and Th17-related cytokines may be affected by immunomodulatory therapeutics employed in MS (70–72). Circumstantial evidence also suggests the possible involvement of Th17 in amyotrophic lateral sclerosis, where reports show increased IL-17 and IL-23 serum and cerebrospinal fluid levels (73), as well as increased IL-17 production by cultured peripheral blood mononuclear cells (74). Finally, involvement of Th17 in Alzheimer's disease is suggested by evidence obtained in rodent models (75) as well as in patients (76, 77).

Pharmacological Modulation of Th17 and IL-17

Additional interest in establishing the possible contribution of Th17 in PD is provided by the increasing opportunities to target the Th17 lineage and its associated cytokines. Several monoclonal antibodies (MoAb) exist which target the IL-17/IL-17R axis (78). While most of them are still in clinical development, the IL-17R-blocking MoAb brodalumab has been recently cleared by FDA for moderate to severe plaque psoriasis (79), and the IL-17-binding MoAb secukinumab has been approved for moderate to severe plaque psoriasis, psoriatic arthritis, and ankylosing spondylitis (80). Despite their efficacy in psoriasis however, brodalumab and secukinumab did not show efficacy (or were even detrimental) in other Th17-related diseases like RA or Crohn's disease [reviewed in Yang et al. (81)], suggesting that Th17 may use mechanisms othr than IL-17 to drive inflammation in different organs and tissues, and/or that targeting the Th17 lineage rather than IL-17 alone could provide better clinical efficacy. Th17-targeting modalities currently under development include small molecule inverse agonists of the Th17 transcription factor RORγt as well as MoAb that block IL-23, which promotes pathogenic Th17 cell function. Ustekinumab is a MoAb targeting the shared IL-12/23 p40 subunit, thus affecting both Th17 and Th1, which received FDA approval for treatment of psoriasis (81). Finally, vitamin D inhibits IL-17 production in rodent and human T cells thus adding to the list of agents potentially affecting Th17 function (82–84).

AIM

In the present review we systematically retrieved and critically evaluated available evidence regarding the contribution of Th17 cells and IL-17 to neuroinflammation and neurodegeneration in PD, to provide a state-of-the-art compendium which will help identifying the future directions that research in this field may take to assess the possible benefits of targeting Th17/IL-17 to develop novel therapeuticts for PD patients.

LITERATURE SEARCH STRATEGY

The database analysis for articles selection was done following the PRISMA statement (85). Briefly, literature review through database searching led to a total of 470 reports, including 23 from PubMed, 391 from Science Direct, and 56 from Scopus. For literature retrieval, the following keywords were used: "Th17," "IL-17," "Parkinson's Disease," "PD." Neither language, nor year restrictions were given, and all reports issued in the period up to and including August 1st, 2018 were included in the screening. After subsequent analysis for relevant titles and abstracts, a total of 417 articles were excluded since they did not specifically focus on PD, Th17 cells or IL-17. Twenty two papers were then selected for full-text eligibility and of those, after full-text reading, 10 were excluded since they were either reviews, or did not deal about PD, Th17 cells and IL-17 together. In the end 12 papers were included in this review. Studies that were considered for inclusion dealt with the changes that Th17 lymphocytes undergo during PD, their effects on neurons in patients and in animal models and the effects/changes of IL-17. A schematic view of the literature selection is given in **Figure 1** while the full list of retrieved records is available as Supplementary File **(Supplementary Table 1)**.

TH17 AND IL-17 IN PD: A CRITICAL APPRAISAL OF THE EVIDENCE

Animal Studies

We identified 3 studies in animals matching the inclusion criteria, two in C57BL/6J mice treated with i.p. injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (86, 87) and one in human *leucine-rich repeat kinase 2* (*LRRK*) *G2019S* gene transgenic rats (88) (**Table 1**). Both Reynolds et al. (86) and Liu et al. (87) show that MPTP-treated mice have increased Th17 in the *substantia nigra*. Liu et al. (87) reports increased Th17 also in the ventral midbrain. In the first study, immunization of mice with nitrated α-synuclein partially resulted in Th1/Th17 polarization of CD4+ T cells and impaired Treg function, and adoptive transfer of nitrated α-synuclein-primed Th17 cells exacerbated MPTP-induced neurodegeneration while adoptive transfer of Treg was completely protective (86). In the second study, experiments in ventral midbrain cell cultures provided direct evidence and mechanistic explanation of Th17-dependent exacerbation of MPP+-induced dopaminergic cell death (87). As a whole, these two studies provide consistent evidence about the pathogenetic role of Th17 in the MPTP mouse model of PD.

The third selected study does not directly address the impact of Th17 cells of neurodegeneration. Indeed, the animal model consisted of human *LRRK2 G2019S* gene transgenic rats (88) which, as many *LRRK2* transgenic or knockin' rodent models, do not exhibit substantial degeneration of brain dopaminergic neurons [revised in Xiong et al. (89)]. *LRRK2* is one of the key PD-associated genes, which is nonetheless also expressed in immune cells, possibly suggesting a role in immunity and inflammation (90, 91), and in particular the *LRRK2 G2019S* mutation is the most frequent known cause of familial and sporadic PD (92). The study by Park et al. (88) shows that rodents carrying such mutation have decreased numbers of Th17 cells in the colon, unchanged levels of Th17 cells in the brain, ad that myeloid cells are defective in supporting Th17 cell differentiation *in vitro*. While not excluding a neurotoxic potential of Th17 cells, results do not suggest a prominent role by Th17 at least in the *LRRK2 G2019S* mutation-associated form of PD. Indirect support to this conclusion comes from the results of recent studies in mice overexpressing human pathogenic *LRRK2* mutations including *LRRK2 G2019S* (93), showing that brain neuroinflammation was not associated with LRRK2 expression and/or activation in brain microglia nor with myeloid and/or T cell infiltration. Serum cytokines were therefore considered to test the hypothesis that neuroinflammation might be triggered through signaling molecules generated outside the CNS. To this end, wild-type and R1441G mice (but not G2019S mice) were challenged with LPS, showing that no genotype-related differences in cytokine concentration occurred in control conditions, however that after LPS R1441G mice showed increased mRNA levels for several cytokines, most of all IFN-γ, IL-6, IL-10, CCL-5, and M-CSF. Unfortunately, the cytokine panel included none of the main Th17-derived cytokines, however it comprised G-CSF, which can be induced by IL-17 (94). Results showed that after LPS mRNA levels of G-CSF were only slightly different in wild-type and R1441G mice, thus providing no support to the hypothesis of a prominent activation of Th17 cells, in possible agreement with the observation that also CD4+ T cell frequency was not different across the two mouse strains (93).

Clinical Studies

Literature search retrieved 9 studies which examined Th17 in PD (**Table 2**). All the studies were performed in sporadic/idiopathic PD patients, compared with healthy subjects (HS) of similar gender distribution and age (although individual matching was actually performed in only one study). In one study (101), subjects were also genotyped for the *LRRK2 G2019S* mutation, however genotypes were used just for patients/HS matching and not for the analysis of Th17 frequency. Five studies, including 193 PD patients and 203 HS, reported increased Th17 frequency in peripheral blood of PD patients, and four, including 215 patients and 165 HS, reported no change or even reduction.

Several issues must be however considered to compare and interpret results across different studies. First of all, from a methodological point of view the assessment of Th17 cells has been performed by means of substantially different approaches. Three studies (30, 95, 101) assessed Th17 by means of classical phenotypic panels based on the surface markers CXCR3, CCR4 and CCR6 (**Table 3**) [see e.g., (102)]. On the contrary, the other six studies (31, 96–100) identified Th17 cells by means of IL-17 intracellular staining after short-term cell stimulation with phorbol 12-myristate 13-acetate (PMA)/ionomycin to induce detectable levels of IL-17 [**Table 3**; see e.g., (103, 104)]. Remarkably, studies identifying Th17 by means of intracellular IL-17 staining reported increased Th17 in PD patients, with the only exception of Cen et al. (98) who found no differences between patients and HS, while studies identifying Th17 cells by

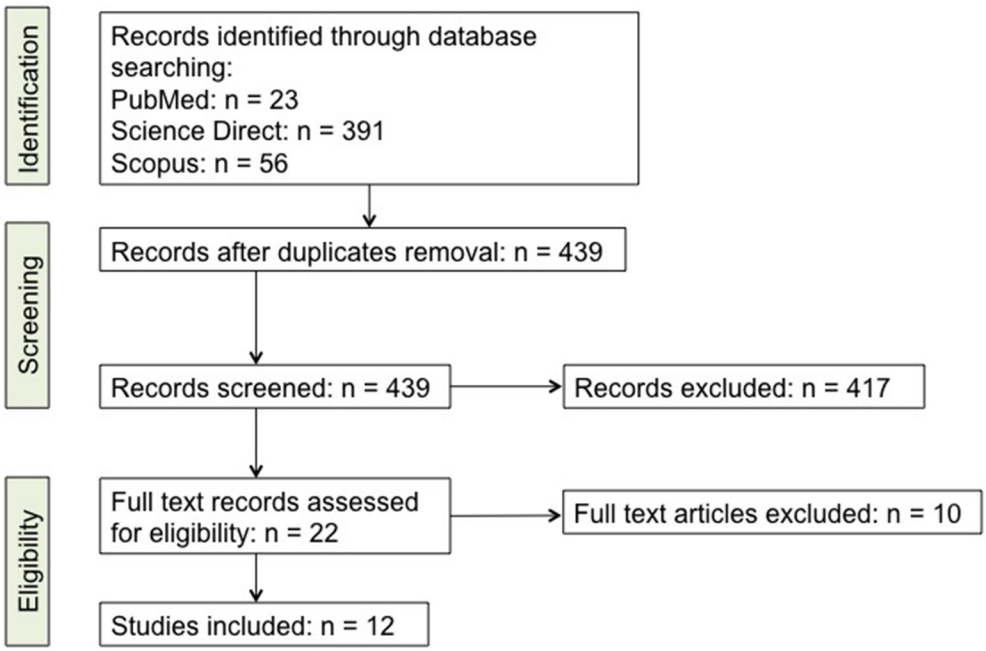


FIGURE 1 | Detailed scheme of the literature screening. A list of the studies is included as Supplementary online data **(Supplementary Table 1)**.

means of surface markers found similar or even reduced Th17 in patients in comparison to HS.

Another critical issue is that in most of the studies Th17 are quantified just as percentage of a reference cell population and not as absolute number of cells per volume of blood. The only two studies providing this latter information are Chen et al. (100) and Kustrimovic et al. (30). The former, in which Th17 were found apparently increased also as absolute numbers, however did not actually perform a direct count of Th17 but just obtained an absolute number using percentages. In other terms, they did not report the actual number of Th17 cells per volume of blood but rather subsequently referred to blood the percentage of IL-17+ cells found in cultured PBMC after separation from whole blood and stimulation with PMA/ionomycin. Kustrimovic et al. (30) on the contrary reported absolute numbers referred to whole blood, and found reduced Th17 in PD patients. This finding is in keeping with the decreased numbers of CD3+ and CD4+ T lymphocytes which have been consistently reported in peripheral blood of PD patients across several studies (28), and which according to Kustrimovic et al. (30) are likely due to decreased Th2, Th17, and Treg. It cannot be excluded therefore that percentage increase of IL17+ T cells may occur in a context where the absolute amounts of circulating Th17 cells is actually decreased. Would this be the case, then direct assessment of IL-17 released from T cells and/or present in serum or tissues becomes of critical importance to establish whether increased Th17/IL-17 function is really enhanced.

Unfortunately, data on IL-17 production by T cells have been provided so far only by Sommer et al. (31) and Kustrimovic et al. (30). In the first study, IL-17 was measured in supernatants after 3-days co-culture of human induced pluripotent stem cells (hiPSCs)-derived neurons with autologous T lymphocytes from 3 patients and 3 HS, finding increased amounts of IL-17 in the supernatants of PD co-cultures. The study also includes pharmacological experiments suggesting the involvement of IL-17/IL-17R signaling in T cell-induced cell death of PD patients' hiPSC-derived mesencephalic brain neurons (31). In the second study, IL-17 was assayed in the supernatants of CD4+ T cells cultured for 48 h in resting conditions and activated with phytohaemagglutinin (PHA). Cells from 6 PD patients on antiparkinson drugs, 4 PD patients who never received dopaminergic substitution treatment and 4 HS were tested, and no difference was observed in IL-17 production either in resting conditions or after PHA, while other cytokines such as IFN-γ and TNF-α were hugely increased in cells from patients (30). The possible contribution of differences in the experimental models used in these studies in the discrepancy regarding IL-17 production by T cells of PD patients remains an unresolved issue.

In summary, while the frequency of Th17 identified by means of their established surface markers may not differ between PD patients and controls, increased frequency of IL-17-producing cells has been consistently reported. *Ex vivo* evidence showing increased secretion of IL-17 from T cells of PD patients is however scarce and contradictory, as only two studies exist which used profoundly different experimental models, one showing enhanced production in samples from just 3 patients in comparison to 3 controls (31) and another one showing no difference in 11 patients in comparison to 4 controls (30). Last but not least, no studies found correlations between Th17/IL-17 and clinically relevant measures of disease state and/or

TABLE 1 | Evidence from animal models.

Animals	Treatment	Main findings	References
C57BL/6J mice	Four i.p. injections of MPTP 16 mg/kg at 2 h intervals.	• MPTP-intoxicated mice had increased infiltration of CD4+ cells (8.5- and 10.3-fold, respectively) within the SN at 7 days-post-intoxication; • immunization of mice with nitrated recombinant α-synuclein (N-4YSyn) partially polarized CD4+ T cells *in vivo* toward either a Th1 or Th17 phenotype, while producing a deficiency in Treg function; • Adoptive transfer of N-4YSyn Th17 cells exacerbated the MPTP-induced loss of striatal TH density; • Adoptive transfer of Treg provided 100% protection of TH+ nigral dopaminergic neurons.	(86)
C57BL/6J mice	Four i.p. injections of MPTP 20 mg/kg at 2 h intervals.	In MPTP-treated mice: • Increased Th17 (identified as CD4/RORγt-immunoreactive cells) in the SNpc; • Increased Th17 (identified as CD4+IL-17+ cells) in the ventral midbrain; In VM cell cultures, Th17 cells exacerbated MPP+-induced dopaminergic neuronal loss and pro-inflammatory/neurotrophic factor disorders through LFA-1/ICAM-1-mediated Th17-VM neurons communication.	(87)
Human *LRRK2 G2019S* gene transgenic rats	*LRRK2* gene polymorphisms are a risk factor for PD *LRRK2 G2019S* is the most prevalent *LRRK2* mutation found in PD patients.	*LRRK2 G2019S* transgenic rats show: • Decreased numbers of bone marrow myeloid progenitors; • Decreased numbers of Th17 cells in the colon, but not in the brain, during TNBS- or DSS-induced colitis or LPS-induced systemic inflammation; • Myeloid cells defective in supporting Th17 cell differentiation *in vitro*.	(88)

progression, such as disease duration, disability scores, intensity of dopaminergic substitution therapy, etc. Clinical evidence regarding dysregulated frequency and/or function of Th17 cells in PD patients remains therefore scarce and inconclusive.

DISCUSSION

Evidence about the involvement of Th17 in PD is so far limited and controversial, as preclinical studies in animal models are just a few while clinical studies provide inconclusive results. While the involvement of Th17 in the MPTP mouse model of PD has been confirmed by two studies (86, 87), no data exist in other models, based either on treatment of animals with different neurotoxins or on genetic modifications. Indeed, neurotoxin-based models are best recapitulating nigrostriatal degeneration, but for instance MPTP-treated mice do not exhibit Lewy bodies and/or α-synuclein-like pathology, while genetic models may better simulate genetic forms of PD (105). The only other animal study available was based on human *LRRK2 G2019S* gene transgenic rats, which however show only mild behavioral alterations and no brain damage (88). Remarkably, in this study the presence of *LRRK2 G2019S*, one of the major PD-linked gene mutations, was associated with unchanged brain Th17 cells and even decreased Th17 in periphery, as well as with defective myeloid cells-induced Th17 differentiation *in vitro*.

On the other side, only few clinical studies included some mechanistic evidence in addition to the mere enumeration of circulating Th17. Possibly, the main apparently conflictual evidence arising is that Th17 are not different between PD patients and controls when assessed by means of Th17-associated surface markers, while they are increased if assayed as IL-17+ cells (**Tables 2**, **3**). Th17 subsets identified by these two methods indeed do not coincide. Evidence exists that Th17-associated surface markers—and in particular CCR6—are expressed on virtually all IL-17+ T lymphocytes. IL-17 is however usually undetectable in freshly isolated cells, which require activation to express measurable IL-17 (as in 5 out of the 6 studies which used intracellular IL-17 staining to identify Th17 cells; **Table 3**). Following activation however IL-17+ T cells may usually represent on average just 7–8% of total Th17 cells, depending on the specific protocol of isolation, culture, and stimulation of T cells (106, 107), although at least a few studies exist showing that they may correlate with Th17 frequency assessed by means of surface markers (108).

TABLE 2 | Evidence from clinical studies.

Subjects	Gender (m/f)	Age (years)	UPDRS III score	H&Y stage	Antiparkinson treatment or LED (mg/day)	Th17	Comment	References
29 patients with sporadic PD vs. 30 HS	PD: 17/12 HS: 16/14	PD: 70.4 ± 7.2 HS: 68.9 ± 5.1	23.9 ± 18.5	2.6 ± 0.9	LED: 419.2 ± 237.1	10.0 ± 2.9% in PD patients vs. 11.3 ± 3.2% in HS ($P > 0.05$)	No difference in Th17 frequency between PD patients and HS. PD patients had however lower CD3+, CD4+ T cells, lower Th1 cells, higher B cells and NK cells, while there was no difference in CD+ T cells, Th2, NK-T cells, and Treg.	(95)
45 patients with idiopathic PD vs. 55 HS[a]	PD: 28/17 HS: 28/27	Mean (range) PD: 61.4 (40–74) HS: 62(40–70)	Not specified	Range: 1–4	Madopar (levodopa+benserazide)	0.892 ± 0.195% and 0.764 ± 0.151% in PD patients with and without constipation, respectively vs. 0.516±0.157% in HS ($P < 0.001$ in both cases)	Higher Th17 frequency in PD patients in comparison to HS. PD patients had also lower Treg frequency in comparison to HS. Th17 were higher and Treg were lower in PD patients with constipation in comparison to those without constipation.	(96)
40 primary PD patients vs. 40 HS	PD: 22/18 HS: 24/16	PD: 60.4 ± 5.8 HS: 58.6 ± 6.2	Not specified	Not specified	Untreated	0.69 ± 0.11% in PD patients vs. 0.34 ± 0.03% in HS ($P < 0.001$)	Higher Th17 frequency in PD patients in comparison to HS. Compared to HS, PD patients had also lower Treg frequency, lower Treg/Th17 ratio, higher serum IL-17, and similar levels of RORγt mRNA in peripheral blood mononuclear cells.	(97)
64 sporadic PD patients vs. 46 HS	PD: 44/20 HS: 18/28	Not specified	Not specified	Not specified	Not specified	Not shown	No difference in Th17 frequency between PD patients and HS. No difference also in Treg frequency, however when stratified by gender, female PD patients had higher Treg/Th17 ratio in comparison to HS.	(98)
80 initial stage PD patients vs. 80 age/gender matched HS	PD: 40/40 HS: 40/40	PD: 66.2 ± 6.5 HS: 66.7 ± 5.3	13.5±5.3	1.2 ± 0.2	Untreated	1.56 ± 1.38% in PD patients vs. 0.13 ± 0.08% in HS ($P < 0.001$)	Higher Th17 frequency in PD patients in comparison to HS. PD patients had also higher frequency of myeloid-derived suppressor cells (MDSC). Th17 and MDSC showed a positive correlation in PD patients but not in HS.	(99)
18 initial stage PD patients vs. 18 age/gender matched HS	PD: 9/9 HS: 9/9	PD: 68.3 ± 6.0 HS: 64.0 ± 7.0	15.5 ± 6.5	1.1 ± 0.3	Not specified	2.3 ± 1.2 x 10^4/mL (1.17 ± 0.61%) in PD patients vs. 0.4 ± 0.2 x 10^4/mL (0.19 ± 0.10%) in HS ($P < 0.001$)	Higher Th17 absolute count and frequency in PD patients in comparison to HS. PD patients had also higher absolute count and frequency of myeloid-derived suppressor cells. Th17 and MDSC showed no correlation either in PD patients or in HS.	(100)

(Continued)

TABLE 2 | Continued

Subjects	Gender (m/f)	Age (years)	UPDRS III score	H&Y stage	Antiparkinson treatment or LED (mg/day)	Th17	Comment	References
40 PD patients vs. 32 G2019S LRRK2 mutation matched HS	PD: 29/11 HS: 8/24	PD: 67.4 ± 1.3 HS: 65.7 ± 1.9	Not specified	Not specified	Not specified	14.2 ± 1.2% of CD4+ (mean ± SEM) in PD patients vs. 12.9 ± 0.8% in HS [b] ($P > 0.05$)	No difference in Th17 frequency between PD patients and HS. PD patients had less CD4+ T cells, however the frequencies of Th1, Th2, Th17 and Treg were the same between PD patients and HS.	(101)
82 idiopathic PD patients (25 drug naive [dn] and 56 on antiparkinson drugs [dt]) vs. 47 HS	PD: 49/33 HS: 25/22	dn: 68.0 ± 9.3 dt: 70.0 ± 8.5 HS:66.8 ± 10.3	dn: 13.6 ± 7.2 dt: 15.3 ± 6.6	dn: 1.3 ± 0.5 dt: 1.9 ± 0.7	11 taking l-DOPA alone, 34 taking l-DOPA + DA agonists without (19) or with rasagiline (15), and 9 taking DA agonists alone (4) or with rasagiline (5). LED: 533.2 ± 360.1	57.1 ± 22.7 x 10^6/L (8.8 ± 3.9% of CD4+ T cells) in PD-dn patients, 56.9 ± 30.3 x 10^6/L (7.4 ± 2.5%) in PD-dt patients, and 89.3 ± 48.5 x 10^6/L (9.2 ± 4.1%) in HS $P < 0.01$ for absolute counts and $P > 0.05$ for % in PD-dn or PD-dt patients vs. HS	Lower Th17 absolute count and no difference in Th17 frequency between PD patients and HS. PD patients had less CD4+ T cells (both as absolute count and as % of total lymphocytes). less Th1/17 and Treg absolute count but same frequency among CD4+ T cells. PD-dt patients had lower Th2 absolute count and frequency and increased Th1 frequency. PD patients had lower levels of RORC mRNA in CD4+ T cells. *Ex vivo* experiments show that: • PD patients CD4+ T cells isolated and cultured for 48 h in resting conditions and activated with PHA produce more IFN-γ and TNF-α (but same amounts of IL-17) in comparison to HS cells; • PD patients naïve CD4+ T cells differentiate more toward Th1 and less toward Th17 in comparison to HS cells.	(30)
10 idiopathic PD patients vs. 10 age/gender matched HS	PD: 9/1 HS: 8/2	PD: 62.5 ± 11.5 HS: 61.9 ± 5.8	14.7 ± 8.3	2.2 ± 0.7	LED: 555.0 ± 325.3	1.67 ± 0.75% of CD3+ T cells in PD patients and 0.75 ± 0.35% in HS ($P < 0.002$)	Higher Th17 absolute count and frequency in PD patients in comparison to HS. *In vitro* experiments show that: • T lymphocytes from PD patients produce more IL-17; • IL-17/IL-17R signaling is involved in T cell-induced cell death of PD patients hiPSC-derived MBNs [c].	(31)

Data are means±SD unless otherwise stated.

[a] *Total patients were 102, however only 45 agreed to provide blood samples for T cell subset analysis.*

[b] *Estimated from Supplementary Figure S3 in Cook et al. (101).*

[c] *hiPSC: fibroblast-derived human induced pluripotent stem cells; MBNs: midbrain neurons.*

TABLE 3 | Flow cytometric panels used in clinical studies.

CD3	CD4	CD8	CD45RO	CXCR3	CCR4	CCR6	IL-17A	Sample *pretreatment*	Th17 phenotype	Th17 changes in PD patients	References
	×		×			×		Whole blood *n/a*	CD4+/CD45RO+/CCR6+	=	(95)
×	×						×	Whole blood *PMA 2 μg/mL + ionomycin 50 μg/mL*	CD3+/CD4+/IL-17A+	↑	(96)
×		×					×	PBMC *PMA 1 μg/mL + ionomycin 50 μg/mL*	CD3+/CD8-/IL-17A+	↑	(97)
×		×					×	PBMC *leukocyte activator cocktail (?)*	CD3+/CD8-/IL-17A+	=	(98)
×		×					×	PBMC *PMA 50 ng/mL + ionomycin 500 ng/mL*	CD3+/CD8-/IL-17A+	↑	(99)
×		×					×	PBMC *PMA 50 ng/mL + ionomycin 500 ng/mL*	CD3+/CD8-/IL-17A+	↑	(100)
×	×		×	×		×		PBMC *n/a*	CD3+/CD4+/CD45RO+/CXCR3-/CCR6+	=	(101)
	×			×	×	×		whole blood *n/a*	CD4+/CXCR3-/CCR4+/CCR6+	=/↓	(30)
	×						×	CD3+ T cells *PMA 20 ng/mL + ionomycin 1 μM*	CD4+/IL-17A+	↑	(31)

n/a, not applicable; PMA, phorbol 12-myristate 13-acetate; PBMC, peripheral blood mononuclear cells; =: unchanged; ↑: increased; ↓: reduced.

A provisional interpretation which might possibly reconcile available evidence is that PD patients have similar percentages of Th17 cells, which however may harbor an increased proportion of cells ready to express IL-17 upon activation. Whether this increased percentage of IL-17-producing cells may actually result in increased Th17/IL-17 activity remains however to be established, in view of the limited and conflicting evidence about IL-17 amounts actually secreted by PD patients lymphocytes, which is unchanged according to Kustrimovic et al. (30) and increased based on Sommer et al. (31), both unfortunately performing experiments in cell preparations from very few subjects. In addition, Kustrimovic et al. (30) reported reduced absolute numbers of Th17 cells per volume of blood in PD patients, thus highlighting the possibility that even increased percentages of IL-17-producing cells might not necessarily result in enhanced Th17/IL-17-dependent systemic proinflammatory effects.

As a whole, evidence about Th17 contribution in PD remains thus circumstantial and awaits further confirmation and in-depth investigation. Before driving provisional conclusions from available studies directly addressing Th17 and IL-17 in PD patients and in animal models of the disease, a few additional indirect lines of evidence about Th17/IL-17 and PD deserve consideration to put the issue in full context, namely: (i) the correlation between PD and immune-related disease, (ii) recent studies about gut microbiome in PD and (iii) on vitamin D, and (iv) emerging evidence regarding dopaminergic modulation of Th17 cells and (v) the influence of Th17 on glial cells.

PD and Immune-Related Disease

There is extensive literature about the association between PD and immune-related disease such as autoimmune disease and cancer. Epidemiological studies strongly suggest that PD patients are at lower risk for most cancers, with the notable exception of breast cancer and melanomas, which may occur more frequently in PD patients as compared with controls (109). Th1 lymphocytes are responsible for cell-mediated immunity to intracellular pathogens and tumor cells (110), thus a relative increase in Th1 in PD patients [as reported by Kustrimovic et al. (30)] might well contribute to lower susceptibility to cancers. Such hypothesis is further supported by evidence in PD patients regarding increased CD4+ T cell production of IFN-γ and TNF-α, two Th1 cytokines critical for antitumor immunity, and their insensitivity to Treg, as well as by the preferential differentiation of naive CD4+ T cells from PD patients toward the Th1 lineage (30). Th17 cells on the contrary play controversial roles in antitumor immunity (111), nonetheless, at least in mice Th17 cells eradicate melanoma tumors to a greater extent than Th1 cells (112). Therefore, increased frequency of melanomas in PD might eventually stand against a systemic increase of Th17 activity in PD.

Immune-related disease associated with PD also include psoriasis, IBD and RA. Patients with psoriasis may have on average 38% increased risk to develop PD, possibly as a result of chronic inflammation (113). The relationship between PD and IBD is much more controversial, since although gene association studies suggest that functional variants in the *LRRK2* gene may

confer shared effects on risk for Crohn's disease and PD, it has been also reported that PD as a whole is associated with lower risk of IBD (−15%) as well as of Crohn's disease (−17%) and ulcerative colitis (−12%) (114). Another study however showed that IBD may increase by 28% the risk of developing PD, while treatment of IBD with anti-TNF-α therapy reduced PD incidence rate among IBD patients by 78% (115). **RA** as well may be associated with a reduced risk of developing PD (−35%) (116). It must be also considered however that in other population-based studies no association between PD and autoimmune disease has been reported, with the only remarkable exception in rheumathoid arthritis patients of a reduced risk of developing PD (−30%, (117)). Psoriasis, IBD and RA are however complex diseases currently seen as supported by both Th1 and Th17-mediated inflammation (118–120), thus evidence for association of any of these immune-mediated diseases with PD provides just a few if any contribution to unravel the specific role of Th17 in PD.

Clues From Gut Microbiome Studies

The gut microbiome attracts increasing attention as key regulator of brain development and homeostasis, possibly acting also through the immune system (121), and as a consequence the gut microbiome in PD represents a rapidly growing area of research. A recent study compared the fecal microbiomes of PD patients and controls, showing in feces of PD patients nearly 80% decreased abundance of *Prevotellaceae*, and increased abundance of other families including *Lactobacillaceae* (122). *Prevotellaceae* abundance may be associated with increased Th17-mediated mucosal inflammation (123), while *Lactobacillaceae* may induce Th1-type immune responses (124). Modifications of the intestinal microbiome in PD might be thus related to reduced Th17 cells and the Th1-biased immunity which we found in PD patients (30).

Th17, Vitamin D, and PD

Vitamin D exerts direct and indirect effects on T lymphocytes, and its deficiency has been linked to autoimmune and cardiovascular disease, hypertension, and even cancer. Remarkably, in both rodent and human T cells vitamin D has been shown to inhibit production of IL-17 and IFN-γ and to promote Treg differentiation and function (82–84). Several lines of evidence suggest that PD patients may be low in vitamin D and that vitamin D supplementation prevents dopaminergic neuron loss in animal models and may be beneficial in patients (125, 126). It cannot be excluded that at least part of vitamin D-induced benefits in PD might depend on its immune effects. Studies are needed however to assess the eventual occurrence and the relative contribution of vitamin D-dependent modulation of Th1, Th17, and Treg in PD.

Dopaminergic Modulation of Human Th17

No therapies are available for PD, and symptomatic treatments rely on dopamine substitution treatments (including the DA precursor l-DOPA, DA agonists and indirect dopaminergic agents) (12). Besides its role as neurotransmitter, DA is however a key transmitter between the nervous and the immune system as well as among immune cells and peripheral tissues (127–129). Although information on DA and Th17/IL-17 is still limited and fragmentary, in rodent models it has been shown that D_1-like D5 DR expressed on DC may contribute to Th17 differentiation and severity of EAE (130). Recently, Melnikov et al. (69) reported that DA may inhibit IL-17 and IFN-γ production in cultured peripheral blood mononuclear cells from HS and MS patients. The ability of DA to inhibit IFN-γ production in peripheral blood mononuclear cells (PBMC) was previously reported by Ferreira et al. (131), who however did not observe any effect of DA on IL-17 or on the Th17-related cytokines. Remarkably, in a subsequent study Ferreira et al. (132) confirmed no effect by DA on IL-17 produced by PBMC but, in apparent contrast with Melnikov et al. (69), reported the ability of DA to increase IL-17 from cells of MS patients. No evidence was provided about the receptor pathways involved, anyway such observations highlight the need for further studies. Melnikov et al. (69) showed that in their experiments the inhibitory effect of DA on IL-17 was antagonized by the D_2-like DR antagonist sulpiride but not by the D_1-like DR antagonist SCH-23390. Unfortunately, pharmacological experiments were not performed on IFN-γ production. Such results deserve careful consideration for their potential implications concerning PD. Indeed, DA agonists used as antiparkinson drugs act mainly on D_2-like DR (for instance, rotigotine is a D_1-like/D_2-like DR mixed agonist, while pramipexole is a D_2-like DR agonist). Would ropinirole or pramipexole modulate IL-17 and IFN-γ production by T cells of PD patients at therapeutically relevant concentrations, DA agonists in PD could possibly shift from mere DA substitutes to immunomodulating drugs targeting Th17 and Th1 mechanisms potentially involved in neuroinflammation underlying PD neurodegeneration.

Influence of Th17 on Glial Cells

The pathogenic role of Th17 cells in the CNS has received extensive attention on the pathogenesis of autoimmune demyelinating diseases, where their contribution is well established (133), and at least one proof-of-concept study provided evidence that secukinumab, which blocks IL-17A, may reduce lesions in MS patients (134). In this context, the ability of Th17 cells to affect glia has received specific attention. Evidence in animal models of MS suggests that Th17 cells preferentially affect astrocytes rather than microglia. In integrin α4-deficient mice, where trafficking of Th1 but not Th17 cells into the CNS is compromised, induction of experimental autoimmune encephalomyelitis results in microglial activation but comparable astrogliosis in comparison to wild-type mice (135). Indeed, while Th1-derived secretions trigger proinflammatory responses in microglia, neither Th17-derived secretions nor increased expression of IL-17A in the brain apparently affect microglial function (135). While miroglia has an established role in neuroinflammation and neurodegeneration in PD (136), the involvement of astrocytes is still a matter of debate (137). As for IL-17, it has been shown that exposure of microglia to IL-17A results in activation and increased production of proinflammatory cytokines, and

IL-17A-neutralizing antibodies prevented neuroinflammation and cognitive impairment in rodents (138). On the other side, at least *in vitro* TLR-dependent activation of microglia has been shown to polarize γδ T cells toward neurotoxic IL-17+ γδ T cells (139).

CONCLUSIONS

Critical appraisal of evidence retrieved after a systematic revision of the literature available about Th17/IL-17 and PD do not allow to reach definite conclusions. Both animal, as well as clinical studies, are limited and in particular the latter are mainly concerned with Th17 frequency rather than function and relationship with PD pathology and clinical progression.

On the other side, indirect evidence potentially may stand in favor of a contribution of Th17 in PD (as suggested by studies on vitamin D) but also against it (in the case of recent studies addressing gut microbiome in PD patients), or may be also once more conflicting (such as studies correlating PD with immune-related disease).

Future research on Th17 in PD patients should thus first of all thoroughly assess circulating Th17 using both surface markers and intracellular IL-17 staining, considering not only cell frequency but also absolute numbers per volume of blood. It would be also necessary to include investigation of IL-17 (as well as of other Th17-related cytokines) secretion and serum/tissue levels.

As suggested in **Figure 2**, key questions to be answered include:

1. Which is the relationship between Th17/IL-17 and PD pathology and clinics (such as disease duration, disability scores, intensity of dopaminergic substitution therapy, etc.)?
2. Which are the mechanisms and the cellular targets (including neurons, microglia, and astrocytes) underlying Th17/IL-17 contribution to PD pathogenesis and progression (in this regard the study by Sommer et al. (31) may be a primer, however results await reproduction and extension)?
3. Do dopaminergic agonists currently used in PD therapeutics affect Th17/IL-17, as reported by *in vitro* evidence for D2-like DR-dependent modulation of human Th17 (69)?

In this last regard, it should be taken in mind that therapy with dopaminergic agonists (and possibly with L-DOPA and other indirect dopaminergic agents) could be also a confounding factor in studies of Th17/IL-17 in PD patients.

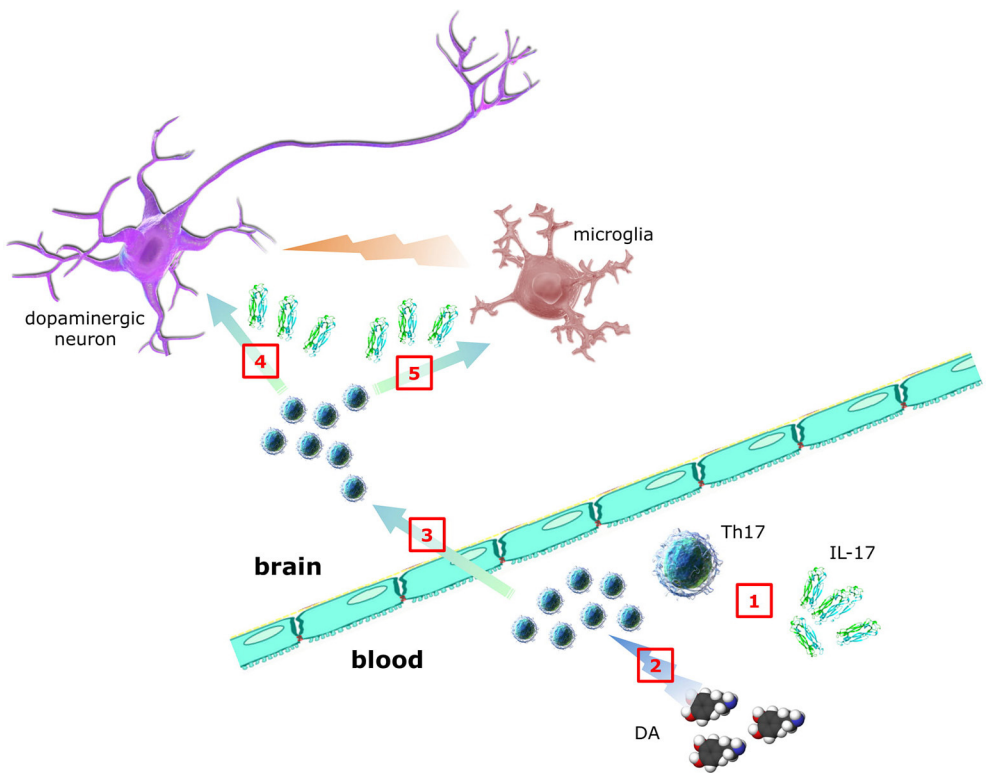


FIGURE 2 | Contribution of Th17 lymphocytes and IL-17 to PD. Whether Th17 and IL-17 in peripheral blood of PD patients (1) are increased, decreased or unchanged is still debated, despite many studies addressed the issue. DA itself may also affect Th17 function, however whether dopaminergic substitution therapy results in any Th17/IL-17 changes is presently unknown (2). In addition, although it is established that T cells infiltrate brains of PD patients, direct demonstration of Th17 has not yet been provided (3). In the same way, although *in vitro* Th17/IL-17 have been shown to exert neurotoxic effects, the clinical relevance of such observations awaits confirmation (4). Finally, despite circumstantial evidence suggesting Th17-glial cells interplay, no data exist so far in PD (5) (individual parts of the figure have been taken and modified from the Wikimedia Commons - http://commons.wikimedia.org).

Careful assessment of Th17 in PD is anyway a priority in the context of the emerging area of peripheral immunity in PD, also in view of the increasing number of therapeutics targeting Th17/IL-17 pathways approved for clinical indications and which might therefore represent potentially novel antiparkinson drugs.

AUTHOR CONTRIBUTIONS

FM and MC: study conception and design. ES, NC, and ER: acquisition of data. ES, NC, ER, FM, and MC: analysis and interpretation of data. All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved, and declare to have confidence in the integrity of the contributions of their co-authors.

FUNDING

ES was supported by a fellowship grant from the University of Insubria for the development of *in vitro* models of immune modulation in neurodegenerative disease.

ACKNOWLEDGMENTS

We gratefully acknowledge the support provided by Fondazione CARIPLO (http://www.fondazionecariplo.it - Project 2011-0504) and by Fondazione UBI per Varese Onlus (grant 18/7/2017), which contributed to the development of some of the ideas and of the experimental research reviewed in the text.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2019.00013/full#supplementary-material

REFERENCES

1. Monahan AJ, Warren M, Carvey PM. Neuroinflammation and peripheral immune infiltration in Parkinson's Disease: an autoimmune hypothesis. *Cell Transplant*. (2008) 17:363–72.
2. Tysnes OB, Storstein A. Epidemiology of Parkinson's disease. *J Neural Transm.* (2017) 124:901–5. doi: 10.1007/s00702-017-1686-y
3. Eriksen JL, Wszolek Z, Petrucelli L. Molecular pathogenesis of Parkinson disease. *Arch Neurol.* (2005) 62:353–7. doi: 10.1001/archneur.62.3.353
4. Schapira AH, Jenner P. Etiology and pathogenesis of Parkinson's disease. *Mov Disord.* (2011) 26:1049–55. doi: 10.1002/mds.23732
5. Kalia LV, Lang AE. Parkinson's disease. *Lancet* (2015) 386:896–912. doi: 10.1016/S0140-6736(14)61393-3
6. Dehay B, Bourdenx M, Gorry P, Przedborski S, Vila M, Hunot S, et al. Targeting α-synuclein for treatment of Parkinson's disease: mechanistic and therapeutic considerations. *Lancet Neurol.* (2015) 14:855–66. doi: 10.1016/S1474-4422(15)00006-X
7. More SV, Kumar H, Kim IS, Song SY, Choi DK. Cellular and molecular mediators of neuroinflammation in the pathogenesis of Parkinson's disease. *Mediators Inflamm.* (2013) 2013:952375. doi: 10.1155/2013/952375
8. McGeer PL, McGeer EG. Inflammation and neurodegeneration in Parkinson's Disease. *Parkinsonism Relat Disord.* (2004) 1:S3–7. doi: 10.1016/j.parkreldis.2004.01.005
9. Boland DF, Stacy M. The economic and quality of life burden associated with Parkinson's disease: a focus on symptoms. *Am J Manag Care* (2012) 18:S168–75.
10. Kowal SL, Dall TM, Chakrabarti R, Storm MV, Jain A. The current and projected economic burden of Parkinson's disease in the United States. *Mov Disord.* (2013) 28:311–8. doi: 10.1002/mds.25292
11. Johnson SJ, Diener MD, Kaltenboek A, Birnbaum HG, Siderowf AD. An economic model of Parkinson's disease: implications for slowing progression in the United States. *Mov Disord.* (2013) 28:319–26. doi: 10.1002/mds.25328
12. Connolly BS, Lang AE. Pharmacological treatment of Parkinson disease: a review. *JAMA* (2014) 311:1670–83. doi: 10.1001/jama.2014.3654
13. Fox SH. Non-dopaminergic treatments for motor control in Parkinson's disease. *Drugs* (2013) 73:1405–15. doi: 10.1007/s40265-013-0105-4
14. Nonnekes J, Timmer MH, de Vries NM, Rascol O, Helmich RC, Bloem BR. Unmasking levodopa resistance in Parkinson's disease. *Mov Disord.* (2016) 31:1602–9. doi: 10.1002/mds.26712
15. Przedborski S. Inflammation and Parkinson's disease pathogenesis. *Mov Disord.* (2010) 25:S55–7. doi: 10.1002/mds.22638
16. Mosley RL, Hutter-Sauders JA, Stone DK, Gendelman HE. Inflammation and adaptive immunity in Parkinson's disease. *Cold Spring Harb Perspect Med.* (2012) 2012:a009381. doi: 10.1101/cshperspect.a009381
17. Cappellano G, Carecchio M, Fleetwood T, Magistrelli L, Cantello R, Dianzani U, et al. Immunity and inflammation in neurodegenerative diseases. *Am J Neurodegener Dis.* (2013) 2:89–107.
18. Gonzàlez H, Elgueta D, Montoya A, Pacheco R. Neuroimmune regulation of microglial activity involved in neuroinflammation and neurodegenerative diseases. *J Neuroimmunol.* (2014) 274:1–13. doi: 10.1016/j.jneuroim.2014.07.012
19. Lee JK, Tran T, Tansey MG. Neuroinflammation in Parkinson's disease. *J Neuroimmune Pharmacol.* (2009) 4:419–29. doi: 10.1007/s11481-009-9176-0
20. McGeer PL, Itagaki S, Akiyama H, McGeer EG. Rate of cell death in parkinsonism indicates active neuropathological process. *Ann Neurol.* (1988) 24:574–6. doi: 10.1002/ana.410240415
21. Mosley RL, Benner EJ, Kadiu I, Thomas M, Boska MD, Hasan K, et al. Neuroinflammation, oxidative stress and the pathogenesis of Parkinson's disease. *Clin Neurosci Res.* (2006) 6:261–81.
22. Gonzàlez H, Pacheco R. T-cell-mediated regulation of neuroinflammation involved in neurodegenerative diseases. *J Neuroinflamm.* (2014) 11:201. doi: 10.1186/s12974-014-0201-8
23. Brochard V, Combadière B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, et al. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest.* (2009) 119:182–92. doi: 10.1172/JCI36470
24. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations. *Annu Rev Immunol.* (2010) 28:445–89. doi: 10.1146/annurev-immunol-030409-101212
25. O'Shea JJ, Paul WE. Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. *Science* (2010) 327:1098–102. doi: 10.1126/science.1178334
26. Gonzàlez H, Contreras F, Pacheco R. Regulation of the neurodegenerative processassociated to Parkinson disease by CD4+ T-cells. *J Neuroimmune Pharmacol.* (2015) 10:561–75. doi: 10.1007/s11481-015-9618-9
27. Tahmasebinia F, Pourgholaminejad A. The role of Th17 cells in auto-inflammatory neurological disorders. *Prog Neuropsychopharmacol Biol Psychiatry* (2017) 79(Pt B):408–16. doi: 10.1016/j.pnpbp.2017.07.023
28. Jiang S, Gao H, Luo Q, Wang P, Yang X. The correlation of lymphocyte subsets, natural killer cell, and Parkinson's disease: a meta-analysis. *Neurol Sci.* (2017) 38:1373–80. doi: 10.1007/s10072-017-2988-4
29. Kustrimovic N, Rasini E, Legnaro M, Bombelli R, Aleksic I, Blandini F, et al. Dopaminergic receptors on CD4+ T naive and memory lymphocytes

correlate with motor impairment in patients with parkinson's disease. *Sci Rep.* (2016) 22:33738. doi: 10.1038/srep33738
30. Kustrimovic N, Comi C, Magistrelli L, Rasini E, Legnaro M, Bombelli R, et al. Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/Th17 and Treg in drug-naïve and drug-treated patients. *J Neuroinflamm.* (2018) 15:205. doi: 10.1186/s12974-018-1248-8
31. Sommer A, Maxreiter F, Krach F, Fadler T, Grosch J, Maroni M, et al. Th17 lymphocytes induce neuronal cell death in a human iPSC-based model of Parkinson's disease. *Cell Stem Cell* (2018) 23:123–31. doi: 10.1016/j.stem.2018.06.015
32. Sallusto F, Zielinski CE, Lanzavecchia A. Human Th17 subsets. *Eur J Immunol.* (2012) 42:2215–20. doi: 10.1002/eji.201242741
33. Cosmi L, Maggi L, Santarlasci V, Liotta F, Annunziato F. T helper cells plasticity in inflammation. *Cytometry Part A* (2014) 85A:36–42. doi: 10.1002/cyto.a.22348
34. Cosmi L, Santarlasci V, Maggi L, Liotta F, Annunziato F. Th17 plasticity: pathophysiology and treatment of chronic inflammatory disorders. *Curr Opin Pharmacol.* (2014) 17:12.16. doi: 10.1016/j.coph.2014.06.004
35. Chen Y, Thai P, Zhao YH, Ho YS, DeSouza MM, Wu R. Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem.* (2003) 278:17036–43. doi: 10.1074/jbc.M210429200
36. Kao CY, Chen Y, Thai P, Wachi S, Huang F, Kim C, et al. IL-17 markedly up-regulates beta-defensin-2 expression in human airway epithelium via JAK and NF-kappaB signalling pathways. *J Immunol.* (2004) 173:3482–91. doi: 10.4049/jimmunol.173.5.3482
37. Kao CY, Huang F, Chen Y, Thai P, Wachi S, Kim C, et al. Up-regulation of CC chemokine ligand 20 expression in human airway epithelium by IL-17 through a JAKindependent but MEK=NF kappaB-dependent signalling pathway. *J Immunol.* (2005) 175:6676–85. doi: 10.4049/jimmunol.175.10.6676
38. Gaffen SL. Structure and signalling in the IL-17 receptor family. *Nature* (2009) 9:556–67. doi: 10.1038/nri2586
39. Bettelli E, Carrier Y, Gao W, Korn T, Storm TB, Oukka M. Reciprocal developmental pathways for the generation of pathogenic effector Th17 and regulatory T cells. *Nature* (2006) 441:235–8. doi: 10.1038/nature04753
40. Zúñiga LA, Jain R, Haines C, Cua DJ. Th17 cell development: from the cradle to grave. *Immunol Rev.* (2013) 252:78–88. doi: 10.1111/imr.12036
41. McGeachy MJ. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells *in vivo*. *Nat Immunol.* (2009) 10:314–24. doi: 10.1038/ni.1698
42. Ghoreschi K, Laurence A, Yang XP, Tato CM, McGeachy MJ, Konkel JE, et al. Generation of pathogenic T(H) 17 cells in the absence of TGF-beta signalling. *Nature* (2010) 467:967–71. doi: 10.1038/nature09447
43. Zhang X, Jin J, Peng X, Ramgolam VS, Marcovic-Plese S. Simvastatin inhibits IL-17 secretion by targeting multiple IL-17-regulatory cytokines and by inhibiting the expression of IL-17 transcription factor RORC in CD4+ lymphocytes. *J Immunol.* (2008) 180:6988–96. doi: 10.4049/jimmunol.180.10.6988
44. Annunziato F, Cosmi L, Liotta F, Maggi E, Romagnani S. Defining the human T helper 17 cell phenotype. *Cell Press* (2012) 33:505–11. doi: 10.1016/j.it.2012.05.004
45. Maddur SM, Miossec P, Kaveri SV, Bayry J. Th17 cells. Biology, pathogenesis, of autoimmune and inflammatory diseases and therapeutic strategies. *Am J Pathol.* (2012) 181:8–18. doi: 10.1016/j.ajpath.2012.03.044
46. Korn, T., Bettelli, E., Oukka, M., Kuchroo,VK. IL-17 and Th17 cells. *Annu Rev Immunol.* (2009) 27, 485–517. doi: 10.1146/annurev.immunol.021908.132710
47. Peters A, Lee Y, Kuchroo VK. The many faces of Th17 cells. *Curr Opin Immunol.* (2011) 23:702–6. doi: 10.1016/j.coi.2011.08.007
48. ElBehi M, Ciric B, Dai H, Yan Y, Cullimore M, Safavi F, et al. The ecephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. *Nat Immunol.* (2011) 12:568–75. doi: 10.1038/ni.2031
49. Codarri L, Gyulveszi G, Tosevski V, Hesske L, Fontana A, Magnenat L, et al. RORγt drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat Immunol.* (2011) 12:560–7. doi: 10.1038/ni.2027
50. Ghoreschi K, Laurence A, Yang XP, Hirahara K, O'Shea JJ. T helper 17 cell heterogeneity and pathogenicity in autoimmune disease. *Trends Immunol.* (2011) 32:395–401. doi: 10.1016/j.it.2011.06.007
51. Shen H, Goodall JC, Hill Gaston JS. Frequency and phenotype of peripheral blood Th17 cells in ankylosing spondylitis and rheumatoid arthritis. *Arthritis Reum.* (2009) 60:1647–56. doi: 10.1002/art.24568
52. Yang J, Chu Y, Yang X, Gao D, Zhu L, Wan L, et al. Th17 and natural Treg cell prduction dynamics in systemic lupus erythematosus. *Arthritis Reum.* (2009) 60:1472–83. doi: 10.1002/art.24499
53. Lowes MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, Haider AS, et al. Psoriasis vulgaris lesions contain descrete populations of Th1 and Th17 cells. *J Invest Dermatol.* (2008) 128:1207–11. doi: 10.1038/sj.jid.5701213
54. Zenewicz LA, Antov A, Flavell RA. CD4 T-cell differentiation and inflammatory bowel disease. *Trends Mol Med.* (2009) 15:199–207. doi: 10.1016/j.molmed.2009.03.002
55. Hu MH, Huang GS, Wu CT, Lin JJ, Hsia SH, Wang HS, et al. Analysis of plasma multiplex cytokines for children with febrile seizures and severe acute ecephatilis. *J Child Neurol.* (2014) 29:182–6. doi: 10.1177/0883073813488829
56. Kuestner RE, Taft DW, Haran A, Brandt CS, Brender T, Lum K, et al. Identification of the IL-17 receptor related molecule, IL-17RC, as the receptor for IL-17F. *J Immunol.* (2007) 179:5462–73. doi: 10.4049/jimmunol.179.8.5462
57. Jin W, Dong C. IL-17 cytokines in immunity and inflammation. *Emerg Microbes Infect.* (2013) 2:e60. doi: 10.1038/emi.2013.58
58. Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. *Nat Rev Immunol.* (2010) 10:479–89. doi: 10.1038/nri2800
59. Reynolds JM, Angkasewinai P, Dong C. IL-17 family member cytokines: regulation and function in innate immunity. *Cytokine Growth Factor Rev.* (2010) 21:413–23. doi: 10.1016/j.cytogfr.2010.10.002
60. Hatfield JK, Brown MA. Group 3 innate lymphoid cells accumulate and exhibit disease-induced activation in the meninges in EAE. *Cell Immunol.* (2015) 297:69–79. doi: 10.1016/j.cellimm.2015.06.006
61. Ling Y, Puel A. IL-17 and infections. *Actas Dermosifiliorg* (2014) 105:34–40. doi: 10.1016/S0001-7310(14)70016-X
62. Blake SJ, Teng MW. Role of IL-17 and IL-22 in autoimmunity and cancer. *Actas Dermosifiliorg* (2014) 105:41–50. doi: 10.1016/S0001-7310(14)70017-1
63. Tengesdal IW, Kitzenberg D, Li S, Nyuydzefe MS, Chen W, Weiss JM, et al. The selective ROCK2 inhibitor KD025 reduces IL-17 secretion in human peripheral blood mononuclear cells independent of IL-1 and IL-6. *Eur J Immunol.* (2018) 48:1679–86. doi: 10.1002/eji.201847652
64. Amarilyo G, Lourenço EV, Shi FD, La Cava A. IL-17 promotes murine lupus. *J Immunol.* (2014) 193:540–3. doi: 10.4049/jimmunol.1400931
65. Jadidi-Niaragh F, Mirshafiey A. Th17 cell, the new player of neuroinflammatory process in multiple sclerosis. *Scand J Immunol.* (2011) 74:1–13. doi: 10.1111/j.1365-3083.2011.02536.x
66. Legroux L, Arbour N. Multiple sclerosis and T lymphocytes: an entangled story. *J Neuroimmune Pharmacol.* (2015) 10:528–46. doi: 10.1007/s11481-015-9614-0
67. Waisman A, Hauptmann J, Regen T. The role of IL-17 in CNS diseases. *Acta Neuropathol.* (2015) 129:625–37. doi: 10.1007/s00401-015-1402-7
68. Durelli L, Conti L, Clerico M, Boselli M, Contessa G, Ripellino P, et al. T-helper 17 cells expand in multiple sclerosis and are inhibited by interferon-beta. *Ann Neurol.* (2009) 65:499–509. doi: 10.1002/ana.21652
69. Melnikov M, Belousova O, Murugin V, Pashenkov M, Boyko A. The role of dopamine in modulation of Th17 immune response in multiple sclerosis. *J Neuroimmunol.* (2016) 292:97–101. doi: 10.1016/j.jneuroim.2016.01.020
70. Esendagli G, Kurne AT, Sayat G, Kilic AK, Guc D, Karabudak R. Evaluation of Th17-related cytokines and receptors in multiple sclerosis patients under interferon β-1 therapy. *J Neuroimmunol.* (2013) 255:81–4. doi: 10.1016/j.jneuroim.2012.10.009
71. Kürtüncü M, Tüzün E, Türkoglu R, Petek-Balc B, Içöz S, Pehlivan M., et al. Effect of short-term interferon-β treatment on cytokines in multiple sclerosis: significant modulation of IL-17 and IL-23. *Cytokine* (2012) 59:400–2. doi: 10.1016/j.cyto.2012.05.004

72. Sato DK, Nakashima I, Bar-Or A, Misu T, Suzuki C, Nishiyama S, et al. Changes in Th17 and regulatory T cells after fingolimod initiation to treat multiple sclerosis. *J Neuroimmunol*. (2014) 268:95–8. doi: 10.1016/j.jneuroim.2014.01.008
73. Rentzos M, Rombos A, Nikolaou C, Zoga M, Zouvelou V, Dimitrakopoulos A, et al. Interleukin-17 and interleukin-23 are elevated in serum and cerebrospinal fluid of patients with ALS: a reflection of Th17 cells activation? *Acta Neurol Scand*. (2010) 122:425–9. doi: 10.1111/j.1600-0404.2010.01333.x
74. Saresella M, Piancone F, Tortorella P, Marventano I, Gatti A, Caputo D, et al. T helper-17 activation dominates the immunologic milieu of both amyotrophic lateral sclerosis and progressive multiple sclerosis. *Clin Immunol*. (2013) 148:79–88. doi:10.1016/j.clim.2013.04.010
75. Zhang J, Ke KF, Liu Z, Qiu YH, Peng YP. Th17 cell-mediated neuroinflammation is involved in neurodegeneration of aβ1-42-induced Alzheimer's disease model rats. *PLoS ONE* (2013) 10:e75786. doi: 10.1371/journal.pone.0075786
76. Saresella M, Calabrese E, Marventano I, Piancone F, Gatti A, Alberoni M, et al. Increased activity of Th-17 and Th-9 lymphocytes and a skewing of the post-thymic differentiation pathway are seen in Alzheimer's disease. *Brain Behav Immun*. (2011) 25:539–47. doi: 10.1016/j.bbi.2010.12.004
77. Oberstein TJ, Taha L, Spitzer P, Hellstern J, Herrmann M, Kornhuber J, et al. Imbalance of circulating Th17 and regulatory T cells in Alzheimer's disease: a case control study. *Front Immunol*. (2018) 9:1213. doi: 10.3389/fimmu.2018.01213
78. Ibrahim S, Girault A, Ohresser M, Lereclus E, Paintaud G, Lecomte T, et al. Monoclonal antibodies targeting the IL-17/IL-17RA axis: an opportunity to improve the efficiency of anti-VEGF therapy in fighting metastatic colorectal cancer? *Clin Colorectal Cancer* (2018) 17:e109–13. doi: 10.1016/j.clcc.2017.10.003
79. FDA. *FDA Approves New Psoriasis Drug. FDA News Release* (2017). Available online at: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm541981.htm (Accessed February 15, 2017).
80. FDA. *Cosentyx (secukinumab) Injection*. (2017). Available online at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125504s016lbl.pdf
81. Yang J, Sundrud MS, Skepner J, Yamagata T. Targeting Th17 cells in autoimmune diseases. *Trends Pharmacol Sci*. (2014) 35:493–500. doi: 10.1016/j.tips.2014.07.006
82. Hewison M. An update on vitamin D and human immunity. *Clin Endocrinol*. (2012) 76:315–25. doi: 10.1111/j.1365-2265.2011.04261.x
83. Ooi JH, Chen J, Cantorna MT. Vitamin D regulation of immune function in the gut: why do T cells have vitamin D receptors? *Mol Aspects Med*. (2012) 33:77–82. doi: 10.1016/j.mam.2011.10.014
84. Cantorna MT, Snyder L, Lin YD, Yang L. Vitamin D and 1,25(OH)2D regulation of T cells. *Nutrients* (2015) 7:3011–21. doi: 10.3390/nu7043011
85. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med*. (2009) 151:264. doi: 10.7326/0003-4819-151-4-200908180-00135
86. Reynolds AD, Stone DK, Hutter JAL, Benner EJ, Mosley RL, Gendelman HE. Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. *J Immunol*. (2010) 184:2261–71. doi: 10.4049/jimmunol.0901852
87. Liu Z, Huang Y, Cao BB, Qiu YH, Peng YP. Th17 cells induce dopaminergic neuronal death via LFA-1/ICAM-1 interaction in a mouse model of Parkinson's disease. *Mon Neurobiol*. (2017) 54:7762–76. doi: 10.1007/s12035-016-0249-9
88. Park J, Lee JW, Cooper SC, Broxmeyer HE, Cannon JR, Kim CH. Parkinson disease-associated LRRK2 G2019S transgene disrupts marrow myelopoiesis and peripheral Th17 repsonse. *J Leukoc Biol*. (2017) 102. doi: 10.1189/jlb.1A0417-147RR
89. Xiong Y, Neifert S, Karuppagounder SS, Liu Q, Stankowski JN, Lee BD, et al. Robust kinase- and age-dependent dopaminergic and norepinephrine neurodegeneration in LRRK2 G2019S transgenic mice. *Proc Natl Acad Sci USA*. (2018) 115:1635–40. doi: 10.1073/pnas.1712648115
90. Russo I, Bubacco L, Greggio E. LRRK2 and neuroinflammation: partners in crime in Parkinson's disease. *J Neuroinflamm*. (2014) 11:52. doi: 10.1186/1742-2094-11-52
91. Lee H, James WS, Cowley SA. LRRK2 in peripheral and central nervous system innate immunity: its link to Parkinson's disease. *Biochem Soc Trans*. (2017) 45:131–9. doi: 10.1042/BST20160262
92. Correia Guedes L, Ferreira JJ, Rosa MM, Coelho M, Bonifati V, Sampaio C. Worldwide frequency of G2019S LRRK2 mutation in Parkinson's disease: a systematic review. *Parkinsonism Relat Disord*. (2010) 16:237–42. doi: 10.1016/j.parkreldis.2009.11.004
93. Kozina E, Sadasivan S, Jiao Y, Dou Y, Ma Z, Tan H, et al. Mutant LRRK2 mediates peripheral and central immune responses leading to neurodegeneration *in vivo*. *Brain* (2018) 141:1753–69. doi: 10.1093/brain/awy077
94. Sandquist I, Kolls J. Update on regulation and effector functions of Th17 cells. *F1000Res*. (2018) 19:205. doi: 10.12688/f1000research.13020.1
95. Niwa F, Kuriyama N, Nakagawa M, Imanishi J. Effects of peripheral lymphocyte subpopulations and the clinical correlation with Parkinson's disease. *Geriatr Gerontol Int*. (2012) 12:102–7. doi: 10.1111/j.1447-0594.2011.00740.x
96. Chen Y, Yu M, Liu X, Qu H, Chen Q, Qian W, et al. Clinical characteristics and peripheral T cell subsets in Parkinson's disease patients with constipation. *Int J Clin Exp Pathol*. (2015) 8:2495–504.
97. Yang F, Li B, Li L, Zhang H. The clinical significance of the imbalance of Th17 and Treg cells and their related cytokines in peripheral blood of Parkinson's disease patients. *Int J Clin Exp Med*. (2016) 9:17946–51.
98. Cen L, Yang C, Huang S, Zhou M, Tang X, Li K, et al. Peripheral lymphocyte subsets as a marker of Parkinson's disease in a chinese population. *Neurosci Bull*. (2017) 33:493–500. doi: 10.1007/s12264-017-0163-9
99. Yang L, Guo C, Zhu J, Feng Y, Chen W, Feng Z, et al. Increased levels of pro-inflammatory and anti-inflammatory cellular responses in Parkinson's disease patiens: search for a disease indicator. *Med Sci Monit*. (2017) 23:2972–8. doi: 10.12659/msm.904240
100. Chen S, Liu Y, Niu Y, Xu Y, Zhou Q, Xu X, et al. Increased abundance of myeloid-derived suppressor cells and Th17 cells in peripheral blood of newly diagnosed Parkinson's disease patients. *Neurosci Lett*. (2017) 648:21–5. doi: 10.1016/j.neulet.2017.03.045
101. Cook DA, Kannarkat GT, Cintron AF, Butkovich LM, Fraser KB, Chang J, et al. LRRK2 levels in immune cells are increased in Parkinson's disease. *Npj Parkinsons Dis*. (2017) 3:11. doi: 10.1038/s41531-017-0010-8
102. Mahnke YD, Beddall MH, Roederer M. OMIP-017: human CD4(+) helper T-cell subsets including follicular helper cells. *Cytometry A* (2013) 83:439–40. doi: 10.1002/cyto.a.22269
103. Freer G, Rindi L. Intracellular cytokine detection by fluorescence-activated flow cytometry: basic principles and recent advances. *Methods* (2013) 61:30–8. doi: 10.1016/j.ymeth.2013.03.035
104. Gupta S, Maecker H. Intracellular cytokine staining (ICS) on human lymphocytes or peripheral blood mononuclear cells (PBMCs). *Bio Protocol* (2015) 5:7. doi: 10.21769/BioProtoc.1442
105. Blesa J, Przedborski S. Parkinson's disease: animal models and dopaminergic cell vulnerability. *Front Neuroanat*. (2014) 8:155. doi: 10.3389/fnana.2014.00155
106. Singh SP, Zhang HH, Foley JF, Hedrick MN, Farber JM. Human T cells that are able to produce IL-17 express the chemokine receptor CCR6. *J Immunol*. (2008) 180:214–21. doi: 10.4049/jimmunol.180.1.214
107. Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A, et al. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat Immunol*. (2007) 8:639–46. doi: 10.1038/ni1467
108. Dunay GA, Tóth I, Eberhard JM, Degen O, Tolosa E, van Lunzen J, et al. Parallel assessment of Th17 cell frequencies by surface marker co-expression versus ex vivo IL-17 production in HIV-1 infection. *Cytometry B Clin Cytom*. (2016) 90:486–92. doi: 10.1002/cyto.b.21352
109. Inzelberg R, Jankovic J. Are Parkinson disease patients protected from some but not all cancers? *Neurology* (2007) 69:1542–50. doi: 10.1212/01.wnl.0000277638.63767.b8
110. Kennedy R, Celis E. Multiple roles for CD4+T cells in anti-tumor immune responses. *Immunol Rev*. (2008) 222:129–44. doi: 10.1111/j.1600-065X.2008.00616.x

111. Bailey SR, Nelson MH, Himes RA, Li Z, Mehrotra S, Paulos CM. Th17 cells in cancer: the ultimate identity crisis. *Front Immunol.* (2014) 5:276. doi: 10.3389/fimmu.2014.00276
112. Muranski P, Boni A, Antony PA, Cassard L, Inrvine KR, Kaiser A, et al. Tumor-specific Th17-polarized cells eradicate large established melanoma. *Blood* (2008) 122:362–73. doi: 10.1182/blood-2007-11-120998
113. Ungprasert P, Srivali N, Kittanamongkolchai W. Risk of Parkinson's Disease Among Patients with Psoriasis: A Systematic Review and Meta-analysis. *Indian J Dermatol.* (2016) 61:152–6. doi: 10.4103/0019-5154.177771
114. Camacho-Soto A, Gross A, Searles Nielsen S, Dey N, Racette BA. Inflammatory bowel disease and risk of Parkinson's disease in Medicare beneficiaries. *Parkinsonism Relat Disord.* (2018) 50:23–8. doi: 10.1016/j.parkreldis.2018.02.008
115. Peter I, Dubinsky M, Bressman S, Park A, Lu C, Chen N, et al. Anti-tumor necrosis factor therapy and incidence of parkinson disease among patients with inflammatory bowel disease. *JAMA Neurol.* (2018) 75:939–46. doi: 10.1001/jamaneurol.2018.0605
116. Sung YF, Liu FC, Lin CC, Lee JT, Yang FC, Chou YC, et al. Reduced risk of parkinson disease in patients with rheumatoid arthritis: a nationwide population-based study. *Mayo Clin Proc.* (2016) 91:1346–53. doi: 10.1016/j.mayocp.2016.06.023
117. Rugbjerg K, Friis S, Ritz B, Schernhammer ES, Korbo L, Olsen JH. Autoimmune disease and risk for Parkinson disease: a population-based case-control study. *Neurology* (2009) 73:1462–8. doi: 10.1212/WNL.0b013e3181c06635
118. Cai Y, Fleming C, Yan J. New insights of T cells in the pathogenesis of psoriasis. *Cell Mol Immunol.* (2012) 9:302–9. doi: 10.1038/cmi.2012.15
119. Gyires K, Tóth ÉV, Zádori SZ. Gut inflammation: current update on pathophysiology, molecular mechanism and pharmacological treatment modalities. *Curr Pharm Des.* (2014) 20:1063–81. doi: 10.2174/13816128113199990417
120. Boissier MC, Semerano L, Challal S, Saidenberg-Kermanac'h N, Falgarone G. Rheumatoid arthritis: from autoimmunity to synovitis and joint destruction. *J Autoimmun.* (2012) 39:222–8. doi: 10.1016/j.jaut.2012.05.021
121. Fung TC, Olson CA, Hsiao EY. Interactions between the microbiota, immune and nervous systems in health and disease. *Nat Neurosci.* (2017) 20:145–55. doi: 10.1038/nn.4476
122. Scheperjans F, Aho V, Pereira PA, Koskinen K, Paulin L, Pekkonen E, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord.* (2015) 30:350–8. doi: 10.1002/mds.26069
123. Larsen JM. The immune response to Prevotella bacteria in chronic inflammatory disease. *Immunology* (2017) 151:363–74. doi: 10.1111/imm.12760
124. de Azevedo MS, Innocentin S, Dorella FA, Rocha CS, Mariat D, Pontes DS, et al. Immunotherapy of allergic diseases using probiotics or recombinant probiotics. *J Appl Microbiol.* (2013) 115:319–33. doi: 10.1111/jam.12174
125. Peterson AL. A review of vitamin D and Parkinson's disease. *Maturitas.* (2014) 78:40–4. doi: 10.1016/j.maturitas.2014.02.012
126. Rimmelzwaan LM, van Schoor NM, Lips P, Berendse HW, Eekhoff EM. Systematic Review of the relationship between Vitamin, D., and Parkinson's Disease. *J Parkinsons Dis.* (2016) 6:29–37. doi: 10.3233/JPD-150615
127. Sarkar C, Basu B, Chakroborty D, Dasgupta PS, Basu S. The immunoregulatory role of dopamine: an update. *Brain Behav Immun.* (2010) 24:525–8. doi: 10.1016/j.bbi.2009.10.015
128. Cosentino M, Marino F. Adrenergic and dopaminergic modulation of immunity in multiple sclerosis: teaching old drugs new tricks? *J Neuroimmune Pharmacol.* (2013) 8:163–79. doi: 10.1007/s11481-012-9410-z
129. Pinoli M, Marino F, Cosentino M. Dopaminergic Regulation of Innate Immunity: a Review. *J Neuroimmune Pharmacol.* (2017) 12:602–23. doi: 10.1007/s11481-017-9749-2
130. Prado C, Contreras F, González H, Díaz P, Elgueta D, Barrientos M, et al. Stimulation of dopamine receptor D5 expressed on dendritic cells potentiates Th17-mediated immunity. *J Immunol.* (2012) 188:3062–70. doi: 10.4049/jimmunol.1103096
131. Ferreira TB, Kasahara TM, Barros PO, Vieira MM, Bittencourt VC, Hygino J, et al. Dopamine up-regulates Th17 phenotype from individuals with generalized anxiety disorder. *J Neuroimmunol.* (2011) 238:58–66. doi: 10.1016/j.jneuroim.2011.06.009
132. Ferreira TB, Barros PO, Teixeira B, Cassano T, Centurião N, Kasahara TM, et al. Dopamine favors expansion of glucocorticoid-resistant IL-17-producing T cells in multiple sclerosis. *Brain Behav Immun.* (2014) 41:182–90. doi: 10.1016/j.bbi.2014.05.013
133. Volpe E, Battistini L, Borsellino G. Advances in T helper 17 cell biology: pathogenic role and potential therapy in Multiple Sclerosis. *Mediators Inflamm.* (2015) 2015:475158. doi: 10.1155/2015/475158
134. Havrdová E, Belova A, Goloborodko A, Tisserant A, Wright A, Wallstroem E, et al. Activity of secukinumab, an anti-IL17A antibody, on brain lesions in RRMS: results from a randomized, proof-of-concept study. *J Neurol.* (2016) 263:1287–95. doi: 10.1007/s00415-016-8128-x
135. Prajeeth CK, Kronisch J, Khorooshi R, Knier B, Toft-Hansen H, Gudi V, et al. Effectors of Th1 and Th17 cells act on astrocytes and augment their neuroinflammatory properties. *J Neuroinflamm.* (2017) 14:204. doi: 10.11186/s12974-017-0978-3
136. Joers V, Tansey MG, Mulas G, Carta AR. Microglial phenotypes in Parkinson's disease and animal models of the disease. *Prog Neurobiol.* (2017) 155:57–75. doi: 10.1016/j.pneurobio.2016.04.006
137. Booth HDE, Hirst WD, Wade-Martins R. The role of astrocytes dysfunction in Parkinson's disease pathogenesis. *Trends Neurosci.* (2017) 40:358–70. doi: 10.1016/j.tins.2017.04.001
138. Sun J, Zhang S, Zhang X, Zhang X, Dong H, Qian Y. IL-17A is implicated in lipopolysaccharide-induced neuroinflammation and cognitive impairment in aged rats via microglial activation. *J Neuroinflamm.* (2015) 12:165. doi: 10.1186/s12974-015-0394-5
139. Derkow K, Krüger C, Dembny P, Lehnardt S. Microglia induce neurotoxic IL-17+ γδ T cells dependent on TLR2, TLR4, and TLR9 activation. *PLoS ONE* (2015) 10:e0135898. doi: 10.1371/journal.pone.0135898

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

BRIEF RESEARCH REPORT
published: 29 January 2019
doi: 10.3389/fimmu.2019.00080

Peripheral Inflammation Regulates CNS Immune Surveillance Through the Recruitment of Inflammatory Monocytes Upon Systemic α-Synuclein Administration

Javier María Peralta Ramos[1], Pablo Iribarren[1], Luc Bousset[2], Ronald Melki[2], Veerle Baekelandt[3] and Anke Van der Perren[3]**

[1] Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Centro de Investigación en Bioquímica Clínica e Inmunología (CONICET), Universidad Nacional de Córdoba, Córdoba, Argentina, [2] Institut François Jacob (MIRCen), CEA and Laboratory of Neurodegenerative Diseases, CNRS, Fontenay-aux-Roses, France, [3] Laboratory for Neurobiology and Gene Therapy, Department of Neurosciences, KU Leuven, Leuven, Belgium

OPEN ACCESS

Edited by:
Cristoforo Comi,
Università degli Studi del Piemonte Orientale, Italy

Reviewed by:
Selva Rivas-Arancibia,
National Autonomous University of Mexico, Mexico
Éva M. Szego,
Eötvös Loránd University, Hungary

***Correspondence:**
Javier María Peralta Ramos
jperalta@fcq.unc.edu.ar;
javier.peralta.ramos@weizmann.ac.il
Anke Van der Perren
anke.vanderperren@kuleuven.be

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology

Received: 17 October 2018
Accepted: 11 January 2019
Published: 29 January 2019

Citation:
Peralta Ramos JM, Iribarren P, Bousset L, Melki R, Baekelandt V and Van der Perren A (2019) Peripheral Inflammation Regulates CNS Immune Surveillance Through the Recruitment of Inflammatory Monocytes Upon Systemic α-Synuclein Administration. Front. Immunol. 10:80. doi: 10.3389/fimmu.2019.00080

Innate immune activation and chronic neuroinflammation are characteristic features of many neurodegenerative diseases including Parkinson's disease (PD) and may contribute to the pathophysiology of the disease. The discovery of misfolded alpha-synuclein (αSYN) protein aggregates, which amplify in a "prion-like" fashion, has led us to consider that pathogenic αSYN might be hijacking the activation and mobilization mechanism of the peripheral immune system to reach and disseminate within the CNS. Furthermore, our lab and other groups have recently shown that αSYN can adopt distinct fibril conformations or "strains" with varying levels of pathogenic impact. Therefore, the aim of this study was to assess the impact of peripheral inflammation on αSYN spreading in order to better understand the participation of the immune system in the progression of PD. The results presented here show that intraperitoneal LPS injection prior to systemic intravenous recombinant administration of two different αSYN pathogenic strains (fibrils or ribbons) in wild type mice, induces an increase in brain resident microglia and promotes the recruitment of leukocytes toward the brain and the spinal cord. Our findings show for the first time that αSYN can be internalized by LPS-primed inflammatory monocytes, which in turn favors the dissemination from the periphery toward the brain and spinal cord. Further, we found a differential recruitment of $CD4^+$ and $CD8^+$ T cells after LPS priming and subsequent administration of the αSYN ribbons strain. Together, these data argue for a role of the peripheral immune system in αSYN pathology.

Keywords: inflammation, alpha-synuclein, inflammatory monocytes, Parkinson's disease, synucleinopathies

INTRODUCTION

Immunological surveillance of the central nervous system (CNS) has shown to be dynamic, specific, and tightly regulated. Innate immune activation and chronic neuroinflammation are characteristic features of many neurodegenerative diseases including Parkinson's disease (PD) and may contribute to the pathophysiology of the disease (1). During neurodegeneration, peripheral

immune cells can gain access to the brain parenchyma (2). Brain-resident microglia encounter myeloid immune cells that have been previously primed in the periphery, establishing an interplay that aggravates the inflammatory process and potentiates neuropathology (3, 4). The recent discovery of a CNS dural lymphatic system that drains macromolecules from the CNS into cervical lymph nodes, further challenges the established basic assumptions of the CNS as an immune privileged site (5–7). Systemic injection of the endotoxin LPS has been widely used as an inflammatory model (8, 9). These peripherally applied stimuli lead to a cytokine-storm that signals to the brain, triggering an immune response. The discovery of misfolded αSYN protein aggregates with different structural characteristics, that could account for the distinct pathological traits within synucleinopathies and which amplify in a "prion-like" fashion (10–14), has led us to consider that pathogenic αSYN might be hijacking the activation and mobilization mechanism of the peripheral immune system to reach and disseminate within the CNS. Therefore, we assessed the impact of peripheral inflammation on αSYN spreading in order to understand the participation of the immune system in αSYN pathology.

MATERIALS AND METHODS

Animals and LPS/αSYN Administration

All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Bioethical Committee of the KU Leuven (Belgium). Eight-week old female C57BL/6 mice (Janvier, France) were housed under a normal 12 h light/dark cycle with free access to pelleted food and tap water. All surgical procedures were performed using aseptic techniques.

Mice were treated with either (a) 20 μg of LPS i.p., (b) 5 μg of atto-488-labeled pathogenic αSYN fibrils or ribbons i.v. (15), (c) LPS combined with αSYN as aforementioned, or (d) saline, following the administration scheme depicted in **Figure 2A**. Twelve hours after the last injection, mice were euthanised and immune cells were isolated from either whole brain or spinal cord and stained for subsequent flow cytometric analysis. Results are representative of two independent experiments combined ($n = 3$–4 animals per group).

LPS from *Escherichia coli* 055:B5 (purified by gel filtration chromatography) was purchased from Sigma-Aldrich and freshly dissolved in sterile saline prior to i.p. injection. Recombinant αSYN fibrils and ribbons were generated, extensively characterized and labeled with the aminoreactive fluorescent dye atto-488 (ATTO-Tech GmbH) as previously described (13, 15).

Isolation of Immune Cells From Mice Brains and Spinal Cords

Twelve hours after the last injection, mice were weighed and deeply anesthetized with a ketamine (60 mg/kg, Pfizer)/medetomidine (0.4 mg/kg, Pfizer) cocktail according to their weight. Immune brain cells were isolated from whole brain or spinal cord homogenates as follows. Briefly, mice were transcardially perfused with ice-cold PBS (Gibco) and brains or spinal cords were collected in DMEM (Gibco) supplemented with sodium pyruvate (Gibco) and a penicillin, streptomycin and glutamine cocktail (Gibco), gently disaggregated mechanically and resuspended in PBS containing 3 mg/mL collagenase D (Roche Diagnostics) plus 10 μg/mL DNAse (Sigma-Aldrich) for an enzymatic homogenization. After this incubation, brain homogenates were filtered in 40 μm pore size cell strainers (BD Biosciences), centrifuged 8 min at 1,800 r.p.m., washed with PBS and resuspended in 6 mL of 38% isotonic Percoll® (GE Healthcare) before a 25 min centrifugation at 800 G with 0 acceleration and 0 brake. Myelin and debris were discarded. Cell pellets containing total brain immune cells were collected, washed with DMEM supplemented with 10% fetal bovine serum (Gibco) and cell viability was determined by trypan blue exclusion using a Neubauer's chamber. Finally, cells were labeled for subsequent flow cytometric analysis.

Flow Cytometric Analysis

Surface staining of single-cell suspension of isolated brain immune cells was performed using standard protocols and analyzed on a FACSCanto II (BD Biosciences). Flow cytometric analysis was defined based on the expression of CD11b, CD45, Ly6C, CD4, and CD8 as follows: microglial cells, $CD11b^{+}$ $CD45^{lo}$; recruited leukocytes, $CD11b^{+/-}$ $CD45^{hi}$; inflammatory monocytes, $CD11b^{+}$ $CD45^{hi}$ $Ly6C^{hi}$; T cells, $CD11b^{-}$ $CD45^{hi}$ $CD4^{+}/CD8^{+}$. Data analysis was conducted using FCS Express (*De Novo* Software). The following antibodies were used in the procedure: monoclonal anti-mouse CD11b APC (BioLegend, clone M1/70), CD11b FITC (BD Pharmingen, clone M1/70), CD45 APC-Cy7 (BioLegend, clone 30-F11), Ly6C PE-Cy7 (BD Pharmingen, clone AL-21), CD4 APC (BD Pharmingen, clone RM4-5), CD8 PE (BD Pharmingen, clone 53-6.7) or isotype control antibodies (BD Pharmingen, APC, clone R35-95; PE-Cy7, clone G155-178). Multiparametric gating analysis strategy was performed as previously described (8).

Statistical Analysis

Results are expressed as mean ± s.e.m. All statistical analyses were performed using Prism® 7.0 (GraphPad Software). Means between groups were compared with one-way analysis of variance followed by a Tukey's *post-hoc* test. Statistical significance levels were set as follows: */# if $p < 0.05$, **/## if $p < 0.01$, and ***/### if $p < 0.001$. The asterisks indicate the comparison against the saline treated group.

RESULTS AND DISCUSSION

The results presented here show that intraperitoneal LPS injection combined with intravenous administration of two different recombinant αSYN pathogenic strains (fibrils or ribbons) in wild type mice, induces an increase in brain resident microglia and promotes the recruitment of leukocytes toward the brain (**Figure 1A**) and the spinal cord (**Figure 1C**). When further characterizing the phenotypic traits of the peripheral cells trafficking to the CNS, we identified neutrophils and professional antigen presenting dendritic cells among innate myeloid leukocytes (data not shown), as well as a distinct

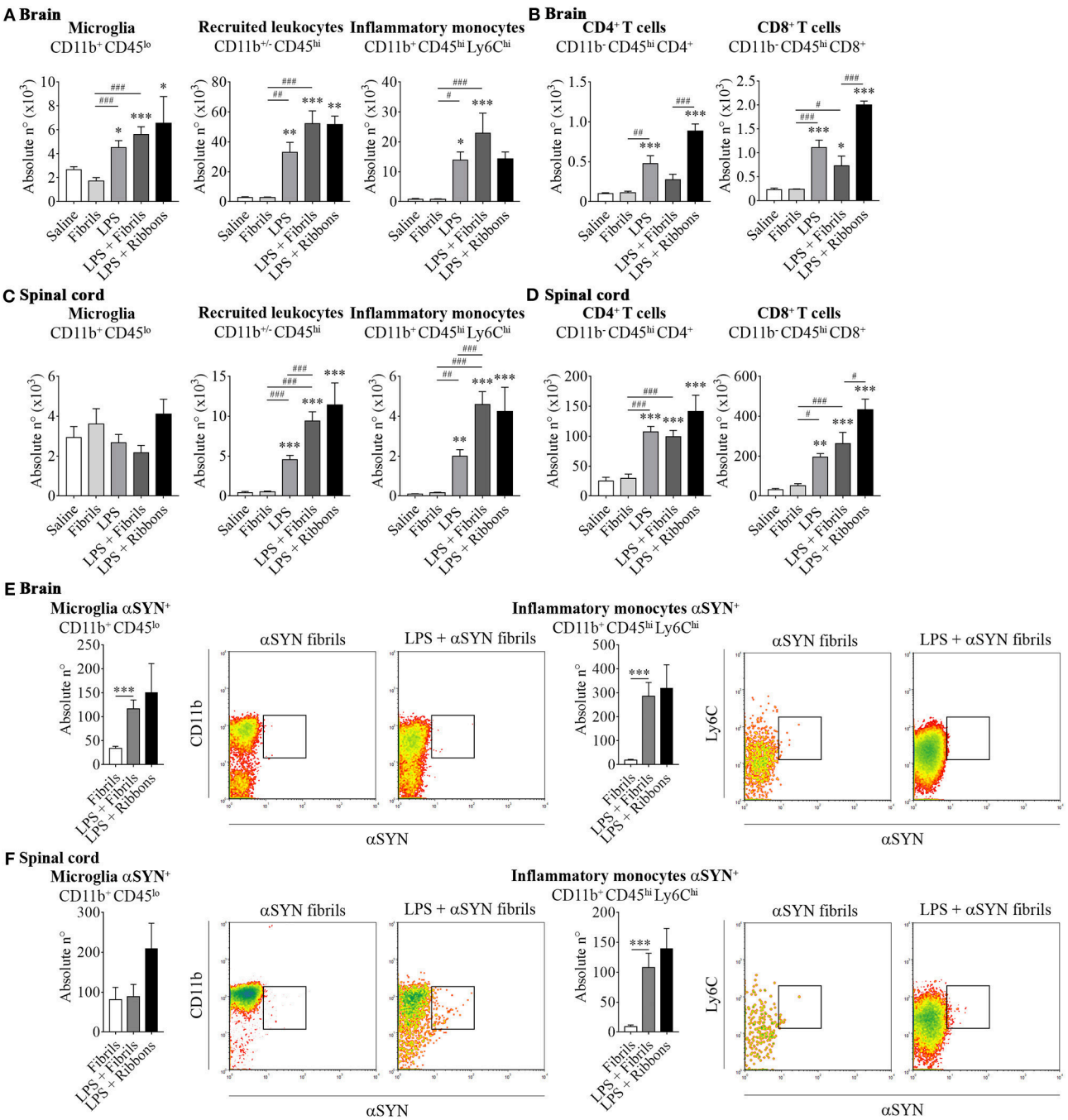


FIGURE 1 | αSYN hijacks the activation and mobilization mechanism of LPS-primed peripheral inflammatory monocytes to disseminate into the CNS**.** Mice were treated with either (a) 20 μg of LPS i.p (LPS group), (b) 5 μg of atto-488-labeled pathogenic αSYN fibrils or ribbons i.v. (αSYN group), (c) LPS combined with αSYN strains (LPS + αSYN group), or (d) saline alone, following the administration scheme depicted in **Figure 2A**. Twelve hours after the last injection, mice were euthanised and immune cells were isolated from either whole brain **(A,B)** or spinal cord **(C,D)** and stained for subsequent flow cytometric analysis. Absolute numbers of $CD11b^+$ $CD45^{lo}$ microglial cells, $CD11b^{+/-}$ $CD45^{hi}$ recruited cells, $CD11b^+$ $CD45^{hi}$ $Ly6C^{hi}$ inflammatory monocytes, $CD11b^-$ $CD45^{hi}$ $CD4^+$ and $CD11b^-$ $CD45^{hi}$ $CD8^+$ lymphocytes were assessed by flow cytometry. Absolute numbers of αSYN-internalized $CD11b^+$ $CD45^{lo}$ microglial cells or $CD11b^+$ $CD45^{hi}$ $Ly6C^{hi}$ inflammatory monocytes purified from brain **(E)** or spinal cord **(F)**, were assessed by flow cytometry. Results are representative of two independent experiments combined ($n = 3$–4 animals per group). Representative CD11b vs. αSYN and Ly6C vs. αSYN density-plots illustrate the gating analysis strategy employed for microglial cells and inflammatory monocytes, when gated in $CD45^{lo}$ or $CD45^{hi}$ cells respectively. Data are expressed as mean ± s.e.m. Means between groups were compared with one-way analysis of variance followed by a Tukey's *post-hoc* test. Statistical significance levels were set as follows: $^{*/\#}$ if $p < 0.05$, $^{**/\#\#}$ if $p < 0.01$, and $^{***/\#\#\#}$ if $p < 0.001$. The asterisks indicate the comparison against the saline treated group.

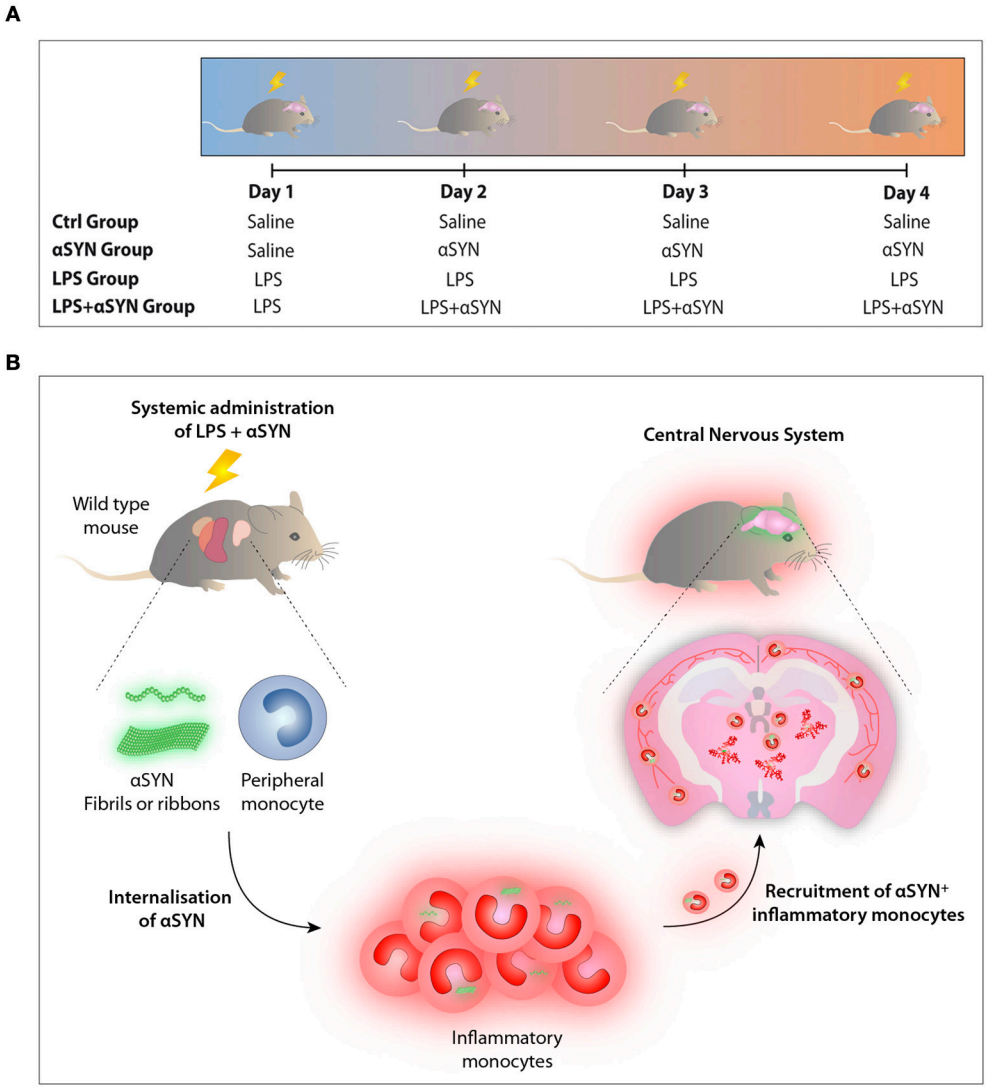


FIGURE 2 | LPS/αSYN administration scheme. **(A)** Mice were treated i.p. with 20 μg of LPS, i.v. with 5 μg of either two atto-488-labeled pathogenic αSYN strains (fibrils or ribbons), with LPS combined with αSYN as aforementioned, or with saline accordingly and following the administration scheme depicted. Twelve hours after the last injection, mice were euthanised and immune cells were isolated from either whole brain or spinal cord and stained for subsequent flow cytometric analysis. **(B)** Proposed model: Inflammatory monocytes, from pivotal sentinels to potential Trojan horses driving the dissemination and propagation of αSYN toward the CNS.

migration of $CD4^+$ and $CD8^+$ T cell subsets after administration of αSYN strains, which was most prominent in the brain for ribbons compared to fibrils **(Figures 1B,D)**. Moreover, LPS-primed inflammatory monocytes proved to be the major source of CNS-associated phagocytes after systemic challenge with αSYN strains. In the brain, fibrils induced a stronger response compared to ribbons, while the effect in the spinal cord was similar for both αSYN strains **(Figures 1A,C)**. Interestingly, we noticed that LPS priming favored αSYN spreading toward the brain and spinal cord, as observed by an upregulation of $αSYN^+$-expressing microglia and inflammatory monocytes **(Figures 1E,F)**.

Mounting evidence supports the notion that the innate immunity has a great capacity of adapting and deploying an innate immune memory upon an inflammatory insult (16, 17), shaping subsequent immune responses in the brain (18). Our findings clearly demonstrate that priming with LPS prior to systemic αSYN challenge, induces an increase in the absolute number of the brain-resident microglia and promotes the recruitment of peripheral leukocytes into the CNS. Similar to other reports (19–21), our results demonstrate that stimulation with LPS of innate immune receptors, such as Toll-like receptor 4, amplifies the inflammation within the CNS.

Sacino and colleagues were the first to describe αSYN pathology in the brain and spinal cord induced by a single peripheral intramuscular injection of αSYN (22). Shortly after that, our group described passage of the blood-brain barrier by recombinant αSYN aggregates and distribution throughout

the CNS after systemic administration (13). Our findings show for the first time that αSYN can be internalized by LPS-primed inflammatory monocytes, which in turn favors the dissemination from the periphery toward the brain and spinal cord. In line with our results, Harms et al. (23) described a recruitment of immune cells toward the CNS prior to neurodegeneration after intracranial αSYN fibril treatment. Additionally, peripheral monocyte entry was recently reported to be required for viral vector-mediated αSYN-induced neuroinflammation and neurodegeneration (24). Together, these data argue for a role of the peripheral immune system in αSYN pathology.

Further, we also found a differential recruitment of $CD4^+$ and $CD8^+$ T cells after LPS priming and subsequent administration of αSYN ribbons compared to fibrils, which was most prominent in the brain. By presenting a different T cell response toward distinct αSYN strains we show the importance of the protein conformation in the capacity to act as an antigenic epitope. Related to this, Sulzer and co-workers recently discovered that PD patient-derived T cells are able to recognize well-defined αSYN peptides (25). The lymphocyte activation gene-3 (LAG3) has been proposed to bind pathogenic αSYN assemblies and to favor their endocytosis and transmission (26). Since effector and regulatory T cells also express LAG3, it will be important to better comprehend the role of this membrane protein since it executes dual roles in autoimmunity and cancer. In this regard, while LAG3 has been shown to play a protective role in autoimmunity by dampening T helper cell responses and promoting regulatory T cell-mediated suppression, it has also been described to bear co-inhibitory features, becoming a target for immune blockade to empower anti-tumor T cell responses (27).

Having identified inflammatory monocytes as potential disease-modifiers, we believe it is necessary to understand the mechanism underlying the internalization and transmission of αSYN as well as its interplay with T cells. Based on our previous report demonstrating that type I interferons are required to induce the selective migration of inflammatory monocytes upon peripheral inflammation (8), targeting these cytokines might hinder the recruitment of these cells and therefore ameliorate the outcome of synucleinopathies like PD. Overall, our findings demonstrate that systemic inflammation induces the recruitment of peripheral leukocytes into the CNS, suggesting that inflammatory monocytes could be turning from pivotal sentinels into potential Trojan horses driving the spreading and propagation of αSYN during disease progression (**Figure 2B**).

MATERIALS AND DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

JPR conceived and designed the research study, performed the experiments, analyzed data, and wrote the manuscript. PI conceived the research study and aided in interpreting the results. LB generated αSYN strains. RM discussed and commented on the manuscript. VB discussed and commented on the manuscript. AVDP designed the research study, performed the experiments, wrote and supervised the manuscript. All authors reviewed the manuscript before submission.

FUNDING

This work was funded in part by the National Scientific and Technical Research Council of Argentina (PhD fellowship to JPR), The Company of Biologists (Disease Model & Mechanisms Travelling Fellowship to JPR), EMBO (Short-Term Fellowship to JPR), the FWO Flanders (G.0927.14, G080517N, and postdoctoral fellowship to AVDP), the KU Leuven (OT/14/120), the CNRS, the ERA-NET JPco-fuND 2015 SYNACTION, and the Queen Elisabeth Medical Foundation for Neurosciences.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Denise Kviatcovsky for kindly designing the artwork. Flow cytometric analysis was performed in the FACS Core of the VIB-KU Leuven.

REFERENCES

1. Gelders G, Baekelandt V, Van der Perren A. Linking Neuroinflammation and Neurodegeneration in Parkinson's Disease. *J Immunol Res.* (2018) 2018:4784268. doi: 10.1155/2018/4784268
2. Baruch K, Deczkowska A, Rosenzweig N, Tsitsou-Kampeli A, Sharif AM, Matcovitch-Natan O, et al. PD-1 immune checkpoint blockade reduces pathology and improves memory in mouse models of Alzheimer's disease. *Nat Med.* (2016) 22:135–7. doi: 10.1038/nm.4022
3. Ousman SS, Kubes P. Immune surveillance in the central nervous system. *Nat Neurosci.* (2012) 15:1096–101. doi: 10.1038/nn.3161
4. Prinz M, Priller J. The role of peripheral immune cells in the CNS in steady state and disease. *Nat Neurosci.* (2017) 20:136–44. doi: 10.1038/nn.4475
5. Aspelund A, Antila S, Proulx ST, Karlsen TV, Karaman S, Detmar M, et al. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. *J Exp Med.* (2015) 212:991–9. doi: 10.1084/jem.20142290
6. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, et al. Structural and functional features of central nervous system lymphatic vessels. *Nature* (2015) 523:337–41. doi: 10.1038/nature 14432
7. Da Mesquita S, Louveau A, Vaccari A, Smirnov I, Cornelison RC, Kingsmore KM, et al. Functional aspects of meningeal lymphatics in ageing and Alzheimer's disease. *Nature* (2018) 560:185–91 doi: 10.1038/s41586-018-0368-8
8. Peralta Ramos JM, Bussi C, Gaviglio EA, Arroyo DS, Baez NS, Rodriguez-Galan MC, et al. Type I IFNs are required to promote central nervous system immune surveillance through the recruitment of inflammatory monocytes upon systemic inflammation. *Front Immunol.* (2017) 8:1666. doi: 10.3389/fimmu.2017.01666
9. Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, et al. Control of microglial neurotoxicity by the fractalkine receptor. *Nat Neurosci.* (2006) 9:917–24. doi: 10.1038/nn1715

10. Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat. Med.* (2008) 14:501–3. doi: 10.1038/nm1746
11. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat. Med.* (2008) 14:504–6. doi: 10.1038/nm1747
12. Olanow CW, Prusiner SB. Is Parkinson's disease a prion disorder? *Proc Natl Acad Sci USA.* (2009) 106:12571–2. doi: 10.1073/pnas.0906759106
13. Peelaerts W, Bousset L, Van der Perren A, Moskalyuk A, Pulizzi R, Giugliano M, et al. α-Synuclein strains cause distinct synucleinopathies after local and systemic administration. *Nature* (2015) 522:340–4. doi: 10.1038/nature14547
14. Peng C, Gathagan RJ, Covell DJ, Medellin C, Stieber A, Robinson JL, et al. Cellular milieu imparts distinct pathological α-synuclein strains in alpha-synucleinopathies. *Nature* (2018) 557:558–63. doi: 10.1038/s41586-018-0104-4
15. Bousset L, Pieri L, Ruiz-Arlandis G, Gath J, Jensen PH, Habenstein B, et al. Structural and functional characterization of two α-synuclein strains. *Nat Commun.* (2013) 4:2575. doi: 10.1038/ncomms3575
16. Netea MG, van der Meer JW. Trained immunity: an ancient way of remembering. *Cell Host Microbe* (2017) 21:297–300. doi: 10.1016/j.chom.2017.02.003
17. Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, et al. Trained immunity: A program of innate immune memory in health and disease. *Science* (2016) 352:aaf1098. doi: 10.1126/science.aaf1098
18. Wendeln AC, Degenhardt K, Kaurani L, Gertig M, Ulas T, Jain G, et al. Innate immune memory in the brain shapes neurological disease hallmarks. *Nature* (2018) 556:332–8. doi: 10.1038/s41586-018-0023-4
19. Cunningham C, Wilcockson DC, Campion S, Lunnon K, Perry VH. Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. *J Neurosci.* (2005) 25:9275–84. doi: 10.1523/JNEUROSCI.2614-05.2005
20. Field R, Campion S, Warren C, Murray C, Cunningham C. Systemic challenge with the TLR3 agonist poly I:C induces amplified IFNalpha/beta and IL-1beta responses in the diseased brain and exacerbates chronic neurodegeneration. *Brain Behav Immun.* (2010) 24:996–1007. doi: 10.1016/j.bbi.2010.04.004
21. Murray CL, Skelly DT, Cunningham C. Exacerbation of CNS inflammation and neurodegeneration by systemic LPS treatment is independent of circulating IL-1beta and IL-6. *J Neuroinflammation* (2011) 8:50. doi: 10.1186/1742-2094-8-50
22. Sacino AN, Brooks M, Thomas MA, McKinney AB, Lee S, Regenhardt RW, et al. Intramuscular injection of alpha-synuclein induces CNS α-synuclein pathology and a rapid-onset motor phenotype in transgenic mice. *Proc Natl Acad Sci USA.* (2014) 111:10732–7. doi: 10.1073/pnas.1321785111
23. Harms AS, Delic V, Thome AD, Bryant N, Liu Z, Chandra S, et al. α-Synuclein fibrils recruit peripheral immune cells in the rat brain prior to neurodegeneration. *Acta Neuropathol Commun.* (2017) 5:85. doi: 10.1186/s40478-017-0494-9
24. Harms AS, Thome AD, Yan Z, Schonhoff AM, Williams GP, Li X, et al. Peripheral monocyte entry is required for α-Synuclein induced inflammation and Neurodegeneration in a model of Parkinson disease. *Exp Neurol.* (2018) 300:179–87. doi: 10.1016/j.expneurol.2017.11.010
25. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize α-synuclein peptides. *Nature* (2017) 546:656–61. doi: 10.1038/nature22815
26. Mao X, Ou MT, Karuppagounder SS, Kam TI, Yin X, Xiong Y, et al. Pathological α-synuclein transmission initiated by binding lymphocyte-activation gene 3. *Science* (2016) 353: aah3374. doi: 10.1126/science.aah3374
27. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: Co-inhibitory receptors with specialized functions in immune regulation. *Immunity* (2016) 44:989–1004. doi: 10.1016/j.immuni.2016.05.001

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 15 February 2019
doi: 10.3389/fimmu.2019.00239

T-Cell-Driven Inflammation as a Mediator of the Gut-Brain Axis Involved in Parkinson's Disease

Javier Campos-Acuña[1], *Daniela Elgueta*[1] *and Rodrigo Pacheco*[1,2]*

[1] *Laboratorio de Neuroinmunología, Fundación Ciencia and Vida, Ñuñoa, Santiago, Chile,* [2] *Departamento de Ciencias Biológicas, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile*

Parkinson's disease (PD) is a neurodegenerative disorder affecting mainly the dopaminergic neurons of the nigrostriatal pathway, a neuronal circuit involved in the control of movements, thereby the main manifestations correspond to motor impairments. The major molecular hallmark of this disease corresponds to the presence of pathological protein inclusions called Lewy bodies in the midbrain of patients, which have been extensively associated with neurotoxic effects. Importantly, different research groups have demonstrated that $CD4^+$ T-cells infiltrate into the substantia nigra of PD patients and animal models. Moreover, several studies have consistently demonstrated that T-cell deficiency results in a strong attenuation of dopaminergic neurodegeneration in animal models of PD, thus indicating a key role of adaptive immunity in the neurodegenerative process. Recent evidence has shown that $CD4^+$ T-cell response involved in PD patients is directed to oxidised forms of α-synuclein, one of the main constituents of Lewy bodies. On the other hand, most PD patients present a number of non-motor manifestations. Among non-motor manifestations, gastrointestinal dysfunctions result especially important as potential early biomarkers of PD, since they are ubiquitously found among confirmed patients and occur much earlier than motor symptoms. These gastrointestinal dysfunctions include constipation and inflammation of the gut mucosa and the most distinctive pathologic features associated are the loss of neurons of the enteric nervous system and the generation of Lewy bodies in the gut. Moreover, emerging evidence has recently shown a pivotal role of gut microbiota in triggering the development of PD in genetically predisposed individuals. Of note, PD has been positively correlated with inflammatory bowel diseases, a group of disorders involving a T-cell driven inflammation of gut mucosa, which is strongly dependent in the composition of gut microbiota. Here we raised the hypothesis that T-cell driven inflammation, which mediates dopaminergic neurodegeneration in PD, is triggered in the gut mucosa. Accordingly, we discuss how structural components of commensal bacteria or how different mediators produced by gut-microbiota, including short-chain fatty acids and dopamine, may affect the behaviour of T-cells, triggering the development of T-cell responses against Lewy bodies, initially confined to the gut mucosa but later extended to the brain.

Keywords: $CD4^+$ T-cell mediated immunity, neo-antigens, gut microbiota, gut-brain axis, Parkinson's disease, neuroinflammation

OPEN ACCESS

Edited by:
Thomas G. Forsthuber, University of Texas at San Antonio, United States

Reviewed by:
Esther Melamed, University of Texas at Austin, United States
Stefan Brocke, University of Connecticut Health Center, United States

***Correspondence:**
Rodrigo Pacheco
rpacheco@cienciavida.org;
rodrigo.pacheco@unab.cl

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology

Received: *09 October 2018*
Accepted: *28 January 2019*
Published: *15 February 2019*

Citation:
Campos-Acuña J, Elgueta D and Pacheco R (2019) T-Cell-Driven Inflammation as a Mediator of the Gut-Brain Axis Involved in Parkinson's Disease. Front. Immunol. 10:239. doi: 10.3389/fimmu.2019.00239

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the world, which involves the progressive death of dopaminergic neurons in the nigrostriatal pathway, a midbrain circuit responsible for the control of voluntary movements. Accordingly, this disorder is characterised by motor symptoms such as bradykinesia, tremor, and postural abnormalities (1). In addition to the motor symptoms, one of the main hallmarks of PD is the presence of protein aggregates in the brain of patients, which are known as Lewy bodies. Importantly, α-synuclein, a central molecular player involved in the physiopathology of PD, has been found to be the main component of Lewy bodies (2). The process of α-synuclein aggregation to form Lewy bodies and the generation of intermediaries oligomers have been associated with the neurotoxic mechanisms involved in PD (3). Of note, one of the main causes of Lewy bodies generation is the oxidative stress, which promotes the covalent modifications of α-synuclein (i.e., by nitration) which strongly favour aggregation (4). In this regard, it has been proposed that aggregation represents an aberrant folding that competes with the proper healthy folding of α-synuclein favoured by high chaperone activity and low oxidative stress. Thereby, an overload of α-synuclein, decreased redox capability or reduced chaperone activity would make neurons more prone to α-synuclein aggregation (5, 6).

Intriguingly, most PD patients present a number of non-motor manifestations such as insomnia, loss of smell, anxiety, depression, apathy, and gastrointestinal dysfunctions, which commonly precede motor symptoms by several years (7, 8). Among non-motor manifestations, gastrointestinal dysfunctions result especially important as potential early biomarkers of PD, since they are ubiquitously found among confirmed patients and occurs much earlier than motor symptoms (9). In this regard, several lines of evidence have suggested a causal relationship between the gut and the brain in PD (10–13). The hypothesis of the involvement of a gut-brain axis was initially proposed by Braak and collaborators in which they suggested that environmental pathogens would be able to cross the intestinal epithelium and to induce misfolding and aggregation of α-synuclein in specific neurons of the enteric nervous system and subsequently, aggregated α-synuclein would propagate to the brain by migrating through the vagus nerve (14). Supporting this idea, enteric neurons have been found to be able to secrete α-synuclein (15). Moreover, experimental evidence obtained in rodents has shown that aggregated α-synuclein administered in the gut might promote aggregation of endogenous α-synuclein and the propagation of these aggregates through the vagus nerve contributing to the accumulation of aggregated α-synuclein in the brain (16). Notably, aggregated α-synuclein reaching the central nervous system might further spread transneuronally to different areas of the brain (17). It has also been observed the loss of neurons of the myenteric and submucosal plexi at early stages of PD, which is associated with apparition of Lewy bodies in the dorsal motor nucleus of the vagus nerve and decreased gastrointestinal motility (18–20). Furthermore, according to the Braak's hypothesis, it has been found that vagotomy in humans results in a reduced risk of PD (21). Importantly, studies performed in rodent models have shown that direct lesion of the nigrostriatal pathway results in altered colonic physiology and, on the other hand, colon inflammation triggers disturbance of nigrostriatal homeostasis, thus indicating a bidirectional communication between central dopaminergic neurons and the enteric nervous system (12).

Increased gut permeability, which corresponds to one of the main triggers of gut inflammation, has been also found in early diagnosed PD patients, a process that correlates with enhanced accumulation of α-synuclein in the gut mucosa (22). Accordingly, it has been described that PD patients display high levels of pro-inflammatory cytokine expression (TNF-α, IFN-γ, and IL-6) and glial activation markers (GFAP and Sox-10) in the ascending colon, thereby indicating an association between PD and colonic inflammation (23). In this regard, a recent study developed with more than 23 million individuals has shown that inflammatory bowel diseases (IBD) represent a risk factor to develop PD (24). Moreover, subsequent studies have shown a significant reduction in the risk to develop PD in those IBD patients that received early treatments with anti-inflammatory therapies such as anti-TNF-α or underwent surgery where tissue with high concentrations of α-synuclein aggregates was removed, thus limiting Lewy bodies spreading to the brain (25, 26).

Bacterial products also play a key role in the development of inflammation in the gut and the brain. Increased gut permeability promotes the leakage of bacteria and their products into the blood circulation leading to the maturation of antigen-presenting cells and the consequent stimulation of inflammatory pathways, thus triggering oxidative stress and favouring the accumulation of aggregated α-synuclein in the enteric nervous system (22, 27). Moreover, systemic inflammation triggered by bacterial products in the blood stream may induce a strong production of pro-inflammatory cytokines by cells from the innate and the adaptive immune system, which can spread through the blood, favour the permeabilization of the blood-brain-barrier and reach the brain. The subsequent stimulation of glial cells by pro-inflammatory cytokines coming from the periphery can trigger neuroinflammation and consequent neuronal death, such as the case of neurodegeneration induced by intraperitoneal administration of lipopolysaccharide (LPS) (28). Furthermore, it is important to consider that several studies performed

Abbreviations: ASO, transgenic mice over-expressing human α-synuclein; α-syn, α-synuclein; APCs, antigen-presenting cells; BBB, blood-brain-barrier; CD, Crohn's disease; CD*n*, cluster of differentiation *n*; DRD*n*, dopamine receptor D*n*; Foxp3, forkhead box P3; GFAP, Glial Fibrillary Acidic Protein; GPR*n*, G-protein coupled receptor *n*; HLA, human leukocyte antigen; HSV1, herpes simplex virus 1; IBD, inflammatory bowel diseases; IFN-γ, interferon γ; IL-*n*, interleukin *n*; IRBP, interphotoreceptor retinoid binding protein; LPR-1, Low-density lipoprotein receptor-related protein 1; LPS, lipopolysaccharide; MHC, Major Histocompatibility Complex; MLN, mesenteric lymph nodes; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MS, Multiple Sclerosis; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PD, Parkinson's disease; RAG1, recombination-activating-gen-1; RAG1KO, RAG1 knockout; RNS, reactive nitrogen species; ROS, reactive oxygen species; SCFAs, short-chain fatty acids; SCID, severe combined immunodeficiency; SFB, segmented filamentous bacteria; TCR, T-cell receptor; Th*n*, T helper *n*; TLRs, Toll like receptors; TLR*n*, Toll like receptor *n*; TNF-α, Tumor Necrosis Factor α; Tregs, regulatory T cells; UC, ulcerative colitis.

in recent years have shown a strong influence of intestinal microbiota in the control of gut inflammation (29–32). Of note, emerging evidence has shown that gut microbiota may control inflammation in two ways: (1). By producing a milieu of mediators that exert direct effects stimulating their receptors in eukaryotic cells of the host, such as short chain fatty acids (SCFAs), neurotransmitters and other metabolites (29, 33); and (2). By providing structures with molecular mimicry with self-antigens, which trigger activation of T-cells with autoreactive potential (34, 35). Importantly, a number of studies have shown relevant association of intestinal microbiota composition with the development of PD (10, 11, 13). However, it is still not clear whether these changes in the composition of the gut microbiota involve the generation of a milieu of microbiota-derived mediators that promote inflammatory behaviour in T-cells, the presence of molecular components with mimicry with self-antigens (i.e., Lewy bodies) or both.

Taken together these findings we propose here the hypothesis that $CD4^+$ T-cell response to Lewy bodies derived antigens is involved in the connection between inflammation in the gut and inflammation in the brain in the context of PD. Accordingly, in this review we analyse how commensal microbiota and its metabolites in the gut may participate triggering α-synuclein aggregation, the main source of antigens driving T-cell-mediated inflammation in PD. We also discuss the evidence indicating that microbiota-derived metabolites may affect the inflammatory and the suppressive function of T-cells, thus suggesting that the precise composition of the microbial consortium in the gut might break the tolerance to self-antigens, triggering T-cell-driven autoimmunity. Finally, we analyse the mechanisms of how dopamine and SCFAs, two key mediators strongly affected by the composition of gut microbiota, may affect T-cell behaviour, and how the alteration in the levels of these mediators might be involved in the development of T-cell mediated autoimmunity associated to PD.

T-CELL DRIVEN INFLAMMATION PLAYS A FUNDAMENTAL ROLE IN THE PHYSIOPATHOLOGY OF IBD AND PD

Evidence in human and animal models has shown the generation of oxidised forms of α-synuclein, especially nitrated α-synuclein, in the substantia nigra of individuals with PD (36–38), which constitutes a major component of Lewy bodies. Of note, the nitration of α-synuclein, which is a consequence of the oxidative stress, results in the generation of neo-antigens (4). Furthermore, studies in mice and recently in humans, have shown that oxidised α-synuclein constitutes a major antigen for the T-cell-mediated immune response involved in PD (37, 39–41). In this regard, it has been shown that nitrated α-synuclein generated in the substantia nigra is captured and presented by antigen-presenting-cells (APCs) in cervical lymph nodes to naive $CD4^+$ T-cells with specificity to this neo-antigen. Once activated, $CD4^+$ T-cells acquire inflammatory phenotypes, such as T-helper-1 (Th1) and Th17, they infiltrate the substantia nigra where microglial cells act as local APCs presenting peptide-antigens derived from nitrated α-synuclein on class II MHC, thus re-stimulating T-cells (37, 42–44). Restimulated $CD4^+$ T-cells produce high local levels of IFN-γ and TNF-α, thus promoting further inflammatory activation of microglial cells (M1 microglia) (45–48). Activated M1 microglia produces high levels of glutamate, TNF-α and reactive oxygen and nitrogen species (ROS/RNS), which in turn induce neuronal death and further generation of oxidised and nitrated proteins, including nitrated α-synuclein (4). Initial microglial activation makes blood-brain barrier (BBB) permissive for leukocyte entrance, and cytokines produce by Th1 and Th17 cells recruit and activate peripheral monocytes/macrophages and neutrophils which produce further neuronal damage (48). Thus, this mechanism involving the innate and adaptive immune system constitutes a vicious cycle, which results in chronic neuroinflammation and constitutes the engine of the progression of neurodegeneration. Of note, several studies performed with different T-cell deficient mouse strains, including TCR-β-chain deficient mice, severe combined immunodeficiency (SCID) mice and recombination-activating-gen-1 (RAG1) knockout (RAG1KO) mice, have shown that T-cell deficiency results in a complete protection of neurodegeneration in mouse models of PD (37, 46, 49). Furthermore, additional analyses have shown that whereas CD8-deficiency does not affect the extent of dopaminergic neurodegeneration, CD4-deficiency results in a strong attenuation of neurodegeneration in a mouse model of PD induced by MPTP (49), thus suggesting that inflammatory $CD4^+$ T-cell response plays a fundamental role promoting neurodegeneration of the nigrostriatal pathway.

Similar to the case of PD, several studies performed with inflammatory colitis mouse models and with samples obtained from patients with Ulcerative Colitis (UC) and with Crohn's disease (CD) have consistently indicated that gut inflammation in IBD is driven mainly by the inflammatory effector $CD4^+$ T-cell subsets Th1 and Th17 (50, 51). In addition, regulatory $CD4^+$ T-cells (Tregs), a suppressive subset of lymphocytes, seem to play a crucial role in maintaining intestinal homeostasis. These cells can suppress inflammation induced by effector T-cells (Th1 and Th17) in a mouse model of chronic inflammatory colitis induced by T-cell transfer into lymphopenic mice (52); and one of the main suppressive mechanisms relies on IL-10 secretion by these cells. In humans, Tregs are increased in the inflamed lamina propria of CD and UC patients compared to uninflamed mucosa and mucosa from healthy controls, and after isolation they retain their ability to suppress effector T-cell response *in vitro*, suggesting that Tregs function could be attenuated just *in situ* by mediators produced by the inflamed gut mucosa (53). Similar to the beneficial role of Tregs in mucosal immunity, the Th22 subset of CD4+ T-cells has been shown to promote homeostasis. In this regard, IL-22 produced by these cells induces the expression of tight junction proteins (i.e., claudin 1 and ZO-1) in epithelial cells, thus increasing the integrity of the mucosal epithelial barrier and protecting it from inflammation (54). Accordingly, it has been shown that the administration of anti-TNF-α therapy (infliximab) in CD patients, which ameliorates gut inflammation, upregulates IL-22 production contributing to intestinal epithelial barrier repair (54). Regarding the antigens recognised by the adaptive immune system in IBD, several autoantigens and

microbiota-derived antigens have been described in both CD and UC (55, 56). In the case of animal models of inflammatory colitis induced by different approaches, the main antigens recognised by adaptive immune system have been shown to correspond to microbiota-derived antigens. For instance, colitis induced by administration of chemicals such as dextran sodium sulfate or 2,4,6-trinitrobenzene sulfonic acid involve the disruption of epithelial layer of gut mucosa, resulting in an acute inflammatory response against microbiota-derived antigens (57, 58). In the case of genetic deficiency of IL-10, the inflammatory response in the gut is caused by the lack of the main suppressive mechanism used by gut Tregs to maintain mucosal homeostasis (59). The model of inflammatory colitis induced by T-cell transfer involves the administration of naive $CD4^+$ T-cells into lymphopenic recipient mice (60). In these conditions, most naive $CD4^+$ T-cells become activated in the gut-associated secondary lymphoid organs by recognising microbiota-derived antigens in the absence of Tregs. Activated $CD4^+$ T-cells differentiate in Th1 and Th17 cells, infiltrate the colonic lamina propria and release IFN-γ, IL-17, and other inflammatory mediators that recruit and stimulate neutrophils and macrophages, thus inducing chronic inflammation in gut mucosa (60). Considering the significant association between PD and IBD, it is likely that Lewy bodies derived antigens might be important targets for the adaptive immune system in IBD as well. According to this notion, it has been hypothesised that upon disruption of the epithelial layer of gut mucosa some microorganisms might induce inflammation, thus promoting oxidative stress and the consequent aggregation of α-synuclein produced by neurons of the enteric nervous system (14, 15). Another possible mechanism to explain how microenvironmental microorganisms might trigger an adaptive immune response against Lewy bodies is by molecular mimicry. In this regard, it has been shown that herpes simplex virus 1 (HSV1) derived antigens trigger the activation of homologous T-cells and B-cells that recognise α-synuclein derived antigens (61, 62). Furthermore, a study that analysed the seropositivity of PD patients and healthy controls to common infectious agents showed that the infection burden of HSV1 and some other pathogens is associated with PD (63). Thus, HSV1 infection might represent an environmental factor triggering PD and/or IBD in genetically susceptible individuals with proper MHC molecules able to present HSV1-derived peptides with molecular mimicry with α-synuclein-derived peptides. We further develop the discussion about potential involvement of molecular mimicry in the section Involvement of Gut-Microbiota in Autoimmunity. Taken together, the evidence indicates that T-cell driven inflammation represents a central process in both, PD and IBD, and suggests that Lewy bodies derived antigens might be important targets leading this T-cell mediated immunity.

LEWY BODIES AS TRIGGERS OF T-CELL MEDIATED IMMUNITY

As stated above, pathological inclusions of α-synuclein appear in early stages of PD, forming Lewy bodies in cells of the enteric nervous system (9). It has been proposed that initial α-synuclein aggregation and consequent Lewy bodies generation would take place in neurons of sites exposed to hostile environmental factors such as the olfactory bulb and gastrointestinal tract (64). Afterward, these α-synuclein inclusions would be transported from the peripheral nervous system to the brain by axonal retrograde movements, a hypothesis supported by experimental data obtained in rodents (16). In this regard, it has been shown that after the injection of human α-synuclein in the gut of rats, this protein is transported through the vagus nerve, reaching the brainstem (16). Furthermore, a number of mouse models of PD that recapitulate the accumulation of aggregated α-synuclein show similar spatiotemporal patterns of Lewy bodies formation as those observed in PD patients, beginning with Lewy bodies generation in the gut several months before the manifestation of motor symptoms (10, 65, 66). The observation that Lewy bodies appearance takes place early in the gut mucosa, even before Lewy bodies formation in the brain suggests that the generation of Lewy bodies would be triggered by environmental factors present in the gut, such as the gut microbiota. Supporting this idea, it has been shown that transgenic mice overexpressing human α-synuclein generate aggregates of α-synuclein in the gut and the brain and develop several Parkinsonian symptoms when housed in specific pathogen free conditions. Nevertheless, these mice display a strong attenuation in α-synuclein aggregation and motor impairment when microbiota is depleted by treatment with broad-spectrum antibiotics or when they are bred in germ free conditions (10). Another study supporting the idea that Lewy bodies aggregation is induced by environmental factors in genetically susceptible individuals has been performed in transgenic mice expressing a A53T mutant form of α-synuclein treated with Paraquat. This drug is a pesticide that is mainly ingested through airways and exerts the inhibition of mitochondrial respiration promoting oxidative stress (67). Oral administration of paraquat triggers expression of aggregated α-synuclein in the olfactory bulb and the enteric nervous system of transgenic mice earlier than in the brain and manifestation of motor impairment (68).

Importantly, several lines of evidence have shown that aggregated α-synuclein might act as a neo-antigen able to trigger adaptive immunity. Accordingly, Lewy bodies have been shown to stimulate Toll-like receptors 2 (TLR2) and TLR4 in local glial cells as well as in infiltrating APCs (i.e., monocyte/macrophages and dendritic cells), thus inducing an initial inflammation in the microenvironment where aggregated α-synuclein is generated (4). Of note, TLRs signalling induces NF-κB activation, which triggers the acquisition of inflammatory phenotypes by glial cells and the acquisition of immunogenic features by dendritic cells involving high expression of class II MHC and strong co-stimulation (47). In line with the idea that PD is initiated in the gut, it has been shown that oral administration of paraquat in transgenic A53T mice triggers an increased expression of Glial fibrillary acidic protein (GFAP; a classic activation marker of astrocytes) in glial cells of the enteric nervous system before neuropathology manifestation in the brain (68). Moreover, a study performed with endoscopic biopsies of children with gut inflammation shows a significant correlation between the levels of α-synuclein accumulation in neurites of the enteric nervous

system and the degree of inflammation of intestinal wall (69). Of note, an equivalent local inflammatory reaction is observed in the substantia nigra upon direct delivery of aggregated α-synuclein. In this regard, the stereotaxic injection of α-synuclein fibrils in the substantia nigra of rats induces an increased expression of class II MHC (an activation marker of glial cells) in local microglial cells of the nigrostriatal pathway as well as the recruitment of peripheral APCs and lymphocytes (70). Taken together these findings indicate that gut microbiota plays an important role as an environmental factor inducing local α-synuclein aggregation, which in turn triggers the stimulation of TLRs in innate immune cells, thus promoting an initial inflammation in the gut mucosa.

Importantly, one of the main consequences of TLR stimulation in APCs is an increased expression of class II MHC molecules on the cell surface (4, 70). Thus, mucosal dendritic cells present in those zones of the gut where α-synuclein aggregation is initiated would receive TLR-stimulation and concomitantly would capture Lewy bodies, process them intracellularly to yield small peptides, and subsequently would present Lewy bodies derived peptides on class II MHC. TLR-stimulation also induces a fast migration of dendritic cells into the draining lymph nodes (MLN in the case of colonic mucosa) and thus, Lewy bodies derived antigens would be presented on class II MHC to naïve $CD4^+$ T-cells, which normally keep patrolling though these lymphoid tissues (71). In this way, when naïve $CD4^+$ T-cells bearing TCR specific for the recognition of Lewy bodies-derived antigens appear and recognise their antigen presented by dendritic cells, they would become activated, proliferate, and then would migrate to the site where inflammation has been initiated, in this case the gut mucosa (72). Supporting this notion, it has recently been described the presence of inflammatory $CD4^+$ T-cells with specificity by different Lewy bodies derived antigens in PD patients (41). Since dendritic cells would acquire an immunogenic phenotype upon TLR-stimulation, including the production of inflammatory cytokines and high expression of class II MHC and co-stimulation, Lewy bodies derived antigens presentation should induce inflammatory effector phenotypes in antigen-specific $CD4^+$ T-cells. Accordingly, recent studies analysing the peripheral immune system of PD patients have shown a biased Th1 (73) and Th17 immunity (74). Th1 and Th17 have been extensively involved in autoimmunity, where they infiltrate the target tissue and promote: (i) The recruitment of monocytes and neutrophils, respectively and; (ii) The microbicide and oxidative activity of local macrophages and infiltrated monocytes and neutrophils, thus favouring local inflammation and tissue damage (75). Therefore, the ROS and RNS induced in local phagocytes by Th1 and Th17 immunity would promote further aggregation of α-synuclein in the neurons of the enteric nervous system. This mechanism would represent a vicious cycle, which results in chronic inflammation, further generation of Lewy bodies in the gut, and the subsequent spreading of Lewy bodies to the brainstem as suggested before (14). Once Lewy bodies reach the brain, they might stimulate TLR signalling in microglial cells, thus favouring the permeabilization of the BBB and the subsequent recruitment of Lewy bodies-specific Th1 and Th17 cells, promoting brain damage (4). Considering all of these factors, we propose the following model: the initial aggregation of α-synuclein in the gut mucosa would trigger Th1 and Th17 immunity and further generation of Lewy bodies that would migrate to the brain in later stages. In this way, T-cell mediated inflammation would represent the engine of tissue damage, first in the gut and later in the brain (**Figure 1**).

According to the coordinate production of autoantibodies commonly associated to T-cells mediated autoimmunity and to the autoimmune nature of PD, autoreactive B-cells have also been involved in this pathology (4). Accordingly, the presence of autoantibodies in the serum and infiltrated within the brain parenchyma has been described in PD (76, 77). Furthermore, analyses performed in necropsies of PD patients have shown that immunoreactivity associated to autoantibodies is located specifically in dopaminergic neurons and Lewy bodies (77). Moreover, the high degree of FcγRs detected on microglia and mononuclear cells infiltrating the substantia nigra of PD patients (77) suggests that the infiltration of these autoantibodies into the central nervous system may strongly contribute to neuroinflammation and neurodegeneration. Despite there are available evidence involving autoantibodies directed to Lewy bodies in the central nervous system of PD patients, there are not studies addressing the presence of autoantibodies to Lewy bodies in gut inflammation.

GUT MICROBIOTA AS A MASTER REGULATOR OF T-CELL MEDIATED IMMUNITY

Intestinal microbiota is in close contact with the gut epithelial barrier, which isolates and separates the intestinal lumen from the rest of the organism. Interestingly, hosts organisms have evolved together with commensal bacteria to establish a symbiotic relationship by mean of synthesising and responding to several common mediators (78). In this regard, mammals have evolved to take advantage of the presence of commensal microbiota in the gastrointestinal tract far beyond the simple degradation of nutrients by some types of bacteria, as mediators synthesised by gut microbiota, including neurotransmitters, metabolites, and fatty acids can strongly affect host metabolism, neural circuits, hormone secretion, behaviour, and the immune response (78, 79). Accordingly, pathologic alterations in the composition of gut microbiota (dysbiosis) have been strongly involved in the development of cancer as well as neuropsychiatric, metabolic, autoimmune and neurodegenerative disorders (80–82). Three mechanisms have been proposed to explain the influence that gut microbiota exerts in the host physiology: (i) The first one involves the secretion of neurotransmitters, neuropeptides and metabolites that might directly stimulate their receptors in neurons of the enteric nervous system, thus triggering and/or modulating neural signals that affect directly the gut physiology or migrate through vagal transmission to the central nervous system affecting behaviour (78). (ii) The second mechanism proposed involves metabolites and hormones produced by microbiota in the intestinal tract that might diffuse through

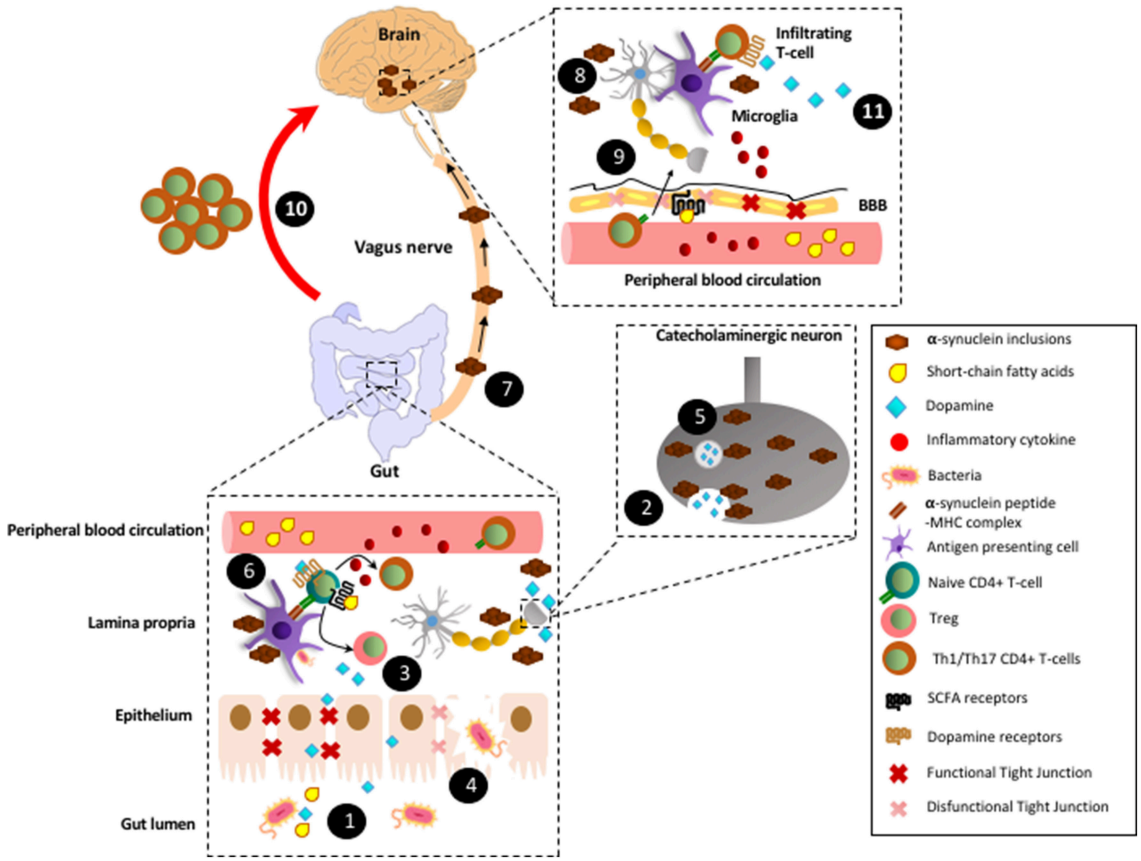


FIGURE 1 | Proposed model by which $CD4^+$ T-cell response involved in Parkinson's disease are triggered in the gut mucosa. (1) In healthy conditions, gut microbiota produces SCFAs and high levels of dopamine. (2) In addition to the gut microbiota, catecholaminergic neurons of the enteric nervous system also contribute to the secretion of high dopamine levels into the gut mucosa and lumen. (3) SCFAs and dopamine stimulate GPR41, GPR43, and GPR109A and low-affinity dopamine receptors (i.e., DRD2), respectively in $CD4^+$ T-cells, favouring their differentiation into Tregs and their suppressive activity, thereby promoting tolerance to food-derived and microbiota-derived antigens. (4) Under some circumstances, such as dysbiosis, some tight junctions components become down-regulated and thereby epithelial layer of gut mucosa might be disrupted. Consequently, some strains of gut microbiota trigger an initial inflammation mediated by innate immune cells, which promote local oxidative stress with the covalent modification of self-proteins. (5) The oxidative environment promotes the generation of α-synuclein inclusions, which impair vesicular secretion by neurons of the enteric nervous system and thereby reduction in dopamine levels. (6) In addition, α-synuclein inclusions are captured by mucosal APCs and presented to naïve $CD4^+$ T-cells specific for Lewy bodies derived antigens. Moreover, α-synuclein inclusions stimulates TLRs in macrophages and dendritic cells, triggering thus inflammation, oxidative stress and thereby further generation of α-synuclein inclusions, which constitutes a vicious cycle of chronic inflammation and generation of Lewy bodies. (7) According to Braak's hypothesis, after a long period of time (years) with chronic inflammation, Lewy bodies generated in the enteric nervous system would be transported by retrograde movement through vagus nerve until reaching the brain stem. (8) Lewy bodies in the brain would stimulate TLRs in microglial cells inducing the production of inflammatory cytokines and thus favouring the permeabilization of the BBB. (9) Inflammatory cytokines coming from peripheral blood circulation would also contribute to BBB permeabilization. In addition, a reduction in SCFAs (i.e., induced by a dysbiosis in gut microbiota) might alter GPR41-signalling in the BBB, thus promoting disassembling of tight junctions and further permeabilization of the BBB. (10) Inflammatory $CD4^+$ T-cells (Th1 and Th17) generated years ago in response to Lewy bodies in the gut mucosa would migrate through the blood and infiltrate the brain (red arrow; this is the main hypothesis raised here). (11) Microglial cells would capture Lewy bodies and subsequently they would present Lewy bodies-derived antigens to Th1 and Th17 infiltrating the brain. Thus, microglial cells would restimulate Lewy body-specific $CD4^+$ T-cells promoting further neuroinflammation and neurodegeneration of the dopaminergic neurons of the nigrostriatal pathway.

the gut wall, entering into the portal circulation and then exert their effects far away by stimulating their receptors expressed in other organs such as adrenal gland, liver, or others (78). (iii) The third mechanism proposed involves the stimulation of receptors expressed in immune cells by mediators produced by gut microbiota, such as short-chain fatty acids (SCFAs), neurotransmitters and other metabolites (29, 83), thus shaping the immune response (see sections Dopaminergic Regulation of T-cell Mediated Immunity and Short-Chain Fatty Acids as Regulators of T-cell Mediated Immunity).

Mucosal immunity involves a tight equilibrium between inflammatory responses against orally administered dangerous foreign antigens and the generation of tolerance to food-derived and commensal microbiota-derived antigens. Regarding the role of gut-microbiota in the immune system, several studies have extensively shown a key role of intestinal segmented

filamentous bacteria (SFB) in the induction of Th17 cells in the gut mucosa (84, 85). Interestingly, it has been shown that signals triggered by SFB to induce Th17 differentiation does not depend on receptors of the innate immune system (i.e., TLRs), but strongly depends on the adhesion of SFB to the intestinal epithelial cells (86). In homeostatic conditions this inflammatory subset of $CD4^+$ T-cells, controls the invasion of gut mucosa by several pathogenic bacterial species by inducing the secretion of IgA by plasma cells into the colonic lumen. Besides mediating immunity against pathogenic bacteria, the induction of Th17 cells in the gut mucosa has also been associated with the development of autoimmune disorders, such as multiple sclerosis, arthritis and uveitis (34, 87, 88), making them a double-edged sword if not controlled properly. In addition to the control of inflammatory Th17 cells, gut microbiota plays also an important role favouring the activity of immunosuppressive T-cells to promote tolerance to innocuous antigens derived from food and commensal bacteria (29). For instance, it has been shown that the commensal bacterium *Bacteroides fragilis* induces the generation of extra-thymic Foxp3+ Tregs in the intestine, thus favouring a tolerogenic environment in the gut mucosa. It is noteworthy that mono-colonization of germ-free animals with *Bacteroides fragilis* significantly increase the IL-10 production and the suppressive activity of Tregs (exclusively in the Foxp3+ population) in the gut mucosa, an effect mediated by the stimulation of TLR2 in mucosal APCs by polysaccharide-A expressed on *Bacteroides fragilis* (89). Thus, these examples illustrate how individual components of the gut microbiota can exert strong changes in the outcome of mucosal immunity.

Addressing the relevance of commensal bacteria in the development of Parkinson's disease, a recent study was carried out using a transgenic mice over-expressing human α-synuclein (ASO mice) in which microbiota was depleted. ASO mice spontaneously develop neuroinflammation, generation of α-synuclein inclusions in the nigrostriatal pathway and several parkinsonian sympoms after 12 weeks of age, including motor impairment and intestinal dysfunction (90). Strikingly, it was shown that depletion of microbiota, by the treatment of mice with broad-spectrum antibiotics or by breeding them in germ-free conditions, results in a nearly complete abolition of the Parkinsonism manifestation, including the attenuation of α-synuclein inclusions generation, neuroinflammation, and motor and intestinal impairment (10). Moreover, when germ-free ASO mice were repopulated with gut microbiota obtained from PD patients they developed a stronger parkinsonian phenotype than when repopulated with gut microbiota obtained from healthy human individuals (10). Interestingly, a distinctive product from microbiota obtained from PD patients in comparison with microbiota obtained from healthy controls was the production of SCFAs, including butyrate, propionate, and acetate. Notably, treatment of germ-free ASO mice with SCFAs recapitulated the pathogenic effect of gut microbiota triggering the development of parkinsonian phenotype (10). A later study addressed the question of whether there is a particular commensal bacterium responsible for triggering the development of PD. Interestingly, the authors found that *Proteus mirabilis* was particularly increased in the gut microbiota of a number of different PD mouse models, including the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model, the MPTP plus probenecid model and the 6-OHDA (6-hydroxydopamine) model (11). The oral administration of *Proteus mirabilis* promotes α-synuclein aggregation in the gut and the brain, favours neuroinflammation and neurodegeneration of dopaminergic neurons of the nigrostriatal pathway and exacerbates the motor impairment (11). The analysis of the molecular mechanism underlying revealed that LPS expressed by *Proteus mirabilis* induced a down-regulation of occludin expression in the gut mucosa, thus disassembling tight junctions in the colon and favouring the disruption of epithelial intestinal layer (11), which triggers an inflammatory process as described in the section Lewy Bodies as Triggers of T-cell Mediated Immunity. According to these results, another study has shown that fecal microbiota transplantation from healthy controls significantly reduces the dysbiosis in PD animals and attenuates the extent of neurodegeneration, neuroinflammation, and motor impairment (13). These findings together indicate that some particular components of the gut microbiota might be the triggers of α-synuclein aggregation and subsequent PD development. However, is important to keep in mind that gut commensal microbiota is composed by more than 1,000 different bacterial species and thereby it is expected that they together should produce complex milieu of mediators that might affect the behaviour of the immune system. In this regard, next sections focus in the analysis of two kind of molecular cues strongly affected by the microbiota composition and that exert key effects on the adaptive immune response: dopamine and SCFAs.

DOPAMINERGIC REGULATION OF T-CELL MEDIATED IMMUNITY

Gut mucosa, which plays a critical role in the induction of tolerance to dietary antigens and to commensal microbiota, constitutes a major source of dopamine available for immune cells (91–93). Importantly, dopamine-mediated regulation of immunity in the gut mucosa seems to be critical for maintaining the tolerance to innocuous antigens, as gut dopamine levels are strongly reduced in patients with CD and UC and in animal models of inflammatory colitis (91, 92). Gut dopamine might be produced from different sources, including the intrinsic enteric nervous system, the intestinal epithelial layer (94), some components of the gut microbiota (95), and certain immune cells, including dendritic cells and Tregs (96–98). Nevertheless, the evidence indicates that one of the main sources of dopamine present in the gut mucosa is given by the commensal gut microbiota (93). In this regard, it has been described that most dopamine arrives to the gut mucosa as glucuronide conjugated, which is biologically inactive. Nevertheless, *Clostridium* species present in the gut microbiota express β-glucuronidase activity, which catalyses the production of free dopamine in the gut mucosa (93). In addition, recent studies have shown *in vitro* evidence indicating that some components of gut microbiota, including *Bacillus cereaus*, *Bacillus mycoides, Bacillus subtilis*, *Proteus vulgaris, Serratia marcescens, S. aureus, E. coli* K-12,

Morganella morganii, Klebisella pneumonia, and *Hafnia alvei*, can also produce dopamine (95). Interestingly, similar to the situation observed in IBD, striatal dopamine levels are also significantly reduced in PD (49), a process that can be observed even before the degeneration of dopaminergic neurons of the nigrostriatal pathway (99). Of note, both IBD and PD involve a local inflammation driven by $CD4^+$ T-cells (as discussed in section Lewy Bodies as Triggers of T-cell Mediated Immunity), cells that are thereby exposed to these changes in dopamine levels. Dopamine exerts its effects by stimulating DRs, termed DRD1-DRD5; all of them belonging to the superfamily of G-protein coupled receptors. All these receptors have been found in $CD4^+$ T-cells from human and mouse origin (100). It is important to consider that each DR displays different affinities for dopamine: DRD3>DRD5>DRD4>DRD2>DRD1 (Ki(nM) = 27, 228, 450, 1,705, 2,340, respectively), thereby their functional relevance depend on dopamine levels (83). Regarding the role of DRs expressed in $CD4^+$ T-cells upon inflammation, our recent studies showed that DRD3-deficient naïve $CD4^+$ T-cells display impaired Th1 differentiation and reduced expansion of Th17 cells and consequently an attenuated manifestation of inflammatory colitis (101, 102). Taking into account the reduction in intestinal dopamine levels [≈1,000 nM in healthy individuals; ≈50 nM in CD and UC patients (91, 93)] and the fact that DRD3 may be selectively stimulated at low dopamine concentrations, these results suggest that low dopamine levels present in the inflamed gut mucosa favour the inflammatory potential of $CD4^+$ T-cells, thus promoting chronic inflammation. Accordingly, DRD3-deficiency in $CD4^+$ T-cells results in a significant attenuation in disease manifestation in a mouse model of inflammatory colitis (102). Of note, equivalent to the situation of inflammatory colitis, we have shown that DRD3-deficiency in $CD4^+$ T-cells results in a complete attenuation of MPTP-induced neurodegeneration and the treatment of wild-type mice with a selective DRD3-antagonist significantly reduces the development of PD in two different animal models (46, 103). Conversely, high dopamine concentrations in the gut of healthy individuals would stimulate DRD2, favouring the production of the anti-inflammatory cytokine IL-10 by $CD4^+$ T-cells (104) and suppressing both increased motility and ulcer development (105). Indeed, a genetic polymorphism of DRD2 gene, which results in decreased receptor expression, has been reported as a risk factor for IBD (106). In this regard, although the frequency of Tregs was not changed in the gut, suppressor function of intestinal Tregs was compromised in inflammatory colitis (107), a condition associated to decreased dopamine levels (92). Interestingly, the impairment of suppressive Tregs function was abolished by the administration of cabergoline, a DRD2 agonist (107). Taken together these findings suggest that, whereas DRD2-signalling in $CD4^+$ T-cells would promote suppressive activity and tolerance in a healthy gut mucosa containing high dopamine levels, the selective DRD3-signalling in $CD4^+$ T-cells promotes the inflammatory potential of T-cell mediated immunity in the inflamed gut mucosa containing low dopamine levels.

Beside the involvement of dopaminergic dysregulation in gut inflammation, arises the question of why dopamine levels are reduced in these conditions. In this regard, different non-exclusive mechanisms might be involved, including changes in the composition of gut microbiota (dysbiosis; see section Gut Microbiota as a Master Regulator of T-cell Mediated Immunity), the loss of catecholaminergic neurons of the enteric nervous system (see section Introduction), and the limited synthesis and secretion of dopamine as a consequence of α-synuclein aggregation in the neurons of the enteric nervous system. According to the latter mechanism involved in the reduction of dopamine levels, it has been described that healthy α-synuclein plays a role in the transport of presynaptic vesicles to nerve terminals. However, the aggregation of this protein, results in impaired secretion of presynaptic vesicles (99). Thus, similar to the reduction of dopamine levels observed in the striatum of PD patients as a consequence of Lewy bodies formation in the dopaminergic neurons of the nigrostriatal pathway, dopaminergic neurons of the enteric nervous system might result in impaired secretion of dopamine upon Lewy bodies formation in the gut mucosa. Moreover, a recent study performed with endoscopic biopsies and blood samples obtained from children with documented gut inflammation show that α-synuclein expression in enteric neurites correlated with the degree of the gut wall inflammation and that both monomeric or oligomeric forms of this protein induced dendritic cells maturation and triggered the recruitment of $CD11b^+$ neutrophils and monocytes, suggesting a role of α-synuclein in the activation of innate immunity in the gastrointestinal tract (69). Taken together, these findings suggest that not only microbiota dysbiosis and the loss of neurons of the enteric nervous system would be involved in the reduction of dopamine levels associated to gut inflammation, but also the aggregation of α-synuclein should play a relevant contribution to this issue. Moreover, α-synuclein seems to play a direct role stimulating innate immunity in the gut mucosa. Thus, it seems that different mechanisms affecting gut homeostasis converge in the upregulation of α-synuclein and the reduction of dopamine levels in the gut mucosa, which plays a key role as a danger-signal stimulating high-affinity dopamine receptors expressed in T-cells, promoting inflammation.

SHORT-CHAIN FATTY ACIDS AS REGULATORS OF T-CELL MEDIATED IMMUNITY

A major class of mediators produced by gut microbiota corresponds to the SCFAs derived from bacterial fermentation products, including acetate, propionate, and butyrate, among others. These mediators might act on T-cell physiology either by stimulating G-protein coupled receptors or by modifying the activity of epigenetic enzymes that regulate gene transcription (108). Regarding the SCFAs effects mediated by G-protein coupled receptors, a number of studies have shown GPR41, GPR43, and GPR109A as the main SCFAs receptors present in T-cells. For instance, the stimulation of GPR41 by propionate, and with lower affinity by butyrate, has been shown to attenuate Th2 responses. Thereby GPR41 stimulation exerts a protective effect

in allergic inflammation in the airways (109). In addition, the stimulation of GPR43, which recognizes acetate and propionate with similar affinities, has been described to exert a potent immunosuppressive effect attenuating gut inflammation. In this regard, it has been shown that GPR43 expression is favoured in colonic Tregs and its stimulation induces the expansion and promotes the suppressive activity of these cells (30). Similarly, the GPR109A, which is stimulated by butyrate and niacin with similar affinity, induces anti-inflammatory features in colonic macrophages and dendritic cells, thus favouring the expansion and suppressive activity of Tregs and concomitantly attenuates the pro-inflammatory potential of Th17 cells (110). Importantly, one of the mechanisms by which SCFAs shape T-cell behaviour is based on the ability of these mediators to inhibit histone deacetylase and thus modifying the epigenetic landscape of T-cells chromatin (111). In this regard, it has been shown that butyrate and propionate increase the acetylation of the *foxp3* locus, favouring a higher expression of Foxp3 and consequently an enhanced Tregs differentiation and stronger suppressive activity (29). In the same direction, it has been described that SCFAs increase the mucosal barrier function in the duodenum by reducing epithelium permeability and increasing the secretion of bicarbonate to the lumen, thus avoiding the immune recognition of luminal bacteria and the consequent inflammation (112). Furthermore, a recent study has shown that Clostridium butyricum B1, by producing butyrate, favours the differentiation of CD4+ T-cells into Th22 (113), a subset of T-cells that produce IL-22, upregulating tight junctions expression in epithelial cells of the gut mucosa, thus increasing the barrier function (54). Taken together these studies indicate that SCFAs derived from intestinal commensal bacteria exert an anti-inflammatory effect in the mucosal immunity of the gut by both, promoting Tregs function and increasing barrier function.

As stated above, recent studies have consistently found a dysbiosis in the gut microbiota of PD patients as well as in a number of animal models of PD (10, 11, 114). In this regard, Unger and collaborators analysed the SCFAs contained in fecal samples obtained from 34 PD patients and 34 age-matched controls and found a significant reduction of SCFAs in PD patients (114). According to these results, reduced production of SCFAs has been related with dysfunctional Tregs activity and consequent gut inflammation (29, 110). Furthermore, it has been recently shown that germ-free mice present an altered organization of tight junctions in the BBB involving a down-regulation of occludin and claudin 5 and consequently an increased permeability of this barrier. Of note, the proper occludin and claudin 5 expression and reduced BBB permeability was restored by monocolonization with different SCFAs-producer bacterial strains (115). Moreover, another study has recently shown that GPR41 stimulation by propionate in the BBB attenuates the expression of the LPR-1 transporter, thus providing protection of the BBB from oxidative stress (116). In apparent controversy with the study performed by Unger and collaborators, as stated above (section Dopaminergic Regulation of T-cell Mediated Immunity) the study performed by Sampson and collaborators found that when microbiota was depleted from ASO mice, several parkinsonian manifestations were significantly reduced or even disappeared, a condition associated with reduced SCFAs production (10). However, when germ-free ASO mice were reconstituted with microbiota obtained from PD patients, they showed decreased levels of acetate but increased levels of propionate and butyrate in fecal samples, which was associated with stronger parkinsonian manifestations in comparison with animals reconstituted with microbiota obtained from healthy individuals (10). Thus, together these studies demonstrate that dysbiosis associated to PD involves an alteration in the production of SCFAs. The precise alterations in the level of SCFAs might subsequently trigger the loss of immune tolerance in the gut mucosa and the failure of BBB functions. Thereby, the dysbiosis involved in PD might be a key factor triggering T-cell autoimmunity directed to Lewy bodies-derived antigens.

INVOLVEMENT OF GUT-MICROBIOTA IN AUTOIMMUNITY

Since PD physiopathology involves an adaptive immune response mediated by $CD4^+$ T-cells, and these cells exert an inflammatory effect in response to self-antigens (i.e., lewy bodies), PD gathers the characteristics to be considered an autoimmunity (4). Accordingly, it has been shown that PD development requires both autoreactive $CD4^+$ T-cells (37, 41, 49, 117) and also proper class II MHC able to present auto-antigens-derived peptides to autoreactive $CD4^+$ T-cells (118). At this point it is important to note that different human leukocyte antigen (HLA) alleles involve different peptide-binding preferences and affinities and consequently activation of different T-cell clones and different quality of activation of pro-inflammatory or anti-inflammatory T-cells (119). Thereby a key genetic factor to take in consideration for autoimmune disorders is the HLA polymorphism. According to the autoimmune nature of PD, alleles HLA-DQB1*06 and HLA-DRB1*0301 have been shown to be significantly more frequent in PD patients (120, 121). Notably, the allele HLA-DRB1*0301 (121) has also been associated to the genetic susceptibility of some classical autoimmune diseases such as diabetes (122) and multiple sclerosis (MS) (123). In addition, a recent study analysed the binding of different alleles of class II MHC to peptides derived from autoantigens associated to PD and found a positive association between alleles HLA-DRB1*1501, HLA-DRB1*0304, and HLA-DRB5*0101 and the binding to autoantigens-derived peptides in PD patients (41). Together these findings indicate that, in addition to the classical polymorphism of components of the mitochondrial and autophagy machinery associated to PD risk (124), the HLA haplotype constitutes a key genetic factor associated to the susceptibility to develop PD.

As indicated in section Dopaminergic Regulation of T-cell Mediated Immunity, gut microbiota has been shown to play a key role in triggering PD development, however the underlying mechanism is still unclear. On the other hand, gut microbiota has been described to be also a fundamental factor in the development of other autoimmune disorders (34, 87, 88, 125). Thereby the understanding of the mechanisms involved in how microbiota trigger autoimmune responses in other

disorders might give the clues to understand how microbiota may induce the development of PD in susceptible individuals. An interesting example is the study of how microbiota promotes the development of uveitis carried out by Horai and collaborators (34). In this study the authors used transgenic mice (R161H mice) bearing $CD4^+$ T-cells expressing a TCR specific for the recognition of the interphotoreceptor retinoid binding protein (IRBP), a component of the retina. These animals spontaneously developed uveitis, an autoimmune response to the retina, which constitutes a major cause of blindness in humans. However, when the authors depleted microbiota by treatment with broad-spectrum antibiotics or by breeding these animals in germ-free conditions, the development of uveitis was strongly attenuated. Interestingly, the activation and acquisition of the Th17 inflammatory phenotype of transgenic $CD4^+$ T-cells took place early in the small intestine, before the infiltration of these autoreactive T-cells into the eye. Strikingly, when authors developed R161H mice knockout for the cognate-antigen of transgenic T-cells (IRBP), these animals still displayed a vigorous activation and acquisition of the Th17 phenotype of transgenic $CD4^+$ T-cells in the small intestine, a process that was dependent on the presence of SFB in the intestine. Thus, this study demonstrated that the activation of autoreactive T-cells is induced by non-cognated antigens present in the SFB or alternatively by antigens coming from SFB with molecular mimicry with retinal antigens. Importantly, this study constitutes an example illustrating how microbiota can trigger autoimmunity by molecular mimicry or by cross-reactivity between autoantigens and bacterial antigens present in the gut microbiota.

Due to its relevance in the development of autoreactive T-cell mediated responses, gut microbiota represents an important environmental factor able to trigger autoimmunity in susceptible individuals. An illustrative example for this, is a recent study performed with 34 monozygotic twin pairs discordant for MS. In this study Berer and collaborators show that when microbiota from the MS twin is transplanted into a transgenic mouse model of spontaneous brain autoimmunity, mice developed autoimmunity with higher incidence than when transplanted with microbiota coming from the healthy twin (125). Importantly, when mice were transplanted with microbiota obtained from MS twins, immune cells present in the gut mucosa produced significantly lower levels of the anti-inflammatory cytokine IL-10 than those immune cells of animals receiving microbiota coming from healthy twins. In addition, the analysis in the composition of gut microbiota shows clear differences between healthy and MS twins (125). Thus, this study represents an illustrative example of how the precise composition of gut microbiota might play an important role favouring the induction of an immunosuppressive environment in the gut mucosa and how changes in the microbiota composition may induce the loss of this environment triggering the development of inflammatory and autoimmune disorders.

Considering the previous example, the parallelism between MS and PD results intriguing: (i) both inflammatory disorders involve an autoimmune component mediated by $CD4^+$ T-cells specific for central nervous system antigens, (ii) in both cases gut microbiota seems to play a key role triggering the activation of autoreactive T-cells in the gut mucosa, and (iii) the risk to develop both pathologies involve a genetic association with HLA-polymorphism. Since the autoimmune nature of MS and its relationship with gut-microbiota have been much longer explored than in PD, maybe we should take advantage from the knowledge about the mechanistic involvement of microbial consortium and adaptive immunity in the physiopathology of MS and from the successful immunotherapies developed in MS to better understand and to fight against PD. In this regard, several studies in MS and animal models (EAE) have shown that microbial organisms might trigger the activation of autoreactive T-cells specific for central nervous system antigens either through molecular mimicry or via bystander activation. Moreover, several gut microbiota-derived metabolites and bacterial products have been described to interact with the immune system to modulate central nervous system autoimmunity (126). In addition, the involvement of some accessory immune cells that results key players in the $CD4^+$ T-cell mediated inflammation associated to MS should be considered in PD, such as γδT-cells, which strongly inhibit the suppressive activity of Tregs in the central nervous system (127), or peripheral macrophages infiltrating the brain, which results much more relevant than microglia promoting neuroinflammation in response to GM-CSF (128). Finally, it is important to mention that there is already some evidence showing significant therapeutic effects in animal models of PD, induced by MPTP, 6-OHDA or rotenone, when treated with therapies used in MS to block the infiltration of autoreactive $CD4^+$ T-cells into the brain, such as Fingolimod/FTY720 (129–131). These findings encourage to further explore the mechanistic parallelism between both pathologies and also to evaluate the efficacy of other immunotherapies used in MS as a potential treatment for PD, such as rituximab (anti-CD20 monoclonal antibody geared to deplete B-cells) or natalizumab (monoclonal antibody mediating the blockade of α4-integrin, required by T-cells to infiltrate the brain).

CONCLUSIONS

Several lines of evidence point to the hypothesis that PD development is triggered in the intestine, including the early loss of neurons of the enteric nervous system, mucosal inflammation, and the generation of α-synuclein inclusions in the gut. Importantly, PD is positively associated to IBD and both disorders involve a $CD4^+$ T-cell driven chronic inflammation. Furthermore, the development of both PD and IBD has been found to be strongly dependent on the composition of gut microbiota. Notably, some components of gut microbiota might trigger the generation of α-synuclein inclusions in the gut, which constitutes the main source of autoantigens driving the $CD4^+$ T-cell response in PD. Furthermore, gut microbiota produces several mediators

in the gut mucosa, such as SCFAs, dopamine and other metabolites, which stimulate their receptors in T-cells, thus shaping the adaptive immune response. In addition, some gut microbiota strains have been shown to trigger autoimmunity by providing molecular mimicry or cross-reactivity with self-antigens. Thus, we propose here that $CD4^+$ T-cell response to Lewy bodies-derived antigens is triggered initially by gut microbiota, inducing an early gut inflammation and later PD (**Figure 1**).

AUTHOR CONTRIBUTIONS

RP designed the study. JC-A, DE, and RP acquired data, analysed data, and wrote the manuscript.

FUNDING

This work was supported by grants FONDECYT-1170093 (to RP), from Fondo Nacional de Desarrollo Científico y Tecnológico de Chile, AFB-170004 (to RP) from Comisión Nacional de Investigación Científica y Tecnológica de Chile (CONICYT) and MJFF-15076 and MJFF-10332.01 (to RP) from Michael J. Fox Foundation for Parkinson's Research. JC-A holds a fellowship (folio 21180205) from the Programa de Formación de Capital Humano Avanzado–Doctorado Nacional from CONICYT.

ACKNOWLEDGMENTS

We thank Laura Quezada for her help in drawing **Figure 1**.

REFERENCES

1. Thomas B. Parkinson's disease: from molecular pathways in disease to therapeutic approaches. *Antioxid Redox Signal.* (2009) 11:2077–82. doi: 10.1089/ars.2009.2697
2. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature* (1997) 388:839–40. doi: 10.1038/42166
3. Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, et al. *In vivo* demonstration that alpha-synuclein oligomers are toxic. *Proc Natl Acad Sci USA.* (2011) 108:4194–9. doi: 10.1073/pnas.1100976108
4. Gonzalez H, Contreras F, Pacheco R. Regulation of the Neurodegenerative Process Associated to Parkinson's Disease by CD4+ T-cells. *J Neuroimmune Pharmacol.* (2015) 10:561–75. doi: 10.1007/s11481-015-9618-9
5. Kim C, Lee SJ. Controlling the mass action of alpha-synuclein in Parkinson's disease. *J Neurochem.* (2008) 107:303–16. doi: 10.1111/j.1471-4159.2008.05612.x
6. Lim KL, Zhang CW. Molecular events underlying Parkinson's disease - an interwoven tapestry. *Front Neurol.* (2013) 4:33. doi: 10.3389/fneur.2013.00033
7. Rana AQ, Ahmed US, Chaudry ZM, Vasan S. Parkinson's disease: a review of non-motor symptoms. *Expert Rev Neurother.* (2015) 15:549–62. doi: 10.1586/14737175.2015.1038244
8. Di Battista ME, Cova I, Rubino A, Papi CP, Alampi G, Purcaro C, et al. Intercepting Parkinson disease non-motor subtypes: a proof-of-principle study in a clinical setting. *J Neurol Sci.* (2018) 388:186–91. doi: 10.1016/j.jns.2018.03.024
9. Poirier AA, Aube B, Cote M, Morin N, Di Paolo T, Soulet D. Gastrointestinal dysfunctions in parkinson's disease: symptoms and treatments. *Parkinsons Dis.* (2016) 2016:6762528. doi: 10.1155/2016/6762528
10. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell* (2016) 167:1469–1480 e1412. doi: 10.1016/j.cell.2016.11.018
11. Choi JG, Kim N, Ju IG, Eo H, Lim SM, Jang SE, et al. Oral administration of Proteus mirabilis damages dopaminergic neurons and motor functions in mice. *Sci Rep.* (2018) 8:1275. doi: 10.1038/s41598-018-19646-x
12. Garrido-Gil P, Rodriguez-Perez AI, Dominguez-Meijide A, Guerra MJ, Labandeira-Garcia JL. Bidirectional Neural Interaction Between Central Dopaminergic and Gut Lesions in Parkinson's Disease Models. *Mol Neurobiol.* (2018). doi: 10.1007/s12035-018-0937-8
13. Sun MF, Zhu YL, Zhou ZL, Jia XB, Xu YD, Yang Q, et al. Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: gut microbiota, glial reaction and TLR4/TNF-alpha signaling pathway. *Brain Behav Immun.* (2018) 70:48–60. doi: 10.1016/j.bbi.2018.02.005
14. Braak H, de Vos RA, Bohl J, Del Tredici K. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neurosci Lett.* (2006) 396:67–72. doi: 10.1016/j.neulet.2005.11.012
15. Paillusson S, Clairembault T, Biraud M, Neunlist M, Derkinderen P. Activity-dependent secretion of alpha-synuclein by enteric neurons. *J Neurochem.* (2013) 125:512–7. doi: 10.1111/jnc.12131
16. Holmqvist S, Chutna O, Bousset L, Aldrin-Kirk P, Li W, Bjorklund T, et al. Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathol.* (2014) 128:805–20. doi: 10.1007/s00401-014-1343-6
17. Rey NL, Steiner JA, Maroof N, Luk KC, Madaj Z, Trojanowski JQ, et al. Widespread transneuronal propagation of alpha-synucleinopathy triggered in olfactory bulb mimics prodromal Parkinson's disease. *J Exp Med.* (2016) 213:1759–78. doi: 10.1084/jem.20160368
18. Dubow JS. Autonomic dysfunction in Parkinson's disease. *Dis Mon.* (2007) 53:265–74. doi: 10.1016/j.disamonth.2007.02.004
19. Park A, Stacy M. Non-motor symptoms in Parkinson's disease. *J Neurol.* (2009) 256(Suppl 3):293–8. doi: 10.1007/s00415-009-5240-1
20. Stacy M. Nonmotor symptoms in Parkinson's disease. *Int J Neurosci.* (2011) 121(Suppl 2):9–17. doi: 10.3109/00207454.2011.620196
21. Svensson E, Horvath-Puho E, Thomsen RW, Djurhuus JC, Pedersen L, Borghammer P, et al. Vagotomy and subsequent risk of Parkinson's disease. *Ann Neurol.* (2015) 78:522–9. doi: 10.1002/ana.24448
22. Forsyth CB, Shannon KM, Kordower JH, Voigt RM, Shaikh M, Jaglin JA, et al. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS ONE* (2011) 6:e28032. doi: 10.1371/journal.pone.0028032
23. Devos D, Lebouvier T, Lardeux B, Biraud M, Rouaud T, Pouclet H, et al. Colonic inflammation in Parkinson's disease. *Neurobiol Dis.* (2013) 50:42–8. doi: 10.1016/j.nbd.2012.09.007
24. Lin JC, Lin CS, Hsu CW, Lin CL, Kao CH. Association between Parkinson's disease and inflammatory bowel disease: a nationwide taiwanese retrospective cohort study. *Inflamm Bowel Dis.* (2016) 22:1049–55. doi: 10.1097/MIB.0000000000000735
25. Camacho-Soto A, Gross A, Searles Nielsen S, Dey N, Racette BA. Inflammatory bowel disease and risk of Parkinson's disease in Medicare beneficiaries. *Parkinsonism Relat Disord.* (2018) 50:23–8. doi: 10.1016/j.parkreldis.2018.02.008
26. Peter I, Dubinsky M, Bressman S, Park A, Lu C, Chen N, et al. Anti-tumor necrosis factor therapy and incidence of Parkinson disease among patients with inflammatory bowel disease. *JAMA Neurol.* (2018) 2018:605. doi: 10.1001/jamaneurol.2018.0605
27. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell* (2010) 140:918–34. doi: 10.1016/j.cell.2010.02.016
28. Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, et al. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia* (2007) 55:453–62. doi: 10.1002/glia.20467

29. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* (2013) 504:451–5. doi: 10.1038/nature12726
30. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* (2013) 341:569–73. doi: 10.1126/science.1241165
31. Kim CH, Park J, Kim M. Gut microbiota-derived short-chain Fatty acids, T cells, and inflammation. *Immune Netw.* (2014) 14:277–88. doi: 10.4110/in.2014.14.6.277
32. Park J, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, et al. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol.* (2015) 8:80–93. doi: 10.1038/mi.2014.44
33. Clemmensen C, Muller TD, Woods SC, Berthoud HR, Seeley RJ, Tschop MH. Gut-Brain Cross-Talk in Metabolic Control. *Cell* (2017) 168:758–74. doi: 10.1016/j.cell.2017.01.025
34. Horai R, Zarate-Blades CR, Dillenburg-Pilla P, Chen J, Kielczewski JL, Silver PB, et al. Microbiota-dependent activation of an autoreactive T cell receptor provokes autoimmunity in an immunologically privileged site. *Immunity* (2015) 43:343–53. doi: 10.1016/j.immuni.2015.07.014
35. Sanchez B, Hevia A, Gonzalez S, Margolles A. Interaction of Intestinal Microorganisms with the human host in the framework of autoimmune diseases. *Front Immunol.* (2015) 6:594. doi: 10.3389/fimmu.2015.00594
36. Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, et al. Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. *Science* (2000) 290:985–9. doi: 10.1126/science.290.5493.985
37. Benner EJ, Banerjee R, Reynolds AD, Sherman S, Pisarev VM, Tsiperson V, et al. Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. *PLoS ONE* (2008) 3:e1376. doi: 10.1371/journal.pone.0001376
38. Yu Z, Xu X, Xiang Z, Zhou J, Zhang Z, Hu C, et al. Nitrated alpha-synuclein induces the loss of dopaminergic neurons in the substantia nigra of rats. *PLoS ONE* (2010) 5:e9956. doi: 10.1371/journal.pone.0009956
39. Reynolds AD, Stone DK, Hutter JA, Benner EJ, Mosley RL, Gendelman HE. Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. *J Immunol.* (2010) 184:2261–71. doi: 10.4049/jimmunol.0901852
40. Christiansen JR, Olesen MN, Otzen DE, Romero-Ramos M, Sanchez-Guajardo V. alpha-Synuclein vaccination modulates regulatory T cell activation and microglia in the absence of brain pathology. *J Neuroinflamm.* (2016) 13:74. doi: 10.1186/s12974-016-0532-8
41. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize alpha-synuclein peptides. *Nature* (2017) 546:656–61. doi: 10.1038/nature22815
42. Reynolds AD, Glanzer JG, Kadiu I, Ricardo-Dukelow M, Chaudhuri A, Ciborowski P, et al. Nitrated alpha-synuclein-activated microglial profiling for Parkinson's disease. *J Neurochem.* (2008) 104:1504–25. doi: 10.1111/j.1471-4159.2007.05087.x
43. Reynolds AD, Kadiu I, Garg SK, Glanzer JG, Nordgren T, Ciborowski P, et al. Nitrated alpha-synuclein and microglial neuroregulatory activities. *J Neuroimmune Pharmacol.* (2008) 3:59–74. doi: 10.1007/s11481-008-9100-z
44. Reynolds AD, Stone DK, Mosley RL, Gendelman HE. Nitrated {alpha}-synuclein-induced alterations in microglial immunity are regulated by CD4+ T cell subsets. *J Immunol.* (2009) 182:4137–49. doi: 10.4049/jimmunol.0803982
45. Barcia C, Ros CM, Annese V, Gomez A, Ros-Bernal F, Aguado-Llera D, et al. IFN-gamma signaling, with the synergistic contribution of TNF-alpha, mediates cell specific microglial and astroglial activation in experimental models of Parkinson's disease. *Cell Death Dis.* (2012) 3:e379. doi: 10.1038/cddis.2012.123
46. Gonzalez H, Contreras F, Prado C, Elgueta D, Franz D, Bernales S, et al. Dopamine receptor D3 expressed on CD4+ T cells favors neurodegeneration of dopaminergic neurons during Parkinson's disease. *J Immunol.* (2013) 190:5048–56. doi: 10.4049/jimmunol.1203121
47. Gonzalez H, Elgueta D, Montoya A, Pacheco R. Neuroimmune regulation of microglial activity involved in neuroinflammation and neurodegenerative diseases. *J Neuroimmunol.* (2014) 274:1–13. doi: 10.1016/j.jneuroim.2014.07.012
48. Gonzalez H, Pacheco R. T-cell-mediated regulation of neuroinflammation involved in neurodegenerative diseases. *J Neuroinflamm.* (2014) 11:201. doi: 10.1186/s12974-014-0201-8
49. Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, et al. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest.* (2009) 119:182–92. doi: 10.1172/JCI36470
50. Olsen T, Rismo R, Cui G, Goll R, Christiansen I, Florholmen J. TH1 and TH17 interactions in untreated inflamed mucosa of inflammatory bowel disease, and their potential to mediate the inflammation. *Cytokine* (2011) 56:633–40. doi: 10.1016/j.cyto.2011.08.036S1043-4666(11)00716-2
51. Granlund A, Flatberg A, Ostvik AE, Drozdov I, Gustafsson BI, Kidd M, et al. Whole genome gene expression meta-analysis of inflammatory bowel disease colon mucosa demonstrates lack of major differences between Crohn's disease and ulcerative colitis. *PLoS ONE* (2013) 8:e56818. doi: 10.1371/journal.pone.0056818PONE-D-12-29699
52. Powrie F, Mason D. OX-22high CD4+ T cells induce wasting disease with multiple organ pathology: prevention by the OX-22low subset. *J Exp Med.* (1990) 172:1701–8. doi: 10.1084/jem.172.6.1701
53. Saruta M, Yu QT, Fleshner PR, Mantel PY, Schmidt-Weber CB, Banham AH, et al. Characterization of FOXP3+CD4+ regulatory T cells in Crohn's disease. *Clin Immunol.* (2007) 125:281–90. doi: 10.1016/j.clim.2007.08.003
54. Fang L, Pang Z, Shu W, Wu W, Sun M, Cong Y, et al. Anti-TNF therapy induces CD4+ T-cell production of IL-22 and promotes epithelial repairs in patients with crohn's disease. *Inflamm Bowel Dis.* (2018) 24:1733–44. doi: 10.1093/ibd/izy126
55. Herszenyi L, Tulassay Z. The role of autoantibodies in inflammatory bowel disease. *Dig Dis.* (2012) 30:201–7. doi: 10.1159/000336702
56. Zhou G, Song Y, Yang W, Guo Y, Fang L, Chen Y, et al. ASCA, ANCA, ALCA and many more: are they useful in the diagnosis of inflammatory bowel disease? *Dig Dis.* (2016) 34:90–7. doi: 10.1159/000442934
57. He Q, Wang L, Wang F, Li Q. Role of gut microbiota in a zebrafish model with chemically induced enterocolitis involving toll-like receptor signaling pathways. *Zebrafish* (2014) 11:255–64. doi: 10.1089/zeb.2013.0917
58. Hernandez-Chirlaque C, Aranda CJ, Ocon B, Capitan-Canadas F, Ortega-Gonzalez M, Carrero JJ, et al. Germ-free and antibiotic-treated mice are highly susceptible to epithelial injury in DSS colitis. *J Crohns Colitis* (2016) 2016:96. doi: 10.1093/ecco-jcc/jjw096
59. Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* (2008) 28:546–58. doi: 10.1016/j.immuni.2008.02.017S1074-7613(08)00113-1
60. Ostanin DV, Bao J, Koboziev I, Gray L, Robinson-Jackson SA, Kosloski-Davidson M, et al. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. *Am J Physiol Gastrointest Liver Physiol.* (2009) 296:G135–46. doi: 10.1152/ajpgi.90462.2008
61. Caggiu E, Paulus K, Arru G, Piredda R, Sechi GP, Sechi LA. Humoral cross reactivity between alpha-synuclein and herpes simplex-1 epitope in Parkinson's disease, a triggering role in the disease? *J Neuroimmunol.* (2016) 291:110–4. doi: 10.1016/j.jneuroim.2016.01.007
62. Caggiu E, Paulus K, Galleri G, Arru G, Manetti R, Sechi GP, et al. Homologous HSV1 and alpha-synuclein peptides stimulate a T cell response in Parkinson's disease. *J Neuroimmunol.* (2017) 310:26–31. doi: 10.1016/j.jneuroim.2017.06.004
63. Bu XL, Wang X, Xiang Y, Shen LL, Wang QH, Liu YH, et al. The association between infectious burden and Parkinson's disease: a case-control study. *Parkinsonism Relat Disord.* (2015) 21:877–81. doi: 10.1016/j.parkreldis.2015.05.015
64. Del Tredici K, Braak H. Review: Sporadic Parkinson's disease: development and distribution of alpha-synuclein pathology. *Neuropathol Appl Neurobiol.* (2016) 42:33–50. doi: 10.1111/nan.12298
65. Wang L, Magen I, Yuan PQ, Subramaniam SR, Richter F, Chesselet MF, et al. Mice overexpressing wild-type human alpha-synuclein display alterations in colonic myenteric ganglia and defecation. *Neurogastroenterol Motil.* (2012) 24:e425–36. doi: 10.1111/j.1365-2982.2012.01974.x

66. Bencsik A, Muselli L, Leboidre M, Lakhdar L, Baron T. Early and persistent expression of phosphorylated alpha-synuclein in the enteric nervous system of A53T mutant human alpha-synuclein transgenic mice. *J Neuropathol Exp Neurol.* (2014) 73:1144–51. doi: 10.1097/NEN.0000000000000137
67. Blesa J, Phani S, Jackson-Lewis V, Przedborski S. Classic and new animal models of Parkinson's disease. *J Biomed Biotechnol.* (2012) 2012:845618. doi: 10.1155/2012/845618
68. Naudet N, Antier E, Gaillard D, Morignat E, Lakhdar L, Baron T, et al. Oral Exposure to Paraquat Triggers Earlier Expression of Phosphorylated alpha-Synuclein in the Enteric Nervous System of A53T Mutant Human alpha-Synuclein Transgenic Mice. *J Neuropathol Exp Neurol.* (2017) 76:1046–57. doi: 10.1093/jnen/nlx092
69. Stolzenberg E, Berry D, Yang E, Lee Y, Kroemer A, Kaufman S, et al. A Role for neuronal alpha-synuclein in gastrointestinal immunity. *J Innate Immun.* (2017) 9:456–63. doi: 10.1159/000477990
70. Harms AS, Delic V, Thome AD, Bryant N, Liu Z, Chandra S, et al. alpha-Synuclein fibrils recruit peripheral immune cells in the rat brain prior to neurodegeneration. *Acta Neuropathol Commun.* (2017) 5:85. doi: 10.1186/s40478-017-0494-9
71. Rivollier A, He J, Kole A, Valatas V, Kelsall BL. Inflammation switches the differentiation program of Ly6Chi monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon. *J Exp Med.* (2012) 209:139–55. doi: 10.1084/jem.20101387
72. Kurmaeva E, Boktor M, Zhang S, Bao R, Berney S, Ostanin DV. Roles of T cell-associated L-selectin and beta7 integrins during induction and regulation of chronic colitis. *Inflamm Bowel Dis.* (2013) 19:2547–59. doi: 10.1097/MIB.0b013e3182a8df0a
73. Kustrimovic N, Comi C, Magistrelli L, Rasini E, Legnaro M, Bombelli R, et al. Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/T17 and Treg in drug-naive and drug-treated patients. *J Neuroinflamm.* (2018) 15:205. doi: 10.1186/s12974-018-1248-8
74. Chen S, Liu Y, Niu Y, Xu Y, Zhou Q, Xu X, et al. Increased abundance of myeloid-derived suppressor cells and Th17 cells in peripheral blood of newly-diagnosed Parkinson's disease patients. *Neurosci Lett.* (2017) 648:21–5. doi: 10.1016/j.neulet.2017.03.045
75. Dardalhon V, Korn T, Kuchroo VK, Anderson AC. Role of Th1 and Th17 cells in organ-specific autoimmunity. *J Autoimmun.* (2008) 31:252–6. doi: 10.1016/j.jaut.2008.04.017
76. Chen S, Le WD, Xie WJ, Alexianu ME, Engelhardt JI, Siklos L, et al. Experimental destruction of substantia nigra initiated by Parkinson disease immunoglobulins. *Arch Neurol.* (1998) 55:1075–80. doi: 10.1001/archneur.55.8.1075
77. Orr CF, Rowe DB, Mizuno Y, Mori H, Halliday GM. A possible role for humoral immunity in the pathogenesis of Parkinson's disease. *Brain* (2005) 128(Pt 11):2665–74. doi: 10.1093/brain/awh625
78. Lyte M. Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. *PLoS Pathog.* (2013) 9:e1003726. doi: 10.1371/journal.ppat.1003726
79. Cox LM, Weiner HL. Microbiota signaling pathways that influence neurologic disease. *Neurotherapeutics* (2018) 15:135–45. doi: 10.1007/s13311-017-0598-8
80. Cani PD. Human gut microbiome: hopes, threats and promises. *Gut* (2018) 2018:316723. doi: 10.1136/gutjnl-2018-316723
81. Chu F, Shi M, Lang Y, Shen D, Jin T, Zhu J, et al. Gut microbiota in multiple sclerosis and experimental autoimmune encephalomyelitis: current applications and future perspectives. *Mediators Inflamm.* (2018) 2018:8168717. doi: 10.1155/2018/8168717
82. Stefano GB, Pilonis N, Ptacek R, Raboch J, Vnukova M, Kream RM. Gut, microbiome, and brain regulatory axis: relevance to neurodegenerative and psychiatric disorders. *Cell Mol Neurobiol.* (2018) 2018:2. doi: 10.1007/s10571-018-0589-2
83. Pacheco R. Targeting dopamine receptor D3 signalling in inflammation. *Oncotarget* (2017) 8:7224–5. doi: 10.18632/oncotarget.14601
84. Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, Mulder I, Lan A, Bridonneau C, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* (2009) 31:677–89. doi: 10.1016/j.immuni.2009.08.020
85. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* (2009) 139:485–98. doi: 10.1016/j.cell.2009.09.033
86. Atarashi K, Tanoue T, Ando M, Kamada N, Nagano Y, Narushima S, et al. Th17 cell induction by adhesion of microbes to intestinal epithelial cells. *Cell* (2015) 163:367–80. doi: 10.1016/j.cell.2015.08.058
87. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity* (2010) 32:815–27. doi: 10.1016/j.immuni.2010.06.001
88. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA.* (2011) 108(Suppl 1):4615–22. doi: 10.1073/pnas.1000082107
89. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci USA.* (2010) 107:12204–9. doi: 10.1073/pnas.0909122107
90. Chesselet MF, Richter F, Zhu C, Magen I, Watson MB, Subramaniam SR. A progressive mouse model of Parkinson's disease: the Thy1-aSyn ("Line 61") mice. *Neurotherapeutics* (2012) 9:297–314. doi: 10.1007/s13311-012-0104-2
91. Magro F, Vieira-Coelho MA, Fraga S, Serrao MP, Veloso FT, Ribeiro T, et al. Impaired synthesis or cellular storage of norepinephrine, dopamine, and 5-hydroxytryptamine in human inflammatory bowel disease. *Dig Dis Sci.* (2002) 47:216–24. doi: 10.1023/A:1013256629600
92. Magro F, Fraga S, Ribeiro T, Soares-da-Silva P. Decreased availability of intestinal dopamine in transmural colitis may relate to inhibitory effects of interferon-gamma upon L-DOPA uptake. *Acta Physiol Scand.* (2004) 180:379–86. doi: 10.1111/j.1365-201X.2004.01260.x
93. Asano Y, Hiramoto T, Nishino R, Aiba Y, Kimura T, Yoshihara K, et al. Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. *Am J Physiol Gastrointest Liver Physiol.* (2012) 303:G1288–95. doi: 10.1152/ajpgi.00341.2012
94. Pacheco R, Contreras F, Zouali M. The dopaminergic system in autoimmune diseases. *Front Immunol.* (2014) 5:117. doi: 10.3389/fimmu.2014.00117
95. Clark A, Mach N. Exercise-induced stress behavior, gut-microbiota-brain axis and diet: a systematic review for athletes. *J Int Soc Sports Nutr.* (2016) 13:43. doi: 10.1186/s12970-016-0155-6
96. Cosentino M, Fietta AM, Ferrari M, Rasini E, Bombelli R, Carcano E, et al. Human CD4+CD25+ regulatory T cells selectively express tyrosine hydroxylase and contain endogenous catecholamines subserving an autocrine/paracrine inhibitory functional loop. *Blood* (2007) 109:632–42. doi: 10.1182/blood-2006-01-028423
97. Prado C, Contreras F, Gonzalez H, Diaz P, Elgueta D, Barrientos M, et al. Stimulation of dopamine receptor D5 expressed on dendritic cells potentiates Th17-mediated immunity. *J Immunol.* (2012) 188:3062–70. doi: 10.4049/jimmunol.1103096
98. Prado C, Bernales S, Pacheco R. Modulation of T-cell mediated immunity by dopamine receptor d5. *Endocr Metab Immune Disord Drug Targets* (2013) 13:184–94. doi: 10.2174/1871530311313020007
99. Abeliovich A, Gitler AD. Defects in trafficking bridge Parkinson's disease pathology and genetics. *Nature* (2016) 539:207–16. doi: 10.1038/nature20414
100. Pacheco R, Prado CE, Barrientos MJ, Bernales S. Role of dopamine in the physiology of T-cells and dendritic cells. *J Neuroimmunol.* (2009) 216:8–19. doi: 10.1016/j.jneuroim.2009.07.018
101. Franz D, Contreras F, Gonzalez H, Prado C, Elgueta D, Figueroa C, et al. Dopamine receptors D3 and D5 regulate CD4(+)T-cell activation and differentiation by modulating ERK activation and cAMP production. *J Neuroimmunol.* (2015) 284:18–29. doi: 10.1016/j.jneuroim.2015.05.003
102. Contreras F, Prado C, Gonzalez H, Franz D, Osorio-Barrios F, Osorio F, et al. Dopamine receptor D3 signaling on CD4+ T cells favors Th1- and Th17-mediated immunity. *J Immunol.* (2016) 196:4143–9. doi: 10.4049/jimmunol.1502420
103. Elgueta D, Aymerich MS, Contreras F, Montoya A, Celorrio M, Rojo-Bustamante E, et al. Pharmacologic antagonism of dopamine receptor D3 attenuates neurodegeneration and motor impairment in a mouse model of Parkinson's disease. *Neuropharmacology* (2017) 113(Pt A):110–123. doi: 10.1016/j.neuropharm.2016.09.028
104. Besser MJ, Ganor Y, Levite M. Dopamine by itself activates either D2, D3 or D1/D5 dopaminergic receptors in normal human T-cells and triggers

the selective secretion of either IL-10, TNFalpha or both. *J Neuroimmunol.* (2005) 169:161–71. doi: 10.1016/j.jneuroim.2005.07.013
105. Miyazawa T, Matsumoto M, Kato S, Takeuchi K. Dopamine-induced protection against indomethacin-evoked intestinal lesions in rats–role of anti-intestinal motility mediated by D2 receptors. *Med Sci Monit.* (2003) 9:BR71–77. Available online at: https://www.medscimonit.com/download/index/idArt/4766
106. Magro F, Cunha E, Araujo F, Meireles E, Pereira P, Dinis-Ribeiro M, et al. Dopamine D2 receptor polymorphisms in inflammatory bowel disease and the refractory response to treatment. *Dig Dis Sci.* (2006) 51:2039–44. doi: 10.1007/s10620-006-9168-3
107. Wu W, Sun M, Zhang HP, Chen T, Wu R, Liu C, et al. Prolactin mediates psychological stress-induced dysfunction of regulatory T cells to facilitate intestinal inflammation. *Gut* (2014) 63:1883–92. doi: 10.1136/gutjnl-2013-306083
108. Koh A, De Vadder F, Kovatcheva-Datchary P, Backhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* (2016) 165:1332–45. doi: 10.1016/j.cell.2016.05.041
109. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med.* (2014) 20:159–66. doi: 10.1038/nm.3444
110. Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, Shi H, et al. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* (2014) 40:128–39. doi: 10.1016/j.immuni.2013.12.007
111. Waldecker M, Kautenburger T, Daumann H, Busch C, Schrenk D. Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. *J Nutr Biochem* (2008) 19:587–93. doi: 10.1016/j.jnutbio.2007.08.002
112. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* (2011) 469:543–7. doi: 10.1038/nature09646
113. Zhou D, Pan Q, Liu XL, Yang RX, Chen YW, Liu C, et al. *Clostridium butyricum* B1 alleviates high-fat diet-induced steatohepatitis in mice via enterohepatic immunoregulation. *J Gastroenterol Hepatol.* (2017) 32:1640–8. doi: 10.1111/jgh.13742
114. Unger MM, Spiegel J, Dillmann KU, Grundmann D, Philippeit H, Burmann J, et al. Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinsonism Relat Disord.* (2016) 32:66–72. doi: 10.1016/j.parkreldis.2016.08.019
115. Michel L, Prat A. One more role for the gut: microbiota and blood brain barrier. *Ann Transl Med.* (2016) 4:15. doi: 10.3978/j.issn.2305-5839.2015.10.16
116. Hoyles L, Snelling T, Umlai UK, Nicholson JK, Carding SR, Glen RC, et al. *Microbiome*-host systems interactions: protective effects of propionate upon the blood-brain barrier. *Microbiome* (2018) 6:55. doi: 10.1186/s40168-018-0439-y
117. Sanchez-Guajardo V, Febbraro F, Kirik D, Romero-Ramos M. Microglia acquire distinct activation profiles depending on the degree of alpha-synuclein neuropathology in a rAAV based model of Parkinson's disease. *PLoS ONE* (2010) 5:e8784. doi: 10.1371/journal.pone.0008784
118. Harms AS, Cao S, Rowse AL, Thome AD, Li X, Mangieri LR, et al. MHCII is required for alpha-synuclein-induced activation of microglia, CD4 T cell proliferation, and dopaminergic neurodegeneration. *J Neurosci.* (2013) 33:9592–600. doi: 10.1523/JNEUROSCI.5610-12.2013
119. Kaufman JF, Auffray C, Korman AJ, Shackelford DA, Strominger J. The class II molecules of the human and murine major histocompatibility complex. *Cell* (1984) 36:1–13. doi: 10.1016/0092-8674(84)90068-0
120. Lampe JB, Gossrau G, Herting B, Kempe A, Sommer U, Fussel M, et al. HLA typing and Parkinson's disease. *Eur Neurol.* (2003) 50:64–8. doi: 10.1159/000072500
121. Sun C, Wei L, Luo F, Li Y, Li J, Zhu F, et al. HLA-DRB1 alleles are associated with the susceptibility to sporadic Parkinson's disease in Chinese Han population. *PLoS ONE* (2012) 7:e48594. doi: 10.1371/journal.pone.0048594
122. Manan H, Angham AM, Sitelbanat A. Genetic and diabetic auto-antibody markers in Saudi children with type 1 diabetes. *Hum Immunol.* (2010) 71:1238–42. doi: 10.1016/j.humimm.2010.09.008
123. de la Concha EG, Cavanillas ML, Cenit MC, Urcelay E, Arroyo R, Fernandez O, et al. DRB1*03:01 haplotypes: differential contribution to multiple sclerosis risk and specific association with the presence of intrathecal IgM bands. *PLoS ONE* (2012) 7:e31018. doi: 10.1371/journal.pone.0031018
124. Winklhofer KF, Haass C. Mitochondrial dysfunction in Parkinson's disease. *Biochim Biophys Acta* (2010) 1802:29–44. doi: 10.1016/j.bbadis.2009.08.013
125. Berer K, Gerdes LA, Cekanaviciute E, Jia X, Xiao L, Xia Z, et al. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. *Proc Natl Acad Sci USA.* (2017) 114:10719–24. doi: 10.1073/pnas.1711233114
126. Berer K, Krishnamoorthy G. Microbial view of central nervous system autoimmunity. *FEBS Lett.* (2014) 588:4207–13. doi: 10.1016/j.febslet.2014.04.007
127. Petermann F, Rothhammer V, Claussen MC, Haas JD, Blanco LR, Heink S, et al. gammadelta T cells enhance autoimmunity by restraining regulatory T cell responses via an interleukin-23-dependent mechanism. *Immunity* (2010) 33:351–63. doi: 10.1016/j.immuni.2010.08.013
128. Codarri L, Gyulveszi G, Tosevski V, Hesske L, Fontana A, Magnenat L, et al. RORgammat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat Immunol.* (2011) 12:560–7. doi: 10.1038/ni.2027
129. Ren M, Han M, Wei X, Guo Y, Shi H, Zhang X, et al. FTY720 Attenuates 6-OHDA-associated dopaminergic degeneration in cellular and mouse parkinsonian models. *Neurochem Res.* (2017) 42:686–96. doi: 10.1007/s11064-016-2125-4
130. Zhao P, Yang X, Yang L, Li M, Wood K, Liu Q, et al. Neuroprotective effects of fingolimod in mouse models of Parkinson's disease. *FASEB J.* (2017) 31:172–9. doi: 10.1096/fj.201600751R
131. Motyl J, Przykaza L, Boguszewski PM, Kosson P, Strosznajder JB. Pramipexole and Fingolimod exert neuroprotection in a mouse model of Parkinson's disease by activation of sphingosine kinase 1 and Akt kinase. *Neuropharmacology* (2018) 135:139–50. doi: 10.1016/j.neuropharm.2018.02.023

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 19 February 2019
doi: 10.3389/fneur.2019.00122

Inflammation, Infectious Triggers, and Parkinson's Disease

*Elisa Caggiu[1], Giannina Arru[1], Sepideh Hosseini[1], Magdalena Niegowska[1], GianPietro Sechi[2], Ignazio Roberto Zarbo[2] and Leonardo A. Sechi[1]**

[1] Microbiology Section, Department of Biomedical Sciences, University of Sassari, Sassari, Italy, [2] Department of Clinical, Surgical and Experimental Medicine, Neurological Clinic, University of Sassari, Sassari, Italy

Parkinson's disease is a neurodegenerative disorder characterized by progressive loss of dopaminergic neurons of the substantia nigra pars compacta with a reduction of dopamine concentration in the striatum. The complex interaction between genetic and environmental factors seems to play a role in determining susceptibility to PD and may explain the heterogeneity observed in clinical presentations. The exact etiology is not yet clear, but different possible causes have been identified. Inflammation has been increasingly studied as part of the pathophysiology of neurodegenerative diseases, corroborating the hypothesis that the immune system may be the nexus between environmental and genetic factors, and the abnormal immune function can lead to disease. In this review we report the different aspects of inflammation and immune system in Parkinson's disease, with particular interest in the possible role played by immune dysfunctions in PD, with focus on autoimmunity and processes involving infectious agents as a trigger and alpha-synuclein protein (α-syn).

Keywords: Parkinson's disease, neurodegenerative disease, neuroinflammation, immune system, alpha-synuclein, autoimmunity, microglia activation, autoantibodies

OPEN ACCESS

Edited by:
Cristoforo Comi, Università degli Studi del Piemonte Orientale, Italy

Reviewed by:
Corinne Lasmezas, The Scripps Research Institute, United States
Jill Baird, VA Portland Health Care System, United States

****Correspondence:***
Leonardo A. Sechi
sechila@uniss.it

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *02 August 2018*
Accepted: *29 January 2019*
Published: *19 February 2019*

Citation:
Caggiu E, Arru G, Hosseini S, Niegowska M, Sechi G, Zarbo IR and Sechi LA (2019) Inflammation, Infectious Triggers, and Parkinson's Disease. Front. Neurol. 10:122. doi: 10.3389/fneur.2019.00122

INTRODUCTION

Parkinson's disease (PD) is a common disorder of the central nervous system (CNS) which determines postural instability, bradykinesia, resting tremor and muscle rigidity. The reduction of dopamine concentration in the striatum is related to the progressive death of neurons located on the substantia nigra pars compacta (SNpc) (1). Although many theories attempted to explain the causes of neuronal death in this region and to identify possible triggers, the exact PD etiology remains unknown. A growing body of evidence indicates that the nervous and immune systems act in synergy and maintain extensive communication (2–5). This interplay seems to underlie neuroinflammation which, apart from PD, is a constant feature of numerous neurodegenerative diseases such as Alzheimer's disease, dementia with Lewy bodies, amyotrophic lateral sclerosis, frontotemporal dementia or Huntington's disease (6) and may have multiple causes, including deficient regulation of immune responses associated with age advancement, infectious agents (bacteria or viruses), exotoxins (e.g., pesticides or MPTP), or deposition of insoluble protein fibrils (e.g., alpha-synuclein). In light of hypotheses seeing inflammation as the basis of neurodegenerative processes, dysfunction of the immune systems adds to the list of other PD contributors linking genetic mutations and environmental factors (**Figure 1**).

In this review, we aim at analyzing different aspects of inflammation and the immune system in PD providing a brief summary about the general characteristics of inflammatory responses with focus on a potential role of alpha-synuclein (α-syn), then moving forward to the analysis of innate

immunity through an overview of microglial activity, and finally describing roles of the adaptive cell-mediated immunity in the disease. In addition, the hypothesis of PD as an autoimmune dysfunction is also discussed.

INFLAMMATION IN PD

Already in 1988 McGeer's research team suggested that inflammation could be the first pathogenic mechanism of PD (7). At the same time, it has been observed that the use of non-steroidal anti-inflammatory drugs (NSAID) decreases the risk of PD, and this could be considered as a proof of inflammogenic characteristics of the disease (8). While neuronal death has been described as evidence of the ongoing CNS inflammation (9), several scientific reports documented microglial activation, cytokine production and the presence of autoantibodies univocally indicating inflammatory processes in PD (10–13). *In vitro* assays employing a dopaminergic neuron model showed some membrane proteins to be targeted by antibodies present in CFS of affected patients (14). A research performed on post-mortem excised brains revealed higher concentrations of cytokines and proapototic proteins in the striatum and cerebrospinal fluid (CSF) of PD patients compared to levels found in healthy controls, pointing at inflammation as a constant element of the disease (15). Through a further immunohistological study, McGeer et al. discovered several alterations in striatal microglial cells of patients with PD that appeared to be activated by an increased synthesis of proinflammatory cytokines (16). Nonetheless, it remains to be explained whether inflammation represents the first cause determining neurodegeneration or if it results from a selective damage process and cell degeneration.

Anthropogenic pollutants account for a significant part of neurotoxic agents. It's enough to think about 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as the most striking example followed by certain pesticides released to the environment. MPTP, which may be accidentally produced during the manufacture of the analgesic opioid drug desmethylprodine (MPPP), causes irreversible neuronal damage and parkinsonian syndromes. Autopsies executed on subjects previously exposed to MPTP showed the activation of microglia persisting for even 16 years (17). These results provided a further evidence that an initial neuronal damage may lead to a neuroinflammatory process and have been confirmed by studies conducted on animal models, several of which demonstrated the ability of MPTP (18), rotenone insecticide (19, 20), and 6-hydroxydopamine (6-OHDA) (21) to activate microglial cells. In the same way, death of dopaminergic neurons has been observed both *in vitro* and *in vivo* after stimulation of microglia with lipopolysaccharides (LPS) (22–27).

ALPHA-SYNUCLEIN AND NEUROINFLAMMATION IN PD

A-syn is a soluble protein highly conserved among vertebrates, with α-helical lipid-binding motif common to all synucleins.

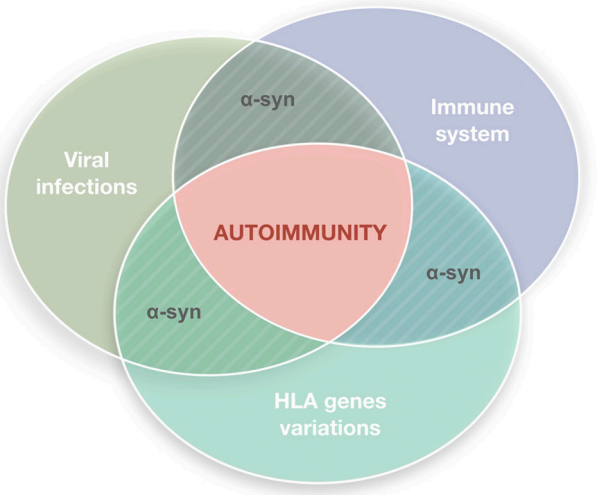


FIGURE 1 | Autoimmune dysfunction in the etiology of Parkinson's disease (PD). The etiology of PD is multifactorial. It has been hypothesized that inflammation may underly the neurodegenerative process, with the immune system playing a key role. Viral infections are plausible triggers able to stimulate the immune system in genetically susceptible individuals inducing reactions that lead to autoimmune responses.

Even though the physiological role of α-syn is not well understood, it is known to carry out crucial functions in synaptic plasticity (28) and in the release of neurotransmitters and synaptic vesicles (29, 30), thereby in regulating synaptic transmission through the stabilization of the SNARE protein complex, whose assembly and disassembly is essential for a correct membrane fusion on neuron terminals (30, 31). Consequently, α-syn is a key protein in the pathogenesis of PD. Although the scientific literature provides countless studies often yielding promising results, the reasons behind the accumulation of α-syn along with its causal role in neurodegeneration are still unresolved. However, it is ascertained that a higher expression of wild-type protein leads to formation of α-syn inclusions in neurons followed by cellular damage (32, 33).

According to post-mortem histological examinations of PD patients, alteration and aggregation of α-syn have been suggested to occur as an epiphenomenon probably mediated by other conditions, such as neuroinflammation (34). It has also been hypothesized that secreted extracellular α-syn can immediately activate glial cells and subsequently induce neuronal inflammation. Glial cells are able to capture and degrade α-syn masses in an effective way similar to neurons (35). The activation of microglia could encourage the production of some protective molecules including brain-derived neurotrophic factor (BDNF) but also proinflammatory cytokines, reactive oxygen and nitrogen species (36) which favor the progression of this neurodegenerative disease. In a study on murine models, Harms et al. observed the recruitment of peripheral innate immune cells such as monocytes and macrophage induced by injection of α-syn fibrils into the SNpc (37).

Additionally, the authors found that the activation of MHC-II is as a primary step preceding the neurodegenerative process. Wild type α-syn is prone to post-translational nitrate modifications which enhance its propensity to aggregate (38). Moreover, nitrated α-syn, not recognized as a self-protein, can indirectly stimulate the maturation of harmful subsets of T helper lymphocytes capable of eliciting profound neural damages (39).

The maintenance of a perfect balance in the homeostasis of extracellular α-syn is essential for the wellbeing of the brain. Recently, a possible role of α-syn as a natural antimicrobial peptide (AMP) has been outlined. AMPs belong to an ancient family of proteins able to generate oligomers and fibrils similar to α-syn and constitute the first line of defense against pathogens acting as potent broad-spectrum antibiotics and immunomodulators (40). The expression of AMPs has not been confined to the brain but detected also in other tissues where the intervention of the adaptive immune system is limited (41). However, when dysregulated, the protective action of AMPs may lead to various toxic effects (42, 43). Some authors highlighted that α-syn exhibits antibacterial activity against *Escherichia coli* and *Staphylococcus aureus,* antifungal activity against pathogenic strains such as *Aspergillus flavus*, *Aspergillus fumigatus* and *Rhizoctonia solani*, and antiviral activity against West Nile Virus (WNV) (44, 45).

The alterations of bidirectional signaling within the gut-brain axis has been intensely studied in the context of the CNS inflammation involving microbial agents. Recently, *Proteus mirabilis* commonly overrepresented in the gut microbiota of PD mouse models has been shown to significantly induce motor deficits, to selectively cause dopaminergic neuronal damage and inflammation in substantia nigra and striatum, and to stimulate α-syn aggregation in the brains and colons of PD mice (46). The degree of acute and chronic inflammation in the intestinal wall has been positively correlated with the expression of α-syn in the enteric neurites of the upper gastrointestinal tract in pediatric patients (47).

The role of viral infections in diverging signaling pathways which regulate the establishment of innate immunity, such as those including proinflammatory molecules and DNA sensing, has been long hypothesized in PD pathogenesis. Herpes simplex virus 1 (HSV-1) encodes a ubiquitin-specific protease (UL36USP) which subverts type I IFN-mediated signaling, in particular IFN-β-induced signaling, independently from its deubiquitinase (DUB) activity (48). HSV-1 UL24 has the ability to inhibit the activation of IFN-β and interleukin-6 (IL-6) promoters mediated by cyclic GMP-AMP synthase (cGAS)—a newly identified foreign DNA sensor, and the interferon-stimulatory DNA-mediated IFN-β and IL-6 production during HSV-1 infection. Moreover, UL24 was shown to selectively block nuclear factor κB (NF-κB) without altering IFN-regulatory factor 3 promoter activation (49).

Chronic neuroinflammation flanked by production of cytokines probably doesn't represent the initiating event of PD but, if lasting, this phenomenon could lead to disease progression through the involvement of microglia and astrocytes. It has been observed that cytokines such as TNF and IFN-γ have a high affinity to dopaminergic neurons (50, 51). In the CNS, these cytokines are mostly produced by microglia that could induce dopaminergic neurons with higher sensitivity (52). Several studies confirmed that PD patients display higher concentrations of TGF-β, IL-1β, IL-6, IFN-γ, and IL-1 in their CSF and striatum than the healthy controls (51, 53, 54). Similarly, a direct correlation between the raised levels of peripheral inflammatory cytokines and the degree of disability has been observed (55). According to a genetic screening for polymorphisms of DNA encoding proinflammatory cytokines such as IL-6, iNOS, IL-1β, and IL-1α (as shown in **Figure 2**), elevated quantities of these molecular mediators increase the risk of developing PD (56, 57). Schröder et al. (58) in their work reported increased levels of IL-2, IL-6, and TNFα and of the monocyte chemoattractant protein 1 (MCP-1) in the CSF of the PD patients whereas no differences were found in sera, confirming previous work (59).

INNATE IMMUNITY IN PD: MICROGLIA ACTIVATION

Microglial cells are the principal actors of innate immunity in the CNS responsible for the protection and restoration of neurons (60). They can be activated by various external or internal insults such as neuronal dysfunction, trauma or certain toxin. Also, a wide range of molecules including viral or bacterial proteins, α-syn, cytokines and antibodies are able to induce the activation of microglia (61). Consequently, microglial cells produce different molecular mediators (e.g., reactive oxygen species, prostanoids and cytokines) with chemotactic and immunomodulatory functions. One of them is tumor necrosis factor (TNF) which in PD plays important roles contributing to the regulation of synaptic plasticity (62–64). PD brains are characterized by the presence of HLA-DR^{+} microglial cells and raised levels of CD68, an activation marker for microglia and macrophages, having a direct relation with α-syn aggregations and the duration of disease (7, 65). Moreover, an increased expression of MHC-II molecules in microglial cells has been observed in chronic neuroinflammation but not in the CNS of healthy subjects (66). Individuals with single nucleotide polymorphism (SNPs) at MCH-II locus are prone to develop PD, which indirectly proves the importance of adaptive immunity in these patients (67).

Microglia can be activated by numerous factors such as α-syn aggregates, neuromelanin, MMP-3, fibrinogen or environmental LPS toxins, MPTP, pesticides (rotenone, paraquat), proteasome and heavy metals, leading ultimately to neuroinflammation, and destruction of dopaminergic neurons (68). Studies employing positron emission tomography (PET) confirmed this phenomenon to occur in PD (7, 61, 69).

The activation of microglia and astrocytes by viruses has been shown to involve DNA-dependent activator of IFN regulatory factor (DAI) which specifically acts as an intracellular sensor for DNA viruses. DAI and its effector molecules are constitutively expressed in microgl cells and astrocytes with upregulation following viral challenge. In a DAI knockdown murine model,

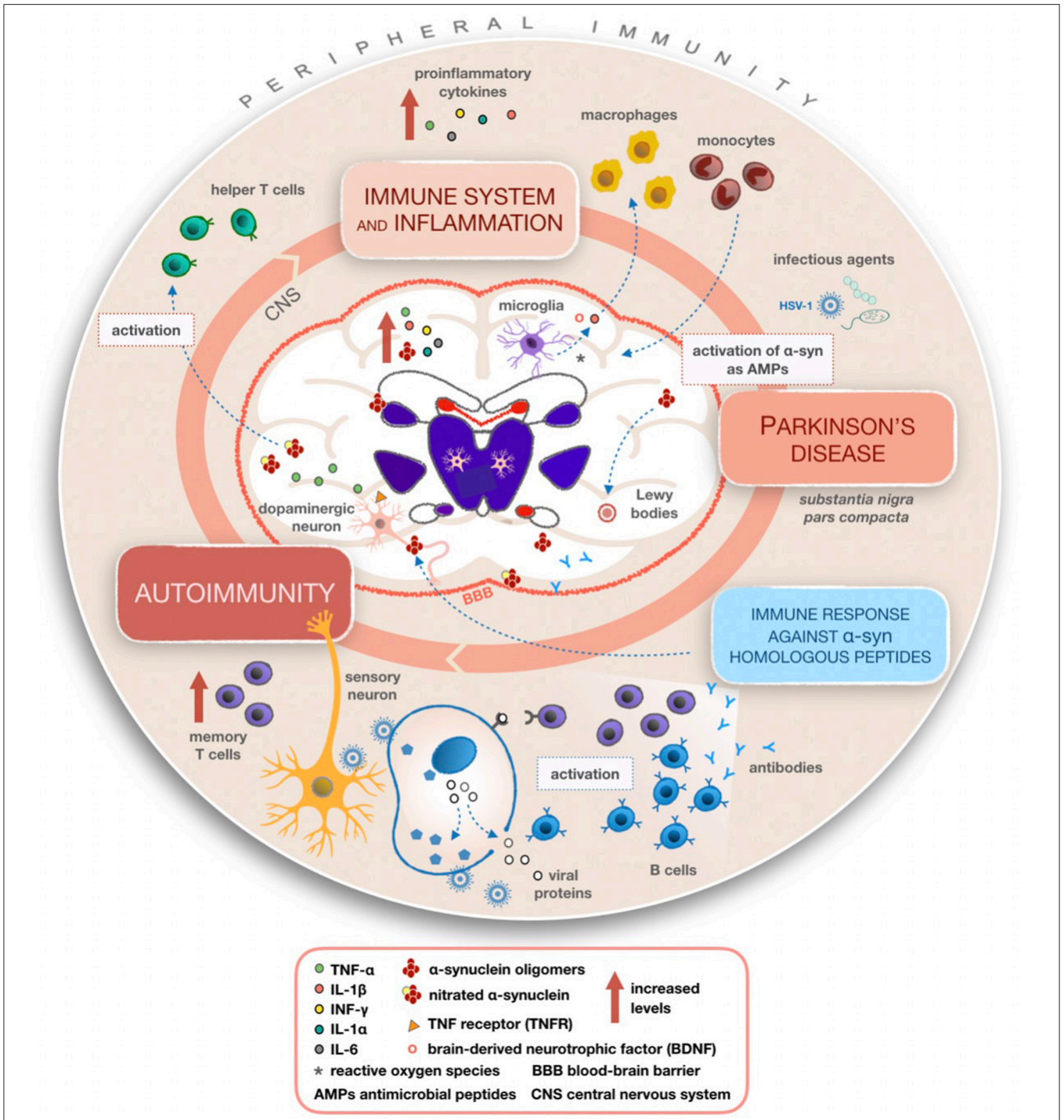


FIGURE 2 | Mechanisms summarizing the involvement of inflammatory and immune processes in Parkinson's disease (PD). Once activated, microglial cells produce cytokines able to recruit macrophages and monocytes from peripheral compartments to the CNS, leading to altered peripheral immunity and various inflammatory processes within the CNS in PD patients. A possible mechanism of action giving rise to autoimmunity involves the reactivation of latent HSV-1 on infected sensory neurons and production of antibodies targeting alpha-synuclein (α-syn) fragments homologous to viral proteins. It is plausible that α-syn acting as an AMP becomes dysregulated during recurring infections with its consequent accumulation in the CNS.

the release and production of neurotoxic mediators by HSV-1 challenged microglia and astrocytes was significantly attenuated. These findings suggest that DAI-mediated pathways may be crucial in the mechanisms of innate immunity activated against potentially lethal inflammation associated with neurotropic DNA virus infection (70).

ADAPTIVE IMMUNITY: ACTIVATION OF CELL-MEDIATED AND HUMORAL IMMUNITY IN PD

The adaptive immune system shows specific responses against foreign antigens activating different T or B lymphocytes (71). The surveillance of homeostasis in the CNS is guaranteed by naïve and memory T cells (72, 73). T cell infiltration has been discovered in post-mortem brain sections of PD patients (74). The analysis of T cell subsets in peripheral blood mononuclear cells (PBMC) of affected patients showed altered immune responses and a decrease in the overall number of lymphocytes, but not in their frequency (75, 76). What is more, PD presents a particular immunological profile unseen in other neurological diseases (OND), where increased numbers of memory T cells and a reduced quantity of naïve T cells have been registered (77). As well, low $CD4^+$:$CD8^+$ ratio and a shift to more IFN-γ^- vs. IL-4-producing T cells have suggested the presence of cytotoxic T cell responses in PD patients (**Figure 2**) (75, 76, 78).

While a few specific proteins such as β-fibrinogen and transaldolase have been identified as possible biomarkers within T cells (79), it has been recorded that $CD8^+$ subsets of PD subjects express Vβ8 receptors at lower frequency than healthy people (80). Moreover, several pathogenic alterations have been found in peripheral blood lymphocytes (PBL) of PD patients, for instance the presence of gaps in the DNA structure of lymphocytes and oxidation in purine b, high level of apoptosis, Cu/Zn superoxide dismutase activity, and the presence of micronuclei (81, 82). Interestingly, DNA damage has significantly declined after treatment with levodopa (83). Research on the overexpression of human α-syn through a recombinant adeno-associated virus vector serotype 2 (AVV2-SYN) system in SNpc of a murine model showed the infiltration of B and T cells alongside the activation of microglia suggesting that α-syn can recall the cells of adaptive immunity and stimulate inflammation (84). Recently, an important reduction in the number of T and B lymphocytes in mice knocked out for α-syn compared to wild type animals has been observed (85). A multiparameter flow cytometry analysis in patients with PD revealed a strong phenotypical shift of intrathecal monocytes and an elevated percentage of activated T lymphocytes coupled with an increase of proinflammatory cytokines in the CFS of PD patients (58).

Recently, Sulzer et al. (86) published a seminal work reporting selected peptides derived from two regions of α-syn which were highly recognized by specific T cell sets in PD patients. This response was predominantly mediated by IL-4 or IFNγ-producing $CD4^+$ T cells, with likely contributions from CD8+/IFNγ producing T cells. Moreover, both α-syn epitopes originating from the natural processing of extracellular native α-syn present in blood and the fibrilized α-syn associated with PD triggered T cell responses. These epitopes were displayed by two MHC class II beta chain alleles, DRB5*01:01 and DRB1*15:01, associated with PD and by others not specific to PD (α-syn is not endogenously expressed by MHC class II expressing cells). The authors concluded that around 40% of the PD patients displayed immune responses to α-syn epitopes which may reflect varying trends in disease progression or impact from environmental factors.

Humoral immunity plays an important role in the etiopathogenesis of PD and many other neurodegenerative diseases. Given a reduction in the number of B cells as a frequent condition in PD patients (75, 87), it has been suggested that the proliferation of lymphocytes might be influenced by levodopa treatment, however some studies did not confirm such a correlation (76, 78). On the other hand, PD patients bear elevated levels of antibodies against dopamine (DA) neurons in comparison to healthy subjects (14, 88) while further investigations showed higher concentrations of antibodies targeting several peptides of α-syn and their homologs derived from HSV-1. It has been hypothesized that, in genetically predisposed individuals, previous HSV-1 infections may induce the production of autoantibodies through the molecular mimicry mechanism (13). Neurohistological studies disclosed the presence of immunoglobulins near dopaminergic neurons in the brains of patients with PD (89) which indicates a possible interaction between microglia and B lymphocytes. Finally, research on mouse models transfected with AVV-α-syn vector showed a significant deposition of IgG in the midbrain, suggesting humoral immunity to exert a remarkable function in the process of neurodegeneration in PD (84).

AUTOIMMUNITY IN PD

Environmental agents and the exposure to vectors (people, animals) may increase the risk of developing PD through transmission of viral infections or bacterial toxins. A case-control study conducted on a large number of PD patients proved a strong association between the disease and previous severe influenza, whereas an inverse association was observed regarding childhood infections, in particular red measles. Furthermore, an occupational exposure to domesticated animals increased the risk of PD (90). Viral infections most likely are not the primary cause but may act as triggers inducing the attack by the immune system against the CNS, dopaminergic neurons in particular. Numerous infectious agents are able to overcome the blood-brain barrier (BBB) and elicit inflammatory processes of the brain parenchyma, such as encephalitis. It is currently known that HSV-1 is one of the etiological agents responsible for sporadic viral encephalitis that often brings to neurological deficit in surviving patients. In murine models, HSV-1 determined a persistent viral lithic gene expression in ependyma during latency determining a chronic inflammatory response that the memory T cells were unable to counteract (91). Other studies in rodents showed that the H5N1 avian influenza virus passed the BBB inducing neurological signs, while a viral infection determined phosphorylation and aggregation of α-syn along with a substantial loss of dopaminergic neurons (92). An analogous study underlines that the highly pathogenic CA/09 H1N1 subtype was able to undermine microglial activation even without reaching the CNS (93). It is therefore conceivable that infectious agents do not act directly causing neuronal damage but, through secondary mechanisms such as the activation of the

immune system trigger reactions leading to typical PD lesions. Other authors documented that people infected with hepatitis C virus (HCV) had a 30% greater likelihood of developing PD than healthy subjects (94). Similarly, a possible association between herpes simplex virus type 1 (HSV-1) infections and PD as higher antibody titers against HSV-1 were observed in the serum of PD patients but not in negative controls (95–97). This trend has been further confirmed through studies employing the micro-indirect hemagglutination (IHA) technique (98), however no increased production of antibodies against HSV-1 was observed in the CSF of PD when compared to controls (97, 98). The hypothesis that some viral triggers are related to the occurrence of CNS disorders such as Alzheimer's disease (AD) or PD has been further confirmed by investigations conducted *in vivo* (99, 100) and *in vitro* (101). The authors demonstrated that in cultured mouse cortical neurons, HSV-1 infection reduced the expression of synaptic proteins along with synaptic transmission through activation of glycogen synthase kinase (GSK)-3 and intracellular accumulation of amyloid beta protein (Aβ) determining synaptic dysfunctions which underlies cognitive impairment in AD. The above-mentioned findings have paved the way for a new branch of research aimed at unraveling the role of autoimmunity in PD and its implication in the loss of dopaminergic neurons typical to this pathology. Many efforts have been made in defining the extent to which autoimmunity is triggered by environmental variables, e.g., infective agents, metals, or other sources of inflammation. Cebrian and co-authors reported that human catecholaminergic substantia nigra and locus coeruleus neurons express MHC-I, therefore they may present antigens in response to exogenous agents and be particularly susceptible to T cell-mediated cytotoxic attack (102).

The importance of HSV-1 infection in triggering autoimmunity of PD has been further highlighted in connection with the mechanism of molecular mimicry and an immunologic cross-reactivity between HSV-1 and human α-syn leading in turn to the destruction of dopaminergic neurons of the substantia nigra (13). This study showed that the level of antibodies against HSV-1 peptides in PD patients was statistically higher than in healthy volunteers; the same trend was seen against human α-syn peptides homologous to viral epitopes. Similarly, molecular mimicry has been observed between a repeat region in the C-terminal half of the latent membrane protein 1 (LMP1) of Epstein-Barr virus (EBV) and the C-terminal region of α-syn. The authors hypothesized that antibodies directed against LMP1 present in genetically susceptible individuals cross-react with the homologous epitope on α-syn inducing its oligomerization (103).

A possible implication of HSV-1 in autoimmunity has been evaluated through another study conducted using the intracellular cytokine (ICC) method which showed that, alongside an alteration of cell patterns, the percentages of CD3, CD4, CD8, and CD56 lymphocytes were lower in PD patients compared to healthy subjects (87). The same authors reported the result of flow cytometry analysis which illustrates that human α-syn peptides and their HSV-1 homologs could remarkably induce the production of NK, CD4, CD8, and cells producing TNF-α in PD patients (87). The two homologous epitopes similarly stimulated T cell responses in a strongly correlated fashion. In addition, the immunogenic properties of these peptides were seen in cells secreting TNF-α which may play an important role in the pathogenesis of PD (87). In other studies, TNF-α exerted an effect on the plasticity of dopaminergic neurons which are particularly susceptible to this proinflammatory cytokine. The ligation of TNF-α with its receptors (TNFRs) is known to cause neuronal death under certain circumstances (62–64).

Further investigation confirmed the presence of autoimmune processes in PD without, however, indicating the triggering agents (86). Blood flow cytometry analysis performed in order to see how T cells respond against different α-syn portions showed a strong response against two specific peptides of this protein, namely Y39 and S129, in PD patients. In parallel, a relation between T cell responses and HLA risk alleles demonstrated that the main responses against α-syn epitope Y39 were expressed by four specific risk alleles. This study asserts the hypothesis that α-syn may activate T cell responses implicated in cell-mediated immunity, particularly autoimmunity, of PD.

A similar scenario is observed in the experimental autoimmune encephalitis model of multiple sclerosis (MS), as myelin proteins used to produce autoimmunity are not endogenous to MHC class II expressing cells but are accumulated and processed for MHC class II to be displayed by antigen presenting cells and microglia. In other autoimmune disorders, MHC class II response may precede MHC class I response (104). Moreover, as in T1D which features epitopes derived from both preproinsulin and additional proteins, it is plausible that PD-related epitopes derived from α-syn and supplementary peptides including molecules of infectious origin may be characterized by sequence homology (105). T cell responses in MS and T1D were shown to recognize self epitopes homologous to antigens from infectious microrganisms associated with the diseases. In MS, epitopes of EBV and *Mycobacterium avium* subsp. *paratuberculosis* homologous to IRF5 induced both humoral and cellular immune responses (106, 107).

It remains ambiguous whether autoimmunity is the primary cause or a consequence of the neurodegenerative process during progression of the disease. A substantial body of data suggest the possibility that autoimmunity may have an important role in the pathogenesis of PD and, if confirmed, a considerable revolution in terms of diagnostic and therapeutic approaches (e.g., immunotherapies and using T cells as biomarkers) should be expected in the near future.

AUTHOR CONTRIBUTIONS

EC, GA, and MN conceived the study and wrote the manuscript. SH performed bibliographic search. GS and IZ read the manuscript. LS conceived, organized the study, and critically reviewed the manuscript.

FUNDING

The study was supported by Micromap project and funds of LS.

REFERENCES

1. Kalia LV, Lang AE. Parkinson's disease. *Lancet*. (2015) 386:896–912. doi: 10.1016/S0140-6736(14)61393-3
2. Amor S, Woodroofe MN. Innate and adaptive immune responses in neurodegeneration and repair. *Immunology* (2014) 141:287–91. doi: 10.1111/imm.12134
3. Cardoso V, Chesné J, Ribeiro H, Garcia-Cassani B, Carvalho T, Bouchery T, et al. Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. *Nature* (2017) 549:277–81. doi: 10.1038/nature23469
4. Kipnis, J. Multifaceted interactions between adaptive immunity and the central nervous system. *Science* (2016) 353:766–71. doi: 10.1126/science.aag2638
5. Steinman L. Elaborate interactions between the immune and nervous systems. *Nat Immunol*. (2004) 5:575–81. doi: 10.1038/ni1078
6. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. *Nat Rev Immunol.* (2014) 14:463–77. doi: 10.1038/nri3705
7. McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* (1988) 38:1285–91.
8. Chen H, Zhang SM, Hernan MA, Schwarzschild MA, Willett WC, Colditz GA, et al. Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease. *Arch Neurol.* (2003) 60:1059–64. doi: 10.1001/archneur.60.8.1059
9. Orr CF, Rowe DB, Halliday GM. An inflammatory review of Parkinson's disease. *Prog Neurobiol.* (2002) 68:325–40. doi: 10.1016/S0301-0082(02)00127-2
10. Mogi M, Harada M, Kondo T, Riederer P, Inagaki H, Minami M, et al. Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from Parkinsonian patients. *Neurosci Lett*. (1994) 180:147–50. doi: 10.1016/0304-3940(94)90508-8
11. Müller T, Blum-Degen D, Przuntek H, Kuhn W. Interleukin-6 levels in cerebrospinal fluid inversely correlate to severity of Parkinson's disease. *Acta Neurol Scand.* (1998) 98:142–44.
12. McGeer PL, Itagaki S, McGeer EG. Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. *Acta Neuropathol*. (1988) 76:550–7.
13. Caggiu E, Paulus K, Arru G, Piredda R, Sechi GP, Sechi LA. Humoral cross reactivity between a α-synuclein and Herpes simplex-1 epitope in Parkinson's disease, a triggering role in the disease? *J Neuroimmunol.* (2016) 291:110–14. doi: 10.1016/j.jneuroim.2016.01.007
14. Carvey PM, McRae A, Lint TF, Ptak LR, Lo ES, Goetz CG, et al. The potential use of a dopamine neuron antibody and a striatal-derived neurotrophic factor as diagnostic markers in Parkinson's disease. *Neurology.* (1991) 41:53–8; discussion: 59–60.
15. Nagatsu T, Mogi M, Ichinose H, Togari A. Cytokines in Parkinson's disease. *J Neural Transm* (2000) 58:143–51.
16. McGeer PL, McGeer EG. Inflammation and neurodegeneration in Parkinson's disease. *Parkinsonism Relat. Disord.* (2004) 10:S3–7. doi: 10.1016/j.parkreldis.2004.01.005
17. Langston JW, Forno LS, Tetrud J, Reeves AG, Kaplan JA, Karluk D. Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine exposure. *Ann Neurol.* (1999) 46:598–605. doi: 10.1002/1531-8249(199910)46:4<598::AID-ANA7>3.0.CO;2-F
18. Członkowska A, Kohutnicka M, Kurkowska-Jastrzebska I, Członkowski A. Microglial reaction in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced Parkinson's disease mice model. *Neurodegeneration* (1996) 5:137–43.
19. Gao HM, Hong JS, Zhang W, Liu B. Distinct role for microglia in rotenone-induced degeneration of dopaminergic neurons. *J Neurosci.* (2002) 22:782–90. doi: 10.1523/JNEUROSCI.22-03-00782.2002
20. Sherer TB, Betarbet R, Greenamyre JT. Environment, mitochondria, and Parkinson's disease. *Neuroscientist* (2002) 8:192–7. doi: 10.1177/1073858402008003004
21. Cicchetti F, Brownell AL, Williams K, Chen YI, Livni E, Isacson O. Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging. *Eur J Neurosci.* (2002) 15:991–8. doi: 10.1046/j.1460-9568.2002.01938.x
22. Arimoto T, Bing G. Up-regulation of inducible nitric oxide synthase in the substantia nigra by liposaccharide causes microglial activation and neurodegeneration. *Neurobiol Dis.* (2003) 12:35–45. doi: 10.1016/S0969-9961(02)00017-7
23. Castrano A, Hererra AJ, Cano J, Machado A. Lipopolysaccharide intranigral injection induces inflammatory reaction damage in nigrostriatal dopaminergic system. *J Neurochem.* (1998) 70:1584–92. doi: 10.1046/j.1471-4159.1998.70041584.x
24. Gao HM, Jiang J, Wilson B, Zhang W, Hong JS, Liu B. Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. *J Neurochem.* (2002) 81:1285–97. doi: 10.1046/j.1471-4159.2002.00928.x
25. Gayle DA, Ling Z, Tong C, Landers T, Lipton JW, Carvey PM. Lipopolysacchride (LPS)-induced dopamine cell loss in culture: roles of tumor necrosis factor-alpha, interleukin-1beta, and nitric oxide. *Brain Res Dev.* (2002) 133:27–35. doi: 10.1016/S0165-3806(01)00315-7
26. Hererra AJ, Castano A, Venero JL, Cano J, Machado A. The single intranigral injection of LPS as a new model for studying the selective effects of inflammatory reactions on dopaminergic system. *Neuorobiol Dis.* (2000) 7:429–47. doi: 10.1006/nbdi.2000.0289
27. Liu Y, Qin L, Li G, Zhang W, An L, Liu B, et al. Dextromethorplan protects dopaminergic neurons against inflammation-mediated degeneration through inhibition of microglial activation. *J Pharmacol Exp Ther.* (2003) 305:212–18. doi: 10.1124/jpet.102.043166
28. Watson JB, Hatami A, David H, Masliah E, Roberts K, Evans CE, et al. Alterations in corticostriatal synaptic plasticity in mice overexpressing human α-synuclein. *Neuroscience* (2009) 159: 501–13. doi: 10.1016/j.neuroscience.2009.01.021
29. Chandra S, Gallardo G, Fernández-Chacón R, Schlüter OM, Südhof TC. α-Synuclein cooperates with CSPα in preventing neurodegeneration. *Cell* (2005) 123:383–96. doi: 10.1016/j.cell.2005.09.028
30. Burré J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Südhof TC. α-Synuclein promotes SNARE-complex assembly *in vivo* and *in vitro*. *Science* (2010) 329:1663–67. doi: 10.1126/science.1195227
31. Murphy DD, Rueter SM, Trojanowski JQ, Lee VM. Synucleins are developmentally expressed, and alphasynuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. *J Neurosci.* (2000) 20:3214–20. doi: 10.1523/JNEUROSCI.20-09-03214.2000
32. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* (1997) 276:2045–47. doi: 10.1126/science.276.5321.2045
33. Tagliaferro L, Chiba-Falek O. Up-regulation of SNCA gene expression: implications to synucleinopathies. *Neurogenetics* (2016) 17:145–57. doi: 10.1007/s10048-016-0478-0
34. Hirsch EC, Hunot S. Neuroinflammation in Parkinson's disease: a target for neuroprotection? *Lancet Neurol.* (2009) 8:382–97. doi: 10.1016/S1353-8020(11)70065-7
35. Lee HJ, Suk JE, Bae EJ, Lee SJ. Clearance and deposition of extracellular alpha-synuclein aggregates in microglia. *Biochem Biophys Res Commun.* (2008) 372:423–28. doi: 10.1016/j.bbrc.2008.05.045
36. Phani S, Loike JD, Przedborski S. Neurodegeneration and inflammation in Parkinson's disease. *Parkinsonism Relat Disord*. (2012). 18:207–9. doi: 10.1016/S1353-8020(11)70064-5
37. Harms AS, Delic V, Thome AD, Bryant N, Liu Z, Chandra S, et al. α-Synuclein fibrils recruit peripheral immune cells in the rat brain prior to neurodegeneration. *Acta Neuropathol Commun.* (2017) 5:85. doi: 10.1186/s40478-017-0494-9
38. Uversky VN, Yamin G, Munishkina LA, Karymov MA, Millett IS, Doniach S. et al. Effects of nitration on the structure and aggregation of alpha-synuclein. *Brain Res Mol Brain Res.* (2005) 134:84–102. doi: 10.1016/j.molbrainres.2004.11.014
39. Benner EJ, Banerjee R, Reynolds AD, Sherman S, Pisarev VM, Tsiperson V. Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. *PLoS ONE* (2008) 3:e1376. doi: 10.1371/journal.pone.0001376

40. Wiesner J, Vilcinskas A. Antimicrobial peptides: the ancient arm of the human immune system. *Virulence* (2010) 1:440–64. doi: 10.4161/viru.1.5.12983
41. Skovronsky DM, Wang J, Lee VM, Doms RW. Quantifying Aβ (1-40) and Aβ (1-42) using sandwich-ELISA. *Methods Mol Med.* (2000). 32:79–89. doi: 10.1385/1-59259-195-7:79
42. Yamaguchi Y, Nagase T, Tomita T, Nakamura K, Fukuhara S, Amano T, et al. Beta-defensin overexpression induces progressive muscle degeneration in mice. *Am J Physiol Cell Physiol.* (2007) 292:C2141–9. doi: 10.1152/ajpcell.00295.2006
43. Paulsen F, Pufe T, Conradi L, Varoga D, Tsokos M, Papendieck J, et al. Antimicrobial peptides are expressed and produced in healthy and inflamed human synovial membranes. *J Pathol.* (2002) 198:369–77. doi: 10.1002/path.1224
44. Park SC, Moon JC, Shin SY, Son H, Jung YJ, Kim NH, et al. Functional characterization of alpha-synuclein protein with antimicrobial activity. *Biochem Biophys Res Commun.* (2016) 478:924–8. doi: 10.1016/j.bbrc.2016.08.052
45. Beatman EL, Massey A, Shives KD, Burrack KS, Chamanian M, Morrison TE, et al. Alpha-synuclein expression restricts RNA viral infections in the brain. *J Virol.* (2015) 90:2767–82. doi: 10.1128/JVI.02949-15
46. Choi JG, Kim N, Ju IG, Eo H, Lim SM, Jang SE, et al. Oral administration of *Proteus mirabilis* damages dopaminergic neurons and motor functions in mice. *Sci Rep.* (2018) 8:1275. doi: 10.1038/s41598-018-19646-x
47. Stolzenberg E, Berry D, Yang Lee EY, Kroemer A, Kaufman S, Wong GCL, et al. A role for neuronal alpha-synuclein in gastrointestinal immunity. *J Innate Immun.* (2017) 9:456–63. doi: 10.1159/000477990
48. Yuan H, You J, You H, Zheng C. Herpes Simplex Virus 1 UL36USP antagonizes type I interferon-mediated antiviral innate immunity. *J Virol.* (2018) 92:e01161-18. doi: 10.1128/JVI.01161-18
49. Xu H, Su C, Pearson A, Mody CH, Zheng C. Herpes Simplex Virus 1 UL24 abrogates the DNA sensing signal pathway by inhibiting NF-κB activation. *J Virol.* (2017) 91:JVI.00025-17. doi: 10.1128/JVI.00025-17
50. McGuire SO, Ling ZD, Lipton JW, Sortwell CE, Collier TJ, Carvey PM. Tumor necrosis factor alpha is toxic to embryonic mesencephalic dopamine neurons. *Exp Neurol.* (2001) 169:219–30. doi: 10.1006/exnr.2001.7688
51. Mount MP, Lira A, Grimes D, Smith PD, Faucher S, Slack R, et al. Involvement of interferon gamma in microglial-mediated loss of dopaminergic neurons. *J Neurosci.* (2007) 27:3328–37. doi: 10.1523/JNEUROSCI.5321-06.2007
52. Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* (1990) 39:151–70. doi: 10.1016/0306-4522(90)90229-W
53. Blum-Degen D, Müller T, Kuhn W, Gerlach M, Przuntek H, Riederer P. Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and *de novo* Parkinson's disease patients. *Neurosci Lett.* (1995) 202:17–20.
54. Mogi M, Harada M, Kondo T, Riederer P, Nagatsu T. Brain beta 2-microglobulin levels are elevated in the striatum in Parkinson's disease. *J Neural Transm Park Dis Dement Sect.* (1995) 9:87–92.
55. Koziorowski D, Tomasiuk R, Szlufi KS, Friedman A. Inflammatory cytokines and NT-proCNP in Parkinson's disease patients. *Cytokine* (2012) 60:762–6. doi: 10.1016/j.cyto.2012.07.030
56. Schulte T, Schols L, Muller T, Woitalla D, Berger K, Kruger R. Polymorphisms in the interleukin- 1 alpha and beta genes and the risk for Parkinson's disease. *Neurosci Lett.* (2002) 326:70–2. doi: 10.1016/S0304-3940(02)00301-4
57. Hakansson A, Westberg L, Nilsson S, Buervenich S, Carmine A, Holmberg B, et al. Interaction of polymorphisms in the genes encoding interleukin-6 and estrogen receptor beta on the susceptibility to Parkinson's disease. *Am J Med Genet B Neuropsychiatr Genet.* (2005) 133B:88–92. doi: 10.1002/ajmg.b.30136
58. Schröder JB, Pawlowski M, Meyer zu Horste G, Gross CC, Wiendl H, Meuth SG, et al. Immune cell activation in the cerebrospinal fluid of patients with Parkinson's disease. *Front. Neurol.* (2018) 9:1081. doi: 10.3389/fneur.2018.01081
59. Jiménez-Jiménez FJ, Alonso-Navarro H, García-Martín E, Agúndez JAG. Cerebrospinal fluid biochemical studies in patients with Parkinson's disease: toward a potential search for biomarkers for this disease. *Front Cell Neurosci.* (2014) 8:369. doi: 10.3389/fncel.2014.00369
60. Ransohoff RM, Brown MA. Innate immunity in the central nervous system. *J Clin Invest.* (2012) 122:1164–71. doi: 10.1172/JCI58644
61. Croisier E, Moran LB, Dexter DT, Pearce RK, Graeber MB. Microglial inflammation in the Parkinsonian substantia nigra: relationship to alpha-synuclein deposition. *J Neuroinflammation.* (2005) 2:14. doi: 10.1186/1742-2094-2-14
62. Aloisi F. Immune function of microglia. *Glia* (2001) 36:165–79. doi: 10.1002/glia.1106
63. Marin-Teva JL, Cuadros MA, Martin-Oliva D, Navascues J. Microglia and neuronal cell death. *Neuron Glia Biol.* (2011) 7:25–40. doi: 10.1017/S1740925X12000014
64. Stellwagen D, Malenka RC. Synaptic scaling mediated by glial TNF-alpha. *Nature* (2006) 440:1054–59. doi: 10.1038/nature04671
65. SSanchez-Guajardo V, Barnum CJ, Tansey MG, Romero-Ramos M. Neuroimmunological processes in Parkinson's disease and their relation to α-synuclein: microglia as the referee between neuronal processes and peripheral immunity. *ASN Neuro.* (2013) 5:113–39. doi: 10.1042/AN20120066
66. Getts MT, Miller SD. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: triggering of autoimmune diseases by infections. *Clin Ex Immunol.* (2010) 160:15–21. doi: 10.1111/j.1365-2249.2010.04132.x
67. Hamza TH, Zabetian CP, Tenesa A, Laederach A, Montimurro J, Yearout D, et al. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nat Genet.* (2010) 42:781–85. doi: 10.1038/ng.642
68. Mahdavi T. Inflammation in Parkinson's disease. In: *Scientific and Clinical Aspects.* Thomas M, editor. Springer (2014). p. 105–26.
69. Gerhard A, Pavese N, Hotton G, Turkheimer F, Es M, Hammers A, et al. *In vivo* imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol Dis.* (2006) 21:404–12. doi: 10.1016/j.nbd.2005.08.002
70. Furr SR, Chauhan VS, Moerdyk-Schauwecker MJ, Marriott I. A role for DNA-dependent activator of interferon regulatory factor in the recognition of Herpes Simplex Virus type 1 by glial cells. *J Neuroinflammation* (2011) 8:99. doi: 10.1186/1742-2094-8-99
71. Krogsgaard M, Davis MM. How T cells 'see' antigen. *Nat Immunol.* (2005) 26:239–45. doi: 10.1038/ni1173
72. Cose S, Brammer C, Khanna KM, Masopust D, Lefrancois L. Evidence that a significant number of naive T cells enter non-lymphoid organs as part of a normal migratory pathway. *Eur J Immunol.* (2006) 36:1423–33. doi: 10.1002/eji.200535539
73. Van der Most RG, Murali-Krishna K, Ahmed R. Prolonged presence of effector-memory CD8 T cells in the central nervous system after dengue virus encephalitis. *Int Immunol.* (2003) 15:119–25. doi: 10.1093/intimm/dxg009
74. Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, et al. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest.* (2009) 119:182–92. doi: 10.1172/JCI36470
75. Stevens CH, Rowe D, Morel-Kopp MC, Orr C, Russell T, Ranola M, et al. Reduced T helper and B lymphocytes in Parkinson's disease. *J Neuroimmunol.* (2012) 252:95–99. doi: 10.1016/j.jneuroim.2012.07.015
76. Bas J, Calopa M, Mestre M, Mollevi DG, Cutillas B, Ambrosio S, et al. Lymphocyte populations in Parkinson's disease and in rat models of Parkinsonism. *J Neuroimmunol.* (2001) 113:146–52. doi: 10.1016/S0165-5728(00)00422-7
77. Fiszer U, Mix E, Fredrikson S, Kostulas V, Link H. Parkinson's disease and immunological abnormalities: increase of HLA-DR expression on monocytes in cerebrospinal fluid and of CD45RO + T cells in peripheral blood. *Acta Neurol Scand.* (1994) 90:160–66.
78. Baba Y, Kuroiwa A, Uitti RJ, Wszolek ZK, Yamada T. Alterations of T-lymphocyte populations in Parkinson disease. *Parkinsonism Relat Disord.* (2005) 11:493–98. doi: 10.1016/j.parkreldis.2005.07.005
79. Alberio T, Pippione AC, Zibetti M, Olgiati S, Cecconi D, Comi C, et al. Discovery and verification of panels of T-lymphocyte proteins as biomarkers of Parkinson's disease. *Sci Rep.* (2012) 2:953. doi: 10.1038/srep00953

80. Fiszer U, Fredrikson S, Mix E, Olsson T, Link H. V region T cell receptor repertoire in Parkinson's disease. *Acta Neurol Scand.* (1996) 93:25–9.
81. Migliore L, Petrozzi L, Lucetti C, Gambaccini G, Bernardini S, Scarpato R, et al. Oxidative damage and cytogenetic analysis in leukocytes of Parkinson's disease patients. *Neurology* (2002) 58:1809–15. doi: 10.1212/WNL.58.12.1809
82. Blandini F, Mangiagalli A, Cosentino M, Marino F, Samuele A, Rasini E, et al. Peripheral markers of apoptosis in Parkinson's disease: the effect of dopaminergic drugs. *Ann N Y Acad Sci.* (2003) 1010:675–8. doi: 10.1196/annals.1299.123
83. Cornetta T, Palma S, Aprile I, Padua L, Tonali P, Testa A, et al. Levodopa therapy reduces DNA damage in peripheral blood cells of patients with Parkinson's disease. *Cell Biol Toxicol.* (2009) 25:321–30. doi: 10.1007/s10565-008-9086-6
84. Theodore S, Cao S, McLean PJ, Standaert DG. Targeted overexpression of human alpha- synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. *J Neuropathol Exp Neurol.* (2008) 67:1149–58. doi: 10.1097/NEN.0b013e31818e5e99
85. Shameli A, Xiao W, Zheng Y, Shyu S, Sumodi J, Meyerson HJ, et al. A critical role for alpha-synuclein in development and function of T lymphocytes. *Immunobiology* (2016) 221:333–40. doi: 10.1016/j.imbio.2015.10.002
86. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize α-synuclein peptides. *Nature* (2017). 546:656–61. doi: 10.1038/nature22815
87. Caggiu E, Paulus K, Galleri G, Arru G, Manetti R, Sechi GP, et al. Homologous HSV1 and Alpha- synuclein peptides stimulate a T cell response in Parkinson's disease. *J Neuroimmunol.* (2017) 310:26–31. doi: 10.1016/j.jneuroim.2017.06.004
88. Han M, Nagele E, DeMarshall C, Acharya N, Nagele R. Diagnosis of Parkinson's disease based on disease-specific autoantibody profiles in human sera. *PLoS ONE* (2012) 7:e32383. doi: 10.1371/journal.pone.0032383
89. Orr CF, Rowe DB, Mizuno Y, Mori H, Halliday GM. A possible role for humoral immunity in the pathogenesis of Parkinson's disease. *Brain* (2005) 128:2665–74. doi: 10.1093/brain/awh625
90. Harris MA, Tsui JK, Marion SA, Shen H, Teschke K. Association of Parkinson's disease with infections and occupational exposure to possible vectors. *Mov Disord.* (2012) 27:1111–7. doi: 10.1002/mds.25077
91. Menendez CM, Jinkins JK, Carr DJ. Resident T cells are unable to control Herpes Simplex Virus-1 activity in the brain ependymal region during latency. *J Immunol.* (2016) 197:1262–75. doi: 10.4049/jimmunol.1600207
92. Jang H, Boltz D, Sturm-Ramirez K, Shepherd KR, Jiao Y, Webster R, et al. Highly pathogenic H5N1 influenza virus can enter the central nervous system and induce neuroinflammation and neurodegeneration. *Proc Natl Acad Sci USA.* (2009) 106:14063–68. doi: 10.1073/pnas.0900096106
93. Sadasivan S, Zanin M, O'Brien K, Schultz-Cherry S, Smeyne RJ. Induction of microglia activation after infection with the non-neurotropic A/CA/04/2009 H1N1 influenza virus. *PLoS ONE* (2015) 10:e0124047. doi: 10.1371/journal.pone.0124047
94. Tsai HH, Liou HH, Muo CH, Lee CZ, Yen RF, Kao CH. Hepatitis C virus infection as a risk factor for Parkinson disease: a nationwide cohort study. *Neurology* (2015) 86:840–6. doi: 10.1212/WNL.0000000000002307
95. Marttila RJ, Arstila P, Nikoskelainen J, Halonen PE, Rinne UK. Viral antibodies in the sera from patients with Parkinson disease. *Eur Neurol.* (1977)15:25–33.
96. Marttila RJ, Rinne UK. Herpes Simplex Virus antibodies in patients with Parkinson's disease. *J Neurol Sci.* (1978) 35:375–9.
97. Marttila RJ, Rinne UK, Tiilikainen A. Virus antibodies in Parkinson's disease. Herpes simplex and measles virus antibodies in serum and CSF and their relation to HLA types. *J Neurol Sci.* (1982) 54:227–38. doi: 10.1016/0022-510X(82)90184-8
98. Marttila RJ, Rinne UK, Halonen P, Madden DL, Sever JL. Herpes viruses and parkinsonism. Herpes Simplex Virus types 1 and 2, and cytomegalovirus antibodies in serum and CSF. *Arch Neurol.* (1981) 38:19–21.
99. Bu, X.L, Wang X, Xiang Y, Shen LL, Wang QH, Liu YH, et al. The association between infectious burden and Parkinson's disease: a case-control study. *Parkinsonism Relat Disord.* (2015) 21: 877–81. doi: 10.1016/j.parkreldis.2015.05.015
100. Vlajinac H, Dzoljic E, Maksimovic J, Marinkovic J, Sipetic S, Kostic, V. Infections as a risk factor for Parkinson's disease: a case-control study. *Int J Neurosci.* (2013) 123:329–32. doi: 10.3109/00207454.2012.760560
101. Piacentini R, Li Puma DD, Ripoli C, Marcocci ME, De Chiara G, Garaci E, et al. Herpes Simplex Virus type-1 infection induces synaptic dysfunction in cultured cortical neurons via GSK-3 activation and intraneuronal amyloid-β protein accumulation. *Sci Rep.* (2015) 5:15444. doi: 10.1038/srep 15444
102. Cebrian C, Zucca FA, Mauri P, Steinbeck JA, Studer L, Scherzer CR, et al. MHC-I expression renders catecholaminergic neurons susceptible to T-cell-mediated degeneration. *Nat. Commun.* (2014) 10:1038. doi: 10.1038/ncomms4633
103. Woulfe JM, Gray MT, Gray DA, Munoz DG, Middeldorp JM. Hypothesis: a role for EBV-induced molecular mimicry in Parkinson's disease. *Parkinsonism Relat Disord.* (2014) 20:685–94. doi: 10.1016/j.parkreldis.2014.02.031
104. Marrack P, Kappler JW. Do MHCII-presented neoantigens drive type 1 diabetes and other autoimmune diseases? *Cold Spring Harb Perspect Med.* (2012) 2:a007765. doi: 10.1101/cshperspect.a007765
105. Niegowska M, Rapini N, Piccinini S, Mameli G, Caggiu E, Manca Bitti ML, et al. Type 1 Diabetes at-risk children highly recognize *Mycobacterium avium* subspecies paratuberculosis epitopes homologous to human Znt8 and Proinsulin. *Sci Rep.* (2016) 6:22266. doi: 10.1038/srep22266
106. Cossu D, Mameli G, Galleri G, Cocco E, Masala S, Frau J, et al. Human interferon regulatory factor 5 homologous epitopes of Epstein-Barr Virus and Mycobacterium avium subsp. paratuberculosis induce a specific humoral and cellular immune response in multiple sclerosis patients. *Mult Scler.* (2015) 21:984–95. doi: 10.1177/13524585145 57304
107. Mameli G, Cocco E, Frau J, Marrosu MG, Sechi LA. Epstein Barr Virus and *Mycobacterium avium* subsp. paratuberculosis peptides are recognized in sera and cerebrospinal fluid of MS patients. *Sci Rep.* (2016) 6:22401. doi: 10.1038/srep22401

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 19 March 2019
doi: 10.3389/fneur.2019.00232

Peripheral-Central Neuroimmune Crosstalk in Parkinson's Disease: What Do Patients and Animal Models Tell Us?

Marie Therese Fuzzati-Armentero, Silvia Cerri and Fabio Blandini*

Laboratory of Cellular and Molecular Neurobiology, IRCCS Mondino Foundation, Pavia, Italy

The brain is no longer considered an immune privileged organ and neuroinflammation has long been associated with Parkinson's disease. Accumulating evidence demonstrates that innate and adaptive responses take place in the CNS. The extent to which peripheral immune alterations impacts on the CNS, or vice and versa, is, however, still a matter of debate. Gaining a better knowledge of the molecular and cellular immune dysfunctions present in these two compartments and clarifying their mutual interactions is a fundamental step in understanding and preventing Parkinson's disease (PD) pathogenesis. This review provides an overview of the current knowledge on inflammatory processes evidenced both in PD patients and in toxin-induced animal models of the disease. It discusses differences and similarities between human and animal studies in the context of neuroinflammation and immune responses and how they have guided therapeutic strategies to slow down disease progression. Future longitudinal studies are necessary and can help gain a better understanding on peripheral-central nervous system crosstalk to improve therapeutic strategies for PD.

Keywords: inflammasomes, cytokine, toxin-induced models, LRRK2, lymphocytes, blood-brain barrier, MPTP, 6-OHDA

OPEN ACCESS

Edited by:
Rodrigo Pacheco,
Fundación Ciencia and Vida, Chile

Reviewed by:
Marina Romero-Ramos,
Aarhus University, Denmark
Rishein Gupta,
University of Texas at San Antonio, United States

****Correspondence:***
Marie Therese Fuzzati-Armentero
m.fuzzati@franceparkinson.fr

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *08 October 2018*
Accepted: *22 February 2019*
Published: *19 March 2019*

Citation:
Fuzzati-Armentero MT, Cerri S and Blandini F (2019) Peripheral-Central Neuroimmune Crosstalk in Parkinson's Disease: What Do Patients and Animal Models Tell Us? Front. Neurol. 10:232. doi: 10.3389/fneur.2019.00232

INTRODUCTION

Innate and adaptive immunity are crucial for the survival of all organisms (1). The innate system as we know it today is the result of a long evolutionary period. Innate immunity promotes inflammation as an immediate, non-specific response to infection, insults and/or biological stressors through a limited set of germline-encoded receptors expressed on specialized cells: macrophages, dendritic cells, natural killer cells, or neutrophils (2). While it does not provide long-lasting immunity it is pivotal to the overall immune response. Adaptive immunity possibly developed and evolved as a complementary plug-in system to strengthen innate immunity in complex organisms (3). Adaptive immunity requires activation of the innate system and subsequent antigen presentation to adaptive immune cells and is based on unlimited somatic diversification of receptors present on lymphoid cells and their selective expansion to match pathogens (4). The adaptive immunity repertoire is specific for each individual, shaped by individual life history, and is the foundation of a strong memory response that allows rapid reaction to repeated infections. Combination of innate and adaptive immunity is required for the comprehensive immune protection observed in humans.

The capacities of the central nervous system (CNS), recalling those provided by the adaptive immune system, evolves in response to each individual's life experience. In the timeline of evolution, the CNS has developed amidst the innate and adaptive immune systems, combining characteristics to allow the appearance and development of increasingly more complex organisms. Throughout evolution the CNS and the two arms of the immune system have co-evolved through constant crosstalk and communication, persistently improving their ability to respond and adapt to the environment. Today, the brain is no longer considered an immune privileged organ. Innate and adaptive responses take place in the CNS (5), and peripheral immune alterations can impact on the CNS. Gaining a better knowledge of the molecular and cellular immune dysfunctions present in these two compartments, clarifying and understanding their mutual interactions represents a fundamental step in the development of alternative therapeutic strategies for neurodegenerative diseases, including Parkinson's disease (PD). PD is not considered an immune disease, but it is now widely accepted that inflammation and neuroinflammation are important players in the etiology and/or the progression of the disease; the triad, inflammation, neuroinflammation, and neurodegeneration, likely intervening in a vicious, each one sustaining the other. A possible role of viral infection in PD etiology has been extensively addressed but is still a matter of debate (6).

Here we will review some inflammatory processes that take place both in the central and peripheral compartments. In particular, we will look at communication barriers that limit passage of information, as well as the regulation of inflammatory markers and cells that may transit from the periphery to the brain, and vice e versa. We will summarize data on the inflammasome, an important player in both inflammatory and neuro-inflammatory processes, which has gained considerable attention in the past decade and that may represent a key factor in peripheral-central neuro-immune crosstalk. Due to space limitations, we will not review the fundamental importance of alpha-synuclein in immune and neuro-immune processes and crosstalk but will sometimes introduce it where appropriate. The subject is complex and deserves a space on its own to be properly addressed and has been reviewed elsewhere (7–10). Finally, we will consider the importance of LRRK2, a major genetic risk factor for developing PD that is expressed by both neurons and immune cells. For each point we will present and compare data obtained from human and animal studies and underline how they converge to help us improve our understanding of immune crosstalk.

NEUROINFLAMMATION AND MICROGLIA IN PD

The CNS, long considered an immune-privileged organ, has developed a tightly regulated immune reactivity (11). We now know that insults, such as endogenous danger signals or pathogens, can trigger an immune response in the CNS. Neuroinflammation is the combined response of immune cells present in the brain, including microglia, astrocytes, infiltrating lymphocytes as well as inflammatory factors. Microglial cells and microgliosis seem to play a particular important function in the initiation of neuroinflammation (12, 13). If not controlled or terminated immune reactions may alter brain homeostasis and cause cellular cell death and chronic inflammation (14). Degenerating neurons may themselves release molecules that will spark inflammation (15), triggering a deleterious feedforward loop.

McGeer and collaborators first evidenced the presence of microglial activation in postmortem brains of PD patients suggesting that neuroinflammation may promote neurodegeneration in PD (16, 17). Population-based prospective data has also indicated that the chronic low-dose consumption of non-steroidal anti-inflammatory drugs (NSAID) reduced the risk of developing PD although the protective effect depended on the type of NSAID molecule (18–22). Anti-inflammatory drugs have, however, not yet proven efficacious as anti-symptomatic or disease-modifying treatments (23). Over the past decades neuroinflammatory processes have undeniably been linked to PD but whether they may be a cause, or a consequence of neuronal degeneration remains unanswered (13). Intrinsic damage in degenerating neurons also referred to as cell-autonomous pathological mechanisms may drive their death and was long considered the sole causes of neurodegeneration. Neuronal degeneration may also be a secondary event induced by pathological interactions or signals from neighboring glial cells or immune cell infiltrating from peripheral compartments. The discovery of Lewy bodies containing alpha-synuclein aggregates (24) and the subsequent development of transgenic mouse models expressing alpha-synuclein in astrocytes and displaying PD-like phenotypic dysfunctions (25) supported the existence of non-cell autonomous mechanisms in the disease.

Microglia cells do not originate from blood-derived cells but are established during early prenatal period from yolk-sac-derived progenitors. They remain segregated in and are shaped by the CNS, maintaining self-renewal abilities throughout life (26, 27) without the contribution of peripheral myeloid cells (28, 29). Under physiological conditions microglia constantly surveil the brain parenchyma and provide trophic support to neurons (30–32). Physiological brain homeostasis, including intact barriers that separate CNS from peripheral compartments, regulated expression of soluble factors (TGF-β, Il-4, Il-13, BDNF, NGF) and receptor-mediated cell-cell interactions, all intervene in maintaining microglia under a surveillance-competent phenotype (33). Microglia cells are equipped with receptors to sense endogenous as well as pathogen danger signals (34). Immune mechanisms combine to confer a tight regulation of microglia function in the brain parenchyma. These include, separation from the blood by barriers, soluble factors such as TGF-β, specific interleukins, BDNF etc., direct contact with neighboring cells through receptors, such as the fraktaline receptor CX3CR1, CD200R, MHC II, as well as transcription factors that may regulate activation phenotypes (33). Under pathological conditions, microglia undergo morphological and functional changes and become "activated" (35); they acquire phagocytic phenotype, increase the expression of chemokine and

cytokine receptors and are themselves a constant supplier of inflammatory factors (32). Recent evidence clearly indicates that microglia can assume a large variety of phenotypic changes upon activation and show significant regional variability in terms of gene expression profile and functionality that goes well-beyond the simple definition of M1/M2 classification (36).

PD Patients

Activated microglia express major histocompatibility class II (MHC-II) markers that present peptides to effector cells including T lymphocytes. In humans these include human leukocyte antigen (HLA)-DR, HLA-DQ, and HLA-DP molecules. Presentation is key to the engagement of adaptive immune, which in turn can further sustain inflammatory processes. In postmortem PD brains, HLA-DR$^+$ microglia were observed in the SNc and striatum (16, 17) (**Figure 1**), mainly associated with neurons containing LB and damaged neurons (37). They also expressed intracellular adhesion molecule (ICAM), the scavenger receptor TLR2 and the lysosomal marker CD68 (37, 38). Interestingly, pro-inflammatory factors, including IL-6 and TNF-a, were expressed in MHCII+ cells (37) (**Figure 2**).

Single nucleotide polymorphism in the MHC-II locus has been associated with increased risk of developing PD (39) and genome-wide association studies (GWA) (39) have noticeably implicated HLA cell-surface complexes as fundamental to trigger the adaptive immune system in the frame of PD pathology (40).

Positron emission tomography (PET) using ^{11}C-(R)-PK11195), a radioligand that binds to the 18-kDa translocator protein (TSPO) mainly expressed on "activated" microglia (41), evidenced increased binding in various brain regions in PD patients compared to healthy controls (36, 42–44). Inconsistent data was obtained with a panel of radioligands, possibly as a consequent of polymorphism-linked difference in TSPO binding affinity of the ligands (45, 46). Thus, while PET analysis can be used to confirm microgliosis in PD, ligands do not have the ability to distinguish among the phenotypic diversity of activation states and do not allow correlation between imaging and disease severity or progression.

Astrocytes are the most abundant cells in the brain. They regulate many brain processes including glucose metabolism (47). They play a fundamental role in maintaining brain homeostasis and providing energy and support to neurons (48). Their role in PD pathology still not well-understood but an elevated cell density and phenotypic changes are observed in astrocytes in postmortem PD brains (49, 50). Astrocytes also contribute to the blood-brain-barrier that is disrupted in patients with PD (51).

PD Animal Models

Glial activation, both astrocytes and microglia, has been consistently observed in toxic animal models of PD, including the 6-OHDA rats and mice (52–54), LPS (55), MPTP treated animals (56, 57), and rotenone models (58–60) (**Figure 1**).

Intracerebral injection of the neurotoxin 6-hydroxydopamine (6-OHDA) in rodents (rats and mice) causes nigrostriatal neurodegeneration and induces a strong glial activation (microglia and astrocytes) in the striatum and substantia nigra (SN). Profile, localization and time of neuroinflammation depend on the site of injection. Increased MHC-II and CD68 expression is detected rapidly after 6-OHDA injection in the medial forebrain bundle, in particular in microglia located nearby neuronal cell loss (61, 62). In 6-OHDA models, microgliosis seems to be a transient phenomenon that peaks shortly before neurodegeneration and then slowly reverts to an apparently normal phenotype. Intrastriatal injection of 6-OHDA in mice causes a rapid increase of TNF-α in microglia indicating an inflammatory-prone phenotype (54).

The MPTP neurotoxin induces persistent microgliosis in non-human primates (NHP) evidenced by enhanced HLA-DR$^+$ microglia (56) reminiscent of phenotypes observed in PD brains (16, 17). In MPTP-treated NHP chronic microglial activation is still present years after MPTP intoxication (56, 63).

A more transient activation of microglia cells is observed in rodents depending on the MPTP regimen and paradigm (56). In a chronic rodent model of intoxication microglia activation is detected well-before neurodegeneration when non-motor dysfunctions, including hyposmia are already present and persists for at least 6 months (64). Upregulation of MHC-I, MHC-II, and ICAM-1 are detected transiently in MPTP mice (65). Increased CD68 expression in microglia located close to neuronal cell death is also observed in MPTP mice (66). Interestingly, the neuro-toxin induces a down regulation of anti-inflammatory markers, including CD206, Arg-1, and YM-1, in the SN suggesting a shift toward a more inflammatory-prone phenotype of microglial cells (67).

Rotenone, a naturally occurring substance largely used in organic agriculture until its prohibition in 2008, has been linked to the development of PD (60) and has been used to develop rodent models of the disease through different administration paradigm including intracerebral or intraperitoneal injection and intragastric administration (68, 69). Consistent neuroinflammation and microglial activation is observed in rotenone models (58–60, 70).

Peripheral or intracerebral injection of the bacterial endotoxin lipopolysaccharide (LPS) causes microgliosis that precedes neuronal cell death (71). LPS acts through interaction with the Toll-like receptor 4 (TLR-4) and is a potent inducer of peripheral (72) and central immune cells (73, 74) but has no direct effect on neuron. LPS injection has been used in numerous toxic-induced or transgenic models of PD and accentuates neurodegeneration and neuroinflammation (48, 49). Recent data indicated that peripheral injection of LPS induces microglial activation that precedes and peaked just before neurodegeneration and then slowly decreased. Interestingly, at later time points a shift toward a more anti-inflammatory profile was observed in microglia (Arg-1$^+$ cells) corresponded to cessation of neurodegenerative processes (75).

Numerous transgenic models of PD have been developed in particular model overexpressing wild type or mutant forms of the protein alpha-synuclein using different promoters (25). The thy-1 a-syn transgenic model, the best characterized syn model (61), shows early and progressive increase of activated microglia specifically detected in the SN and striatum and that precedes nigrostriatal neurodegeneration (76, 77). Similarly,

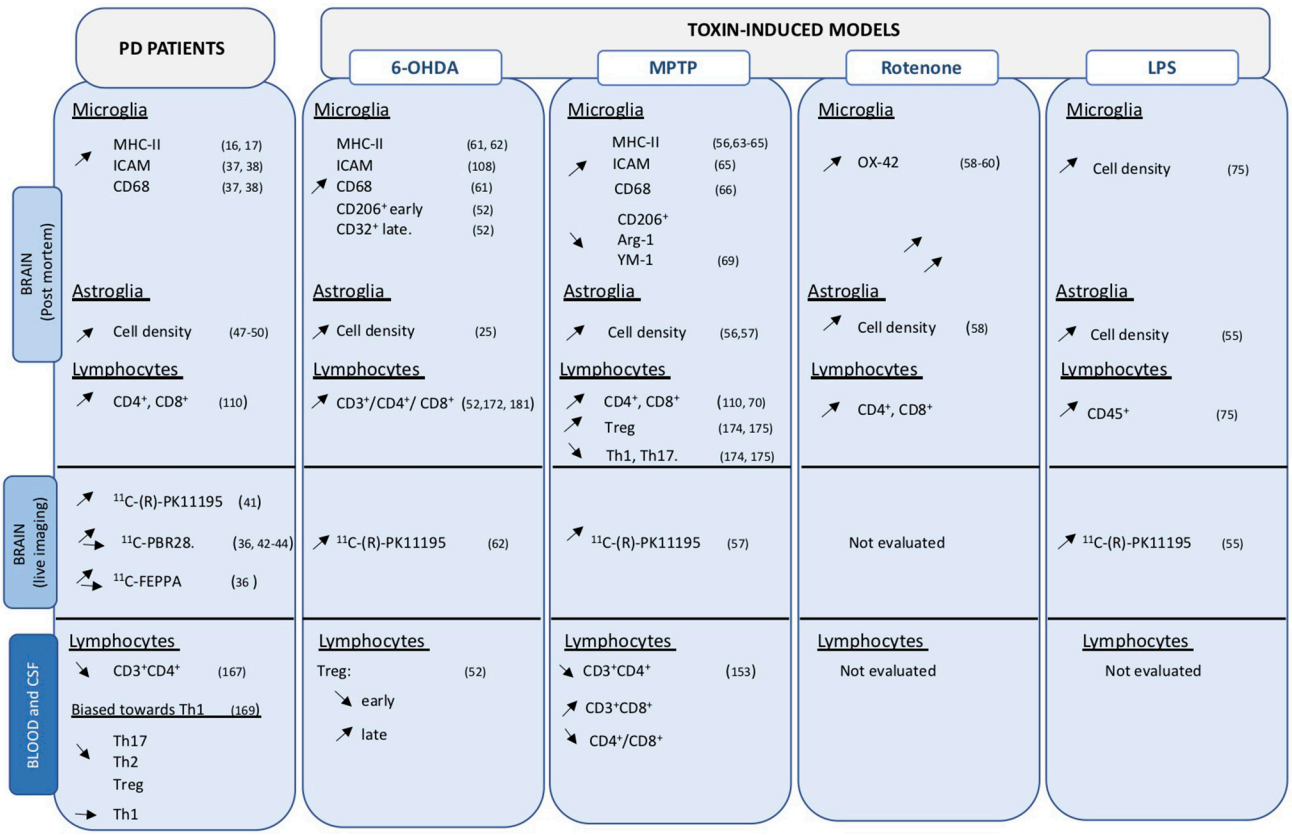


FIGURE 1 | Overview of cellular changes in central and peripheral compartments. Data obtained on PD brain and blood or CSF samples, as well as those from toxin-induced animal models are indicated. Up- and down regulation are indicated by the corresponding arrows.

viral vector-driven overexpression of a-syn models (AAV a-syn models) have been developed in rodents (78) and primates (79). Robust microglial activation is also observed in AAV a-syn models in rodents (80–82) and in primates (83) and are consistent with neuroinflammatory features observed in PD patients including MHC-II upregulation. A paper by Ferreira and Romero-Ramos (84) extensively reviews the role and crosstalk between a-syn and microglia in PD and because a space limitation this topic will not be further addressed here.

Evidence and data obtained in PD patients and animal models of PD (**Figure 1**) clearly converge and sustain the importance of neuroinflammation in the disease.

COMMUNICATION ROUTES BETWEEN THE PERIPHERY AND THE BRAIN

Crosstalk between systems implies and requires the existence of communication routes. Neurons in the brain are protected from adverse effects of peripherally borne insults by several barriers, including the blood-brain barrier (BBB), the blood CSF barrier (BCSFB) and the meninges. They have different permeability to substance and cells. Under physiological conditions only few leukocytes are observed in the CNS. These barriers represent physiological and selective entrance to the CNS, yet at the same time they are also a niche where blood-derived immune cell may distantly modulate or affect brain homeostasis (85).

The BBB is formed of endothelial cell tight junctions and a layer astrocytes end-foot. BBB alterations may be linked to aging, by far the most relevant risk factor for developing PD. Astrocytes are important players in maintaining an intact BBB and age-related changes in astrocytes may modify BBB permeability (86, 87). Astrocytes also release numerous soluble factors, including monocyte chemoattractant protein-1 (MCP-1), which favors the recruitment and infiltration of monocytes from the periphery into the brain. The CSF, filling the space in between, contains self-maintained resident myeloid cells of embryonic origin (88). Under physiological conditions, the BBB allows the passive diffusion of water and lipophilic molecules and the selective transport of molecules necessary for neural function, such as glucose and amino acids, but does not permit cellular infiltration to the brain. Dysfunction or disruption of tight junctions can cause leaky BBB and exposes the brain to blood-borne substances.

The BCSF is formed by the choroid plexus (CP) that produces and distributes the CSF throughout the CNS (89). It is formed of tight junctions and epithelial cells, which express trafficking

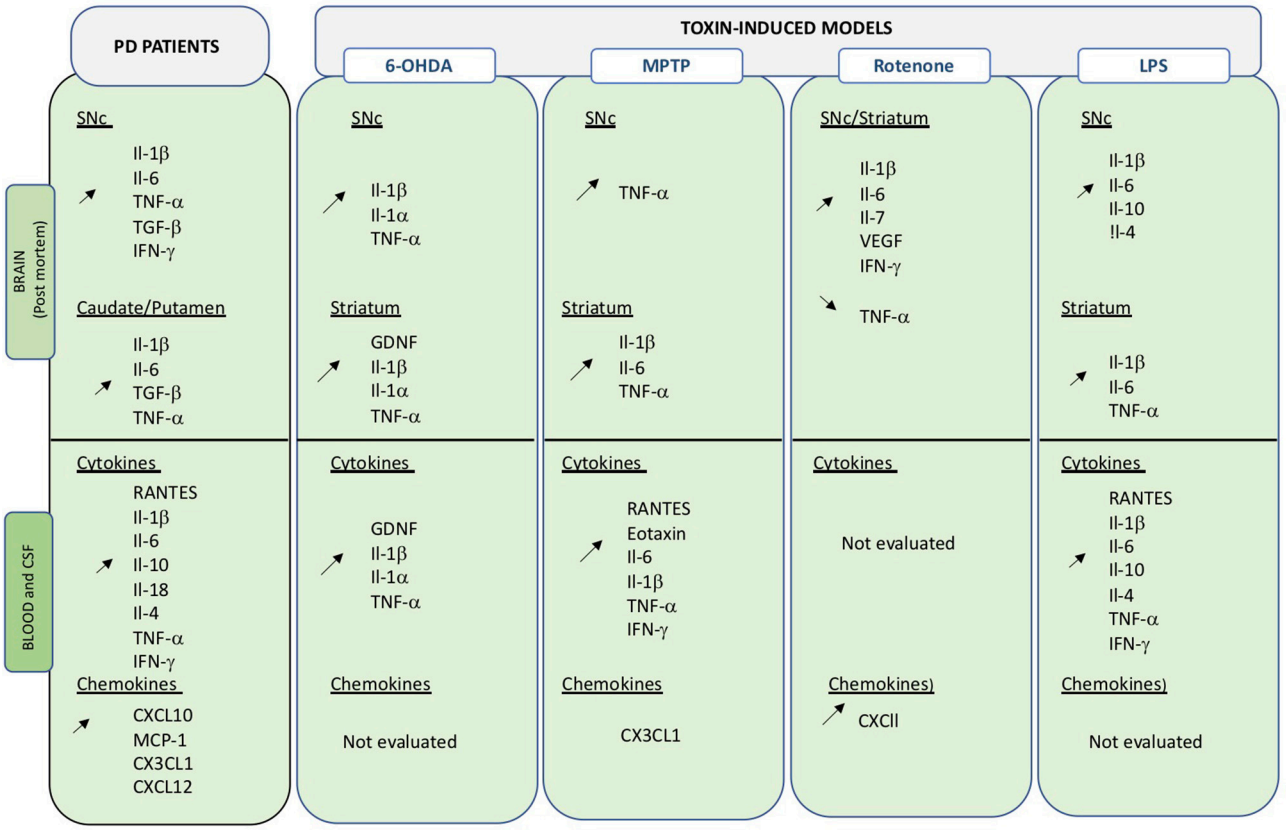


FIGURE 2 | Overview of changes in inflammatory molecules in central and peripheral compartments. Data obtained on PD brain and blood or CSF samples, as well as those from toxin-induced animal models are indicated. Up- and down regulation are indicated by the corresponding arrows.

molecules (85, 90, 91) These may sense and response to signals secreted by immune cells present both in the CNS and the stroma. The presence of epithelial vs. endothelial cells renders the BCSF less impermeable than the BBB and trafficking of a low number of T cells, in particular $CD4^+$ memory T cells, is possible even under physiological conditions (92).

The meninges surround the brain and the spinal cord and contain the myeloid- and T-cell-populated CSF in the subarachnoid space (88, 93). Similarly to the BCSF, they have a different anatomical structure than the BBB that could allow migration of immune cells into the brain parenchyma (94).

Recent evidence indicates that a selected population of resident cells, including T cell subsets, NK cells, B cells and dendritic cells, are present in those brain boundaries, the "brain interface," mostly located in the meninges and the BCSF. These cells may serve as communication bridges to and from the brain (95) and have been defined in depth recently (96). Expression of CD44 is crucial for cell motility and is not present in brain resident myeloid cells (96).

Pathological events, in the CNS or in peripheral systems, may modulate barrier integrity leading to important functional dysregulation and opening of tightly regulated communication routes causing unwanted crosstalk and passage of noxious molecules or cells that may sensitize or worsen existing conditions. Leakage could potentiate existing neuroinflammatory processes by allowing infiltration of peripheral cells into the brain parenchyma. It could also be caused by neuroinflammation itself.

PD Patients

BBB disruption has been reported in PD patients (97, 98). Post-mortem brain samples show accumulation of blood-derived proteins (fibrinogen, IgG) in the striatum and globus pallidus (51, 99). Microvascular degeneration, disrupted and damaged tight junctions, changes in the capillary basement membrane of the subthalamic nucleus, as well as red blood cell extravasation in striatum have been documented. Aberrant angiogenesis in the SN, locus coeruleus and putamen also support alterations in BBB (100, 101). Increased of blood-derived albumin in CSF and of IgG CSF: serum ratio (102, 103) are also indicative of barrier dysfunction. Live neuroimaging studies have evidenced disruption of BBB integrity in basal ganglia (104) and deep cortical gray matter regions and white matter (105), as well as diminished P-glycoprotein function (97).

PD Animal Models

Existing data in animal models confirm the presence of barrier alteration although this pathological data has not been systematically evaluated in all models. Injection of LPS causes

BBB disruption and loss of TH-positive cells in rodents (23, 106). When present, BBB disruption occurs mostly at the site of dopaminergic cell loss but the causes and sequence of events leading to BBB permeability changes are still a matter of debate.

Altered expression of cerebral adhesion molecules, possibly reflecting BBB alterations have been detected in 6-OHDA rats (105, 107) and have been associated with concomitant alterations of peripheral molecules (108). Increased BBB permeability has also been observed in the striatum and SNC following intrastriatal injection of 6-OHDA (109). It has also been suggested that brain accumulation of iron measured in 6-OHDA animals is partly due to altered BBB (107).

Upregulation of adhesion molecules in important for infiltration of cells has been observed in MPTP models (rodents and NHP) (110, 111). Peripheral inflammation itself may cause BBB alterations, thus favoring or sustaining neurodegeneration. Inflammatory mediators, produced systemically, or within the brain, can signal through the endothelial cells causing alteration in tight junctions' structure thus modifying BBB permeability. Increased number of blood vessels and endothelial cells have been described in proximity of degenerating neurons in MPTP-treated NHP (112). Changes in phospho-glycoprotein functionality suggestive of BBB alterations have also been observed in MPTP-treated NHP (113). Therapeutic strategies that prevent BBB leakage through activation of CB2 receptors have been shown to reduce dopamine neuron loss in the MPTP model (114). However, it remains unclear whether increased BBB integrity is a causal effect or the result of reduced neuronal cell loss.

No clear evidence of BBB alteration, measured by fluorescein leakage to the brain, has been detected in the rotenone model (115).

Globally, evidence obtained from human and animal studies clearly points to a dysregulation of barriers between the peripheral and central compartment. It remains to be determined if increased BBB integrity is a causal effect or the result of neuronal cell loss and neuroinflammation.

INFLAMMATORY MARKERS

Peripheral inflammatory markers including cytokines and chemokines are critical signaling molecules in the modulation of the immune system and can affect both peripheral and central systems. Cytokines and chemokines are actively transported across the BBB by saturable transport and any variations in expression levels in the blood may directly or indirectly impact on CNS function (116). Peripheral inflammation could thus be an important contributor in the etiology of PD as well as in disease progress (**Figure 2**).

PD Patients

Increased levels of inflammatory markers were already detected in PD post-mortem samples in the late 1990's (**Figure 2**) (117–121). Changes in cytokine/chemokine levels have then been largely evaluated in PD patients biofluids (blood, serum plasma, CSF) and measurements show divergences of pro-inflammatory and anti-inflammatory profiles compared to healthy subjects (122–126). Qin and collaborators have recently performed a systemic review and meta-analysis of published data to investigate alterations of peripheral cytokines in PD patients (127). Findings from 25 peer-reviewed publications, including 1,547 PD patients, and 1,107 healthy controls, confirmed that patients present an increased inflammatory-prone response and allowed the identification of elevated levels of pro-inflammatory factors, including RANTES Il-1β, Il-2, Il-6, TNF-α, and C-reactive protein. Interestingly, Il-1β is an important central downstream effector of inflammasome activation, an inflammatory mediator that is attracting considerable attention in neurodegenerative diseases (see below section Inflammasome). Altered levels of IFN-γ and Il-8 were also detected, although in a limited number of small studies (122, 128). Similarly, alterations in levels of the anti-inflammatory cytokines Il-4 and Il-10 were detected (118, 122). Il-10 is thought to oppose action of proinflammatory cytokines but may also be involved in B cell survival and activation as well as IFN-γ production. Recently, some of the above-mentioned cytokines have been correlated with specific PD phenotypes (129). High Il-10 was related to non-tremor and late onset PD, while Il-6 correlated with longer disease duration and TNF-α with disease progression. Correlation between RANTEs and disease severity (130) as well as TNF-α blood levels and non-motor symptoms have also been suggested.

Chemokines have been less commonly assessed in blood or CSF of PD patients, but recent data suggest that elevated CXCL10 levels may be related to worsening of cognitive functions in PD patients (131). Similarly, changes in MCP1 (CCL2), CXCL10 and CX3CL1 have been detected (132, 133). Changes are also observed in the CSF, in particular increased levels of Il-2, Il-6, TNF-α and MCP-1 have been measures (134). Importantly, increased levels of Il-1β, Il-6, and TNF-α, have also been detected in PD brains (135, 136), suggesting that changes in both systems may be correlated.

PD Animal Models

Few studies have addressed the modulation of inflammatory factors in peripheral compartments, including blood and CSF, in animal models of PD. Nonetheless, results obtained in animal models also recapitulate the convergence of peripheral and central changes in inflammatory molecules.

In 6-OHDA rodents blockage of TNF-α or Il-4 can reduce neurodegeneration (23, 137), while it is exacerbated by treatment with systemic Il-1β (138, 139). Interestingly, a similar worsening effect was observed in DJ-1 KO (140) and Parkin KO mice (141) for which neurodegeneration of dopaminergic neurons is usually absent (25). A delayed increase of striatal Il-1β mRNA levels is observed following 6-OHDA administration (142). Modulation of Il-1α, Il-1β, Il-6, and GDNF levels is observed at different time points both in serum CSF and brain extract of 6-OHDA rats (53, 143) It is possible that the time course analyses (24 h, 7 days, 4 and 8 weeks after toxic insult) in the various studies does not allow the detection of transient up- or down-regulation of the inflammatory markers.

CSF and serum concentration of TNF-α and IFN-γ are elevated in MPTP-treated NHP, even several years after intoxication (144, 145). Confirming the importance of the two

cytokines, KO mice lacking IFN-γ or TNF-α receptors are protected from MPTP neurotoxicity (144, 146–148). Elevated levels of Il-1β TNF-a and Il-6 are also consistently observed in serum and brain following MPTP administration in mice (67, 149–153).

More recent evidence from the acute MPTP mouse model, indicates a transient increased in both brain and serum levels of RANTES and eotaxin (154, 155). RANTES is known to induce migration and homing of lymphoid cells (156) and as indicated above is also increased in serum of PD patients (127). Eotaxin is an important factor involved in infiltration of mononuclear cells at inflammation sites (157). Biweekly injection of RANTES and eotaxin induced a continuous activation of neuroinflammation and T-cell infiltration as well as persistent neurodegeneration, while functional blocking antibodies to the two factors reduced T-cell infiltration, neuroinflammation and neurodegeneration. Interestingly, increased RANTES and eotaxin levels, as well as T cell infiltration have also been detected in the serum of MPTP-treated NHP (158). Blocking RANTES and eotaxin expression could significantly reduce neuroinflammation. Unfortunately, effect on neurodegeneration of RANTES blockade was not assessed in this study.

Animals that received repeated i.p. injection of LPS presented with a significant loss of dopaminergic neurons that peaked at 19 days and remained stable thereafter. An immediate increase in pro-inflammatory cytokines, Il-1β, Il-6, and TNF-α was detected in the brain. Interestingly, this pro-inflammatory upregulation preceded neurodegeneration, was then reduced when maximal neuronal cell loss was detected, and switch to a more anti-inflammatory profile (Il-10) as cell loss ceased and stabilized (75). This sequential modulation from a pro- to an anti-inflammatory phenotype may represent an interesting target to arrest and resolve chronic neuro-inflammation.

LYMPHOCYTE INFILTRATION

In a normal adult brain, the crosstalk between the peripheral immune system and the brain is transient, and there is no evidence that it may lead to the central neuroinflammation. As mentioned above under physiological conditions, only few leukocytes are observed in the CNS. A growing body of evidence suggests that in chronic neurodegeneration, not only are the brain-resident microglia activated (159, 160), but they may be "primed" by previous or ongoing systemic inflammation, leading to the exaggerated synthesis of pro-inflammatory molecules (161–163). Numerous studies showed that microglial cells can be activated by the chronic infiltration of peripheral inflammatory T cells (164, 165), as well as various toxic molecules circulating from the peripheral tissue to the brain (166). T lymphocytes are an essential component of adaptive immunity and collaborate with B cells to produce an immune response.

PD Patients

T cell populations are altered in peripheral compartments and invade the CNS in PD and may contribute to neuronal degeneration and disease progression. Infiltration of $CD4^+$ and $CD8^+$ cells have been observed in post-mortem analyses of PD brains (110) (**Figure 1**).

In peripheral blood of PD patients, alterations of the adaptive system are detected showing a decrease in both B and T lymphocytes, together with alterations in components of innate immunity including increased natural killer cells and neutrophils levels. In particular, a reduction in $CD3^+/CD4^+$ lymphocytes is consistently described, while the number of $CD8^+$ cells remains largely unchanged in blood of PD patients (167).

$CD4^+$ T cells can acquire different phenotypes corresponding to different inflammatory states. Pro-inflammatory T helper (Th) cells include Th1 that produce IFN-γ and TNF-α, and Th17 cells, producing Il-17 and Il-22. Anti-inflammatory cells include Th2 that release Il-4, Il-5, and IL-13, and regulatory T cells (Treg) that are fundamental modulator of T cell activation. Kustrimovic and collaborators have recently indicated that the balance among different T-cell phenotypes in the blood of PD patients is biased toward a more Th1-response, with a reduction in the number of Th17, Th2, and Treg, but not Th1 cells (168). This imbalance was further reflected by a Th-1-prone polarization in response to specific inflammatory stimuli, observed *in vitro* in lymphocytes from PD patients but not healthy volunteers. The reduced efficacy of PD Treg cells in controlling the release of pro-inflammatory cytokines by effector T cells (169) is a likely contributing factor that further amplifies this Th1-prone profile of peripheral T cells in PD.

PD Animal Models

Evidence in toxin-induced animal models corroborates data obtained in PD patients and sustains the important function of T cell subsets in neurodegenerative processes in PD (**Figure 1**). Infiltration of T cells, in particular $CD4^+$ and $CD8^+$ infiltration in the brain parenchyma, has been documented in numerous animal models of PD, including MPTP mice (110, 170), intragastric rotenone PD model (171), as well as in 6-OHDA PD models (52, 172). Much information on T-cell infiltration has been obtained using the MPTP mouse model combined to a variety of transgenic models. $Rag1^{-/-}$ mice, which lack mature lymphocytes, and $Tcrb^{-/-}$ mice, which lack T cell receptor β, are more resistant to acute MPTP toxicity compared to control mice (173, 174). Similarly, administration of MPTP to $CD4^{-/-}$ mice induced less prominent dopaminergic cell loss compared to that observed in $CD8^{-/-}$ animals (110). Altogether, these data indicate the importance of T lymphocyte infiltration and sustain a prevalent function of $CD4^+$ over $CD8^+$ lymphocytes in the MPTP-induced neurodegeneration processes.

The Th1-prone imbalance together with the reduced Treg efficacy observed in PD patient blood, combined with the importance of anti-inflammatory action and regulation of Treg in neurodegeneration is further sustained by experiments involving adoptive transfer of T cell subsets in MPTP mice. Transfer of Treg cells reduced neuronal cell loss, while transfer of Th1 or Th17 increased neurodegeneration (174, 175). In the same line, immunization with bacillus Calmette-Guerrin that favors Treg activation had a protective potential in MPTP mice insult (176). Chung and collaborators also reported that neuroprotective potential of bee venom immunization in MPTP mice could be

linked to a global reduction of $CD4^+$ infiltration accompanied by a relative increased proportion of Treg cells in the brain parenchyma (177). Reduction in the number of lymphocytes in MPTP mice has been reported as early as 1992 (178) and confirmed by recent data reporting a global reduction in the number of $CD3^+$ with reduced $CD3^+CD4^+$ but increased $CD3^+CD8^+$ cells (153).

Infiltration of T-lymphocytes has also been observed in 6-OHDA mice and rats PD models together with time-dependent neuroinflammation (52, 179). Blood of 6-OHDA animals showed an initial decrease in Treg cells that progressively returned to normal values. Interestingly, reduced Treg levels at the peripheral level corresponded to a phenotypic shift in microglial activation, from an anti-inflammatory phenotype ($CD206^+$) to a more pro-inflammatory ($CD32^+$) phenotype, as well as with the reduction of neuronal cell loss in the SNc, further suggesting an important modulatory role of Treg cells in the neuronal cell loss and neuroinflammatory (53).

Considering the close interrelationship between T cells and microglia cells (180), therapies that change T cells may directly modulate microglial phenotype and vice and versa. For example, stimulation of the regulatory function of $CD4^+$ cells infiltrating the brain may represent and therapeutic strategy to limit neurodegeneration.

MONOCYTE/MACROPHAGES

As described above the presence of infiltrating lymphocytes in the CNS is well-documented both in animal models of PD and in post-mortem analyses of PD brains. Differently, a role for monocytes/macrophages in PD remains unclear but evidence suggests that they may also be contributing actors to the disease. Macrophages and monocytes are important players in the regulation of immune reaction in peripheral compartments and can pass the BBB to enter the brain where they may participate in regulation of central neuroinflammatory process (181).

Monocytes are short-lived myeloid-derived cells that continuously generated from bone marrow precursors (182). Monocytes circulate in the blood and tissues and do not proliferate under physiological conditions. They are key components of the innate immune system, express cell surface receptors as well as pathogen recognition receptors, and can produce cytokines. During inflammation they may migrate to inflamed tissues and differentiate into dendritic cells or macrophages (27, 183). Under physiological conditions, monocytes are constantly renewed from the myeloid repertoire while microglia renew themselves without the contribution of peripheral myeloid cells (28, 35).

Circulating monocytes can be found in the brain parenchyma only following BBB disruption caused for example by irradiation and bone marrow transplantation (184). Parabiosis experiments (26, 185) indicate that there is no infiltration of monocytes in the brain under physiological conditions. In neurodegenerative diseases, monocytes may infiltrate the brain and join microglia. As microglia they also undergo phenotypic changes, rapidly acquire a macrophage-like phenotype but never fully gain a microglial identity (186). Until recently, it was difficult to distinguish infiltration monocytes from endogenous microglia. Recent data indicates that the chemokine receptor CCR2, required for cellular infiltration, is expressed on blood monocytes but not on resting or activated microglia and that the reverse is true for the fraktaline receptor CXC3CR1 (187, 188). This differential expression of cell surface markers favors the distinction between monocytes and microglia population in the brain parenchyma during a short time-window before infiltrating monocytes turn into tissue macrophages and downregulate CCR2 (189).

A specific population of macrophages are located at the brain interface, including meninges and CP; they have the same ontogenetic origin as microglia and differentiate through fine-tuned processes to give rise to separate cellular population with distinct profiles (190). Brain interface macrophages can be replenished by circulating cells but mostly originate from embryonic yolk sac and are maintained by self-renewal (88). They express microglia markers including CD11b, CX3CR1 and are different than circulating monocytes. The role of these perivascular macrophages is still under study.

PD Patients

Recent data indicates that, in early stage PD patients, disease-specific gene expression in peripheral monocytes may correlate with disease severity (191). Interestingly, genes relating to leukocyte migration and regulation of immune responses were found to be enriched in PD monocytes. Of particular interest, LRRK2 expression was highly upregulated in monocytes (see the section LRRK2: a genetic factor and an immune mediator in PD). Different gene expression patterns in monocytes are observed when looking at distinct disease stages (192) suggesting that monocytes may represent an important population to identify disease progression markers in PD.

PD Animal Models

MPTP treatment in mice increases the number of circulating monocytes (193). Infiltration of $CCR2^+$ monocytes has been detected in the brain parenchyma of CCR2-GFP reporter mice following acute MPTP treatment (194). Interestingly, monocyte infiltration was transient and occurred before infiltration of T cells. In this acute model, blocking $CRR2^+$ had no effect on MPTP-induced neurodegeneration. Precise contribution of monocytes infiltration to neuronal cell death still needs to be clearly demonstrated and more chronic states of infiltration may be needed.

INFLAMMASOMES AND PARKINSON'S DISEASE

Innate immunity is the first line of defense of the organism. It has evolved to recognize conserved pathogen molecular sequence (pathogen-associated molecular pattern—PAMP) through a set of receptors, the pattern recognition receptors (PRR). PRR can also be activated by damage-associated molecular patterns (DAMP). Recognition of PAMP or DAMP by PRR normally triggers transcriptional activation and neo-synthesis of proteins.

Inflammasomes react to specific PRR signal, trigger caspase 1 activation, which in turn causes the maturation and release of the pro-inflammatory cytokines Il-1β and Il-18.

The inflammasome is a macromolecular complex formed through the oligomerization of a receptor, an adaptor, and caspase-1, the effector of the complex. Inflammasome receptors belong to several families, including the nucleotide-binding domain and leucine-rich repeats containing receptor family (NLR). In the past years NLRs have emerged as key sensors and regulators responding to PAMP and in particular DAMP produced under non-microbial inflammatory conditions (195). NLRP3 is the most characterized and studied inflammasome receptor and NLRP3 alterations have been linked to several pathologies, including neurodegenerative diseases (196). Evidence indicates that inflammasomes are important players in both peripheral and central innate immunity (197) and need to be tightly controlled to avoid overt inflammatory activation (198, 199).

PD Patients

Post-mortem analysis of confirmed idiopathic PD brains revealed high NLRP3 protein expression in surviving neurons (200). Downstream effectors of NLRP3, namely IL-18 and IL-1β are increased in CSF and serum of PD patients (201). IL-1β is a key cytokine in PD and increased levels are detected in central and peripheral compartments both in PD patients and in animal models of the disease (**Figure 2**). Considering the close link between NLRP3 and IL-1β maturation, inflammasomes may be an important linking bridge between peripheral inflammation and central PD pathology (**Figure 3**).

A SNPs variant (rs7525979) of NLRP3, linked to altered stability of inflammasome assembly, is associated with significantly lower risk of developing PD (200). Interestingly, NLRP3 polymorphism has recently been associated with inflammatory bowel disease [IBD (202)]; IBD being a recently identified risk factor for PD development (203). Sustaining this link, recent retrospective analyses indicate that anti-TNF-α therapy to treat IBD reduces the incidence of PD, with a 78% reduction of risk in treated compared to non-treated patients (204). Thus, peripheral and central inflammation may be linked through activation of inflammasomes. Thus, a specific genetic susceptibility bridges PD and inflammation in the gut. The importance and involvement of the gut-brain axis PD pathogenesis has drawn considerable attention in the past decades. The Braak theory suggests that PD pathology may initially be triggered in the gastrointestinal (GI) tract and then spread to the brain via the vagus nerve. While the Braak theory is still a matter of debate (205, 206), the dysregulation of the gut-brain axis is well-established (207). A majority of patients suffer GI disorders that often appear before the onset of any motor symptoms (208). Today, there is no consensus on whether GI disturbances occur as a consequence of neurodegeneration in the CNS, the enteric nervous system, or both, or because of a yet unknown pathological process. Evidence supporting a contribution of chronic intestinal inflammation in PD has been covered elsewhere (207, 209, 210) and will not be reviewed here.

Several pathologic features characteristic of PD are linked to inflammasome regulation and activation further sustaining its importance. Indeed, dopamine (DA), the key neurotransmitter in PD, acts on almost all peripheral blood cells, which knowingly express dopamine receptors (211). DA has been shown to be an endogenous modulator of inflammasome by promoting NLRP3 ubiquitination and degradation (212). DA can inhibit LPS-induced NLRP3 activation in mice (212). Inflammasome can also be triggered by α-syn (213). Mitochondrial alteration is a common defect normally observed in most cell compartments in PD patients (214). Mitochondrial stress induced by rotenone can prime the activation of NLRP3 pointing out a link between mitochondrial defect and inflammasome activation (215, 216).

PD Animal Models

Evidence in an animal model of PD indicates that IL-1β is primarily induced by NLRP3 activation in brain and microglia (217). Multiple studies in animals have shown that blocking NLPR3 can block development of PD phenotypes. Inhibition of inflammasome in MPTP-treated mice can reduce neuronal cell death (218). NLRP3$^{-/-}$ mice are less susceptible to MPTP compared to wild type animals and show reduced Il-1β and Il-18 production in serum (212). On the same line, mice lacking Caspase-1, the inflammasome effector, are less susceptible to MPTP (219). Inhibition of caspase-1 also reduces susceptibility to intracerebral administration of LPS or 6-OHDA in rats (220). Intragastric and ip exposure to rotenone increases striatal NLRP3 levels in treated mice (171, 221) and leads to caspase 1 cleavage, as well as neuroinflammation. Interestingly, the same treatment in mice lacking NLRP3 does not induce an inflammatory response and reduced neuronal cell death, typically induced by the neurotoxin, is detected (171) (**Figure 3**).

A large number of cytokines can be produced by the liver, which seems to be an active indirect participant in inflammatory processes. Aside from increased NLRP3 activation in the brain, MPTP-treated mice also present NLRP3 changes in liver and bone marrow-derived macrophages (BMDM) (222). In these mice, the selective liver-directed downregulation of NLRP3 reduced hepatic NLRP3 levels, as well as levels of proinflammatory cytokines in serum and brain. Specifically, Il-1β and Il-18, but not the anti-inflammatory Il-4 and Il-10 cytokines, were impacted, suggesting the induction of an anti-inflammatory prone environment. Decreased hepatic inflammasome activation was accompanied by reduced neurodegenerative and neuroinflammatory processes. Interestingly, hepatic alterations have been previously described in 6-OHDA treated animals (143) and it was suggested that brain-liver axis may intervene in a feedforward loop in which neurodegeneration caused hepatic alteration that in turn exacerbated neurodegeneration. Inflammasome components have not been evaluated in this study but could represent an important mediator in peripheral-to-central transmission of inflammation and vice and versa. Interestingly, BMDM isolated from animals receiving hepatic inhibitory vectors displayed reduced release of Il-1β and caspase 1 in the absence of changes in NLRP3 expression suggesting an indirect effect produce by hepatic inhibition.

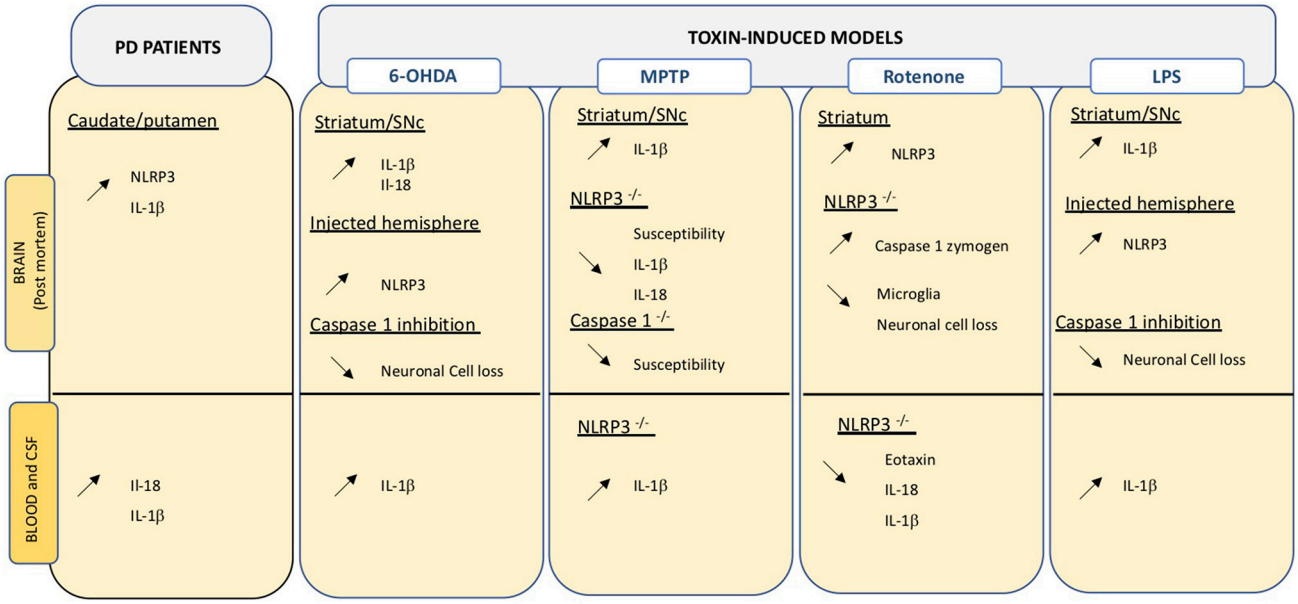


FIGURE 3 | Overview of changes relating the inflammasome in central and peripheral compartments. Data obtained on PD brain and blood or CSF samples, as well as those from toxin-induced animal models are indicated. Up- and down regulation are indicated by the corresponding arrows.

The study of NLRP3 is important because inflammasomes are the core of sterile inflammation associated with exposure to chemicals, proteinopathies as well as stress that are all part of the multifactorial panel influencing incidence of PD. A more thorough understanding of inflammasomes and effects of inhibition of NLRP3 in existing models together with evaluation of peripheral levels of factors is warranted across models and in peripheral blood of PD patients.

LRRK2: A GENETIC FACTOR AND AN IMMUNE MEDIATOR IN PD

Leucine—rich repeat kinase 2 (LRRK2) is a large 286 kDa protein that contains several distinct functional and protein-protein interaction domains. This inherent structure suggests that LRRK2 interacts with different partners in different cells and may modulate numerous cellular functions (223) and pathways. LRRK2 mutations are associated with a dominant form of familial PD that is similar in presentation and age of onset to idiopathic PD. Up to 40% of familial PD is linked to LRKK2 mutation (224). Interestingly, the LRRK2 locus is also a major genetic susceptibility factor in idiopathic PD (225). The two most common PD-related LRRK2 mutations, G2019S, and R1441G/C, cluster within the kinase and GTPase domains that are surrounded by large interactions sites (226) but the physiological importance of LRRK2 itself and the contribution of mutations to PD pathology remain unclear. LRRK2 mutations have an incomplete penetrance in PD patients and most rodent transgenic models developed to date (http://www.neurodegenerationresearch.eu/models-for-parkinsons-disease/in~vivo-mammalian-models/lrrk2/) show no or little evidence of any neuronal cell loss, strongly indicating that other factors, environmental or genetic, must intervene to trigger neuronal cell loss.

PD Patients

Large GWA studies have detected common LRRK2 variants that confer increased (N2081D) or reduced (N551K or R1398H) risk to develop Crohn's disease, a subtype of IBD (227). The N2081D, but not N551K and R1398H variants increased LRRK2 kinase activity. As mentioned above IBD is a recently identified risk factor for PD. Effects of the variants on IBD also correlated with an increased and reduced risk to develop PD. Both diseases share inflammation as a common denominator and may therefore share common disease mechanisms, in which LRRK2 may be an important hub.

LRRK2 is expressed in both neurons and cells of the innate and adaptive systems (228, 229), where it may become upregulated following microbial or viral infection. Expression levels of LRRK2 protein are increased in B and T cells, as well as in monocytes of PD patients compared to control subjects (230). Interestingly, LRKK2 levels were elevated specifically in $CD16^+$ pro-inflammatory monocytes, with a slight increase also observed in T effector cells. *In vitro*, stimulation of PD patient-derived monocytes induced stable and long-lasting activation of HLA-DR, while only short-lived HLA-DR activation was observed in control monocytes. HLA-DR is part of the MHC-II locus that is highly polymorphic and is involved in antigen presentation required for $CD4^+$ cell activation. Opposite correlations between LRKK2 and HLA-DR expression,

positive or negative, were observed in monocytes from PD patients and control subjects, respectively (230). SNPs in the HLA-DR region have been associated with increased risk of developing PD (39, 225, 231, 232). The combination of specific HLA polymorphisms and pesticide exposure seem to favor the induction of a more pro-inflammatory-prone $CD4^+$ activation (233). Thus, LRRK2 may be an important factor that intervenes at the immune interface and may favor a pro-inflammatory-prone environment in PD patients as well as in animal models (**Figure 2**).

PD Animal Models

LRRK2 deficient rats show significantly increased percentage of $CD4^+$ and $CD3^+$ cells, but not $CD8^+$ in the spleen, as well as a reduced percentage of B cells compared to wild type animals (234). Interestingly, LRRK2 KO rats challenged with α-syn overexpression or intracerebral LPS administration are protected from neurodegeneration normally occuring in wild type animals (235). Transgenic mice overexpressing pathogenic LRRK2 mutations, R1441G or G2019S, do not normally present any evidence of neuronal cell loss or neuroinflammation in the SNc. Recently, Kozina and collaborators showed that systemic LPS-induced inflammation triggered significant loss of TH-positive cells only in mice overexpressing a mutant form of the human LRRK2 protein (236). No neurodegeneration was induced by LPS in mice overexpressing the human wild type LRRK2 protein, strongly suggesting a role of LRRK2 mutations in pathological mechanisms leading to cell loss. Interestingly, no lymphocyte infiltration was detected in transgenic LRKK2 mutant animals. Analysis of peripheral immune reaction indicated that systemic LPS administration in the context of mutant LRRK2 triggered significant increase in peripheral cytokines that exacerbated neuroinflammation in the brain, increased LRRK2 expression in neurons and caused neurodegeneration. Interestingly, time-dependent increase of peripheral expression of numerous immune factors, including IL-1β, IL-6, IL-10, RANTES, CXCL1, were detected in LPS-treated mutant LRRK2 mice factors. These factors are reminiscent of alterations observed in the blood of PD patients (see the section "Inflammatory markers" above).

DISCUSSION

The etiology of PD pathogenesis is still largely unknown, and evidence strongly indicates that combinations of multiple factors are involved in triggering neurodegeneration. Neuroinflammation and systemic inflammation have been implicated in PD pathogenesis and appear as key aggravating factors. Yet it remains unknown if inflammation and immune dysfunctions mediate PD or if PD mediate immune dysfunction in both peripheral and central systems. Evidence obtained from patients and animal models of the disease gives clues of pathological alterations but does not as yet clearly answer this question.

Our current knowledge on pathological mechanisms involved in PD etiology and progression in humans is a consequence of the limited access to *in vivo* brain data. Cerebral imaging, while rapidly evolving in more powerful tools, has yet failed to give clear cut correlations between central dysfunctions and disease stage or progression, or with peripheral biological markers. Notwithstanding the development of improved radioligands for in vivo PET analyses, a clear understanding of the role of neuroinflammation in PD progression is still lacking. The comprehension of the complex nature of microglia cells, which can embrace a diversity of phenotype throughout the course of the disease, has guided de development of therapeutic strategies that would help mitigate their deleterious effect and modulate them toward a more "protective" phenotype rather them just inhibiting microglia activation. Exenatide, a GLP-1 receptor agonist, is a perfect example of a promising therapeutic alternative that may slow down disease progression through modulation of inflammation and neuroinflammation. GLP-1 receptors are highly expressed in microglia (237). In animals, exenatide has been shown to reduced MPTP-induced activation of microglia as well as the levels of inflammatory molecules including TNF-α and Il-β (238). Similar effects have been obtained in the rotenone model (239). The molecule, already available in the market for the treatment of insulin-resistant diabetes, has rapidly advanced in clinical trials for PD treatment. After a positive proof of concept open-label study that suggested significant improvement in both motor and non-motor PD features (240), researchers moved to a double-blind, placebo-controlled trial (241). Results indicate a significant improvement in motor scores in exenatide treated patients vs. the placebo group. Improvement in non-motor symptoms have also been reported for the same patients (242, 243). While the action of exenatide and how it may slow down PD progression is not clear, a recent evidence indicates that another GLP-1 agonist, NLY01, may limit microglia activation and reduce release of inflammatory molecules thus limiting neuroinflammation.

Preclinical models, in particular MPTP-treated mice, have also been used to evaluate the effect of minocycline, a tetracycline derivative, and showed that the molecule also possess anti-inflammatory properties, reducing microglia activation and Il-1β production in the SN (244). NET-PD FS 1, a futility trial in early untreated PD patients suggested that prolonged minocycline treatment caused no major safety concerns (245). Another antibiotic tetracycline molecule, doxycycline, has also shown promising results in animal models of PD, including 6-OHDA-treated mice (246), the LPS rat model (247). Doxycycline effect is associated with reduced microglia activation and may regulate inflammasome signaling (248). Today, large efforts are being made to modify the tetracycline molecules and separate their antimicrobial and anti-inflammatory properties to reduce any bacterial resistance that may occur after chronic use of antibiotics.

The inflammasome and its importance in neurodegenerative disease has attracted a lot of attention in the past years. All animal models of PD present upregulated levels of Il-1β both in peripheral and central compartment (**Figure 3**) and Il-1β is a major downstream activator of the inflammasome. Evidence indicates that blocking inflammasome with a small molecule called MCC950, which can readily cross the BBB,

reduced Il-1β and caspase 1 levels and neurodegeneration in 6-OHDA animals and improved motor features in treated animals (249). MCC950 is being developed by a drug company that is hoping to start a Phase 1 clinical trial to determine the safety features in humans. Interestingly, many natural compounds with known anti-inflammatory properties have inhibitory activities on various components of the NLRP3 inflammasome pathway. Curcumin, resveratrol, and quercetin have all been shown to reduce levels of caspace-1 and Il-1β (250–252).

To date, most brain data still refer to postmortem pathology and thus only give a snapshot information, at a given and often advanced disease time point, on processes that may have been ongoing and have been evolving for years or decades. In the search for therapies that may slow down disease progression it is important to grasp the evolution of pathological mechanisms that are likely different at different disease stages. Current challenges and unmet needs in PD is the development of biomarkers that allow assessing ongoing dysregulated process in humans. Animal models largely contribute to the current knowledge of peripheral and central dysregulations that accompany neuronal cell loss. Numerous animal models have been developed in different organisms (25). While none of them fully recapitulate the multifactorial deficits observed in humans, they have, in particular, toxic transgenic rodent models, greatly helped address unanswered questions and are ideal for studying early and progressive neurodegenerative processes.

The perfect animal model that recapitulates all PD features does not exist. Yet animal models allow the evaluation of relevant genetic and environmental factors involved in PD pathology. Many immune alterations detected in PD patients have also been observed in animal models (see **Figures 1**–**3**). They have the advantage of presenting a homogeneous population, all individuals sharing identical genotype, and because they show high anatomical and physiological similarities to humans, they can be readily analyzed to understand the interrelationships and crosstalk between different body compartments. Importantly, animal models allow for relatively easy longitudinal studies. To date, few studies have assessed alterations in systemic compartments in available models. In the future, more systematic time-course evaluations of potential changes in peripheral immune factors are warranted. Similarly, the crossing of existing transgenic models or the combination of genetic and environmental factors in models to generate multiple hit triggers, will improve our knowledge on peripheral-central inflammation crosstalk.

Similarly, studies on patients have mostly concentrated on single point evaluation of immune alterations. Post-mortem studies give clear but static indication of late stage neuroinflammation alterations in PD brain, including infiltration of T or B lymphocytes infiltration or microglial activation. Similarly, analyses of levels of soluble factors and immune cells describe alterations at single time points. These studies have generated valuable information and have advanced our knowledge and understanding of PD. Yet, they represent a time point snapshot and barely or do not take into account the progressive nature of PD or the heterogeneity of the PD patient population. Longitudinal studies and patient stratification are desperately needed to gain a more comprehensive understanding of PD pathogenesis and disease progression.

In the past decades, biorepository resources and studies that allow collecting, processing, storing and distributing biospecimens have been developed to support research. These include the BioFIND study (completed in 2015), the Parkinson's Progression marker Initiative (PPMI), the Parkinson's Disease biomarker program (PDBP), the De Novo Parkinson (DeNoPa) in Germany, the ICEBERG study in France, the Norwegian Parkwest study, COPPADIS 2015 in Spain, the Oxford Parkinson's disease Centre and many more. These repositories aim at establishing a comprehensive follow up and collection of bio samples that will permit a better understanding of factors involved in disease progression, in particular in immune systems. The parallel development of both more precise imaging markers and more potent imaging equipment will allow a more precise *in vivo* quantification of neuroinflammatory processes, in particular microglial activation, that take place in affected brain structures, and allow correlation of ongoing immune alterations taking place in the brain and in peripheral systems.

CONCLUSION

In the past decades, the development of animal models of PD has greatly contributed to expand our understanding of the disease. Animal models, transgenic, toxin- or viral induced, permit the analyses of more specific pathways and their impact on PD genotype. Today, the role of inflammation and neuroinflammation in the etiology and progression of PD, as well as the knowledge that both must closely interact is well-accepted. However, our understanding on how they communicate and combine to trigger and sustain neuronal cell death still needs to be refined. Comparing longitudinal data from patients and models will help us unravel the complicated mechanisms involved in peripheral-central inflammation crosstalk and open new ways of developing alternative therapeutic strategies to slow down disease progression.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

AUTHOR CONTRIBUTIONS

MF-A reviewed the literature and wrote the first draft of the manuscript. SC and FB reviewed the literature and critically reviewed the manuscript.

FUNDING

This study was supported by a grant from the Italian Ministry of Health (Ricerca Corrente 2017).

REFERENCES

1. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nat Immunol.* (2015) 16:343–53. doi: 10.1038/ni.3123
2. Murphy K. *Innate Immunity*: *The First Line of Defense.* Abingdon: Garland Science, Taylor & Francis group (2012).
3. Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol.* (1989) 54 (Pt. 1):1–13. doi: 10.1101/SQB.1989.054.01.003
4. Mosley RL. Adaptive immunity in neurodegenerative and neuropsychological disorders. *J Neuroimmune Pharmacol.* (2015) 10:522–7. doi: 10.1007/s11481-015-9640-y
5. Kannarkat GT, Boss JM, Tansey MG. The role of innate and adaptive immunity in Parkinson's disease. *J Parkinsons Dis.* (2013) 3:493–514. doi: 10.3233/JPD-130250.
6. Olsen L, Dowd E, Mckernan D. A Role for viral infection in Parkinson's etiology–more than jsut "shaky" evidence? *Neuronal Signal.* (2018) 2:NS20180166. doi: 10.1042/NS20170166
7. Brundin P, Melki R. Prying into the Prion Hypothesis for Parkinson's Disease. *J Neurosci.* (2017) 37:9808–18. doi: 10.1523/JNEUROSCI.1788-16.2017
8. Xu L, Pu J. Alpha-Synuclein in Parkinson's Disease: from pathogenetic dysfunction to potential clinical application. *Parkinsons Dis.* (2016) 2016:1720621. doi: 10.1155/2016/1720621
9. George S, Brundin P. Immunotherapy in Parkinson's Disease: micromanaging alpha-synuclein aggregation. *J Parkinsons Dis.* (2015) 5:413–24. doi: 10.3233/JPD-150630
10. Allen Reish HE, Standaert DG. Role of alpha-synuclein in inducing innate and adaptive immunity in Parkinson disease. *J Parkinsons Dis.* (2015) 5:1–19. doi: 10.3233/JPD-140491
11. Galea I, Bechmann I, Perry VH. What is immune privilege (not)? *Trends Immunol.* (2007) 28:12–8. doi: 10.1016/j.it.2006.11.004
12. Li Q, Barres BA. Microglia and macrophages in brain homeostasis and disease. *Nat Rev Immunol.* (2018) 18:225–42. doi: 10.1038/nri.2017.125
13. Tansey MG, Goldberg MS. Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. *Neurobiol Dis.* (2010) 37:510–8. doi: 10.1016/j.nbd.2009.11.004
14. Sochocka M, Diniz BS, Leszek J. Inflammatory response in the CNS: friend or foe? *Mol Neurobiol.* (2017) 54:8071–89. doi: 10.1007/s12035-016-0297
15. Disabato DJ, Quan N, Godbout JP. Neuroinflammation: the devil is in the details. *J Neurochem.* (2016) 139 (Suppl. 2):136–53. doi: 10.1111/jnc.13607
16. Mcgeer PL, Itagaki S, Boyes BE, Mcgeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology.* (1988) 38:1285–91. doi: 10.1212/WNL.38.8.1285
17. Mcgeer PL, Itagaki S, Mcgeer EG. Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. *Acta Neuropathol.* (1988) 76:550–7. doi: 10.1007/BF00689592
18. Chen H, Jacobs E, Schwarzschild MA, Mccullough ML, Calle EE, Thun MJ, et al. Nonsteroidal antiinflammatory drug use and the risk for Parkinson's disease. *Ann Neurol.* (2005) 58:963–7. doi: 10.1002/ana.20682
19. Gagne JJ, Power MC. Anti-inflammatory drugs and risk of Parkinson disease: a meta-analysis. *Neurology.* (2010) 74:995–1002. doi: 10.1212/WNL.0b013e3181d5a4a3
20. Gao X, Chen H, Schwarzschild MA, Ascherio A. Use of ibuprofen and risk of Parkinson disease. *Neurology.* (2011) 76:863–9. doi: 10.1212/WNL.0b013e31820f2d79
21. Poly TN, Islam MMR, Yang HC, Li YJ. Non-steroidal anti-inflammatory drugs and risk of Parkinson's disease in the elderly population: a meta-analysis. *Eur J Clin Pharmacol.* (2019) 75:99–108. doi: 10.1007/s00228-018-2561-y
22. Ren L, Yi J, Yang J, Li P, Cheng X, Mao P. Nonsteroidal anti-inflammatory drugs use and risk of Parkinson disease: a dose-response meta-analysis. *Medicine.* (2018) 97:e12172. doi: 10.1097/MD.0000000000012172
23. Bok E, Cho EJ, Chung ES, Shin WH, Jin BK. Interleukin-4 Contributes to Degeneration of Dopamine Neurons in the Lipopolysaccharide-treated Substantia Nigra *in vivo*. *Exp Neurobiol.* (2018) 27:309–19. doi: 10.5607/en.2018.27.4.309
24. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature.* (1997) 388:839–40. doi: 10.1038/42166
25. Breger LS, Fuzzati Armentero MT. Genetically engineered animal models of Parkinson's disease: from worm to rodent. *Eur J Neurosci.* (2018) 49:533–60. doi: 10.1111/ejn.14300
26. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci.* (2007) 10:1538–43. doi: 10.1038/nn2014
27. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science.* (2010) 330:841–5. doi: 10.1126/science.1194637
28. Askew K, Li K, Olmos-Alonso A, Garcia-Moreno F, Liang Y, Richardson P, et al. Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain. *Cell Rep.* (2017) 18:391–405. doi: 10.1016/j.celrep.2016.12.041
29. Tay TL, Mai D, Dautzenberg J, Fernandez-Klett F, Lin G, Sagar, Datta M, et al. A new fate mapping system reveals context-dependent random or clonal expansion of microglia. *Nat Neurosci.* (2017) 20:793–803. doi: 10.1038/nn.4547
30. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, et al. ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat Neurosci.* (2005) 8:752–8. doi: 10.1038/nn1472
31. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science.* (2005) 308:1314–8. doi: 10.1126/science.1110647
32. Wolf SA, Boddeke HW, Kettenmann H. Microglia in Physiology and Disease. *Annu Rev Physiol.* (2017) 79:619–43. doi: 10.1146/annurev-physiol-022516-034406
33. Deczkowska A, Amit I, Schwartz M. Microglial immune checkpoint mechanisms. *Nat Neurosci.* (2018) 21:779–86. doi: 10.1038/s41593-018-0145-x
34. Hickman SE, Kingery ND, Ohsumi TK, Borowsky ML, Wang LC, Means TK, et al. The microglial sensome revealed by direct RNA sequencing. *Nat Neurosci.* (2013) 16:1896–905. doi: 10.1038/nn.3554
35. Tay TL, Savage JC, Hui CW, Bisht K, Tremblay ME. Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. *J Physiol.* (2017) 595:1929–45. doi: 10.1113/JP272134
36. Joers V, Tansey MG, Mulas G, Carta AR. Microglial phenotypes in Parkinson's disease and animal models of the disease. *Prog Neurobiol.* (2017) 155:57–75. doi: 10.1016/j.pneurobio.2016.04.006
37. Imamura K, Hishikawa N, Sawada M, Nagatsu T, Yoshida M, Hashizume Y. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathol.* (2003) 106:518–26. doi: 10.1007/s00401-003-0766-2
38. Doorn KJ, Moors T, Drukarch B, Van De Berg W, Lucassen PJ, Van Dam AM. Microglial phenotypes and toll-like receptor 2 in the substantia nigra and hippocampus of incidental Lewy body disease cases and Parkinson's disease patients. *Acta Neuropathol Commun.* (2014) 2:90. doi: 10.1186/s40478-014-0090-1
39. Hamza TH, Zabetian CP, Tenesa A, Laederach A, Montimurro J, Yearout D, et al. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nat Genet.* (2010) 42:781–5. doi: 10.1038/ng.642
40. Pierce S, Coetzee GA. Parkinson's disease-associated genetic variation is linked to quantitative expression of inflammatory genes. *PLoS ONE.* (2017) 12:e0175882. doi: 10.1371/journal.pone.0175882
41. Mondelli V, Vernon AC, Turkheimer F, Dazzan P, Pariante CM. Brain microglia in psychiatric disorders. *Lancet Psychiatry.* (2017) 4:563–72. doi: 10.1016/S2215-0366(17)30101-3
42. Gerhard A, Pavese N, Hotton G, Turkheimer F, Es M, Hammers A, et al. *In vivo* imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol Dis.* (2006) 21:404–12. doi: 10.1016/j.nbd.2005.08.002
43. Iannaccone S, Cerami C, Alessio M, Garibotto V, Panzacchi A, Olivieri S, et al. *In vivo* microglia activation in very early dementia with Lewy bodies, comparison with Parkinson's disease. *Parkinsonism Relat Disord.* (2013) 19:47–52. doi: 10.1016/j.parkreldis.2012.07.002

44. Ouchi Y, Yoshikawa E, Sekine Y, Futatsubashi M, Kanno T, Ogusu T, et al. Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann Neurol.* (2005) 57:168–75. doi: 10.1002/ana.20338
45. Owen DR, Yeo AJ, Gunn RN, Song K, Wadsworth G, Lewis A, et al. An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *J Cereb Blood Flow Metab.* (2012) 32:1–5. doi: 10.1038/jcbfm.2011.147
46. Varnas K, Cselenyi Z, Jucaite A, Halldin C, Svenningsson P, Farde L, et al. PET imaging of [(11)C]PBR28 in Parkinson's disease patients does not indicate increased binding to TSPO despite reduced dopamine transporter binding. *Eur J Nucl Med Mol Imaging.* (2019) 46:367–75. doi: 10.1007/s00259-018-4161-6
47. Tsacopoulos M, Magistretti PJ. Metabolic coupling between glia and neurons. *J Neurosci.* (1996) 16:877–85. doi: 10.1523/JNEUROSCI.16-03-00877.1996
48. Mederos S, Gonzalez-Arias C, Perea G. Astrocyte-neuron networks: a multilane highway of signaling for homeostatic brain function. *Front Synaptic Neurosci.* (2018) 10:45. doi: 10.3389/fnsyn.2018.00045
49. Braak H, Sastre M, Del Tredici K. Development of alpha-synuclein immunoreactive astrocytes in the forebrain parallels stages of intraneuronal pathology in sporadic Parkinson's disease. *Acta Neuropathol.* (2007) 114:231–41. doi: 10.1007/s00401-007-0244-3
50. Damier P, Hirsch EC, Zhang P, Agid Y, Javoy-Agid F. Glutathione peroxidase, glial cells and Parkinson's disease. *Neuroscience.* (1993) 52:1–6. doi: 10.1016/0306-4522(93)90175-F
51. Gray MT, Woulfe JM. Striatal blood-brain barrier permeability in Parkinson's disease. *J Cereb Blood Flow Metab.* (2015) 35:747–50. doi: 10.1038/jcbfm.2015.32
52. Ambrosi G, Kustrimovic N, Siani F, Rasini E, Cerri S, Ghezzi C, et al. Complex changes in the innate and adaptive immunity accompany progressive degeneration of the nigrostriatal pathway induced by intrastriatal injection of 6-hydroxydopamine in the rat. *Neurotox Res.* (2017) 32:71–81. doi: 10.1007/s12640-017-9712-2
53. Armentero MT, Levandis G, Nappi G, Bazzini E, Blandini F. Peripheral inflammation and neuroprotection: systemic pretreatment with complete Freund's adjuvant reduces 6-hydroxydopamine toxicity in a rodent model of Parkinson's disease. *Neurobiol Dis.* (2006) 24:492–505. doi: 10.1016/j.nbd.2006.08.016
54. Siani F, Greco R, Levandis G, Ghezzi C, Daviddi F, Demartini C, et al. Influence of estrogen modulation on glia activation in a murine model of Parkinson's Disease. *Front Neurosci.* (2017) 11:306. doi: 10.3389/fnins.2017.00306
55. Dickens AM, Vainio S, Marjamaki P, Johansson J, Lehtiniemi P, Rokka J, et al. Detection of microglial activation in an acute model of neuroinflammation using PET and radiotracers 11C-(R)-PK11195 and 18F-GE-180. *J Nucl Med.* (2014) 55:466–72. doi: 10.2967/jnumed.113.125625
56. Vazquez-Claverie M, Garrido-Gil P, San Sebastian W, Izal-Azcarate A, Belzunegui S, Marcilla I, et al. Acute and chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administrations elicit similar microglial activation in the substantia nigra of monkeys. *J Neuropathol Exp Neurol.* (2009) 68:977–84. doi: 10.1097/NEN.0b013e3181b35e41
57. Belloli S, Pannese M, Buonsanti C, Maiorino C, Di Grigoli G, Carpinelli A, et al. Early upregulation of 18-kDa translocator protein in response to acute neurodegenerative damage in TREM2-deficient mice. *Neurobiol Aging.* (2017) 53:159–68. doi: 10.1016/j.neurobiolaging.2017.01.010
58. Pan-Montojo F, Anichtchik O, Dening Y, Knels L, Pursche S, Jung R, et al. Progression of Parkinson's disease pathology is reproduced by intragastric administration of rotenone in mice. *PLoS ONE.* (2010) 5:e8762. doi: 10.1371/journal.pone.0008762
59. Pan-Montojo F, Funk RH. Implications of Parkinson's disease pathophysiology for the development of cell replacement strategies and drug discovery in neurodegenerative diseases. *CNS Neurol Disord Drug Targets.* (2012) 11:907–20. doi: 10.2174/1871527311201070907
60. Sherer TB, Kim JH, Betarbet R, Greenamyre JT. Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. *Exp Neurol.* (2003) 179:9–16. doi: 10.1006/exnr.2002.8072
61. Marinova-Mutafchieva L, Sadeghian M, Broom L, Davis JB, Medhurst AD, Dexter DT. Relationship between microglial activation and dopaminergic neuronal loss in the substantia nigra: a time course study in a 6-hydroxydopamine model of Parkinson's disease. *J Neurochem.* (2009) 110:966–75. doi: 10.1111/j.1471-4159.2009.06189.x
62. Cicchetti F, Brownell AL, Williams K, Chen YI, Livni E, Isacson O. Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging. *Eur J Neurosci.* (2002) 15:991–8. doi: 10.1046/j.1460-9568.2002.01938.x
63. Mcgeer PL, Schwab C, Parent A, Doudet D. Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration. *Ann Neurol.* (2003) 54:599–604. doi: 10.1002/ana.10728
64. Schintu N, Frau L, Ibba M, Garau A, Carboni E, Carta AR. Progressive dopaminergic degeneration in the chronic MPTPp mouse model of Parkinson's disease. *Neurotox Res.* (2009) 16:127–39. doi: 10.1007/s12640-009-9061-x
65. Yasuda Y, Shinagawa R, Yamada M, Mori T, Tateishi N, Fujita S. Long-lasting reactive changes observed in microglia in the striatal and substantia nigral of mice after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Brain Res.* (2007) 1138:196–202. doi: 10.1016/j.brainres.2006.12.054
66. Kurkowska-Jastrzebska I, Wronska A, Kohutnicka M, Czlonkowski A, Czlonkowska A. The inflammatory reaction following 1-methyl-4-phenyl-1,2,3,6–tetrahydropyridine intoxication in mouse. *Exp Neurol.* (1999) 156:50–61. doi: 10.1006/exnr.1998.6993
67. Pisanu A, Lecca D, Mulas G, Wardas J, Simbula G, Spiga S, et al. Dynamic changes in pro- and anti-inflammatory cytokines in microglia after PPAR-gamma agonist neuroprotective treatment in the MPTPp mouse model of progressive Parkinson's disease. *Neurobiol Dis.* (2014) 71:280–91. doi: 10.1016/j.nbd.2014.08.011
68. Betarbet R, Sherer TB, Mackenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci.* (2000) 3:1301–6. doi: 10.1038/81834
69. Cicchetti F, Drouin-Ouellet J, Gross RE. Environmental toxins and Parkinson's disease: what have we learned from pesticide-induced animal models? *Trends Pharmacol Sci.* (2009) 30:475–83. doi: 10.1016/j.tips.2009.06.005
70. Ojha S, Javed H, Azimullah S, Abul Khair SB, Haque ME. Neuroprotective potential of ferulic acid in the rotenone model of Parkinson's disease. *Drug Des Devel Ther.* (2015) 9:5499–510. doi: 10.2147/DDDT.S90616
71. Dutta G, Zhang P, Liu B. The lipopolysaccharide Parkinson's disease animal model: mechanistic studies and drug discovery. *Fundam Clin Pharmacol.* (2008) 22:453–64. doi: 10.1111/j.1472-8206.2008.00616.x
72. Thomas RC, Bath MF, Stover CM, Lambert DG, Thompson JP. Exploring LPS-induced sepsis in rats and mice as a model to study potential protective effects of the nociceptin/orphanin FQ system. *Peptides.* (2014) 61:56–60. doi: 10.1016/j.peptides.2014.08.009
73. Hauss-Wegrzyniak B, Lukovic L, Bigaud M, Stoeckel ME. Brain inflammatory response induced by intracerebroventricular infusion of lipopolysaccharide: an immunohistochemical study. *Brain Res.* (1998) 794:211–24. doi: 10.1016/S0006-8993(98)00227-3
74. Liu M, Bing G. Lipopolysaccharide animal models for Parkinson's disease. *Parkinsons Dis.* (2011) 2011:327089. doi: 10.4061/2011/327089
75. Beier EE, Neal M, Alam G, Edler M, Wu LJ, Richardson JR. Alternative microglial activation is associated with cessation of progressive dopamine neuron loss in mice systemically administered lipopolysaccharide. *Neurobiol Dis.* (2017) 108:115–27. doi: 10.1016/j.nbd.2017.08.009
76. Su X, Maguire-Zeiss KA, Giuliano R, Prifti L, Venkatesh K, Federoff HJ. Synuclein activates microglia in a model of Parkinson's disease. *Neurobiol Aging.* (2008) 29:1690–701. doi: 10.1016/j.neurobiolaging.2007.04.006
77. Watson MB, Richter F, Lee SK, Gabby L, Wu J, Masliah E, et al. Regionally-specific microglial activation in young mice over-expressing human wildtype alpha-synuclein. *Exp Neurol.* (2012) 237:318–34. doi: 10.1016/j.expneurol.2012.06.025
78. Volpicelli-Daley LA, Kirik D, Stoyka LE, Standaert DG, Harms AS. How can rAAV-alpha-synuclein and the fibril alpha-synuclein models advance our understanding of Parkinson's disease? *J Neurochem.* (2016) 139 (Suppl. 1):131–55. doi: 10.1111/jnc.13627

79. Eslamboli A, Romero-Ramos M, Burger C, Bjorklund T, Muzyczka N, Mandel RJ, et al. Long-term consequences of human alpha-synuclein overexpression in the primate ventral midbrain. *Brain.* (2007) 130:799–815. doi: 10.1093/brain/awl382
80. Harms AS, Cao S, Rowse AL, Thome AD, Li X, Mangieri LR, et al. MHCII is required for alpha-synuclein-induced activation of microglia, CD4 T cell proliferation, and dopaminergic neurodegeneration. *J Neurosci.* (2013) 33:9592–600. doi: 10.1523/JNEUROSCI.5610-12.2013
81. Sanchez-Guajardo V, Febbraro F, Kirik D, Romero-Ramos M. Microglia acquire distinct activation profiles depending on the degree of alpha-synuclein neuropathology in a rAAV based model of Parkinson's disease. *PLoS ONE.* (2010) 5:e8784. doi: 10.1371/journal.pone.0008784
82. Theodore S, Cao S, Mclean PJ, Standaert DG. Targeted overexpression of human alpha-synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. *J Neuropathol Exp Neurol.* (2008) 67:1149–58. doi: 10.1097/NEN.0b013e31818e5e99
83. Barkholt P, Sanchez-Guajardo V, Kirik D, Romero-Ramos M. Long-term polarization of microglia upon alpha-synuclein overexpression in nonhuman primates. *Neuroscience.* (2012) 208:85–96. doi: 10.1016/j.neuroscience.2012.02.004
84. Ferreira SA, Romero-Ramos M. Microglia Response During Parkinson's Disease: alpha-synuclein intervention. *Front Cell Neurosci.* (2018) 12:247. doi: 10.3389/fncel.2018.00247
85. Shechter R, London A, Schwartz M. Orchestrated leukocyte recruitment to immune-privileged sites: absolute barriers versus educational gates. *Nat Rev Immunol.* (2013) 13:206–18. doi: 10.1038/nri3391
86. Cabezas R, Avila M, Gonzalez J, El-Bacha RS, Baez E, Garcia-Segura LM, et al. Astrocytic modulation of blood brain barrier: perspectives on Parkinson's disease. *Front Cell Neurosci.* (2014) 8:211. doi: 10.3389/fncel.2014.00211
87. Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron.* (2008) 57:178–201. doi: 10.1016/j.neuron.2008.01.003
88. Goldmann T, Wieghofer P, Jordao MJ, Prutek F, Hagemeyer N, Frenzel K, et al. Origin, fate and dynamics of macrophages at central nervous system interfaces. *Nat Immunol.* (2016) 17:797–805. doi: 10.1038/ni.3423
89. Falcao AM, Marques F, Novais A, Sousa N, Palha JA, Sousa JC. The path from the choroid plexus to the subventricular zone: go with the flow! *Front Cell Neurosci.* (2012) 6:34. doi: 10.3389/fncel.2012.00034
90. Emerich DF, Skinner SJ, Borlongan CV, Vasconcellos AV, Thanos CG. The choroid plexus in the rise, fall and repair of the brain. *Bioessays.* (2005) 27:262–74. doi: 10.1002/bies.20193
91. Johanson CE, Stopa EG, Mcmillan PN. The blood-cerebrospinal fluid barrier: structure and functional significance. *Methods Mol Biol.* (2011) 686:101–31. doi: 10.1007/978-1-60761-938-3_4
92. Svenningsson A, Andersen O, Edsbagge M, Stemme S. Lymphocyte phenotype and subset distribution in normal cerebrospinal fluid. *J Neuroimmunol.* (1995) 63:39–46. doi: 10.1016/0165-5728(95)00126-3
93. Derecki NC, Cardani AN, Yang CH, Quinnies KM, Crihfield A, Lynch KR, et al. Regulation of learning and memory by meningeal immunity: a key role for IL-4. *J Exp Med.* (2010) 207:1067–80. doi: 10.1084/jem.20091419
94. Benakis C, Llovera G, Liesz A. The meningeal and choroidal infiltration routes for leukocytes in stroke. *Ther Adv Neurol Disord.* (2018) 11:1756286418783708. doi: 10.1177/1756286418783708
95. Herz J, Filiano AJ, Smith A, Yogev N, Kipnis J. Myeloid cells in the central nervous system. *Immunity.* (2017) 46:943–56. doi: 10.1016/j.immuni.2017.06.007
96. Korin B, Ben-Shaanan TL, Schiller M, Dubovik T, Azulay-Debby H, Boshnak NT, et al. High-dimensional, single-cell characterization of the brain's immune compartment. *Nat Neurosci.* (2017) 20:1300–9. doi: 10.1038/nn.4610
97. Kortekaas R, Leenders KL, Van Oostrom JC, Vaalburg W, Bart J, Willemsen AT, et al. Blood-brain barrier dysfunction in Parkinsonian midbrain *in vivo*. *Ann Neurol.* (2005) 57:176–9. doi: 10.1002/ana.20369
98. Bartels AL, Willemsen AT, Kortekaas R, De Jong BM, De Vries R, De Klerk O, et al. Decreased blood-brain barrier P-glycoprotein function in the progression of Parkinson's disease, PSP and MSA. *J Neural Transm.* (2008) 115:1001–9. doi: 10.1007/s00702-008-0030-y
99. Pienaar IS, Lee CH, Elson JL, Mcguinness L, Gentleman SM, Kalaria RN, et al. Deep-brain stimulation associates with improved microvascular integrity in the subthalamic nucleus in Parkinson's disease. *Neurobiol Dis.* (2015) 74:392–405. doi: 10.1016/j.nbd.2014.12.006
100. Desai Bradaric B, Patel A, Schneider JA, Carvey PM, Hendey B. Evidence for angiogenesis in Parkinson's disease, incidental Lewy body disease, and progressive supranuclear palsy. *J Neural Transm.* (2012) 119:59–71. doi: 10.1007/s00702-011-0684-8
101. Wada K, Arai H, Takanashi M, Fukae J, Oizumi H, Yasuda T, et al. Expression levels of vascular endothelial growth factor and its receptors in Parkinson's disease. *Neuroreport.* (2006) 17:705–9. doi: 10.1097/01.wnr.0000215769.71657.65
102. Janelidze S, Lindqvist D, Francardo V, Hall S, Zetterberg H, Blennow K, et al. Increased CSF biomarkers of angiogenesis in Parkinson disease. *Neurology.* (2015) 85:1834–42. doi: 10.1212/WNL.0000000000002151
103. Pisani V, Stefani A, Pierantozzi M, Natoli S, Stanzione P, Franciotta D, et al. Increased blood-cerebrospinal fluid transfer of albumin in advanced Parkinson's disease. *J Neuroinflammation.* (2012) 9:188. doi: 10.1186/1742-2094-9-188
104. Al-Bachari S, Vidyasagar R, Emsley HC, Parkes LM. Structural and physiological neurovascular changes in idiopathic Parkinson's disease and its clinical phenotypes. *J Cereb Blood Flow Metab.* (2017) 37:3409–21. doi: 10.1177/0271678X16688919
105. Ham JH, Yi H, Sunwoo MK, Hong JY, Sohn YH, Lee PH. Cerebral microbleeds in patients with Parkinson's disease. *J Neurol.* (2014) 261:1628–35. doi: 10.1007/s00415-014-7403-y
106. Garcia-Dominguez I, Vesela K, Garcia-Revilla J, Carrillo-Jimenez A, Roca-Ceballos MA, Santiago M, et al. Peripheral inflammation enhances microglia response and nigral dopaminergic cell death in an *in vivo* MPTP Model of Parkinson's Disease. *Front Cell Neurosci.* (2018) 12:398. doi: 10.3389/fncel.2018.00398
107. Olmedo-Diaz S, Estevez-Silva H, Oradd G, Af Bjerken S, Marcellino D, Virel A. An altered blood-brain barrier contributes to brain iron accumulation and neuroinflammation in the 6-OHDA rat model of Parkinson's disease. *Neuroscience.* (2017) 362:141–51. doi: 10.1016/j.neuroscience.2017.08.023
108. Armentero MT, Levandis G, Bazzini E, Cerri S, Ghezzi C, Blandini F. Adhesion molecules as potential targets for neuroprotection in a rodent model of Parkinson's disease. *Neurobiol Dis.* (2011) 43:663–8. doi: 10.1016/j.nbd.2011.05.017
109. Carvey PM, Zhao CH, Hendey B, Lum H, Trachtenberg J, Desai BS, et al. 6-Hydroxydopamine-induced alterations in blood-brain barrier permeability. *Eur J Neurosci.* (2005) 22:1158–68. doi: 10.1111/j.1460-9568.2005.04281.x
110. Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, et al. Infiltration of $CD4^+$ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest.* (2009) 119:182–92. doi: 10.1172/JCI36470
111. Miklossy J, Doudet DD, Schwab C, Yu S, Mcgeer EG, Mcgeer PL. Role of ICAM-1 in persisting inflammation in Parkinson disease and MPTP monkeys. *Exp Neurol.* (2006) 197:275–83. doi: 10.1016/j.expneurol.2005.10.034
112. Barcia C, Bautista V, Sanchez-Bahillo A, Fernandez-Villalba E, Faucheux B, Poza Y Poza M, et al. Changes in vascularization in substantia nigra pars compacta of monkeys rendered Parkinsonian. *J Neural Transm.* (2005) 112:1237–48. doi: 10.1007/s00702-004-0256-2
113. Thiollier T, Wu C, Contamin H, Li Q, Zhang J, Bezard E. Permeability of blood-brain barrier in macaque model of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson disease. *Synapse.* (2016) 70:231–9. doi: 10.1002/syn.21889
114. Chung YC, Shin WH, Baek JY, Cho EJ, Baik HH, Kim SR, et al. CB2 receptor activation prevents glial-derived neurotoxic mediator production, BBB leakage and peripheral immune cell infiltration and rescues dopamine neurons in the MPTP model of Parkinson's disease. *Exp Mol Med.* (2016) 48:e205. doi: 10.1038/emm.2015.100
115. Ravenstijn PG, Merlini M, Hameetman M, Murray TK, Ward MA, Lewis H, et al. The exploration of rotenone as a toxin for inducing Parkinson's disease in rats, for application in BBB transport and PK-PD experiments. *J Pharmacol Toxicol Methods.* (2008) 57:114–30. doi: 10.1016/j.vascn.2007.10.003

116. Banks WA. Blood-brain barrier transport of cytokines: a mechanism for neuropathology. *Curr Pharm Des.* (2005) 11:973–84. doi: 10.2174/1381612053381684
117. Hunot S, Dugas N, Faucheux B, Hartmann A, Tardieu M, Debre P, et al. FcepsilonRII/CD23 is expressed in Parkinson's disease and induces, *in vitro*, production of nitric oxide and tumor necrosis factor-alpha in glial cells. *J Neurosci.* (1999) 19:3440–7. doi: 10.1523/JNEUROSCI.19-09-03440.1999
118. Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T. Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci Lett.* (1996) 211:13–6. doi: 10.1016/0304-3940(96)12706-3
119. Mogi M, Harada M, Kondo T, Narabayashi H, Riederer P, Nagatsu T. Transforming growth factor-beta 1 levels are elevated in the striatum and in ventricular cerebrospinal fluid in Parkinson's disease. *Neurosci Lett.* (1995) 193:129–32. doi: 10.1016/0304-3940(95)11686-Q
120. Mogi M, Harada M, Riederer P, Narabayashi H, Fujita K, Nagatsu T. Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from Parkinsonian patients. *Neurosci Lett.* (1994) 165:208–10. doi: 10.1016/0304-3940(94)90746-3
121. Shimoji M, Pagan F, Healton EB, Mocchetti I. CXCR4 and CXCL12 expression is increased in the nigro-striatal system of Parkinson's disease. *Neurotox Res.* (2009) 16:318–28. doi: 10.1007/s12640-009-9076-3
122. Brodacki B, Staszewski J, Toczylowska B, Kozlowska E, Drela N, Chalimoniuk M, et al. Serum interleukin (IL-2, IL-10, IL-6, IL-4), TNFalpha, and INFgamma concentrations are elevated in patients with atypical and idiopathic parkinsonism. *Neurosci Lett.* (2008) 441:158–62. doi: 10.1016/j.neulet.2008.06.040
123. Choi C, Jeong JH, Jang JS, Choi K, Lee J, Kwon J, et al. Multiplex analysis of cytokines in the serum and cerebrospinal fluid of patients with Alzheimer's disease by color-coded bead technology. *J Clin Neurol.* (2008) 4:84–8. doi: 10.3988/jcn.2008.4.2.84
124. Dursun E, Gezen-Ak D, Hanagasi H, Bilgic B, Lohmann E, Ertan S, et al. The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. *J Neuroimmunol.* (2015) 283:50–7. doi: 10.1016/j.jneuroim.2015.04.014
125. Hall S, Janelidze S, Surova Y, Widner H, Zetterberg H, Hansson O. Cerebrospinal fluid concentrations of inflammatory markers in Parkinson's disease and atypical Parkinsonian disorders. *Sci Rep.* (2018) 8:13276. doi: 10.1038/s41598-018-31517-z
126. Williams-Gray CH, Wijeyekoon R, Yarnall AJ, Lawson RA, Breen DP, Evans JR, et al. Serum immune markers and disease progression in an incident Parkinson's disease cohort (ICICLE-PD). *Mov Disord.* (2016) 31:995–1003. doi: 10.1002/mds.26563
127. Qin XY, Zhang SP, Cao C, Loh YP, Cheng Y. Aberrations in Peripheral Inflammatory Cytokine Levels in Parkinson Disease: a systematic review and meta-analysis. *JAMA Neurol.* (2016) 73:1316–24. doi: 10.1001/jamaneurol.2016.2742
128. Eidson LN, Kannarkat GT, Barnum CJ, Chang J, Chung J, Caspell-Garcia C, et al. Candidate inflammatory biomarkers display unique relationships with alpha-synuclein and correlate with measures of disease severity in subjects with Parkinson's disease. *J Neuroinflammation.* (2017) 14:164. doi: 10.1186/s12974-017-0935-1
129. Karpenko MN, Vasilishina AA, Gromova EA, Muruzheva ZM, Bernadotte A. Interleukin-1beta, interleukin-1 receptor antagonist, interleukin-6, interleukin-10, and tumor necrosis factor-alpha levels in CSF and serum in relation to the clinical diversity of Parkinson's disease. *Cell Immunol.* (2018) 327:77–82. doi: 10.1016/j.cellimm.2018.02.011
130. Tang P, Chong L, Li X, Liu Y, Liu P, Hou C, et al. Correlation between serum RANTES levels and the severity of Parkinson's disease. *Oxid Med Cell Longev.* (2014) 2014:208408. doi: 10.1155/2014/208408
131. Rocha NP, Scalzo PL, Barbosa IG, Souza MS, Morato IB, Vieira EL, et al. Cognitive status correlates with CXCL10/IP-10 Levels in Parkinson's Disease. *Parkinsons Dis.* (2014) 2014:903796. doi: 10.1155/2014/903796
132. Linquist S, Saylor B, Cottenie K, Elliott TA, Kremer SC, Gregory TR. Distinguishing ecological from evolutionary approaches to transposable elements. *Biol Rev Camb Philos Soc.* (2013) 88:573–84. doi: 10.1111/brv.12017
133. Shi M, Bradner J, Hancock AM, Chung KA, Quinn JF, Peskind ER, et al. Cerebrospinal fluid biomarkers for Parkinson disease diagnosis and progression. *Ann Neurol.* (2011) 69:570–80. doi: 10.1002/ana.22311
134. Schroder JB, Pawlowski M, Meyer Zu Horste G, Gross CC, Wiendl H, Meuth SG, et al. Immune cell activation in the cerebrospinal fluid of patients with Parkinson's Disease. *Front Neurol.* (2018) 9:1081. doi: 10.3389/fneur.2018.01081
135. Mogi M, Harada M, Kondo T, Riederer P, Inagaki H, Minami M, et al. Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. *Neurosci Lett.* (1994) 180:147–50. doi: 10.1016/0304-3940(94)90508-8
136. Boka G, Anglade P, Wallach D, Javoy-Agid F, Agid Y, Hirsch EC. Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. *Neurosci Lett.* (1994) 172:151–4. doi: 10.1016/0304-3940(94)90684-X
137. McCoy MK, Martinez TN, Ruhn KA, Szymkowski DE, Smith CG, Botterman BR, et al. Blocking soluble tumor necrosis factor signaling with dominant-negative tumor necrosis factor inhibitor attenuates loss of dopaminergic neurons in models of Parkinson's disease. *J Neurosci.* (2006) 26:9365–75. doi: 10.1523/JNEUROSCI.1504-06.2006
138. Pott Godoy MC, Tarelli R, Ferrari CC, Sarchi MI, Pitossi FJ. Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease. *Brain.* (2008) 131:1880–94. doi: 10.1093/brain/awn101
139. Koprich JB, Reske-Nielsen C, Mithal P, Isacson O. Neuroinflammation mediated by IL-1beta increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease. *J Neuroinflammation.* (2008) 5:8. doi: 10.1186/1742-2094-5-8
140. Chien CH, Lee MJ, Liou HC, Liou HH, Fu WM. Microglia-Derived Cytokines/Chemokines Are Involved in the Enhancement of LPS-Induced Loss of Nigrostriatal Dopaminergic Neurons in DJ-1 Knockout Mice. *PLoS ONE.* (2016) 11:e0151569. doi: 10.1371/journal.pone.0151569
141. Frank-Cannon TC, Tran T, Ruhn KA, Martinez TN, Hong J, Marvin M, et al. Parkin deficiency increases vulnerability to inflammation-related nigral degeneration. *J Neurosci.* (2008) 28:10825–34. doi: 10.1523/JNEUROSCI.3001-08.2008
142. Depino AM, Earl C, Kaczmarczyk E, Ferrari C, Besedovsky H, Del Rey A, et al. Microglial activation with atypical proinflammatory cytokine expression in a rat model of Parkinson's disease. *Eur J Neurosci.* (2003) 18:2731–42. doi: 10.1111/j.1460-9568.2003.03014.x
143. Vairetti M, Ferrigno A, Rizzo V, Ambrosi G, Bianchi A, Richelmi P, et al. Impaired hepatic function and central dopaminergic denervation in a rodent model of Parkinson's disease: a self-perpetuating crosstalk? *Biochim Biophys Acta.* (2012) 1822:176–84. doi: 10.1016/j.bbadis.2011.11.008
144. Barcia C, Ros CM, Annese V, Gomez A, Ros-Bernal F, Aguado-Yera D, et al. IFN-gamma signaling, with the synergistic contribution of TNF-alpha, mediates cell specific microglial and astroglial activation in experimental models of Parkinson's disease. *Cell Death Dis.* (2011) 2:e142. doi: 10.1038/cddis.2011.17
145. Nagatsu T, Mogi M, Ichinose H, Togari A. Changes in cytokines and neurotrophins in Parkinson's disease. *J Neural Transm Suppl.* (2000) 277–90. doi: 10.1007/978-3-7091-6301-6_19
146. Sriram K, Matheson JM, Benkovic SA, Miller DB, Luster MI, O'callaghan JP. Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: implications for Parkinson's disease. *FASEB J.* (2002) 16:1474–6. doi: 10.1096/fj.02-0216fje
147. Mount MP, Lira A, Grimes D, Smith PD, Faucher S, Slack R, et al. Involvement of interferon-gamma in microglial-mediated loss of dopaminergic neurons. *J Neurosci.* (2007) 27:3328–37. doi: 10.1523/JNEUROSCI.5321-06.2007
148. Barcia C, Ros CM, Annese V, Gomez A, Ros-Bernal F, Aguado-Llera D, et al. IFN-gamma signaling, with the synergistic contribution of TNF-alpha, mediates cell specific microglial and astroglial activation in experimental models of Parkinson's disease. *Cell Death Dis.* (2012) 3:e379. doi: 10.1038/cddis.2012.123

149. Bian MJ, Li LM, Yu M, Fei J, Huang F. Elevated interleukin-1beta induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine aggravating dopaminergic neurodegeneration in old male mice. *Brain Res.* (2009) 1302:256–64. doi: 10.1016/j.brainres.2009.07.030
150. Lofrumento DD, Saponaro C, Cianciulli A, De Nuccio F, Mitolo V, Nicolardi G, et al. MPTP-induced neuroinflammation increases the expression of pro-inflammatory cytokines and their receptors in mouse brain. *Neuroimmunomodulation.* (2011) 18:79–88. doi: 10.1159/000320027
151. Yasuda Y, Shimoda T, Uno K, Tateishi N, Furuya S, Yagi K, et al. The effects of MPTP on the activation of microglia/astrocytes and cytokine/chemokine levels in different mice strains. *J Neuroimmunol.* (2008) 204:43–51. doi: 10.1016/j.jneuroim.2008.08.003
152. Luchtman DW, Shao D, Song C. Behavior, neurotransmitters and inflammation in three regimens of the MPTP mouse model of Parkinson's disease. *Physiol Behav.* (2009) 98:130–8. doi: 10.1016/j.physbeh.2009.04.021
153. Zhou T, Zhu M, Liang Z. (-)-Epigallocatechin-3-gallate modulates peripheral immunity in the MPTP-induced mouse model of Parkinson's disease. *Mol Med Rep.* (2018) 17:4883–8. doi: 10.3892/mmr.2018.8470
154. Chandra G, Rangasamy SB, Roy A, Kordower JH, Pahan K. Neutralization of RANTES and eotaxin prevents the loss of dopaminergic neurons in a mouse model of Parkinson Disease. *J Biol Chem.* (2016) 291:15267–81. doi: 10.1074/jbc.M116.714824
155. Chandra G, Roy A, Rangasamy SB, Pahan K. Induction of adaptive immunity leads to nigrostriatal disease progression in MPTP Mouse Model of Parkinson's Disease. *J Immunol.* (2017) 198:4312–26. doi: 10.4049/jimmunol.1700149
156. Appay V, Rowland-Jones SL. RANTES: a versatile and controversial chemokine. *Trends Immunol.* (2001) 22:83–7. doi: 10.1016/S1471-4906(00)01812-3
157. Wada T, Furuichi K, Sakai N, Shimizu M, Segawa C, Kobayashi K, et al. Eotaxin contributes to renal interstitial eosinophilia. *Nephrol Dial Transplant.* (1999) 14:76–80. doi: 10.1093/ndt/14.1.76
158. Roy A, Mondal S, Kordower JH, Pahan K. Attenuation of microglial RANTES by NEMO-binding domain peptide inhibits the infiltration of CD8(+) T cells in the nigra of Hemiparkinsonian monkey. *Neuroscience.* (2015) 302:36–46. doi: 10.1016/j.neuroscience.2015.03.011
159. Roy A, Fung YK, Liu X, Pahan K. Up-regulation of microglial CD11b expression by nitric oxide. *J Biol Chem.* (2006) 281:14971–80. doi: 10.1074/jbc.M600236200
160. Roy A, Jana A, Yatish K, Freidt MB, Fung YK, Martinson JA, et al. Reactive oxygen species up-regulate CD11b in microglia via nitric oxide: Implications for neurodegenerative diseases. *Free Radic Biol Med.* (2008) 45:686–99. doi: 10.1016/j.freeradbiomed.2008.05.026
161. Perry VH, Holmes C. Microglial priming in neurodegenerative disease. *Nat Rev Neurol.* (2014) 10:217–24. doi: 10.1038/nrneurol.2014.38
162. Perry VH. Stress primes microglia to the presence of systemic inflammation: implications for environmental influences on the brain. *Brain Behav Immun.* (2007) 21:45–6. doi: 10.1016/j.bbi.2006.08.004
163. Cunningham C, Campion S, Lunnon K, Murray CL, Woods JF, Deacon RM, et al. Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease. *Biol Psychiatry*. (2009) 65:304–12. doi: 10.1016/j.biopsych.2008.07.024
164. Dasgupta S, Jana M, Liu X, Pahan K. Role of very-late antigen-4 (VLA-4) in myelin basic protein-primed T cell contact-induced expression of proinflammatory cytokines in microglial cells. *J Biol Chem.* (2003) 278:22424–31. doi: 10.1074/jbc.M301789200
165. Dasgupta S, Jana M, Liu X, Pahan K. Myelin basic protein-primed T cells of female but not male mice induce nitric-oxide synthase and proinflammatory cytokines in microglia: implications for gender bias in multiple sclerosis. *J Biol Chem*. (2005) 280:32609–17. doi: 10.1074/jbc.M500299200
166. Jana M, Pahan K. Induction of lymphotoxin-alpha by interleukin-12 p40 homodimer, the so-called biologically inactive molecule, but not IL-12 p70. *Immunology.* (2009) 127:312–25. doi: 10.1111/j.1365-2567.2008.02985.x
167. Jiang S, Gao H, Luo Q, Wang P, Yang X. The correlation of lymphocyte subsets, natural killer cell, and Parkinson's disease: a meta-analysis. *Neurol Sci.* (2017) 38:1373–80. doi: 10.1007/s10072-017-2988-4
168. Kustrimovic N, Comi C, Magistrelli L, Rasini E, Legnaro M, Bombelli R, et al. Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/T17 and Treg in drug-naive and drug-treated patients. *J Neuroinflammation*. (2018) 15:205. doi: 10.1186/s12974-018-1248-8
169. Saunders JA, Estes KA, Kosloski LM, Allen HE, Dempsey KM, Torres-Russotto DR, et al. $CD4^+$ regulatory and effector/memory T cell subsets profile motor dysfunction in Parkinson's disease. *J Neuroimmune Pharmacol.* (2012) 7:927–38. doi: 10.1007/s11481-012-9402-z
170. Levite M. Dopamine and T cells: dopamine receptors and potent effects on T cells, dopamine production in T cells, and abnormalities in the dopaminergic system in T cells in autoimmune, neurological and psychiatric diseases. *Acta Physiol.* (2016) 216:42–89. doi: 10.1111/apha.12476
171. Martinez EM, Young AL, Patankar YR, Berwin BL, Wang L, Von Herrmann KM, et al. Editor's Highlight: Nlrp3 Is required for inflammatory changes and nigral cell loss resulting from chronic intragastric rotenone exposure in mice. *Toxicol Sci.* (2017) 159:64–75. doi: 10.1093/toxsci/kfx117
172. Engler H, Doenlen R, Riether C, Engler A, Niemi MB, Besedovsky HO, et al. Time-dependent alterations of peripheral immune parameters after nigrostriatal dopamine depletion in a rat model of Parkinson's disease. *Brain Behav Immun.* (2009) 23:518–26. doi: 10.1016/j.bbi.2009.01.018
173. Benner EJ, Banerjee R, Reynolds AD, Sherman S, Pisarev VM, Tsiperson V, et al. Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. *PLoS ONE.* (2008) 3:e1376. doi: 10.1371/journal.pone.0001376
174. Reynolds AD, Stone DK, Hutter JA, Benner EJ, Mosley RL, Gendelman HE. Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. *J Immunol.* (2010) 184:2261–71. doi: 10.4049/jimmunol.0901852
175. Reynolds AD, Banerjee R, Liu J, Gendelman HE, Mosley RL. Neuroprotective activities of CD4+CD25+ regulatory T cells in an animal model of Parkinson's disease. *J Leukoc Biol.* (2007) 82:1083–94. doi: 10.1189/jlb.0507296
176. Lacan G, Dang H, Middleton B, Horwitz MA, Tian J, Melega WP, et al. Bacillus Calmette-Guerin vaccine-mediated neuroprotection is associated with regulatory T-cell induction in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *J Neurosci Res.* (2013) 91:1292–302. doi: 10.1002/jnr.23253
177. Chung ES, Kim H, Lee G, Park S, Kim H, Bae H. Neuro-protective effects of bee venom by suppression of neuroinflammatory responses in a mouse model of Parkinson's disease: role of regulatory T cells. *Brain Behav Immun.* (2012) 26:1322–30. doi: 10.1016/j.bbi.2012.08.013
178. Chi DS, Gong L, Daigneault EA, Kostrzewa RM. Effects of MPTP and vitamin E treatments on immune function in mice. *Int J Immunopharmacol.* (1992) 14:739–46. doi: 10.1016/0192-0561(92)90070-2
179. Wheeler CJ, Seksenyan A, Koronyo Y, Rentsendorj A, Sarayba D, Wu H, et al. T-lymphocyte deficiency exacerbates behavioral deficits in the 6-OHDA unilateral lesion rat model for Parkinson's disease. *J Neurol Neurophysiol.* (2014) 5:209. doi: 10.4172/2155-9562.1000209
180. Schetters STT, Gomez-Nicola D, Garcia-Vallejo JJ, Van Kooyk Y. Neuroinflammation: Microglia and T Cells Get Ready to Tango. *Front Immunol.* (2017) 8:1905. doi: 10.3389/fimmu.2017.01905
181. Pare A, Mailhot B, Levesque SA, Juzwik C, Ignatius Arokia Doss PM, Lecuyer MA, et al. IL-1beta enables CNS access to CCR2(hi) monocytes and the generation of pathogenic cells through GM-CSF released by CNS endothelial cells. *Proc Natl Acad Sci USA*. (2018) 115:E1194–E1203. doi: 10.1073/pnas.1714948115
182. Hettinger J, Richards DM, Hansson J, Barra MM, Joschko AC, Krijgsveld J, et al. Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol.* (2013) 14:821–30. doi: 10.1038/ni.2638
183. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. *Science*. (2010) 327:656–61. doi: 10.1126/science.1178331
184. Prinz M, Erny D, Hagemeyer N. Ontogeny and homeostasis of CNS myeloid cells. *Nat Immunol.* (2017) 18:385–92. doi: 10.1038/ni.3703
185. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature*. (2015) 518:547–51. doi: 10.1038/nature13989
186. Bennett FC, Bennett ML, Yaqoob F, Mulinyawe SB, Grant GA, Hayden Gephart M, et al. A combination of ontogeny and CNS environment

establishes microglial identity. *Neuron.* (2018) *98*:1170–83 e1178. doi: 10.1016/j.neuron.2018.05.014
187. Prinz M, Priller J. Tickets to the brain: role of CCR2 and CX3CR1 in myeloid cell entry in the CNS. *J Neuroimmunol.* (2010) 224:80–4. doi: 10.1016/j.jneuroim.2010.05.015
188. Saederup N, Cardona AE, Croft K, Mizutani M, Cotleur AC, Tsou CL, et al. Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. *PLoS ONE.* (2010) 5:e13693. doi: 10.1371/journal.pone.0013693
189. Chu HX, Arumugam TV, Gelderblom M, Magnus T, Drummond GR, Sobey CG. Role of CCR2 in inflammatory conditions of the central nervous system. *J Cereb Blood Flow Metab.* (2014) 34:1425–9. doi: 10.1038/jcbfm.2014.120
190. Lopez-Atalaya JP, Askew KE, Sierra A, Gomez-Nicola D. Development and maintenance of the brain's immune toolkit: Microglia and non-parenchymal brain macrophages. *Dev Neurobiol.* (2018) 78:561–79. doi: 10.1002/dneu.22545
191. Schlachetzki JCM, Prots I, Tao J, Chun HB, Saijo K, Gosselin D, et al. A monocyte gene expression signature in the early clinical course of Parkinson's disease. *Sci Rep.* (2018) 8:10757. doi: 10.1038/s41598-018-28986-7
192. Grozdanov V, Bliederhaeuser C, Ruf WP, Roth V, Fundel-Clemens K, Zondler L, et al. Inflammatory dysregulation of blood monocytes in Parkinson's disease patients. *Acta Neuropathol.* (2014) 128:651–63. doi: 10.1007/s00401-014-1345-4
193. Cote M, Poirier AA, Aube B, Jobin C, Lacroix S, Soulet D. Partial depletion of the proinflammatory monocyte population is neuroprotective in the myenteric plexus but not in the basal ganglia in a MPTP mouse model of Parkinson's disease. *Brain Behav Immun.* (2015) 46:154–67. doi: 10.1016/j.bbi.2015.01.009
194. Parillaud VR, Lornet G, Monnet Y, Privat AL, Haddad AT, Brochard V, et al. Analysis of monocyte infiltration in MPTP mice reveals that microglial CX3CR1 protects against neurotoxic over-induction of monocyte-attracting CCL2 by astrocytes. *J Neuroinflammation.* (2017) 14:60. doi: 10.1186/s12974-017-0830-9
195. Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. *Nat Rev Immunol.* (2016) 16:407–20. doi: 10.1038/nri.2016.58
196. Heneka MT. Inflammasome activation and innate immunity in Alzheimer's disease. *Brain Pathol.* (2017) 27:220–2. doi: 10.1111/bpa.12483
197. Inouye BM, Hughes FM Jr, Sexton SJ, Purves JT. The emerging role of inflammasomes as central mediators in inflammatory bladder pathology. *Curr Urol.* (2018) 11:57–72. doi: 10.1159/000447196
198. Elliott EI, Sutterwala FS. Initiation and perpetuation of NLRP3 inflammasome activation and assembly. *Immunol Rev.* (2015) 265:35–52. doi: 10.1111/imr.12286
199. Seo MJ, Hong JM, Kim SJ, Lee SM. Genipin protects d-galactosamine and lipopolysaccharide-induced hepatic injury through suppression of the necroptosis-mediated inflammasome signaling. *Eur J Pharmacol.* (2017) 812:128–37. doi: 10.1016/j.ejphar.2017.07.024
200. Von Herrmann KM, Salas LA, Martinez EM, Young AL, Howard JM, Feldman MS, et al. NLRP3 expression in mesencephalic neurons and characterization of a rare NLRP3 polymorphism associated with decreased risk of Parkinson's disease. *NPJ Parkinsons Dis.* (2018) 4:24. doi: 10.1038/s41531-018-0061-5
201. Zhang P, Shao XY, Qi GJ, Chen Q, Bu LL, Chen LJ, et al. Cdk5-dependent activation of neuronal inflammasomes in Parkinson's Disease. *Mov Disord.* (2016) 31:366–76. doi: 10.1002/mds.26488
202. Opipari A, Franchi L. Role of inflammasomes in intestinal inflammation and Crohn's disease. *Inflamm Bowel Dis.* (2015) 21:173–81. doi: 10.1097/MIB.0000000000000230
203. Villumsen M, Aznar S, Pakkenberg B, Jess T, Brudek T. Inflammatory bowel disease increases the risk of Parkinson's disease: a Danish nationwide cohort study 1977-2014. *Gut.* (2019) 68:18–24. doi: 10.1136/gutjnl-2017-315666
204. Peter I, Dubinsky M, Bressman S, Park A, Lu C, Chen N, et al. Anti-tumor necrosis factor therapy and incidence of Parkinson Disease among patients with inflammatory bowel disease. *JAMA Neurol.* (2018) 75:939–46. doi: 10.1001/jamaneurol.2018.0605
205. Borghammer P. Is constipation in Parkinson's disease caused by gut or brain pathology? *Parkinsonism Relat Disord.* (2018) 55:6–7. doi: 10.1016/j.parkreldis.2018.08.014
206. Lionnet A, Leclair-Visonneau L, Neunlist M, Murayama S, Takao M, Adler CH, et al. Does Parkinson's disease start in the gut? *Acta Neuropathol.* (2018) 135:1–12.
207. Scheperjans F, Derkinderen P, Borghammer P. The gut and Parkinson's Disease: hype or hope? *J Parkinsons Dis.* (2018) 8:S31–9. doi: 10.3233/JPD-181477
208. Park H, Lee JY, Shin CM, Kim JM, Kim TJ, Kim JW. Characterization of gastrointestinal disorders in patients with parkinsonian syndromes. *Parkinsonism Relat Disord.* (2015) 21:455–60. doi: 10.1016/j.parkreldis.2015.02.005
209. Houser MC, Tansey MG. The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *NPJ Parkinsons Dis.* (2017) 3:3. doi: 10.1038/s41531-016-0002-0
210. Chapelet G, Leclair-Visonneau L, Clairembault T, Neunlist M, Derkinderen P. *Can the gut be the missing piece in uncovering PD pathogenesis? Parkinsonism Relat Disord.* (2018). doi: 10.1016/j.parkreldis.2018.11.014. [Epub ahead of print].
211. Colombo C, Cosentino M, Marino F, Rasini E, Ossola M, Blandini F, et al. Dopaminergic modulation of apoptosis in human peripheral blood mononuclear cells: possible relevance for Parkinson's disease. *Ann N Y Acad Sci.* (2003) 1010:679–82. doi: 10.1196/annals.1299.124
212. Yan Y, Jiang W, Liu L, Wang X, Ding C, Tian Z, et al. Dopamine controls systemic inflammation through inhibition of NLRP3 inflammasome. *Cell.* (2015) 160:62–73. doi: 10.1016/j.cell.2014.11.047
213. Codolo G, Plotegher N, Pozzobon T, Brucale M, Tessari I, Bubacco L, et al. Triggering of inflammasome by aggregated alpha-synuclein, an inflammatory response in synucleinopathies. *PLoS ONE.* (2013) 8:e55375. doi: 10.1371/journal.pone.0055375
214. Bose A, Beal MF. Mitochondrial dysfunction in Parkinson's disease. *J Neurochem.* (2016) 139 (Suppl. 1):216–31. doi: 10.1111/jnc.13731
215. Won JH, Park S, Hong S, Son S, Yu JW. Rotenone-induced impairment of mitochondrial electron transport chain confers a selective priming signal for NLRP3 inflammasome activation. *J Biol Chem.* (2015) 290:27425–37. doi: 10.1074/jbc.M115.667063
216. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature.* (2011) 469:221–5. doi: 10.1038/nature09663
217. Zhou K, Shi L, Wang Y, Chen S, Zhang J. Recent Advances of the NLRP3 Inflammasome in Central Nervous System Disorders. *J Immunol Res.* (2016) 2016:9238290. doi: 10.1155/2016/9238290
218. Xu LL, Wu YF, Yan F, Li CC, Dai Z, You QD, et al. 5-(3,4-Difluorophenyl)-3-(6-methylpyridin-3-yl)-1,2,4-oxadiazole (DDO-7263), a novel Nrf2 activator targeting brain tissue, protects against MPTP-induced subacute Parkinson's disease in mice by inhibiting the NLRP3 inflammasome and protects PC12 cells against oxidative stress. *Free Radic Biol Med.* (2019) 134:288–303. doi: 10.1016/j.freeradbiomed.2019.01.003
219. Qiao C, Zhang LX, Sun XY, Ding JH, Lu M, Hu G. Caspase-1 deficiency alleviates dopaminergic neuronal death via inhibiting Caspase-7/AIF Pathway in MPTP/p Mouse Model of Parkinson's Disease. *Mol Neurobiol.* (2017) 54:4292–302. doi: 10.1007/s12035-016-9980-5
220. Mao Z, Liu C, Ji S, Yang Q, Ye H, Han H, et al. The NLRP3 Inflammasome is Involved in the Pathogenesis of Parkinson's Disease in rats. *Neurochem Res.* (2017) 42:1104–15. doi: 10.1007/s11064-017-2185-0
221. Sarkar S, Malovic E, Harishchandra DS, Ghaisas S, Panicker N, Charli A, et al. Mitochondrial impairment in microglia amplifies NLRP3 inflammasome proinflammatory signaling in cell culture and animal models of Parkinson's disease. *NPJ Parkinsons Dis.* (2017) 3:30. doi: 10.1038/s41531-017-0032-2
222. Qiao C, Zhang Q, Jiang Q, Zhang T, Chen M, Fan Y, et al. Inhibition of the hepatic Nlrp3 protects dopaminergic neurons via attenuating systemic inflammation in a MPTP/p mouse model of Parkinson's disease. *J Neuroinflammation.* (2018) 15:193. doi: 10.1186/s12974-018-1236-z
223. Harvey K, Outeiro TF. The role of LRRK2 in cell signalling. *Biochem Soc Trans.* (2018) 47:197–207. doi: 10.1042/BST20180464

224. Lesage S, Durr A, Tazir M, Lohmann E, Leutenegger AL, Janin S, et al. LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. *N Engl J Med.* (2006) 354:422–3. doi: 10.1056/NEJMc055540
225. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genet.* (2009) 41:1303–7. doi: 10.1038/ng.485
226. Greggio E. Role of LRRK2 kinase activity in the pathogenesis of Parkinson's disease. *Biochem Soc Trans.* (2012) 40:1058–62. doi: 10.1042/BST20120054
227. Hui KY, Fernandez-Hernandez H, Hu J, Schaffner A, Pankratz N, Hsu NY, et al. Functional variants in the LRRK2 gene confer shared effects on risk for Crohn's disease and Parkinson's disease. *Sci Transl Med.* (2018) 10:eaai7795. doi: 10.1126/scitranslmed.aai7795
228. Gardet A, Benita Y, Li C, Sands BE, Ballester I, Stevens C, et al. LRRK2 is involved in the IFN-gamma response and host response to pathogens. *J Immunol.* (2010) 185:5577–85. doi: 10.4049/jimmunol.1000548
229. Hakimi M, Selvanantham T, Swinton E, Padmore RF, Tong Y, Kabbach G, et al. Parkinson's disease-linked LRRK2 is expressed in circulating and tissue immune cells and upregulated following recognition of microbial structures. *J Neural Transm.* (2011) 118:795–808. doi: 10.1007/s00702-011-0653-2
230. Cook DA, Kannarkat GT, Cintron AF, Butkovich LM, Fraser KB, Chang J, et al. LRRK2 levels in immune cells are increased in Parkinson's disease. *NPJ Parkinsons Dis.* (2017) 3:11. doi: 10.1038/s41531-017-0010-8
231. Hill-Burns EM, Factor SA, Zabetian CP, Thomson G, Payami H. Evidence for more than one Parkinson's disease-associated variant within the HLA region. *PLoS ONE.* (2011) 6:e27109. doi: 10.1371/journal.pone.0027109
232. Ahmed I, Tamouza R, Delord M, Krishnamoorthy R, Tzourio C, Mulot C, et al. Association between Parkinson's disease and the HLA-DRB1 locus. *Mov Disord.* (2012) 27:1104–10. doi: 10.1002/mds.25035
233. Kannarkat GT, Cook DA, Lee JK, Chang J, Chung J, Sandy E, et al. Common genetic variant association with Altered HLA Expression, Synergy with Pyrethroid Exposure, and Risk for Parkinson's Disease: An Observational and Case-Control Study. *NPJ Parkinsons Dis.* (2015) 1:15002. doi: 10.1038/npjparkd.2015.2
234. Ness D, Ren Z, Gardai S, Sharpnack D, Johnson VJ, Brennan RJ, et al. Leucine-rich repeat kinase 2 (LRRK2)-deficient rats exhibit renal tubule injury and perturbations in metabolic and immunological homeostasis. *PLoS ONE.* (2013) 8:e66164. doi: 10.1371/journal.pone.0066164
235. Daher JP, Volpicelli-Daley LA, Blackburn JP, Moehle MS, West AB. Abrogation of alpha-synuclein-mediated dopaminergic neurodegeneration in LRRK2-deficient rats. *Proc Natl Acad Sci USA.* (2014) 111:9289–94. doi: 10.1073/pnas.1403215111
236. Kozina E, Sadasivan S, Jiao Y, Dou Y, Ma Z, Tan H, et al. Mutant LRRK2 mediates peripheral and central immune responses leading to neurodegeneration *in vivo*. *Brain.* (2018) 141:1753–69. doi: 10.1093/brain/awy077
237. Yun SP, Kam TI, Panicker N, Kim S, Oh Y, Park JS, et al. Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease. *Nat Med.* (2018) 24:931–8. doi: 10.1038/s41591-018-0051-5
238. Kim S, Moon M, Park S. Exendin-4 protects dopaminergic neurons by inhibition of microglial activation and matrix metalloproteinase-3 expression in an animal model of Parkinson's disease. *J Endocrinol.* (2009) 202:431–9. doi: 10.1677/JOE-09-0132
239. Nassar NN, Al-Shorbagy MY, Arab HH, Abdallah DM. Saxagliptin: a novel antiparkinsonian approach. *Neuropharmacology.* (2015) 89:308–17. doi: 10.1016/j.neuropharm.2014.10.007
240. Aviles-Olmos I, Dickson J, Kefalopoulou Z, Djamshidian A, Ell P, Soderlund T, et al. Exenatide and the treatment of patients with Parkinson's disease. *J Clin Invest.* (2013) 123:2730–6. doi: 10.1172/JCI68295
241. Athauda D, Maclagan K, Skene SS, Bajwa-Joseph M, Letchford D, Chowdhury K, et al. Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. *Lancet.* (2017) 390:1664–75. doi: 10.1016/S0140-6736(17)31585-4
242. Athauda D, Maclagan K, Budnik N, Zampedri L, Hibbert S, Aviles-Olmos I, et al. Post hoc analysis of the Exenatide-PD trial-Factors that predict response. *Eur J Neurosci.* (2018) 49:410–21. doi: 10.1111/ejn.14096
243. Athauda D, Maclagan K, Budnik N, Zampedri L, Hibbert S, Skene SS, et al. What effects might exenatide have on non-motor symptoms in Parkinson's Disease: a post hoc analysis. *J Parkinsons Dis.* (2018) 8:247–58. doi: 10.3233/JPD-181329
244. Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, et al. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J Neurosci.* (2002) 22:1763–71. doi: 10.1523/JNEUROSCI.22-05-01763.2002
245. Investigators NN-P. A pilot clinical trial of creatine and minocycline in early Parkinson disease: 18-month results. *Clin Neuropharmacol.* (2008) 31:141–50. doi: 10.1097/WNF.0b013e3181342f32
246. Lazzarini M, Martin S, Mitkovski M, Vozari RR, Stuhmer W, Bel ED. Doxycycline restrains glia and confers neuroprotection in a 6-OHDA Parkinson model. *Glia.* (2013) 61:1084–100. doi: 10.1002/glia.22496
247. Zhang GB, Feng YH, Wang PQ, Song JH, Wang P, Wang SA. A study on the protective role of doxycycline upon dopaminergic neuron of LPS-PD rat model rat. *Eur Rev Med Pharmacol Sci.* (2015) 19:3468–74.
248. Bortolanza M, Nascimento GC, Socias SB, Ploper D, Chehin RN, Raisman-Vozari R, et al. Tetracycline repurposing in neurodegeneration: focus on Parkinson's disease. *J Neural Transm.* (2018) 125:1403–15. doi: 10.1007/s00702-018-1913-1
249. Coll RC, Robertson AA, Chae JJ, Higgins SC, Munoz-Planillo R, Inserra MC, et al. A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. *Nat Med.* (2015) 21:248–55. doi: 10.1038/nm.3806
250. Yin H, Guo Q, Li X, Tang T, Li C, Wang H, et al. Curcumin Suppresses IL-1beta Secretion and Prevents Inflammation through Inhibition of the NLRP3 Inflammasome. *J Immunol.* (2018) 200:2835–46. doi: 10.4049/jimmunol.1701495
251. Zou P, Liu X, Li G, Wang Y. Resveratrol pretreatment attenuates traumatic brain injury in rats by suppressing NLRP3 inflammasome activation via SIRT1. *Mol Med Rep.* (2018) 17:3212–7.
252. Domiciano TP, Wakita D, Jones HD, Crother TR, Verri WA Jr, Arditi M, et al. Quercetin Inhibits Inflammasome Activation by Interfering with ASC oligomerization and prevents interleukin-1 mediated mouse vasculitis. *Sci Rep.* (2017) 7:41539. doi: 10.1038/srep41539

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 01 May 2019
doi: 10.3389/fimmu.2019.00981

Dopamine Receptor D3 Expression Is Altered in CD4$^+$ T-Cells From Parkinson's Disease Patients and Its Pharmacologic Inhibition Attenuates the Motor Impairment in a Mouse Model

*Daniela Elgueta[1,2], Francisco Contreras[1], Carolina Prado[1], Andro Montoya[1], Valentina Ugalde[1], Ornella Chovar[1], Roque Villagra[3], Claudio Henríquez[3], Miguel A. Abellanas[4], María S. Aymerich[4], Rarael Franco[5,6] and Rodrigo Pacheco[1,2]**

[1] Laboratorio de Neuroinmunología, Fundación Ciencia & Vida, Santiago, Chile, [2] Departamento de Ciencias Biológicas, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile, [3] Departamento de Ciencias Neurológicas Oriente, Facultad de Medicina, Universidad de Chile, Santiago, Chile, [4] Departamento de Bioquímica y Genética, Programa de Neurociencias, Centro de Investigación Médica Aplicada (CIMA), Universidad de Navarra, Pamplona, Spain, [5] Department of Biochemistry and Molecular Biomedicine, University of Barcelona, Barcelona, Spain, [6] Centro de Investigación en Red, Enfermedades Neurodegenerativas CiberNed, Instituto de Salud Carlos III, Madrid, Spain

OPEN ACCESS

Edited by:
Fabienne Brilot,
University of Sydney, Australia

Reviewed by:
Tyler Cutforth,
Columbia University Irving Medical Center, United States
Rene Luis Vidal,
Centro de Biología Integrativa, Universidad Mayor, Chile

***Correspondence:**
Rodrigo Pacheco
rpacheco@cienciavida.org

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology

Received: 08 September 2018
Accepted: 16 April 2019
Published: 01 May 2019

Citation:
Elgueta D, Contreras F, Prado C, Montoya A, Ugalde V, Chovar O, Villagra R, Henríquez C, Abellanas MA, Aymerich MS, Franco R and Pacheco R (2019) Dopamine Receptor D3 Expression Is Altered in CD4$^+$ T-Cells From Parkinson's Disease Patients and Its Pharmacologic Inhibition Attenuates the Motor Impairment in a Mouse Model. Front. Immunol. 10:981. doi: 10.3389/fimmu.2019.00981

Neuroinflammation constitutes a fundamental process involved in Parkinson's disease (PD). Microglial cells play a central role in the outcome of neuroinflammation and consequent neurodegeneration of dopaminergic neurons in the substantia nigra. Current evidence indicates that CD4$^+$ T-cells infiltrate the brain in PD, where they play a critical role determining the functional phenotype of microglia, thus regulating the progression of the disease. We previously demonstrated that mice bearing dopamine receptor D3 (DRD3)-deficient CD4$^+$ T-cells are completely refractory to neuroinflammation and consequent neurodegeneration induced by the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In this study we aimed to determine whether DRD3-signalling is altered in peripheral blood CD4$^+$ T-cells obtained from PD patients in comparison to healthy controls (HC). Furthermore, we evaluated the therapeutic potential of targeting DRD3 confined to CD4$^+$ T-cells by inducing the pharmacologic antagonism or the transcriptional inhibition of DRD3-signalling in a mouse model of PD induced by the chronic administration of MPTP and probenecid (MPTPp). *In vitro* analyses performed in human cells showed that the frequency of peripheral blood Th1 and Th17 cells, two phenotypes favoured by DRD3-signalling, were significantly increased in PD patients. Moreover, naïve CD4$^+$ T-cells obtained from PD patients displayed a significant higher Th1-biased differentiation in comparison with those naïve CD4$^+$ T-cells obtained from HC. Nevertheless, DRD3 expression was selectively reduced in CD4$^+$ T-cells obtained from PD patients. The results obtained from *in vivo* experiments performed in mice show that the transference of CD4$^+$ T-cells treated *ex vivo* with the DRD3-selective antagonist PG01037 into MPTPp-mice resulted in a significant reduction of motor impairment, although without significant effect in neurodegeneration. Conversely, the transference of

$CD4^+$ T-cells transduced *ex vivo* with retroviral particles codifying for an shRNA for DRD3 into MPTPp-mice had no effects neither in motor impairment nor in neurodegeneration. Notably, the systemic antagonism of DRD3 significantly reduced both motor impairment and neurodegeneration in MPTPp mice. Our findings show a selective alteration of DRD3-signalling in $CD4^+$ T-cells from PD patients and indicate that the selective DRD3-antagonism in this subset of lymphocytes exerts a therapeutic effect in parkinsonian animals dampening motor impairment.

Keywords: neuroinflammation, neurodegeneration, Parkinson's disease patients, MPTP mouse model, dopamine receptors, CD4+ T-cells

INTRODUCTION

Several lines of evidence have indicated that neuroinflammation plays a pivotal role in the development of Parkinson's disease (PD) (1). Microglial cells constitute the central players in neuroinflammation, thereby their functional phenotype determines whether surrounding neurons survive or die. In this regard, depending on the integration of molecular cues, microglial cells may acquire either a neurotoxic or a neuroprotective phenotype, which are known as M1 and M2, respectively (2).

Growing evidence in human and animal models has shown the generation of nitrated forms of α-synuclein in the substantia nigra (SN) of individuals with PD (3–5), which is mainly contained in protein inclusions called Lewy bodies. Of note, the nitration of α-synuclein, which is a consequence of the oxidative stress, results in the generation of neo-antigens (1). Furthermore, studies in mice and recently in humans, have shown that oxidised α-synuclein constitutes a major antigen for the T-cell-mediated immune response involved in PD (4, 6–8). In this regard, it has been shown that nitrated α-synuclein generated in the SN is captured and presented by antigen-presenting-cells (APCs) in cervical lymph nodes to naive $CD4^+$ T-cells with specificity to this neo-antigen. Once activated, $CD4^+$ T-cells acquire inflammatory phenotypes, such as T-helper-1 (Th1) and Th17, then they infiltrate the SN where microglial cells act as local APCs presenting nitrated α-synuclein-derived antigens on class II MHC, thus re-stimulating T-cells (4, 9–11). Re-stimulated $CD4^+$ T-cells produce high local levels of IFN-γ and TNF-α, thus promoting further inflammatory activation of microglial cells (M1-microglia) (2, 12, 13). Activated M1-microglia produces several neurotoxic and inflammatory mediators, including reactive oxygen and nitrogen species, which in turn induce neuronal death and further generation of oxidised and nitrated proteins (1). Thus, this mechanism constitutes a vicious cycle, which results in chronic neuroinflammation and represents the engine of the progression of neurodegeneration. Of note, several studies have shown that $CD4^+$ T-cells deficiency results in a complete protection of neurodegeneration in mouse models of PD (4, 13, 14), thus indicating that inflammatory $CD4^+$ T-cell response is required to promote neurodegeneration of the nigrostriatal pathway.

During last 15 years, several studies have shown dopamine as a major regulator of inflammation (15–17). In this regard, it has been consistently demonstrated that dopaminergic signalling mediated by low-affinity dopamine receptors, including dopamine receptors D1 (DRD1) and DRD2 exerts anti-inflammatory effects in several experimental systems (15, 16, 18). On the other hand, recent studies addressing the role of dopaminergic regulation of $CD4^+$ T-cells have shown genetic and pharmacologic evidence indicating that the stimulation of high-affinity dopamine receptors, including DRD3 and DRD5, favours the acquisition of Th1 and Th17 phenotypes, respectively, thus promoting inflammation (19–21).

Since dopaminergic neurons are the main cells affected in PD, dopamine levels are strongly reduced in the brain of PD patients and animal models (14, 22). Thereby, dopaminergic signalling mediated by low-affinity dopamine receptors is favoured in the nigrostriatal pathway of healthy individuals, whilst the selective stimulation of high-affinity dopamine receptors is promoted in PD (17). In this regard, our previous results have shown that DRD3-signalling in $CD4^+$ T-cells plays a fundamental role in the development of neurodegeneration in a mouse model of PD induced by the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (13). Accordingly, the genetic deficiency of DRD3 strongly limited the acquisition of the inflammatory potential of $CD4^+$ T-cells infiltrating the SN of MPTP-treated mice, abrogating neurodegeneration of the nigrostriatal pathway. Furthermore, the systemic administration of a DRD3-antagonist resulted in a significant attenuation of both neurodegeneration and motor impairment in MPTP-treated mice (23). According to the relevance of DRD3-signalling in animal models of PD, it has been shown a significant association of PD-progression with the reduction of the *Drd3*-transcription in peripheral blood mononuclear cells (PBMCs) obtained from PD patients (24). Thus, current evidence

Abbreviations: Ab, Antibody; APC, antigen-presenting cells; DRD*n*, Dopamine receptor *n*; Foxp3, forkhead box P3; GFP, green fluorescent protein; GFAP, Glial Fibrillary Acidic Protein; IFN-γ, interferon γ; IL-*n*, interleukin *n*; mAb, monoclonal Ab; MFI, mean fluorescence intensity; MoCA, Montreal Cognitive Assessment; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; pAb, polyclonal Ab; PBMCs, peripheral blood mononuclear cells; PE, phycoerythrin; PD, Parkinson's disease; PMA, phorbol 12-myristate 13-acetate; RAG1, recombination-activating-gen-1; RAG1KO, RAG1 knockout; SN, substantia nigra; SNpc, SN *pars compacta*; Th*n*, T helper *n*; TLRs, Toll like receptors; TLR*n*, Toll like receptor *n*; TNF-α, Tumor Necrosis Factor α; Tregs, regulatory T cells; UPDRS, Unified Parkinson's Disease Rating Scale.

suggests that DRD3-signalling in lymphocytes plays a relevant role favouring the development of PD in animal models and human individuals.

In this study, we addressed the question of whether DRD3 expression is altered in $CD4^+$ T-cells obtained from PD patients and how it is associated with the inflammatory phenotypes of these cells. Furthermore, we evaluated the therapeutic potential of the inhibition of DRD3-signalling confined to $CD4^+$ T-cells using an animal model of PD induced by the chronic administration of MPTP.

MATERIALS AND METHODS

Human Subjects

Forty-one patients from both genders (22 females and 19 males) who meet Diagnostic Criteria of the Brain Bank of the Society of Parkinson's Disease in the UK (UK PDSBB) were recruited from Hospital del Salvador. Demographic information was collected and summarized in **Table 1**. Cognitive impairment was evaluated by Montreal Cognitive Assessment (MoCA) test and disease severity was determined with both Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn and Yahr stages. The functional capacity was obtained as the Schwab and England score. Patients with acute inflammatory or infectious diseases, with chronic inflammatory or autoimmune diseases, with hepatic damage, renal damage, fasting hyperglycemia, or cancer and other diseases that might produce altered immunity were excluded from this study. Thirty-eight age-matched healthy controls (HC) chosen under the same exclusion criteria were included in the study. Venous blood samples were obtained in universal tubes containing heparin. Tubes were subsequently coded and stored at room temperature until processing, which occurred within 2 h after collection.

Flow Cytometry Analysis of Peripheral Blood Immune Cells From Human Individuals

Human PBMCs were obtained immediately after the extraction of heparinized blood from HC or PD patients using Ficoll-PaqueTM Plus (Ge Healthcare). PBMCs were immediately analysed or activated with anti-human CD3 monoclonal antibody (mAb; 2 μg/ml, Biolegend) and anti-human CD28 mAb (2 μg/ml, Biolegend) in medium XVIVO-10 (Lonza) containing 1% autologous serum for 3d at 37°C and 5% CO_2. The expression of DRD3 was analysed in different $CD4^+$ T-cell subsets, including total $CD4^+$ T-cells ($CD3^+CD4^+$), naive $CD4^+$ T-cells ($CD3^+CD4^+CD45RA^+CD45RO^-$) and effector/memory ($CD3^+CD4^+CD45RA^-CD45RO^+$). DRD3 expression was also analysed in B cells and natural killer (NK). For this purpose immunostaining for surface markers was performed using the following fluorophore-conjugated mAbs: Brilliant violet 421-conjugated anti-CD3 mAb (1:100, Biolegend), FITC-conjugated anti-CD4 mAb (1:100, Biolegend), PECy7-conjugated anti-CD45RO mAb (1:100), Biolegend), APC-Cy7-conjugated anti-CD45RA mAb (1:100, Biolegend), PE-Cy7-conjugated anti-CD56 mAb (1:100, Biolegend), APC-Cy7-conjugated anti-CD19 mAb (1:100, Biolegend) and PE-Cy5-conjugated anti-CD25 mAb (1:100, Biolegend). To determine DRD3 expression we used a primary polyclonal antibody (pAb) anti-DRD3 IgG antibody developed in rabbit (2 μg/ml, Abcam), and a secondary PE-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology). As an isotype control, irrelevant rabbit polyclonal IgG (2μg/ml, Abcam) was used instead the anti-DRD3 pAb. To study T-cell phenotypes, resting or activated PBMCs were re-stimulated in the presence of 50 ng/ml of phorbol 12-myristate 13-acetate (PMA), 1 μg/ml ionomycin and 5 μg/ml Brefeldin A for 3 h at 37°C and intracellular cytokine or transcription factor staining was analysed in $CD4^+$ T-cells. To analyse the extent of T-cell differentiation to the Th1 phenotype, naive $CD4^+$ T-cells were purified from PBMCs with the Naive T Cell isolation Kit, Human MACS (Miltenyi Biotec). Afterward, cells were incubated (10^6 cells/ml) with anti-human CD3 mAb (1μg/ml), anti-human CD28 mAb (2μg/ml), recombinant IL-2 (5μg/ml), recombinant IL-12 (2.5 ng/ml) and anti-human IL-4 mAb (12.5 ng/ml) all from Biolegend, in XVIVO-10 medium containing 1% autologous serum for 5d. Then, cells were re-stimulate with PMA, ionomycin and brefeldin A during 3 h and the frequency of Th1, Th17, and Tregs phenotypes was analysed by intracellular immunostaining of IFN-γ, IL-17, and Foxp3 respectively in the $CD4^+$ T-cell population. For intracellular staining the following mAbs were used: PE-Cy7-conjugated anti-IFN-γ mAb (1:100, Biolegend), APC-Cy7-conjugated anti-IL-17 mAb (1:100, Biolegend) and PE-conjugated anti-Foxp3 mAb (1:100, Biolegend).

TABLE 1 | Demographic features and disease activity of Parkinson's disease patients.

Gender (Female/Male)	41 (22/19)
Age	65.6 ± 12.2
MoCA test[a]	27 ± 3.2
UPDRS[b]	31 ± 21.2
Hoehn and Yahr[c]	2.1 ± 0.97
Schwab and England[d]	80 ± 18.8

[a] *Montreal Cognitive Assessment (scale 0–30).*
[b] *Unified Parkinson's Disease Rating Scale (scale 0–199).*
[c] *Modified Hoehn and Yahr scale (scale 0–5).*
[d] *Schwab and England activities of daily living scale (%).*

Animals

Ten-to-twelve weeks old C57BL/6 mice were used for all *in vivo* experiments. Wild-type (WT) and *Foxp3*^{gfp} reporter C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). C57BL/6 *Drd3*$^{-/-}$(DRD3KO) mice were kindly donated by Dr. Marc Caron (25). *Foxp3*^{gfp} *Drd3*$^{-/-}$ were generated by crossing parental mouse strains. We confirmed this new strain to be transgenic and Drd3-deficient by flow cytometry analysis of blood cells and PCR of genomic DNA, respectively. Five mice per cage were housed at 21°C in a humidity-controlled environment, on a12/12h light/dark cycle with lights on at 8 a.m., with *ad libitum* access to food and water. All mice were maintained and manipulated according to institutional guidelines at the pathogen-free facility of the Fundación Ciencia & Vida.

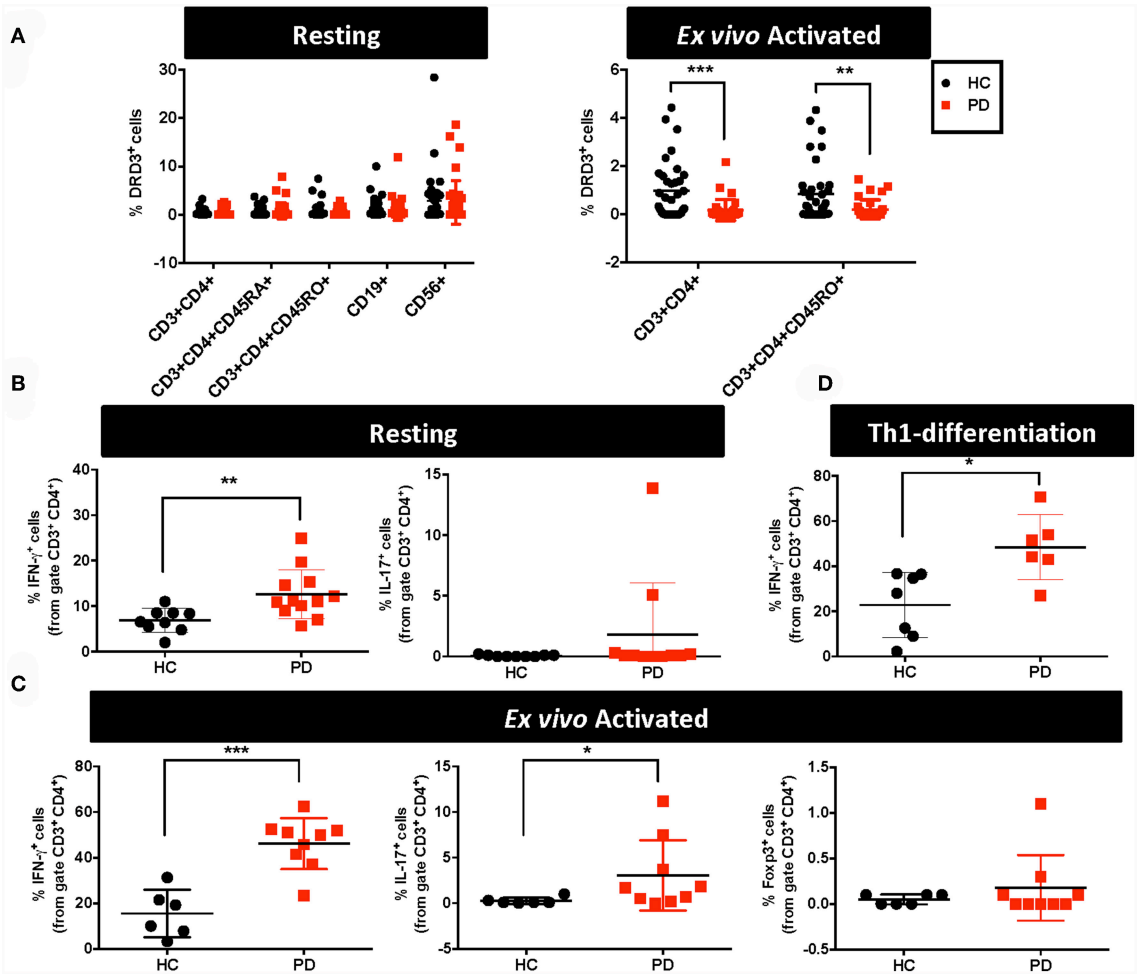


FIGURE 1 | Alterations in the functional phenotype and DRD3 expression in $CD4^+$ T-cells obtained from Parkinson's disease patients. **(A–C)** PBMCs were isolated from healthy donors (HC) and Parkinson's Disease patients (PD) and they were immediately analysed or activated with anti-CD3 and anti-CD28 antibodies for 72 h. **(A)** Cells were immunostained for several surface markers and DRD3 expression was analysed in different lymphocyte populations by flow cytometry. Left panel shows the frequency of $DRD3^+$ cells in total $CD4^+$ T-cells ($CD3^+$ $CD4^+$), naïve $CD4^+$ T-cells ($CD3^+$ $CD4^+$ $CD45RA^+$), memory $CD4^+$ T-cells ($CD3^+$ $CD4^+$ $CD45RO^+$), B-cells ($CD19^+$), and NK cells ($CD56^+$) in resting conditions. Right panel shows the frequency of total $CD4^+$ T-cells ($CD3^+$ $CD4^+$) and memory/effector $CD4^+$ T-cells ($CD3^+$ $CD4^+$ $CD45RO^+$) after T-cell activation. Two-tailed unpaired Student *t*-test was used for comparisons between HC ($n = 38$) and PD ($n = 41$). $^{**}p < 0.01$; ***, $p < 0.0001$. **(B)** Resting T-cells or **(C)** T-cells activated with anti-CD3 and anti-CD28 antibodies for 72 h were stimulated with PMA and ionomycin in the presence of brefeldin A for 4 h and IFN-γ (left panels), IL-17 (middle panels), and Foxp3 (right panels) expression was determined by intracellular immunostaining in the $CD3^+$ $CD4^+$ gated population. $Foxp3^+$ cells were undetectable in resting conditions. Two-tailed unpaired Student *t*-test was used for comparisons between HC [$n = 9$ in **(B)**; $n = 6$ in **(C)**] and PD [$n = 11$–12 in **(B)**; $n = 9$ in **(C)**]. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.0001$. **(D)** Naïve $CD4^+$ T-cells ($CD3^+$ $CD4^+$ $CD45RA^+$) T-cells were isolated from PBMCs obtained from HC and PD individuals and then they were incubated in Th1-biased conditions for 5 days. Afterwards, cells were stimulated with PMA and ionomycin in the presence of brefeldin A for 3 h and IFN-γ expression was determined by intracellular immunostaining in the $CD3^+$ $CD4^+$ gated population. Two-tailed unpaired Student *t*-test was used for comparisons between HC ($n = 7$) and PD ($n = 6$). $^*p < 0.05$.

MPTPp Intoxication and Treatments With PG01037

Animals were treated as outlined in **Figures 2A**, **5A**. Groups received 10 intraperitoneal (i.p.) injections of MPTP hydrochloride (20 mg/kg in saline; Toronto Research Chemicals INC, Toronto, ON, Canada) and probenecid (250 mg/kg in saline; Life Technologies, Oregon, USA), administered twice a week throughout 5 weeks. In all groups receiving MPTP (or the vehicle) and probenecid, both compounds were administered in two consecutive injections during the early morning. Some experimental groups received the i.v. transference of *ex vivo* manipulated $CD4^+$ T-cells (as described below) and in other cases mice received the i.p. administration of PG01037 (30 mg/kg; Tocris Bioscience) as indicated in figure legends.

Viral Transduction

For initial testing of the efficacy of different short hairpin RNA (shRNA) directed to *Drd3* transcription, we generated HEK293T cells overexpressing stably DRD3. For this purpose, HEK293T cells were transfected with lentiviral vectors codifying

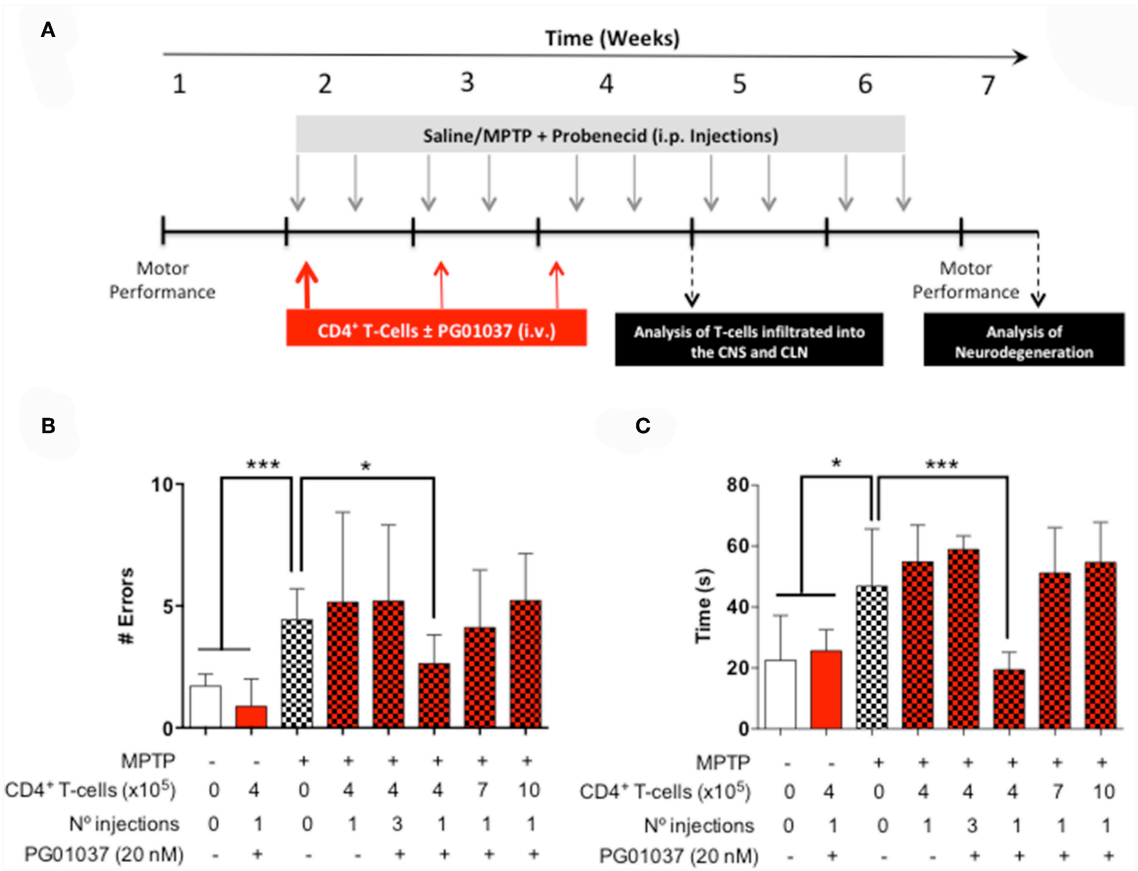


FIGURE 2 | Evaluation of the therapeutic potential of $CD4^+$ T-cells treated *ex vivo* with a DRD3 antagonist in the motor performance of MPTPp-treated mice. **(A)** Experimental design: Control animals (without MPTPp treatment) were treated with saline, probenecid, and with or without the i.v. injection of $CD4^+$ T-cells treated *ex vivo* with PG01037. MPTPp animals received 10 i.p. injections with MPTP (20 mg/kg) and probenecid (250 mg/kg) during weeks 2–6 (grey arrows). $CD4^+$ T-cells (4×10^5, 7×10^5, or 10×10^5 per mouse) were treated with or without PG01037 (20 nM) and then i.v. injected in experimental animals 1 day after the first MPTPp injection (bold red arrow). In some cases, animals received 3 injections of $CD4^+$ T-cells separated by 1 week intervals (bold and thin red arrows). T-cell infiltration was analysed after 3 weeks of MPTPp-treatment. Neurodegeneration was analysed 1 week after the last MPTPp injection. Motor performance was analysed the week before beginning with MPTPp administration to distribute experimental groups with homogeneous motor performance and then it was evaluated again 16 h after the last MPTPp injection in the Beam-test **(B)** and in the coat-hanger test **(C)**. Experimental groups receiving i.v. injections of $CD4^+$ T-cells are indicated in red bars. Data represents the mean with the SEM. One-way ANOVA followed by Tukey's multiple comparison *post hoc* test were used to determine statistical differences: $^*p < 0.05$ $^{***}p < 0.001$, $n = 5$–17 mice per group.

for the reporter gene red fluorescent protein (RFP) followed by a 2A sequence, puromycin resistance gene and *Drd3*; the whole construct under the control of the CMV promoter (pLenti-GIII-CMV-RFP-2A-Puro-DRD3). Cells were transfected in the presence of turbofect (Thermo Scientific) and 48h later, puromycin (3 μg) was added and cells were grown for 28d. RFP^+ cells were isolated by cell-sorting and then used to test the efficacy of four different shRNA for *Drd3* transcription (shDrd3 1-4). Afterward, HEK293T overexpressing DRD3 (3.5×10^5 cells per point) were transfected with lentiviral vectors codifying for different versions of shDrd3 or an scrambled shRNA, followed by green fluorescent protein (GFP) reporter gene (piLenti-shRNA-GFP). Forty-eight hours later, cells were lysed and the levels of *Drd3* transcripts were quantified by qRT-PCR.

For silencing DRD3 expression in $CD4^+$ T-cells, we used the retroviral vector pBullet (26), which was kindly provided by Dr. Hinrich Abken. We inserted a region encoding GFP, U6 promoter, shRNA against DRD3 (shDrd3-3; 5′-TGC CCT CTC CTC TTT GGT TTC AAC ACA AC-3′) and H1 promoter, into pBullet vector via NcoI and SalI restriction sites (Genscript, *Pisca*taway, NJ). pBullet vector drives the expression of the entire construct by the CMV promoter upstream the NcoI site. This vector was transfected into Phoenix-AMPHO cells and GFP^+ cells were purified by cell sorting to generate a stable cell line producing shDRD3 retrovirus (RV-shDRD3) in the supernatant as described before (20). Total $CD4^+$ T-cells were activated with α-CD3ε mAb 1 μg/ ml, α-CD28 mAb 1 μg/ ml, IL-2 10 ng/ ml in RPMI medium containing 5% FBS in 6-well plates at 37°C and 5% CO_2 for 96 h. Cells were infected with retroviral particles at 24

and 48 h of incubation. Infection was carried out by spinoculating cells with retrovirus in retronectin-coated plates (Takara Bio, Japan). As a non-silencing control, we transduced $CD4^+$ T-cells with a control vector (RV-Control) codifying just for GFP. At day 5 of culture, cells were restimulated and transduction efficiency was determined (GFP^+ cells) in $CD4^+$ T-cells by flow cytometry.

Quantitative RT-PCR

Levels of *Drd3* transcripts were quantified as described previously (20). Briefly, total RNA extracted from cells using the Total RNA EZNA kit (Omega Bio-Tek), was DNase-digested using the TURBO DNA-free kit (Ambion) and 1 μg of RNA was used to synthesize cDNA utilizing M-MLV reverse transcriptase (Life Technologies). Quantitative gene expression analysis was performed using Brilliant II SYBR Green QPCR Master Mix (Agilent). Primers were used at a concentration of 0.5 μM. Expression of *Drd3* was normalised to *Gapdh.* The sequences of the primers used are the following: *Drd3,* forward 5n′-GAA CTC CTT AAG CCC CAC CAT-3′ and reverse 5′-GAA GGC CCC GAG CAC AAT-3′; and *Gapdh,* forward 5′-TCC GTG TTC CTA CCC CCA ATG-3′ and reverse 5′-GAG TGG GAG TTG CTG TTG AAG-3′.

Transference of *ex vivo* Manipulated $CD4^+$ T-Cells

Anti-CD3 mAb (100 ng/well) in PBS was pre-incubated in 96-well plates during 16h at 4°C and then washed twice with PBS. Total splenic $CD4^+$ T-cell were isolated by using a negative selection kit (Miltenyi) and incubated (3×10^6 cells per well) in anti-CD3-coated 96-well plates containing soluble anti-CD28 mAbs (100 ng/well). Immediately after inducing T-cell activation, in some cases cells were treated with 20 nM PG01037 for 24 h and then i.v. injected (4×10^5, 7×10^5, or 1×10^6 cells/mouse) into recipient mice. In other cases, $CD4^+$ T-cells were transduced with RV-shDRD3 or RV-Control and then GFP^+ cells were purified by cell-sorting and subsequently i.v. transferred (4×10^5 cells/mouse) into recipient mice.

Coat-Hanger Test

To determine the motor performance, we used the coat-hanger test, which has been validated for detection of motor dysfunctions (27, 28). Briefly, we used a steel coat hanger (diameter: 2 mm, length: 40 cm) suspended at a height of 35 cm from a cushioned surface. The surface of the coat hanger was marked with regular sections of 5 cm each. The mice were placed in the middle of the hanger and the time taken to move from the middle of the hanger to an extreme was recorded (extreme latency). In addition, the number of sections by which mice moved though after the first 60 seconds was also determined (# sections).

Beam Test

As a second test to evaluate motor performance we used a simplified version of the beam test previously described (29). Briefly, we used a horizontal beam 25 cm length and 3 cm width. The beam surface was covered by a metallic grid (1 cm^2). Mice were videotaped while traversing the grid-surface beam from one of the extreme of the beam to the opposite extreme, where the home-cage was located. Two days of training were performed for habituation to the task. The number of errors (# errors) was quantified by watching the videos in slow-motion mode. An error was defined as when a forelimb or hindlimb slipped through the grid and became visible between the grid and the beam surface or on the side of the grid during a forward movement.

Tissue Processing

Animals were sacrificed by transcardial perfusion 48 h after the last MPTPp injection. For histological techniques, mice were anesthetised with an overdose of 5% isoflurane (Sigma-Aldrich) and transcardially perfused with 4% paraformaldehyde (Merck, Darmstadt, Germany) in 0.125 M phosphate buffered saline (PBS, pH 7.4). Brains were removed and cryoprotected for 48 h in 20% glycerin and 2% DMSO in PBS. For flow cytometry analysis, mice were transcardially perfused with PBS instead paraformaldehyde, brains were rapidly removed, dissected, and immediately processed for flow cytometry as indicated below.

Histological Techniques and Quantification

Immunohistochemistry was performed on free-floating sections (40 μm thick) and for a given experiment all sections were processed at the same time with the respective primary antibody. Sections were washed with PBS and endogenous peroxidase activity was inactivated by 30 min incubation with 0.03% H_2O_2 in methanol (Sigma-Aldrich). After washing three times with PBS, the tissue was incubated for 40 min with blocking solution [4% goat serum, 0.05% Triton X-100 (Sigma-Aldrich) and 4% BSA (Merck, Darmstadt, Germany) in PBS], and exposed overnight to the primary antibodies diluted in blocking solution at room temperature. The primary antibodies used were: rabbit anti-tyrosine hydroxylase pAb (TH, 1:1000; Millipore, Temecula, CA, USA), rat anti-dopamine transporter pAb (anti-DAT, 1:500; Millipore), rabbit anti-GFAP antibody (1:500; abcam [EPR1034Y], Cambridge, UK) and rabbit anti-Iba1 antibody (1:1000; abcam [EPR16588], Cambridge, UK). For colorimetric immunohistochemistry, antibody binding was detected by incubating sections with biotinylated goat anti-rabbit pAb (1:500; Jackson ImmunoResearch Laboratories, West Gore, PA, USA) or biotinylated goat anti–rat pAb (1:500; Jackson ImmunoResearch Laboratories, West Gore, PA, USA) in blocking solution for 2 h at room temperature. The biotinylated antibodies were detected with peroxidase-conjugated avidin (1:5000; Sigma-Aldrich) for 90 min at room temperature followed by incubation with 0.05% diaminobenzidine (Sigma-Aldrich) in 0.03% H_2O_2/Trizma-HCl buffer (pH 7.6). Sections were mounted on glass slides in a 0.2% solution of gelatin in 0.05 M Tris (pH 7.6) (Sigma-Aldrich). The mean number of TH^+ neurons from six SN *pars compacta* (SNpc) sections (separated by 120 μm between each other) per mouse was quantified under light microscopy at a magnification of 200X, and the total area of SNpc was calculated using ImageJ software (National Institutes of Health, Bethesda, MD). Density of dopaminergic neurons was expressed as the number of TH^+ neurons per area (mm^2) in the SNpc. The intensity of immunostaining of dopaminergic terminals in the striatum was evaluated within the TH- or DAT–immunoreactive area (optical density) and was quantified using ImageJ software. To

evaluate the extent of astrogliosis, the mean of GFAP-associated immunoreactivity was analysed in areas of interest (660 μm x 877 μm) in five striatum sections per mouse and quantified as the integrated density using the Image-J software. To determine the extent of activated microglia, the mean number of Iba-1^{high} reactive microglia displaying ameboid shape was quantified in areas of interest of 660 μm x 877 μm in five striatum sections per mouse.

Flow Cytometry Analysis of Mouse T-Cells

Deep cervical lymph nodes and brain sections that contain mid-brain and striatum from MPTPp-treated mice were minced and then disaggregated using Collagenase Type IV 1mg/ml (Gibco, New York, USA) and DNase I 0.25 mg/mL (Roche, Mammheim, Germany). After enzymatic disaggregation, cells were passage through 70 μm-pore cell-strainer to obtain a single-cell suspension. The cell suspension was centrifuged in a gradient of Percoll TM GE Healthcare (Fermelo Biotec) 70%/40%. The mononuclear cells were extracted from the interface and resuspended in RPMI 1640 medium supplemented with 10% FBS. Then, cells were re-stimulated with 50 ng/ml PMA; 1 μg/ml ionomycin and 5 μg/ml Brefeldin A for 3 h at 37°C. To evaluate the phenotype of T-cells, the mononuclear cells were stained with fluorochromes-coupled mAbs directed to surface markers, fixed with formaldehyde 1% and permeabilized with Staining Buffer Factor Set eBioscience™ Foxp3/Transcriptm (Thermo Fisher Scientific). Afterwards, cells were stained with fluorochromes-coupled mAbs directed to cytokines and transcriptional factors. The expression of cytokines and transcription factors in different T-cell subsets was analysed by flow cytometry (FACSCantoII, BD Bioscience). For surface or intracellular immunostaining, anti-CD4, anti-TCRγδ, anti-IFN-γ, anti-IL-17, anti-Foxp3, and anti-RORγt fluorochrome-conjugated mAbs were used at a dilution of 1:300 (all from Biolegend). For *in vitro* T-cell activation assays, effector $CD4^+$ T-cells (Teff; GFP^-) and regulatory $CD4^+$ T-cells (Treg; GFP^+) were isolated from the spleen of *Foxp3*^{gfp} reporter mice by cell sorting using a FACS Aria II (BD), obtaining purities over 98%. All *in vitro* experiments were performed using complete RPMI medium (supplemented with 10% FBS, 2 mM L-Glutamine, 100 U/mL Penicillin, 100 μg/mL Streptomycin and 50 μM β-mercaptoethanol). To assess activation, cells were stimulated for 6d with 50 ng/well of plate-bound anti-CD3 mAb and 2 μg/mL soluble anti-CD28 mAb on flat-bottom 96-well plates (Thermo Scientific). IL-2 (10 ng/mL) was added to the culture at days 0, 3, and 5. To force the Th1 differentiation naive $CD25^-CD4^+$ T-cells were activated in the presence of 20 ng/mL IL-12, 10 ng/mL IL-2, and 5 μg/mL anti-IL-4 for 4d. All analyses were performed in living cells using the Zombie Aqua (Zaq) fixable viability kit (Biolegend) in the ZAq^- population. Flow cytometry analysis was performed using a FACS Canto II (BD). Data were analysed using the FlowJo software (Tree Star).

Statistical Analysis

All values were expressed as mean ± SEM. Differences in means between PD patients and HC groups were analysed by 2-tailed unpaired Student's *t*-test. Comparisons between different experimental groups in MPTPp experiments were performed by one-way ANOVA followed by the multi-comparison Tukey's *post-hoc* test. Correlations between different parameters were analysed by Pearson's test when data was normally distributed or by Spearman's test when data was not normally distributed. Normal distribution was determined by Shapiro–Wilk test. *P*-value ≤ 0.05 was considered significant. Analyses were performed with GraphPad Prism 6 software.

Study Approval

The study performed with human individuals conforms to the principles outlined in the Declaration of Helsinki, the study protocol was approved by the local Ethics Committee of the Hospital del Salvador, Santiago (Chile), and all the participants signed a written informed consent before enrollment. All procedures performed in animals were approved by and complied with regulations of the Institutional Animal Care and Use Committee at Fundación Ciencia & Vida.

RESULTS

DRD3 Expression Is Reduced in $CD4^+$ T-Cells Obtained From PD Patients

As stated above, emerging evidence has shown that DRD3-signalling in $CD4^+$ T-cells plays a pivotal role favouring the development of PD in animal models (13, 23). Since a previous study found a significant reduction in the levels of *drd3*-transcripts in PBMCs obtained from PD (24), we addressed here the question of whether DRD3 expression, at the level of protein, was altered in $CD4^+$ T-cells obtained from PD patients. For this purpose, we analysed DRD3 expression in different lymphocyte populations from PD patient samples displaying different stages of PD progression. Accordingly, we analysed blood samples from 38 HC and 41 PD patients. In these samples we determined DRD3 expression in different $CD4^+$ T-cells subsets, including naive $CD4^+$ T-cells, memory $CD4^+$ T-cells, effector $CD4^+$ T-cells and total $CD4^+$ T-cells. In addition, we also included the expression of DRD3 in B cells and in natural killer (NK) cells, as we found high levels of DRD3 expression in these lymphocyte populations obtained from healthy donors samples (**Figure 1A**, left panel). We analysed DRD3 expression in the different lymphocyte subsets by flow cytometry using the gating strategy indicated (**Supplementary Figure 1**). Interestingly, when DRD3 expression was compared in HC and PD patients, we did not find differences in DRD3 expression in total $CD4^+$ T-cells ($CD3^+$ $CD4^+$), naive $CD4^+$ T-cells ($CD3^+$ $CD45RA^+$ $CD4^+$), memory/effector $CD4^+$ T-cells ($CD3^+$ $CD45RO^+$ $CD4^+$), B-cells ($CD19^+$) and NK cells ($CD56^+$) upon resting conditions (**Figure 1A**, left panel). Conversely, when DRD3 expression was evaluated after *ex vivo* T-cell activation, DRD3 expression was significantly reduced in total $CD4^+$ T-cells ($CD3^+$ $CD4^+$) and in memory/effector $CD4^+$ T-cells ($CD3^+$ $CD4^+$ $CD45RO^+$) obtained from PD patients (**Figure 1A**, right panel). This data indicates a significant down-regulation of DRD3 expression in *ex vivo* activated $CD4^+$ T-cells obtained from PD patients. We next attempted to evaluate whether DRD3 down-regulation was associated with PD activity. For this purpose, we analysed the potential correlations between

DRD3 expression and the clinical score of PD patients evaluated by different tests, including the UPDRS, the Modified Hoehn and Yahr scale, the Schwab and England score and the MoCA test. Interestingly, we found a significant correlation between DRD3 down-regulation in naïve $CD4^+$ T-cells and the UPDRS clinical score (**Supplementary Figure 2**), although we did not find significant associations of disease activity with the DRD3 down-regulation in activated total or memory/effector $CD4^+$ T-cells (**Supplementary Figure 3**).

$CD4^+$ T-Cells Obtained From PD Patients Display an Increased Percentage of Pro-inflammatory Phenotypes and Biased Th1-Differentiation

DRD3-signalling in $CD4^+$ T-cells has been consistently associated to Th1 and Th17 mediated immunity (13, 19, 20). Furthermore, Th1 and Th17 have been proven to be the inflammatory phenotypes of $CD4^+$ T-cells driving neuroinflammation and consequent neurodegeneration of dopaminergic neurons in animal models of PD (6, 13). On the other hand, it has been demonstrated that suppressive activity of Tregs is able to dampen T-cell mediated inflammation in PD models, thus attenuating neurodegeneration (6, 30, 31). For these reasons, we next aimed to determine potential alterations in the percentage of inflammatory T-cell phenotypes Th1 and Th17 as well as in the extent of the anti-inflammatory T-cell phenotype, Treg, obtained from PD patients in comparison to HC. Accordingly, in a subgroup of PD and HC we first analysed the functional phenotypes in resting T-cells. For this purpose, immediately after isolation from fresh blood samples, PBMC were stimulated with PMA and ionomycin for 4h and cytokine production and the expression of key transcription factors were analysed by intracellular immunostaining followed by flow cytometry analysis. These analyses in "resting" conditions (after just a short period of stimulation) were performed to have an idea of the frequency of inflammatory and anti-inflammatory phenotypes contained in the population of memory and effector T-cells. The results show that PD individuals presented 2-fold higher Th1 frequency in resting $CD4^+$ T-cells in comparison with HC (**Figure 1B**). On the other hand, Th17 frequency in resting $CD4^+$ T-cells was similar in PD and HC, whilst Tregs were not detectable in these conditions (**Figure 1B** and data not shown). To allow the expansion of T-cells, we next analysed the frequency of relevant functional phenotypes of $CD4^+$ T-cells after the activation with anti-CD3 and anti-CD28 antibodies for 3d. Then, activated T-cells were re-stimulated with PMA and ionomycin during the last 4h and cytokine production and the expression of transcription factors were quantified by intracellular immunostaining followed by flow cytometry analysis. The results show about 3-fold increase in the frequency of both Th1 and Th17 phenotypes in *ex vivo* activated $CD4^+$ T-cells obtained from PD patients in comparison with those obtained from HC (**Figure 1C**). Of note, in these conditions Tregs were detectable, although no differences were observed between PD patients and HC (**Figure 1C**). We also analysed the potential association of Th1, Th17, Treg, or total $CD4^+$ T-cells frequencies in peripheral blood of PD patients with the severity of the disease, however we did not find any significant correlation (**Supplementary Figure 4**). Since we observed a higher difference in Th1 frequency between PD and HC after *ex vivo* T-cell activation (**Figure 1C**) than when compared in resting conditions (**Figure 1B**), we wondered whether naive $CD4^+$ T-cells differentiating to Th1 phenotype could be contributing to this higher Th1 frequency. To address this possibility, we performed experiments in which naive $CD4^+$ T-cells were first isolated by cell-sorting, cultured in Th1-skewed conditions and then the extent of Th1 differentiation was compared between PD patients and HC. Interestingly, these results show a 2-fold increase of Th1 differentiation in PD patients in comparison with HC (**Figure 1D**). Taken together these results indicate that $CD4^+$ T-cells in PD patients present higher frequencies of pro-inflammatory phenotypes and naive cells display a skewed Th1-differentiation.

The Transference of $CD4^+$ T-Cells Treated *ex vivo* With a Selective DRD3-Antagonist Exerts a Therapeutic Effect Attenuating Motor Impairment in MPTPp-Treated Mice

Since a prominent role of DRD3-signalling in $CD4^+$ T-cells has been observed in the development of PD in mouse models (13, 32), and the systemic DRD3-antagonsim has been proven to attenuate neurodegeneration and motor impairment in different animal models of PD (23), we next aimed to test the therapeutic potential of the selective inhibition of DRD3-confined to $CD4^+$ T-cells. For this purpose, we used an animal model of PD induced by the chronic administration of MPTP and probenecid (MPTPp), which results in both, loss of dopaminergic neurons of the nigrostriatal pathway and a significant motor impairment (33). To exert a selective DRD3-antagonism in $CD4^+$ T-cells, these cells were pre-incubated *ex vivo* with PG01037 (hereinafter called $CD4^+$/PG01037) (34) and then transferred into MPTPp-treated mice. Of note, DRD3-antagonism mediated by PG01037 20 nM attenuated the potentiation of Th1-differentiation exerted by the selective stimulation of DRD3 with dopamine 50 nM (**Supplementary Figure 5**). Accordingly, we performed a titration of the number of injections and the number of $CD4^+$ T-cells per injection able to exert a significant therapeutic effect on MPTPp-treated mice. For this purpose, $CD4^+$ T-cells were incubated with PG01037 20 nM *ex vivo* and then, $4x10^5$, $7x10^5$ or $10x10^5$ $CD4^+$ T-cells per mouse were transferred in a single i.v. injection into MPTPp mice. Moreover, another group of mice was treated with three injections (separated by 7d between) of $4x10^5$ $CD4^+$ T-cells per mouse each (**Figure 2A**). We also used a control group of mice that received $CD4^+$ T-cells without *ex vivo* treatment with PG01037. In all experimental groups we determined the therapeutic potential at the level of motor impairment and neurodegeneration and the extent of participation of different T-cell subsets into the midbrain and cervical lymph nodes (CLN). Notably, the results show that only a single injection of 4×10^5 $CD4^+$/PG01037, but not single injections of 7×10^5 or 10×10^5 or three injections of 4×10^5 $CD4^+$/PG01037 per mouse, exerted

significant attenuation of motor impairment as determined by the beam test (# errors; **Figure 2B**) and the coat-hanger test (extreme latency; **Figure 2C**). Thus, these results indicate that the treatment of MPTPp-intoxicated mice with a single injection of 4×10^5 $CD4^+$/PG01037 exerts a therapeutic effect at the level of motor impairment in this animal model.

To determine how the different regime of treatment of MPTPp mice with the transfer of *ex vivo* manipulated $CD4^+$ T-cells affected the participation of different T-cell subsets into the midbrain and CLN, we first determine the time-point in which neuroinflammation was already evident in this animal model. Accordingly, we evaluated the dynamics of M1 and M2 phenotypes in microglial cells in the brain and the extent of T-cell infiltration in meningeal vessels at different time-points in MPTPp animals. We observed that both, meningeal $CD4^+$ T-cells and M1-microglia were already increased after 3 weeks of MPTPp treatment (**Supplementary Figure 6**) and thereby we chose this time-point to analyse the phenotypes of $CD4^+$ T-cells infiltrating the midbrain and CLN. Interestingly, the results show that the single injection of 4×10^5 $CD4^+$/PG01037 was the only treatment that did not reduce the number of mononuclear cells infiltrating the midbrain of MPTPp mice (**Figures 3A,B**), coinciding with the therapeutic effect observed at the level of motor impairment. Conversely, at the level of T-cells infiltrating CLN, all the therapeutic regime of transfer of $CD4^+$/PG01037 in MPTPp-intoxicated mice resulted in significant reduction of alive lymph nodes cells (**Figures 3C,D**). Moreover, single injections of 4×10^5 or 10×10^5 or three injections of 4×10^5 $CD4^+$/PG01037, but not the single injection of 7×10^5 $CD4^+$/PG01037 significantly attenuated the number of $CD4^+$ T-cells infiltrating the CLN in MPTPp mice (**Figures 3C,D**). Interestingly, only a single injection of 10×10^5 or three injections of 4×10^5 $CD4^+$/PG01037 resulted in attenuated number of Th1 and Th17 in the CLN of MPTPp-intoxicated mice

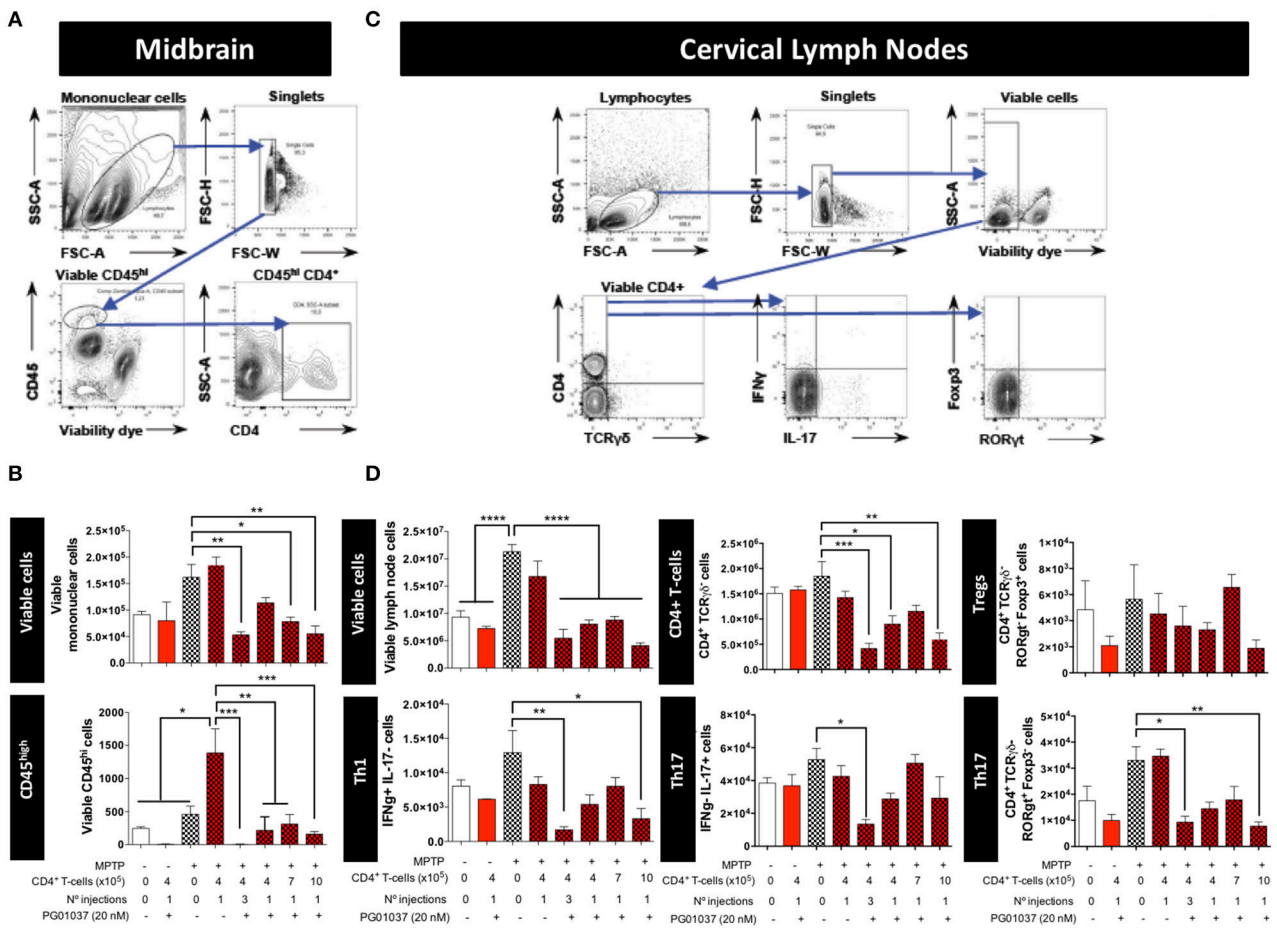


FIGURE 3 | Analysis of T-cell infiltration into the brain and cervical lymph nodes in MPTPp-intoxicated mice after transference of $CD4^+$ T-cells treated with PG01037 *ex vivo*. Animals were treated as described in **Figure 2A** and sacrificed after 3 weeks of MPTPp intoxication (at the end of week number 4 in the scheme of **Figure 2A**). The frequencies of different inflammatory and anti-inflammatory lymphocyte subsets infiltrating the midbrain **(A,B)** and cervical lymph nodes **(C,D)** were analysed by flow cytometry. **(A,C)** Representative dot plots showing the gating strategy. **(B,D)** absolute numbers per animal of different lymphocyte subsets obtained from the different experimental groups were quantified. Data represent the mean with the SEM. One-way ANOVA followed by Tukey's multiple comparison *post-hoc* test was used to determine statistical differences: $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$; $n = 3$ mice per group.

(**Figures 3C,D**). In addition, any of the treatments proven show a significant difference in the extent of Tregs infiltrating CLN in MPTPp mice (**Figures 3C,D**). Thus, unexpectedly, the only difference at the level of T-cells infiltrating midbrain and CLN that was associated with the selective therapeutic effect at the level of motor impairment was the absence of reduction in the number of total mononuclear cells infiltrating the midbrain of MPTPp mice. To address a potential effect of DRD3-signalling in the viability of $CD4^+$ T-cells, we isolated $CD4^+$ T-cells from the spleen of WT or DRD3-defficient mice and then were activated for 6d and subsequently the viability was analysed in Teff and Treg cells by flow cytometry. The results show no significant differences in the frequency of living Treg or Teff between both genotypes (**Supplementary Figure 7**), thus ruling out the possibility that DRD3-signalling affected $CD4^+$ T-cells viability.

Finally, to determine the therapeutic potential of the different regime of T-cell transfer at the level of neurodegeneration, we next quantified the loss of dopaminergic neurons in the SN and the extent of dopaminergic terminals in the striatum of experimental mice. For this purpose, after the determination of motor performance, mice were sacrificed and dopaminergic neurons were quantified by immunohistochemical analysis of tyrosine hydroxylase (TH) in the SN *pars compacta* (SNpc) and the density of dopaminergic terminals was evaluated in the striatum by immunohistochemical analysis of dopamine transporter (DAT). Quite unexpected, the results show that the extent of neurodegeneration was not significantly attenuated by any of the regime of *ex vivo* manipulated T-cell transfer (**Figure 4**). Taken together these results indicate that a single injection of 4×10^5 $CD4^+$/PG01037 exerts a therapeutic effect in MPTPp mice attenuating motor impairment but without effect in the extent of neurodegeneration.

The *ex vivo* Transcriptional Inhibition of *Drd3* in $CD4^+$ T-Cells Does Not Exert Any Therapeutic Effect in MPTPp-Treated Mice

Because the pharmacologic DRD3-antagonism of $CD4^+$ T-cells *ex vivo* exerted a therapeutic effect only at the level of motor impairment but not at the level of neurodegeneration we attempted to improve the therapeutic power inducing a more sustained inhibition of DRD3-signalling in $CD4^+$ T-cells. For this purpose, we generated retroviral particles codifying for an shRNA to interfere with *Drd3*-transcription (shDRD3). After setting up the transduction protocols, we confirmed that retroviral transduction with the shDRD3 (RV-shDRD3) actually reduced the levels of *Drd3*-transcripts and resulted in impaired production of IFN-γ by $CD4^+$ T-cells (**Supplementary Figure 8**), as described before (20). Afterwards, we performed a set of experiments aimed to compare the therapeutic potency of the transfer of $CD4^+$/PG01037, the transfer of $CD4^+$ T-cells *ex vivo* transduced with RV-shDRD3 (hereinafter called $CD4^+$/RV-shDRD3) or the systemic DRD3-antagonism. For this purpose, MPTPp-intoxicated mice received a single injection of 4×10^5 $CD4^+$/PG01037, a single injection of 4×10^5 $CD4^+$/RV-shDRD3 or the i.p. administration of PG01037 at 30 mg/kg and the extent of neurodegeneration, motor impairment and T-cell phenotypes in midbrain and CLN were determined (**Figure 5A**). The results show that only the transfer of $CD4^+$/PG01037 or the systemic administration of PG01037, but not the transfer of $CD4^+$/RV-shDRD3 exerted a significant attenuation of motor impairment in MPTPp-intoxicated mice reducing the number of errors in the beam test (**Figure 5B**) and increasing the number of sections travelled in the coat-hanger test (**Figure 5C**). It is noteworthy that in a previous study using the same animal model of PD, we show that a single i.p. injection of 30 mg/kg PG01037 had no effect in motor impairment (23). Since the remaining PG01037 concentration present in $CD4^+$/PG01037 (after the *ex vivo* treatment with 20 nM PG01037 followed by cells washing) is much lower than that present in a single i.p. injection of 30 mg/kg PG01037, it is tempting to rule out that the therapeutic effect exerted by $CD4^+$/PG01037 in motor impairment is independent of $CD4^+$ T-cells and just due to PG01037. The analysis of T-cell phenotypes in midbrain and CLN did not give any significant differences, despite they show interesting trends for the treatment with systemic PG01037 in decreasing Th1 and Th17 frequencies and increasing Tregs frequency in CLN of MPTPp-intoxicated mice (**Supplementary Figure 9**). Consistently with previous results (23), the quantification of dopaminergic neurons of the nigrostriatal pathway shows a significant reduction in neuronal loss only when MPTPp-intoxicated mice were treated with systemic PG01037, but not when received the transfer of $CD4^+$/PG01037 or $CD4^+$/RV-shDRD3 (**Figure 6**). Together, these results indicate that whereas the systemic DRD3-antagonism exerts a therapeutic effect attenuating neurodegeneration and motor impairment, the transfer of $CD4^+$/PG01037 exerts a therapeutic effect only confined to the motor impairment but without effect in neuronal loss. Quite unexpectedly, the transfer of $CD4^+$/RV-shDRD3 did not exert any detectable therapeutic effect. A summary of the different therapeutic effects observed for the different treatments in different set of experiments carried out in this study is shown in **Table S1**.

The Systemic DRD3-Antagonism as Well as the Transference of $CD4^+$ T-Cells Treated *ex vivo* With a Selective DRD3-Antagonist Reduce the Extent of Microglial Activation in MPTPp-Treated Mice

To gain a deeper insight in the mechanism involved in the therapeutic effect exerted by the systemic DRD3-antagonism and by the transfer of $CD4^+$/PG01037 in MPTPp-treated mice, we next attempted to analyse how was affected astrocyte and microglial activation. For this purpose, MPTPp-intoxicated mice received a single injection of 4×10^5 $CD4^+$/PG01037, or the i.p. administration of PG01037 at 30 mg/kg (as indicated in **Figure 5A**) and the extent of astrogliosis and microglial activation were evaluated in the striatum by immunohistochemical analyses of GFAP and Iba1, respectively. Unexpectedly, the results show no differences in the degree of astrogliosis among the different experimental groups (**Figure 7**). On the other hand, both the systemic DRD3-antagonism and

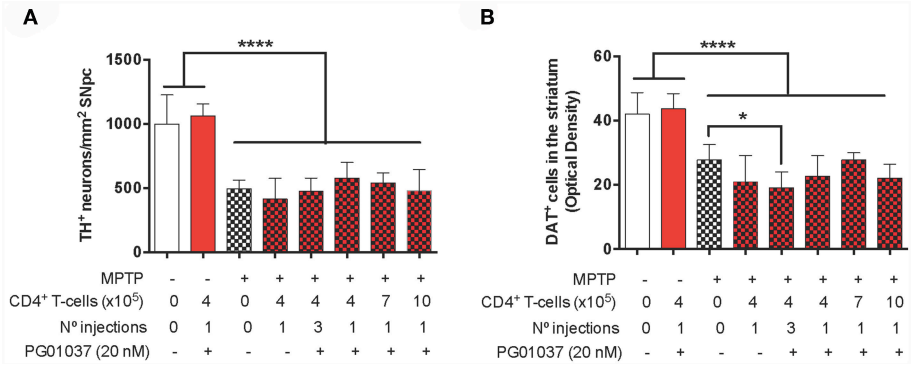


FIGURE 4 | Evaluation of the therapeutic potential at the level of neurodegeneration of DRD3 inhibition in CD4+ T-cells by treatment with a selective antagonist *ex vivo* in MPTPp-treated mice. $CD4^+$ T-cells (4×10^5, 7×10^5, or 10×10^5 per mouse) were treated with or without PG01037 (20 nM) and then i.v. injected in experimental animals 1 day after the first MPTPp injection (see **Figure 2A**). In some cases, animals received 3 injections of $CD4^+$ T-cells separated by 1 week intervals. Neurodegeneration was analysed 1 week after the last MPTPp injection. Dopaminergic neurons were quantified by immunohistochemical analysis of tyrosine hydroxylase (TH) in the substantia nigra pars compacta (SNpc) **(A)** and dopamine transporter (DAT) in the striatum **(B)**. Data represent the mean with the SEM. One-way ANOVA followed by Tukey's multiple comparison *post-hoc* test was used to determine statistical differences: $^*p < 0.05$; $^{****}p < 0.0001$; $n = 5$–8 mice per group.

the transfer of $CD4^+$/PG01037 induced a marked reduction in the extent of microgliosis in MPTPp-treated mice (**Figure 7**). Thus, these results show that the therapeutic effect observed for systemic DRD3-antagonism at the level of neurodegeneration and motor impairment and for the transfer of $CD4^+$/PG01037 at the level of motor impairment involve an attenuation in microglial activation.

DISCUSSION

Our data here demonstrates a significant and selective reduction of DRD3-expression confined to $CD4^+$ T-cells obtained from PD patients. Moreover, our results indicate that the pharmacologic DRD3-antagonism, but not the interference of *Drd3*-trascription in $CD4^+$ T-cells *ex vivo* resulted in a therapeutic effect at the level of motor impairment. However, only systemic DRD3-antagonism, but not the transfer of $CD4^+$/PG01037, reduced the extent of neurodegeneration of the dopaminergic neurons of the nigrostriatal pathway.

Interestingly, our results show a significant reduction of DRD3 expressed in $CD4^+$ T-cells, which could be due to a compensatory mechanism attempting to decrease the inflammatory effect induced by DRD3-stimulation in this lymphocyte population (13). Moreover, this alteration on DRD3 expression in $CD4^+$ T-cells obtained from PD patients could represent a useful marker for diagnostic analysis. Of note, Nagai et al., described before that the levels of *Drd3* mRNA were decreased in total PBMCs obtained from PD patients and the degree of this down-regulation was correlated with the stage of disease progression (24). Accordingly, we found a significant correlation between DRD3 down-regulation (at the protein level) in naive $CD4^+$ T-cells and the degree of disease activity (see the UPDRS score in **Supplementary Figure 2**). Of note, we did not find significant associations of disease activity with the DRD3 down-regulation in any other lymphocyte subset analysed, including B-cells, NK cells, resting or activated total $CD4^+$ T-cells and activated memory/effector $CD4^+$ T-cells (**Supplementary Figure 2**, **3**). Thus, these results suggest a selective association of clinical PD progression with the extent of down-regulation of DRD3 expression selectively on naive $CD4^+$ T-cells.

Despite our previous results obtained in mouse models indicate that DRD3-signalling in $CD4^+$ T-cells favours the development of PD (13), our results obtained from PD patients show a significant and selective reduction of DRD3 expression in $CD4^+$ T-cells obtained from peripheral blood in comparison to those obtained from HC (**Figure 1**). Thus, this reduction of DRD3 expression in $CD4^+$ T-cells obtained from PD patients could be interpreted as an adaptive down-regulation of this pro-inflammatory receptor after a long-term period of chronic inflammation in these patients. Nevertheless, another plausible explanation for this fact can be that simply, $CD4^+$ T-cells specific for relevant antigens associated to PD [i.e., nitrated α-synuclein; (1, 35)] would acquire inflammatory phenotypes with high DRD3-expression (20) and they would be just infiltrating the site of inflammation (into the SNpc) but not recirculating in the periphery. This fact would explain why $CD4^+$ T-cells expressing low levels of DRD3 would be selectively found in the periphery. This latter hypothesis highlights the advantages that should have an antigen-specific therapy based in $CD4^+$ T-cells as a treatment for PD. In this regard, it is expected that a therapy involving the inhibition of DRD3 confined only to those $CD4^+$ T-cells specific for relevant antigens associated to PD (i.e., nitrated α-synuclein) would exert a stronger therapeutic potential than those therapeutic approaches tested here involving the inhibition of DRD3-signalling in $CD4^+$ T-cells irrespective of their antigen-specificity. Furthermore, an antigen-specific $CD4^+$ T-cell based therapy for PD would avoid the multiple potential side-effects exerted by systemic administration of dopaminergic drugs, such as PG01037.

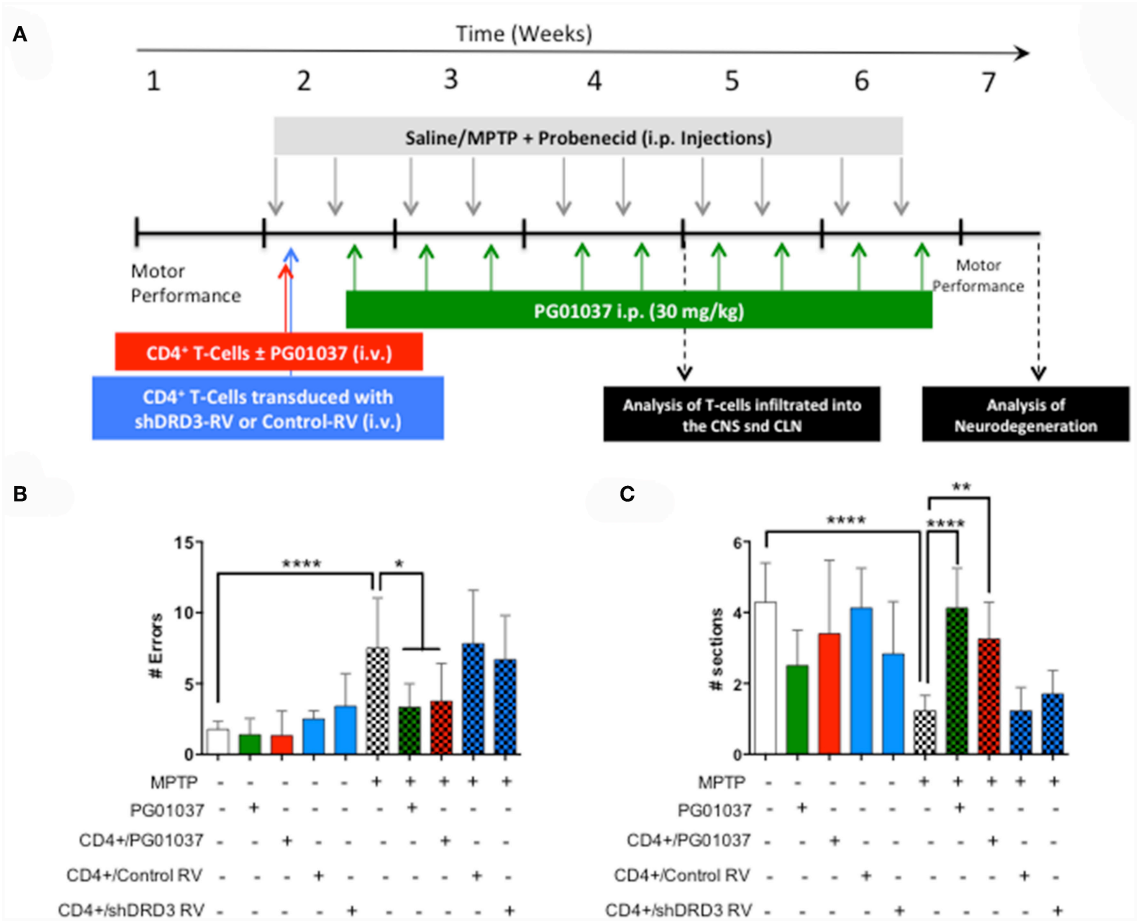


FIGURE 5 | Comparison of the therapeutic potential of the systemic DRD3-antagonistm or the intravenous transference of $CD4^+$ T-cells transduced with shRNA for DRD3 or treated with a DRD3 antagonist on the motor performance of MPTPp-treated mice. **(A)** Experimental design: Control animals (without MPTPp treatment) were treated with saline and probenecid alone, or with i.p. PG01037, or the i.v. transference of $CD4^+$ T-cells treated with PG01037, transduced with RV-Control or transduced with RV-shDRD3. MPTPp animals received 10 i.p. injections with MPTP (20 mg/kg) and probenecid (250 mg/kg) during weeks 2–6 (grey arrows). $CD4^+$ T-cells (4×10^5 cells per mouse) were treated with 20 nM PG01037 (red) or transduced with retroviral particles (MOI 1:1) codifying for RV-Control or RV-shDRD3 (blue) and then i.v. injected in experimental animals 1 day after the first MPTPp injection. Mice treated with systemic DRD3-antagonism (green) received 9 i.p. injections of PG01037 (30 mg/kg) administered 1 day after MPTPp injections starting after the second MPTPp administration. T-cell infiltration was analysed after 3 weeks of MPTPp-treatment. Neurodegeneration was analysed 1 week after the last MPTPp injection. Motor performance was analysed the week before beginning with MPTPp administration to distribute experimental groups with homogeneous motor performance and then it was evaluated again 16 h after the last MPTPp injection in the Beam-test **(B)** and in the coat-hanger test **(C)**. Data represents the mean with the SEM. One-way ANOVA followed by Tukey's multiple comparison *post hoc* test were used to determine statistical differences: $^*p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$, $n = 5$–12 mice per group.

Importantly, we found that naive $CD4^+$ T-cells obtained from Chilean PD patients display an increased differentiation toward Th1, a functional phenotype that has been involved in the inflammatory response associated to neurodegeneration in animal models (6, 13). In the same direction, in an study performed in a cohort of 82 Italian PD patients, Kustrimovic *et al.*, have performed an analysis of the different functional T-cell phenotypes and have described an Th1-biased immune signature in both, drug-naive or drug-treated PD (36). In addition, another previous study carried out with 40 Italian PD patients has shown a positive correlation between the degree of PD progression with the levels of IFN-γ produced by PBMCs (37). Thereby, together these results suggest that this Th1-skewed differentiation of naive $CD4^+$ T-cells is a general feature of PD patients, irrespective of their ethnicity and independent on the administration of dopaminergic drugs.

The present findings indicate that both, the systemic DRD3-antagonism as well as the transfer of $CD4^+$/PG01037 attenuated the motor impairment induced by MPTPp-intoxication, however the systemic treatment with PG01037 was the only therapeutic approach that reduced the loss of dopaminergic neurons in the SNpc. This differential therapeutic effect exerted by the systemic DRD3-antagonism in comparison to the transfer of $CD4^+$/PG01037 could be due to that systemic administration of PG01037 would be able to block DRD3-signalling not only in $CD4^+$ T-cells, but also in astrocytes. In this regard, we

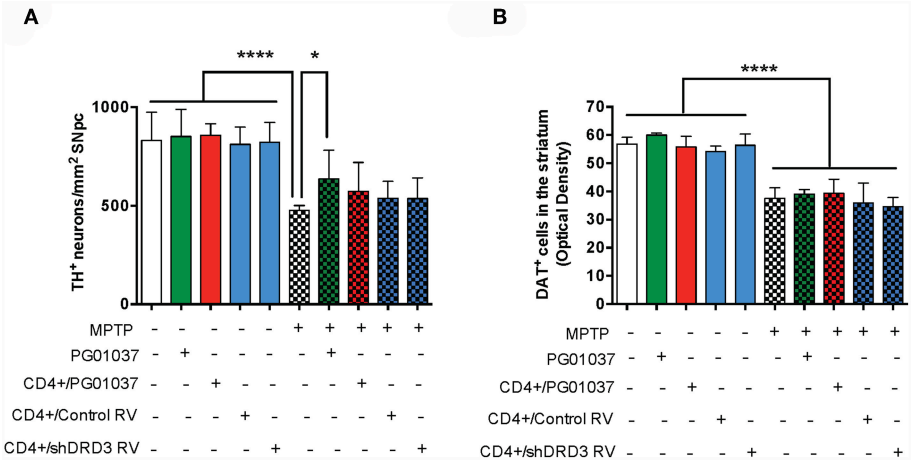


FIGURE 6 | Comparison of the therapeutic potential of the systemic DRD3-antagonistm or the intravenous transference of $CD4^+$ T-cells transduced with shRNA for DRD3 or treated with a DRD3 antagonist on neurodegeneration of MPTPp-treated mice. $CD4^+$ T-cells (4×10^5 cells per mouse) were treated with 20 nM PG01037 (red) or transduced with retroviral particles (MOI = 1) codifying for RV-Control or RV-shDRD3 (blue) and then i.v. injected in experimental animals 1 day after the first MPTPp injection. Mice treated with systemic DRD3-antagonism (green) received 9 i.p. injections of PG01037 (30 mg/kg) administered 1 day after MPTPp injections starting after the second MPTPp administration (see scheme in **Figure 5A**). Neurodegeneration was analysed 1 week after the last MPTPp injection. Dopaminergic neurons were quantified by immunohistochemical analysis of tyrosine hydroxylase (TH) in the substantia nigra pars compacta (SNpc) **(A)** and dopamine transporter (DAT) in the striatum **(B)**. Data represent the mean with the SEM. One-way ANOVA followed by Tukey's multiple comparison *post-hoc* test were used to determine statistical differences: $^*p < 0.05$; $^{****}p < 0.0001$ $n = 5$–7 **(A)** or $n = 5$–11 **(B)** mice per group.

have obtained evidence suggesting that DRD3-inhibition in astrocytes favours an anti-inflammatory astrogliosis, which was associated with reduced number of inflammatory microglia (23). Accordingly, in the present study we observed that both the systemic DRD3-antagonsim and the transfer of $CD4^+$/PG01037 decreased the number of inflammatory microglia, but without apparent effect in astrocyte activation (**Figure 7**). In addition to astrocytes and $CD4^+$ T-cells, DRD3 has been described to be expressed in other subsets of immune cells that could play a relevant role in neuroinflammation associated to PD, including B-cells, NK cells, and neutrophils (38). In this regard, auto-antibodies recognising Lewy bodies and dopaminergic neurons have been detected in the serum and infiltrated in the brain parenchyma of PD patients (39). Furthermore, the stereotaxic delivery of IgG purified from PD patients induces a significant loss of dopaminergic neurons of the SN in rats in comparison with the effect observed for IgG purified from HC, thus suggesting a relevant role of B-cells in the physiopathology of PD (40). On the other hand, it has been described a significant reduction in the expression of the NK inhibitory receptor NKG2A in PD (41). Moreover, a recent meta-analysis performed with 943 patients indicated an association of PD with increased number of NK cells (42), suggesting a role for NK cells in the physiopathology of this disorder. In addition, Th17 cells, which have been consistently involved in PD (6, 43, 44) exert their effector function mainly by recruiting neutrophils to the site of inflammation, where they release cytotoxic granules inducing directly the death of target cells (45). Thus, DRD3 expressed in neutrophils, B-cells or NK cells could potentially also play a role promoting PD development and progression, nevertheless, further studies are necessaries to address experimentally the relevance and relative contribution of these potential mechanisms in PD.

Intriguingly, the i.v. transfer of $CD4^+$/PG01037 into MPTPp-treated mice, reduced the motor impairment but without significant effects in neurodegeneration. This discrepancy could be due to that, by affecting IL-4 and IFN-γ production (20), DRD3-signalling in $CD4^+$ T-cells might be involved in the cross-talk between T-cell function and neuronal tasks, irrespective of the neurodegenerative process. In this regard, it has been previously shown that IL-4-produced by $CD4^+$ T-cells might regulate the acquisition of spatial memory in the hippocampus (46). Whether DRD3-signalling in $CD4^+$ T-cells may affect neural circuitry involved in motor performance or not should be addressed in further studies.

Intriguingly, when we tested the therapeutic potential of the transfer of increasing number of $CD4^+$/PG01037 into MPTPp-mice, we did not observe a dose-response curve. Unexpectedly, we obtained a therapeutic effect only with the lower dose of $CD4^+$/PG01037 (4×10^5 cells per mouse), but not with higher doses of T-cells (7×10^5 or 10×10^5 cells per mouse). According to these results, it has been previously shown that the transfer of low dose of $CD4^+$ T-cells (5×10^4 cells per mouse) exerts a stronger effect than higher doses of $CD4^+$ T-cells in an anti-tumour therapy (47). In this regard, it is though that the transfer of lower doses of therapeutic $CD4^+$ T-cells allows a stronger expansion *in vivo* after antigen-recognition.

Unexpectedly, whereas the DRD3-antagonsim confined to $CD4^+$ T-cells exerted a significant attenuation of motor impairment in MPTPp-treated mice, the transcriptional inhibition of *Drd3* in $CD4^+$ T-cells had no effect in motor impairment. This apparent controversy among our results

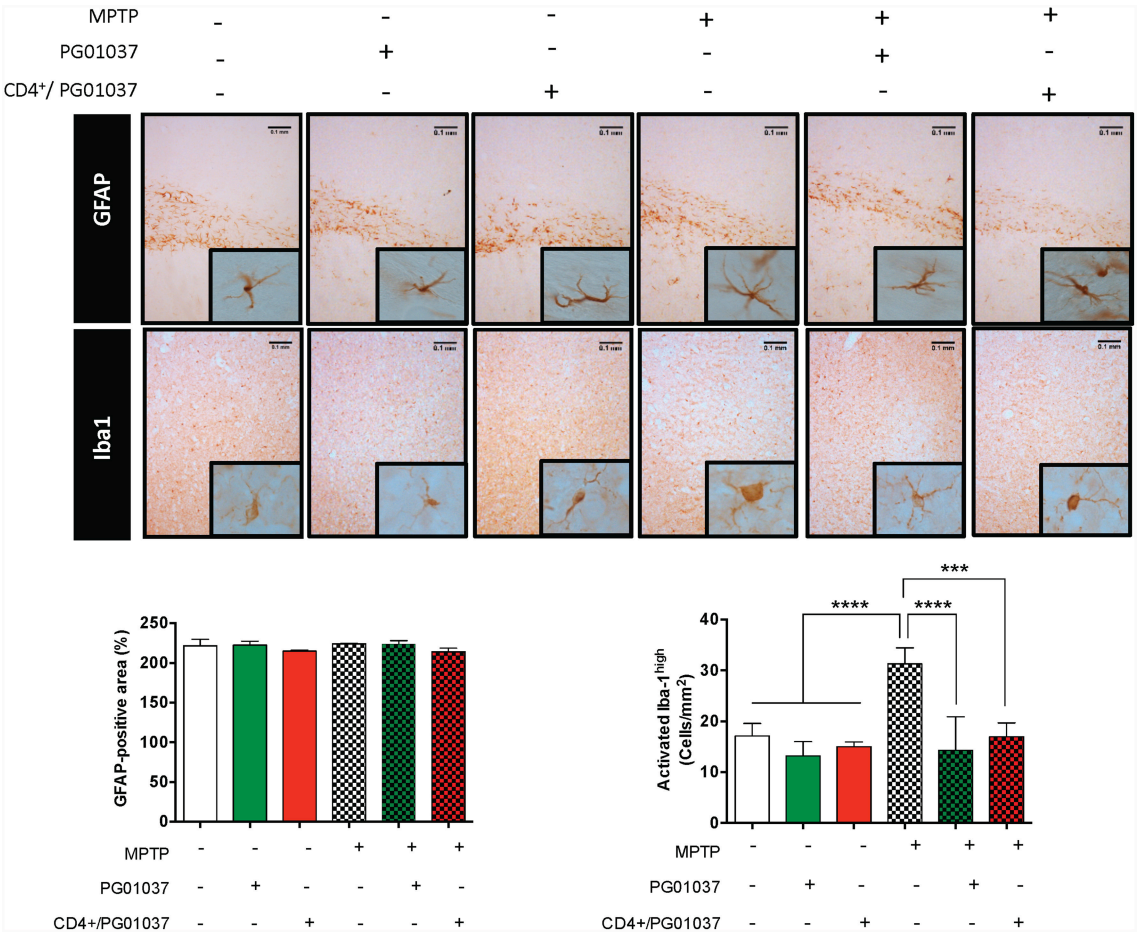


FIGURE 7 | Analysis of neuroinflammation in MPTPp-intoxicated mice upon treatment with systemic DRD3-antagonism or with the intravenous transference of $CD4^+$ T-cells treated with a DRD3 antagonist. $CD4^+$ T-cells (4×10^5 cells per mouse) were treated with 20 nM PG01037 (red) and then i.v. injected in experimental animals 1 day after the first MPTPp injection. Mice treated with systemic DRD3-antagonism (green) received 9 i.p. injections of PG01037 (30 mg/kg) administered 1 day after MPTPp injections starting after the second MPTPp administration (see scheme in **Figure 5A**). Neuroinflammation was analysed 1 week after the last MPTPp injection. Astrogliosis was quantified by immunohistochemical analysis of GFAP and microglial activation was quantified by immunohistochemical analysis of Iba1 in the striatum. Representative overview images of GFAP (top panel) and Iba1 (middle panel) immunostaining are shown. Quantification of GFAP-associated density (bottom-left panel) and the number of $Iba1^{high}$ cells per area (bottom-right panel) are shown in the bottom panels. Data represent the mean with the SEM from 3 to 5 mice per group. One-way ANOVA followed by Tukey's multiple comparison *post-hoc* test were used to determine statistical differences: $^{***}p < 0.001$; $^{****}p < 0.0001$.

could be due to the different extent of DRD3 inhibition. In this regard, DRD3-antagonism in $CD4^+$ T-cells was performed with 20 nM PG01037, a concentration that represents approximately 28-fold its K_i (34). Thereby, the conditions used to promote DRD3-antagonism ensure the inhibition of nearly all DRD3 expressed in $CD4^+$ T-cells. On the other hand, despite $CD4^+$/RV-shDRD3 displayed a significant impairment in the production of IFN-γ (**Supplementary Figure 8E**), the reduction exerted in the levels of *drd3*-transcripts was about 50% (**Supplementary Figure 8D**). Thereby, it is likely that this milder effect in DRD3-inhibition exerted by $CD4^+$ T-cells transduction with RV-shDRD3 would explain why we could not observe any detectable therapeutic effect at the level of motor impairment or neurodegeneration. At this point, it is important to note that upon T-cell activation, DRD3 expression was sharply reduced in $CD4^+$ T-cells (**Supplementary Figure 10**), although the levels of *drd3* transcripts were increased (20). These results suggest that DRD3-mediated effects are triggered in resting conditions or early after $CD4^+$ T-cell activation, but with consequences later in T-cell response. Moreover, since T-cell activation is required to promote retroviral transduction in $CD4^+$ T-cells, we could not observe a significant effect of shDRD3 transduction on DRD3 expression in activated $CD4^+$ T-cells (**Supplementary Figure 10**). Thus, a plausible explanation for the lack of therapeutic effect observed for the transfer of $CD4^+$ T-cells transduced *ex vivo* with RV-shDRD3 is that an early inhibition of DRD3-signalling in resting $CD4^+$ T-cells is necessary to evoke the beneficial effects induced in motor impairment in MPTPp-treated mice.

It is intriguing that our previous study evaluating the therapeutic potential of systemic administration of PG01037 shows a significant increase in anti-inflammatory astrogliosis and

attenuation of neurodegeneration of the nigrostriatal pathway at the level of neuronal bodies in the SNpc and at the level of dopaminergic terminals in the striatum (23), however the present study shows just a therapeutic effect at the level of SNpc, but not in the striatum and without effect in astrocyte activation (**Figures 4**, **7**). This discrepancy could be explained by the fact that these two set of experiments were performed in two different animal facilities, and therefore the microbiota composition of experimental mice should be different as well. In this regard, it has been recently demonstrated that gut microbiota has a strong impact in the susceptibility of individuals to develop neurodegeneration and the motor impairment associated to PD in humans and animal models (48).

Finally, it is important to note that the treatments that exerted therapeutic effects here, the systemic PG01037 administration and the i.v. transfer of $CD4^+$/PG01037, were administered early during the induction of the disease (24 h after the second MPTPp injection and 24 h after the first MPTPp injection, respectively). Despite these treatments were given before the motor onset, early diagnosis of PD in humans is currently quickly evolving. Indeed, there are some key early symptoms, including REM sleep disorder, olfactory loss (49), and gut-associated issues (50) that together might predict PD manifestation and the development of motor impairment with many years in advance. In this regard, drug-design is currently pointing to stop the progression of neurodegeneration in PD at early stages of diagnosis, earlier than the onset of motor impairment.

ETHICS STATEMENT

The study performed with human individuals conforms to the principles outlined in the Declaration of Helsinki, the study protocol was approved by the local Ethics Committee of the Hospital del Salvador, Santiago (Chile), and all the participants signed a written informed consent before enrollment. All procedures performed in animals were approved by and complied with regulations of the Institutional Animal Care and Use Committee at Fundación Ciencia & Vida.

AUTHOR CONTRIBUTIONS

RP designed the study. DE, FC, CP, AM, VU, and OC conducted experiments. DE, FC, CP, AM, VU, OC, CH, and RV acquired data. DE, FC, CP, AM, OC, MAA, MSA, RF, and RP analysed data. MAA and MSA provided new reagents. DE and RP wrote the manuscript.

FUNDING

This work was supported by *Programa de Apoyo a Centros con Financiamiento Basal* AFB-170004 (to Fundación Ciencia & Vida) from Comisión Nacional de Investigación Científica y Tecnológica de Chile (CONICYT) and by grants FONDECYT-1170093 (to RP), and FONDECYT-3160383 (to CP) from Fondo Nacional de Desarrollo Científico y Tecnológico de Chile, MJFF-10332 and MJFF-15076 (to RP) from Michael J. Fox Foundation for Parkinson Research. DE holds a fellowship from the Universidad Andres Bello.

ACKNOWLEDGMENTS

We thank Dr. Marc Caron for providing *Drd3*$^{-/-}$mice. We thank Dr. Hinrich Abken for providing us with the pBullet vector, Miss María José Fuenzalida for her technical assistance in cell-sorting and Dr. Sebastián Valenzuela for his valuable veterinary assistance in our animal facility. We also thank to Elvira Inapaimilla Vergara and Carolina Toledo for their helpful assistance in the extraction of blood samples and thank all the HC and PD patients that contributed with their blood samples to this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.00981/full#supplementary-material

REFERENCES

1. Gonzalez H, Contreras F, Pacheco R. Regulation of the neurodegenerative process associated to Parkinson's Disease by CD4+ T-cells. *J Neuroimmune Pharmacol.* (2015) 10:561–75. doi: 10.1007/s11481-015-9618-9
2. Gonzalez H, Elgueta D, Montoya A, Pacheco R. Neuroimmune regulation of microglial activity involved in neuroinflammation and neurodegenerative diseases. *J Neuroimmunol.* (2014) 274:1–13. doi: 10.1016/j.jneuroim.2014.07.012
3. Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, et al. Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. *Science.* (2000) 290:985–9. doi: 10.1126/science.290.5493.985
4. Benner EJ, Banerjee R, Reynolds AD, Sherman S, Pisarev VM, Tsiperson V, et al. Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. *PLoS ONE.* (2008) 3:e1376. doi: 10.1371/journal.pone.0001376
5. Yu Z, Xu X, Xiang Z, Zhou J, Zhang Z, Hu C, et al. Nitrated alpha-synuclein induces the loss of dopaminergic neurons in the substantia nigra of rats. *PLoS ONE.* (2010) 5:e9956. doi: 10.1371/journal.pone.0009956
6. Reynolds AD, Stone DK, Hutter JA, Benner EJ, Mosley RL, Gendelman HE. Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. *J Immunol.* (2010) 184:2261–71. doi: 10.4049/jimmunol.0901852
7. Christiansen JR, Olesen MN, Otzen DE, Romero-Ramos M, Sanchez-Guajardo V. alpha-Synuclein vaccination modulates regulatory T cell activation and microglia in the absence of brain pathology. *J Neuroinflammation.* (2016) 13:74. doi: 10.1186/s12974-016-0532-8
8. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize alpha-synuclein peptides. *Nature.* (2017) 546:656–61. doi: 10.1038/nature22815
9. Reynolds AD, Glanzer JG, Kadiu I, Ricardo-Dukelow M, Chaudhuri A, Ciborowski P, et al. Nitrated alpha-synuclein-activated microglial profiling for Parkinson's disease. *J Neurochem.* (2008) 104:1504–25. doi: 10.1111/j.1471-4159.2007.05087.x
10. Reynolds AD, Kadiu I, Garg SK, Glanzer JG, Nordgren T, Ciborowski P, et al. Nitrated alpha-synuclein and microglial neuroregulatory activities.

J Neuroimmune Pharmacol. (2008) 3:59–74. doi: 10.1007/s11481-008-9100-z
11. Reynolds AD, Stone DK, Mosley RL, Gendelman HE. Nitrated alpha-synuclein-induced alterations in microglial immunity are regulated by CD4+ T cell subsets. *J Immunol.* (2009) 182:4137–49. doi: 10.4049/jimmunol.0803982
12. Barcia C, Ros CM, Annese V, Gomez A, Ros-Bernal F, Aguado-Llera D, et al. IFN-gamma signaling, with the synergistic contribution of TNF-alpha, mediates cell specific microglial and astroglial activation in experimental models of Parkinson's disease. *Cell Death Dis.* (2012) 3:e379. doi: 10.1038/cddis.2012.123
13. Gonzalez H, Contreras F, Prado C, Elgueta D, Franz D, Bernales S, et al. Dopamine Receptor D3 Expressed on CD4+ T cells favors neurodegeneration of dopaminergic neurons during Parkinson's Disease. *J Immunol.* (2013) 190:5048–56. doi: 10.4049/jimmunol.1203121
14. Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, et al. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest.* (2009) 119:182–92. doi: 10.1172/JCI36470
15. Torres-Rosas R, Yehia G, Pena G, Mishra P, del Rocio Thompson-Bonilla M, Moreno-Eutimio MA, et al. Dopamine mediates vagal modulation of the immune system by electroacupuncture. *Nat Med.* (2014) 20:291–5. doi: 10.1038/nm.3479
16. Yan Y, Jiang W, Liu L, Wang X, Ding C, Tian Z, et al. Dopamine controls systemic inflammation through inhibition of NLRP3 inflammasome. *Cell.* (2015) 160:62–73. doi: 10.1016/j.cell.2014.11.047
17. Pacheco R. Targeting dopamine receptor D3 signalling in inflammation. *Oncotarget.* (2017) 8:7224–5. doi: 10.18632/oncotarget.14601
18. Shao W, Zhang SZ, Tang M, Zhang XH, Zhou Z, Yin YQ, et al. Suppression of neuroinflammation by astrocytic dopamine D2 receptors via alphaB-crystallin. *Nature.* (2013) 494:90–4. doi: 10.1038/nature11748
19. Franz D, Contreras F, Gonzalez H, Prado C, Elgueta D, Figueroa C, et al. Dopamine receptors D3 and D5 regulate CD4(+)T-cell activation and differentiation by modulating ERK activation and cAMP production. *J Neuroimmunol.* (2015) 284:18–29. doi: 10.1016/j.jneuroim.2015.05.003
20. Contreras F, Prado C, Gonzalez H, Franz D, Osorio-Barrios F, Osorio F, et al. Dopamine Receptor D3 Signaling on CD4+ T Cells Favors Th1- and Th17-Mediated Immunity. *J Immunol.* (2016) 196:4143–9. doi: 10.4049/jimmunol.1502420
21. Osorio-Barrios F, Prado C, Contreras F, Pacheco R. Dopamine receptor D5 signaling plays a dual role in experimental autoimmune encephalomyelitis potentiating Th17-mediated immunity and favoring suppressive activity of regulatory T-cells. *Front Cell Neurosci.* (2018) 12:192. doi: 10.3389/fncel.2018.00192
22. Ehringer H, Hornykiewicz O. [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system]. *Klin Wochenschr.* (1960) 38:1236–9.
23. Elgueta D, Aymerich MS, Contreras F, Montoya A, Celorrio M, Rojo-Bustamante E, et al. Pharmacologic antagonism of dopamine receptor D3 attenuates neurodegeneration and motor impairment in a mouse model of Parkinson's disease. *Neuropharmacology.* (2017) 113(Pt A):110–23. doi: 10.1016/j.neuropharm.2016.09.028
24. Nagai Y, Ueno S, Saeki Y, Soga F, Hirano M, Yanagihara T. Decrease of the D3 dopamine receptor mRNA expression in lymphocytes from patients with Parkinson's disease. *Neurology.* (1996) 46:791–5.
25. Joseph JD, Wang YM, Miles PR, Budygin EA, Picetti R, Gainetdinov RR, et al. Dopamine autoreceptor regulation of release and uptake in mouse brain slices in the absence of D(3) receptors. *Neuroscience.* (2002) 112:39–49. doi: 10.1016/S0306-4522(02)00067-2
26. Weijtens ME, Willemsen RA, Hart EH, Bolhuis RL. A retroviral vector system 'STITCH' in combination with an optimized single chain antibody chimeric receptor gene structure allows efficient gene transduction and expression in human T lymphocytes. *Gene Ther.* (1998) 5:1195–203. doi: 10.1038/sj.gt.3300696
27. Brooks SP, Dunnett SB. Tests to assess motor phenotype in mice: a user's guide. *Nat Rev Neurosci.* (2009) 10:519–29. doi: 10.1038/nrn2652
28. Cutando L, Busquets-Garcia A, Puighermanal E, Gomis-Gonzalez M, Delgado-Garcia JM, Gruart A, et al. Microglial activation underlies cerebellar deficits produced by repeated cannabis exposure. *J Clin Invest.* (2013) 123:2816–31. doi: 10.1172/JCI67569
29. Fleming SM, Salcedo J, Fernagut PO, Rockenstein E, Masliah E, Levine MS, et al. Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein. *J Neurosci.* (2004) 24:9434–40. doi: 10.1523/JNEUROSCI.3080-04.2004
30. Reynolds AD, Banerjee R, Liu J, Gendelman HE, Mosley RL. Neuroprotective activities of CD4+CD25+ regulatory T cells in an animal model of Parkinson's disease. *J Leukoc Biol.* (2007) 82:1083–94. doi: 10.1189/jlb.0507296
31. Sanchez-Guajardo V, Annibali A, Jensen PH, Romero-Ramos M. α-Synuclein vaccination prevents the accumulation of Parkinson disease-like pathologic inclusions in striatum in association with regulatory T cell recruitment in a rat model. *J Neuropathol Exp Neurol.* (2013) 72:624–45. doi: 10.1097/NEN.0b013e31829768d2
32. Chen Y, Ni YY, Liu J, Lu JW, Wang F, Wu XL, et al. Dopamine receptor 3 might be an essential molecule in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. *BMC Neurosci.* (2013) 14:76. doi: 10.1186/1471-2202-14-76
33. Fernandez-Suarez D, Celorrio M, Riezu-Boj JI, Ugarte A, Pacheco R, Gonzalez H, et al. The monoacylglycerol lipase inhibitor JZL184 is neuroprotective and alters glial cell phenotype in the chronic MPTP mouse model. *Neurobiol Aging.* (2014) 35:2603–16. doi: 10.1016/j.neurobiolaging.2014.05.021
34. Grundt P, Carlson EE, Cao J, Bennett CJ, McElveen E, Taylor M, et al. Novel heterocyclic trans olefin analogues of N-{4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl}arylcarboxamides as selective probes with high affinity for the dopamine D3 receptor. *J Med Chem.* (2005) 48:839–48. doi: 10.1021/jm049465g
35. Gonzalez H, Pacheco R. T-cell-mediated regulation of neuroinflammation involved in neurodegenerative diseases. *J Neuroinflammation.* (2014) 11:201. doi: 10.1186/s12974-014-0201-8
36. Kustrimovic N, Comi C, Magistrelli L, Rasini E, Legnaro M, Bombelli R, et al. Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/T17 and Treg in drug-naive and drug-treated patients. *J Neuroinflammation.* (2018) 15:205. doi: 10.1186/s12974-018-1248-8
37. Reale M, Iarlori C, Thomas A, Gambi D, Perfetti B, Di Nicola M, et al. Peripheral cytokines profile in Parkinson's disease. *Brain Behav Immun.* (2009) 23:55–63. doi: 10.1016/j.bbi.2008.07.003
38. McKenna F, McLaughlin PJ, Lewis BJ, Sibbring GC, Cummerson JA, Bowen-Jones D, et al. (2002). Dopamine receptor expression on human T- and B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. *J Neuroimmunol.* 132, 34–40. doi: 10.1016/S0165-5728(02)00280-1
39. Orr CF, Rowe DB, Mizuno Y, Mori H, Halliday GM. A possible role for humoral immunity in the pathogenesis of Parkinson's disease. *Brain.* (2005) 128(Pt 11):2665–74. doi: 10.1093/brain/awh625
40. Chen S, Le WD, Xie WJ, Alexianu ME, Engelhardt JI, Siklos L, et al. Experimental destruction of substantia nigra initiated by Parkinson disease immunoglobulins. *Arch Neurol.* (1998) 55:1075–80.
41. Mihara T, Nakashima M, Kuroiwa A, Akitake Y, Ono K, Hosokawa M, et al. Natural killer cells of Parkinson's disease patients are set up for activation: a possible role for innate immunity in the pathogenesis of this disease. *Parkinsonism Relat Disord.* (2008) 14:46–51. doi: 10.1016/j.parkreldis.2007.05.013
42. Jiang S, Gao H, Luo Q, Wang P, Yang X. The correlation of lymphocyte subsets, natural killer cell, and Parkinson's disease: a meta-analysis. *Neurol Sci.* (2017) 38:1373–80. doi: 10.1007/s10072-017-2988-4
43. Liu Z, Huang Y, Cao BB, Qiu YH, Peng YP. Th17 cells induce dopaminergic neuronal death via LFA-1/ICAM-1 interaction in a mouse model of Parkinson's Disease. *Mol Neurobiol.* (2017) 54:7762–76. doi: 10.1007/s12035-016-0249-9
44. Wagle Shukla A, Fox SH. Th17 lymphocyte spearheads the immune attack in Parkinson's disease: new evidence for neuronal death. *Mov Disord.* (2018) 33:1560. doi: 10.1002/mds.27496

45. Dardalhon V, Korn T, Kuchroo VK, Anderson AC. Role of Th1 and Th17 cells in organ-specific autoimmunity. *J Autoimmun.* (2008) 31:252–6. doi: 10.1016/j.jaut.2008.04.017
46. Derecki NC, Cardani AN, Yang CH, Quinnies KM, Crihfield A, Lynch KR, et al. Regulation of learning and memory by meningeal immunity: a key role for IL-4. *J Exp Med.* (2010) 207:1067–80. doi: 10.1084/jem.20091419
47. Quezada SA, Simpson TR, Peggs KS, Merghoub T, Vider J, Fan X, et al. Tumor-reactive CD4(+) T cells develop cytotoxic activity and eradicate large established melanoma after transfer into lymphopenic hosts. *J Exp Med.* (2010) 207:637–50. doi: 10.1084/jem.20091918
48. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's Disease. *Cell.* (2016) 167:1469–80 e1412. doi: 10.1016/j.cell.2016.11.018
49. Prashanth R, Roy SD, Mandal PK, Ghosh S. Parkinson's disease detection using olfactory loss and REM sleep disorder features. *Conf Proc IEEE Eng Med Biol Soc.* (2014) 2014:5764–7. doi: 10.1109/EMBC.2014.6944937
50. Campos JA, Elgueta D, Pacheco R. T-cell-driven inflammation as a mediator of the gut-brain axis involved in Parkinson's Disease. *Fron Immunol.* (2019) 10:239. doi: 10.3389/fimmu.2019.00239

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any financial or non-financial competing interests as defined by this journal, with the exception of a pending patent application describing therapeutic use of selective DRD3-antagonist in Parkinson's disease, and which could be construed as a potential conflict of interest. Authors of said patent present in this paper are: RP, DE, FC, and VU.

frontiers in Immunology

ORIGINAL RESEARCH
published: 07 May 2019
doi: 10.3389/fimmu.2019.00969

Probiotics May Have Beneficial Effects in Parkinson's Disease: *In vitro* Evidence

Luca Magistrelli[1,2], *Angela Amoruso*[3], *Luca Mogna*[3], *Teresa Graziano*[3], *Roberto Cantello*[2], *Marco Pane*[3] *and Cristoforo Comi*[2]*

[1] PhD Program in Clinical and Experimental Medicine and Medical Humanities, University of Insubria, Varese, Italy, [2] Neurology Unit, Department of Translational Medicine, Interdisciplinary Research Centre of Autoimmune Diseases, Movement Disorders Centre, University of Piemonte Orientale, Novara, Italy, [3] Biolab Research Srl, Research and Development, Novara, Italy

Background: Parkinson's disease (PD) is characterized by loss of dopaminergic neurons and intraneuronal accumulation of alpha-synuclein, both in the basal ganglia and in peripheral sites, such as the gut. Peripheral immune activation and reactive oxygen species (ROS) production are important pathogenetic features of PD. In this context, the present study focused on the assessment of *in vitro* effects of probiotic bacterial strains in PBMCs isolated from PD patients vs. healthy controls.

Methods: 40 PD patients and 40 matched controls have been enrolled. Peripheral blood mononuclear cells (PBMCs) were isolated and co-cultured with a selection of probiotics microorganisms belonging to the *lactobacillus* and *bifidobacterium* genus. *In vitro* release of the major pro- (Tumor Necrosis Factor-alpha and Interleukin-17A and 6) and anti-inflammatory (Interleukin 4 and 10) cytokines by PBMCs, as well as the production of ROS was investigated. Furthermore, we assessed the ability of probiotics to influence membrane integrity, antagonize the growth of potential pathogen bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae* and encode tyrosine decarboxylase genes (*tdc*).

Results: All probiotic strains were able to inhibit inflammatory cytokines and ROS production in both patients and controls. The most striking results were obtained in PD subjects with *L. salivarius* LS01 and *L. acidophilus* which significantly reduced pro-inflammatory and increased the anti-inflammatory cytokines ($p < 0.05$). Furthermore, most strains determined restoration of membrane integrity and inhibition of *E. coli* and *K. pneumoniae*. Finally, we also showed that all the strains do not carry *tdc* gene, which is known to decrease levodopa bioavailability in PD patients under treatment.

Conclusions: Probiotics exert promising *in vitro* results in decreasing pro-inflammatory cytokines, oxidative stress and potentially pathogenic bacterial overgrowth. *In vivo* longitudinal data are mandatory to support the use of bacteriotherapy in PD.

Keywords: probiotic, neuroinflammation, Parkinson's disease (PD), oxidative stress, cytokines

OPEN ACCESS

Edited by:
Letizia Leocani,
San Raffaele Hospital (IRCCS), Italy

Reviewed by:
Clara Ballerini,
University of Florence, Italy
Mahmoud Salami,
Kashan University of Medical Sciences, Iran

***Correspondence:**
Cristoforo Comi
comi@med.uniupo.it
orcid.org/0000-0002-6862-9468

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology

Received: *06 September 2018*
Accepted: *15 April 2019*
Published: *07 May 2019*

Citation:
Magistrelli L, Amoruso A, Mogna L, Graziano T, Cantello R, Pane M and Comi C (2019) Probiotics May Have Beneficial Effects in Parkinson's Disease: In vitro Evidence. Front. Immunol. 10:969. doi: 10.3389/fimmu.2019.00969

INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disease, characterized by loss of dopaminergic neurons and intracellular accumulation of alpha-synuclein (α-syn) in the surviving neurons (1). Involvement of inflammatory mechanisms, with an imbalance between detrimental and protective immune functions (2), as well as neurotoxicity of reactive oxygen species (ROS) have been documented by several studies (3, 4). Both neuroinflammation and ROS may favor α-syn aggregation which may in turn increase pro-inflammatory cytokines and oxidative stress, thus triggering a vicious circle (5). Clinical presentation of PD is classically defined by the presence of motor symptoms such as bradykinesia, rest tremor, and rigidity. On the other hand, patients often complain of non-motor symptoms like hyposmia, constipation, pain and psychiatric conditions (e.g., anxiety, depression) that in many cases may precede the onset of clinically established disease (6, 7). Seminal work by Braak et al. hypothesized an initial aggregation of α-syn in the gut with subsequent propagation along the vagus nerve to the brain to reach the substantia nigra in the mesencephalon (8). Moreover, constipation represents a relevant symptom of PD, affecting about 70–80% of patients (9) and may precede motor symptoms by 20 years (10). The loss of enteric dopaminergic neurons determines an impairment of gastric mobility with an increased dopaminergic content and overexpression of dopaminergic receptors in the stomach (11). Furthermore, PD patients present an increased intestinal permeability and higher expression of colonic pro-inflammatory cytokines (12). Accordingly, in an α-syn overexpressing murine model of PD, gut microbiota is necessary for both microglia activation and motor impairment (13). In addition, a direct correlation between gut bacterial count and disease progression was found in PD patients (14). PD patients have a different composition of gut microbiota compared to healthy subjects (15) with reduced levels of *Prevotellaceae* and abundance of *Enterobacteriaceae* (16). *Prevotella* are in fact involved in the production of thiamine and folate, both of which are important for proper intestinal homeostasis (17). On the contrary, increased levels of *Enterobacteriaceae* have been associated to a severe PD phenotype with postural instability and gait difficulty. (16).

Of note, there is evidence that probiotics may modulate not only inflammation through cytokines production (18, 19), but also oxidative damage through a down-modulation of ROS (20). Another relevant aspect of the host-microbial interaction is the established role of infections in accelerating clinical decline in PD patients (21). In a recent investigation, focused on the clinical features and therapeutic outcomes of infected patients with or without PD, the incidence of respiratory tract and urinary tract infections was higher in PD than in age and sex-matched non-PD patients. Additionally, a longer mean hospitalization time was observed in the PD group (22). In this regard, specific probiotic strains may potentially counteract the growth of common pathogens, such as *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae (K. pneumoniae)* (23, 24). Furthermore, Van Kessel et al. recently reported that some probiotic strains produce tyrosine decarboxylase (TDC) (25). This bacterial enzyme efficiently converts levodopa to dopamine in the gut, even in the presence of human decarboxylase inhibitors or tyrosine, a competitive substrate. Accordingly, *in situ* levels of levodopa in PD patients are decreased by significant abundance of gut bacterial TDC (25). TDC genes (*tdc*) have been detected in particular in the genome of numerous bacterial species within the genera *Lactobacillus* and *Enterococcus* (26, 27). Abundance of bacterial *tdc* in stool specimens of PD patients was indeed correlated with increased daily dosage requirement of levodopa (25).

On this background, the aim of our study was to investigate the *in vitro* effects of probiotics on samples from a group of PD patients compared to healthy subjects. To do that, we assessed cytokine and reactive oxygen species (ROS) release by peripheral blood mononuclear cells (PBMCs), and restoration of artificial membrane permeability. In addition, we investigated the ability of the selected probiotics to directly inhibit *E. coli* and *K. pneumoniae*. Finally, we verified the absence of *tdc* within the genome of the selected probiotic strains.

PATIENTS AND METHODS

Patients

We enrolled 40 patients with PD (15 women and 25 men, mean age 70 ± 8 years) and 40 age-matched healthy donors (HD, 18 women and 22 men, mean age 68 ± 7 years). PD diagnosis was performed according to the Movement Disorders Society (MDS) diagnostic criteria, e.g., when: (a) subjects presented with a parkinsonism, defined as bradykinesia, associated to rest tremor or rigidity without signs of atypical parkinsonism; (b) exclusion criteria, red flags and supportive criteria were assessed (28).

Patients were regularly followed-up at the Movement Disorder Center of Maggiore Hospital in Novara (Italy). For each patient the following parameters were considered: gender, age at onset, disease duration, Hoehn & Yahr stage (29), UPDRS III score (30), and PD therapy calculating the levodopa equivalent doses (LED) of each drug according to Tomlinson et al. (31).

Subjects with past or concomitant autoimmune disease and with a previous or ongoing immune-modulating or suppressive therapy were excluded. All subjects underwent a complete blood cell (CBC) analysis including C-Reactive Protein (CRP) and erythrocyte sedimentation rate in order to exclude both defects or activation of the immune system. All subjects were of Italian origin.

This study was approved by the local Ethics Committee (CE 65/16). Patients were included in the study after having read and signed an informed consent form for research purpose.

Cell Cultures

Twenty milliliters of blood were drawn by venipuncture in vacuum tubes containing heparin on the same day of the clinical assessment. In order to rule out any confounding factors caused by circadian rhythm, all samples were collected at the same time of the day. Human PBMCs were isolated from heparinized blood by Healthy Donors (HD-PBMCs) and PD patients (PD-PBMCs). For cell isolation, standard techniques of dextran sedimentation and Histopaque (density = 1.077 g/cm^3) gradient centrifugation

(400 × g, 30 min, room temperature) were used. Cells were then recovered by thin suction at the interface. Isolated cells were then re-suspended in RPMI 1640 medium supplemented with 5% fetal bovine serum (FBS) and 2 mM glutamine. Cell viability (trypan blue dye exclusion) was usually >98%.

Bacteria and Growth Conditions

Six probiotic strains (*Lactobacillus salivarius* LS01 DSM 22775, *Lactobacillus plantarum* LP01 LMG P-21021, *Lactobacillus acidophilus* LA02 DSM 21717, *Lactobacillus rhamnosus* LR06 DSM 21981, *Bifidobacterium animalis* subsp. *lactis* BS01 LMG P-21384, *Bifidobacterium breve* BR03 DSM 16604), from the Probiotical SpA collection, have been used in the present study: probiotic strains were stored in 20% glycerol at −80°C. More than 90% of the cells were alive upon thawing. Before use, microorganisms were grown in anaerobic conditions with CO_2-generating kits (Anaerocult A; Merck, Darmstadt, Germany) overnight at 37°C in de Man-Rogosa-Sharpe (MRS) broth containing 0.05% cysteine hydrochloride, and then sub-cultured until the mid-log phase. For the enumeration of live bacteria, the BD Cell Viability Kit (BD Biosciences, Milan, Italy) were used as instructed by the manufacturer. For stimulation experiments, bacteria were suspended in RPMI-1640 medium [Invitrogen, Italy] and added to PBMCs cultures.

Cytokine Release

Cytokine release by PBMCs was measured with an enzyme-linked immunoassay kit according to the manufacturer's instructions (ELISA Ready-SET-Go! Affymetrix eBioscience, USA). Interleukin 17A (IL-17A), tumor necrosis factor α (TNF-α), and IL-10 were assessed in both healthy controls and PD patients, whereas IL-6 and IL-4 were assessed only in PD-PBMCs (before and after probiotic stimulation). The levels of each cytokine were calculated in ρg/ml, in accordance with the manufacturer's instructions. For these experiments, HD and PD PBMCs (1×10^6 cells/plate) were pre-treated for 24 h with the indicated probiotic strains in 1:1 ratio. Only HD PBMCs were treated previously with purified lipopolysaccharide (LPS) from *E. coli* 055:B5 (Sigma Chemicals, Milan, Italy) at a concentration of 10 g/mL.

Superoxide Anion (O_2^-) Production

HD- and PD-PBMCs (1×10^6 cells/plate) were treated for 24 h with probiotic strains in 1:1 ratio. O_2^- production was evaluated by the superoxide dismutase-sensitive cytochrome C reduction assay and calculated as nmol reduced cytochrome C/10^6 cells/30 min, using an extinction coefficient of 21.1 mM. To avoid interference with spectrophotometrical recordings, cells were incubated with RPMI 1,640 without phenol red and FBS. Basal values (O_2^- production from unstimulated PBMCs) in HD were 2.2 ± 0.4 nmol reduced cytochrome C/10^6 cells/30', and in PD patient-PBMCs were 140 ± 12 nmol reduced cytochrome C/10^6 cells/30'. PMA is a stimulus known to induce a strong and significant respiratory burst. In line with this, PMA 10^{-7} M exposure determined a significant increase of cytochrome C levels in HD-PBMCs: 52 ± 4.5 nmol reduced cytochrome C/10^6 cells/30′.

Measurements of TransEpithelial Electrical Resistance (TEER)

Caco-2 cells are human colonic adenocarcinoma cells that form confluent, polarized epithelial monolayers with well-differentiated intercellular tight junctions structures. The integrity of the barrier function can be measured with TransEpithelial Electrical Resistance (TEER). TEER is an *in vitro* measurement of the movement of ions across the paracellular pathway.

A reduction in TEER may represent an early expression of cell damage and indicates that the barrier function of the intestine is decreased. Caco-2 cell lines have been extensively used over the last 20 years as a model of the intestinal barrier. The experiment was performed with an inflammatory stressor (a combination of TNF-α and IL1-ß), which is known to reduce the relative TEER of Caco-2 cells. The monolayer of Caco-2 cells was first exposed to the probiotic bacteria for 1 h, followed by exposure to the inflammatory stressor in the presence of the same probiotic bacteria, also for 1 h. After a recovery time of 24 h the TEER of the monolayer was measured. The results were compared to the TEER of a monolayer that was exposed to the stressor alone and to an unexposed sample. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum. Caco-2 (1×10^6 cells/well) differentiated at 20 days were provided by the Anemocyte s.r.l. (Gerenzano, Varese, Italy). TEER was measured in each monolayer before adding 10^6 AFU [Active Fluorescent Units were evaluated with cytofluorimetric analytical method ISO 19344:2015 (E)-IDF 232:2015 (E)] of probiotic strains onto the apical surface for 24 h prior to treatment of the basolateral medium with TNF-α and IL1-ß (10 ng/ml; Thermo Scientific, USA). It was determined that 10^6 AFU of probiotic strains did not cause deleterious effects on epithelial cells over the time-course of the experiments and that the medium used in this experiment did not cause bacterial overgrowth.

Spot-on Lawn Antimicrobial Assay/Agar Spot Antimicrobial Assay

The antimicrobial activity against *E. coli* and *K. pneumoniae* was assessed according to the protocol described by Santini C. (32). Briefly, 5 µl of probiotic overnight fresh cultures with an optical density (OD) at 600 nm close to 1 were spotted on the surface of MRS agar plates and incubated anaerobically for 5 h at 37°C to allow strain development (spot). The *E. coli* ATCC 8739 strain or *K. pneumoniae* ATCC 13883 strain was inoculated in Brain Heart Infusion (BHI) soft agar and dispensed onto spot plates. When the top agar was solid, the plates were inverted and incubated in conditions of anaerobiosis at 37°C for 48 h. At the end of incubation, plates were examined for the appearance of clear zones showing the antagonistic activity. The plate inhibition technique experiments were carried out on triplicates, and the

TABLE 1 | Modulation of cytokines production by the probiotic strains in PD patients.

Probiotic strain		IL10		TNF-α		IL17-A		IL4		IL6	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LS01	Baseline	140.12	18.01	255.52	29.55	114.08	15.41	102.00	16.4	197.2	18.2
	After stimulus	194.24	15.42	146.69	28.67	52.48	9.25	149.6	34.7	135.2	4.2
	P	**<0.001**		**<0.001**		**<0.001**		**<0.01**		**<0.001**	
LP01	Baseline	121.81	20.17	221.80	16.18	120.04	16.00	110.2	12.3	178.2	22.8
	After stimulus	161.70	20.17	264.10	100.41	73.22	11.32	135.2	21.3	114.9	11.4
	P	**<0.001**		**0.01**		**<0.001**		n.s.		**0.01**	
LA02	Baseline	165.33	20.53	228.90	26.89	112.81	15.28	26.3	2.5	180.2	18.2
	After stimulus	192.18	23.22	195.15	33.52	87.99	12.80	45.6	4.9	92.5	5.9
	P	**<0.001**		**<0.001**		**<0.001**		**<0.05**		**<0.001**	
LR06	Baseline	196.71	23.67	128.90	16.89	112.81	15.28	60.00	1.15	188.0	22.30
	After stimulus	241.63	18.16	123.54	46.35	81.22	12.12	88.2	21.1	112.5	11.4
	P	**<0.001**		n.s		**<0.001**		**<0.05**		**<0.001**	
BS01	Baseline	186.71	22.67	255.74	29.57	119.44	15.94	71.2	15.3	196.4	29.6
	After stimulus	236.34	33.63	297.82	38.78	109.88	14.99	99.2	22.4	96.3	21.4
	P	**<0.001**		**<0.001**		**0.007**		n.s		**<0.001**	
BR03	Baseline	141.12	18.11	117.19	15.72	116.43	15.64	110.7	18.11	190.8	16.7
	After stimulus	147.38	17.74	82.30	13.14	72.19	11.22	147.38	18.7	81.9	18.1
	P	n.s.		**<0.001**		**<0.001**		**<0.05**		**<0.001**	

All data are expressed in pg/ml; n.s., not statistically significant.

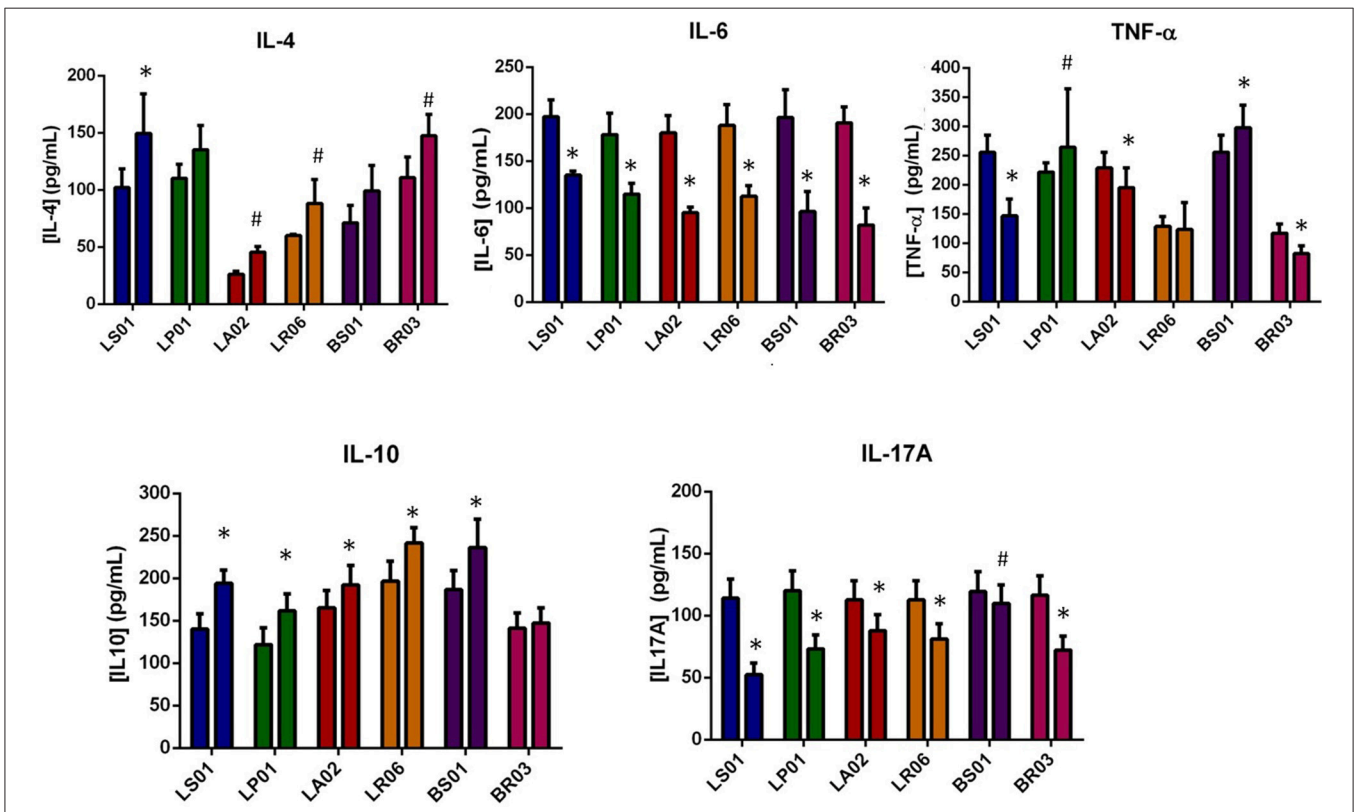


FIGURE 1 | Modulation of cytokine production by probiotic strains. For each probiotic strain the first column indicates the baseline values, while the second after probiotic administration. *$p < 0.001$; #$p < 0.05$ post vs. pre-exposure from PD-PBMCs.

mean values of growth inhibition zones around the disks were measured using a ruler (mm) and recorded.

Search for Tyrosine Decarboxylase (TDC) Genes

To identify whether the genomes of the probiotic strains employed encoded *tdc*, the TDC protein sequence (EOT87933) from *Enterococcus faecalis* v583 was used as a query along with *E. faecalis* v583 as a positive control using the BLAST program of PATRIC suite (www.patricbrc.org). *E. faecalis* V583 TDC protein sequence (NCBI accession: EOT87933) was downloaded in FASTA format. PATRIC suite was used for the Annotation of the six bacterial strains using the RAST tool kit encoded within the software. Annotated genomes were grouped together and BLASTed against the TDC protein sequences.

Statistical Analysis

Results are expressed as the means ± SEM of duplicate measures determined in three independent experiments. Differences between unstimulated and stimulated samples were tested using the *t* test with GraphPad Prism 6.0 software. Correlations between probiotic strain and clinical-demographic variables were calculated using Spearman test. Values of $p < 0.05$ were considered significant.

RESULTS

Cytokine Release

IL-10 and IL-4 are important cytokines in the regulation of immune responses, counterbalancing the pro-inflammatory effects of TNF-α, IL-6 and IL-17A. Cytokines modulation by different probiotic strains was measured in PBMCs from both PD patients and healthy donors (**Table 1** and **Figure 1**; **Table S1**). In PD-PBMCs, most probiotic strains determined a statistically significant reduction of pro-inflammatory cytokines production (TNF-α, IL-6, and IL-17A) and an increase of the anti-inflammatory IL-4 and IL-10. The most striking results were obtained with LS01 (TNF-α: baseline 255.52 ± 29.55 pg/ml, after stimulus 146.69 ± 28.67 pg/ml, $p < 0.001$; IL-6 baseline 197.2 ± 18.2 pg/ml, after stimulus 132.5 ± 4.2 pg/ml, $p < 0.001$; IL17-A baseline 114.08 ± 15.41 pg/ml, after stimulus 52.48 ± 9.25 pg/ml, $p < 0.001$; IL-4 baseline 102.00 ± 16.4 pg/ml, after stimulus 149.6 ± 34.7 pg/ml, $p < 0.001$; IL-10: baseline 140.12 ± 18.01 pg/ml, after stimulus 194.24 ± 15.42 pg/ml, $p < 0.001$) and LA02 (TNF-α: baseline 228.90 ± 26.89 pg/ml, after stimulus 195.15 ± 33.52 pg/ml, $p < 0.001$; IL-6 baseline 180.2 ± 92.5 pg/ml, after stimulus 92.5 ± 5.9 pg/ml, $p < 0.001$; IL17-A baseline 112.81 ± 15.28 pg/ml, after stimulus 87.99 ± 12.80 pg/ml, $p < 0.001$; IL-4 baseline 26.3 ± 2.5 pg/ml, after stimulus 45.6 ± 4.9 pg/ml, $p < 0.05$; IL-10: baseline 165.33 ± 20.53 pg/ml, after stimulus 192.18 ± 23.22 pg/ml, $p < 0.001$). The remaining data, including all *p* values, are shown in **Table 1**.

Superoxide Anion (O_2^-) Production

First, we found a statistically significant difference in baseline O^{2-} production from unstimulated PBMCs between patients and controls. In fact, reduced cytochrome C in HD was 2.2 ± 0.4 nmol vs. 140 ± 12 nmol/10^6 cells/30' in PD-PBMCs ($p < 0.001$). To test the antioxidant effects of probiotics on HD-PBMCs we induced a toxic condition using PMA, a stimulus known to determine a strong and significant respiratory burst. Consistently, PMA exposure in HD-PBMCs increased levels of

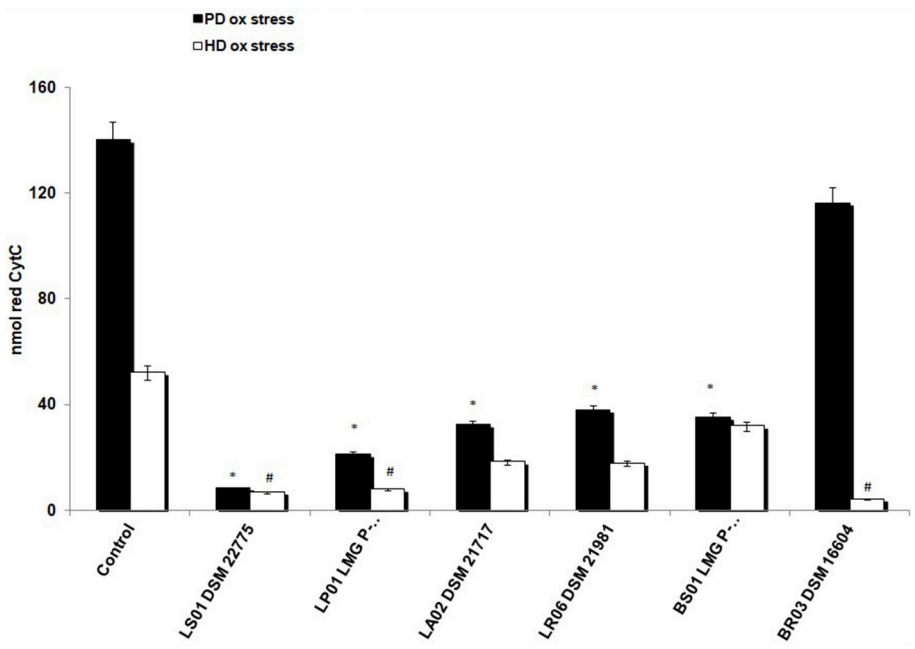


FIGURE 2 | Reduction of superoxide anion (O_2^-) production. *$p < 0.01$ post vs. pre-exposure from PD-PBMCs. #$p < 0.05$ post vs. pre-exposure from PMA-exposed HD-PBMCs.

TABLE 2 | TERR evaluation using different probiotic strains.

	LA02	LP01	LR06	LS01	BR03	BS01
Baseline integrity	100%	100%	100%	100%	100%	100%
Integrity after damaging stimulus	40%	40%	40%	40%	40%	40%
Integrity after probiotic strain	91%	100%	100%	81%	94%	84%
P value	<0.05	<0.001	<0.001	<0.05	<0.05	<0.001

TABLE 3 | Inhibition of bacterial overgrowth exerted by probiotic strains.

Probiotic strain [inhibition (mm)]	*E. coli*		*K. pneumoniae*	
	Mean	SEM	Mean	SEM
LS01	0.43	0.15	0.58	0.17
LP01	0.57	0.14	1.42	0.56
LA02	0.30	0.11	0.23	0.10
LR06	0.48	0.10	0.63	0.37
BS01	0.01	0.004	0.11	0.05
BR03	0.01	0.004	0.15	0.01

Results are expressed in mm.

reduced cytochrome c (52 ± 4.5 nmol). As depicted in **Figure 2**, after exposure to probiotic strains, we found an overall decrease of O_2^- production in unstimulated PBMCs from PD patients and in PMA-stimulated PBMCs from HD. In detail, LS01, LP01, LA02, LR06, BS01 caused a robust decrease of O_2^- from PD-PBMCs ($p < 0.01$ post vs. pre-exposure). A weaker, though statistically significant, effect was obtained from PMA-stimulated HD-PBMCs after exposure to LS01, LP01, BR03 ($p < 0.05$ post vs. pre-exposure).

TEER Evaluation

Three strains (LP01, LR06, and BR03) provided higher protection of epithelial cells against the cytokine-induced barrier dysfunction ($p < 0.001$), whereas three others (LS01, LA02, and BS01) had a lower, though still significant, effect ($p < 0.05$). The TEER ratio was measured before adding the bacterial inoculum (CTR) and in a damage-tissue model and after addition and incubation of the probiotic strains on Caco-2 monolayer. The results are shown in **Table 2** and **Figure S1**.

Inhibition of *E. coli* and *K. pneumoniae*

As shown in **Table 3**, most probiotic strains showed a robust inhibitory capacity against the target pathogen strains *E. coli* and *K. pneumoniae* involved in the comorbidities of PD. Particularly, LP01 and LR06 exerted the highest inhibition. Moreover, such inhibition was not observed when a negative control (MRS acidified at pH 4.3 but without any probiotic strains; data not shown) was tested, thus confirming the specificity of the detected antagonistic activity.

TABLE 4 | Comparison of ROS production after each probiotic stimulation between female and male patients.

	LA02	LP01	LR06	LS01	BR03	BS01
Female	0.29	0.16	0.40	0.06	0.82	0.16
Male	0.20	0.14	0.19	0.06	0.83	0.31
p value	0.17	0.67	**0.005**	0.97	0.97	**0.03**

Data are expressed as the value after probiotic strain exposure from a baseline conventionally established as 1. The statistically significant differences are shown in bold.

Tyrosine Decarboxylase (TDC) Genes

Only *E. faecalis* V583 gave an Identity value equal to 100% with a Query Cover of 100%, whilst the other probiotic strains scored below 35% of Identity, thus excluding the expression of a tyrosine decarboxylase activity within the six tested probiotic strains (data not shown).

Clinical Analysis

Demographic Results

Mean age at PD onset was 65 ± 8 years. In detail, at study enrollment, 4 patients were drug naïve, 26 were taking levodopa (one of them was on Duodopa) and 10 were taking dopaminergic treatment other than levodopa (dopamine agonists and MAO inhibitors). Mean LED was 469.9 mg/day ± 354. Mean UPDRS III was 12.45 ± 6.9 points.

Gender

Comparing responses to probiotics in relation to gender, we found a statistically significant difference between male and female PD patients in ROS production from PBMCs. The effect of LR06 was more pronounced in samples from male vs. female donors (81 and 60% reduction compared to baseline levels, respectively; $p < 0.05$; **Table 4**). On the contrary, BS01 was more effective in samples from female than male patients (84 and 69% reduction compared to baseline levels, respectively; $p < 0.05$; **Table 4**).

Disease Duration

We analyzed ROS and cytokine levels from PD-PBMCs exposed to different probiotic strains in relation to disease duration. We found that LA02 provided a robust anti-oxidant effect, which decreased significantly in samples of patients with longer disease duration (rho = 0.22, $p < 0.05$ **Table 5**, **Figure 3**). Such correlation was not detected with other strains. Furthermore, we found no other statistically significant correlation between the effect of the different strains and the remaining clinical variables (H&Y stage, UPDRS score, LED, data not shown).

DISCUSSION

In this study we showed that probiotic strains modulate the release of cytokines and ROS by PBMCs of PD patients and healthy controls. Particularly, *L. salivarius* (LS01) and *L. acidophilus* (LA02) showed the best profiles in PD-PBMCs, being able to significantly decrease all the pro-inflammatory cytokines

TABLE 5 | Correlations between probiotic strains modulation of ROS production and clinical characteristics of PD patients.

Probiotic strain	Disease duration	H&Y stage	UPDRS III score	LED
LA02 (rho; p)	**0.22; 0.002**	0.18; 0.94	0.04; 0.55	0.03; 0.22
LP01	0.05; 0.19	−0.03; 0.53	0.07; 0.53	−0.01; 0.60
LR06	0.08; 0.88	−0.08; 0.83	−0.16; 0.40	0.06; 0.80
LS01	0.17; 0.61	0.25; 0.55	0.28; 0.63	0.05; 0.12
BR03	−0.01; 0.83	0.08; 0.84	−0.10; 0.88	0.01; 0.73
BS01	−0.05; 0.45	0.32; 0.21	0.06; 0.33	0.14; 0.48

Data are expressed as the value after probiotic strain exposure from a baseline conventionally established as 1. The statistically significant differences are shown in bold.

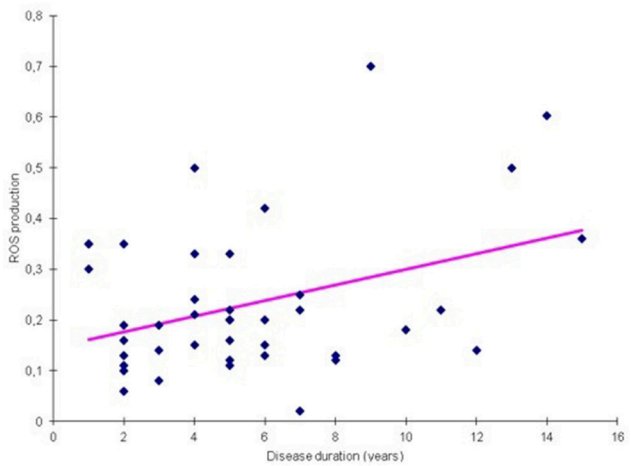


FIGURE 3 | Correlation between ROS production and disease duration for probiotic LA02 ($p = 0.002$).

and increase the anti-inflammatory ones. The same strains were also able to significantly reduce ROS production in both PD and HD-PBMCs. In addition, all the tested probiotic strains restored epithelial damage in Caco-2 cells. Finally, the tested probiotic strains exerted a robust capacity of inhibiting *E. coli* and *K. pneumoniae*. These Gram-negative bacteria are frequently detected in blood cultures of septic PD patients (22). Such antagonistic activity might be ascribed to the active bacteriocins secreted by probiotic strains. Probiotics have been studied in PD for their potential symptomatic effect on constipation (33) and probiotic strains BS01, LP01, and BR03 have shown improvement in constipation and associated symptoms in healthy adults (34) and more interestingly in chronically constipated elderly (35). However, despite the great interest that recently arose around the gut-brain axis in health and disease, our study is the first to specifically address the effect of probiotics on mediators of inflammation and oxidative damage in PBMCs of PD patients. On the other hand, experimental evidence on the anti-inflammatory and anti-oxidative effects of probiotics is rapidly growing. *L. plantarum* displayed the capacity of decreasing the histopathological damages in a murine model of Alzheimer's disease (AD) leading to increased production of acetylcholine with consequent clinical improvement (36). Accordingly, it was shown that probiotics administration was effective in modulating cognitive functions in a group of AD patients (37). Probiotics may also be helpful in other contexts of neuroinflammation such as post-traumatic stress disorder, amyotrophic lateral sclerosis and cognitive dysfunction after surgery (38–40). Of note, in a murine model of stress, modification of gut microbiota provided both behavioral and immunological beneficial effects (41).

Altogether, such findings suggest that probiotics may represent a promising strategy to counteract the detrimental immune activation that takes place in PD. In fact, work by independent groups is indicating that peripheral and central immune responses are strictly interconnected in PD and the study of such mechanisms may provide relevant advances in both diagnostic and therapeutic areas (42, 43). An important breakthrough in this area was the demonstration of the antigenic role of α-syn on peripheral T cells: such epitopes can in fact drive T helper and cytotoxic responses in PD patients (44). Moreover, recent work by Kustrimovic et al. showed that PD patients display a predominance of Th1 mediated responses compared to healthy controls (45). A pro-inflammatory profile, with increased production of IL-1α, IL-1β, and CXCL8 was also detected in stool samples of PD patients, further supporting the involvement of intestinal immunity in PD (46). The results of the present study support the concept of a predominantly pro-inflammatory environment in the periphery, since pro-inflammatory cytokines production was significantly increased in PD patients vs. controls. Notably, the probiotic strains tested in our *in vitro* experiments were able to counterbalance such pro-inflammatory response. Our results also suggest the involvement of IL17A in PD: patients present in fact higher levels of IL17A than healthy controls. The role of IL17 producing T helper cells (Th17) in the context of neurodegeneration has not yet been completely elucidated (47). There is evidence showing that Th17 cause cell death in a human iPSC-based model of PD and also evidence that Th17 can be induced and regulated by the intestinal microbiota (48, 49).

Our study has indeed some limitations: sample size is relatively small, and the cross-sectional design suggests caution in the interpretation of results. Furthermore, our data derive from *in vitro* experiments, which might not reflect precisely the complex pathophysiological dynamics of PD and should therefore be reproduced *in vivo*. Possible strategies may involve a study on an animal model of PD, or alternatively the direct evaluation of the clinical and biological effects of probiotics administration in PD patients. In both cases, a longitudinal study in which biomarkers and clinical findings are collected before and after probiotics administration would likely provide important responses.

Our data also suggest that the effect of probiotics might be different with respect to disease stage and gender. Accordingly, we found that LA02 provided a down-modulation of ROS that was more pronounced in the early stages of disease. These data need further confirmation since previous studies did not detect any correlations between clinical or biological variables and disease stage in PD patients treated with probiotics

(46). Moreover, LR06 and BS01 displayed different anti-inflammatory and anti-oxidant activities in PBMCs from male compared to female PD patients. Of note, it was previously reported that microbiota composition may differ between male and female subjects and that this in turn may influence immune functions (50). One last open question regards the relationships between probiotics, peripheral immune function and dopaminergic therapy, especially considering that, to date, the influence of PD treatment on peripheral immunity is still controversial (51–53). Definite answers to this question, as well as to whether and how probiotic administration should be personalized, will likely come from longitudinal *in vivo* studies.

Overall, our preliminary findings suggest a potential role for probiotic strains in modulating inflammation and oxidative stress and protecting the epithelium from gut permeability. Further relevant findings include a possible inhibitory effect against *E. coli* and K. *pneumoniae,* which might be exerted without interfering with levodopa levels.

ETHICS STATEMENT

This study was approved by the local Ethics Committee (CE 65/16). Patients were included in the study after having read and signed an informed consent form for research purpose.

AUTHOR CONTRIBUTIONS

All authors contributed to manuscript revision, read and approved the submitted version. CC, MP, LMo, and RC contributed to manuscript revision. CC, MP, LMa, and AA contributed to conception and design of the study. LMa and AA organized the database, performed the statistical analysis, wrote the first draft of the manuscript. MP, TG, and AA contributed acquisition, analysis or interpretation of data.

FUNDING

This study was funded by the AGING PROJECT—Department of Excellence—Università del Piemonte Orientale.

ACKNOWLEDGMENTS

We thank Dr. Thomas Fleetwood for proofreading the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.00969/full#supplementary-material

REFERENCES

1. Srivanitchapoom P, Pitakpatapee Y, Suengtaworn A. Parkinsonian syndromes: a review. *Neurol India*. (2018) 66:S15–25. doi: 10.4103/0028-3886.226459
2. Comi C, Tondo G. Insights into the protective role of immunity in neurodegenerative disease. *Neural Regen Res*. (2017) 12:64–5. doi: 10.4103/1673-5374.198980
3. Cappellano G, Carecchio M, Fleetwood T, Magistrelli L, Cantello R, Dianzani U, et al. Immunity and inflammation in neurodegenerative diseases. *Am J Neurodegener Dis*. (2013) 21:89–107.
4. Liu X, Yamada N, Maruyama W, Osawa T. Formation of dopamine adducts derived from brain polyunsaturated fatty acids: mechanism for Parkinson disease. *J Biol Chem*. (2008) 283:34887–95. doi: 10.1074/jbc.M805682200
5. Dias V, Junn E, Mouradian JJ. The role of oxidative stress in Parkinson disease. *J Parkinsons Dis*. (2013) 3:461–91. doi: 10.3233/JPD-130230
6. Cerosimo MG, Raina GB, Pecci C, Pellene A, Calandra Cr, Gutiérrez C, et al. Gastrointestinal manifestations in Parkinson's disease: prevalence and occurrence before motor symptoms. *J Neurol*. (2013) 260:1332–8. doi: 10.1007/s00415-012-6801-2
7. Comi C, Magistrelli L, Oggioni GD, Carecchio M, Fleetwood T, Cantello R, et al. Peripheral nervous system involvement in Parkinson's disease: evidence and controversies. *Parkinsonism Relat Disord*. (2014) 20:1329–34. doi: 10.1016/j.parkreldis.2014.10.010
8. McCann H, Cartwright H, Halliday GM. Neuropathology of asynuclein propagation and Braak hypothesis. *Mov Disord*. (2016) 31:152–60. doi: 10.1002/mds.26421
9. Fasano A, Visanji NP, Liu LW, Lang AE, Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. *Lancet Neurol*. (2015) 14:625–39. doi: 10.1016/S1474-4422(15)00007-1
10. Felice VD, Quigley EM, Sullivan AM, Gerard AM, O'Keeffe GW, O'Mahony SM. Microbiota-gut-brain signalling in Parkinson's disease: implications for non-motor symptoms. *Parkinsonism Relat Disord*. (2016) 27:1–8. doi: 10.1016/j.parkreldis.2016.03.012
11. Zheng LF, Song J, Fan RF, Chen QZ, Ren XL, Zhang XL, et al. The role of vagal pathway and gastric dopamine in the rats after a 6-hydroxydopamine microinjection in the substantia nigra. *Acta Physiol Oxf*. (2014) 211:434–46. doi: 10.1111/apha.12229
12. Forsyth CB, Shannon KM, Kordower JH, Voigt RM, Shaikh M, Jaglin JA, et al. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS ONE*. (2011) 6:e28032. doi: 10.1371/journal.pone.0028032
13. Sampson TR, Debelius JW Thron T, Janssen S, Shastri GG, Esra Ilhan Z, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell*. (2016) 167:1469–80. doi: 10.1016/j.cell.2016.11.018
14. Minato T, Maeda T, Fujisawa Y, Tsuji H, Nomonoto K, Ohno K, et al. Progression of Parkinson's disease is associated with gut dysbiosis:two-year follow-up study. *PLoS ONE*. (2017) 12:e0187307. doi: 10.1371/journal.pone.0187307
15. Keshavarzian A, Green SJ, Engen PA, Voigt RM, Naqib A, Forsyth CB. Colonic bacterial composition in Parkinson's disease. *Mov Disord*. (2015) 30:1351–60. doi: 10.1002/mds.26307
16. Sheperijans F, Aho V, Pereira PAB, Koskinen K, Paulin L, Pekkonen E, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord*. (2015) 30:350–8. doi: 10.1002/mds.26069
17. Nair AT, Ramachandran V, Jorghee NM, Antony S, Ramalingam G. Gut microbiota dysfunction as reliable non-invasive early diagnosis biomearkers in the pathophysiology of Parkinson's disease: a critical review. *J Neurogastroenterol Motil*. (2018) 1:30–42. doi: 10.5056/jnm17105
18. Nicola S, Amoruso A, Deidda F, Pane M, Allesina S, Mogna L, et al. Searching for the perfect homeostasis: five strains of *Bifidobacterium longum* from centenarians have a similar behavior in the production of cytokines. *J Clin Gastroenterol*. (2016) 50 (Suppl 2):S126–30. doi: 10.1097/MCG.0000000000000678
19. Fujiwara D, Inoue S, Wakabayashi H, Fujii T. The anti-allergic effects of lactic acid bacteria are strain dependent and mediated effects on both Th1/Th2 cytokine expression and balance. *Int Arch Allergy Immunol*. (2004) 135:205–15. doi: 10.1159/000081305
20. Nowak A, Paliwoda A, Blasiak J. Anti-proliferative, pro-apoptotic and anti-oxidative activity of *Lactobacillus* and *Bifidobacterium* strains: a review of mechanisms and therapeutic perspectives. *Crit Rev Food Sci Nutr*. (2018) 16:1–42. doi: 10.1080/10408398.2018.1494539

21. Kelly B, Blake C, Lennon O. Acute hospital admissions of individuals with a known Parkinson's disease diagnosis in Ireland 2009-2012: a short report. *J Parkinson Dis.* (2016) 6:709–16. doi: 10.3233/JPD-160839
22. Su CM, Kung CT, Chen FC, Cheng HH, Hsiao SY, Lai YR, et al. Manifestations and outcomes of patients with Parkinson's disease and serious infection in the emergency department. *Biomed Res Int*. (2018) 17:6014896. doi: 10.1155/2018/6014896
23. Mogna L, Deidda F, Nicola S, Amoruso A, Del Piano M, Mogna G. *In vitro* inhibition of *Klebsiella pneumoniae* by *Lactobacillus delbrueckii* subsp. delbrueckii LDD01 (DSM 22106): an innovative strategy to possibly counteract such infections in humans? *J Clin Gastroenterol.* (2016) 50 (Suppl 2):S136–9. doi: 10.1097/MCG.0000000000000680
24. Mogna L, Del Piano M, Deidda F, Nicola S, Soattini L, Debiaggi R, et al. Assessment of the *in vitro* inhibitory activity of specific probiotic bacteria against different *Escherichia coli* strains. *J Clin Gastroenterol.* (2012) 46 Suppl:S29–32. doi: 10.1097/MCG.0b013e31826852b7
25. van Kessel SP, Frye AK, El-Gendy AO, Castejon M, Keshavarzian A, van Dijk G, et al. Gut bacterial tyrosine decarboxylases restrict levels of levodopa in the treatment of Parkinson's disease. *Nat Commun*. (2019) 10:310. doi: 10.1038/s41467-019-08294-y
26. Perez M, Calles-Enríquez M, Nes I, Martin MC, Fernandez M, Ladero V, et al. Tyramine biosynthesis is transcriptionally induced at low pH and improves the fitness of *Enterococcus faecalis* in acidic environments. *Appl Microbiol Biotechnol.* (2015) 99:3547–58. doi: 10.1007/s00253-014-6301-7
27. Perez M, Ladero V, Del Rio B, Redruello B, de Jong A, Kuipers OP, et al. Transcriptome profiling of TDC cluster deletion mutant of Enterococcus faecalis V583. *Genom Data.* (2016) 9:67–9. doi: 10.1016/j.gdata.2016.06.012
28. Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel, et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord.* (2015) 30:1591–601. doi: 10.1002/mds.26424
29. Goetz CG, Poewe W, Rascol O, Sampaio C, Stebbins GT, Counsell C, et al. Movement disorder. society task force on rating scales for Parkinson's disease. Movement disorder society task force report on the Hoehn and Yahr staging scale: status and recommendations. *Mov Disord.* (2004) 19:1020. doi: 10.1002/mds.20213
30. Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, et al. Movement disorder society UPDRS revision task force. Movement disorder society sponsored revision of the unified Parkinson's disease rating scale (MDSUPDRS): scale presentation and clinimetric testing results. *Mov Disord*. (2008) 23:2129. doi: 10.1002/mds.22340
31. Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Mov Disord.* (2010) 25:2649–53. doi: 10.1002/mds.23429
32. Tharmaraj N, ShahNP. Antimicrobial effects of probiotics against selected pathogenic and spoilage bacteria in cheese-based dips. *Int Food Res J*. (2009) 16:261–76.
33. Barichella M, Pacchetti C, Bolliri C, Cassani E, Iorio L, Pusani C, et al. Probiotics and prebiotic fiber for constipation associated with Parkinson's disease. *Neurology*. (2016) 87:1274–80. doi: 10.1212/WNL.0000000000003127
34. Del Piano M, Carmagnola S, Anderloni A, Andorno S, Ballarè M, Balzarini M, et al. The use of probiotics in healthy volunteers with evacuation disorders and hard stools: a double-blind, randomized, placebo-controlled study. *J Clin Gastroenterol.* (2010) 44 (Suppl 1):S30–4. doi: 10.1097/MCG.0b013e3181ee31c3
35. Del Piano M, Montino F, Carmagnola S, Anderloni AM, Orsello E, Garello F, et al. The use of probiotics in the treatment of constipation in the elderly. *Cibus*. (2005) 1:23–30.
36. Nimgampalle M, Kuna Y. Anti-Alzheimer properties of probiotic, *Lactobacillus plantarum* MTCC 1325 in Alzheimer's disease induced albino rats. *J Clin Diagn Res*. (2017) 11:KC01–KC05. doi: 10.7860/JCDR/2017/26106.10428
37. Akbari E, Asemi Z, Daneshvar Kakhaki R, Bahmani F, Kouchaki E, Tamtaji OR. Effect of probiotic supplementation on cognitive function and metabolic status in Alzheimer's disease: a randomized, double-blind and controlled trial. *Front Aging Neurosci.* (2016) 8:256. doi: 10.3389/fnagi.2016.00256
38. Frank MG, Fonken LK, Watkins LR, Maier SF, Lowry CA. Could probiotics be used to mitigate neuroinflammation? *ACS Chem Neurosci.* (2018) 10:13–5. doi: 10.1021/acschemneuro.8b00386
39. Mazzini L, Mogna L, De Marchi F, Amoruso A, Pane M, Aloisio I, et al Potential role of gut microbiota in ALS pathogenesis and possible novel therapeutic strategies. *J Clin Gastroenterol.* (2018) 52 (Suppl 1):S68–70. doi: 10.1097/MCG.0000000000001042
40. Skvarc DR, Berk M, Byrne LK, Dean OM, Dodd S, Lewis M, et al. Post-operative cognitive dysfunction: an exploration of the inflammatory hypothesis and novel therapies. *Neurosci Biobehav Rev*. (2018) 84:116–33. doi: 10.1016/j.neubiorev.2017.11.011
41. Reber SO, Siebler PH, Donner NC, Morton JT, Smith DG, Kopelman JM, et al. Immunization with a heat-killed preparation of the environmental bacterium *Mycobacterium vaccae* promotes stress resilience in mice. *Proc Natl Acad Sci USA*. (2016) 113:E3130–9. doi: 10.1073/pnas.1600324113
42. Sommer A, Winner B, Prots I. The Trojan horse - neuroinflammatory impact of T cells in neurodegenerative diseases. *Mol Neurodegener.* (2017) 12:78. doi: 10.1186/s13024-017-0222-8
43. Alberio T, Pippione AC, Zibetti M, Olgiati S, Cecconi D, Comi C, et al. Discovery and verification of panels of T-lymphocyte proteins as biomarkers of Parkinson's disease. *Sci Rep*. (2012) 2:953. doi: 10.1038/srep00953
44. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize α-synuclein peptides. *Nature*. (2017) 546:656–61. doi: 10.1038/nature22815
45. Kustrimovic N, Comi C, Magistrelli L, Rasini E, Legnaro M, Bombelli R, et al. Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of Th1/Th17/Th2 and Treg in drug-naïve and drug-treated patients. *J Neuroinflamm*. (2018) 15:205. doi: 10.1186/s12974-018-1248-8
46. Houser MC, Chang J, Factor SA, Molho ES, Zabetian CP, Hill-Burns EM, et al. Stool immune profiles evince gastrointestinal inflammation in Parkinson's disease. *Mov Disord.* (2018) 33:793–804. doi: 10.1002/mds.27326
47. Storelli E, Cassina N, Rasini E, Marino F, Cosentino M. Do Th17 Lymphocytes and IL-17 contribute to Parkinson's disease? A systematic review of available evidence. *Front Neurol.* (2019) 10:13. doi: 10.3389/fneur.2019.00013
48. Sommer A, Maxreiter F, Krach F, Fadler T, Grosch J, Maroni M, et al. Th17 lymphocytes induce neuronal cell death in a human iPSC-based model of Parkinson's disease. *Cell Stem Cell.* (2018) 23:123–31. doi: 10.1016/j.stem.2018.06.015
49. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. (2009) 139:485–98. doi: 10.1016/j.cell.2009.09.033
50. Fransen F, van Beek AA, Borghuis T, Meijer B, Hugenholtz F, van der Gaast-de Jongh C, et al. The impact of gut microbiota on gender-specific differences in immunity. *Front Immunol.* (2017) 8:754. doi: 10.3389/fimmu.2017.00754
51. Kustrimovic N, Rasini E, Legnaro M, Bombelli R, Aleksic I, Blandini F, et al. Dopaminergic receptors on CD4+ T naive and memory lymphocytes correlate with motor impairment in patients with Parkinson's disease. *Sci Rep*. (2016) 6:33738. doi: 10.1038/srep33738
52. Alberio T, Pippione AC, Comi C, Olgiati S, Cecconi D, Zibetti M, et al. Dopaminergic therapies modulate the T-CELL proteome of patients with Parkinson's disease. *IUBMB Life*. (2012) 64:846–52. doi: 10.1002/iub.1073
53. Soreq L, Bergman H, Goll Y, Greenberg DS, Israel Z, Soreq H. Deep brain stimulation induces rapidly reversible transcript changes in Parkinson's leucocytes. *J Cell Mol Med*. (2012) 16:1496–507. doi: 10.1111/j.1582-4934.2011.01444.x

Conflict of Interest Statement: AA, LMo, TG, and MP were employed by company Biolab Research srl, Novara, Italy.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership