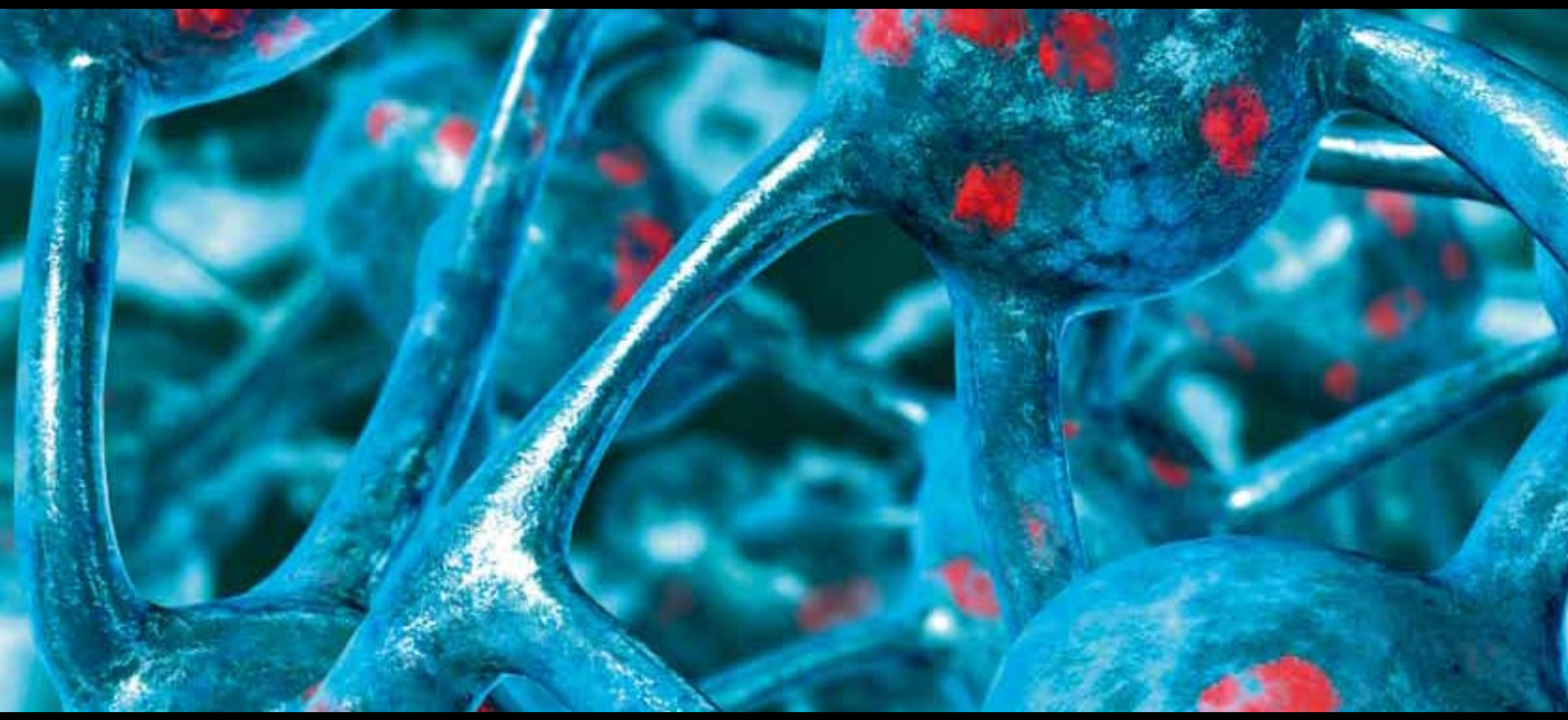
Inflammation and Parkinson's Disease

Guest Editors: Carlos Barcia, Stéphane Hunot, Gilles J. Guillemin, and Fernando Pitossi



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Editorial Board

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Editorial

Inflammation and Parkinson's Disease

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After more than two decades of research, the latest published studies regarding the protective effects of anti-inflammatory drugs in Parkinson's disease (PD) indicate that only a subset of nonsteroidal anti-inflammatory drugs (NSAIDs) may be efficient in decreasing the risk of PD. In particular, recent epidemiology studies and meta-analysis have shown that, among these NSAIDS, the inhibitors of the enzyme cyclooxygenase (COX) were the most potent compounds reaching the highest rate of PD prevention. These data clearly support a COX-specific mechanism of neuroprotection and reinforce the idea that neuroinflammation in PD comprises specific features that should be unraveled. Such that knowledge should help the development of specific drugs targeting inflammatory mediators.

Several clinical trials currently ongoing have focused their goals in evaluating *in vivo* potential imaging biomarkers for inflammatory changes in neurodegeneration. [18F] FEPPA and [(11) C] PBR28 are being evaluated for their capacity in detecting neuroinflammation by single photon emission computed tomography (SPECT). This is an important step regarding the safe monitoring of neuroinflammation in patients. If successful, these *in vivo* imaging methods will be valuable not only to determine precisely the right therapeutic window but also to accurately measure the biological outcomes of neuroprotective treatments. Most importantly, this also means that the inflammatory component of PD is having significant attention among researchers and it will probably be assumed in the clinical scenario in the coming years. In addition, there are numerous preclinical trials testing the protective effects of anti-inflammatory drugs in animal models of PD and hopefully some of them will soon be brought into Phase I trials. However, further research and new perspectives are needed to understand the specific aspects of inflammation in PD.

In the present special issue, we present 9 review articles that explore new insights into the inflammatory reaction associated with PD. D. Litteljohn et al. show an excellent review of how the toxin-based models of PD have contributed significantly to the study of the mechanisms underlying neuroinflammatory processes in Parkinsonism and outline the role of TNF-α and IFN-γ, two cytokines critically involved in glial cell activation and dopaminergic degeneration. Then, T. Farooqui and A. A. Farooqui describe in a comprehensive review how lipid-derived factors are able to induce cellular stress and inflammation, which may be involved in PD pathogenesis. In line with this, M. Liu and G. Bing show in their revision how lipopolysaccharide (LPS), a cell-wall component of Gram-negative bacteria and prototypical inflammogen, induces dopaminergic cell death indicating that the inflammatory response is by itself detrimental. Importantly, inflammation-induced toxicity seems to be highly specific for dopaminergic cells and with very special distinctiveness in some dopaminergic areas. In fact, V. Roca et al. nicely described, in their review, the unique susceptibility to inflammation of the Substantia Nigra, the prime locus of dopaminergic cell death in PD.

On the other hand, C. C. Ferrari and R. Tarelli describe in their complete study how systemic inflammation may impact

central inflammation and dopaminergic cell death. Then, they explore the possibility that peripheral inflammation could be a contributing factor in PD development. This view is further complemented by the review from A. Machado et al., which gives a different perspective of this particular topic but focused on studies performed in animal models of PD.

Finally, in a last series of review, authors consider new perspectives and therapeutic targets to avert toxic inflammation in PD. First, A. R. Carta et al. discuss an emerging strategy to block neuroinflammation in PD using PPAR-γ agonists. This is particularly interesting in light of the above discussion since ibuprofen but not aspirin or acetaminophen has been shown to display PPAR-γ agonistic activity. P. M. Flood et al. proposed new ways for targeting NF-kB through IKK complex inhibition, a promising route that may be a useful approach in a close future in the treatment of PD. Finally, in a last review article, A. Zinger et al. describe how the kynurenine pathway, a metabolic pathway involved in the production of nicotinamide adenine dinucleotide, may be critically involved in the neuroinflammatory process in PD and how this pathway could be controlled for therapeutic purposes.

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Review Article

Inflammatory Mechanisms of Neurodegeneration in Toxin-Based Models of Parkinson's Disease

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Parkinson's disease (PD) has been associated with exposure to a variety of environmental agents, including pesticides, heavy metals, and organic pollutants; and inflammatory processes appear to constitute a common mechanistic link among these insults. Indeed, toxin exposure has been repeatedly demonstrated to induce the release of oxidative and inflammatory factors from immunocompetent microglia, leading to damage and death of midbrain dopamine (DA) neurons. In particular, proinflammatory cytokines such as tumor necrosis factor-α and interferon-γ, which are produced locally within the brain by microglia, have been implicated in the loss of DA neurons in toxin-based models of PD; and mounting evidence suggests a contributory role of the inflammatory enzyme, cyclooxygenase-2. Likewise, immune-activating bacterial and viral agents were reported to have neurodegenerative effects themselves and to augment the deleterious impact of chemical toxins upon DA neurons. The present paper will focus upon the evidence linking microglia and their inflammatory processes to the death of DA neurons following toxin exposure. Particular attention will be devoted to the possibility that environmental toxins can activate microglia, resulting in these cells adopting a "sensitized" state that favors the production of proinflammatory cytokines and damaging oxidative radicals.

1. Introduction

Parkinson's disease (PD) is the most common neurodegenerative disorder of motor functioning, affecting nearly six million people worldwide. The disorder is particularly prevalent in the elderly population, with a typical clinical onset after 60−65 years of age. Notwithstanding the rare familial forms of PD that appear to have a strong genetic component, the vast majority of PD cases (upwards of 90%) are idiopathic in nature. Regardless of etiology, PD is characterized primarily by the progressive degeneration of dopamine (DA) neurons within the substantia nigra pars compacta (SNc) region of the midbrain, resulting in the diminished monoamine release at downstream striatal nerve terminals. Clinically, the Parkinsonian syndrome, which typically becomes manifest following 50−60% SNc DA neuron loss, comprises a constellation of well-defined motor symptoms, including bradykinesia, hypokinesia (or akinesia), cogwheel rigidity, resting tremor, and postural instability [1]. In addition to the motor impairment evident in all PD cases, a substantial number of PD patients also display prominent "nonmotor" symptoms (many of which manifest before the onset of motor decline and PD diagnosis), including autonomic and olfactory problems (e.g., sleep disorders, hyposmia), as well as cognitive and psychological disturbances (e.g., anxiety, depression) [2]. While striatal DA denervation may influence the development of at least some of these symptoms (e.g., memory and attention problems [3]), it is likely that multi-neurotransmitter dysfunction in brain regions important for autonomic, emotional and psychological functioning (e.g., locus coeruleus, prefrontal cortex, hippocampus) is important in this regard (perhaps stemming from parallel inflammatory and neurodegenerative processes) [4, 5].

Epidemiological studies have implicated exposure to pesticides and other potential environmental toxins (e.g., heavy metals and even immune infections) in the evolution of PD [6, 7]. Parallel work in rodents has likewise revealed that administration of certain pesticides, most notably

paraquat and rotenone, recapitulates many of the characteristic neuropathological and behavioral features of PD [8, 9]. Over the past few decades it has become clear that neuroinflammatory factors, including proinflammatory cytokines produced by glial cells, are involved in many aspects of the neurodegenerative process in PD. Indeed, manipulation of cytokines and associated inflammatory signaling pathways was reported to affect DA neuronal survival in response to a host of different toxins [10, 11]. Moreover, alterations of microglial cell reactivity have been routinely demonstrated during early and late phases of the degenerative process in animal models of PD [12, 13]. Correspondingly, postmortem PD brains typically display signs of heightened inflammatory and oxidative distress, including increased proinflammatory cytokines and microglial activation [14, 15], as well as augmented oxidative and inflammatory enzyme expression (e.g., nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2)) [16, 17].

Substantial recent interest has focused on microglial cells as potential mediators of pathology in PD; however, it remains to be determined whether these cells are primary players in disease progression or are secondarily recruited following damage. Alternatively, it might be the case that microglia are involved in all stages of PD but that their role changes (e.g., neuroprotective versus neurodestructive) as the disease progresses through different stages. Indeed, during normal physiological conditions microglial cells are constantly detecting and reacting to modifications in their local environment and attempting to maintain proper tissue homeostasis [18, 19]. When sufficiently stressed by insults, neurons release ATP into the extracellular space and microglia migrate along these ATP gradients and facilitate the removal of the dead/sick cells through phagocytosis [18, 20]. However, in the case of PD, these "danger" signals released from injured and dying cells (e.g., ATP, heat-shock proteins) may be subtle and occur over a prolonged period of time [21], essentially placing microglia in a chronically active state.

The reactivity state of microglia varies along a spectrum ranging from resting to hyperactive and is under the strict control of several regulatory proteins [22]. Some evidence suggests that microglial cells can perform neuroprotective functions in PD, at least in the short term, by secreting trophic factors such as nerve growth factor, neurotrophin-3 and brain-derived neurotrophic factors (BDNFs) [23, 24]. However, as the disease progresses, there is compelling evidence to indicate that microglia undergo significant elevations in cell surface activation/adhesion molecules and adopt a more hyperactive state that is morphologically similar to peripheral macrophages [25]. In this state, microglia are capable of upregulating the synthesis and release of a host of proinflammatory and prooxidant factors, including cytokines, prostaglandins (PGs) and reactive oxygen species (ROS) [26]. Indeed, following toxin exposure, chronically activated microglia can produce large quantities of superoxide (e.g., via the NADPH oxidase enzyme), which, in turn, can lead to the damage and death of adjacent DA neurons [27, 28].

The present paper will, (1) cover the evidence linking exposure to environmental toxins and the development of PD; (2) review the mechanisms by which inflammatory cytokines affect central nervous system (CNS) functioning; and (3) evaluate the possibility that cytokines and inflammatory and oxidative enzymes are involved in the PD-like neurodegenerative process induced by environmental toxins.

2. Environmental Toxin Exposure and PD

Although familial forms of PD are relatively rare, certain genetic mutations have been reported to enhance susceptibly to environmental insults and hence, might contribute to the more common idiopathic cases of the disease. In fact, a recent study revealed that individuals possessing a combination of mutations of the DA transporter (DAT) and who had substantial life-long pesticide exposure were at greater risk for developing PD than individuals with either the genetic factor or pesticide exposure alone [6]. Moreover, the recent findings that polymorphism within certain environment responsive genes encoding effector proteins critical for cellular detoxification and xenobiotic metabolism (e.g., CYP2D64, GSTT1 and P1) modified the risk of developing PD, suggests that environmental toxicants might contribute to PD in genetically vulnerable individuals [29, 30]. However, another report indicated that pesticide exposure was a significant predictor of PD incidence among individuals with a negative family history but not those with a positive family history of the disease [31]. In effect, it is likely that the role of genetics depends upon the particular "subtype" of PD. Indeed, PD appears to be a highly heterogeneous disorder with corresponding heterogeneity in etiological origins. Whereas autosomal dominant/recessive familial forms of PD (e.g., LRRK2, DJ-1, Parkin) appear to be at one end of the spectrum, purely environmental "toxic exposure" cases may represent the other end. Hence, the bulk of "idiopathic" PD cases falls in the middle and will likely involve a mix of genetic and environmental influences. Indeed, there is a very low penetrance of LRRK2 heterozygotic carriers that actually express the PD phenotype; yet, a significant proportion of PD patients carry a LRRK2 mutation, suggesting that such genes might be seen as susceptibility factors [32].

While genetic vulnerability may be seen as providing a backdrop for disease provocation, several compelling lines of evidence suggest that environmental agents, including commonly used pesticides, can act as triggers for the development of PD. In fact, a progressively greater odds ratio for developing PD was associated with pesticide exposure [6], and several other epidemiological studies have implicated specific pesticides, including rotenone (an organic insecticide) and paraquat (a chemical herbicide still widely used throughout the world), in the development of parkinsonism [33, 34]. Indeed, a sharp increase of PD incidence was seen in agricultural areas that use these pesticides [35, 36]. In particular, the nonselective herbicide, paraquat (N,N′-dimethyl-4,4′-bipyridylium ion), significantly augmented the risk of developing PD as a function of cumulative pesticide exposure [37].

Animal studies also demonstrate that the pesticides, paraquat and rotenone, which are chemically similar to the established DA neurotoxin, MPTP, can reliably induce PD-like pathology, and hence, are becoming widely used to produce a parkinsonian syndrome in animals. Indeed, systemic exposure to paraquat provoked a dose-dependent loss of DA neurons in the SNc [8, 38], coupled with a reduction in the density of striatal DA fibres expressing tyrosine hydroxylase, the rate-limiting enzyme in DA synthesis [39]. The pesticide was also shown to diminish striatal DA concentration and to reproduce certain aspects of the PD phenotype in rodents (bradykinesia, motor coordination deficits, depressive-like symptoms, memory impairment) [39–43]. However, it is worth noting that the impact of paraquat upon the striatum appears to be somewhat less pronounced than the effects of the pesticide upon SNc DA neuronal soma [44]. As well, some authors have failed to find changes in striatal DA levels or behavioral impairment, even in the presence of loss of DA soma [45]. It is conceivable that compensatory downstream processes provoked by soma loss (e.g., changes in dendritic branching patterns, upregulation of proneuroplastic peptides or neurotrophins, or alterations of brain monoamine systems) could account for such discrepancies between SNc pathology and striatal functioning. Also, variations in experimental design (e.g., route of administration, dosing regimen, sacrifice interval, striatal subregions tested, age of mice) probably contribute to some of the inconsistency in findings across studies [46–48].

Of course, paraquat is not alone in producing sometimes discrepant research findings, as virtually all of the most common toxin models of PD have engendered controversy with respect to selective DA neuron loss, variable striatal DA depletion and behavioral impairment, and/or the generation of Lewy body-like α-synuclein inclusions (see Table 1) [49–51]. Of these PD mimetics, MPTP is perhaps the most widely used and well characterized, producing consistent and reproducible PD-like pathology in several animal species (e.g., DA lesion, reactive microgliosis, motor deficit). Yet, pesticides such as paraquat and rotenone, in addition to having greater ecological relevance than the MPTP model of PD, have been shown to provoke histopathological changes that more closely resemble the disease, particularly the deposition of α-synuclein aggregates in neuronal Lewy body-like inclusions (see Table 1) [9, 52, 53]. In fact, Drolet and colleagues [54] recently found that rats treated with systemic rotenone displayed marked α-synuclein pathology in small intestine myenteric neurons that was reminiscent of the enteric Lewy body pathology commonly seen in PD patients. Moreover, it was reported that exposure to certain combinations of heavy metals and pesticides may synergistically provoke conformational changes in α-synuclein, favoring the development of PD-like pathology [55]. In fact, recent work revealed that exposure to a combination of iron and paraquat synergistically increased α-synuclein aggregation and fibrillization, and augmented the extent of microglia-induced oxidative stress and neurodegeneration [56, 57]. Similarly, although the dithiocarbamate pesticide, maneb, had no effect on SNc DA neurons alone, when coadministered with paraquat it synergistically enhanced nigrostriatal damage and associated glial reactivity [58].

Pesticides can adversely affect neuronal survival by impairing mitochondrial functioning and overstimulating microglial cells, causing an accumulation of oxidative free radicals (e.g., superoxide, hydroxyl radicals) and inflammatory factors (particularly cytokines). Indeed, as will be discussed in ensuing sections, we and others showed that paraquat and rotenone enhanced the expression of proinflammatory cytokines and elicited oxidative-nitrosative stress through activation of the microglial inflammatory enzyme, NADPH oxidase. In fact, as was reported for MPTP [27], paraquat was demonstrated to preferentially damage midbrain DA neurons through direct microglial-dependent NADPH oxidase activity in neuron-microglia cultures [59]. However, the role of microglia is not without controversy, with one recent report indicating that neither rotenone nor paraquat directly activated cultured microglia (in terms of morphology, nitric oxide synthesis and cytokine release) [60]. Similarly, accumulating evidence suggests that the PG synthase, COX-2, may contribute to the neurodegenerative effects of numerous DA toxins.

3. Inflammatory Cytokines in Relation to Central Nervous System Functioning

Until fairly recently the brain was believed to function more or less independently of the immune system. However, it is now accepted that circulating T lymphocytes, macrophages and other peripheral immune cells routinely enter cerebrum, albeit in limited concentrations, and perform a variety of "housekeeping" tasks that are essential for immunosurveillance of the CNS [61]. The converse is also true in that changes in neural transmission, such as those provoked by drug administration, can affect immune cell activity in the periphery. Yet, it should be noted that the blood brain barrier (BBB), as well as several endogenous inhibitory and apoptotic mechanisms operating within the brain itself, normally tightly regulate the transmigratory flow of immune factors into the CNS, and how long they persist therein. For instance, immune cells entering the brain typically are removed or die via apoptosis relatively rapidly; this is essential since postmitotic neurons are especially sensitive to immune attack. However, increasing evidence indicates that a range of neurological diseases, including Amyotrophic Lateral Sclerosis, Alzheimer's disease (AD) and PD have a prominent neuroinflammatory component, involving increased infiltration of immune cells, coupled with activation of resident brain glial cells [17, 62].

Nonetheless, it should be underscored that not all CNS inflammation is uniformly "bad"; indeed, transient neuroinflammatory responses are a natural consequence of injury or infection and may actually preserve viable brain tissue in a manner analogous to a short-lived immune response in the periphery (e.g., removal of cellular debris, release of trophic factors) [63, 64]. Moreover, neuronal degeneration itself provokes secondary inflammation that may or may not

Table 1: A comparison of some of the most common toxin-based animal models of Parkinson's disease.

Toxin model	Mode of action	Advantages	Disadvantages
6-OHDA	(i) DAT substrate (ii) Cytotoxic quinone and ROS formation	(i) Full DA depletion (ii) Mimics late-stage PD	(i) Does not cross BBB (ii) DA degeneration is not progressive (iii) No Lewy body-like inclusions (iv) Lacks external validity
MPTP	(i) Converted into MPP^+ (ii) DAT substrate (iii) Inhibits mitochondrial complex I (iv) Strong inflammatory component	(i) Highly reproducible (ii) Induces substantial DA loss and motor impairment	(i) DA degeneration is not progressive (ii) Does not provoke Lewy body-like inclusions (iii) Systemic toxicity (iv) Lacks external validity
Paraquat	(i) Potent redox cycler (ii) Neuroinflammatory component	(i) Progressive loss of DA neurons (ii) Lewy-body like α-synuclein inclusions (iii) Potential ecological validity	(i) Inconsistent striatal DA loss and motor impairment (ii) Induces only moderate DA cell loss when administered alone (ii) Systemic toxicity
Rotenone	(i) Readily crosses DA neuron membrane (ii) Inhibits mitochondrial complex I (iii) Neuroinflammatory component	(i) Progressive loss of DA neurons (ii) Lewy body-like inclusions (iii) Potential ecological validity	(i) Variable reproducibility (ii) Systemic toxicity (iii) Nonspecific accumulation within the CNS
LPS	Immune system activation	(i) Progressive loss of DA neurons (ii) Strong inflammatory component (iii) Sensitizes DA neurons to later treatment with LPS or other toxins	No Lewy body-like inclusions

come to influence the primary degenerative process. Thus, it is difficult to assign valence to neuroinflammatory events occurring in the PD brain on the basis of clinical autopsy studies alone. In this context, toxin-based animal models of PD provide a suitable and ecologically relevant means of assessing the role and disease-modulating capability of inflammatory responses that are either mounted or sustained by the CNS.

In addition to the infiltration of immune cells into the brain parenchyma, substantial evidence has revealed that immune factors can influence CNS functioning through activation of receptors located on peripheral organs or the BBB. These can, in turn, promote second messenger cascades or stimulate neural afferents that innervate the CNS [65]. Indeed, one of the primary mechanisms facilitating neuroimmune communication is the release of soluble glycoprotein messengers called cytokines. Although cytokines are typically produced by peripheral immune cells, evidence in recent decades has also convincingly uncovered their production from CNS glial cells [66]. In particular, immunocompotent microglia produce several cytokines and bear receptors for these immunotransmitters, which can act locally in an autocrine or paracrine manner to regulate functioning of the originating or neighboring cells, respectively [67].

The list of polypeptides that comprise the rapidly growing family of cytokine immunotransmitters include; the interferons (IFN), interleukins (IL), tumor necrosis factors (TNF), chemokines (subclass of chemoattractant cytokines), and growth and cell stimulating factors. Historically, the classification of cytokines has been based upon their molecular structure, as well as common physiological actions they possess, including the production of inflammation (i.e., swelling and irritation resulting from leukocyte infiltration) or fever (pyrogenicity) [67]. IL-1β, TNF-α and IL-6, which are all released from activated macrophages, are potent proinflammatory cytokines, whereas IL-4 and IL-10, which are released from T-cells, have antiinflammatory actions.

Cytokines may gain entry to the brain through sites where the BBB is somewhat compromised (i.e., areas with fewer or less complex tight junctions), namely at circumventricular organs such as the median eminence and area postrema [65]. As well, saturable carrier-mediated transport mechanisms capable of moving IL-1β and TNF-α may allow for limited penetration of cytokines into the brain [68, 69]. Once cytokines gain entry to the brain, they interact with receptors on cells lining the BBB, around the meninges, as well as at vascular areas of the brain [70]. Through volume diffusion, infiltrating cytokines may ultimately penetrate deep within the brain parenchyma [71, 72] where they can influence, among other things, neuronal Ca^{2+} channels and MAP kinase and COX-2 signaling [73, 74].

Pro- and- antiinflammatory cytokine levels are markedly increased by immune and traumatic insults [75]. In this regard, endothelial cells that line the interior surface of blood vessels and the brain ventricles produce IL-1β and IL-6, and infection or injury augments their concentration [76]. Further, microglia, which serve as the brain's own specialized immune cells, are primary cytokine producers, and the synthesis of these cytokines was augmented by head injury, stroke and neurotoxins [77–79].

4. Neuroinflammatory Mechanisms of PD: Microglia, Cytokines, and Inflammatory Enzymes

Systemic infection may interact with environmental insults to induce exaggerated neuroinflammatory, degenerative and behavioural changes in neurological patients [80]. Indeed, exposure to pathogens or cytokines might have especially marked CNS consequences when encountered in the context of concomitant chemical toxin, traumatic head injury, or psychological stressor exposure, each of which can contribute to a breakdown of the BBB, hence favoring entry of peripheral immune pathogens into the CNS. In this regard, the bacterial endotoxin, LPS, synergistically augmented DA loss in midbrain-microglia cocultures exposed to pesticides, such as rotenone [81] and these effects may be related to enhanced NADPH oxidase-mediated release of the superoxide radical [27]. Our own work has similarly shown that a low dose of LPS enhanced the neurotoxic effects of the herbicide, paraquat, such that a substantial number of DA-producing neurons were destroyed (i.e., more than was observed with paraquat alone) and PD-like symptoms emerged [82]. The augmented neurodegenerative response was observed when paraquat administration occurred at a time of maximal LPS-induced microglial activation (after 2 days), suggesting that the inflammatory priming sensitized microglial responding, thereby contributing to the degenerative effects of later paraquat exposure. Importantly, although relatively high concentrations of LPS alone had neurodegenerative consequences on DA neurons [83, 84], our studies involved relatively low concentrations of the endotoxin that alone activated microglia but had no effect upon DA neuronal survival.

The possibility exists that environmental or inflammatory toxins might promote a sensitization of neuronal processes across the lifespan, such that exposure to an immune/chemical toxin at one point in life enhances vulnerability to the behavioural and neurodestructive effects of these challenges when subsequently encountered months or even years later. In particular, at *in utero* and early life stages when neuronal migration and synaptic pruning are occurring, neurons are especially sensitive to perturbations caused by environmental agents. At the same time, biological detoxification systems involved in metabolism and clearance of toxic substances are not fully developed in fetuses, infants and young children. Indeed, prenatal exposure to LPS induced a relatively permanent elevation of inflammatory factors within the nigrostriatal system and reduced the number of mature DA neurons in adulthood [85, 86].

Exposure to LPS during critical developmental times was also found to have protracted consequences that involve a dramatic long-term sensitization of the inflammatory immune response, such that the neuroinflammatory and neurodegenerative actions of pesticides applied during adulthood were greatly enhanced [87, 88]. As well, bacterial vaginosis, a common infection during pregnancy, has been linked to both the development of neurological disorders, including cerebral hemorrhage and cerebral palsy, and with enhanced levels of several proinflammatory cytokines, including IL-1β, IL-6, and TNF-α in adulthood [89, 90]. Consistent with these findings, our contention has been that early immunogenic exposure may provoke mild neuroinflammation that, over time, renders neurons vulnerable to the effects of normally low-grade insults later in life. It may also be that early toxin exposure causes modest neuronal damage (or a silent lesion) that only becomes "un-masked" upon later multiple toxin exposures, again resulting in some threshold of neuronal vulnerability eventually being breached.

4.1. Role of Proinflammatory Cytokines in PD. Cytokines primarily act through either of three molecular pathways, involving activation of: (1) NFκB, (2) c-Jun N terminal kinase (JNK), or (3) janus kinase (JAK) and signal transducer and activator of transcription (STAT). The latter two pathways involve the sequential phosphorylation of a series of intracellular proteins following administration of several cytokines, including IL-6, IL-10 and IFN-γ, resulting in the production of factors important for inflammatory and neuronal processes [91]. Similarly, the production of immune and CNS factors, including the inflammatory enzyme, COX-2, occurs following NFκB activation. In particular, IL-1β and TNF-α trigger the phosphorylation and degradation of the inhibitory factor, IκB, which normally holds NFκB in an inactive state, resulting in its translocation to the nucleus where it influences (inflammatory) gene expression. In fact, we found that COX-2 deletion markedly influenced the production of cytokines following stressor and endotoxin exposure [92].

Increasingly, cytokines have been implicated in acute and chronic neuronal demise [91]. Indeed, clinical studies revealed augmented levels of proinflammatory cytokines (TNF-α, IL-6, IL-1β, IFN-γ) in postmortem brain as well as in the blood and/or cerebral spinal fluid (CSF) of patients with stroke, head injury, AD and PD [62, 93–95]. A further recent study found that PD patients had elevated basal and LPS-induced blood levels of numerous proinflammatory cytokines, including MCP-1, RANTES, MIP-1α, IL-8, IFN-γ, IL-1β and TNF-α; and significant correlations were observed between cytokine levels and severity of parkinsonism [96]. Although many of these findings have been recapitulated in animal models, it is still uncertain whether these cytokines primarily play a neuroprotective or neurodestructive role. It may be that relatively low endogenous cytokine levels act in a protective capacity to buffer against damage related to death processes, whereas relatively high levels of these factors contribute to neuronal damage [97]. Indeed, low levels of cytokines can provoke the release of potentially beneficial trophic factors (BDNF, GDNF) and free radical scavengers (MnSOD), but elevated levels can activate oxidative-inflammatory cascades or even induce apoptotic death (self-destructive programmed death mechanism) [98, 99]. For instance, mice genetically lacking TNF-α receptors (thereby removing the influence of low endogenous levels of TNF-α) were more susceptible to ischemic injury [97]; yet, administration of exogenous TNF-α at the time of ischemia exacerbated neuronal death [100]. Likewise, administration of the endogenous IL-1 antagonist, IL-1ra, reduced infarct size in response to middle cerebral artery occlusion and

prevented the accumulation of inflammatory infiltrates within the area of damage [101], suggesting a prominent destructive role for IL-1 in acute cerebrovascular insults. In effect, the concentration as well as timing of cytokine exposure likely determines whether primarily protective or deleterious consequences arise from these immunotransmitters.

4.1.1. Interferons in PD. Interferons (IFNs) are broadly divided into either type I IFNs, including the IFN-α and IFN-β isoforms, which originated from a common ancestral gene, or the structurally unrelated type II form, IFN-γ (formerly called macrophage activating factor). The main signaling pathways utilized by the IFNs involve the sequential phosphorylation of STATs by intracellular JAK protein kinases (stimulated by ligand-receptor binding). IFN-γ is secreted predominantly from type 1 helper T lymphocytes (Th1) and natural killer (NK) cells; yet, recent reports indicate that the cytokine is also synthesized de novo within the brain by activated microglia [102]. In contrast, the production of IFN-α and IFN-β does not appear to be under the control of specific cell types, and indeed, most cells appear to be able to secrete these cytokines in response to viral insult [103]. Although IFNs were originally believed to be exclusively antiviral substances, it has become apparent that the cytokine family is involved in a broad array of immunoregulatory functions that may either inhibit or promote disease states within the periphery or CNS (e.g., cancer, chronic microbial or parasitic infection) [104, 105].

Cancer and hepatitis C patients receiving IFN-α immunotherapy have been observed in many instances to develop a PD-like syndrome, including tremors, muscle rigidity and a generalized paucity of movement [106, 107]; and postmortem examination of PD brains revealed the presence of MxA (type I IFN-inducible GTPase) in SNc Lewy bodies and neuronal swellings [108, 109]. Similarly, recent data suggest an important role for IFN-γ in MPTP and paraquat animal models of PD [42, 110]. In corroboration of these results, IFN-γ levels are elevated in the blood [110, 111] and postmortem SNc brain tissue [112, 113] of PD patients; and a polymorphism in the gene coding for IFN-γ differentially modified the risk of developing early- or late-onset PD [114]. In addition, levels of serum and CSF neopterin, a pteridine marker of IFN-γ-associated immune system activation, are elevated in PD patients and tend to be highest among those with more severe symptoms [115]. Indeed, PD patients exhibit fewer infectious episodes and malignancies [116, 117], possibly stemming from enhanced proinflammatory IFN signaling.

Interestingly, a recent study [118] indicated that IFN-γ is capable of inflicting direct excitotoxic neuronal damage by signaling through a distinct, neuron-specific receptor complex formed by the IFN-γ receptor and the AMPA receptor GluR1 subunit. In this way, IFN-γ was observed to induce dendritic beading in mouse cortical neurons secondary to an increase in Ca^{2+} influx, nitric oxide (NO) generation and ATP depletion [118]. However, most available evidence suggests that IFN-γ likely influences neuronal survival and functioning through its actions on glial cells, particularly microglia.

While the microglial gene network subject to regulatory control by IFN-γ is both extensive and diverse (reflecting the pleiotropic nature of IFN-γ and cytokines in general), a number of positively regulated IFN-γ-responsive genes (i.e., those containing GAS (gamma activation sequence), IRF-E (interferon regulatory factor element), or ISRE (interferon-stimulated response element) binding sites) encode proteins implicated in immunoinflammatory processes [119, 120]; and hence, may be of particular relevance for neurological disorders such as PD. For instance, IFN-γ-associated microglial JAK/STAT signaling arbitrates (either directly or indirectly via secondary transcription factors such as IRF-1) the upregulated or de novo expression of several genes encoding proteins critical for antigen presentation to lymphocytes (e.g., MHC class I/II, immunoproteasome subunits LMP-2 and LMP-7), recruitment and activation of T cells (i.e., chemokines and adhesion molecules), and classical pathway-dependent complement deposition [119, 120]. Importantly, many of these same immunologically relevant factors have been localized to microglia in the SNc of postmortem PD brains or animals exposed to DA-targeting neurotoxins [114, 121–123], suggesting that IFN-γ may be a critical determinant of prospective adaptive immune responses in PD.

While the pathogenic relevance of adaptive immune activation in PD has long been debated, a recent study demonstrated that mice genetically lacking mature CD4+ T lymphoctyes (but not CD8+ T cells) were protected against MPTP-induced neurodegeneration [124]. However, in this study, CD4+ T cell-mediated DA neuronal loss was found to be dependent on the presence of the TNF ligand family member, FasL, and not IFN-γ. Of course, these results do not necessarily preclude a role of IFN-γ in T cell-mediated dopaminergic neurodegeneration; indeed, FasL is capable of augmenting inflammatory cytokine cascades from microglia (in addition to directly mediating neuronal apoptosis), and Fas receptor expression is potently upregulated in activated microglia following inflammatory insult [125].

In addition to facilitating communication between microglia and peripheral immune cells, IFN-γ plays a key role in the activation of oxidative and inflammatory microglial enzyme systems that evolved to protect the host against pathogenic (and possibly xenobiotic) threats to the CNS. Indeed, IFN-γ in combination with TNF-α induces microglial expression of iNOS and several key subunits of NADPH oxidase [126], as well as the IFN-inducible double-stranded RNA-activated kinase, PKR [127]. Of course, NADPH oxidase and iNOS are important mediators of oxidative-nitrosative stress, and PKR, through its actions on NFκB, is capable of inducing the PG- and- ROS-producing enzyme, COX-2 [128]. In fact, pretreatment with the indole hormone, melatonin, attenuated IFN-γ- and- LPS-mediated expression of COX-2 (and iNOS), and this effect was attributed to the inhibition of NFκB activation [129]. Moreover, we recently found that IFN-γ was critical for the induction of oxidative (iNOS, NADPH oxidase subunits) and

inflammatory (COX-2, NFκB) factors following paraquat treatment in a mouse model of PD [44]. Importantly, many of these factors were localized to microglia and their downregulation in the absence of IFN-γ was associated with marked neuroprotection against paraquat [44]. Accordingly, IFN-γ may impact neuronal survival by way of its downstream effects on key microglial enzymes implicated in the elaboration of deleterious inflammatory factors (i.e., NO, ROS, prostanoids).

Likewise, the proinflammatory interleukins, IL-7, IL-15, IL-12, IL1-α, and IL-1β, are subject to upregulation by IFN-γ at the gene level in microglia (either directly or indirectly; e.g., IFN-γ upregulates caspase-1, which in turn activates IL-1β) [120, 130], suggesting that type II IFN may be an early mover of proinflammatory cytokine cascades. Further, some of these cytokines (including IFN-γ itself) can skew CD4+ T cell development towards a Th1/proinflammatory phenotype, which has, in fact, been described in PD [131]. In addition, IFN-γ stimulates TNF-α production in microglia, presumably through the sensitization of these cells to antigens (e.g., LPS) and, potentially, xenobiotic agents (e.g., pesticides) [132]. Importantly, our laboratory observed that the loss of DA neurons induced by paraquat treatment was associated with enhanced IL-1β and TNF-α mRNA within the SNc [44]. Moreover, IFN-γ-deficient mice failed to show such cytokine elevations and DA neuronal degeneration in response to the pesticide [44], indicating once again that IFN-γ might be a pivotal mediator of toxin-induced inflammatory and degenerative pathology.

IFN-γ signaling may also drive the downregulation of several ostensibly neuroprotective species in microglial cells, which could increase neuronal vulnerability to oxidative and inflammatory damage. For instance, IFN-γ dampened microglial expression of the antiinflammatory cytokine, IL-10 [120], as well as the soluble trophic factor, insulin-like growth factor (IGF)-1 [119], both of which have been shown to exert neuroprotective effects in toxin-based animal models of PD [133–135]. Similarly, Moran and colleagues [130] reported that the expression levels of osteopontin, a secretory phosphoprotein with antiapoptotic properties that can attenuate the neurodegenerative consequences of stroke [136] and MPTP (at least in common marmosets) [137], were suppressed in IFN-γ-activated microglia [130]. It ought to be mentioned that genetic ablation of osteopontin actually mitigated the SNc neuronal loss and striatal DA denervation following MPTP intoxication in mice, suggesting that osteopontin may, in fact, contribute to DA neurodegeneration [138]. Yet, IFN-γ activity was not directly assessed in this study (although the MPTP-treated wild-type mice displayed osteopontin-positive reactive microglia [138]), and interspecies variability in MPTP sensitivity could conceivably account for the discrepancy between the studies. In essence, IFN-γ may contribute to the neurodegenerative response in PD and its toxin-based animal models by mediating not only the activation of critical immune effector mechanisms, but also the suppression of microglial processes more closely aligned with antiinflammation and immune resolution.

In addition to microglia, recent evidence suggests that astrocytes may mediate some of the central immunomodulatory actions of IFN-γ, potentially through STAT1-independent signal transduction pathways. For instance, Hashioka and colleagues [139] found that IFN-γ (but not LPS, TNF-α, or IL-1β) caused astroctyes to become neurotoxic in vitro, reducing the viability of cultured neuroblastoma cells. Moreover, inhibition of STAT3 reduced the neurotoxic potential of these IFN-γ-activated astrocytic cells [140]. In contrast, several other reports indicated that IFN-γ signaling in astrocytes mediates primarily neuroprotective events. Indeed, IFN-γ-induced activation of astrocytes attenuated hippocampal neuronal damage after status epilepticus (SE) in rats, while neutralization of astrocytic IFN-γ receptors aggravated SE-induced neuronal pathology [141]. Likewise, combined IFN-γ and LPS treatment reduced apoptosis of hippocampal neurons induced by in vitro application of beta-amyloid protein, but only in the presence of astrocytes [142]. In fact, Ramírez et al. [142] provided evidence linking this antiapoptotic effect to the upregulated secretion of the antiinflammatory cytokine, transforming growth factor (TGF)-β, from IFN-γ- and LPS-activated astrocytes. Thus, while there is much still to be elucidated regarding the complex nature of brain IFN signaling in health and disease (e.g., cellular targets, effector molecules), a large body of evidence suggests a potentially central role for this cytokine group, particularly IFN-γ, in mediating aspects of the inflammatory repertoire and neurodegenerative process of PD.

4.1.2. Interleukins and Tumor Necrosis Factor-α in PD. The cysteine protease, interleukin-converting enzyme (caspase-1), cleaves the 31–33 kDa precursor, proIL-1, to form the mature and biologically active IL-1α and IL-1β cytokines [143]. Some of the synthesized IL-1 is secreted in a soluble form, but a proportion is retained within the cell membrane [144]. Both the soluble and membrane-bound forms of IL-1 are biologically active, particularly with respect to lymphocyte activation [144]. IL-1 signaling is dependent upon its type I receptor and the IL-1 receptor accessory protein, which are located on adjacent portions of the membrane [145]. Much like IL-1β, TNF-α is a pleiotropic cytokine, which exerts a wide array of actions on numerous cell types. For instance, it has physiological actions on bone osteoclasts (important for rheumatoid arthritis), mononuclear and polymorphonuclear blood cells, fibroblasts, skin keratinocytes, insulin sensitive adipocytes, as well as brain neurons and glial cells [146]. Like other cytokines, TNF-α typically acts locally at the site of generation; however, small amounts of the cytokine are found circulating in the bloodstream.

As in the case of IFN-γ, mounting evidence suggests a role for ILs and TNF-α in PD. Specifically, postmortem analyses of PD brain tissue revealed increased expression of TNF-α and its related Fas receptor, as well as the cytokines IFN-γ, IL-1β and IL-6 [15]. Likewise, in animals, MPTP induced alterations of proinflammatory cytokine genes, including those encoding IL-1β and TNF-α [147, 148]; and the DA neurotoxin, 6-OHDA, increased levels

of these cytokines within the SNc and striatum [149]. Indeed, an increasing number of studies are beginning to assess the impact of cytokine manipulations on PD-like pathology. In this regard, both systemic and central administration of IL-1β was reported to affect SNc DA neuronal survival. Indeed, pharmacological inhibition of IL-1β attenuated the loss of DA neurons provoked by intra-SNc infusion of LPS together with 6-OHDA injection [150]. Moreover, direct application of IL-1β augmented the neurodestructive effects of 6-OHDA upon cultured midbrain neurons [151]. Somewhat surprisingly, chronic adenoviral induced expression of IL-1β in the striatum also induced a loss of SNc DA neurons [152], suggesting that the cytokine can exert damaging effects upon DA terminals that result in the retrograde destruction of upstream soma. Importantly, the IL-1β induced loss of neurons was associated with motor impairment and an enhanced microglial response; and antiinflammatory treatment prevented these effects [152]. Yet, other older studies reported that central infusion of IL-1β protected DA neurons from 6-OHDA and MPTP toxicity and induced dendritic branching from residual neurons following SNc lesion [153, 154]. The discrepancies between the studies remain to be explained but likely stem from dose and timing considerations, since, as already mentioned, some cytokines might have both protective and deleterious effects depending on their concentration and the state of the microenvironment in which they act.

Involvement of TNF-α in PD, like IL-1β, is somewhat controversial, with two conflicting reports indicating that TNF-α deletion either protected striatal terminals and normalized DA levels in MPTP-treated mice [155, 156] or increased DA metabolism, without necessarily affecting neuronal survival [157]. Interestingly, in one study there was no effect of intra-SNc infusion of TNF-α or IL-1β either alone or together upon neuronal survival [84], but the source for this outcome is uncertain. More recently, adenoviral vector mediated long-term expression of TNF-α within the SNc was reported to provoke a progressive loss of DA neurons over 28 days that was associated with irreversible akinesia [158]. Likewise, overexpression of a dominant negative TNF-α protein (inhibits endogenous TNF-α) in the SNc ameliorated the loss of DA neurons and motor impairment induced by 6-OHDA treatment [159].

The cytokines IL-1β and TNF-α typically influence central processes through NFκB, a transcription factor that plays a critical role in the regulation of innate and adaptive immune reactions, including the mobilization of inflammatory chemokines and lymphocyte proliferative responses following infection or traumatic injury [160, 161]. Indeed, NFκB signaling occurs ubiquitously throughout the brain, and IL-1β infusion into the lateral ventricles induced the translocation of NFκB to the nucleus at several brain regions distal to the site of infusion, including the choroid plexus, ependymal cells, cerebral vasculature and meninges [162].

NFκB is composed of five subunits, together with a nuclear localization signal, which are normally held in an inactive state by an endogenous inhibitory factor, IκB. However, exposure to inflammatory stimuli triggers the phosphorylation and consequent degradation of IκB, resulting in the translocation of NFκB to the nucleus where it promotes gene expression [160]. Immunological insults may initiate this NFκB cascade through the provocation of cytokines, particularly IL-1β and TNF-α, which, after binding to their cell surface receptors, stimulate kinases that target IκB for ubiquitination and subsequent proteasomal degradation [160]. As well, these cytokines may also affect CNS processes by stimulating NFκB signaling cascades.

NFκB appears to have potent effects upon CNS processes important for neuronal survival and plasticity. The transcription factor may have a neuroprotective role through the induction of antiapoptotic proteins, such as Bcl-2 and the antioxidant enzyme, manganese superoxide dismutase (MnSOD) [163]. Yet, NFκB signaling may also result in the synthesis or upregulation of inflammatory cytokines and enzymes, ROS, and excitotoxins that can contribute to neurodegeneration. For instance, iNOS expression within microglia and astrocytes is readily provoked by NFκB activation following exposure to cytokines, such as IL-1β or IL-12 [164, 165]. Similarly, stressor exposure may contribute to neurological pathology by affecting NFκB-mediated production of oxidative radicals given that restraint stress was shown to promote neuronal excitotoxicity in rats that was associated with enhanced TNF-α release and NFκB mediated activation of iNOS and COX-2 [166]. Ultimately, a host of factors, including the chronicity and type of inducing stimulus, likely influence whether NFκB activation has protective or detrimental effects upon neuronal survival or functioning.

4.2. Cyclooxygenase-2 in PD. Cyclooxygenase, present in the CNS as COX-1, COX-2 and COX-3 isoforms, is an integral plasma membrane glycoprotein critically involved in the production of PGs from arachidonic acid (AA). The first step in PG biosynthesis involves the conversion of glycerophospholipid into free AA by phospholipase A2, which is ubiquitously present in all brain tissues and whose expression is upregulated by infection or injury [167]. Thereafter, COX metabolizes AA into PGG2 and then PGH2, which is transformed further by terminal synthases into specific PG species. AA is preferentially metabolized by COX-2 to PGE2 (the most abundant PG), whereas COX-1 produces only small amounts of this prostanoid [168]. In addition to these biologically active lipid mediators, substantial amounts of ROS are formed during the COX-mediated peroxidative reduction of PGG2 to PGH2.

Within the CNS, all three COX isozymes are expressed and heterogeneously distributed in several discrete neural populations where they mediate a diverse range of functions in health and disease. COX-1 may be generally described as a constitutive "housekeeping" enzyme, supplying PGs at (low) levels relevant for the regulation of myriad homeostatic brain processes (e.g., cerebral blood flow) [167]. Much less is known regarding the functions of COX-3 (which appears to be splice variant of COX-1); however, preliminary evidence suggests that the recently discovered COX isozyme may be important for species-specific febrile responses and the processing of painful stimuli [169, 170]. Contrastingly, under normal physiological conditions COX-2 partakes

in a diverse array of response-related activities, including synaptic plasticity and signaling, neurotransmission, memory consolidation during rapid eye movement (REM) sleep, membrane excitability, and gene expression [167, 171]. Indeed, the COX-2 gene promoter contains multiple regulatory elements (e.g., that recognize glucocorticoids, cytokines, NFκB, cAMP, and CREB) that either enhance or suppress COX-2 transcription [171, 172].

Not surprisingly, COX-2 is subject to induction by a variety of inflammatory stimuli, many of which (e.g., cytokines, ROS) have been implicated in the generalized activation of microglial cells in response to and as a corollary of acute inflammation associated with infection, brain injury or neurodegeneration. For instance, administration of the bacterial endotoxin, LPS, or the DA toxin, MPTP, elicited a marked increase in microglial COX-2 expression [173, 174]. Yet, COX-2 is also present in neurons, and is similarly induced during inflammatory episodes [17].

As is the case for proinflammatory cytokines, substantial evidence indicates that COX-2 may play an important role in the neurodegenerative process of PD and its animal models. In this regard, COX-2 expression was elevated in microglial cells [175] and DA neurons [176] within the SNc of postmortem PD brain (although the latter study failed to detect increased COX-2 in microglia). Likewise, MPTP-intoxicated mice displayed augmented COX-2 immunoreactivity within both SNc neurons [176] and microglia [174], and pharmacologic inhibition or genetic ablation of COX-2 prevented the loss of DA neurons following exposure to MPTP or 6-OHDA [177, 178]. Similarly, Yang and colleagues [179] recently demonstrated the crucial role of COX-2 in paraquat-induced neurotoxicity in vitro, and our group found that mice genetically lacking COX-2 were resistant to the PD-like neurological (nigrostriatal DA transmission) and behavioural (bradykinesia) effects of the pesticide [180].

Interestingly, several epidemiological reports indicated that NSAIDs, which act primarily to inhibit COX-2 (but also scavenge ROS and RNS [181, 182]), might either prevent or delay PD onset [183–185]. Yet, numerous other contemporaneous studies have failed to find compelling evidence of a protective role of such drugs in PD [186–188]. Although consensus remains elusive, a recent meta-analysis evaluating the impact of NSAIDs on PD risk revealed that regular, long-term use of nonaspirin NSAIDs (but not aspirin or acetamenophen) reduced PD incidence by roughly 15% [189].

Inflammatory PG signaling, which is mediated in large part by PGE2, constitutes a primary mechanism by which COX-2 might come to influence neuronal functioning and survival in neurological illness. For instance, PGE2 signaling through the EP1 receptor provoked cAMP-dependent apoptosis of hippocampal neurons [190], and pharmacological blockade of EP1 receptors completely prevented DA neuron loss following 6-OHDA treatment in embryonic rat mesencephalic primary neuronal cultures [191]. Emerging evidence indicates that PGE2 may act in an autocrine or paracrine manner to augment the COX-2-dependent microglial (and possibly neuronal) production of further prostanoid species [192, 193]. Indeed, PGE2 is capable of promoting the inherent transcriptional activities of NFκB [194], which can then exert trans-activational control over the COX-2 gene promoter [195]. In this way, PG signaling between neural cells might serve in the recruitment of otherwise quiescent microglia and augment the synthesis and release of inflammatory mediators, including further PG species, from heretofore activated microglial cells [174, 196]. This, in turn, would of course be expected to exacerbate ongoing DA neurodegeneration.

It ought to be underscored, however, that despite the evidence seemingly linking microglial COX-2 to DA neuronal death, there remains considerable controversy surrounding the relative contribution (and functional relevance) of microglial versus neuronal COX-2 in PD. Indeed, several reports indicated that a JNK-mediated induction of COX-2 in neurons but not microglia is critical for DA neuron cytotoxicity following MPTP treatment [197, 198]. Moreover, Teismann and colleagues [176] provided compelling evidence favoring the importance of cell-autonomous oxidative processes (i.e., COX-2-derived ROS, DA-quinone formation) over PG-mediated inflammatory ones in COX-2-dependent neurodegeneration. Similarly, increased neuronal COX-2 activity has been implicated in paraquat-induced neurotoxicity [179]; and, while the regulatory mechanisms subserving neuronal COX-2 induction by paraquat have yet to be defined, there is reason to believe that JNK pathway activation may be critical, given the importance of JNK in mediating the ROS-dependent in vitro and in vivo neurodegenerative effects of paraquat upon DA neurons [199, 200]. In contrast, the findings of a recent study in monkeys suggested that neuronal COX-2 expression was not associated with increased susceptibility to MPTP-induced neurodegeneration [201]; and Boyd et al. [202] observed robust strain-specific differences in neuronal COX-2 responses to MPTP in mice. In short, while the cellular and molecular determinants of brain COX-2 expression in neurological illness remain somewhat controversial, it is relatively certain that inflammatory and/or oxidative responses mediated by inducible COX-2 activity contribute to the neurodegenerative process of PD and its animal models.

It is also worth noting that COX-2, in addition to mediating potentially deleterious proinflammatory and prooxidative responses, appears to promote inflammatory immune resolution both in the CNS and the periphery. Consistent with this notion, even as PGE2 signaling through EP1 receptors is implicated in COX-2-mediated neurotoxic events, EP2/EP4 receptor signaling often mediates prosurvival responses. For instance, stimulation of EP2 and/or EP4 receptors prevented neuronal death following excitotoxic/ischemic insult [203, 204] and antagonized the neurotoxic effects of beta-amyloid [205], 6-OHDA [206] and LPS [207]. More generally, multiple COX-2-derived prostanoids seem able to promote central immunosuppressive/antiinflammatory responses by directing a reduction in proinflammatory factors (e.g., TNF-α, NO) or an increase in antiinflammatory ones (e.g., IL-10, BDNF) [208, 209]. Further, other nonprostanoid COX (and lipoxygenase) derived lipid mediators, particularly the recently discovered

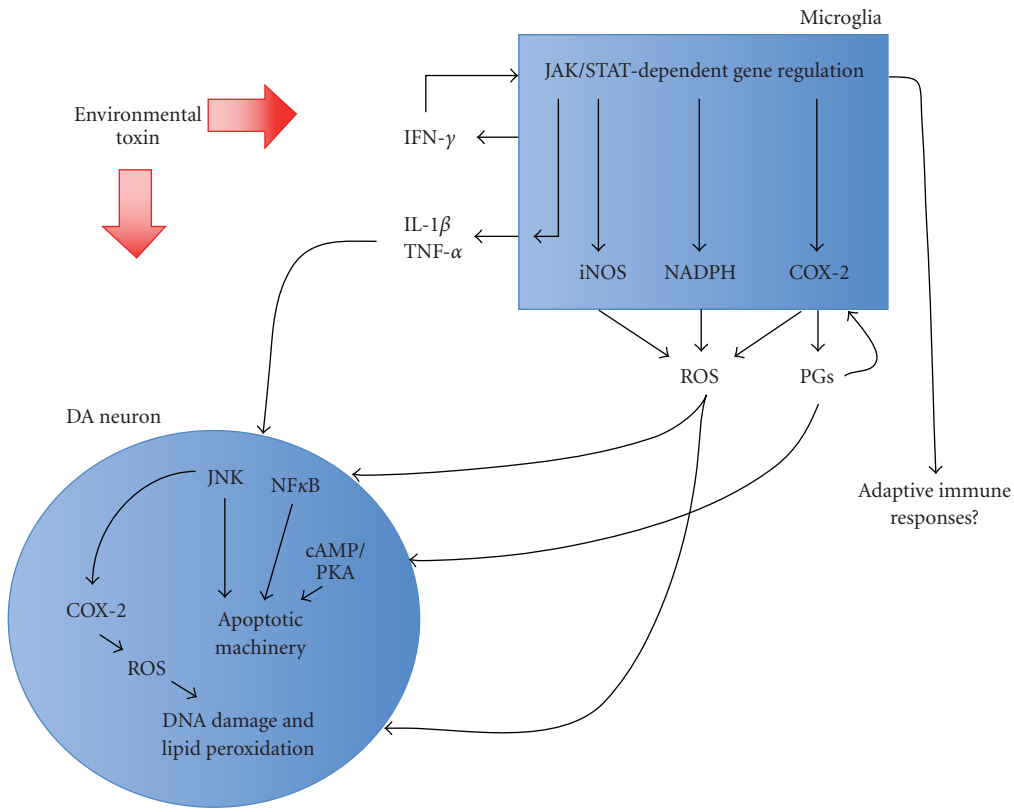

Figure 1: Conceptual overview of how environmental toxins may provoke DA neurodegeneration in Parkinson's disease and its animal models. Chronically activated microglia are integral mediators of pathology, synthesizing and secreting a plethora of prooxidant and proinflammatory factors, several of which (e.g., IFN-γ, PGs) may form positive feedback loops to stimulate the production of further inflammatory/oxidative factors (e.g., ROS, PGs) by microglial cells. Several mutually nonexclusive mechanisms exist whereby toxin-induced microglial release of prooxidant/inflammatory agents may lead to DA neurodegeneration; these include lipid peroxidation, DNA damage and the activation of intracellular apoptotic pathways. Additionally, there is evidence to suggest that both adaptive immune responses (e.g., T cell-dependent) and cell-autonomous oxidative processes (e.g., DA-quinone formation) may contribute to DA neuronal loss in PD.

DHA-derived docosanoids (resolvins and neuroprotectins), appear to antagonize the inflammatory actions of COX-2 and curb proinflammatory CNS responses more generally (e.g., inhibit NFκB, up/downregulate anti/proapoptotic proteins, suppress cytokine synthesis, modulate leukocyte trafficking) [210, 211]. It is conceivable that variations in COX-2 signaling that favor EP2/EP4 receptor involvement, and hence DA neuronal survival, might occur in conjunction with certain cytokine profiles and inflammatory responses following DA neuron injury. It might even be the case that the nature of the inflammatory response (involving COX-2 and cytokines) might vary over time following insult, such that there may be a waxing and waning of neuroprotective versus neurotoxic mechanisms engaged.

5. Conclusions and Future Directions

The findings discussed in this paper provide support for a role of proinflammatory factors, particularly cytokines (IFN-γ, IL-1β, TNF-α) and inducible enzymes (COX-2), as well as their associated inflammatory signaling pathways (e.g., JAK-STAT, NFκB, and MAP kinases), in the prodeath processes operating in PD (see Figure 1); and hence, support the contention that antiinflammatory treatments might have general clinical utility for PD and other neurodegenerative conditions. Given the complexities of the inflammatory response, future efforts would be wise to focus on developing more selective immune modulatory agents that target specific cytokines (and other inflammatory mediators) at certain stages of PD. This is not to say that such antiinflammatory agents should replace conventional treatments, rather these novel drugs might be useful as adjuncts or "add-ons" to existing therapies. Indeed, owing to the multifaceted nature of and likely multiple mechanisms involved in neurological illnesses such as PD, a multipronged drug approach seems reasonable.

While it seems undeniable that the inflammatory immune response plays a crucial role in dopaminergic loss and the clinical symptoms observed in PD, it remains to be determined whether neuroinflammation is most relevant to disease process genesis or, rather, is secondarily induced following neuronal injury and thus more critically aligned with shaping the evolution of pathology over time. The emerging picture does suggest, however, that one important mechanism underlying DA neuronal loss in toxin-based animal models of PD involves the (chronic) activation

of microglial cells, which through the actions of proinflammatory cytokines and inducible enzymes, mediates the production of damaging oxidative radicals and soluble inflammatory mediators.

While excessive microglial and proinflammatory cytokine driven inflammation can mediate profoundly deleterious CNS consequences, including DA neurodegeneration in PD and its toxin-based animal models, it should be underscored that many aspects of immune surveillance and glial activity are essential for brain health. Indeed, routine trafficking of T lymphocytes into the CSF and microglia-neuron and microglia-astrocyte interactions are critical for protecting the CNS from invading pathogens, regulating extracellular fluid composition, removing potentially harmful cellular debris, and promoting adaptive neuroplastic responses to CNS challenge. Hence, a delicate balance exists between the positive and negative aspects of immune-inflammatory signaling in the CNS. Problems likely arise when environmental toxins or other external challenges overwhelm and usurp the natural plasticity of neuroinflammatory responses to promote an abnormal, hyperactive microglial state that encourages overzealous oxidative/inflammatory factor release.

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References

[1] T. Klockgether, "Parkinson's disease: clinical aspects," *Cell and Tissue Research*, vol. 318, no. 1, pp. 115–120, 2004.

[2] M. Menza, R. D. Dobkin, and H. Marin, "Treatment of depression in parkinson's disease," *Current Psychiatry Reports*, vol. 8, no. 3, pp. 234–240, 2006.

[3] R. M. Marié, L. Barré, B. Dupuy, F. Viader, G. Defer, and J. C. Baron, "Relationships between striatal dopamine denervation and frontal executive tests in Parkinson's disease," *Neuroscience Letters*, vol. 260, no. 2, pp. 77–80, 1999.

[4] E. C. Wolters and H. Braak, "Parkinson's disease: premotor clinico-pathological correlations," *Journal of Neural Transmission, Supplement*, no. 70, pp. 309–319, 2006.

[5] V. S. Kostić, F. Agosta, I. Petrović et al., "Regional patterns of brain tissue loss associated with depression in Parkinson disease," *Neurology*, vol. 75, no. 10, pp. 857–863, 2010.

[6] F. D. Dick, G. de Palma, A. Ahmadi et al., "Environmental risk factors for Parkinson's disease and parkinsonism: the Geoparkinson study," *Occupational and Environmental Medicine*, vol. 64, no. 10, pp. 666–672, 2007.

[7] M. G. Weisskopf, J. Weuve, H. Nie et al., "Association of cumulative lead exposure with Parkinson's disease," *Environmental Health Perspectives*, vol. 118, no. 11, pp. 1609–1613, 2010.

[8] A. L. McCormack, M. Thiruchelvam, A. B. Manning-Bog et al., "Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat," *Neurobiology of Disease*, vol. 10, no. 2, pp. 119–127, 2002.

[9] J. R. Cannon, V. Tapias, H. M. Na, A. S. Honick, R. E. Drolet, and J. T. Greenamyre, "A highly reproducible rotenone model of Parkinson's disease," *Neurobiology of Disease*, vol. 34, no. 2, pp. 279–290, 2009.

[10] L. M. Bolin, I. Strycharska-Orczyk, R. Murray, J. W. Langston, and D. Di Monte, "Increased vulnerability of dopaminergic neurons in MPTP-lesioned interleukin-6 deficient mice," *Journal of Neurochemistry*, vol. 83, no. 1, pp. 167–175, 2002.

[11] K. Sriram, J. M. Matheson, S. A. Benkovic, D. B. Miller, M. I. Luster, and J. P. O'Callaghan, "Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: role of TNF-α," *FASEB Journal*, vol. 20, no. 6, pp. 670–682, 2006.

[12] M. G. Purisai, A. L. McCormack, S. Cumine, J. Li, M. Z. Isla, and D. A. Di Monte, "Microglial activation as a priming event leading to paraquat-induced dopaminergic cell degeneration," *Neurobiology of Disease*, vol. 25, no. 2, pp. 392–400, 2007.

[13] V. Sanchez-Guajardo, F. Febbraro, D. Kirik, and M. Romero-Ramos, "Microglia acquire distinct activation profiles depending on the degree of α-synuclein neuropathology in a rAAV based model of Parkinson's disease," *PLoS ONE*, vol. 5, no. 1, article e8784, 2010.

[14] P. L. McGeer, S. Itagaki, B. E. Boyes, and E. G. McGeer, "Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains," *Neurology*, vol. 38, no. 8, pp. 1285–1291, 1988.

[15] T. Nagatsu, M. Mogi, H. Ichinose, and A. Togari, "Changes in cytokines and neurotrophins in Parkinson's disease," *Journal of Neural Transmission, Supplement*, no. 60, pp. 277–290, 2000.

[16] S. Hunot, F. Boissière, B. Faucheux et al., "Nitric oxide synthase and neuronal vulnerability in Parkinson's disease," *Neuroscience*, vol. 72, no. 2, pp. 355–363, 1996.

[17] E. C. Hirsch and S. Hunot, "Neuroinflammation in Parkinson's disease: a target for neuroprotection?" *Lancet Neurology*, vol. 8, no. 4, pp. 382–397, 2009.

[18] D. Davalos, J. Grutzendler, G. Yang et al., "ATP mediates rapid microglial response to local brain injury in vivo," *Nature Neuroscience*, vol. 8, no. 6, pp. 752–758, 2005.

[19] A. Nimmerjahn, F. Kirchhoff, and F. Helmchen, "Neuroscience: resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo," *Science*, vol. 308, no. 5726, pp. 1314–1318, 2005.

[20] M. L. Cotrina, J. H. C. Lin, J. C. López-García, C. C. G. Naus, and M. Nedergaard, "ATP-mediated glia signaling," *Journal of Neuroscience*, vol. 20, no. 8, pp. 2835–2844, 2000.

[21] P. G. Popovich and E. E. Longbrake, "Can the immune system be harnessed to repair the CNS?" *Nature Reviews Neuroscience*, vol. 9, no. 6, pp. 481–493, 2008.

[22] A. Michelucci, T. Heurtaux, L. Grandbarbe, E. Morga, and P. Heuschling, "Characterization of the microglial phenotype under specific pro-inflammatory and anti-inflammatory conditions: effects of oligomeric and fibrillar amyloid-β," *Journal of Neuroimmunology*, vol. 210, no. 1-2, pp. 3–12, 2009.

[23] Z. C. Baquet, P. C. Bickford, and K. R. Jones, "Brain-derived neurotrophic factor is required for the establishment of the proper number of dopaminergic neurons in the substantia nigra pars compacta," *Journal of Neuroscience*, vol. 25, no. 26, pp. 6251–6259, 2005.

[24] A. L. Peterson and J. G. Nutt, "Treatment of Parkinson's disease with trophic factors," *Neurotherapeutics*, vol. 5, no. 2, pp. 270–280, 2008.

[25] H. M. Gao and J. S. Hong, "Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression," *Trends in Immunology*, vol. 29, no. 8, pp. 357–365, 2008.

[26] J. Anrather, G. Racchumi, and C. Iadecola, "NF-κB regulates phagocytic NADPH oxidase by inducing the expression of gp91phox," *Journal of Biological Chemistry*, vol. 281, no. 9, pp. 5657–5667, 2006.

[27] H. M. Gao, B. Liu, W. Zhang, and J. S. Hong, "Critical role of microglial NADPH oxidase-derived free radicals in the in vitro MPTP model of Parkinson's disease," *FASEB Journal*, vol. 17, no. 13, pp. 1954–1956, 2003.

[28] D. C. Wu, P. Teismann, K. Tieu et al., "NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 10, pp. 6145–6150, 2003.

[29] R. Vilar, H. Coelho, E. Rodrigues et al., "Association of A313 G polymorphism (GSTP1*B) in the glutathione-S-transferase P1 gene with sporadic Parkinson's disease," *European Journal of Neurology*, vol. 14, no. 2, pp. 156–161, 2007.

[30] M. Singh, A. J. Khan, P. P. Shah, R. Shukla, V. K. Khanna, and D. Parmar, "Polymorphism in environment responsive genes and association with Parkinson disease," *Molecular and Cellular Biochemistry*, vol. 312, no. 1-2, pp. 131–138, 2008.

[31] D. B. Hancock, E. R. Martin, G. M. Mayhew et al., "Pesticide exposure and risk of Parkinson's disease: a family-based case-control study," *BMC Neurology*, vol. 8, article 6, 2008.

[32] V. Bonifati, "LRRK2 low-penetrance mutations (Gly2019Ser) and risk alleles (Gly2385Arg)—linking familial and sporadic Parkinson's disease," *Neurochemical Research*, vol. 32, no. 10, pp. 1700–1708, 2007.

[33] J. A. Firestone, T. Smith-Weller, G. Franklin, P. Swanson, W. T. Longstreth Jr., and H. Checkoway, "Pesticides and risk of Parkinson disease: a population-based case-control study," *Archives of Neurology*, vol. 62, no. 1, pp. 91–95, 2005.

[34] S. Costello, M. Cockburn, J. Bronstein, X. Zhang, and B. Ritz, "Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California," *American Journal of Epidemiology*, vol. 169, no. 8, pp. 919–926, 2009.

[35] I. Baldi, A. Cantagrel, P. Lebailly et al., "Association between Parkinson's disease and exposure to pesticides in southwestern France," *Neuroepidemiology*, vol. 22, no. 5, pp. 305–310, 2003.

[36] A. S. Dhillon, G. L. Tarbutton, J. L. Levin et al., "Pesticide/environmental exposures and Parkinson's disease in East Texas," *Journal of Agromedicine*, vol. 13, no. 1, pp. 37–48, 2008.

[37] H. H. Liou, M. C. Tsai, C. J. Chen et al., "Environmental risk factors and Parkinson's disease: a case-control study in Taiwan," *Neurology*, vol. 48, no. 6, pp. 1583–1588, 1997.

[38] X. Li, J. Yin, C. M. Cheng, J. L. Sun, Z. Li, and Y. L. Wu, "Paraquat induces selective dopaminergic nigrostriatal degeneration in aging C57BL/6 mice," *Chinese Medical Journal*, vol. 118, no. 16, pp. 1357–1361, 2005.

[39] A. I. Brooks, C. A. Chadwick, H. A. Gelbard, D. A. Cory-Slechta, and H. J. Federoff, "Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss," *Brain Research*, vol. 823, no. 1-2, pp. 1–10, 1999.

[40] T. Chanyachukul, K. Yoovathaworn, W. Thongsaard, S. Chongthammakun, P. Navasumrit, and J. Satayavivad, "Attenuation of paraquat-induced motor behavior and neurochemical disturbances by L-valine in vivo," *Toxicology Letters*, vol. 150, no. 3, pp. 259–269, 2004.

[41] P. Chen, Z. Chen, A. Li et al., "Catalytic metalloporphyrin protects against paraquat neurotoxicity in vivo," *Biomedical and Environmental Sciences*, vol. 21, no. 3, pp. 233–238, 2008.

[42] D. Litteljohn, E. Mangano, N. Shukla, and S. Hayley, "Interferon-γ deficiency modifies the motor and co-morbid behavioral pathology and neurochemical changes provoked by the pesticide paraquat," *Neuroscience*, vol. 164, no. 4, pp. 1894–1906, 2009.

[43] Q. Chen, Y. Niu, R. Zhang et al., "The toxic influence of paraquat on hippocampus of mice: involvement of oxidative stress," *NeuroToxicology*, vol. 31, no. 3, pp. 310–316, 2010.

[44] E. N. Mangano, D. Litteljohn, R. So et al., "Interferon-gamma plays a crucial role in paraquat-induced neurodegeneration involving oxidative and pro-inflammatory pathways," *Neurobiology of Aging*. In press.

[45] M. Thiruchelvam, A. McCormack, E. K. Richfield et al., "Age-related irreversible progressive nigrostriatal dopaminergic neurotoxicity in the paraquat and maneb model of the Parkinson's disease phenotype," *European Journal of Neuroscience*, vol. 18, no. 3, pp. 589–600, 2003.

[46] A. I. Rojo, C. Cavada, M. R. de Sagarra, and A. Cuadrado, "Chronic inhalation of rotenone or paraquat does not induce Parkinson's disease symptoms in mice or rats," *Experimental Neurology*, vol. 208, no. 1, pp. 120–126, 2007.

[47] K. Prasad, E. Tarasewicz, J. Mathew et al., "Toxicokinetics and toxicodynamics of paraquat accumulation in mouse brain," *Experimental Neurology*, vol. 215, no. 2, pp. 358–367, 2009.

[48] M. J. Kang, S. J. Gil, J. E. Lee, and H. C. Koh, "Selective vulnerability of the striatal subregions of C57BL/6 mice to paraquat," *Toxicology Letters*, vol. 195, no. 2-3, pp. 127–134, 2010.

[49] G. E. Meredith, P. K. Sonsalla, and M. F. Chesselet, "Animal models of Parkinson's disease progression," *Acta Neuropathologica*, vol. 115, no. 4, pp. 385–398, 2008.

[50] F. Cicchetti, J. Drouin-Ouellet, and R. E. Gross, "Environmental toxins and Parkinson's disease: what have we learned from pesticide-induced animal models?" *Trends in Pharmacological Sciences*, vol. 30, no. 9, pp. 475–483, 2009.

[51] J. T. Greenamyre, J. R. Cannon, R. Drolet, and P. G. Mastroberardino, "Lessons from the rotenone model of Parkinson's disease," *Trends in Pharmacological Sciences*, vol. 31, no. 4, pp. 141–142, 2010.

[52] V. N. Uversky, J. Li, and A. L. Fink, "Pesticides directly accelerate the rate of α-synuclein fibril formation: a possible factor in Parkinson's disease," *FEBS Letters*, vol. 500, no. 3, pp. 105–108, 2001.

[53] A. B. Manning-Bog, A. L. McCormack, J. Li, V. N. Uversky, A. L. Fink, and D. A. Di Monte, "The herbicide paraquat causes up-regulation and aggregation of α-synuclein in mice: paraquat and α-synuclein," *Journal of Biological Chemistry*, vol. 277, no. 3, pp. 1641–1644, 2002.

[54] R. E. Drolet, J. R. Cannon, L. Montero, and J. T. Greenamyre, "Chronic rotenone exposure reproduces Parkinson's disease gastrointestinal neuropathology," *Neurobiology of Disease*, vol. 36, no. 1, pp. 96–102, 2009.

[55] V. N. Uversky, J. Li, K. Bower, and A. L. Fink, "Synergistic effects of pesticides and metals on the fibrillation of α-synuclein: implications for Parkinson's disease," *NeuroToxicology*, vol. 23, no. 4-5, pp. 527–536, 2002.

[56] J. Peng, L. Peng, F. F. Stevenson, S. R. Doctrow, and J. K. Andersen, "Iron and paraquat as synergistic environmental risk factors in sporadic Parkinson's disease accelerate age-related neurodegeneration," *Journal of Neuroscience*, vol. 27, no. 26, pp. 6914–6922, 2007.

[57] J. Peng, F. F. Stevenson, M. L. Oo, and J. K. Andersen, "Iron-enhanced paraquat-mediated dopaminergic cell death due to increased oxidative stress as a consequence of microglial activation," *Free Radical Biology and Medicine*, vol. 46, no. 2, pp. 312–320, 2009.

[58] M. Thiruchelvam, E. K. Richfield, R. B. Baggs, A. W. Tank, and D. A. Cory-Slechta, "The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: Implications for Parkinson's disease," *Journal of Neuroscience*, vol. 20, no. 24, pp. 9207–9214, 2000.

[59] X. F. Wu, M. L. Block, W. Zhang et al., "The role of microglia in paraquat-induced dopaminergic neurotoxicity," *Antioxidants and Redox Signaling*, vol. 7, no. 5-6, pp. 654–661, 2005.

[60] H. Klintworth, G. Garden, and Z. Xia, "Rotenone and paraquat do not directly activate microglia or induce inflammatory cytokine release," *Neuroscience Letters*, vol. 462, no. 1, pp. 1–5, 2009.

[61] E. H. Wilson, W. Weninger, and C. A. Hunter, "Trafficking of immune cells in the central nervous system," *Journal of Clinical Investigation*, vol. 120, no. 5, pp. 1368–1379, 2010.

[62] C. K. Glass, K. Saijo, B. Winner, M. C. Marchetto, and F. H. Gage, "Mechanisms underlying inflammation in neurodegeneration," *Cell*, vol. 140, no. 6, pp. 918–934, 2010.

[63] T. Wyss-Coray and L. Mucke, "Inflammation in neurodegenerative disease—a double-edged sword," *Neuron*, vol. 35, no. 3, pp. 419–432, 2002.

[64] M. G. Tansey, M. K. McCoy, and T. C. Frank-Cannon, "Neuroinflammatory mechanisms in Parkinson's disease: potential environmental triggers, pathways, and targets for early therapeutic intervention," *Experimental Neurology*, vol. 208, no. 1, pp. 1–25, 2007.

[65] L. E. Goehler, A. Erisir, and R. P. A. Gaykema, "Neural-immune interface in the rat area postrema," *Neuroscience*, vol. 140, no. 4, pp. 1415–1434, 2006.

[66] A. Suzumura, M. Sawada, and T. Takayanagi, "Production of interleukin-12 and expression of its receptors by murine microglia," *Brain Research*, vol. 787, no. 1, pp. 139–142, 1998.

[67] E. N. Benveniste, "Cytokine actions in the central nervous system," *Cytokine and Growth Factor Reviews*, vol. 9, no. 3-4, pp. 259–275, 1998.

[68] W. A. Banks, L. Ortiz, S. R. Plotkin, and A. J. Kastin, "Human interleukin (IL) 1α, murine IL-1α and murine IL-1β are transported from blood to brain in the mouse by a shared saturable mechanism," *Journal of Pharmacology and Experimental Therapeutics*, vol. 259, no. 3, pp. 988–996, 1991.

[69] E. G. Gutierrez, W. A. Banks, and A. J. Kastin, "Murine tumor necrosis factor alpha is transported from blood to brain in the mouse," *Journal of Neuroimmunology*, vol. 47, no. 2, pp. 169–176, 1993.

[70] S. Rivest, "How circulating cytokines trigger the neural circuits that control the hypothalamic-pituitary-adrenal axis," *Psychoneuroendocrinology*, vol. 26, no. 8, pp. 761–788, 2001.

[71] B. Schobitz, J. M. H. M. Reul, and F. Holsboer, "The role of the hypothalamic-pituitary-adrenocortical system during inflammatory conditions," *Critical Reviews in Neurobiology*, vol. 8, no. 4, pp. 263–291, 1994.

[72] S. Nadeau and S. Rivest, "Effects of circulating tumor necrosis factor on the neuronal activity and expression of the genes encoding the tumor necrosis factor receptors (p55 and p75) in the rat brain: a view from the blood-brain barrier," *Neuroscience*, vol. 93, no. 4, pp. 1449–1464, 1999.

[73] V. Tancredi, G. D'Arcangelo, F. Grassi et al., "Tumor necrosis factor alters synaptic transmission in rat hippocampal slices," *Neuroscience Letters*, vol. 146, no. 2, pp. 176–178, 1992.

[74] F. Wuchert, D. Ott, S. Rafalzik, J. Roth, and R. Gerstberger, "Tumor necrosis factor-α, interleukin-1β and nitric oxide induce calcium transients in distinct populations of cells cultured from the rat area postrema," *Journal of Neuroimmunology*, vol. 206, no. 1-2, pp. 44–51, 2009.

[75] K. Kamm, W. Vanderkolk, C. Lawrence, M. Jonker, and A. T. Davis, "The effect of traumatic brain injury upon the concentration and expression of interleukin-1beta and interleukin-10 in the rat," *Journal of Trauma*, vol. 60, no. 1, pp. 152–157, 2006.

[76] W. A. Banks, "Cytokines, CVSs, and the blood-brain-barrier," in *Psychoneuroimmunology*, R. Ader, D. L. Felten, and N. Cohen, Eds., pp. 483–498, Academic Press, New York, NY, USA, 2001.

[77] F. Clausen, A. Hånell, M. Björk et al., "Neutralization of interleukin-1β modifies the inflammatory response and improves histological and cognitive outcome following traumatic brain injury in mice," *European Journal of Neuroscience*, vol. 30, no. 3, pp. 385–396, 2009.

[78] B. H. Clausen, K. L. Lambertsen, A. A. Babcock, T. H. Holm, F. Dagnaes-Hansen, and B. Finsen, "Interleukin-1 beta and tumor necrosis factor-alpha are expressed by different subsets of microglia and macrophages after ischemic stroke in mice," *Journal of Neuroinflammation*, vol. 5, article 46, 2008.

[79] Y.-Q. Shen, G. Hebert, L.-Y. Lin et al., "Interleukine-1β and interleukine-6 levels in striatum and other brain structures after MPTP treatment: influence of behavioral lateralization," *Journal of Neuroimmunology*, vol. 158, no. 1-2, pp. 14–25, 2005.

[80] V. H. Perry, C. Cunningham, and C. Holmes, "Systemic infections and inflammation affect chronic neurodegeneration," *Nature Reviews Immunology*, vol. 7, no. 2, pp. 161–167, 2007.

[81] H. M. Gao, J. S. Hong, W. Zhang, and B. Liu, "Synergistic dopaminergic neurotoxicity of the pesticide rotenone and inflammogen lipopolysaccharide: relevance to the etiology of Parkinson's disease," *Journal of Neuroscience*, vol. 23, no. 4, pp. 1228–1236, 2003.

[82] E. N. Mangano and S. Hayley, "Inflammatory priming of the substantia nigra influences the impact of later paraquat exposure: neuroimmune sensitization of neurodegeneration," *Neurobiology of Aging*, vol. 30, no. 9, pp. 1361–1378, 2009.

[83] W. G. Kim, R. P. Mohney, B. Wilson, G. H. Jeohn, B. Liu, and J. S. Hong, "Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia," *Journal of Neuroscience*, vol. 20, no. 16, pp. 6309–6316, 2000.

[84] A. Castaño, A. J. Herrera, J. Cano, and A. Machado, "The degenerative effect of a single intranigral injection of LPS on the dopaminergic system is prevented by dexamethasone, and not mimicked by rh-TNF-α IL-1β IFN-γ," *Journal of Neurochemistry*, vol. 81, no. 1, pp. 150–157, 2002.

[85] Z. Ling, Y. Zhu, C. W. Tong, J. A. Snyder, J. W. Lipton, and P. M. Carvey, "Progressive dopamine neuron loss following supra-nigral lipopolysaccharide (LPS) infusion into rats

exposed to LPS prenatally," *Experimental Neurology*, vol. 199, no. 2, pp. 499–512, 2006.

[86] B. K. Barlow, D. A. Cory-Slechta, E. K. Richfield, and M. Thiruchelvam, "The gestational environment and Parkinson's disease: evidence for neurodevelopmental origins of a neurodegenerative disorder," *Reproductive Toxicology*, vol. 23, no. 3, pp. 457–470, 2007.

[87] P. M. Carvey, Q. Chang, J. W. Lipton, and Z. Ling, "Prenatal exposure to the bacteriotoxin lipopolysaccharide leads to long-term losses of dopamine neurons in offspring: a potential, new model of Parkinson's disease," *Frontiers in Bioscience*, vol. 8, pp. s826–s837, 2003.

[88] Z. Ling, Q. A. Chang, C. W. Tong, S. E. Leurgans, J. W. Lipton, and P. M. Carvey, "Rotenone potentiates dopamine neuron loss in animals exposed to lipopolysaccharide prenatally," *Experimental Neurology*, vol. 190, no. 2, pp. 373–383, 2004.

[89] H. Hagberg, C. Mallard, and B. Jacobsson, "Role of cytokines in preterm labour and brain injury," *British Journal of Obstetrics and Gynaecology*, vol. 112, supplement 1, pp. 16–18, 2005.

[90] E. Ribiani, A. Rosati, M. Romanelli, L. Cruciani, F. Incalza, and G. C. Di Renzo, "Perinatal infections and cerebral palsy," *Minerva Ginecologica*, vol. 59, no. 2, pp. 151–157, 2007.

[91] H. Anisman, Z. Merali, and S. Hayley, "Neurotransmitter, peptide and cytokine processes in relation to depressive disorder: comorbidity between depression and neurodegenerative disorders," *Progress in Neurobiology*, vol. 85, no. 1, pp. 1–74, 2008.

[92] S. Hayley, E. Mangano, M. Strickland, and H. Anisman, "Lipopolysaccharide and a social stressor influence behaviour, corticosterone and cytokine levels: divergent actions in cyclooxygenase-2 deficient mice and wild type controls," *Journal of Neuroimmunology*, vol. 197, no. 1, pp. 29–36, 2008.

[93] V. Basic Kes, A. M. Simundic, N. Nikolac, E. Topic, and V. Demarin, "Pro-inflammatory and anti-inflammatory cytokines in acute ischemic stroke and their relation to early neurological deficit and stroke outcome," *Clinical Biochemistry*, vol. 41, no. 16-17, pp. 1330–1334, 2008.

[94] B. Brodacki, J. Staszewski, B. Toczyłowska et al., "Serum interleukin (IL-2, IL-10, IL-6, IL-4), TNFα, and INFγ concentrations are elevated in patients with atypical and idiopathic parkinsonism," *Neuroscience Letters*, vol. 441, no. 2, pp. 158–162, 2008.

[95] J. C. Goodman, M. Van, S. P. Gopinath, and C. S. Robertson, "Pro-inflammatory and pro-apoptotic elements of the neuroinflammatory response are activated in traumatic brain injury," *Acta neurochirurgica. Supplement*, vol. 102, pp. 437–439, 2008.

[96] M. Reale, C. Iarlori, A. Thomas et al., "Peripheral cytokines profile in Parkinson's disease," *Brain, Behavior, and Immunity*, vol. 23, no. 1, pp. 55–63, 2009.

[97] A. J. Bruce, W. Boling, M. S. Kindy et al., "Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors," *Nature Medicine*, vol. 2, no. 7, pp. 788–794, 1996.

[98] R. J. Rogers, J. M. Monnier, and H. S. Nick, "Tumor necrosis factor-α selectively induces MnSOD expression via mitochondria-to-nucleus signaling, whereas interleukin-1β utilizes an alternative pathway," *Journal of Biological Chemistry*, vol. 276, no. 23, pp. 20419–20427, 2001.

[99] G. B. T. von Boyen, M. Steinkamp, I. Geerling et al., "Proinflammatory cytokines induce neurotrophic factor expression in enteric glia: a key to the regulation of epithelial apoptosis in Crohn's disease," *Inflammatory Bowel Diseases*, vol. 12, no. 5, pp. 346–354, 2006.

[100] F. C. Barone, B. Arvin, R. F. White et al., "Tumor necrosis factor-α: a mediator of focal ischemic brain injury," *Stroke*, vol. 28, no. 6, pp. 1233–1244, 1997.

[101] F. C. Barone, R. F. White, P. A. Spera et al., "Ischemic preconditioning and brain tolerance: temporal histological and functional outcomes, protein synthesis requirement, and interleukin-1 receptor antagonist and early gene expression," *Stroke*, vol. 29, no. 9, pp. 1937–1950, 1998.

[102] J. Kawanokuchi, T. Mizuno, H. Takeuchi et al., "Production of interferon-γ by microglia," *Multiple Sclerosis*, vol. 12, no. 5, pp. 558–564, 2006.

[103] K. Schroder, P. J. Hertzog, T. Ravasi, and D. A. Hume, "Interferon-γ: an overview of signals, mechanisms and functions," *Journal of Leukocyte Biology*, vol. 75, no. 2, pp. 163–189, 2004.

[104] T. Decker, M. Müller, and S. Stockinger, "The Yin and Yang of type I interferon activity in bacterial infection," *Nature Reviews Immunology*, vol. 5, no. 9, pp. 675–687, 2005.

[105] M. Rayamajhi, J. Humann, K. Penheiter, K. Andreasen, and L. L. Lenz, "Induction of IFN-$\alpha\beta$ enables Listeria monocytogenes to suppress macrophage activation by IFN-γ," *Journal of Experimental Medicine*, vol. 207, no. 2, pp. 327–337, 2010.

[106] P. Sarasombath, K. Sumida, and D. A. Kaku, "Parkinsonism associated with interferon alpha therapy for chronic myelogenous leukemia," *Hawaii Medical Journal*, vol. 61, no. 3, pp. 48–57, 2002.

[107] M. Kajihara, S. Montagnese, P. Khanna et al., "Parkinsonism in patients with chronic hepatitis C treated with interferon-α2b: a report of two cases," *European Journal of Gastroenterology and Hepatology*, vol. 22, no. 5, pp. 628–631, 2010.

[108] T. Yamada, M. A. Horisberger, N. Kawaguchi, I. Moroo, and T. Toyoda, "Immunohistochemistry using antibodies to α-interferon and its induced protein, MxA, in Alzheimer's and Parkinson's disease brain tissues," *Neuroscience Letters*, vol. 181, no. 1-2, pp. 61–64, 1994.

[109] T. Yamada, "Further observations on MxA-positive Lewy bodies in Parkinson's disease brain tissues," *Neuroscience Letters*, vol. 195, no. 1, pp. 41–44, 1995.

[110] M. P. Mount, A. Lira, D. Grimes et al., "Involvement of interferon-γ in microglial-mediated loss of dopaminergic neurons," *Journal of Neuroscience*, vol. 27, no. 12, pp. 3328–3337, 2007.

[111] I. E. Gribova, B. B. Gnedenko, V. V. Poleshchuk, and S. G. Morozov, "Content of interferon-gamma, alpha tumor necrosis factor and autoantibodies to them in blood serum during Parkinson's disease," *Biomeditsinskaya Khimiya*, vol. 49, no. 2, pp. 208–212, 2003.

[112] S. Hunot, N. Dugas, B. Faucheux et al., "FcεRII/CD23 is expressed in Parkinson's disease and induces, in vitro, production of nitric oxide and tumor necrosis factor-α in glial cells," *Journal of Neuroscience*, vol. 19, no. 9, pp. 3440–3447, 1999.

[113] M. Mogi, T. Kondo, Y. Mizuno, and T. Nagatsu, "p53 protein, interferon-γ, and NF-κB levels are elevated in the parkinsonian brain," *Neuroscience Letters*, vol. 414, no. 1, pp. 94–97, 2007.

[114] I. Mizuta, M. Nishimura, E. Mizuta et al., "Relation between the high production related allele of the interferon-γ(IFN-γ) gene and age at onset of idiopathic Parkinson's disease in Japan," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 71, no. 6, pp. 818–819, 2001.

[115] B. Widner, F. Leblhuber, and D. Fuchs, "Increased neopterin production and tryptophan degradation in advanced Parkinson's disease," *Journal of Neural Transmission, Supplement*, vol. 109, no. 2, pp. 181–189, 2002.

[116] N. Kawaguchi, T. Yamada, M. Takahashi, and T. Hattori, "Expression of MxA mRNA in peripheral blood mononuclear cells in Parkinson's disease," *Parkinsonism and Related Disorders*, vol. 5, no. 1-2, pp. 43–47, 1999.

[117] M. D'Amelio, P. Ragonese, L. Morgante et al., "Tumor diagnosis preceding Parkinson's disease: a case-control study," *Movement Disorders*, vol. 19, no. 7, pp. 807–811, 2004.

[118] T. Mizuno, G. Zhang, H. Takeuchi et al., "Interferon-γ directly induces neurotoxicity through a neuron specific, calcium-permeable complex of IFN-γ receptor and AMPA GluR1 receptor," *FASEB Journal*, vol. 22, no. 6, pp. 1797–1806, 2008.

[119] L. B. Moran, D. C. Duke, F. E. Tarkheimer, R. B. Banati, and M. B. Graeber, "Towards a transcriptome definition of microglial cells," *Neurogenetics*, vol. 5, no. 2, pp. 95–108, 2004.

[120] R. B. Rock, S. Hu, A. Deshpande et al., "Transcriptional response of human microglial cells to interferon-γ," *Genes and Immunity*, vol. 6, no. 8, pp. 712–719, 2005.

[121] I. Kurkowska-Jastrzebska, A. Wrońska, M. Kohutnicka, A. Członkowski, and A. Członkowska, "MHC class II positive microglia and lymphocytic infiltration are present in the substantia nigra and striatum in mouse model of Parkinson's disease," *Acta Neurobiologiae Experimentalis*, vol. 59, no. 1, pp. 1–8, 1999.

[122] K. Imamura, N. Hishikawa, M. Sawada, T. Nagatsu, M. Yoshida, and Y. Hashizume, "Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains," *Acta Neuropathologica*, vol. 106, no. 6, pp. 518–526, 2003.

[123] D. A. Loeffler, D. M. Camp, and S. B. Conant, "Complement activation in the Parkinson's disease substantia nigra: an immunocytochemical study," *Journal of Neuroinflammation*, vol. 3, article 29, 2006.

[124] V. Brochard, B. Combadière, A. Prigent et al., "Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease," *Journal of Clinical Investigation*, vol. 119, no. 1, pp. 182–192, 2009.

[125] K. R. Ju, H. S. Kim, J. H. Kim, N. Y. Lee, and C. K. Park, "Retinal glial cell responses and Fas/FasL activation in rats with chronic ocular hypertension," *Brain Research*, vol. 1122, no. 1, pp. 209–221, 2006.

[126] M. Mir, V. J. Asensio, L. Tolosa et al., "Tumor necrosis factor alpha and interferon gamma cooperatively induce oxidative stress and motoneuron death in rat spinal cord embryonic explants," *Neuroscience*, vol. 162, no. 4, pp. 959–971, 2009.

[127] M. Jana, X. Liu, S. Koka, S. Ghosh, T. M. Petro, and K. Pahan, "Ligation of CD40 stimulates the induction of nitric-oxide synthase in microglial cells," *Journal of Biological Chemistry*, vol. 276, no. 48, pp. 44527–44533, 2001.

[128] M. Zamanian-Daryoush, T. H. Mogensen, J. A. DiDonato, and B. R. G. Williams, "NF-κB activation by double-stranded-RNA-activated protein kinase (PKR) is mediated through NF-κB-inducing kinase and IκB kinase," *Molecular and Cellular Biology*, vol. 20, no. 4, pp. 1278–1290, 2000.

[129] E. Esposito, A. Iacono, C. Muià et al., "Signal transduction pathways involved in protective effects of melatonin in C6 glioma cells," *Journal of Pineal Research*, vol. 44, no. 1, pp. 78–87, 2008.

[130] L. B. Moran, D. C. Duke, and M. B. Graeber, "The microglial gene regulatory network activated by interferon-gamma," *Journal of Neuroimmunology*, vol. 183, no. 1-2, pp. 1–6, 2007.

[131] Y. Baba, A. Kuroiwa, R. J. Uitti, Z. K. Wszolek, and T. Yamada, "Alterations of T-lymphocyte populations in Parkinson disease," *Parkinsonism and Related Disorders*, vol. 11, no. 8, pp. 493–498, 2005.

[132] J. Durbin, L. Doughty, K. Nguyen, M. Caligiuri, J. van Deusen, and C. Biron, "The role of STAT1 in viral sensitization to LPS," *Journal of Endotoxin Research*, vol. 9, no. 5, pp. 313–316, 2003.

[133] L. Qian, M. L. Block, S. J. Wei et al., "Interleukin-10 protects lipopolysaccharide-induced neurotoxicity in primary midbrain cultures by inhibiting the function of NADPH oxidase," *Journal of Pharmacology and Experimental Therapeutics*, vol. 319, no. 1, pp. 44–52, 2006.

[134] L. C. Johnston, X. Su, K. Maguire-Zeiss et al., "Human interleukin-10 gene transfer is protective in a rat model of Parkinson's disease," *Molecular Therapy*, vol. 16, no. 8, pp. 1392–1399, 2008.

[135] A. Quesada, B. Y. Lee, and P. E. Micevych, "PI3 kinase/Akt activation mediates estrogen and IGF-1 nigral DA neuronal neuroprotection against a unilateral rat model of Parkinson's disease," *Developmental Neurobiology*, vol. 68, no. 5, pp. 632–644, 2008.

[136] R. Meller, S. L. Stevens, M. Minami et al., "Neuroprotection by osteopontin in stroke," *Journal of Cerebral Blood Flow and Metabolism*, vol. 25, no. 2, pp. 217–225, 2005.

[137] J. Iczkiewicz, M. J. Jackson, L. A. Smith, S. Rose, and P. Jenner, "Osteopontin expression in substantia nigra in MPTP-treated primates and in Parkinson's disease," *Brain Research*, vol. 1118, no. 1, pp. 239–250, 2006.

[138] W. Maetzler, D. Berg, N. Schalamberidze et al., "Osteopontin is elevated in Parkinson's disease and its absence leads to reduced neurodegeneration in the MPTP model," *Neurobiology of Disease*, vol. 25, no. 3, pp. 473–482, 2007.

[139] S. Hashioka, A. Klegeris, C. Schwab, and P. L. McGeer, "Interferon-γ-dependent cytotoxic activation of human astrocytes and astrocytoma cells," *Neurobiology of Aging*, vol. 30, no. 12, pp. 1924–1935, 2009.

[140] S. Hashioka, A. Klegeris, H. Qing, and P. L. McGeer, "STAT3 inhibitors attenuate interferon-γ-induced neurotoxicity and inflammatory molecule production by human astrocytes," *Neurobiology of Disease*. In press.

[141] H. J. Ryu, J.-E. Kim, M.-J. Kim et al., "The protective effects of interleukin-18 and interferon-γ on neuronal damages in the rat hippocampus following status epilepticus," *Neuroscience*, vol. 170, no. 3, pp. 711–721, 2010.

[142] G. Ramírez, R. Toro, H. Döbeli, and R. von Bernhardi, "Protection of rat primary hippocampal cultures from Aβ cytotoxicity by pro-inflammatory molecules is mediated by astrocytes," *Neurobiology of Disease*, vol. 19, no. 1-2, pp. 243–254, 2005.

[143] N. A. Thornberry, H. G. Bull, J. R. Calaycay et al., "A novel heterodimeric cysteine protease is required for interleukin-1β processing in monocytes," *Nature*, vol. 356, no. 6372, pp. 768–774, 1992.

[144] C. A. Dinarello, "Biology of interleukin 1," *FASEB Journal*, vol. 2, no. 2, pp. 108–115, 1988.

[145] N. J. Rothwell and S. J. Hopkins, "Cytokines and the nervous system II: actions and mechanisms of action," *Trends in Neurosciences*, vol. 18, no. 3, pp. 130–136, 1995.

[146] A. Eigler, B. Sinha, G. Hartmann, and S. Endres, "Taming TNF: strategies to restrain this proinflammatory cytokine," *Immunology Today*, vol. 18, no. 10, pp. 487–492, 1997.

[147] S. Mandel, E. Grünblatt, and M. B. H. Youdim, "cDNA microarray to study gene expression of dopaminergic neurodegeneration and neuroprotection in MPTP and 6-hydroxydopamine models: implications for idiopathic Parkinson's disease," *Journal of Neural Transmission, Supplement*, no. 60, pp. 117–124, 2000.

[148] M. J. Bian, L. M. Li, M. Yu, J. Fei, and F. Huang, "Elevated interleukin-1β induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine aggravating dopaminergic neurodegeneration in old male mice," *Brain Research*, vol. 1302, pp. 256–264, 2009.

[149] F. Jin, Q. Wu, Y. F. Lu, Q. H. Gong, and J. S. Shi, "Neuroprotective effect of resveratrol on 6-OHDA-induced Parkinson's disease in rats," *European Journal of Pharmacology*, vol. 600, no. 1–3, pp. 78–82, 2008.

[150] J. B. Koprich, C. Reske-Nielsen, P. Mithal, and O. Isacson, "Neuroinflammation mediated by IL-1β increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease," *Journal of Neuroinflammation*, vol. 5, article 8, 2008.

[151] M. C. Pott Godoy, R. Tarelli, C. C. Ferrari, M. I. Sarchi, and F. J. Pitossi, "Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease," *Brain*, vol. 131, no. 7, pp. 1880–1894, 2008.

[152] M. C. Pott Godoy, C. C. Ferrari, and F. J. Pitossi, "Nigral neurodegeneration triggered by striatal AdIL-1 administration can be exacerbated by systemic IL-1 expression," *Journal of Neuroimmunology*, vol. 222, no. 1-2, pp. 29–39, 2010.

[153] J. Wang, K. S. Bankiewicz, R. J. Plunkett, and E. H. Oldfield, "Intrastriatal implantation of interleukin-1. Reduction of parkinsonism in rats by enhancing neuronal sprouting from residual dopaminergic neurons in the ventral tegmental area of the midbrain," *Journal of Neurosurgery*, vol. 80, no. 3, pp. 484–490, 1994.

[154] J. Saura, M. Parés, J. Bové et al., "Intranigral infusion of interleukin-1β activates astrocytes and protects from subsequent 6-hydroxydopamine neurotoxicity," *Journal of Neurochemistry*, vol. 85, no. 3, pp. 651–661, 2003.

[155] K. Sriram, J. M. Matheson, S. A. Benkovic, D. B. Miller, M. I. Luster, and J. P. O'Callaghan, "Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: implications for Parkinson's disease," *FASEB Journal*, vol. 16, no. 11, pp. 1474–1476, 2002.

[156] B. Ferger, A. Leng, A. Mura, B. Hengerer, and J. Feldon, "Genetic ablation of tumor necrosis factor-alpha (TNF-α) and pharmacological inhibition of TNF-synthesis attenuates MPTP toxicity in mouse striatum," *Journal of Neurochemistry*, vol. 89, no. 4, pp. 822–833, 2004.

[157] E. Rousselet, J. Callebert, K. Parain et al., "Role of TNF-α receptors in mice intoxicated with the parkinsonian toxin MPTP," *Experimental Neurology*, vol. 177, no. 1, pp. 183–192, 2002.

[158] A. L. de Lella Ezcurra, M. Chertoff, C. Ferrari, M. Graciarena, and F. Pitossi, "Chronic expression of low levels of tumor necrosis factor-α in the substantia nigra elicits progressive neurodegeneration, delayed motor symptoms and microglia/macrophage activation," *Neurobiology of Disease*, vol. 37, no. 3, pp. 630–640, 2010.

[159] M. K. McCoy, K. A. Ruhn, T. N. Martinez, F. E. McAlpine, A. Blesch, and M. G. Tansey, "Intranigral lentiviral delivery of dominant-negative TNF attenuates neurodegeneration and behavioral deficits in hemiparkinsonian rats," *Molecular Therapy*, vol. 16, no. 9, pp. 1572–1579, 2008.

[160] P. C. Lucas, L. M. McAllister-Lucas, and G. Nunez, "NF-κB in signaling in lymphocytes: a new cast of characters," *Journal of Cell Science*, vol. 117, no. 1, pp. 31–39, 2004.

[161] M. E. Poynter, R. Cloots, T. van Woerkom et al., "NF-κB activation in airways modulates allergic inflammation but not hyperresponsiveness," *Journal of Immunology*, vol. 173, no. 11, pp. 7003–7009, 2004.

[162] J. P. Konsman, V. Tridon, and R. Dantzer, "Diffusion and action of intracerebroventricularly injected interleukin-1 in the CNS," *Neuroscience*, vol. 101, no. 4, pp. 957–967, 2000.

[163] M. P. Mattson, "NF-κB in the survival and plasticity of neurons," *Neurochemical Research*, vol. 30, no. 6-7, pp. 883–893, 2005.

[164] M. Hartlage-Rübsamen, R. Lemke, and R. Schliebs, "Interleukin-1β, inducible nitric oxide synthase, and nuclear factor- κB are induced in morphologically distinct microglia after rat hippocampal lipopolysaccharide/interferon-γ injection," *Journal of Neuroscience Research*, vol. 57, no. 3, pp. 388–398, 1999.

[165] K. Pahan, F. G. Sheikh, X. Liu, S. Hilger, M. McKinney, and T. M. Petro, "Induction of nitric-oxide synthase and activation of NF-κB by interleukin-12 p40 in microglial cells," *Journal of Biological Chemistry*, vol. 276, no. 11, pp. 7899–7905, 2001.

[166] J. L. M. Madrigal, B. García-Bueno, J. R. Caso, B. G. Pérez-Nievas, and J. C. Leza, "Stress-induced oxidative changes in brain," *CNS and Neurological Disorders—Drug Targets*, vol. 5, no. 5, pp. 561–568, 2006.

[167] L. Minghetti, "Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases," *Journal of Neuropathology and Experimental Neurology*, vol. 63, no. 9, pp. 901–910, 2004.

[168] T. G. Brock, R. W. McNish, and M. Peters-Golden, "Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E," *Journal of Biological Chemistry*, vol. 274, no. 17, pp. 11660–11666, 1999.

[169] N. V. Chandrasekharan, H. Dai, K. L.T. Roos et al., "COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 21, pp. 13926–13931, 2002.

[170] N. M. Davies, R. L. Good, K. A. Roupe, and J. A. Yáñez, "Cyclooxygenase-3: axiom, dogma, anomaly, enigma or splice error?—Not as easy as 1, 2, 3," *Journal of Pharmacy and Pharmaceutical Sciences*, vol. 7, no. 2, pp. 217–226, 2004.

[171] J. W. Phillis, L. A. Horrocks, and A. A. Farooqui, "Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: their role and involvement in neurological disorders," *Brain Research Reviews*, vol. 52, no. 2, pp. 201–243, 2006.

[172] S. B. Appleby, A. Ristimaki, K. Neilson, K. Narko, and T. Hla, "Structure of the human cyclo-oxygenase-2 gene," *Biochemical Journal*, vol. 302, no. 3, pp. 723–727, 1994.

[173] J. K. Elmquist, C. D. Breder, J. E. Sherin et al., "Intravenous lipopolysaccharide induces cyclooxygenase 2-like immunoreactivity in rat brain perivascular microglia and meningeal macrophages," *Journal of Comparative Neurology*, vol. 381, no. 2, pp. 119–128, 1997.

[174] R. Vijitruth, M. Liu, D. Y. Choi, X. V. Nguyen, R. L. Hunter, and G. Bing, "Cyclooxygenase-2 mediates microglial activation and secondary dopaminergic cell death in the mouse MPTP model of Parkinson's disease," *Journal of Neuroinflammation*, vol. 3, article 6, 2006.

[175] C. Knott, G. Stern, and G. P. Wilkin, "Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2," *Molecular and Cellular Neuroscience*, vol. 16, no. 6, pp. 724–739, 2000.

[176] P. Teismann, K. Tieu, D. K. Choi et al., "Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 9, pp. 5473–5478, 2003.

[177] Z. H. Feng, T. G. Wang, D. D. Li et al., "Cyclooxygenase-2-deficient mice are resistant to 1-methyl-4-phenyl1, 2, 3, 6-tetrahydropyridine-induced damage of dopaminergic neurons in the substantia nigra," *Neuroscience Letters*, vol. 329, no. 3, pp. 354–358, 2002.

[178] R. Sánchez-Pernaute, A. Ferree, O. Cooper, M. Yu, A. L. Brownell, and O. Isacson, "Selective COX-2 inhibition prevents progressive dopamine neuron degeneration in a rat model of Parkinson's disease," *Journal of Neuroinflammation*, vol. 1, no. 1, article 6, 2004.

[179] W. Yang, E. Tiffany-Castiglioni, M.-Y. Lee, and I.-H. Son, "Paraquat induces cyclooxygenase-2 (COX-2) implicated toxicity in human neuroblastoma SH-SY5Y cells," *Toxicology Letters*, vol. 199, no. 3, pp. 239–246, 2010.

[180] D. Litteljohn, E. N. Mangano, and S. Hayley, "Cyclooxygenase-2 deficiency modifies the neurochemical effects, motor impairment and co-morbid anxiety provoked by paraquat administration in mice," *European Journal of Neuroscience*, vol. 28, no. 4, pp. 707–716, 2008.

[181] M. Asanuma, S. Nishibayashi-Asanuma, I. Miyazaki, M. Kohno, and N. Ogawa, "Neuroprotective effects of non-steroidal anti-inflammatory drugs by direct scavenging of nitric oxide radicals," *Journal of Neurochemistry*, vol. 76, no. 6, pp. 1895–1904, 2001.

[182] D. Costa, L. Moutinho, J. L. F. C. Lima, and E. Fernandes, "Antioxidant activity and inhibition of human neutrophil oxidative burst mediated by arylpropionic acid non-steroidal anti-inflammatory drugs," *Biological and Pharmaceutical Bulletin*, vol. 29, no. 8, pp. 1659–1670, 2006.

[183] H. Chen, E. Jacobs, M. A. Schwarzschild et al., "Nonsteroidal antiinflammatory drug use and the risk for Parkinson's disease," *Annals of Neurology*, vol. 58, no. 6, pp. 963–967, 2005.

[184] M. A. Hernán, G. Logroscino, and L. A. G. Rodríguez, "Nonsteroidal anti-inflammatory drugs and the incidence of Parkinson disease," *Neurology*, vol. 66, no. 7, pp. 1097–1099, 2006.

[185] A. D. Wahner, J. M. Bronstein, Y. M. Bordelon, and B. Ritz, "Nonsteroidal anti-inflammatory drugs may protect against Parkinson disease," *Neurology*, vol. 69, no. 19, pp. 1836–1842, 2007.

[186] T. G. Ton, S. R. Heckbert, W. T. Longstreth Jr. et al., "Non-steroidal anti-inflammatory drugs and risk of Parkinson's disease," *Movement Disorders*, vol. 21, no. 7, pp. 964–969, 2006.

[187] M. Bornebroek, L. M. L. de Lau, M. D. M. Haag et al., "Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease," *Neuroepidemiology*, vol. 28, no. 4, pp. 193–196, 2007.

[188] M. Etminan, B. C. Carleton, and A. Samii, "Non-steroidal anti-inflammatory drug use and the risk of Parkinson disease: a retrospective cohort study," *Journal of Clinical Neuroscience*, vol. 15, no. 5, pp. 576–577, 2008.

[189] J. J. Gagne and M. C. Power, "Anti-inflammatory drugs and risk of Parkinson disease: a meta-analysis," *Neurology*, vol. 74, no. 12, pp. 995–1002, 2010.

[190] T. Kawano, J. Anrather, P. Zhou et al., "Prostaglandin E2 EP1 receptors: downstream effectors of COX-2 neurotoxicity," *Nature Medicine*, vol. 12, no. 2, pp. 225–229, 2006.

[191] E. Carrasco, D. Casper, and P. Werner, "PGE2 receptor EP1 renders dopaminergic neurons selectively vulnerable to low-level oxidative stress and direct PGE2 neurotoxicity," *Journal of Neuroscience Research*, vol. 85, no. 14, pp. 3109–3117, 2007.

[192] F. S. Shie, K. S. Montine, R. M. Breyer, and T. J. Montine, "Microglial EP2 is critical to neurotoxicity from activated cerebral innate immunity," *GLIA*, vol. 52, no. 1, pp. 70–77, 2005.

[193] V. Vichai, C. Suyarnsesthakorn, D. Pittayakhajonwut, K. Sriklung, and K. Kirtikara, "Positive feedback regulation of COX-2 expression by prostaglandin metabolites," *Inflammation Research*, vol. 54, no. 4, pp. 163–172, 2005.

[194] B. Poligone and A. S. Baldwin, "Positive and negative regulation of NF-κB by COX-2. Roles of different prostaglandins," *Journal of Biological Chemistry*, vol. 276, no. 42, pp. 38658–38664, 2001.

[195] A. Nadjar, V. Tridon, M. J. May et al., "NFκB activates in vivo the synthesis of inducible Cox-2 in the brain," *Journal of Cerebral Blood Flow and Metabolism*, vol. 25, no. 8, pp. 1047–1059, 2005.

[196] T. Wang, Z. Pei, W. Zhang et al., "MPP+-induced COX-2 activation and subsequent dopaminergic neurodegeneration," *FASEB Journal*, vol. 19, no. 9, pp. 1134–1136, 2005.

[197] S. Hunot, M. Vila, P. Teismann et al., "JNK-mediated induction of cyclooxygenase 2 is required for neurodegeneration in a mouse model of Parkinson's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 2, pp. 665–670, 2004.

[198] Y. Wang, Y. Zhang, Z. Wei et al., "JNK inhibitor protects dopaminergic neurons by reducing COX-2 expression in the MPTP mouse model of subacute Parkinson's disease," *Journal of the Neurological Sciences*, vol. 285, no. 1-2, pp. 172–177, 2009.

[199] J. Peng, X. O. Mao, F. F. Stevenson, M. Hsu, and J. K. Andersen, "The herbicide paraquat induces dopaminergic nigral apoptosis through sustained activation of the JNK pathway," *Journal of Biological Chemistry*, vol. 279, no. 31, pp. 32626–32632, 2004.

[200] W.-S. Choi, G. Abel, H. Klintworth, R. A. Flavell, and Z. Xia, "JNK3 mediates paraquat-and rotenone-induced dopaminergic neuron death," *Journal of Neuropathology and Experimental Neurology*, vol. 69, no. 5, pp. 511–520, 2010.

[201] M. Vázquez-Claverie, P. Garrido-Gil, W. San Sebastián et al., "1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) does not elicit long-lasting increases in cyclooxygenase-2 expression in dopaminergic neurons of monkeys," *Journal of Neuropathology and Experimental Neurology*, vol. 68, no. 1, pp. 26–36, 2009.

[202] J. D. Boyd, H. Jang, K. R. Shepherd et al., "Response to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) differs in mouse strains and reveals a divergence in JNK signaling and COX-2 induction prior to loss of neurons in the substantia nigra pars compacta," *Brain Research*, vol. 1175, no. 1, pp. 107–116, 2007.

[203] L. McCullough, L. Wu, N. Haughey et al., "Neuroprotective function of the PGE2 EP2 receptor in cerebral ischemia," *Journal of Neuroscience*, vol. 24, no. 1, pp. 257–268, 2004.

[204] A. S. Ahmad, H. Zhuang, V. Echeverria, and S. Doré, "Stimulation of prostaglandin EP2 receptors prevents NMDA-induced excitotoxicity," *Journal of Neurotrauma*, vol. 23, no. 12, pp. 1895–1903, 2006.

[205] V. Echeverria, A. Clerman, and S. Doré, "Stimulation of PGE2 receptors EP2 and EP4 protects cultured neurons against oxidative stress and cell death following β-amyloid exposure," *European Journal of Neuroscience*, vol. 22, no. 9, pp. 2199–2206, 2005.

[206] E. Carrasco, P. Werner, and D. Casper, "Prostaglandin receptor EP2 protects dopaminergic neurons against 6-OHDA-mediated low oxidative stress," *Neuroscience Letters*, vol. 441, no. 1, pp. 44–49, 2008.

[207] J. Shi, J. Johansson, N. S. Woodling, Q. Wang, T. J. Montine, and K. Andreasson, "The prostaglandin E2 E-prostanoid 4 receptor exerts anti-inflammatory effects in brain innate immunity," *Journal of Immunology*, vol. 184, no. 12, pp. 7207–7218, 2010.

[208] S. F. Tzeng, H. Y. Hsiao, and O. T. Mak, "Prostaglandins and cyclooxygenases in glial cells during brain inflammation," *Current Drug Targets: Inflammation and Allergy*, vol. 4, no. 3, pp. 335–340, 2005.

[209] A. J. Hutchinson, C. L. Chou, D. D. Israel, W. Xu, and J. W. Regan, "Activation of EP2 prostanoid receptors in human glial cell lines stimulates the secretion of BDNF," *Neurochemistry International*, vol. 54, no. 7, pp. 439–446, 2009.

[210] N. G. Bazan, V. L. Marcheselli, and K. Cole-Edwards, "Brain response to injury and neurodegeneration: endogenous neuroprotective signaling," *Annals of the New York Academy of Sciences*, vol. 1053, pp. 137–147, 2005.

[211] A. A. Farooqui, L. A. Horrocks, and T. Farooqui, "Modulation of inflammation in brain: a matter of fat," *Journal of Neurochemistry*, vol. 101, no. 3, pp. 577–599, 2007.

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Review Article

Lipid-Mediated Oxidative Stress and Inflammation in the Pathogenesis of Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative movement disorder of unknown etiology. PD is characterized by the progressive loss of dopaminergic neurons in the substantia nigra, depletion of dopamine in the striatum, abnormal mitochondrial and proteasomal functions, and accumulation of α-synuclein that may be closely associated with pathological and clinical abnormalities. Increasing evidence indicates that both oxidative stress and inflammation may play a fundamental role in the pathogenesis of PD. Oxidative stress is characterized by increase in reactive oxygen species (ROS) and depletion of glutathione. Lipid mediators for oxidative stress include 4-hydroxynonenal, isoprostanes, isofurans, isoketals, neuroprostanes, and neurofurans. Neuroinflammation is characterized by activated microglial cells that generate proinflammatory cytokines, such as TNF-α and IL-1β. Proinflammatory lipid mediators include prostaglandins and platelet activating factor, together with cytokines may play a prominent role in mediating the progressive neurodegeneration in PD.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder of unknown etiology. PD is characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, which project to the striatum, the output of which governs locomotor behavior [1, 2]. While 90–95% of PD cases have no known genetic basis, approximately 5–10% arise from inherited mutations [3]. Roughly half of early-onset PD is caused by loss-of-function mutations in the *parkin* gene [4], which encodes an E3 ubiquitin ligase. Although the molecular mechanism of vulnerability of dopaminergic neurons in the substantia nigra pars compacta is not known, it is suggested that monoamine oxidase-mediated abnormal dopamine metabolism, hydrogen peroxide generation, abnormal mitochondrial and proteasomal dysfunctions along with microglial cell activation may be closely associated with neurodegenerative process [5]. Monoamine oxidase catalyzes the oxidative deamination of dietary amines and monoamine neurotransmitters, such as serotonin, norepinephrine, dopamine, β-phenylethylamine, and other trace amines. The rapid degradation of these molecules ensures the proper functioning of synaptic neurotransmission and is critically important not only for the regulation of emotional behaviors, but also for other neural functions. PD is accompanied by abnormalities in synaptic neurotransmission in the basal ganglia. The loss of dopaminergic neurons in the substantia nigra pars compacta may be related to resting tremor, rigidity, bradykinesia, postural instability, and gait disturbance in PD patients. The neuropathological hallmarks of PD include the presence of Lewy bodies mostly composed of α-synuclein, a presynaptic protein that not only plays an important role in neuropathology of PD, but is also known to bind Cu^{2+}, a divalent metal ion, which accelerates the aggregation of α-synuclein to form various toxic aggregates *in vitro* [5, 6]. Neurochemically, PD is characterized by the mitochondrial dysfunction, reactive oxygen species (ROS) generation, nitric oxide (NO) production, excitotoxicity, inflammation, accumulation of aberrant or misfolded proteins, and ubiquitin-proteasome system dysfunction (Figure 1) [1, 2].

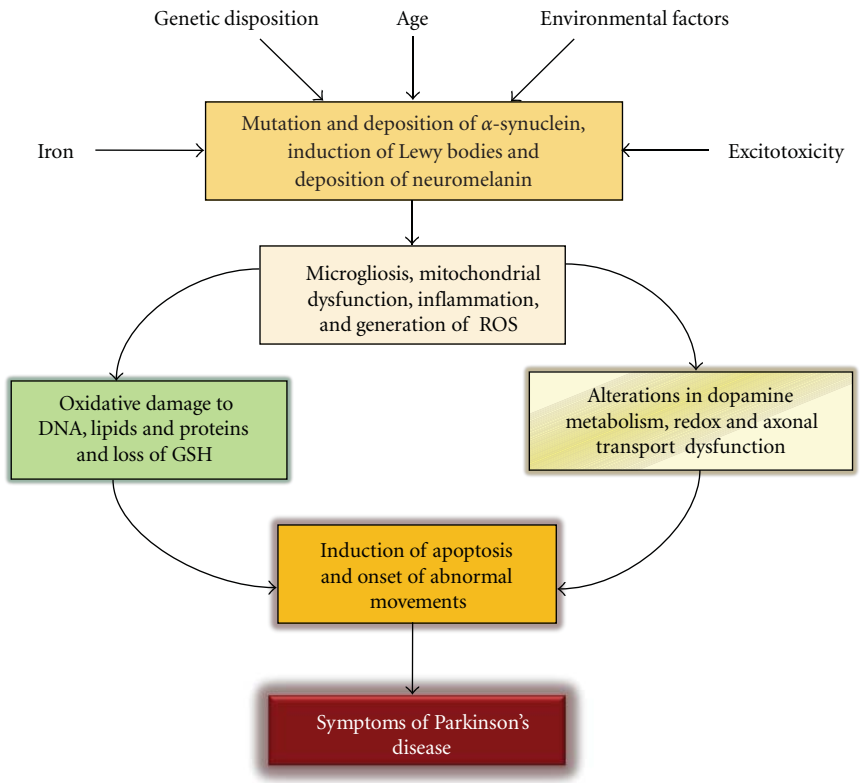

Figure 1: Potential factors and events associated with the pathogenesis of PD.

2. Oxidative Stress and Its Consequences in Brain

Oxidative stress is a cytotoxic condition that occurs in the tissue when antioxidant mechanisms are overwhelmed by ROS [7]. Thus, oxidative stress is a threshold phenomenon characterized by a major increase in the amount of oxidized cellular components. ROS include superoxide anions, hydroxyl, alkoxyl, and peroxyl radicals, and hydrogen peroxide. The major sources of ROS are the mitochondrial respiratory chain, an uncontrolled arachidonic acid (ARA) cascade, and NADPH oxidase (Figure 2) [8]. These processes utilize molecular oxygen and produce ROS, which include superoxide anion (O_2) and H_2O_2. Superoxide is rapidly converted to H_2O_2 by superoxide dismutase (SOD), and in turn H_2O_2 is converted to H_2O by catalase [9]. In the presence of metal ions, such as Fe^{2+} and Cu^{2+}, H_2O_2 can be further converted to hydroxyl radical ($^{\bullet}OH$) through the Fenton reaction. Hydroxyl radicals can attack polyunsaturated fatty acids in membrane phospholipids forming the peroxyl radical ($ROO^{\bullet}$) and then propagate the chain reaction of lipid peroxidation [5].

Low levels of ROS are needed for normal cellular functions including, but not restricted to, the regulation of neuronal excitability via redox-sensitive ion channels, synaptic plasticity, gene transcription, and for the activity of enzymes controlling protein phosphorylation [10]. At higher concentrations, ROS cause neural membrane damage. The biological targets of ROS include membrane proteins, unsaturated lipids, and DNA [11]. Although neurodegeneration in neurological disorders is a multifactorial process [5, 12], it is becoming increasingly evident that the major underlying factor in the neurological disorders is the increased oxidative stress substantiated by the findings that the protein side-chains are modified either directly or indirectly by ROS. The reaction between ROS and proteins or unsaturated lipids in the plasma membrane also results in the chemical cross-linking of membrane proteins and lipids and a reduction in membrane unsaturation. The depletion of unsaturation in membrane lipids is associated with decreased membrane fluidity and decreased activity of membrane-bound enzymes, ion-channels, and receptors [13].

ROS also attack DNA bases causing damage through hydroxylation, ring opening, and fragmentation [14]. This attack generates 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 2, 6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) [15]. ROS may also attack the sugar phosphate backbone of DNA [7]. An indication of this DNA damage comes from the presence of free bases in urine. Abstraction of hydrogen by ROS at the C-4 position of the sugar moiety also produces single-strand breaks in DNA. This is accompanied by a second sugar oxidation on the complementary strand, causing a double strand break in DNA. These reactions may be responsible for the mutagenic effects of ROS in brain tissue [14].

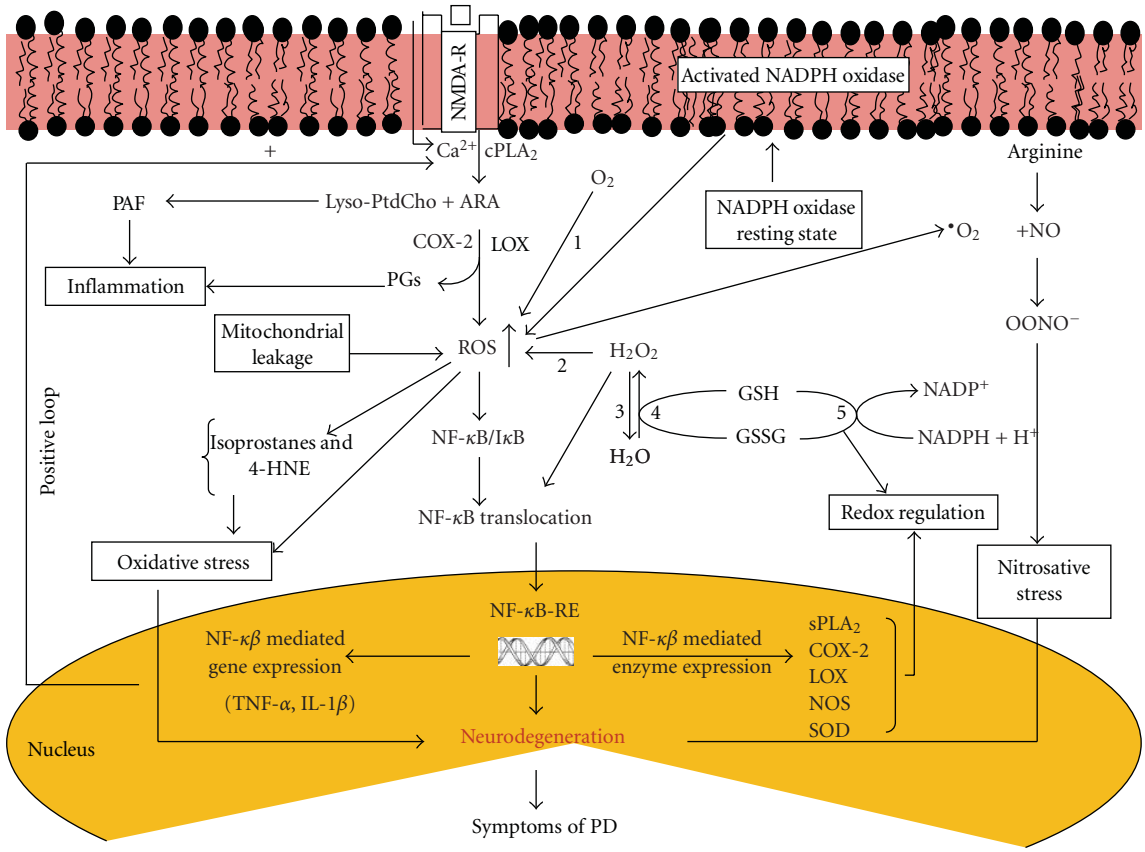

Figure 2: Generation of ROS, activation of NF-κB, redox status, and gene expression in Parkinson's disease. (1) NADPH oxidase; (2) superoxide dismutase; (3) catalase; (4) glutathione peroxidase; (5) glutathione reductase; cytosolic phospholipase A_2 ($cPLA_2$); secretory phospholipase A_2 ($sPLA_2$); cyclooxygenase-2 (COX), lipoxygenase (LOX); SOD; nitric oxide synthase (NOS); cytokines, TNF-α and IL-1β; reduced glutathione (GSH); oxidized glutathione (GSSG) and hydrogen peroxide (H_2O_2). Increase in oxidative stress-mediated expression of NF-κB induces transcription of $sPLA_2$. COX-2, NOS, and SOD in the nucleus as well as proinflammatory cytokines (TNF-α and IL-1β) that further upregulate activities of $cPLA_2$, $sPLA_2$, NOS through a positive loop mechanism in cytoplasm and neural membranes.

Activation of nitric oxide synthase (NOS) generates nitric oxide (NO), which reacts with superoxide to form peroxynitrite. This molecule oxidatively modifies nucleic acid, lipid, sugar, and protein, leading to nuclear damage, mitochondrial damage, proteasome inhibition, and endoplasmic reticulum (ER) stress [16]. NO and peroxynitrite not only decrease glutathione but also S-nitrosylate many proteins. Excessive nitrosative stress contributes to the hyperactivation of the N-methyl-D-aspartate (NMDA)-type glutamate receptor, mitochondrial dysfunction, and cellular aging. Excessive generation of free radicals and related molecules (ROS) and NO species have been reported to trigger pathological production of misfolded proteins, abnormal mitochondrial dynamics (comprised of mitochondrial fission and fusion events), and apoptotic pathways in neuronal cells [17, 18]. Emerging evidence suggests that excessive NO production can contribute to these pathological processes, specifically by S-nitrosylation of specific target proteins, such as protein disulfide isomerase (PDI), ubiquitin protein ligase, parkin (forming SNO-parkin), and mitochondrial fragmentation through β-amyloid-related S-nitrosylation of dynamin-related protein-1 [18]. Among these proteins, PDI is responsible for normal protein folding in the endoplasmic reticulum (ER). S-nitrosylation of PDI compromises its function and induces misfolding not only in cell cultures systems, but also in animal models of neurodegenerative diseases [17, 18]. In addition, NO-mediated effects on dopaminergic neurons may also include the inhibition of cytochrome oxidase, ribonucleotide reductase, mitochondrial complexes I, II, and IV in the respiratory chain, superoxide dismutase, glyceraldehyde-3-phosphate dehydrogenase, activation or initiation of DNA strand breakage, poly (ADP-ribose) synthase, lipid peroxidation, protein oxidation, release of iron, and increased generation of toxic radicals such as hydroxyl radicals and peroxynitrite [19]. Accumulating evidence suggests that excessive ROS/RNS formation by above-mentioned processes may induce UPS-impairment and/or misfolding of molecular chaperons, thus resulting in protein aggregation and neuronal damage [18]. This suggestion supports the possible occurrence of cross-talk between mitochondria and UPS controlling organelles (proteasomes). Thus, the production of excessive ROS by mitochondria may adversely affect UPS activity leading to neurodegeneration in neurological disorders [12, 20].

Among neural cells, neurons are particularly vulnerable to oxidative damage not only because of mitochondrial dysfunction [21], but also due to inactivation of glutamine synthetase, which reduces the uptake of glutamate by glial cells and increases glutamate availability at the synapse producing excitotoxicity [9]. In addition, neuronal membrane peroxidative injury may lead to the depletion of unsaturated fatty acids in neural membranes, which not only causes changes in membrane fluidity but also affects activities of membrane-bound enzymes, ion channels, and receptors [10].

Glial cell's response to oxidative stress-mediated neurodegenerative process is extremely complex. On one side, astrocytes protect neurons from excitotoxicity through glutamate uptake system, and on the other side astrocytes contribute to the extracellular glutamate via reversed glutamate transporter [22]. In addition, astrocytes may undergo astrocytosis after dopaminergic cell loss and contribute to the inflammatory response [7]. Microglial cells respond to oxidative stress-mediated neurodegenerative process by transforming themselves into activated microglia. They not only change their shape into "ameboid" morphology, but also release matrix metalloproteinases, ROS, RNS, prostaglandin E_2, and proinflammatory cytokines such as TNF-α and IL-β1 [10].

3. Oxidative Stress, Nitrosative Stress and Their Consequences in PD

Involvement of oxidative stress in the pathogenesis of PD is supported by both postmortem studies and by studies showing the increased level of oxidative stress in the substantia nigra pars compacta. A plausible source of oxidative stress in nigral dopaminergic neurons is the redox reactions that specifically involve dopamine and producing various toxic quinone species (DAQ), such as dopamine-o-quinone (DQ), aminochrome (AC), and indole-quinone (IQ). Oxidation products of dopamine have been shown to alter mitochondrial function, including mitochondrial swelling and decrease in electron transport chain activity [23, 24]. Studies on toxic effects of dopamine-derived DAQ on mitochondria, specifically on NADH and GSH pools, indicate that the generation of DAQ in isolated respiring mitochondria induces the opening of the permeability transition pore most probably by inducing oxidation of NADH, while GSH levels are not affected. It is proposed that studies on diverse reactivity for the different DAQ may provide information on the complex molecular mechanisms underlying oxidative stress and mitochondria dysfunction in PD. Markers of lipid peroxidation include 4-hydroxy-trans-2-nonenal (4-HNE), 4-oxo-trans-2-nonenal (4-ONE), acrolein, isoprostanes, and isofurans are significantly increased in PD (Table 1 and Figure 3). These markers are derived from arachidonic acid (ARA), which is released from neural membrane glycerophospholipids through the activation of cytosolic phospholipases A_2 ($cPLA_2$). This enzyme is coupled with NMDA receptors through G protein independent mechanism [10]. This suggestion is supported by studies on $cPLA_2$ deficient mice. These mice are resistant to a specific dopaminergic neurotoxicity induced by the toxin 1-methyl,4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) [25]. In the brain, MPTP is converted to its toxic metabolite, 1-methyl-4-phenylpyridinium ion (MPP^+), in the presence of monoamine oxidase B. MPP^+ is actively taken up into nigrostriatal neurons where it inhibits mitochondrial oxidative phosphorylation leading to neuronal cell death [26]. In MPTP-induced model of Parkinsonism, PLA_2 inhibitors (quinacrine and arachidonyltrifluoromethyl ketone) protect dopaminergic neurons from neurodegeneration [27, 28]. In addition, the inhibition of COX-2, the enzyme responsible for the production of proinflammatory prostaglandin (PGE_2), not only decreases the lesions caused by MPTP but also protects dopaminergic neurons in the substantia nigra. However, the molecular mechanism associated with COX-2-mediated neurodegeneration in animal model of PD remains unknown. However, COX-2 inhibition may prevent the formation of the oxidant species of reactive quinones. These metabolites are involved in the pathogenesis of PD [29–31].

Nitric oxide (NO) plays multiple roles in the brain and spinal cord tissues. In addition to regulating proliferation, survival and differentiation of neurons, it is involved in synaptic activity, neural plasticity, and memory function [39]. The evidence for the involvement of NO in neurotoxic processes associated with PD comes from studies using experimental models of this disease. NOS inhibitors can prevent MPTP-mediated dopaminergic neurotoxicity.

Table 1: Levels of mediators, proteins, and factors, which facilitate and maintain oxidative stress and inflammatory responses in PD.

(a)

(A) Lipid mediator	Effect	Reference
Prostaglandins	Increased	[10]
Lipid peroxidation	Increased	[10]
4-Hydroxynonenal	Increased	[10]
Hydroxycholesterol	Increased	[32]
8-OHdGua	Increased	[32]
PINK	Increased	[33]
Aggregated α-synuclein	Increased	[34]
NF-κB activity	Increased	[35]
Oxidative stress	Increased	[12]
Neuroinflammation	Increased	[12]
Neurodegeneration	Increased	[12]

(b)

(B) Factors		
TNF-α	Increased	[36]
IL-1β	Increased	[36]
IL-4	Increased	[37]
IL-6	Increased	[37]
Epidermal growth factor	Increased	[36, 37]
Transforming growth factor	Increased	[36, 37]
Brain-derived neurotrophic factor	Decreased	[38]
Nerve growth factor	Decreased	[38]

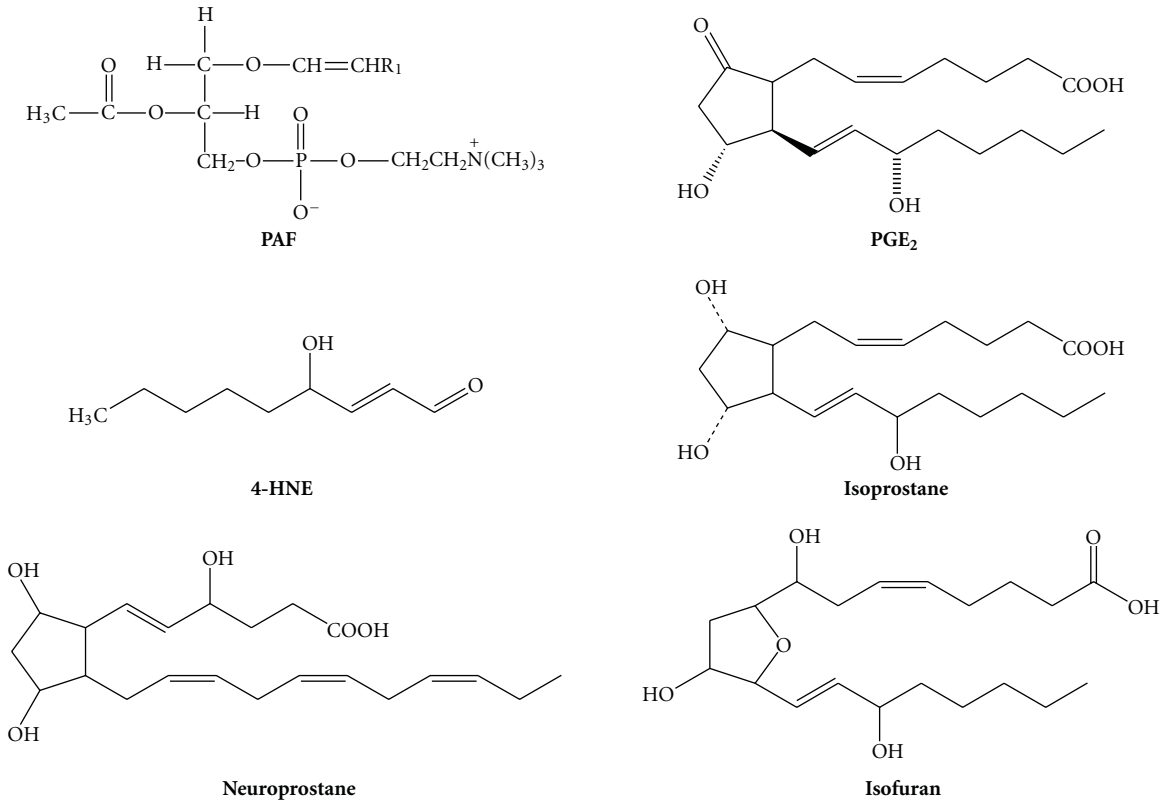

Figure 3: Chemical structures of biomarkers for oxidative stress.

Furthermore, NO not only fosters dopamine depletion, but NO-mediated neurotoxicity is averted by 7-nitroindazole, a nNOS inhibitor, in the substantia nigra pars compacta [19]. Moreover, mutant mice lacking the nNOS gene are more resistant to MPTP neurotoxicity when compared with wild-type littermates. Selegiline, an irreversible inhibitor of monoamine oxidase B, produces beneficial effects in PD by enhancing dopaminergic function. Selegiline and its metabolite (desmethylselegiline) also act by reducing apoptotic cell death by modulating the expression of number of genes, including superoxide dismutase, Bcl-2, Bcl-xl, NOS, c-Jun, and nicotinamide adenine nucleotide dehydrogenase. It is likely that selegiline-mediated antiapoptotic activity may also be involved in the prevention of a progressive reduction of mitochondrial membrane potential in preapoptotic neurons [19]. The neuroprotective effects of selegiline may also involve neurotrophic factors (nerve growth factor, brain-derive neurotrophic factor, neurotrophin 3) and ligands of glial cell line-derived neurotrophic factor [5, 12], which contribute to neurogenesis in the damaged brains. Excessive generation of NO may also facilitate the pathological production of misfolded proteins, abnormal mitochondrial dynamics (comprised of mitochondrial fission and fusion events), and apoptotic pathways in neuronal cells [40]. Thus, in animal models of PD, S-nitrosylation targets include parkin, a ubiquitin E3 ligase and neuroprotective molecule, and protein-disulfide isomerase (PDI), a chaperone enzyme associated with nascent protein folding. S-nitrosylation of parkin and PDI compromises its ubiquitin E3 ligase and PDI activities and their protective function suggesting that nitrosative stress is an important factor in regulating neuronal survival during the pathogenesis of PD [41, 42]. Significant S-nitrosylation of above proteins may contribute to abnormal mitochondrial fragmentation, resulting in synaptic damage that is found not only in PD but also in other neurodegenerative diseases, such as AD and ALS [40].

4. Inflammatory Responses in Brain

Inflammation is a protective process that not only isolates the injured brain tissue from uninjured area but also destroys affected cells, and repairs the extracellular matrix [31, 43]. Without a strong inflammatory response, brain would be prone to neurotraumatic and neurodegenerative diseases. The main mediators of neuroinflammation are microglial cells. They participate in repair and resolution processes after injury to restore normal tissue homeostasis. Microglial cells also play an important role as resident immunocompetent after neural cell injury and disease. As stated above, during neurodegenerative process, the resting microglial cells are transformed into activated microglia, which are characterized by amoeboid morphology [44]. Activated microglial cells not only migrate rapidly to the site where neurodegenerative process is taking place, but also engulf dead cells, and clear cellular debris [45, 46].

The chronic activation of microglia in PD may cause neuronal damage through the release of potentially cytotoxic molecules such as proinflammatory cytokines, ROS, proteinases and complement proteins [5, 11, 44]. Although very little is known about signaling mechanisms associated with modulation of microglial activation in PD, it is proposed that low levels of cytokines and chemokines released by microglial cells and to lesser extent astrocytes not only facilitate modulation of neurogenesis through the release of trophic factors that protect against ROS, and glutamate, but also promote the removal of dead and damaged neuron [11, 44–46]. In contrast, high levels of glial cell-secreted cytokines and chemokines promote neurodegeneration through the activation of phospholipases A_2 and COX-2 [44]. Emerging evidence suggests that microglia have a specialized immune surveillance role and mediate innate immune responses to neurodegenerative process by secreting a myriad of factors that include cytokines, chemokines, prostaglandins, ROS, RNS, and growth factors. Some of these factors have neuroprotective and trophic activities and aid in brain repair processes, while others enhance oxidative stress and trigger neurodegeneration [47].

Two types of inflammatory responses (acute and chronic) occur in brain tissue. Acute inflammation response develops rapidly and may be accompanied by pain, whereas chronic inflammation develops slowly and remains below the threshold of pain perception. As a result, the immune system continues to attack the brain tissue and chronic inflammation lingers for years, ultimately reaching the threshold of detection [31]. Inflammatory response also involves recruitment and migration of polymorphonuclear leukocytes (PMN) and lymphocytes from the blood stream into brain tissue. This is followed by a process called resolution, a turning off mechanism by neural cells to limit tissue injury. Acute inflammation normally resolves spontaneously, but the mechanism associated with this process remains elusive [48].

The chronic activation of microglia may not only cause neuronal damage through the release of proinflammatory cytokines and chemokines, but also ROS, proteinases and complement proteins. Very little is known about molecular mechanisms and internal and external factors that control and modulate the dynamics of acute and chronic neuroinflammation. Collective evidence suggests that inflammatory response involves the interplay not only among microglia, astrocytes, neurons, PMN, and endothelial cells, but also among various lipid mediators that originate from enzymatic and nonenzymatic degradation of neural membrane glycerophospholipids sphingolipids and cholesterol [44, 49]. In addition, transcription factors such as peroxisome proliferator-activated receptor (PPAR) and NF-κB also play an important role in modulation of inflammatory responses.

During inflammatory response expression and activities of a number of enzymes including secretory phospholipase A_2 ($sPLA_2$), cytosolic phospholipase A_2 ($cPLA_2$), cyclooxygenase-2 (COX-2), and lipoxygenases (LOX), that release ARA and convert it to proinflammatory prostaglandin (PGE_2) and leukotriene B_4, are markedly increased [44]. Lysophospholipids, the other product of PLA_2 catalyzed reaction is converted to proinflammatory lipid mediator, the platelet-activating factor (PAF). Generation of prostaglandins and PAF increases the intensity of inflammatory response [12]. During inflammatory response, upregulation of inducible NOS (iNOS) generates high levels of nitric oxide and peroxynitrite ($ONOO^-$), which not only nitrates proteins but breaks down into hydroxyl radicals promoting further intensification of inflammatory response. In addition, at the site of neurodegenerative process, neural and nonneural cells express and secrete cytokines, chemokine, and complement proteins, which also play important roles in induction, propagation, and maintenance of inflammatory response [12, 44].

Cytokines are major effectors of the inflammatory response. Cytokines produce their effects by interacting with specific membrane-associated receptors. Cytokines play an-important role in neural cell response to neurodegenerative processes [50]. Although physiological levels of cytokines are needed for normal neural cell function and survival, but increased secretion of cytokines during neurodegenerative process can be detrimental to neurons [51]. Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are major cytokines that are upregulated in the brain tissue during inflammatory response (Table 1). In addition, interleukin-1α (IL-1α), interleukin-3, interleukin-6 (IL-6), and tumor and growth factors (TGF-α and β) are also secreted by both microglia and astrocytes during inflammatory response. Accumulating evidence suggests that secretion and interactions of various cytokines during inflammatory response may result in their synergistic or antagonistic activities through a complex network that not only involves their feedback loops, but also modulates levels of various lipid mediators by regulating activities of isoforms of PLA_2, COX-2, NOS [51, 52]. The consequences of excessive inflammatory response include secretion of high levels of proinflammatory cytokines and chemokines and production of more free radical causing more oxidative stress, which can not only damage neurons through the downregulation of neurotrophins and their receptors but also by blocking neurogenesis. In addition, the interactions of cytokines with their receptors result in activation of cascades of protein kinases, which may lead to the activation of transcription factor, nuclear factor kappa B (NF-κB). Activated NF-κB migrates to the nucleus where it mediates the transcription of many genes implicated in inflammatory and immune responses [44]. These genes include COX-2, intracellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), E-selectin, TNF-α, IL-1β, IL-6, $sPLA_2$, inducible nitric oxide synthase (iNOS), and matrix metalloproteinases (MMPs). It is not known whether inflammatory response in neurodegenerative diseases is the consequence or the cause of neurodegeneration [12].

5. Inflammation in PD Brain

Aging, genetic disposition, increase in iron levels, and environmental factors may trigger the abnormality in proteasomal function leading to the initiation and deposition of

mutated α-synuclein and induction of Lewy body formation in the brain (Figure 1). Neuromelanin (NM), an iron binding complex polymer pigment, interacts with α-synuclein and contributes to its aggregation. Neuromelanin (NM) occurs in catecholaminergic neurons of the substantia nigra and locus coeruleus in human brains and in brains of different animal species [53]. In brain, the conversion of dopamine to NM through the generation of aminochrome and polymerization is facilitated by iron, and this process is blocked by iron chelator, desferrioxamine [53, 54]. The interactions between iron and NM not only promote NM synthesis but also play an important role in intraneuronal iron homeostasis [53]. In PD, where nigral iron levels are increased, saturation of high-affinity iron-binding sites on NM may saturate the protective capacity of this molecule, leading instead to an increase in redox-active iron, and subsequent cellular damage both *in vitro* and *in vivo* [53, 55]. The increase in the release of iron from NM modulates the ubiquitin-proteasome system in mitochondria, leading to the failure to clear proteins such as α-synuclein and to the development of abnormal α-synuclein-immunopositive Lewy bodies that may facilitate the degeneration of dopaminergic neurons in PD [55]. During neurodegenerative process, microgliosis may also play a crucial role. It is proposed that NM acts as a stimulus and triggers microgliosis in animal and cell culture models of PD [56, 57]. This proposal is supported by studies on injections of NM in rat brain cerebral cortex and substantia nigra to monitor microglial cell activation (Iba-1 and/or GFAP antibody) and neurodegeneration (tyrosine hydroxylase) [57]. In this study, LPS injections are used as positive controls and PBS injections are used as negative controls. The injections of LPS induce a strong inflammatory response in the cortex as well in the substantia nigra. Similar results have been obtained in NM injected brains, and PBS injections induce only moderate or no glial activation. However, the inflammatory response declines during the time course when LPS and NM were different. In the NM injected group strong microglia activation is accompanied by a significant dopaminergic cell loss after 1 week of survival time whereas in LPS-injected, brains inflammatory response declines during the time course. These results clearly indicate that extracellular NM may be one of the key molecules leading to microglial activation and neuronal cell death in the substantia nigra [57]. It is proposed that extraneuronal melanin may trigger microgliosis, microglial chemotaxis and microglial activation in PD with subsequent release of neurotoxic mediators. The addition of human NM to microglial cell cultures not only produces positive chemotactic effects, but activates the proinflammatory transcription factor nuclear factor-κB (NF-κB) via phosphorylation and degradation of the inhibitor protein κB (I-κB), and induces an upregulation of TNF-α, IL-6 and NO [56]. In addition, microglial cells secrete a myriad of factors, such as cytokines, chemokines, prostaglandins, ROS and RNS, and growth factors [58]. Some of these factors produce neuroprotective and trophic effects and promote in brain repair processes, while others increase oxidative stress and induce and mediate apoptotic cascades in neurons. Therefore, pro- and anti-inflammatory responses must be in balance to prevent the potential detrimental effects of prolonged or unregulated inflammation-induced oxidative stress on vulnerable neuronal populations. Accumulating evidence suggests that in PD degeneration of dopaminergic neurons in substantia nigra is accompanied not only by the progressive loss of NM but also by the induction of microgliosis [59]. Although NM particles are phagocytized and degraded by microglial cells within minutes, but extracellular NM particle-mediated microglial activation results in the generation of superoxide, nitric oxide, hydrogen peroxide, and other proinflammatory factors including cytokines and chemokines, which support oxidative stress and inflammation in the brain [36, 37, 59].

6. Conclusions

PD is a common neurodegenerative movement disorder, which affects increasing number of elderly population. The disorder is caused by a selective degeneration of dopaminergic neurons in the substantia nigra pars compacta. Although the molecular mechanism associated with neurodegeneration in PD is not known, it is becoming increasingly evident that neurodegeneration in PD is a multifactorial process that may involve monoamine oxidase-mediated abnormal dopamine metabolism, increase in iron levels, hydrogen peroxide generation, abnormal mitochondrial and proteasomal function along with microgliosis may be closely associated with the pathogenesis of PD. Microglial cells play a critical role in forming a self-propelling cycle leading to sustained chronic neuroinflammation and driving the progressive neurodegeneration in PD. The above-mentioned processes have been shown to contribute to the oxidative stress, accumulation of α-synuclein, and neuroinflammation not only in cell culture and animal models of PD, but also in brains of PD patients.

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References

[1] M. F. Beal, "Mitochondrial dysfunction in neurodegenerative diseases," *Biochimica et Biophysica Acta*, vol. 1366, no. 1-2, pp. 211–223, 1998.

[2] P. Jenner and C. W. Olanow, "The pathogenesis of cell death in Parkinson's disease," *Neurology*, vol. 66, no. 10, supplement 4, pp. S24–S36, 2006.

[3] M. J. Farrer, K. Haugarvoll, O. A. Ross et al., "Genomewide association, Parkinson disease, and PARK10," *American Journal of Human Genetics*, vol. 78, no. 6, pp. 1084–1088, 2006.

[4] C. B. Lücking, A. Dürr, V. Bonifati et al., "Association between early-onset Parkinson's disease and mutations in the parkin gene," *New England Journal of Medicine*, vol. 342, no. 21, pp. 1560–1567, 2000.

[5] A. A. Farooqui, *Neurochemical Aspects of Neuroinflammation in Brain in Molecular Aspects of Neurodegeneration and Neuroprotection*, Bentham Science, Oak Park, Ill, USA, 2010, A. A. Farooqui and T. Farooqui, Eds.

[6] C. Wang, L. Liu, L. Zhang, Y. Peng, and F. Zhou, "Redox reactions of the α-synuclein-Cu^{2+} complex and their effects on neuronal cell viability," *Biochemistry*, vol. 49, no. 37, pp. 8134–8142, 2010.

[7] B. Halliwell, "Oxidative stress and neurodegeneration: where are we now?" *Journal of Neurochemistry*, vol. 97, no. 6, pp. 1634–1658, 2006.

[8] G. Y. Sun, L. A. Horrocks, and A. A. Farooqui, "The roles of NADPH oxidase and phospholipases A_2 in oxidative and inflammatory responses in neurodegenerative diseases," *Journal of Neurochemistry*, vol. 103, no. 1, pp. 1–16, 2007.

[9] A. A. Farooqui, W. Y. Ong, and L. A. Horrocks, *Neurochemical Aspects of Excitotoxicity*, Springer, New York, NY, USA, 2008.

[10] A. A. Farooqui and L. A. Horrocks, *Glycerophospholipids in Brain*, Springer, New York, NY, USA, 2007.

[11] M. Valko, H. Morris, and M. T. D. Cronin, "Metals, toxicity and oxidative stress," *Current Medicinal Chemistry*, vol. 12, no. 10, pp. 1161–1208, 2005.

[12] A. A. Farooqui, *Neurochemical Aspects of Neurotraumatic and Neurodegenerative Disease*, Springer, New York, NY, USA, 2010.

[13] J. D. Fernstrom, "Effects of dietary polyunsaturated fatty acids on neuronal function," *Lipids*, vol. 34, no. 2, pp. 161–169, 1999.

[14] A. Buisson, N. Lakhmeche, C. Verrecchia, M. Plotkine, and R. G. Boulu, "Nitric oxide: an endogenous anticonvulsant substance," *NeuroReport*, vol. 4, no. 4, pp. 444–446, 1993.

[15] A. M. Jenkinson, A. R. Collins, S. J. Duthie, K. W. J. Wahle, and G. G. Duthie, "The effect of increased intakes of polyunsaturated fatty acids and vitamin E on DNA damage in human lymphocytes," *FASEB Journal*, vol. 13, no. 15, pp. 2138–2142, 1999.

[16] N. Shibata and M. Kobayashi, "The role for oxidative stress in neurodegenerative diseases," *Brain and Nerve*, vol. 60, no. 2, pp. 157–170, 2008.

[17] S. A. Lipton, Z. Gu, and T. Nakamura, "Inflammatory mediators leading to protein misfolding and uncompetitive/fast off-rate drug therapy for neurodegenerative disorders," *International Review of Neurobiology*, vol. 82, pp. 1–27, 2007.

[18] T. Nakamura and S. A. Lipton, "Emerging roles of S-nitrosylation in protein misfolding and neurodegenerative diseases," *Antioxidants and Redox Signaling*, vol. 10, no. 1, pp. 87–101, 2008.

[19] M. Ebadi and S. K. Sharma, "Peroxynitrite and mitochondrial dysfunction in the pathogenesis of Parkinson's disease," *Antioxidants and Redox Signaling*, vol. 5, no. 3, pp. 319–335, 2003.

[20] D. M. Branco, D. M. Arduino, A. R. Esteves, D. F. Silva, S. M. Cardoso, and C. R. Oliveira, "Cross-talk between mitochondria and proteasome in Parkinson's disease pathogenesis," *Frontiers in Aging Neuroscience*, vol. 2, article 17, 2010.

[21] A. Atlante, P. Calissano, A. Bobba, A. Azzariti, E. Marra, and S. Passarella, "Cytochrome c is released from mitochondria in a reactive oxygen species (ROS)-dependent fashion and can operate as a ROS scavenger and as a respiratory substrate in cerebellar neurons undergoing excitotoxic death," *Journal of Biological Chemistry*, vol. 275, no. 47, pp. 37159–37166, 2000.

[22] M. A. Dronne, E. Grenier, T. Dumont, M. Hommel, and J. P. Boissel, "Role of astrocytes in grey matter during stroke: a modelling approach," *Brain Research*, vol. 1138, no. 1, pp. 231–242, 2007.

[23] F. H. Khan, T. Sen, A. K. Maiti, S. Jana, U. Chatterjee, and S. Chakrabarti, "Inhibition of rat brain mitochondrial electron transport chain activity by dopamine oxidation products during extended in vitro incubation: implications for Parkinson's disease," *Biochimica et Biophysica Acta*, vol. 1741, no. 1-2, pp. 65–74, 2005.

[24] M. Bisaglia, M. E. Soriano, I. Arduini, S. Mammi, and L. Bubacco, "Molecular characterization of dopamine-derived quinones reactivity toward NADH and glutathione: implications for mitochondrial dysfunction in Parkinson disease," *Biochimica et Biophysica Acta*, vol. 1802, no. 9, pp. 699–706, 2010.

[25] P. Klivenyi, M. F. Beal, R. J. Ferrante et al., "Mice deficient in group IV cytosolic phospholipase A_2 are resistant to MPTP neurotoxicity," *Journal of Neurochemistry*, vol. 71, no. 6, pp. 2634–2637, 1998.

[26] T. P. Singer, N. Castagnoli Jr., R. R. Ramsay, and A. J. Trevor, "Biochemical events in the development of parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine," *Journal of Neurochemistry*, vol. 49, no. 1, pp. 1–8, 1987.

[27] M. Tariq, H. A. Khan, K. A. Moutaery, and S. A. Deeb, "Protective effect of quinacrine on striatal dopamine levels in 6-OHDA and MPTP models of Parkinsonism in rodents," *Brain Research Bulletin*, vol. 54, no. 1, pp. 77–82, 2001.

[28] N. Yoshinaga, Y. Yasuda, T. Murayama, and Y. Nomura, "Possible involvement of cytosolic phospholipase A_2 in cell death induced by 1-methyl-4-phenylpyridinium ion, a dopaminergic neurotoxin, in GH3 cells," *Brain Research*, vol. 855, no. 2, pp. 244–251, 2000.

[29] Z. Feng, D. Li, P. C. W. Fung, Z. Pei, D. B. Ramsden, and S. L. Ho, "COX-2-deficient mice are less prone to MPTP-neurotoxicity than wild-type mice," *NeuroReport*, vol. 14, no. 15, pp. 1927–1929, 2003.

[30] P. Teismann, M. Vila, D. K. Choi et al., "COX-2 and neurodegeneration in Parkinson's disease," *Annals of the New York Academy of Sciences*, vol. 991, pp. 272–277, 2003.

[31] P. L. Wood, *Neuroinflammation: Mechanisms and Managements*, Humana Press, Totowa, NJ, USA, 1998.

[32] R. C. S. Seet, C. Y. J. Lee, E. C. H. Lim et al., "Oxidative damage in Parkinson disease: measurement using accurate biomarkers," *Free Radical Biology and Medicine*, vol. 48, no. 4, pp. 560–566, 2010.

[33] H. Büeler, "Impaired mitochondrial dynamics and function in the pathogenesis of Parkinson's disease," *Experimental Neurology*, vol. 218, no. 2, pp. 235–246, 2009.

[34] K. Beyer, "Mechanistic aspects of Parkinson's disease: α-synuclein and the biomembrane," *Cell Biochemistry and Biophysics*, vol. 47, no. 2, pp. 285–299, 2007.

[35] A. Ghosh, A. Roy, X. Liu et al., "Selective inhibition of NF-κB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 47, pp. 18754–18759, 2007.

[36] M. Sawada, K. Imamura, and T. Nagatsu, "Role of cytokines in inflammatory process in Parkinson's disease," *Journal of Neural Transmission, Supplement*, no. 70, pp. 373–381, 2006.

[37] T. Nagatsu and M. Sawada, "Inflammatory process in Parkinson's disease: role for cytokines," *Current Pharmaceutical Design*, vol. 11, no. 8, pp. 999–1016, 2005.

[38] T. Nagatsu, M. Mogi, H. Ichinose, and A. Togari, "Changes in cytokines and neurotrophins in Parkinson's disease," *Journal of Neural Transmission, Supplement*, no. 60, pp. 277–290, 2000.

[39] V. Calabrese, C. Cornelius, E. Rizzarelli, J. B. Owen, A. T. Dinkova-Kostova, and D. A. Butterfield, "Nitric oxide in cell survival: a janus molecule," *Antioxidants & Redox Signaling*, vol. 11, no. 11, pp. 2717–2739, 2009.

[40] T. Nakamura and S. A. Lipton, "S-nitrosylation of critical protein thiols mediates protein misfolding and mitochondrial dysfunction in neurodegenerative diseases," *Antioxidants and Redox Signaling*. In press.

[41] A. H. K. Tsang and K. K. K. Chung, "Oxidative and nitrosative stress in Parkinson's disease," *Biochimica et Biophysica Acta*, vol. 1792, no. 7, pp. 643–650, 2009.

[42] K. K. K. Chung, B. Thomas, X. Li et al., "S-nitrosylation of parkin regulates ubiquitination and compromises Parkin's protective function," *Science*, vol. 304, no. 5675, pp. 1328–1331, 2004.

[43] J. Correale and A. Villa, "The neuroprotective role of inflammation in nervous system Injuries," *Journal of Neurology*, vol. 251, no. 11, pp. 1304–1316, 2004.

[44] A. A. Farooqui, L. A. Horrocks, and T. Farooqui, "Modulation of inflammation in brain: a matter of fat," *Journal of Neurochemistry*, vol. 101, no. 3, pp. 577–599, 2007.

[45] E. C. Hirsch and S. Hunot, "Neuroinflammation in Parkinson's disease: a target for neuroprotection?" *The Lancet Neurology*, vol. 8, no. 4, pp. 382–397, 2009.

[46] C. M. Long-Smith, A. M. Sullivan, and Y. M. Nolan, "The influence of microglia on the pathogenesis of Parkinson's disease," *Progress in Neurobiology*, vol. 89, no. 3, pp. 277–287, 2009.

[47] M. G. Tansey and M. S. Goldberg, "Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention," *Neurobiology of Disease*, vol. 37, no. 3, pp. 510–518, 2010.

[48] C. N. Serhan and J. Savill, "Resolution of inflammation: the beginning programs the end," *Nature Immunology*, vol. 6, no. 12, pp. 1191–1197, 2005.

[49] A. A. Farooqui, L. A. Horrocks, and T. Farooqui, "Interactions between neural membrane glycerophospholipid and sphingolipid mediators: a recipe for neural cell survival or suicide," *Journal of Neuroscience Research*, vol. 85, no. 9, pp. 1834–1850, 2007.

[50] S. Amor, F. Puentes, D. Baker, and P. van der Valk, "Inflammation in neurodegenerative diseases," *Immunology*, vol. 129, no. 2, pp. 154–169, 2010.

[51] C. J. Wilson, C. E. Finch, and H. J. Cohen, "Cytokines and cognition—the case for a head-to-toe inflammatory paradigm," *Journal of the American Geriatrics Society*, vol. 50, no. 12, pp. 2041–2056, 2002.

[52] Z. Xing, J. Gauldie, G. Cox et al., "IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses," *Journal of Clinical Investigation*, vol. 101, no. 2, pp. 311–320, 1998.

[53] D. Sulzer, J. Bogulavsky, K. E. Larsen et al., "Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 22, pp. 11869–11874, 2000.

[54] I. Paris, J. Lozano, C. Perez-Pastene, P. Muñoz, and J. Segura-Aguilar, "Molecular and neurochemical mechanisms in PD pathogenesis," *Neurotoxicity Research*, vol. 16, no. 3, pp. 271–279, 2009.

[55] M. Gerlach, P. Riederer, and K. L. Double, "Neuromelanin-bound ferric iron as an experimental model of dopaminergic neurodegeneration in Parkinson's disease," *Parkinsonism and Related Disorders*, vol. 14, supplement 2, pp. S185–S188, 2008.

[56] H. Wilms, L. Zecca, P. Rosenstiel, J. Sievers, G. Deuschl, and R. Lucius, "Inflammation in Parkinson's diseases and other neurodegenerative diseases: cause and therapeutic implications," *Current Pharmaceutical Design*, vol. 13, no. 18, pp. 1925–1928, 2007.

[57] L. Zecca, H. Wilms, S. Geick et al., "Human neuromelanin induces neuroinflammation and neurodegeneration in the rat substantia nigra: implications for Parkinson's disease," *Acta Neuropathologica*, vol. 116, no. 1, pp. 47–55, 2008.

[58] M. G. Tansey and M. S. Goldberg, "Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention," *Neurobiology of Disease*, vol. 37, no. 3, pp. 510–518, 2010.

[59] W. Zhang, K. Phillips, A. R. Wielgus et al., "Neuromelanin activates microglia and induces degeneration of dopaminergic neurons: implications for progression of Parkinson's disease," *Neurotoxicity Research*, vol. 19, no. 1, pp. 63–72, 2011.

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Review Article

Lipopolysaccharide Animal Models for Parkinson's Disease

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Lipopolysaccharide (LPS), an endotoxin from Gram-negative bacteria, acts as a potent stimulator of microglia and has been used to study the inflammatory process in the pathogenesis of Parkinson's disease (PD) and anti-inflammatory therapy for PD treatment. Here, we review the growing body of literature on both in vitro and in vivo LPS PD models. Primary cell cultures from mesencephalic tissue were exposed to LPS in vitro; LPS was stereotaxically injected into the substantia nigra, striatum, or globus pallidus of brain or injected into the peritoneal cavity of the animal in vivo. In conclusion, the LPS PD models are summarized as (1) local and direct LPS treatment and (2) systemic LPS treatment. Mechanisms underlying the PD models are investigated and indicated that LPS induces microglial activation to release a variety of neurotoxic factors, and damaged neurons may trigger reactive microgliosis, which lead to progressive dopaminergic neurodegeneration.

1. Introduction

Parkinson's disease (PD) is the most prevalent neurodegenerative movement disorder. In PD, clinical symptoms including tremor, rigidity, and bradykinesia are primarily resulted from the loss of dopamine-containing neurons in the substantia nigra pars compacta. Although the etiology and pathogenesis of PD remain not fully elucidated, many interacting pathological processes appear to contribute to dopaminergic neuron degeneration in the disease. Recently, inflammatory processes have been implicated as one of the active contributors to dopaminergic neuron damage in the development and progression of the disease [1, 2]. In the central nervous system, microglia, the resident innate immune cells, play a major role in the inflammatory process. Typically microglia exist in a resting state characterized by ramified morphology and monitor the brain environment [3]. In response to various pathogenic stimuli including inflammation, microglia are readily activated and undergo a transformation to amoeboid morphology with an upregulated catalogue of surface molecules [3–5]. Activated microglia can serve diverse beneficial functions essential to neuron survival, which include cellular maintenance and innate immunity [6]. However, uncontrolled activated microglia produces a variety of neurotoxic factors such as proinflammatory cytokines (interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6)), nitro oxide (NO), prostaglandin E2, and superoxide, which lead to neuronal damage or death [1, 7–10]. Additionally, damaged neurons may emit injury signals to cause microglia activation, which used to be defined as reactive microgliosis [11]. This microglial-neuronal interaction will be reinforced and become a self-amplifying cycle of neuronal injury and microglial activation, which finally leads to more neuronal damage and death. Importantly, clinical researches have reported that microglial activation was found in the nigrostriatal system of PD patients [12–14]. Therefore, it is essential to study the inflammatory process in PD, which may help us understand the pathogenesis of the disease and eventually develop an effective therapeutic strategy.

Over the last two decades, studies in animal models have demonstrated that inflammation induced by lipopolysaccharide (LPS) can replicate some characteristics of PD, including extensive activation of microglia and selective loss of dopaminergic neurons in the nigrostriatal system [15–19]. The history of understanding LPS starts in the late nineteenth century. LPS is found in the outer membrane of Gram-negative bacteria and acts as endotoxin. LPS from many Gram-negative bacteria species initiates acute inflammatory responses in mammals and induces a diverse range of effects, ranging from pyrexia to Gram-negative septic shock [20]. Thus, using different serotypes of LPS and their different

application routes may cause different outcomes [21]. Moreover, LPSs from different bacteria species share common features in their basic architecture, which consists of three covalently linked segments, a surface carbohydrate polymer (O-specific chain), a core oligosaccharide featuring an outer and inner region, and an acylated glycolipid (termed lipid A). The O-specific chain shows the most diversity and is the basis for serological specificity, while lipid A, which anchors the LPS molecule in the Gram-negative outer membrane, is the most conserved biochemical structure across different bacterial species [20]. There is wide acceptance that the lipid A moiety is the innate immune stimulating or endotoxic component of LPS [22]. In addition, it is documented that LPS-associated pathology results from the stimulation of host cell responses, in which LPS binds to specific receptors in order to elicit the release of cytokines and other inflammatory mediators. Several membrane-bound and soluble proteins have been shown to bind LPS; the most important appear to be CD14 and LPS-binding protein (LBP) and the toll-like receptor (TLR) family which is a recently discovered group of transmembrane receptors [23, 24]. In the central nervous system, it is found that systemic LPS injection upregulated its membrane CD14 receptor within specific cellular populations including microglia in the brain [25]. Thereafter, microglia were identified as the major LPS-responsive cell in the brain. LPS binds to TLR4 on microglia and induces microglial activation that results in neuronal damage [26, 27].

LPS acts as an endotoxin and elicits multiple pathological effects in human beings. One case report may uncover a potential link between LPS infection and the development of Parkinsonism. A 22-year-old laboratory worker was accidentally exposed to 10 μg *Salmonella minnesota* LPS through an open wound and developed Parkinson's syndrome with bradykinesia, rigidity, tremor, and cogwheel phenomenon three weeks later; damage to the substantia nigra and cerebral cortex was shown by positron emission tomography a few years after the accident [28]. However, it is known that LPS from many bacterial species such as *Salmonella, Pseudomonas, Vibrio, and Rhizobium* can initiate acute inflammatory responses in mammals and induce a large and diverse range of effects, ranging from pyrexia and Gram-negative septic shock [29]. There is another case report regarding the *Salmonella* endotoxin exposure. One middle-aged laboratory worker was self-administered intravenously a single large dose of endotoxin (1 mg *Salmonella minnesota* LPS) and immediately developed a severe septic shock syndrome with multiple-organ dysfunction. The patient was successfully rescued in the emergency room, and there has been no follow-up report to date [30]. Thus, further investigation and more epidemiologic data are needed to exploit the relationship between endotoxin and PD.

In the current paper, we present a summary of a variety of LPS PD models and discuss their strengths and limitations, which may be helpful for the future LPS PD study.

2. In Vitro Studies of LPS PD Model

2.1. LPS Treatment to Cell Culture from Mesencephalic Tissue. Bronstein et al. in 1995 reported the comparison study between the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA) and LPS in rat mesencephalic cultures [31]. Investigators found that, in the neuron-enriched cultures, 6-OHDA killed 89% of the tyrosine hydroxylase- (TH-) immunopositive neurons, but LPS (50 μg/mL) was not neurotoxic; however, in the mixed neuron-glial cultures, 6-OHDA killed only 27% of the TH-immunopositive neurons, but LPS killed 70% of the TH-immunopositive neurons. This early experiment suggested that the dopaminergic neurotoxicity of LPS is dependent on the presence of microglia. Subsequently, dopaminergic neurotoxicity of LPS was confirmed by the other groups on rat mesencephalic mixed neuron-glial cultures and demonstrated that LPS induced microglial activation, and activated microglia released the proinflammatory and cytotoxic factors: NO, TNF-α, and IL-1β, which lead to dopaminergic neuron damage [32, 33]. In addition, the dopaminergic neurotoxicity of LPS was studied on mouse mesencephalic neuron-glial culture, and it was found that the neurotoxicity was mainly mediated through LPS-induced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation on microglia, which generated reactive oxygen species production, which are neurotoxic factors [34].

In the studies of LPS-treated primary cultures generated from forebrains of embryonic day 17 mice, the investigators found that the LPS neurotoxicity occurred through binding the signal-transducing receptor, TLR4; microglia are the major cells in the central nervous system that express TLR4. However, it is emphasized that the toxic effect of LPS on neurons is a general phenomenon, independent of neuronal subtype [26]. Thus, LPS may cause neurotoxicity without selectivity for neuronal types. Since previous studies from primary microglia cultures have found LPS treatment induced the release of proinflammatory and cytotoxic factors from microglia [35, 36], the investigators suggested that the activation of TLR4 on microglia may initiate the intracellular signaling pathway of microglia, and result in the release of proinflammatory mediators which cause neuronal damage [26].

3. In Vivo Studies of LPS PD Model

3.1. Intranigral Injection of LPS. In order to study the response of the nigrostriatal system to inflammation, Bing et al. and Castaño et al. independently reported the PD model of LPS intranigral injection in 1998 [15, 16]. After LPS was stereotaxically injected into the nigral area of rats, investigators found that LPS induced microglia activation and dopaminergic neuron loss in the substantia nigra [15, 16]. In a following study, it was reported that LPS-induced dopaminergic neuronal damage was permanent, as observed one year postinjection. Moreover, there was no detectable damage to either the GABAergic or the serotoninergic neurons in the striatum and nigra after LPS injection, indicating that LPS selectively induced dopaminergic neuron death in the nigrostriatal system [37]. Thereafter, more studies confirmed the results and also found the increased level of proinflammatory cytokines including IL-1β, TNF-α, IL-6, and NO in the substantia nigra after LPS injection,

which may be causal factor for LPS-induced neuronal damage [38–40]. In addition, the effects of intranigral LPS injection on behavior and dopamine content and turnover were investigated and showed that LPS treatment enhanced locomotor activity 2- to 3-fold and increased dopamine turnover ratios in comparison with control subjects. This suggests that LPS insult may induce a compensatory response of dopaminergic system [41].

3.2. Intrapallidal Injection of LPS. The globus pallidus is a major integrative nucleus within the basal ganglia, with neurons projecting to striatum, subthalamic nucleus, entopeduncular nucleus, and substantia nigra. Thus, the globus pallidus is positioned to influence the nigrostriatal pathway and function of the basal ganglia as a whole. LPS was injected into the globus pallidus of young and middle-aged rats. The results showed that microglial activation was found in both globus pallidus and substantia nigra, dopaminergic neurons were significantly and progressively decreased in the substantia nigra, and locomotor deficits were detected in animal after LPS injection [17]. Moreover, the following study reported an increased level of proinflammatory cytokines including IL-1β, TNF-α, and IL-6, the elevated expression of inducible nitric oxide synthase, and the enhanced α-synuclein nitration and oligomerization in the substantia nigra of LPS-injected animal [42]. Interestingly, the above pathological changes were much severer in middle-aged animals when compared with the younger animals after LPS treatment, supporting the view that aging itself is a risk factor for PD development [42]. Inflammation promotes the release of neurotoxic factors and the development of synucleinopathy lesions that finally lead to dopaminergic neurodegeneration in PD model of LPS intrapallidal injection. Additionally, the finding of abnormal α-synuclein may help us to explore the mechanisms underlying progressive loss of dopaminergic neurons in LPS PD models. It is reported that aggregated α-synuclein induced microglial activation in a primary mesencephalic neuron-glia culture system [43], thus the pathological process of reactive microgliosis may be triggered and microglial activation may become uncontrolled, which eventually result in progressive dopaminergic neurotoxicity.

3.3. Intrastriatal Injection of LPS. In the nigrostriatal system, the cell bodies of dopaminergic neurons are located in the substantia nigra and their dopamine-containing terminals are distributed in the striatum. After LPS was injected into the striatum of rats, we detected a progressive degeneration of dopamine cell bodies in the substantia nigra and their axonal terminals in the striatum, a depletion of dopamine content in the striatum, cytoplasmic accumulation of α-synuclein and ubiquitin in the nigral dopamine neurons, and behavioral deficits assessed by cylinder test and amphetamine-induced rotational behavior behavioral test [19, 44–46]. Molecular mechanisms underlying the neurotoxicity of LPS intrastriatal injection included activation of microglia, impairment of mitochondria state III and state V respiration, and an increased release of proinflammatory mediators: IL-1β, TNF-α, IL-6, IL-1α, and NO, in both the substantia nigra and the striatum. This indicates that the inflammatory insult or stimuli in the striatum not only directly damaged the terminals of dopaminergic neurons in the striatum, but also indirectly damaged the cell bodies of dopaminergic neurons in the substantia nigra through an unknown retrograde signal transduction pathway [19, 44, 45].

3.4. Intraperitoneal/Systemic Injection of LPS. To study how infectious disease through blood transmission affects the development of neurodegenerative disease in the central nervous system, LPS was systemically injected into animals. Early work reported that after systemic (intraperitoneal or intravenous) injection of LPS, LPS has the ability to target the brain in upregulating its membrane CD14 receptor within specific cellular populations including microglia, which is likely to be responsible for the transcription of proinflammatory cytokines: first within accessible structures from the blood and thereafter through scattered parenchymal cells during severe sepsis [25]. In addition, early work also showed that intraperitoneal endotoxin even at a high dose (2 mg/kg of LPS, which has cardiovascular effects, e.g., a decrease in blood pressure) into rats did not disrupt blood-brain barrier (BBB) permeability, suggesting that intraperitoneal LPS administration is unlikely to contribute to the observed central nervous system mediated effects of endotoxin [47]. However, other studies have found that some cytokines including TNF-α and IL-1 can be transported across the BBB by saturable transport systems, which are able to directly affect central nervous system functions [48, 49]. Using the intraperitoneal injection of LPS in mice, Qin et al. reported that increased cytokine TNF-α due to LPS insult was critical for the transfer of inflammation from the periphery to the central nervous system to induce microglial activation and dopaminergic neuron loss in the substantia nigra at 7 and 9 months posttreatment [18]. Nevertheless, Byler et al. found that systemic LPS injection alone did not affect dopamine levels or Parkinsonian behavioral tests in mice whereas systemic LPS plus MPTP in combination induced the depletion of dopamine in the striatum and Parkinsonian behavioral deficits (reduced stride length) at 4 months postinjection [50]. In addition, MPTP treatment alone reduced striatal dopamine levels quickly but they recovered to normal levels later, addressing the point that nigrostriatal dopamine neurons may succumb after time to multiple toxic agents [50].

4. Implication Using the Animal LPS PD Models

A number of studies have suggested that microglial activation plays a key role in the initiation and progression of PD [8, 12, 13, 51, 52]. LPS PD models provide us with a good tool to investigate the inflammatory process in PD development and anti-inflammation therapy for PD treatment. For example, naloxone, an antagonist of opioid receptors, provided the dopaminergic neuroprotective effects against LPS damage [32, 38, 53]. Interleukin-10, a natural immune modulator, reduced LPS-induced dopaminergic neurotoxicity by inhibiting microglial activation [40, 54]. Pioglitazone, an agonist of peroxisome proliferator-activated

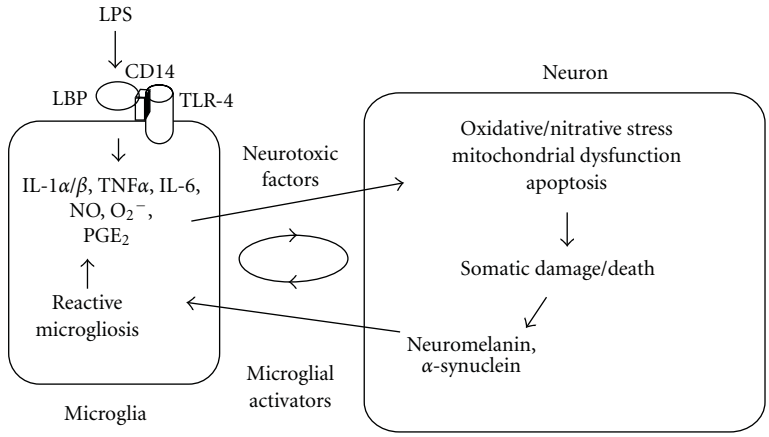

Figure 1: LPS induces progressive neurotoxicity. In response to LPS stimuli, microglial cells are readily activated. It is demonstrated that LPS binds to specific receptors, for example, CD14/TLR4/LBP receptor complex on the microglia, to induce microglial activation. Uncontrolled microglial activation produce a variety of neurotoxic factors such as proinflammatory cytokines (IL-1, TNF-α, IL-6), NO, PGE_2, and O_2^-, which lead to neuronal damage or death through a cascade of events such as oxidative/nitrative stress, mitochondrial dysfunction, and apoptosis. Moreover, damaged neurons may emit injury signals to cause microglia activation, which is defined as reactive microgliosis. The injury signals could be neuromelanin and α-synuclein released by injured dopaminergic neurons. This microglial-neuronal interaction will be reinforced and become a self-amplifying cycle of neuronal injury and microglial activation, which may finally result in the neurodegenerative disease.

receptor gamma, improved dopaminergic neuron survival by restoring mitochondrial function, decreasing the release of proinflammatory mediators and suppressing the oxidative stress [33, 45, 55, 56]. Minocycline, a semisynthetic second-generation tetracycline, exerts potential neuroprotective effects by reducing the inflammatory response and inhibiting apoptotic cell death [57–59]. Among all of these, minocycline is receiving a great deal of attention for its potent antiinflammatory and anti-apoptosis effects. It has been demonstrated that minocycline has few safety concerns and that it should be considered for a large phase III efficacy trial after phase II clinical trials in early PD patients [60, 61]. Currently, minocycline is used in many ongoing clinical trials for various diseases including PD [62].

5. Discussion of LPS PD Models

Mechanisms underlying the LPS PD models are investigated and indicated that LPS induces microglial activation, activated microglia release proinflammatory and neurotoxic factors such as IL-1, TNF-α, IL-6, and NO to cause neuronal damage [40, 42, 63], and damaged neuron may emit injury signals such as neuromelanin and abnormal α-synuclein to trigger reactive microgliosis [43, 64, 65]. This neuronal-microglial interaction may be reinforced and become a self-amplifying cycle to result in progressive dopaminergic neurodegeneration (Figure 1). Based on the application routes in these LPS PD models, we summarize them as follows: (1) LPS is directly and locally applied into the nigrostriatal system and its related structures, such as LPS treatment in mesencephalic cell culture systems in vitro and stereotaxic injection of LPS in nigra, striatum or globus pallidus in vivo; (2) LPS is systemically administered and selectively affects the nigrostriatal system, such as intraperitoneal injection of LPS in vivo. First, let us discuss the local and direct LPS treatment of PD models. Many studies have suggested that dopaminergic neurons are more vulnerable than others in the nigrostriatal system to inflammation-mediated neurotoxicity owing to their precarious redox equilibrium and colocalization with a large population of microglia [2, 66]. Thus, inflammatory responses induced by direct and local LPS treatment may selectively cause dopaminergic neuron damage in mesencephalic tissue in vitro and in the nigrostriatal system in vivo. For stereotaxic injection of LPS in nigra, striatum, or globus pallidus in vivo, because of the smaller size of the nigral area compared with the striatum/globus pallidus area and the dense distribution of dopaminergic neurons in the nigra, intranigral injection itself may cause severe mechanical injury to neurons and glial cells in the nigral area whereas intrastriatal/intrapallidal LPS injection has the advantage of keeping intact the structure of the nigra for enabling the study of the toxic effect of inflammation on neurons. Moreover, intrastriatal/intrapallidal LPS treatment not only induces progressive dopaminergic neuron loss, but also leads to behavioral deficits in animal studies. Thus intrastriatal/intrapallidal LPS injection may be a better PD model in vivo. Next, let us discuss the systemic LPS treatment of PD model. There remains a puzzle how systemic treatment of LPS selectively induced dopaminergic neuron death in the nigrostriatal system of brain. We know that LPS acts as a potent stimulator of microglia and microglia density varies by brain region in human and animals. It has been reported that the level of microglial cells was high in the medulla oblongata and pons in comparison with that in the substantia nigra, hippocampus, thalamus, basal ganglia, and pedunculus cerebri in an adult normal human brain study [67]. Likewise, microglial cells are not uniformly distributed in the normal adult mouse brain. Lawson et al.

reported that microglial densely populated areas include the hippocampus, olfactory telencephalon, basal ganglia, and substantia nigra in the adult mouse brain [68]. Importantly, these studies demonstrate that the density of microglial cells in the substantia nigra is similar to that in the hippocampus, basal ganglia, and so on for both the human and mouse brains. In other words, microglia activation and subsequent proinflammatory cytokines release due to LPS insult may occur in several brain regions, but not in nigral area alone. For example, LPS is also widely used in experimental in vitro and in vivo models of inflammation and amyloidosis for Alzheimer's disease [69, 70]. Thus, it needs further investigation for the selective dopaminergic neurodegeneration in the substantia nigra after systemic LPS treatment. In summary, bacterial endotoxin LPS used as a potent stimulator of glial cells, especially microglia, help us to study the molecular mechanism underlying inflammatory processes in neurodegenerative diseases in the central nervous system. Direct and local LPS treatment in the nigrostriatal system and its related structures may be better PD models to study the etiology and therapeutic strategies for inflammation in PD.

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References

[1] Y. S. Kim and T. H. Joh, "Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease," *Experimental and Molecular Medicine*, vol. 38, no. 4, pp. 333–347, 2006.

[2] M. L. Block, L. Zecca, and J. S. Hong, "Microglia-mediated neurotoxicity: uncovering the molecular mechanisms," *Nature Reviews Neuroscience*, vol. 8, no. 1, pp. 57–69, 2007.

[3] A. Nimmerjahn, F. Kirchhoff, and F. Helmchen, "Neuroscience: resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo," *Science*, vol. 308, no. 5726, pp. 1314–1318, 2005.

[4] D. Davalos, J. Grutzendler, G. Yang et al., "ATP mediates rapid microglial response to local brain injury in vivo," *Nature Neuroscience*, vol. 8, no. 6, pp. 752–758, 2005.

[5] B. P. Cho, D. Y. Song, S. Sugama et al., "Pathological dynamics of activated microglia following medial forebrain bundle transection," *Glia*, vol. 53, no. 1, pp. 92–102, 2006.

[6] W. J. Streit, "Microglia as neuroprotective, immunocompetent cells of the CNS," *Glia*, vol. 40, no. 2, pp. 133–139, 2002.

[7] R. B. Banati, S. E. Daniel, and S. B. Blunt, "Glial pathology but absence of apoptotic nigral neurons in long-standing Parkinson's disease," *Movement Disorders*, vol. 13, no. 2, pp. 221–227, 1998.

[8] J. W. Langston, L. S. Forno, J. Tetrud, A. G. Reeves, J. A. Kaplan, and D. Karluk, "Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure," *Annals of Neurology*, vol. 46, no. 4, pp. 598–605, 1999.

[9] C. F. Orr, D. B. Rowe, Y. Mizuno, H. Mori, and G. M. Halliday, "A possible role for humoral immunity in the pathogenesis of Parkinson's disease," *Brain*, vol. 128, no. 11, pp. 2665–2674, 2005.

[10] M. L. Block and J. S. Hong, "Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism," *Progress in Neurobiology*, vol. 76, no. 2, pp. 77–98, 2005.

[11] W. J. Streit, S. A. Walter, and N. A. Pennell, "Reactive microgliosis," *Progress in Neurobiology*, vol. 57, no. 6, pp. 563–581, 1999.

[12] P. L. McGeer, S. Itagaki, H. Akiyama, and E. G. McGeer, "Rate of cell death in parkinsonism indicates active neuropathological process," *Annals of Neurology*, vol. 24, no. 4, pp. 574–576, 1988.

[13] P. L. McGeer, S. Itagaki, B. E. Boyes, and E. G. McGeer, "Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains," *Neurology*, vol. 38, no. 8, pp. 1285–1291, 1988.

[14] K. Imamura, N. Hishikawa, M. Sawada, T. Nagatsu, M. Yoshida, and Y. Hashizume, "Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains," *Acta Neuropathologica*, vol. 106, no. 6, pp. 518–526, 2003.

[15] G. Bing, X. Lu, N. A. Zheng, L. Jin, Y. Qi, and H.-C. Kim, "Microglia mediated dopaminergic cell death in the substantia nigra: a new animal model for Parkinson's disease," *Neuroscience Abstracts*, vol. 24, p. 44, 1998.

[16] A. Castaño, A. J. Herrera, J. Cano, and A. Machado, "Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system," *Journal of Neurochemistry*, vol. 70, no. 4, pp. 1584–1592, 1998.

[17] J. Zhang, D. M. Stanton, X. V. Nguyen et al., "Intrapallidal lipopolysaccharide injection increases iron and ferritin levels in glia of the rat substantia nigra and induces locomotor deficits," *Neuroscience*, vol. 135, no. 3, pp. 829–838, 2005.

[18] L. Qin, X. Wu, M. L. Block et al., "Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration," *Glia*, vol. 55, no. 5, pp. 453–462, 2007.

[19] D. Y. Choi, M. Liu, R. L. Hunter et al., "Striatal neuroinflammation promotes parkinsonism in rats," *PLoS One*, vol. 4, no. 5, Article ID e5482, 2009.

[20] J. Schletter, H. Heine, A. J. Ulmer, and E. T. Rietschel, "Molecular mechanisms of endotoxin activity," *Archives of Microbiology*, vol. 164, no. 6, pp. 383–389, 1995.

[21] T. Nedrebø and R. K. Reed, "Different serotypes of endotoxin (lipopolysaccharide) cause different increases in albumin extravasation in rats," *Shock*, vol. 18, no. 2, pp. 138–141, 2002.

[22] R. J. Ulevitch and P. S. Tobias, "Recognition of Gram-negative bacteria and endotoxin by the innate immune system," *Current Opinion in Immunology*, vol. 11, no. 1, pp. 19–22, 1999.

[23] K. Takeda, T. Kaisho, and S. Akira, "Toll-like receptors," *Annual Review of Immunology*, vol. 21, pp. 335–376, 2003.

[24] C. A. Janeway Jr. and R. Medzhitov, "Innate immune recognition," *Annual Review of Immunology*, vol. 20, pp. 197–216, 2002.

[25] S. Lacroix, D. Feinstein, and S. Rivest, "The bacterial endotoxin lipopolysaccharide has the ability to target the brain in upregulating its membrane CD14 receptor within specific cellular populations," *Brain Pathology*, vol. 8, no. 4, pp. 625–640, 1998.

[26] S. Lehnardt, L. Massillon, P. Follett et al., "Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 14, pp. 8514–8519, 2003.

[27] K. Hoshino, O. Takeuchi, T. Kawai et al., "Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide evidence for TLR4 as the Lps gene product," *Journal of Immunology*, vol. 162, no. 7, pp. 3749–3752, 1999.

[28] I. Niehaus and J. H. Lange, "Endotoxin: is it an environmental factor in the cause of Parkinson's disease?" *Occupational and Environmental Medicine*, vol. 60, no. 5, p. 378, 2003.

[29] I. Stewart, P. J. Schluter, and G. R. Shaw, "Cyanobacterial lipopolysaccharides and human health—a review," *Environmental Health*, vol. 5, article 7, 2006.

[30] A. M. T. da Silva, H. C. Kaulbach, F. S. Chuidian, D. R. Lambert, A. F. Suffredini, and R. L. Danner, "Brief report: shock and multiple-organ dysfunction after self-administration of salmonella endotoxin," *The New England Journal of Medicine*, vol. 328, no. 20, pp. 1457–1461, 1993.

[31] D. M. Bronstein, I. Perez-Otano, V. Sun et al., "Glia-dependent neurotoxicity and neuroprotection in mesencephalic cultures," *Brain Research*, vol. 704, no. 1, pp. 112–116, 1995.

[32] B. Liu, L. Du, and J. S. Hong, "Naloxone protects rat dopaminergic neurons against inflammatory damage through inhibition of microglia activation and superoxide generation," *Journal of Pharmacology and Experimental Therapeutics*, vol. 293, no. 2, pp. 607–617, 2000.

[33] B. Xing, T. Xin, R. L. Hunter, and G. Bing, "Pioglitazone inhibition of lipopolysaccharide-induced nitric oxide synthase is associated with altered activity of p38 MAP kinase and PI3K/Akt," *Journal of Neuroinflammation*, vol. 5, article 4, 2008.

[34] L. Qin, Y. Liu, T. Wang et al., "NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia," *Journal of Biological Chemistry*, vol. 279, no. 2, pp. 1415–1421, 2004.

[35] S. C. Lee, W. Liu, D. W. Dickson, C. F. Brosnan, and J. W. Berman, "Cytokine production by human fetal microglia and astrocytes: differential induction by lipopolysaccharide and IL-1β," *Journal of Immunology*, vol. 150, no. 7, pp. 2659–2667, 1993.

[36] C. C. Chao, S. Hu, T. W. Molitor, E. G. Shaskan, and P. K. Peterson, "Activated microglia mediate neuronal cell injury via a nitric oxide mechanism," *Journal of Immunology*, vol. 149, no. 8, pp. 2736–2741, 1992.

[37] A. J. Herrera, A. Castaño, J. L. Venero, J. Cano, and A. Machado, "The single intranigral injection of LPS as a new model for studying the selective effects of inflammatory reactions on dopaminergic system," *Neurobiology of Disease*, vol. 7, no. 4, pp. 429–447, 2000.

[38] X. Lu, G. Bing, and T. Hagg, "Naloxone prevents microglia-induced degeneration of dopaminergic substantia nigra neurons in adult rats," *Neuroscience*, vol. 97, no. 2, pp. 285–291, 2000.

[39] M. D. C. Hernández-Romero, S. Argüelles, R. F. Villarán et al., "Simvastatin prevents the inflammatory process and the dopaminergic degeneration induced by the intranigral injection of lipopolysaccharide," *Journal of Neurochemistry*, vol. 105, no. 2, pp. 445–459, 2008.

[40] T. Arimoto, D. Y. Choi, X. Lu et al., "Interleukin-10 protects against inflammation-mediated degeneration of dopaminergic neurons in substantia nigra," *Neurobiology of Aging*, vol. 28, no. 6, pp. 894–906, 2007.

[41] P. F. Hsieh, L. G. Chia, D. R. Ni et al., "Behavior, neurochemistry and histology after intranigral lipopolysaccharide injection," *NeuroReport*, vol. 13, no. 3, pp. 277–280, 2002.

[42] D. Y. Choi, J. Zhang, and G. Bing, "Aging enhances the neuroinflammatory response and α-synuclein nitration in rats," *Neurobiology of Aging*, vol. 31, no. 9, pp. 1649–1653, 2010.

[43] W. Zhang, T. Wang, Z. Pei et al., "Aggregated α-synuclein activates microglia: a process leading to disease progression in Parkinson's disease," *FASEB Journal*, vol. 19, no. 6, pp. 533–542, 2005.

[44] R. L. Hunter, B. Cheng, D. Y. Choi et al., "Intrastriatal lipopolysaccharide injection induces Parkinsonism in C57/B6 mice," *Journal of Neuroscience Research*, vol. 87, no. 8, pp. 1913–1921, 2009.

[45] R. L. Hunter, N. Dragicevic, K. Seifert et al., "Inflammation induces mitochondrial dysfunction and dopaminergic neurodegeneration in the nigrostriatal system," *Journal of Neurochemistry*, vol. 100, no. 5, pp. 1375–1386, 2007.

[46] R. L. Hunter, D. Y. Choi, J. F. Kincer, W. A. Cass, G. Bing, and D. M. Gash, "Fenbendazole treatment may influence lipopolysaccharide effects in rat brain," *Comparative Medicine*, vol. 57, no. 5, pp. 487–492, 2007.

[47] U. Bickel, B. Grave, Y. S. Kang, A. Del Rey, and K. Voigt, "No increase in blood-brain barrier permeability after intraperitoneal injection of endotoxin in the rat," *Journal of Neuroimmunology*, vol. 85, no. 2, pp. 131–136, 1998.

[48] W. Pan and A. J. Kastin, "TNFα transport across the blood-brain barrier is abolished in receptor knockout mice," *Experimental Neurology*, vol. 174, no. 2, pp. 193–200, 2002.

[49] W. A. Banks, "Blood-brain barrier transport of cytokines: a mechanism for neuropathology," *Current Pharmaceutical Design*, vol. 11, no. 8, pp. 973–984, 2005.

[50] S. L. Byler, G. W. Boehm, J. D. Karp et al., "Systemic lipopolysaccharide plus MPTP as a model of dopamine loss and gait instability in C57Bl/6J mice," *Behavioural Brain Research*, vol. 198, no. 2, pp. 434–439, 2009.

[51] P. L. McGeer, C. Schwab, A. Parent, and D. Doudet, "Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration," *Annals of Neurology*, vol. 54, no. 5, pp. 599–604, 2003.

[52] Y. Ouchi, E. Yoshikawa, Y. Sekine et al., "Microglial activation and dopamine terminal loss in early Parkinson's disease," *Annals of Neurology*, vol. 57, no. 2, pp. 168–175, 2005.

[53] B. Liu, J. W. Jiang, B. C. Wilson et al., "Systemic infusion of naloxone reduces degeneration of rat substantia nigral dopaminergic neurons induced by intranigral injection of lipopolysaccharide," *Journal of Pharmacology and Experimental Therapeutics*, vol. 295, no. 1, pp. 125–132, 2000.

[54] L. Qian, M. L. Block, S. J. Wei et al., "Interleukin-10 protects lipopolysaccharide-induced neurotoxicity in primary midbrain cultures by inhibiting the function of NADPH oxidase," *Journal of Pharmacology and Experimental Therapeutics*, vol. 319, no. 1, pp. 44–52, 2006.

[55] R. L. Hunter, D. Y. Choi, S. A. Ross, and G. Bing, "Protective properties afforded by pioglitazone against intrastriatal LPS in Sprague-Dawley rats," *Neuroscience Letters*, vol. 432, no. 3, pp. 198–201, 2008.

[56] B. Xing, M. Liu, and G. Bing, "Neuroprotection with pioglitazone against LPS insult on dopaminergic neurons may be associated with its inhibition of NF-κB and JNK activation and suppression of COX-2 activity," *Journal of Neuroimmunology*, vol. 192, no. 1-2, pp. 89–98, 2007.

[57] M. Tomás-Camardiel, I. Rite, A. J. Herrera et al., "Minocycline reduces the lipopolysaccharide-induced inflammatory reaction, peroxynitrite-mediated nitration of proteins, disruption

of the blood-brain barrier, and damage in the nigral dopaminergic system," *Neurobiology of Disease*, vol. 16, no. 1, pp. 190–201, 2004.

[58] L. W. Fan, YI. Pang, S. Lin et al., "Minocycline reduces lipopolysaccharide-induced neurological dysfunction and brain injury in the neonatal rat," *Journal of Neuroscience Research*, vol. 82, no. 1, pp. 71–82, 2005.

[59] S. M. Lee, T. Y. Yune, S. J. Kim et al., "Minocycline inhibits apoptotic cell death via attenuation of TNF-α expression following iNOS/NO induction by lipopolysaccharide in neuron/glia co-cultures," *Journal of Neurochemistry*, vol. 91, no. 3, pp. 568–578, 2004.

[60] B. Ravina, "A randomized, double-blind, futility clinical trial of creatine and minocycline in early Parkinson disease," *Neurology*, vol. 66, no. 5, pp. 664–671, 2006.

[61] K. Kieburtz, B. Tilley, B. Ravina et al., "A pilot clinical trial of creatine and minocycline in early Parkinson disease: 18-month results," *Clinical Neuropharmacology*, vol. 31, no. 3, pp. 141–150, 2008.

[62] M. O. Griffin, E. Fricovsky, G. Ceballos, and F. Villarreal, "Tetracyclines: a pleitropic family of compounds with promising therapeutic properties. Review of the literature," *American Journal of Physiology*, vol. 299, no. 3, pp. C539–C548, 2010.

[63] B. Xing, T. Xin, R. L. Hunter, and G. Bing, "Pioglitazone inhibition of lipopolysaccharide-induced nitric oxide synthase is associated with altered activity of p38 MAP kinase and PI3K/Akt," *Journal of Neuroinflammation*, vol. 5, article 4, 2008.

[64] L. Zecca, H. Wilms, S. Geick et al., "Human neuromelanin induces neuroinflammation and neurodegeneration in the rat substantia nigra: implications for Parkinson's disease," *Acta Neuropathologica*, vol. 116, no. 1, pp. 47–55, 2008.

[65] H. M. Gao, P. T. Kotzbauer, K. Uryu, S. Leight, J. Q. Trojanowski, and V. M. Y. Lee, "Neuroinflammation and oxidation/nitration of α-synuclein linked to dopaminergic neurodegeneration," *Journal of Neuroscience*, vol. 28, no. 30, pp. 7687–7698, 2008.

[66] L. Zecca, A. Stroppolo, A. Gatti et al., "The role of iron and molecules in the neuronal vulnerability of locus coeruleus and substantia nigra during aging," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 26, pp. 9843–9848, 2004.

[67] M. Mittelbronn, K. Dietz, H. J. Schluesener, and R. Meyermann, "Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude," *Acta Neuropathologica*, vol. 101, no. 3, pp. 249–255, 2001.

[68] L. J. Lawson, V. H. Perry, P. Dri, and S. Gordon, "Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain," *Neuroscience*, vol. 39, no. 1, pp. 151–170, 1990.

[69] J. Miklossy, "Chronic inflammation and amyloidogenesis in Alzheimer's disease—role of spirochetes," *Journal of Alzheimer's Disease*, vol. 13, no. 4, pp. 381–391, 2008.

[70] A. D. Roth, G. Ramírez, R. Alarcón, and R. von Bernhardi, "Oligodendrocytes damage in Alzheimer's disease: beta amyloid toxicity and inflammation," *Biological Research*, vol. 38, no. 4, pp. 381–387, 2005.

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Review Article

The Degenerating Substantia Nigra as a Susceptible Region for Gene Transfer-Mediated Inflammation

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Parkinson's disease (PD) is characterized by the progressive degeneration of neurons in the substantia nigra pars compacta (SN). The naïve SN is highly susceptible to inflammation. In addition, microglial activation in the degenerating SN displays distinct characteristics that increase the reactivity of the region towards inflammatory stimuli. On the other hand, gene therapy for PD has recently move forward into clinical settings, with PD being the neurodegenerative disorder with the highest number of Phase I/II gene therapy clinical trials approved and completed. These clinical trials are not targeting the SN, but this region is a certain candidate for future gene therapy interventions. Here, the unique immune-related properties of the degenerating SN in the context of a putative gene therapy intervention are reviewed. Several variables affecting the host response to gene delivery such as vector type and dosage, age and stage of disease of patients, and method of gene delivery and transgene used are discussed. Finally, approaches to diminish the risk of immune-mediated toxicity by gene transfer in the SN are presented.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterised by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SN) (reviewed in [1]). The aetiology of the most common forms of PD remains unknown. Current therapeutic treatments comprise pharmacological strategies to compensate for dopamine deficiency or surgical interventions that reduce the hyperactivity of specific regions within the basal ganglia (reviewed in [2]). However, dopamine replacement can lead to undesired side-effects 5–10 years after the beginning of treatment [3]. As no treatment is available that can prevent disease progression, the search for new therapeutic interventions is intense. In particular, gene therapy approaches have successfully reached the clinical trial stage in a number of cases [4]. Approved gene therapy clinical trials are based on restoring the activity of the basal ganglia by providing growth factors, inhibiting hyperactive regions, or enhancing dopamine synthesis [4].

Viral gene delivery seems to be the method of choice for gene therapy for PD due to its high efficiency for gene transduction. A drawback to the delivery of genes via viral vectors comes by introducing an antigenic load into the brain. These antigens will invariably elicit a transient innate immune response [5]. The nature and functional (toxic or protective) consequences of this response will vary depending on a number of variables but of utmost importance is the region of gene transfer, the viral dose used, and the state of microglial activation in that region [5]. Importantly, the SN is highly susceptible to the toxic effects of inflammation [6, 7]. In addition, microglial activation during neurodegeneration in this region possesses particular features that could exacerbate disease progression if a proinflammatory stimulus hits the SN [8].

This paper will focus on the properties of microglial activation in the degenerating SN in PD. In addition, the immune reaction after gene delivery by adenoviral, adeno-associated, and lentiviral vectors in the CNS will be discussed. Finally, a list of risk factors and parameters that could be considered when assessing the possible influence of gene transfer to the SN is presented as well as alternative approaches to circumvent inflammation-mediated toxicity.

2. Inflammation in the Central Nervous System

Inflammation in the Central Nervous System (CNS) has different features according to (i) the region in which it occurs, (ii) the stimulus, and (iii) the molecular and cellular milieu at the time of the response. For example, inflammation in the brain parenchyma is usually restricted to certain leukocyte populations, harder to initiate, and less widespread than inflammation when it occurs in the ventricles, meninges, and choroid plexus [9]. At these sites the characteristics are more reminiscent of a typical systemic inflammatory response. This difference is mainly due to the absence of dendritic cells, conventional lymphatics, the downregulation of major histocompatibility complex (MHC) molecules within the CNS parenchyma, and the presence of local immunosuppressive factors (reviewed in [4, 9–11]). In addition, the innate inflammatory response in the CNS parenchyma does not always lead to an activation of the adaptive arm of the immune system (reviewed in [12]).

By origin and function, microglial cells can be regarded as the resident macrophages of the brain and are the main innate immune cells in the CNS. Microglial activation is a highly dynamic process [13–15] and involves phenotypic and reversible transitions that have been categorized into at least 4 stages according to Kreutzberg [16] (see Figure 1). Microglial activation is a patho-physiological feature of many brain diseases and for many years its key function was thought to be solely the removal of cellular debris [13]. Overwhelming evidence now shows that microglial activation is a phenomenon actively involved in neurodegeneration or neuroprotection (reviewed in [13, 17]).

Despite the many differences among animal models of PD and among PD patients, a common feature found in the SN in PD is the presence of microglial activation (reviewed in [13, 17–20]). Since the first description of microglial activation in the SN of PD brains by McGeer and colleagues in 1988 [21], numerous studies (more than 30) have repeated this observation in animal models and PD patients (reviewed in [13, 19]). Microglial-secreted factors that are associated with PD pathology include Interleukin-(IL) 1β, Tumor necrosis Factor α (TNF), IL-6, IL-2, Interferon-γ (IFN-γ), prostaglandins, and reactive oxygen and nitrogen species (for a comprehensive review see [22]). This is unlike astrogliosis, which is not as pronounced nor so consistently present in PD patients or animal models [23, 24]. Therefore, the discussion will be focused on microglial activation. The role of astrocytes in PD has been recently discussed in [19].

3. Microglial Activation and PD-Animal Models

In the 6-OHDA model of nigrostriatal neurodegeneration, microglial activation in the SN was morphological defined as stage II and III, but not IV [24], see Figure 1. During the neurodegenerative process, transcription but not translation of key proinflammatory cytokines, was markedly increased [24]. In this way no proinflammatory environment was generated as a consequence of neuronal cell death. Therefore, microglial activation during neurodegeneration is not associated with the production of a proinflammatory milieu, as previously presupposed [8, 24]. This observation concurs with the physiological role of macrophages during the clearance of apoptotic cells in the periphery where such macrophage activation does not promote inflammation [25]. An example of this would be the clearance of neutrophils: it is estimated that 10^{10} neutrophils/day enter apoptosis and that macrophages are responsible for removing them in humans. Were this process to be proinflammatory, the human body would be permanently inflamed. In PD, most if not all neuronal loss in the SN is supposed to be apoptotic [26] and thus, even though activated microglia are essential to remove neuronal cell debris, a proinflammatory milieu should not be expected from this activation.

Activated microglial cells with higher proinflammatory cytokine mRNA but not protein expression have been described as being in a "primed" state, ready to produce an outburst of proinflammatory cytokines if a second stimulus appears (see Figure 1). Indeed, it has been demonstrated that if a subtoxic dose of a proinflammatory stimulus, such as bacterial endotoxin, is delivered to the degenerating SN, the translation of increased levels of mRNA coding for IL-1 takes place and an intense proinflammatory environment is generated [8]. Interestingly, this effect can also be elicited systemically by the sustained expression of circulating IL-1 [8]. Of utmost importance was the observation that this displacement of the equilibrium towards a proinflammatory milieu in the SN exacerbated disease progression and triggered earlier and more pronounced motor signs [8] (see Figure 1). Reversing the order of the stimuli could lead to a similar or a different observation. It has been described that previous exposure to LPS rendered the animals more susceptible to the neurotoxic effects of 6-OHDA [27]. On the other hand, the prior inoculation of IL-1 has a neuroprotective effects on the nigral neurons [28]. In addition, if this preexposition to inflammation was performed during pregnancy, the adult offspring were not only more susceptible to 6-OHDA administration, but had fewer dopaminergic cells in the SN at postnatal day 10 compared to controls [29, 30].

Neurons in the SN have been shown to be particularly susceptible to microglial-mediated toxicity *in vitro* and *in vivo* [6, 7], and anti-inflammatory interventions have been shown to be neuroprotective in animal models of PD [31–35]. By contrast, some early work has reported neuroprotective effects of inflammatory mediators. Variables such as duration and amount of expression of a specific cytokine seem to be important to anticipate the final effect of a given cytokine on neuronal viability. For example, the acute injection of IL-1β in the SN was not toxic for dopaminergic neurons *in vivo* if the cytokine was injected alone (10 ng or 1000 Units) or in combination with 1000 Units of TNF and 100 Units of IFN-γ in the SN [36, 37]. If, however, the expression of IL-1 or TNF in the SN was sustained between 14 and 21 days, it caused neuronal death, motor symptoms, and microglial activation to Stage IV [38–40]. Similarly, long-term inhibition of IL-1 or TNF attenuated loss of dopaminergic neurons in PD models [8, 41–43].

It can be concluded from these data that dosage and duration of expression are important to predict an effect of

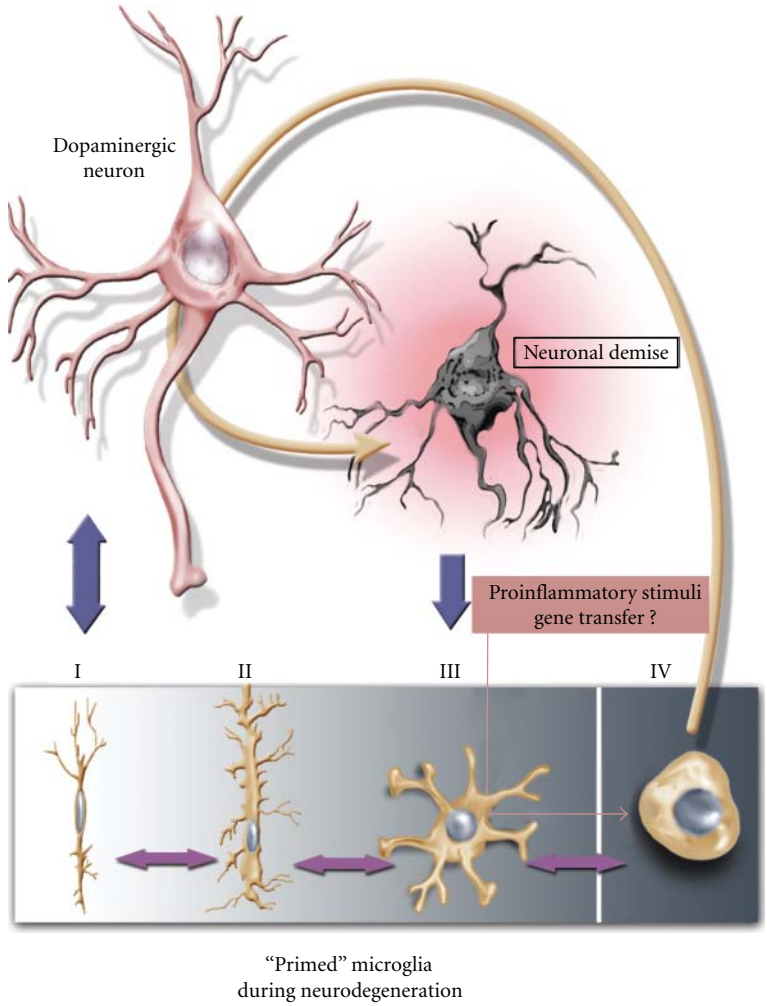

Figure 1: Schematic representation of the interactions between microglial activation and gene transfer. During neuronal death, microglial cells are activated to a "primed" state, where no proinflammatory cytokines are secreted. After receiving an additional (proinflammatory) stimulus, microglia activation changes into an exacerbated proinflammatory state that leads to increased neurodegeneration. Depending on variables such as type and dosage of vector used, inoculation of viral vectors might provide this second proinflammatory stimulus.

a given cytokine on neuronal viability in the SN. However, some univocal effects could be defined. For example, if a subtoxic inflammatory stimulus is present in the degenerating SN where "primed" microglia are present, neuronal death could be exacerbated. Similarly, if the expression of IL-1 or TNF in the SN at proinflammatory levels is sustained, neuronal cell death is likely to occur.

4. Microglial Activation and PD-Clinical Data

As explained above, microglial activation has been found in the SN of PD patients (reviewed in [13, 19]) in postmortem tissue sample and also by noninvasive imaging [20, 44]. In addition, higher expression levels of IL-1, IL-2, IL-6, and TNF were found in striatal postmortem samples of PD patients [45–47]. Using the peripheral benzodiazepine receptor as ligand (PK-11195) in a positron emission tomography study, neuroinflammatory processes have been verified in the pons, basal ganglia, and frontal cortex of PD patients [44]. The resolution of this technique has not allowed an accurate study of the SN. It should also be noted that evidence from PD patients indicates that microglial activation is not restricted to the SN, but it can also involve the putamen, hippocampus, brain stem, and cingulate and temporal cortex [44, 48].

5. Possible Effects of Inflammation-Eliciting Gene Transfer on the Degenerating SN

Viral gene delivery will introduce an antigenic load into the brain [49]. These antigens will invariably elicit at least, a transient innate immune response [49]. As stated before, in a naïve CNS, the innate immune response can be dissociated from the adaptive immune response in the brain parenchyma; for example, the systemic immune system can remain ignorant of a first antigenic challenge into the brain parenchyma. However, what happens if this viral load hits a brain region in which on-going inflammation or "primed"

microglial activation (as most likely will occur in the SN of PD patients) is present? Several variables need to be considered to answer this complex question, including vector type, vector dosage, method of delivery, patients' age, stage of disease progression, and transgene used.

5.1. Vector Type and Dosage. The three main types of vector that emerge as solid candidates for gene therapy clinical trials for PD are adenoviral, adeno-associated, and lentiviral vectors.

Adenoviral vectors are among the most studied vectors for gene delivery in the CNS. In particular, the immune response to this virus in the brain has been extensively investigated. First-generation adenoviral vectors (Fg-Ad) allow transgene expression in the naïve brain for up to one year, which appears to be independent of a transient and initial inflammatory response [8, 12, 38, 39, 49–53]. However, preexisting or subsequent systemic immune response to adenovirus in the host abolish, or at least reduce by half, the transgene expression and can lead to cytotoxicity ([49–52], reviewed in [5, 11]). Direct inoculation of $5 \times 10^6 - 1 \times 10^7$ infective particles of control adenovectors in the SN, caused between 10–25% of neurodegeneration per se [39, 40], reenforcing the idea of an increased susceptibility of this region to inflammation. As is the case with any viral vector, dosage determines the response detected after vector administration. A threshold of more than 10^7 infective particles of adenoviral vectors in the periphery was determined to be needed to eliminate transgene expression in the brain [52]. This drawback is not seen when high capacity adenoviral vectors (hc-Ad) are used for gene transfer in the CNS. Therefore, hcAd seem to be better vectors for gene transfer in the brain than Fg-Ad. Nevertheless, the viral capsid is identical for both vectors and will elicit a response that, within a degenerating SN, may cause an increase in inflammation with toxic effects. In addition, internalized adenoviral DNA activates an inflammasome-dependent maturation of pro-IL-1 to the active form of IL-1 in macrophages. Thus, it is not unlikely that any type of adenoviral vector injected in the degenerating SN will contribute to drive the environment to a proinflammatory milieu [54].

Adeno-associated vectors (AAV) are the vectors of choice for the vast majority of gene therapy clinical trials against PD [4]. The intrastriatal injection of relatively low titers of AAV ($2 - 4 \times 10^8$ i.p.) in naïve animals provokes a low innate immune response with no mononuclear cell infiltrate or cuffing of nearby blood vessels [55, 56]. However, at higher doses, a transient but significant astrogliosis can be detected [57]. In addition, in animal models previously exposed to systemic AAV, immunization inhibited AAV serotype 2 (AAV-2) gene transfer in the CNS [56], and readministration of AAV in the brain induced a greater inflammatory response [56, 58].

Results from Phase I and II clinical trials for PD have not reported severe adverse effects related to AAV administration [59–63]. Gene transfer was performed in the putamen or in the subthalamic nucleus. Despite these encouraging results, long-term analysis of a bigger cohort is needed to provide robust data on the degree of safety of these vectors during gene transfer in the brain. Unfortunately, the possible toxic effects of inflammation that could mask the potential beneficial effect of the treatment were apparently not studied. Recently, a Phase I/II clinical trial has been approved to inoculate patients with AAV vectors expressing neurturin not only in the putamen, but also in the SN (http://www.ClinicalTrials.gov identifier: NCT00985517). According to the data on the susceptibility of the SN to inflammation discussed above, it will be valuable to study the inflammatory response to the treatment in each patient to draw conclusions on safety and possibly efficacy in this trial.

Lentiviral vectors have been approved as vehicles for clinical gene transfer of aromatic amino-acid decarboxylase, Tyrosine Hydroxylase (TH) and GTP-cyclohydrolase 1, all three genes necessary for dopamine synthesis [64]. Stimulation of dendritic cells by lentiviral vectors is weak compared with other single-stranded RNA viruses [65]. In addition, a reduced immune response has been detected after brain administration of multiple-deleted lentiviral vectors [66]. Worryingly, time-and dose-dependent downregulation of TH, the rate limiting enzyme in dopamine synthesis, has been reported after lentiviral-delivery of neurturin [67]. In addition, in the periphery, lentiviral delivery of reporter genes into the lung triggered T-cell mediated immune responses against the transgene [68]. Results from the above-mentioned clinical trial are awaited to verify the seemingly low-level immune response against lentiviral vectors in the brain. Finally, the phenomenon of gene silencing that depends of vector and promoter used and state of differentiation of the target cell should be considered [69]. For example, it could be tempting to try to compensate loss of expression by gene silencing with increased dosage of vector administered, increasing the probability of eliciting a proinflammatory response and therefore toxic effects on the SN.

5.2. Method of Gene Delivery. Independently of the vector of choice, the method of gene delivery may dramatically influence the magnitude and characteristics of the immune reaction against the gene delivery. For example, it is crucial that the method of vector delivery is accurate enough to prevent antigens from reaching the brain ventricular system and the deep cervical lymph nodes so as to keep the systemic immune system ignorant of the antigenic challenge produced by gene delivery in the CNS [5]. If this cannot be achieved, a systemic immune reaction against the viral vector and/or transgene delivered is likely to be generated [5]. At the very least, it will foreshorten the temporal expression profile and, at worst it will promote neurotoxic immune-mediated effects in the brain (reviewed in [11]). In conclusion, a delivery method of high accuracy is needed to prevent antigen diffusion into undesired brain regions.

5.3. Age and Disease Progression. In parallel, age-related changes in immune reactivity include enhanced Blood-Brain Barrier permeability and increased microglial and astroglial reactivity [70–72]. Therefore, changes in the

immune response against viral gene transfer can be expected in a manner that is dependent on the age of the treated subject.

L-Dopa therapy is quite effective in the early stages of most PD patients. Therefore, most clinical trials, including those related to gene therapy, are usually target to late-stage PD patients. It has been proposed that neuroprotective strategies will not be beneficial to these patients since at that stage of the disease there are limited amounts of dopaminergic neurons to protect. Likewise, late-stage PD patients will have encountered more opportunities for neuroinflammation in the SN to start and therefore have a higher risk of vector-mediated toxicity by gene transfer in the SN.

5.4. Transgene Used. Immune responses against the transgene are not infrequent and depend on whether there has been a prior exposure to it and whether it is syn- or xenogenic to the host [11]. In addition, it should also be borne in mind that chronic inflammation can facilitate dendritic cell infiltration into the CNS, which can facilitate antigen presentation to naïve T cells [73]. Again this is a plausible situation in the degenerating SN.

6. How Can All These Risks Be Minimized?

A better understanding of the immunological component of the SN in PD patients together with studies on the possible beneficial effects of complementary anti-inflammatory treatments, changes in vector serotype, novel chemical formulations, and novel vector design will all help to design the best scenario to avoid undesired effects of an inflammatory response to gene delivery in the CNS. Alternatively, taking advantage of certain intrinsic properties of viral vectors might help to circumvent the risk of inflammation in the SN. For example, vectors can be used for the retrograde delivery of genes (e.g., adenoviral vectors could be administered in the striatal terminals of nigral neurons to deliver genes in the SN [38]). Certainly, this strategy has the disadvantage that it can reduce the amount of transgene delivered to the SN as seems to be the case in the Phase II trial with AAV-neurturin [63]. Nevertheless, it is a useful strategy to be considered when planning future gene therapy strategies using different vectors or transgenes. In addition, analyzing risk factors for each treatment and patient (age, method of gene delivery, immunogenicity of vector used, immunological status of the brain area to treat, influence of the transgene to be transferred, dosage, previous exposure to the virus used as vector) will certainly reduce the risk of immune-derived toxicity during gene transfer protocols against PD. In the future, this immunological risk analysis could even be used as an inclusion or exclusion criterium. Unfortunately, nowadays knowledge is still lacking to define parameters with univocal effects on the immunological response of gene transfer into the SN, and technology is behind to determine the immunological status of the SN at the time of gene transfer. Therefore, the most reasonable measurement is to design a clinical trial protocol to reduce the risk of inflammation-mediated toxicity as much as possible.

For example, we would like to propose that if a constellation of risk factors (increased age, late stage of disease, previous exposed to the virus, high viral dose) is present, an anti-inflammatory therapy could be considered [44]. Anti-inflammatory treatments such as COX-2 inhibition, minocycline, and naloxone have promising effects on animal models of PD [31–35]. In the context of a possible inflammatory reaction in a gene therapy protocol in the SN, these anti-inflammatory treatments may be reconsidered as complementary treatments. In addition, not only conventional anti-inflammatory therapies could be helpful to reduce the inflammatory risk of a PD patient, but anti-inflammatory molecules could be delivered by gene transfer in addition to other therapeutic genes. In particular, the viral delivery of Interleukin-10 or IL-1ra has been shown to be neuroprotective in the 6-OHDA rat model of PD [8, 74].

7. Conclusions

The SN is the main area of neurodegeneration in PD. Microglial activation and proinflammatory cytokine production have special characteristics in the degenerating SN that should not be underestimated when designing a gene transfer protocol in that area. It is expected that "primed" microglia or an on-going inflammatory response will be present at the time of gene transfer in PD patients. Numerous variables are in play that could change the expected outcome of gene delivery. An exhaustive analysis of the status of risks factors known to lead to inflammation at the moment of clinical intervention, leading to complementary anti-inflammatory treatments and/or alternative gene delivery strategies or additional genes delivered (e.g., IL-10) is proposed to reduce undesired, inflammation-driven side effects. Gene therapy against PD has reached maturity with eight clinical trials approved. It is of importance to consider, with the limitations of the available technology and knowledge, all variables affecting the immunological status of the PD patient and the possible interactions with the inflammatory component of gene delivery. This analysis should increase the probability of providing safe gene transfer in the SN and reduce inflammation-biased results that can obscure the efficacy of a given gene transfer protocol.

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References

[1] T. Hatano, S. I. Kubo, S. Sato, and N. Hattori, "Pathogenesis of familial Parkinson's disease: new insights based on monogenic forms of Parkinson's disease," *Journal of Neurochemistry*, vol. 111, no. 5, pp. 1075–1093, 2009.

[2] A. E. Lang and A. M. Lozano, "Parkinson's disease: second of two parts," *The New England Journal of Medicine*, vol. 339, no. 16, pp. 1130–1143, 1998.

[3] A. L. Benabid, "Gene therapy for Parkinson's disease: do we have the cure?" *Lancet Neurology*, vol. 9, no. 12, pp. 1142–1143, 2010.

[4] M. M. McMenamin and M. J. A. Wood, "Progress and prospects: immunobiology of gene therapy for neurodegenerative disease: Prospects and risks," *Gene Therapy*, vol. 17, no. 4, pp. 448–458, 2010.

[5] P. R. Lowenstein, K. Kroeger, and M. G. Castro, "Immunology of neurological gene therapy: how T cells modulate viral vector-mediated therapeutic transgene expression through immunological synapses," *Neurotherapeutics*, vol. 4, no. 4, pp. 715–724, 2007.

[6] W. G. Kim, R. P. Mohney, B. Wilson, G. H. Jeohn, B. Liu, and J. S. Hong, "Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia," *Journal of Neuroscience*, vol. 20, no. 16, pp. 6309–6316, 2000.

[7] A. Castaño, A. J. Herrera, J. Cano, and A. Machado, "Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system," *Journal of Neurochemistry*, vol. 70, no. 4, pp. 1584–1592, 1998.

[8] M. C. P. Godoy, R. Tarelli, C. C. Ferrari, M. I. Sarchi, and F. J. Pitossi, "Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease," *Brain*, vol. 131, no. 7, pp. 1880–1894, 2008.

[9] V. H. Perry, "A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation," *Journal of Neuroimmunology*, vol. 90, no. 2, pp. 113–121, 1998.

[10] V. H. Perry, M. D. Bell, H. C. Brown, and M. K. Matyszak, "Inflammation in the nervous system," *Current Opinion in Neurobiology*, vol. 5, no. 5, pp. 636–641, 1995.

[11] P. R. Lowenstein, R. J. Mandel, W. D. Xiong, K. Kroeger, and M. G. Castro, "Immune responses to adenovirus and adeno-associated vectors used for gene therapy of brain diseases: the role of immunological synapses in understanding the cell biology of neuroimmune interactions," *Current Gene Therapy*, vol. 7, no. 5, pp. 347–360, 2007.

[12] P. R. Lowenstein and M. G. Castro, "Inflammation and adaptive immune responses to adenoviral vectors injected into the brain: peculiarities, mechanisms, and consequences," *Gene Therapy*, vol. 10, no. 11, pp. 946–954, 2003.

[13] V. H. Perry, J. A. R. Nicoll, and C. Holmes, "Microglia in neurodegenerative disease," *Nature Reviews Neurology*, vol. 6, no. 4, pp. 193–201, 2010.

[14] M. J. Carson, T. V. Bilousova, S. S. Puntambekar, B. Melchior, J. M. Doose, and I. M. Ethell, "A rose by any other name? The potential consequences of microglial heterogeneity during CNS health and disease," *Neurotherapeutics*, vol. 4, no. 4, pp. 571–579, 2007.

[15] C. A. Colton, "Heterogeneity of microglial activation in the innate immune response in the brain," *Journal of Neuroimmune Pharmacology*, vol. 4, no. 4, pp. 399–418, 2009.

[16] G. W. Kreutzberg, "Microglia: a sensor for pathological events in the CNS," *Trends in Neurosciences*, vol. 19, no. 8, pp. 312–318, 1996.

[17] C. K. Glass, K. Saijo, B. Winner, M. C. Marchetto, and F. H. Gage, "Mechanisms underlying inflammation in neurodegeneration," *Cell*, vol. 140, no. 6, pp. 918–934, 2010.

[18] C. M. Long-Smith, A. M. Sullivan, and Y. M. Nolan, "The influence of microglia on the pathogenesis of Parkinson's disease," *Progress in Neurobiology*, vol. 89, no. 3, pp. 277–287, 2009.

[19] E. C. Hirsch and S. Hunot, "Neuroinflammation in Parkinson's disease: a target for neuroprotection?" *The Lancet Neurology*, vol. 8, no. 4, pp. 382–397, 2009.

[20] Y. Ouchi, S. Yagi, M. Yokokura, and M. Sakamoto, "Neuroinflammation in the living brain of Parkinson's disease," *Parkinsonism and Related Disorders*, vol. 15, supplement 3, pp. S200–S204, 2009.

[21] P. L. McGeer, S. Itagaki, B. E. Boyes, and E. G. McGeer, "Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains," *Neurology*, vol. 38, no. 8, pp. 1285–1291, 1988.

[22] M. G. Tansey and M. S. Goldberg, "Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention," *Neurobiology of Disease*, vol. 37, no. 3, pp. 510–518, 2010.

[23] B. Mirza, H. Hadberg, P. Thomsen, and T. Moos, "The absence of reactive astrocytosis is indicative of a unique inflammatory process in Parkinson's disease," *Neuroscience*, vol. 95, no. 2, pp. 425–432, 1999.

[24] A. M. Depino, C. Earl, E. Kaczmarczyk et al., "Microglial activation with atypical proinflammatory cytokine expression in a rat model of Parkinson's disease," *European Journal of Neuroscience*, vol. 18, no. 10, pp. 2731–2742, 2003.

[25] V. A. Fadok, D. L. Bratton, A. Konowal, P. W. Freed, J. Y. Westcott, and P. M. Henson, "Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF," *Journal of Clinical Investigation*, vol. 110, no. 4, pp. 890–898, 1998.

[26] Y. He, T. Lee, and S. K. Leong, "6-Hydroxydopamine induced apoptosis of dopaminergic cells in the rat substantia nigra," *Brain Research*, vol. 858, no. 1, pp. 163–166, 2000.

[27] J. B. Koprich, C. Reske-Nielsen, P. Mithal, and O. Isacson, "Neuroinflammation mediated by IL-1β increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease," *Journal of Neuroinflammation*, vol. 5, article 8, 2008.

[28] J. Saura, M. Parés, J. Bové et al., "Intranigral infusion of interleukin-1β activates astrocytes and protects from subsequent 6-hydroxydopamine neurotoxicity," *Journal of Neurochemistry*, vol. 85, no. 3, pp. 651–661, 2003.

[29] Z. D. Ling, D. A. Gayle, S. Y. Ma et al., "In utero bacterial endotoxin exposure causes loss of tyrosine hydroxylase neurons in the postnatal rat midbrain," *Movement Disorders*, vol. 17, no. 1, pp. 116–124, 2002.

[30] P. M. Carvey, Q. Chang, J. W. Lipton, and Z. Ling, "Prenatal exposure to the bacteriotoxin lipopolysaccharide leads to long-term losses of dopamine neurons in offspring: a potential, new model of Parkinson's disease," *Frontiers in Bioscience*, vol. 8, pp. s826–s837, 2003.

[31] Y. He, S. Appel, and W. Le, "Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum," *Brain Research*, vol. 909, no. 1-2, pp. 187–193, 2001.

[32] A. Hald and J. Lotharius, "Oxidative stress and inflammation in Parkinson's disease: is there a causal link?" *Experimental Neurology*, vol. 193, no. 2, pp. 279–290, 2005.

[33] R. Sánchez-Pernaute, A. Ferree, O. Cooper, M. Yu, A. L. Brownell, and O. Isacson, "Selective COX-2 inhibition prevents progressive dopamine neuron degeneration in a rat

model of Parkinson's disease," *Journal of Neuroinflammation*, vol. 1, article 6, 2004.

[34] D. C. Wu, V. Jackson-Lewis, M. Vila et al., "Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease," *Journal of Neuroscience*, vol. 22, no. 5, pp. 1763–1771, 2002.

[35] B. Liu, L. Du, and J. S. Hong, "Naloxone protects rat dopaminergic neurons against inflammatory damage through inhibition of microglia activation and superoxide generation," *Journal of Pharmacology and Experimental Therapeutics*, vol. 293, no. 2, pp. 607–617, 2000.

[36] A. Castaño, A. J. Herrera, J. Cano, and A. Machado, "The degenerative effect of a single intranigral injection of LPS on the dopaminergic system is prevented by dexamethasone, and not mimicked by rh-TNF-α IL-1β IFN-γ," *Journal of Neurochemistry*, vol. 81, no. 1, pp. 150–157, 2002.

[37] A. Depino, C. Ferrari, M. C. Pott Godoy, R. Tarelli, and F. J. Pitossi, "Differential effects of interleukin-1β on neurotoxicity, cytokine induction and glial reaction in specific brain regions," *Journal of Neuroimmunology*, vol. 168, no. 1-2, pp. 96–110, 2005.

[38] M. C. Pott Godoy, C. C. Ferrari, and F. J. Pitossi, "Nigral neurodegeneration triggered by striatal AdIL-1 administration can be exacerbated by systemic IL-1 expression," *Journal of Neuroimmunology*, vol. 222, no. 1-2, pp. 29–39, 2010.

[39] A. L. De Lella Ezcurra, M. Chertoff, C. Ferrari, M. Graciarena, and F. Pitossi, "Chronic expression of low levels of tumor necrosis factor-α in the substantia nigra elicits progressive neurodegeneration, delayed motor symptoms and microglia/macrophage activation," *Neurobiology of Disease*, vol. 37, no. 3, pp. 630–640, 2010.

[40] C. C. Ferrari, M. C. Pott Godoy, R. Tarelli, M. Chertoff, A. M. Depino, and F. J. Pitossi, "Progressive neurodegeneration and motor disabilities induced by chronic expression of IL-1β in the substantia nigra," *Neurobiology of Disease*, vol. 24, no. 1, pp. 183–193, 2006.

[41] T. A. Tran, M. K. McCoy, M. B. Sporn, and M. G. Tansey, "The synthetic triterpenoid CDDO-methyl ester modulates microglial activities, inhibits TNF production, and provides dopaminergic neuroprotection," *Journal of Neuroinflammation*, vol. 5, article 14, 2008.

[42] M. K. McCoy, T. N. Martinez, K. A. Ruhn 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," *Journal of Neuroscience*, vol. 26, no. 37, pp. 9365–9375, 2006.

[43] M. K. McCoy, K. A. Ruhn, T. N. Martinez, F. E. McAlpine, A. Blesch, and M. G. Tansey, "Intranigral lentiviral delivery of dominant-negative TNF attenuates neurodegeneration and behavioral deficits in hemiparkinsonian rats," *Molecular Therapy*, vol. 16, no. 9, pp. 1572–1579, 2008.

[44] A. Gerhard, N. Pavese, G. Hotton et al., "In vivo imaging of microglial activation with [C](R)-PK11195 PET in idiopathic Parkinson's disease," *Neurobiology of Disease*, vol. 21, no. 2, pp. 404–412, 2006.

[45] M. Mogi, M. Harada, T. Kondob et al., "Interleukin-1β, interleukin-6, epidermal growth factor and transforming growth factor-α are elevated in the brain from parkinsonian patients," *Neuroscience Letters*, vol. 180, no. 2, pp. 147–150, 1994.

[46] M. Mogi, M. Harada, P. Riederer, H. Narabayashi, K. Fujita, and T. Nagatsu, "Tumor necrosis factor-α (TNF-α) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients," *Neuroscience Letters*, vol. 165, no. 1-2, pp. 208–210, 1994.

[47] M. Mogi, M. Harada, T. Kondo, P. Riederer, and T. Nagatsu, "Interleukin-2 but not basic fibroblast growth factor is elevated in parkinsonian brain. Short communication," *Journal of Neural Transmision*, vol. 103, no. 8-9, pp. 1077–1081, 1996.

[48] K. Imamura, N. Hishikawa, M. Sawada, T. Nagatsu, M. Yoshida, and Y. Hashizume, "Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains," *Acta Neuropathologica*, vol. 106, no. 6, pp. 518–526, 2003.

[49] C. Barcia, M. Jimenez-Dalmaroni, K. M. Kroeger et al., "One-year expression from high-capacity adenoviral vectors in the brains of animals with pre-existing anti-adenoviral immunity: clinical implications," *Molecular Therapy*, vol. 15, no. 12, pp. 2154–2163, 2007.

[50] C. E. Thomas, G. Schiedner, S. Kochanek, M. G. Castro, and P. R. Löwenstein, "Peripheral infection with adenovirus causes unexpected long-term brain inflammation in animals injected intracranially with first-generation, but not with high-capacity, adenovirus vectors: toward realistic long-term neurological gene therapy for chronic diseases," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 13, pp. 7482–7487, 2000.

[51] C. E. Thomas, G. Schiedner, S. Kochanek, M. G. Castro, and P. R. Lowenstein, "Preexisting antiadenoviral immunity is not a barrier to efficient and stable transduction of the brain, mediated by novel high-capacity adenovirus vectors," *Human Gene Therapy*, vol. 12, no. 7, pp. 839–846, 2001.

[52] C. Barcia, C. Gerdes, W. D. Xiong et al., "mmunological thresholds in neurological gene therapy: highly efficient elimination of transduced cells might be related to the specific formation of immunological synapses between T cells and virus-infected brain cells," *Neuron Glia Biology*, vol. 2, no. 4, pp. 309–322, 2006.

[53] C. C. Ferrari, A. M. Depino, F. Prada et al., "Reversible demyelination, blood-brain barrier breakdown, and pronounced neutrophil recruitment induced by chronic IL-1 expression in the brain," *American Journal of Pathology*, vol. 165, no. 5, pp. 1827–1837, 2004.

[54] D. A. Muruve, V. Pétrilli, A. K. Zaiss et al., "The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response," *Nature*, vol. 452, no. 7183, pp. 103–107, 2008.

[55] R. J. Mandel, K. G. Rendahl, S. K. Spratt, R. O. Snyder, L. K. Cohen, and S. E. Leff, "Characterization of intrastriatal recombinant adeno-associated virus- mediated gene transfer of human tyrosine hydroxylase and human GTP- cyclohydrolase I in a rat model of Parkinson's disease," *Journal of Neuroscience*, vol. 18, no. 11, pp. 4271–4284, 1998.

[56] C. S. Peden, C. Burger, N. Muzyczka, and R. J. Mandel, "Circulating anti-wild-type adeno-associated virus type 2 (AAV2) antibodies inhibit recombinant AAV2 (rAAV2)-mediated, but not rAAV5-mediated, gene transfer in the brain," *Journal of Virology*, vol. 78, no. 12, pp. 6344–6359, 2004.

[57] S. Reimsnider, F. P. Manfredsson, N. Muzyczka, and R. J. Mandel, "Time course of transgene expression after intrastriatal pseudotyped rAAV2/1, rAAV2/2, rAAV2/5, and rAAV2/8 transduction in the rat," *Molecular Therapy*, vol. 15, no. 8, pp. 1504–1511, 2007.

[58] M. Y. Mastakov, K. Baer, C. W. Symes, C. B. Leichtlein, R. M. Kotin, and M. J. During, "Immunological aspects of recombinant adeno-associated virus delivery to the mammalian

brain," *Journal of Virology*, vol. 76, no. 16, pp. 8446–8454, 2002.

[59] J. L. Eberling, W. J. Jagust, C. W. Christine et al., "Results from a phase I safety trial of hAADC gene therapy for Parkinson disease," *Neurology*, vol. 70, no. 21, pp. 1980–1983, 2008.

[60] W. J. Marks, J. L. Ostrem, L. Verhagen et al., "Safety and tolerability of intraputaminal delivery of CERE-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial," *The Lancet Neurology*, vol. 7, no. 5, pp. 400–408, 2008.

[61] M. G. Kaplitt, A. Feigin, C. Tang et al., "Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial," *Lancet*, vol. 369, no. 9579, pp. 2097–2105, 2007.

[62] A. Feigin, M. G. Kaplitt, C. Tang et al., "Modulation of metabolic brain networks after subthalamic gene therapy for Parkinson's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 49, pp. 19559–19564, 2007.

[63] W. J. Marks Jr., R. T. Bartus, J. Siffert et al., "Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial," *The Lancet Neurology*, vol. 9, no. 12, pp. 1164–1172, 2010.

[64] B. Jarraya, S. Boulet, G. S. Ralph et al., "Dopamine gene therapy for Parkinson's disease in a nonhuman primate without associated dyskinesia," *Science Translational Medicine*, vol. 1, no. 2, p. 2ra4, 2009.

[65] A. Pichlmair, S. S. Diebold, S. Gschmeissner et al., "Tubulovesicular structures within Vesicular stomatitis virus G protein-pseudotyped lentiviral vector preparations carry DNA and stimulate antiviral responses via toll-like receptor 9," *Journal of Virology*, vol. 81, no. 2, pp. 539–547, 2007.

[66] C. F. Valori, K. Ning, M. Wyles, and M. Azzouz, "Development and applications of non-HIV-based lentiviral vectors in neurological disorders," *Current Gene Therapy*, vol. 8, no. 6, pp. 406–418, 2008.

[67] B. Georgievska, D. Kirik, and A. Björklund, "Overexpression of glial cell line-derived neurotrophic factor using a lentiviral vector induces time- and dose-dependent downregulation of tyrosine hydroxylase in the intact nigrostriatal dopamine system," *Journal of Neuroscience*, vol. 24, no. 29, pp. 6437–6445, 2004.

[68] M. P. Limberis, C. L. Bell, J. Heath, and J. M. Wilson, "Activation of transgene-specific T cells following lentivirus-mediated gene delivery to mouse lung," *Molecular Therapy*, vol. 18, no. 1, pp. 143–150, 2010.

[69] M. Vroemen, N. Weidner, and A. Blesch, "Loss of gene expression in lentivirus- and retrovirus-transduced neural progenitor cells is correlated to migration and differentiation in the adult spinal cord," *Experimental Neurology*, vol. 195, no. 1, pp. 127–139, 2005.

[70] S. Sugama, L. Yang, B. P. Cho et al., "Age-related microglial activation in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration in C57BL/6 mice," *Brain Research*, vol. 964, no. 2, pp. 288–294, 2003.

[71] H. Y. Chung, H. J. Kim, J. W. Kim, and B. P. Yu, "The inflammation hypothesis of aging: molecular modulation by calorie restriction," *Annals of the New York Academy of Sciences*, vol. 928, pp. 327–335, 2001.

[72] C. Pelegrí, A. M. Canudas, J. del Valle et al., "Increased permeability of blood-brain barrier on the hippocampus of a murine model of senescence," *Mechanisms of Ageing and Development*, vol. 128, no. 9, pp. 522–528, 2007.

[73] E. J. McMahon, S. L. Bailey, C. V. Castenada, H. Waldner, and S. D. Miller, "Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis," *Nature Medicine*, vol. 11, no. 3, pp. 335–339, 2005.

[74] L. C. Johnston, X. Su, K. Maguire-Zeiss et al., "Human interleukin-10 gene transfer is protective in a rat model of parkinson's disease," *Molecular Therapy*, vol. 16, no. 8, pp. 1392–1399, 2008.

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Review Article

Parkinson's Disease and Systemic Inflammation

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Peripheral inflammation triggers exacerbation in the central brain's ongoing damage in several neurodegenerative diseases. Systemic inflammatory stimulus induce a general response known as sickness behaviour, indicating that a peripheral stimulus can induce the synthesis of cytokines in the brain. In Parkinson's disease (PD), inflammation was mainly associated with microglia activation that can underlie the neurodegeneration of neurons in the *substantia nigra* (SN). Peripheral inflammation can transform the "primed" microglia into an "active" state, which can trigger stronger responses dealing with neurodegenerative processes. Numerous evidences show that systemic inflammatory processes exacerbate ongoing neurodegeneration in PD patient and animal models. Anti-inflammatory treatment in PD patients exerts a neuroprotective effect. In the present paper, we analyse the effect of peripheral infections in the etiology and progression in PD patients and animal models, suggesting that these peripheral immune challenges can exacerbate the symptoms in the disease.

1. Neurodegenerative Diseases and Systemic Inflammation

Inflammation is a defensive reaction against harmful stimuli that can induce a defensive response in the body. In the central nervous system (CNS), the main innate immune defensive role is played by the immunocompetent resident cells, the microglia [1]. Neurodegenerative diseases present microglia activation as the main hallmark, which can change its morphology from quiescent and ramified (resting) towards a round ameboidal shape (activated) [2]. Resting microglia displays a low-level expression of membrane receptors, such as CD45, CD14, and CD11b [1]. Activated microglia exhibits upregulation of cell surface receptors and proinflammatory and anti-inflammatory cytokines, such as major histocompatibility complex (MHC) class II, CD40, CD80, CD86, CD11b (reviewed by [3]) demonstrating changes in their activity [4, 5] (Figure 1). Microglia can be activated by proinflammatory stimuli, but microglia activation does not always exert a proinflammatory reaction. Microglial activation in some neurodegenerative diseases was not accompanied by proinflammatory cytokine secretions [6, 7]. Depino et al., 2003 demonstrated that microglial cells induced an increase in IL-1β mRNA in the *substantia nigra* (SN) but no translation of this cytokine was observed in an animal model of PD. These observations prompted the idea of "primed microglia" to describe the atypical microglia state, which precedes a further neurotoxic microglial activation as a consequence of a secondary proinflammatory stimulus [8, 9]. Microglia activation increases neurotoxicity and, therefore, contributes to neurodegeneration through the release of free radicals such as superoxide radicals, nitric Oxide (NO), inducible nitric oxide synthase (iNOS) [10–13], and proinflammatory, immunomodulatory and anti-inflammatory cytokines, such as IL-1β, TNF-α, IL-6, IL-8, IL-12, IL-15, and IL-10 [4, 14, 15]. Central or peripheral inflammation can transform the "primed" state of microglia into an "active" state, which can trigger or induce stronger responses dealing with neurodegenerative processes. Therefore, inflammation was mainly associated with microglia activation that can underlie the neurodegeneration of dopaminergic neurons of the SN.

The CNS has been considered as immunologically privileged and protected by the blood brain barrier (BBB) which

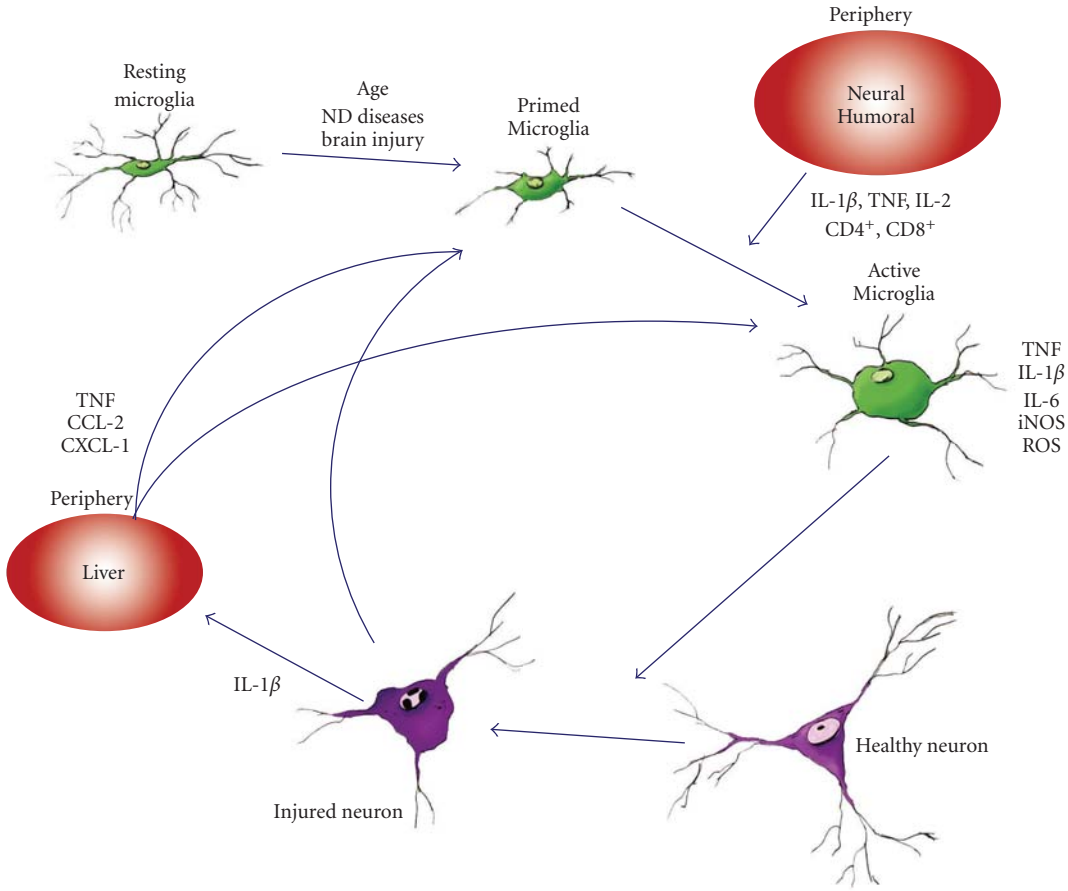

Figure 1: Schematic diagram showing the relationship between peripheral inflammation and neuronal loss in PD. Neurodegenerative diseases present microglial activation as the main hallmark, which can change its morphology from resting (ramified) towards an activated round shape (ameboidal). The intermediate stage, "primed microglia", describes the atypical microglial stage, which precedes a further neurotoxic microglial activation as a consequence of a secondary pro-inflammatory stimulus. This stimulus can come from the periphery, either through neural or humoral pathways. Activated microglia release pro-inflammatory cytokines which can act on neuronal integrity. In addition, acute brain injury induces early hepatic expression of chemokines, which in turn produce recruitment of leukocytes into the blood and subsequently brain and liver inflammation via a chemokines and cytokines web.

prevents entry of pathogens and immune cells into the parenchyma. However, this statement has changed in the last few years, because the communication between central CNS and periphery is more fluid than previously considered. In many neurological disorders, the immune system plays an important role in the progression of these diseases. Indeed, BBB breakdown and inflammation appear to play a major role in the pathology of numerous neurodegenerative diseases compromising the vascular unit and inducing leukocyte migration within the brain parenchyma (reviewed in [16]).

Systemic inflammatory stimuli circulate into the blood and can get into the brain inducing the synthesis of cytokines that, in turn, can induce a general inflammatory response including liver acute phase response and the components to induce sickness behaviour [17–21]. Proinflammatory stimulus would trigger the secretion of proinflammatory molecules in the diseased brain [6] (Figure 1).

Peripheral inflammation sparks off exacerbation in the central brain's ongoing damage in several neurodegenerative diseases, such as Alzheimer's disease (AD), multiple sclerosis, Parkinson's disease, prion disease, stroke, and Wallerian degeneration [8, 9, 22–26]. Indeed, Perry's group has studied the effect of peripheral inflammation on behavioural response, demonstrating a worsening of degenerative processes related to delirium in AD [19, 27]. In particular, PD patients and animal models with ongoing inflammatory neurodegeneration processes evidence exacerbation of the neurodegenerative process after a peripheral inflammatory stimulus [28–33]. Aging was also proposed to prime microglia cells [14]. MHC-II was increased in aged brains and i.p administration of LPS resulted in an increased inflammatory response in elderly patients [15, 34–36].

Previously, we described that the communication between the brain and the periphery as a one way road. However, the central-peripheral relationship is more complex, and the traffic becomes a two way road. Acute brain injury induces early hepatic expression of chemokines, which in turn produce movement of leukocytes into the blood and subsequently brain and liver inflammation [9, 37–40]. The

production of cytokines by the liver as a systemic response to CNS injury is a component of CNS response. The injection of IL-1 into the brain is associated with hepatic expression of CXCL1, which is responsible for neutrophil recruitment to the brain [40]. Hepatic TNF-α is also a component of the systemic response to IL-1β injured brain [39]. TNF-α was also found associated with IL-1β induced sickness behaviour, in addition, the inhibition of peripheral TNF-α can block some components of sickness behaviour induced by centrally injected IL-1β [41]. Peripheral TNF-α appears to be involved in microglial activation and the subsequent recruitment of monocytes into the brain in a model of peripheral liver inflammation resulting from bile duct ligation [42]. Therefore, the hepatic production of cytokines and chemokines may also be considered as a target to neutralize acute and chronic brain injury (Figure 1).

In summary, systemic inflammatory events could influence the aetiology and progression of many ongoing degenerative diseases. Despite previous evidences, the contribution of peripheral inflammation to the progression of neurodegenerative diseases is not fully understood. Analysis of the inflammatory components of the systemic response that influence ongoing damage in the brain should be carefully studied and considered as potential therapeutic targets.

2. Routes of Systemic Inflammation

The circulating cytokines and other inflammatory molecules that are produced by systemically induced insults can affect the brain through several routes, mainly humoral or neural pathways. The humoral route between the nervous and the immune systems has been related to sickness behaviour, characterized by fever, anorexia, and alteration in the behaviour. The humoral mechanisms are mostly related to the presence of the blood brain barrier (BBB). The BBB regulates the passage of substances from the blood to the brain (reviewed in [43]). This barrier can be seriously affected in brain injury. There are several ways of crossing the barrier: (1) the substances can enter through the areas in the CNS that lack BBB, like the circumventricular organs, (2) some molecules may cross the BBB using specific transporters (e.g., cytokines, amines), (3) BBB permeability may be increased as a consequence of the stimuli *per se,* (4) endothelial cells can be activated by the peripheral stimuli, inducing the synthesis of molecules within the CNS [44–46], and (5) the choroidplexus transiently alters its gene expression profile as a response to peripheral LPS stimuli [47]. Systemic injection of LPS can cause BBB damage and allow the entrance of granulocytes from the periphery to the brain [48]. Indeed, BBB breakdown was described in PD patients and animal models [49–53]. The disruption of the BBB allows the extravasation of proinflammatory cytokines and immune cells which can activate microglia in the SN and, therefore, induce neurodegeneration.

The second route of neuroimmune communication, known as the neural pathway, is related to the transmission of peripheral inflammatory signals through the autonomic nervous system. The most important afferent responsible for the neural transmission of peripheral signals is the vagus nerve. Neural pathways are stimulated by peripheral signals that rapidly increase the levels of brain cytokines [2, 54, 55]. Subdiaphragmatic lesion of the vagus nerve and vagotomy attenuate brain cytokine production and behavioural effects after a systemic challenge [56–60]. IL-1β receptors are present on vagal ganglia close to liver and lymphatic nodes [61]. Inflammatory processes in the periphery are conducted to the brain via the vagal afferents and, as a response, the vagal efferents act on the systemic inflammatory events through acetylcholinesterase secretions [25, 54, 62]. In addition, Kamer et al., 2008, suggested that the neural pathway is also involved in the transmission of inflammatory signals from the oral cavity in periodontal diseases, and this mechanism would be related to worsening of AD symptoms [62].

A third potential pathway was recently proposed using a model of inflammatory liver injury [42]. These authors suggested the existence of cellular messengers, activated monocytes, which were recruited into the brain. These activated monocytes secreted messenger molecules, such as TNF-α and MCP-1 (which has been classically defined as humoral) within the brain during systemic inflammatory diseases [42, 63].

3. Parkinson's Disease and Systemic Inflammation: Evidence from the Clinic

Peripheral immunological challenges and chronic inflammatory diseases influence the pathogenesis and progression of PD. The communication between the immune and the nervous system is very fluid, cytokines being the main mediators of inflammation in both brain and periphery. There is evidence that suggests a link between peripheral inflammation and PD. The influenza pandemic during the second world war was associated with an increase in PD in the population [64]. In addition, people infected with Japanese encephalitis virus and H5N1 influenza virus presented a higher risk for developing PD [65, 66].

Activated microglial cells and proinflammatory cytokines, including IL-1β, TNF-α, and IL-6, have been described in SN of postmortem tissue [67, 68], as reviewed in [69]. In vivo studies have also demonstrated that the serum and cerebrospinal fluid of PD patients have higher levels of IL-1β, TNF-α, and IL-2 and also $CD4^+$ and $CD8^+$ T lymphocytes, indicating peripheral activation of lymphocytes [70–74]. The relationship between inflammation and PD has been demonstrated by several authors [3, 69, 75–79]. Subject carriers of IL-1β-511 homozygous variant genotype show a 2-fold increased risk of PD, which induced an increment of susceptibility of dopaminergic neurons to toxicity [80]. On the other hand, increased peripheral cytokine production influences PD progression. PD patients showed elevated serum levels of TNF-α and TNF-α receptor 1 compared to control subjects, which can contribute to PD pathogenesis [72, 81, 82]. Also, elevated plasma concentration of IL-6 correlates with increased risk of PD [52].

Although late onset sporadic PD was recently associated with genetic variation in the HLA DR region, stressing the importance of the immune component in this disease,

[83] PD can also be triggered by diseases that induce systemic infections. Indeed, PD patients often suffer infectious diseases, and the main causes of death are pneumonia and respiratory infections [30–33]. It was described that gastrointestinal infections could contribute to a worsening of PD [84, 85]. However, peripheral immune response in PD patients has shown contradictory results, because some authors have found unaffected levels of cytokines and immune parameters in PD patients [73, 86]. Several studies support a role for the adaptative immune system in PD etiology and progression. The presence of cytotoxic T lymphocyte ($CD4^+$ and $CD8^+$) has been described to infiltrate the SN of patients and animal PD models [87–89]. The influx of these peripheral cells into the brain parenchyma could indicate a BBB dysfunction in PD patients [49, 69]. Indeed, the adaptative immune system might modulate microglia activation in PD pathogenesis [90].

Anti-inflammatory therapies were used in PD patients to decrease the effect of inflammatory reactions. The NSAIDs had an anti-inflammatory and neuroprotective effect in PD. Chronic users of nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, a COX-1 and COX-2 inhibitor, exhibited diminished PD incidence [91, 92]. Minocycline, a derivate of tetracycline that crosses the BBB, also improves neuronal survival in PD. This molecule was described as an inhibitor of microglial activation, proliferation, and release of proinflammatory cytokines [93–95]. It should be taken into consideration that inflammation has a dual action in neurodegeneration, also inducing molecules useful in promoting repair and regeneration of damaged tissue. Therefore, considering the beneficial effects of some of the inflammatory components, restriction of the inflammatory response is not always the best choice. Better alternative therapies should be considered in order to really make progress in this field (reviewed in [96]). These authors propose a multidisciplinary research aimed at protein clearance and immunoprotection induced by T cell regulators.

The studies related to the inflammatory processes in PD should be carefully evaluated in order to develop more suitable tools that will allow us to diminish neurodegeneration and improve the quality of life in PD patients.

4. Parkinson's Disease and Systemic Inflammation: Experimental Evidences

The link between peripheral infection and PD neurodegeneration in both patient and animal models was demonstrated in several studies. LPS causes a systemic inflammatory reaction known as sickness behaviour characterized by fever, anorexia, weight loss, and reduction of activity [97]. A similar response can be obtained with i.p injection of proinflammatory cytokines, such as IL-1β and IL-6 (reviewed in [58]). The resident microglia in the brain responds to these stimuli and generates sickness behaviour. Taking this into consideration, ongoing inflammatory degenerative processes can be accelerated by systemic inflammation. Peripheral inflammatory states, such as infection and injury, can exacerbate neuronal death stimulating "primed microglial cells" towards a more aggressive state.

PD animal models support the previous hypothesis. Pregnant rats exposed to intraperitoneal (i.p.) injection of LPS resulted in a decreased number of dopaminergic neurons in the pups when compared to nonexposed controls [98]. In concordance with Carvey's results, rat foetuses exposed to LPS are more susceptible to 6-OHDA in adulthood [99, 100]. In adult animals, there is also data that strongly suggests the role of peripheral inflammation in the ongoing PD model. Animals with central dopaminergic hypoactivity are associated with an increased peripheral inflammatory response after bacterial LPS injection [101]. Gastrointestinal dysfunctions are related to peripheral inflammation; indeed, ulcerative colitis correlates with increased levels of TNF-α, IL-1β, IL-6, and acute phase proteins in rat serum [102]. Peripheral inflammation induced by ulcerative colitis worsened the effects induced by intranigral LPS, such as loss of dopaminergic neurons, microglial activation, and alteration in BBB permeability [102]. All previous data indicate that the relationship between the peripheral immune system and the central dopaminergic system is very close.

Proinflammatory cytokines, including IL-1β and TNF, have been described as involved in promoting neurodegeneration. These cytokines induced the synthesis of chemokines, producing in turn the recruitment of neutrophils and monocytes from the blood stream. IL-1β alone might induce the cellular recruitment to the brain parenchyma [103–106]. The effects of IL-1β in the progression of neurodegenerative disease have been studied by several groups [9, 23, 28, 29, 37–39, 104, 105, 107]. Systemic challenge with LPS induces CNS IL-1β synthesis and sickness behaviour in animals with ongoing central inflammation [19]. However, systemic inflammation actively inhibits recruitment of leukocytes in the CNS, when LPS is injected 2 hours before the intracerebroventricular injection of IL-1β [108]. Systemic inflammation generated by IL-1β induces BBB disruption and increased brain damage in a model of stroke [9, 23]. In addition, chronic systemic expression of IL-1β was able to exacerbate neuronal demise and microglial activation in the SN of both 6OHDA and an inflammatory PD model [28, 29], increasing the clinical impact of these findings [29]. Exacerbation of neurodegeneration in 6OHDA model was accompanied by an increase in activated microglia in the animals that received IL-1β as peripheral stimulus [28]. The same group has demonstrated that a peripheral systemic stimulus causes exacerbation of the behavioral symptoms and neuronal loss in an inflammatory PD model based on the chronic IL-1β expression in the striatum. These events were accompanied by massive activation of microglia with the concomitant expression of MHC-II [29]. Therefore, the increment in the neurodegenerative process can be correlated with an increase in MHC-II expression induced by a peripheral stimulus [29].

Peripheral induction of TNF-α activates brain microglia that, in turn, produces proinflammatory factors and, as a consequence, induces dopaminergic neuronal loss in the SN [109]. Indeed, dominant negative TNF-α inhibitor displayed neuroprotective properties in both 6OHDA and chronic LPS rat model [110].

However, on the contrary, some inflammatory components of the adaptative immune response were described as immunomodulators of the neurodegenerative process. $CD4^+$ $CD25^+$ regulatory T cells (Tregs) suppress neuroinflammation, attenuate microglia response, and induce nigrostriatal protection in a MPTP model [111, 112]. These cells exert their activity by upregulation of brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), IL-10 and transforming growth factor (TGF-β), and downregulating proinflammatory cytokines and ROS production (reviewed in [111, 113]). These data suggest that Tregs exert their action modulating the immune response, possibly via the interaction between the peripheral and the CNS immune system (Reviewed in [89, 96]). Immunotherapy should be directed towards a Th2 Treg response in order to downregulate the Th1 response [112]. The interaction of Tregs with cells or molecules may modulate the adaptative immune response. Therefore, alterations of the immune system as a consequence of peripheral inflammation could change the immunological properties of Tregs, inducing a phenotype not suitable for the beneficial Treg activation. Further studies should be undertaken before Tregs immunization treatment is to be considered for PD patients with ongoing peripheral infections.

The use of anti-inflammatory drugs has been extensively studied in PD animal models (reviewed in [78]). The role of COX has been studied in an MPTP model demonstrating that indomethacin protects dopaminergic neurons in SN [114]. Treatment with rofecoxib and celecoxib also induces a protective effect in the SN neurons [115–117]. In addition, the use of dexamethasone was demonstrated to reduce the neuronal loss in a 6-OHDA plus LPS exacerbation and, in an inflammatory model of PD, exacerbated with IL-1β as a peripheral inflammatory stimulus [28, 29].

The interaction between brain inflammation and systemic inflammation may be responsible for the progression of neurodegenerative disease. Studying the relationship between CNS and periphery could help find targets for therapeutic treatments.

5. Conclusion

Central or systemic inflammatory insults should be considered as risk factors in the PD aetiology and progression. The clear knowledge of mechanisms implicated in immune/nervous communication and the mechanisms involved in microglia activation and their switch to an aggressive phenotype could help improve the therapeutic tools leading to better patient quality of life, reducing the exacerbation of PD symptoms, and delaying the progression of the disease.

References

[1] G. W. Kreutzberg, "Microglia: a sensor for pathological events in the CNS," *Trends in Neurosciences*, vol. 19, no. 8, pp. 312–318, 1996.

[2] V. H. Perry, J. A. R. Nicoll, and C. Holmes, "Microglia in neurodegenerative disease," *Nature Reviews Neurology*, vol. 6, no. 4, pp. 193–201, 2010.

[3] P. S. Whitton, "Inflammation as a causative factor in the aetiology of Parkinson's disease," *British Journal of Pharmacology*, vol. 150, no. 8, pp. 963–976, 2007.

[4] S. U. Kim and J. de Vellis, "Microglia in health and disease," *Journal of Neuroscience Research*, vol. 81, no. 3, pp. 302–313, 2005.

[5] R. M. Ransohoff and V. H. Perry, "Microglial physiology: unique stimuli, specialized responses," *Annual Review of Immunology*, vol. 27, pp. 119–145, 2009.

[6] V. H. Perry, C. Cunningham, and D. Boche, "Atypical inflammation in the central nervous system in prion disease," *Current Opinion in Neurology*, vol. 15, no. 3, pp. 349–354, 2002.

[7] A. M. Depino, C. Earl, E. Kaczmarczyk et al., "Microglial activation with atypical proinflammatory cytokine expression in a rat model of Parkinson's disease," *European Journal of Neuroscience*, vol. 18, no. 10, pp. 2731–2742, 2003.

[8] C. Cunningham, D. C. Wilcockson, S. Campion, K. Lunnon, and V. H. Perry, "Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration," *Journal of Neuroscience*, vol. 25, no. 40, pp. 9275–9284, 2005.

[9] B. W. McColl, N. J. Rothwell, and S. M. Allan, "Systemic inflammatory stimulus potentiates the acute phase and CXC chemokine responses to experimental stroke and exacerbates brain damage via interleukin-1- and neutrophil-dependent mechanisms," *Journal of Neuroscience*, vol. 27, no. 16, pp. 4403–4412, 2007.

[10] T. Arimoto and G. Bing, "Up-regulation of inducible nitric oxide synthase in the substantia nigra by lipopolysaccharide causes microglial activation and neurodegeneration," *Neurobiology of Disease*, vol. 12, no. 1, pp. 35–45, 2003.

[11] M. M. Iravani, K. Kashefi, P. Mander, S. Rose, and P. Jenner, "Involvement of inducible nitric oxide synthase in inflammation-induced dopaminergic neurodegeneration," *Neuroscience*, vol. 110, no. 1, pp. 49–58, 2002.

[12] A. Czlonkowska, I. Kurkowska-Jastrzebska, A. Czlonkowski, D. Peter, and G. B. Stefano, "Immune processes in the pathogenesis of Parkinson's disease—a potential role for microglia and nitric oxide," *Medical Science Monitor*, vol. 8, no. 8, pp. RA165–RA177, 2002.

[13] L. Minghetti, E. Polazzi, A. Nicolini, A. Greco, and G. Levi, "Possible role of microglial prostanoids and free radicals in neuroprotection and neurodegeneration," *Advances in Experimental Medicine and Biology*, vol. 468, pp. 109–119, 1999.

[14] R. N. Dilger and R. W. Johnson, "Aging, microglial cell priming, and the discordant central inflammatory response to signals from the peripheral immune system," *Journal of Leukocyte Biology*, vol. 84, no. 4, pp. 932–939, 2008.

[15] C. J. Henry, Y. Huang, A. M. Wynne, and J. P. Godbout, "Peripheral lipopolysaccharide (LPS) challenge promotes microglial hyperactivity in aged mice that is associated with exaggerated induction of both pro-inflammatory IL-1β and anti-inflammatory IL-10 cytokines," *Brain, Behavior, and Immunity*, vol. 23, no. 3, pp. 309–317, 2009.

[16] H. B. Stolp and K. M. Dziegielewska, "Review: role of developmental inflammation and blood-brain barrier dysfunction in neurodevelopmental and neurodegenerative diseases," *Neuropathology and Applied Neurobiology*, vol. 35, no. 2, pp. 132–146, 2009.

[17] H. O. Besedovsky and A. del Rey, "Immune-neuro-endocrine interactions: facts and hypotheses," *Endocrine Reviews*, vol. 17, no. 1, pp. 64–102, 1996.

[18] F. Pitossi, A. del Rey, A. Kabiersch, and H. Besedovsky, "Induction of cytokine transcripts in the central nervous system and pituitary following peripheral administration of endotoxin to mice," *Journal of Neuroscience Research*, vol. 48, no. 4, pp. 287–298, 1997.

[19] M. I. Combrinck, V. H. Perry, and C. Cunningham, "Peripheral infection evokes exaggerated sickness behaviour in preclinical murine prion disease," *Neuroscience*, vol. 112, no. 1, pp. 7–11, 2002.

[20] V. H. Perry, "The influence of systemic inflammation on inflammation in the brain: implications for chronic neurodegenerative disease," *Brain, Behavior, and Immunity*, vol. 18, no. 5, pp. 407–413, 2004.

[21] D. Londono and D. Cadavid, "Bacterial lipoproteins can disseminate from the periphery to inflame the brain," *American Journal of Pathology*, vol. 176, no. 6, pp. 2848–2857, 2010.

[22] C. Cunningham, D. C. Wilcockson, D. Boche, and V. H. Perry, "Comparison of inflammatory and acute-phase responses in the brain and peripheral organs of the ME7 model of prion disease," *Journal of Virology*, vol. 79, no. 8, pp. 5174–5184, 2005.

[23] B. W. McColl, N. J. Rothwell, and S. M. Allan, "Systemic inflammation alters the kinetics of cerebrovascular tight junction disruption after experimental stroke in mice," *Journal of Neuroscience*, vol. 28, no. 38, pp. 9451–9462, 2008.

[24] S. J. Spencer, A. Mouihate, and Q. J. Pittman, "Peripheral inflammation exacerbates damage after global ischemia independently of temperature and acute brain inflammation," *Stroke*, vol. 38, no. 5, pp. 1570–1577, 2007.

[25] V. H. Perry, C. Cunningham, and C. Holmes, "Systemic infections and inflammation affect chronic neurodegeneration," *Nature Reviews Immunology*, vol. 7, no. 2, pp. 161–167, 2007.

[26] K. Palin, C. Cunningham, P. Forse, V. H. Perry, and N. Platt, "Systemic inflammation switches the inflammatory cytokine profile in CNS Wallerian degeneration," *Neurobiology of Disease*, vol. 30, no. 1, pp. 19–29, 2008.

[27] C. Cunningham, S. Campion, J. Teeling, L. Felton, and V. H. Perry, "The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C)," *Brain, Behavior, and Immunity*, vol. 21, no. 4, pp. 490–502, 2007.

[28] M. C. P. Godoy, R. Tarelli, C. C. Ferrari, M. I. Sarchi, and F. J. Pitossi, "Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease," *Brain*, vol. 131, no. 7, pp. 1880–1894, 2008.

[29] M. C. Pott Godoy, C. C. Ferrari, and F. J. Pitossi, "Nigral neurodegeneration triggered by striatal AdIL-1 administration can be exacerbated by systemic IL-1 expression," *Journal of Neuroimmunology*, vol. 222, no. 1-2, pp. 29–39, 2010.

[30] Y. Hasegawa, T. Inagaki, M. Sawada, and A. Suzumura, "Impaired cytokine production by peripheral blood mononuclear cells and monocytes/macrophages in Parkinson's disease," *Acta Neurologica Scandinavica*, vol. 101, no. 3, pp. 159–164, 2000.

[31] M. K. Beyer, K. Herlofson, D. Arsland, and J. P. Larsen, "Causes of death in a community-based study of Parkinson's disease," *Acta Neurologica Scandinavica*, vol. 103, pp. 7–11, 2001.

[32] M. D'Amelio, P. Ragonese, L. Morgante et al., "Long-term survival of Parkinson's disease: a population-based study," *Journal of Neurology*, vol. 253, no. 1, pp. 33–37, 2006.

[33] H. Arai, T. Furuya, Y. Mizuno, and H. Mochizuki, "Inflammation and infection in Parkinson's disease," *Histology and Histopathology*, vol. 21, no. 4–6, pp. 673–678, 2006.

[34] J. P. Godbout, J. Chen, J. Abraham et al., "Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system," *FASEB Journal*, vol. 19, no. 10, pp. 1329–1331, 2005.

[35] J. Chen, J. B. Buchanan, N. L. Sparkman, J. P. Godbout, G. G. Freund, and R. W. Johnson, "Neuroinflammation and disruption in working memory in aged mice after acute stimulation of the peripheral innate immune system," *Brain, Behavior, and Immunity*, vol. 22, no. 3, pp. 301–311, 2008.

[36] M. Sawada, H. Sawada, and T. Nagatsu, "Effects of aging on neuroprotective and neurotoxic properties of microglia in neurodegenerative diseases," *Neurodegenerative Diseases*, vol. 5, no. 3-4, pp. 254–256, 2008.

[37] D. C. Wilcockson, S. J. Campbell, D. C. Anthony, and V. H. Perry, "The systemic and local acute phase response following acute brain injury," *Journal of Cerebral Blood Flow and Metabolism*, vol. 22, no. 3, pp. 318–326, 2002.

[38] S. J. Campbell, P. M. Hughes, J. P. Iredale et al., "CINC-1 is an acute-phase protein induced by focal brain injury causing leukocyte mobilization and liver injury," *FASEB Journal*, vol. 17, no. 9, pp. 1168–1170, 2003.

[39] S. J. Campbell, R. M. J. Deacon, Y. Jiang, C. Ferrari, F. J. Pitossi, and D. C. Anthony, "Overexpression of IL-1β by adenoviral-mediated gene transfer in the rat brain causes a prolonged hepatic chemokine response, axonal injury and the suppression of spontaneous behaviour," *Neurobiology of Disease*, vol. 27, no. 2, pp. 151–163, 2007.

[40] S. J. Campbell, V. H. Perry, F. J. Pitossi et al., "Central nervous system injury triggers Hepatic CC and CXC chemokine expression that is associated with leukocyte mobilization and recruitment to both the central nervous system and the liver," *American Journal of Pathology*, vol. 166, no. 5, pp. 1487–1497, 2005.

[41] Y. Jiang, R. Deacon, D. C. Anthony, and S. J. Campbell, "Inhibition of peripheral TNF can block the malaise associated with CNS inflammatory diseases," *Neurobiology of Disease*, vol. 32, no. 1, pp. 125–132, 2008.

[42] C. D'Mello, T. Le, and M. G. Swain, "Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factora signaling during peripheral organ inflammation," *Journal of Neuroscience*, vol. 29, no. 7, pp. 2089–2102, 2009.

[43] B. V. Zlokovic, "The blood-brain barrier in health and chronic neurodegenerative disorders," *Neuron*, vol. 57, no. 2, pp. 178–201, 2008.

[44] R. Dantzer, "Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity," *European Journal of Pharmacology*, vol. 500, no. 1–3, pp. 399–411, 2004.

[45] R. Dantzer and K. W. Kelley, "Twenty years of research on cytokine-induced sickness behavior," *Brain, Behavior, and Immunity*, vol. 21, no. 2, pp. 153–160, 2007.

[46] L. E. Goehler, A. Erisir, and R. P. A. Gaykema, "Neural-immune interface in the rat area postrema," *Neuroscience*, vol. 140, no. 4, pp. 1415–1434, 2006.

[47] F. Marques, J. C. Sousa, G. Coppola et al., "The choroid plexus response to a repeated peripheral inflammatory stimulus," *BMC Neuroscience*, vol. 10, article 135, 2009.

[48] M. Bohatschek, A. Werner, and G. Raivich, "Systemic LPS injection leads to granulocyte influx into normal and injured brain: effects of ICAM-1 deficiency," *Experimental Neurology*, vol. 172, no. 1, pp. 137–152, 2001.

[49] R. Kortekaas, K. L. Leenders, J. C. H. van Oostrom et al., "Blood-brain barrier dysfunction in Parkinsonian midbrain in vivo," *Annals of Neurology*, vol. 57, no. 2, pp. 176–179, 2005.

[50] P. M. Carvey, C. H. Zhao, B. Hendey et al., "6-hydroxydopamine-induced alterations in blood-brain barrier permeability," *European Journal of Neuroscience*, vol. 22, no. 5, pp. 1158–1168, 2005.

[51] C. Barcia, V. Bautista, A. Sanchez-Bahillo et al., "Changes in vascularization in substantia nigra pars compacta of monkeys rendered parkinsonian," *Journal of Neural Transmission*, vol. 112, no. 9, pp. 1237–1248, 2005.

[52] H. Chen, E. J. O'Reilly, M. A. Schwarzschild, and A. Ascherio, "Peripheral inflammatory biomarkers and risk of Parkinson's disease," *American Journal of Epidemiology*, vol. 167, no. 1, pp. 90–95, 2008.

[53] E. Yan, M. Castillo-Melendez, T. Nicholls, J. Hirst, and D. Walker, "Cerebrovascular responses in the fetal sheep brain to low-dose endotoxin," *Pediatric Research*, vol. 55, no. 5, pp. 855–863, 2004.

[54] K. J. Tracey, "The inflammatory reflex," *Nature*, vol. 420, no. 6917, pp. 853–859, 2002.

[55] V. H. Perry, T. A. Newman, and C. Cunningham, "The impact of systemic infection on the progression of neurodegenerative disease," *Nature Reviews Neuroscience*, vol. 4, no. 2, pp. 103–112, 2003.

[56] R. M. Bluthe, B. Michaud, K. W. Kelley, and R. Dantzer, "Vagotomy blocks behavioural effects of interleukin-1 injected via the intraperitoneal route but not via other systemic routes," *NeuroReport*, vol. 7, no. 15–17, pp. 2823–2827, 1996.

[57] R. M. Bluthe, B. Michaud, K. W. Kelley, and R. Dantzer, "Vagotomy attenuates behavioural effects of interleukin-1 injected peripherally but not centrally," *NeuroReport*, vol. 7, no. 9, pp. 1485–1488, 1996.

[58] R. Dantzer, R. M. Bluthe, S. Laye, J. L. Bret-Dibat, P. Parnet, and K. W. Kelley, "Cytokines and sickness behavior," *Annals of the New York Academy of Sciences*, vol. 840, pp. 586–590, 1998.

[59] R. M. Bluthe, V. Walter, P. Parnet et al., "Lipopolysaccharide induces sickness behaviour in rats by a vagal mediated mechanism," *Comptes Rendus de l'Academie des Sciences*, vol. 317, no. 6, pp. 499–503, 1994.

[60] M. K. Hansen, P. Taishi, Z. Chen, and J. M. Krueger, "Vagotomy blocks the induction of interleukin-1β (IL-1β) mRNA in the brain of rats in response to systemic IL-1β," *Journal of Neuroscience*, vol. 18, no. 6, pp. 2247–2253, 1998.

[61] L. E. Goehler, R. P. A. Gaykema, K. T. Nguyen et al., "Interleukin-1β in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems?" *Journal of Neuroscience*, vol. 19, no. 7, pp. 2799–2806, 1999.

[62] A. R. Kamer, R. G. Craig, A. P. Dasanayake, M. Brys, L. Glodzik-Sobanska, and M. J. de Leon, "Inflammation and Alzheimer's disease: possible role of periodontal diseases," *Alzheimer's and Dementia*, vol. 4, no. 4, pp. 242–250, 2008.

[63] W. Rostene, P. Kitabgi, and S. M. Parsadaniantz, "Chemokines: a new class of neuromodulator?" *Nature Reviews Neuroscience*, vol. 8, no. 11, pp. 895–904, 2007.

[64] R. C. Dale, A. J. Church, R. A. H. Surtees et al., "Encephalitis lethargica syndrome: 20 new cases and evidence of basal ganglia autoimmunity," *Brain*, vol. 127, no. 1, pp. 21–33, 2004.

[65] H. Shoji, M. Watanabe, S. Itoh, H. Kuwahara, and F. Hattori, "Japanese encephalitis and parkinsonism," *Journal of Neurology*, vol. 240, no. 1, pp. 59–60, 1993.

[66] H. Jang, D. Boltz, K. Sturm-Ramirez et al., "Highly pathogenic H5N1 influenza virus can enter the central nervous system and induce neuroinflammation and neurodegeneration," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 33, pp. 14063–14068, 2009.

[67] M. Mogi, M. Harada, T. Kondob et al., "Interleukin-1β, interleukin-6, epidermal growth factor and transforming growth factor-α are elevated in the brain from parkinsonian patients," *Neuroscience Letters*, vol. 180, no. 2, pp. 147–150, 1994.

[68] M. Mogi, M. Harada, H. Narabayashi, H. Inagaki, M. Minami, and T. Nagatsu, "Interleukin (IL)-1β, IL-2, IL-4, IL-6 and transforming growth factor-α levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease," *Neuroscience Letters*, vol. 211, no. 1, pp. 13–16, 1996.

[69] E. C. Hirsch and S. Hunot, "Neuroinflammation in Parkinson's disease: a target for neuroprotection?" *Lancet Neurology*, vol. 8, no. 4, pp. 382–397, 2009.

[70] R. J. Dobbs, A. Charlett, A. G. Purkiss, S. M. Dobbs, C. Weller, and D. W. Peterson, "Association of circulating TNF-α and IL-6 with ageing and parkinsonism," *Acta Neurologica Scandinavica*, vol. 100, no. 1, pp. 34–41, 1999.

[71] D. Blum-Degena, T. Muller, W. Kuhn, M. Gerlach, H. Przuntek, and P. Riederer, "Interleukin-1β and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients," *Neuroscience Letters*, vol. 202, no. 1-2, pp. 17–20, 1995.

[72] M. Reale, C. Iarlori, A. Thomas et al., "Peripheral cytokines profile in Parkinson's disease," *Brain, Behavior, and Immunity*, vol. 23, no. 1, pp. 55–63, 2009.

[73] K. Hisanaga, M. Asagi, Y. Itoyama, and Y. Iwasaki, "Increase in peripheral CD4 bright+ CD8 dull+T cells in Parkinson disease," *Archives of Neurology*, vol. 58, no. 10, pp. 1580–1583, 2001.

[74] J. Bas, M. Calopa, M. Mestre et al., "Lymphocyte populations in Parkinson's disease and in rat models of parkinsonism," *Journal of Neuroimmunology*, vol. 113, no. 1, pp. 146–152, 2001.

[75] M. G. Tansey, M. K. McCoy, and T. C. Frank-Cannon, "Neuroinflammatory mechanisms in Parkinson's disease: potential environmental triggers, pathways, and targets for early therapeutic intervention," *Experimental Neurology*, vol. 208, no. 1, pp. 1–25, 2007.

[76] T. C. Frank-Cannon, L. T. Alto, F. E. McAlpine, and M. G. Tansey, "Does neuroinflammation fan the flame in neurodegenerative diseases?" *Molecular Neurodegeneration*, vol. 4, no. 1, article 47, 2009.

[77] Y. C. Chung, H. W. Ko, E. Bok et al., "The role of neuroinflammation on the pathogenesis of Parkinson's disease," *BMB Reports*, vol. 43, no. 4, pp. 225–232, 2010.

[78] E. Esposito, V. Di Matteo, A. Benigno, M. Pierucci, G. Crescimanno, and G. Di Giovanni, "Non-steroidal anti-inflammatory drugs in Parkinson's disease," *Experimental Neurology*, vol. 205, no. 2, pp. 295–312, 2007.

[79] J. B. Koprich, C. Reske-Nielsen, P. Mithal, and O. Isacson, "Neuroinflammation mediated by IL-1β increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease," *Journal of Neuroinflammation*, vol. 5, article 8, 2008.

[80] A. D. Wahner, J. S. Sinsheimer, J. M. Bronstein, and B. Ritz, "Inflammatory cytokine gene polymorphisms and increased risk of Parkinson disease," *Archives of Neurology*, vol. 64, no. 6, pp. 836–840, 2007.

[81] P. Scalzo, A. Kummer, F. Cardoso, and A. L. Teixeira, "Increased serum levels of soluble tumor necrosis factor-α receptor-1 in patients with Parkinson's disease," *Journal of Neuroimmunology*, vol. 216, no. 1-2, pp. 122–125, 2009.

[82] M. Dufek, M. Hamanova, J. Lokaj et al., "Serum inflammatory biomarkers in Parkinson's disease," *Parkinsonism and Related Disorders*, vol. 15, no. 4, pp. 318–320, 2009.

[83] T. H. Hamza, C. P. Zabetian, A. Tenesa et al., "Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease," *Nature Genetics*, vol. 42, no. 9, pp. 781–785, 2010.

[84] H. Przuntek, T. Muller, and P. Riederer, "Diagnostic staging of Parkinson's disease: conceptual aspects," *Journal of Neural Transmission*, vol. 111, no. 2, pp. 201–216, 2004.

[85] C. Weller, N. Oxlade, S. M. Dobbs, R. J. Dobbs, A. Charlett, and I. T. Bjarnason, "Role of inflammation in gastrointestinal tract in aetiology and pathogenesis of idiopathic parkinsonism," *FEMS Immunology and Medical Microbiology*, vol. 44, no. 2, pp. 129–135, 2005.

[86] Y. Baba, A. Kuroiwa, R. J. Uitti, Z. K. Wszolek, and T. Yamada, "Alterations of T-lymphocyte populations in Parkinson disease," *Parkinsonism and Related Disorders*, vol. 11, no. 8, pp. 493–498, 2005.

[87] H. Akiyama, S. Itagaki, and P. L. McGeer, "Major histocompatibility complex antigen expression on rat microglia following epidural kainic acid lesions," *Journal of Neuroscience Research*, vol. 20, pp. 147–157, 1988.

[88] V. Brochard, B. Combadiere, A. Prigent et al., "Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease," *Journal of Clinical Investigation*, vol. 119, no. 1, pp. 182–192, 2009.

[89] D. K. Stone, A. D. Reynolds, R. L. Mosley, and H. E. Gendelman, "Innate and adaptive immunity for the pathobiology of Parkinson's disease," *Antioxidants and Redox Signaling*, vol. 11, no. 9, pp. 2151–2166, 2009.

[90] M. G. Tansey and M. S. Goldberg, "Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention," *Neurobiology of Disease*, vol. 37, no. 3, pp. 510–518, 2010.

[91] H. Chen, S. M. Zhang, M. A. Hernan et al., "Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease," *Archives of Neurology*, vol. 60, no. 8, pp. 1059–1064, 2003.

[92] H. Chen, E. Jacobs, M. A. Schwarzschild et al., "Nonsteroidal antiinflammatory drug use and the risk for Parkinson's disease," *Annals of Neurology*, vol. 58, no. 6, pp. 963–967, 2005.

[93] T. M. Tikka and J. E. Koistinaho, "Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia," *Journal of Immunology*, vol. 166, no. 12, pp. 7527–7533, 2001.

[94] H. S. Kim and Y. H. Suh, "Minocycline and neurodegenerative diseases," *Behavioural Brain Research*, vol. 196, no. 2, pp. 168–179, 2009.

[95] M. Tomas-Camardiel, I. Rite, A. J. Herrera et al., "Minocycline reduces the lipopolysaccharide-induced inflammatory reaction, peroxynitrite-mediated nitration of proteins, disruption of the blood-brain barrier, and damage in the nigral dopaminergic system," *Neurobiology of Disease*, vol. 16, no. 1, pp. 190–201, 2004.

[96] L. M. Kosloski, D. M. Ha, J. A. L. Hutter et al., "Adaptive immune regulation of glial homeostasis as an immunization strategy for neurodegenerative diseases," *Journal of Neurochemistry*, vol. 114, no. 5, pp. 1261–1276, 2010.

[97] M. J. Kluger, "Fever: role of pyrogens and cryogens," *Physiological Reviews*, vol. 71, no. 1, pp. 93–127, 1991.

[98] P. M. Carvey, Q. Chang, J. W. Lipton, and Z. Ling, "Prenatal exposure to the bacteriotoxin lipopolysaccharide leads to long-term losses of dopamine neurons in offspring: a potential, new model of Parkinson's disease," *Frontiers in Bioscience*, vol. 8, pp. s826–s837, 2003.

[99] Z. D. Ling, D. A. Gayle, S. Y. Ma et al., "In utero bacterial endotoxin exposure causes loss of tyrosine hydroxylase neurons in the postnatal rat midbrain," *Movement Disorders*, vol. 17, no. 1, pp. 116–124, 2002.

[100] Z. Ling, Q. A. Chang, C. W. Tong, S. E. Leurgans, J. W. Lipton, and P. M. Carvey, "Rotenone potentiates dopamine neuron loss in animals exposed to lipopolysaccharide prenatally," *Experimental Neurology*, vol. 190, no. 2, pp. 373–383, 2004.

[101] H. Engler, R. Doenlen, C. Riether et al., "Time-dependent alterations of peripheral immune parameters after nigrostriatal dopamine depletion in a rat model of Parkinson's disease," *Brain, Behavior, and Immunity*, vol. 23, no. 4, pp. 518–526, 2009.

[102] R. F. Villarán, A. M. Espinosa-Oliva, M. Sarmiento et al., "Ulcerative colitis exacerbates lipopolysaccharide-induced damage to the nigral dopaminergic system: potential risk factor in Parkinson's disease," *Journal of Neurochemistry*, vol. 114, no. 6, pp. 1687–1700, 2010.

[103] D. C. Anthony, S. J. Bolton, S. Fearn, and V. H. Perry, "Age-related effects of interleukin-1β on polymorphonuclear neutrophil-dependent increases in blood-brain barrier permeability in rats," *Brain*, vol. 120, no. 3, pp. 435–444, 1997.

[104] C. C. Ferrari, A. M. Depino, F. Prada et al., "Reversible demyelination, blood-brain barrier breakdown, and pronounced neutrophil recruitment induced by chronic IL-1 expression in the brain," *American Journal of Pathology*, vol. 165, no. 5, pp. 1827–1837, 2004.

[105] C. C. Ferrari, M. C. Pott Godoy, R. Tarelli, M. Chertoff, A. M. Depino, and F. J. Pitossi, "Progressive neurodegeneration and motor disabilities induced by chronic expression of IL-1β in the substantia nigra," *Neurobiology of Disease*, vol. 24, no. 1, pp. 183–193, 2006.

[106] S. S. Shaftel, T. J. Carlson, J. A. Olschowka, S. Kyrkanides, S. B. Matousek, and M. K. O'Banion, "Chronic interleukin-1β expression in mouse brain leads to leukocyte infiltration and neutrophil-independent blood-brain barrier permeability without overt neurodegeneration," *Journal of Neuroscience*, vol. 27, no. 35, pp. 9301–9309, 2007.

[107] S. J. Campbell, D. C. Wilcockson, A. G. Butchart, V. H. Perry, and D. C. Anthony, "Altered chemokine expression in the spinal cord and brain contributes to differential interleukin-1β-induced neutrophil recruitment," *Journal of Neurochemistry*, vol. 83, no. 2, pp. 432–441, 2002.

[108] S. Ching, L. He, W. Lai, and N. Quan, "IL-1 type I receptor plays a key role in mediating the recruitment of leukocytes into the central nervous system," *Brain, Behavior, and Immunity*, vol. 19, no. 2, pp. 127–137, 2005.

[109] L. Qin, X. Wu, M. L. Block et al., "Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration," *GLIA*, vol. 55, no. 5, pp. 453–462, 2007.

[110] M. K. McCoy, T. N. Martinez, K. A. Ruhn 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," *Journal of Neuroscience*, vol. 26, no. 37, pp. 9365–9375, 2006.

[111] A. D. Reynolds, R. Banerjee, J. Liu, H. E. Gendelman, and R. L. Mosley, "Neuroprotective activities of CD4+CD25+ regulatory T cells in an animal model of Parkinson's disease," *Journal of Leukocyte Biology*, vol. 82, no. 5, pp. 1083–1094, 2007.

[112] A. D. Reynolds, D. K. Stone, J. A. L. Hutter, E. J. Benner, R. L. Mosley, and H. E. Gendelman, "Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease," *Journal of Immunology*, vol. 184, no. 5, pp. 2261–2271, 2010.

[113] X. Huang, A. D. Reynolds, R. L. Mosley, and H. E. Gendelman, "CD 4+ T cells in the pathobiology of neurodegenerative disorders," *Journal of Neuroimmunology*, vol. 211, no. 1-2, pp. 3–15, 2009.

[114] I. Kurkowska-Jastrzebska, M. Babiuch, I. Joniec, A. Przybylkowski, A. Czlonkowski, and A. Czlonkowska, "Indomethacin protects against neurodegeneration caused by MPTP intoxication in mice," *International Immunopharmacology*, vol. 2, no. 8, pp. 1213–1218, 2002.

[115] P. Teismann, K. Tieu, D. K. Choi et al., "Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 9, pp. 5473–5478, 2003.

[116] P. Teismann, M. Vila, D. K. Choi et al., "COX-2 and neurodegeneration in Parkinson's disease," *Annals of the New York Academy of Sciences*, vol. 991, pp. 272–277, 2003.

[117] R. Sanchez-Pernaute, A. Ferree, O. Cooper, M. Yu, A. L. Brownell, and O. Isacson, "Selective COX-2 inhibition prevents progressive dopamine neuron degeneration in a rat model of Parkinson's disease," *Journal of Neuroinflammation*, vol. 1, article 6, 2004.

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Review Article

Peripheral Inflammation Increases the Damage in Animal Models of Nigrostriatal Dopaminergic Neurodegeneration: Possible Implication in Parkinson's Disease Incidence

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Inflammatory processes described in Parkinson's disease (PD) and its animal models appear to be important in the progression of the pathogenesis, or even a triggering factor. Here we review that peripheral inflammation enhances the degeneration of the nigrostriatal dopaminergic system induced by different insults; different peripheral inflammations have been used, such as IL-1β and the ulcerative colitis model, as well as insults to the dopaminergic system such as 6-hydroxydopamine or lipopolysaccharide. In all cases, an increased loss of dopaminergic neurons was described; inflammation in the substantia nigra increased, displaying a great activation of microglia along with an increase in the production of cytokines such as IL-1β and TNF-α. Increased permeability or disruption of the BBB, with overexpression of the ICAM-1 adhesion molecule and infiltration of circulating monocytes into the substantia nigra, is also involved, since the depletion of circulating monocytes prevents the effects of peripheral inflammation. Data are reviewed in relation to epidemiological studies of PD.

1. Introduction

Parkinson's disease (PD) is the second most common aging-related neurodegenerative disease after Alzheimer's disease (AD). The main symptom of PD is a movement disorder called Parkinsonism (muscle rigidity, akinesia, and resting tremor) caused by dopamine (DA) deficiency in the striatum due to DA neuron degeneration in the substantia nigra (SN). Although a small percentage of PD is familial (fPD), most is sporadic (sPD), associated with aging and with no hereditary history. Aetiology of PD probably involves both environmental agents and genetic risk factors [1–3]. The implication of inflammatory process in PD is accepted, since many inflammatory marks have been described in PD and its animal models (for a review of neuroinflammation in PD, see [4]). Consequently, neuroinflammation is now thought to be fundamental for, or even a triggering factor of, the progression of PD pathogenesis.

Activated microglia and reactive human leukocyte antigen-DR (HLA-DR)-positive microglia were found in the SN pars compacta (SNpc) [5, 6] in the postmortem analysis of PD patients. Immunohistochemical studies have shown many activated microglial cells in neurotoxin-treated SNpc in various animal PD models [7], suggesting the presence of inflammatory processes [8–12]. Moreover, levels of proinflammatory substances such as cyclooxygenase 2 (COX2) and cytokines including interleukine-1 beta (IL-1β), interferon-gamma (IFN-γ), and tumour necrosis factor-alpha (TNF-α), are found to be high in postmortem PD brains [13–17]. Similarly, 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal PD models showed increased levels of these inflammatory mediators [10, 18–21], which are secreted from microglia, neurons, and astrocytes [22–25]. Therefore, these molecules may be actively involved in disease progression.

Several studies have tried to correlate polymorphisms in the promoters of several cytokine genes to the risk of developing PD [26–31]. Pattarini et al. [32] have shown protection against MPTP toxicity-measured as attenuation of dopamine depletion in the striatum-after genetic ablation of either TNF-α or its receptors Tnfrsf1 and Tnfrsf2 [18, 21, 33, 34], although neither genetic ablation nor pharmacological manipulation of TNF-α prevents neuronal loss in the SNpc [18, 35, 36]. On the contrary, Il-6 knockout mice are more sensitive to MPTP toxicity [37], which could be in agreement with the neuroprotective effect described for IL-6 [38]. Clinical studies on chronic users of nonsteroidal anti-inflammatory drugs (NSAIDs) suggest that some of these agents could lower the incidence of PD [39–42]. However, no such association was found in other studies [43, 44], although this preventive effect has been described in experiments with animals [45–47]. In the MPTP model of PD [48], the inactivation of microglia also showed to be neuroprotective.

Animal models of degeneration of the nigrostriatal dopaminergic system have been developed by intranigral injection of proinflammogens [11, 49–60]. Furthermore, other features support the implication of inflammation in the development or progression of PD. Chronic traumatic brain injury associated with boxing has been etiologically linked to PD with the well-known "punch-drunk syndrome" or "dementia pugilistica" that sometimes develops in boxers as a result of long-term subclinical concussions [61–66]. This is in agreement with the fact that inflammation through microglial activation accompanies the CNS tissue's response to injury (for review see Loane and Byrnes [67]).

The brain is considered an immunologically privileged organ, free from immune reactions, since it is protected by the blood-brain barrier (BBB). However, accumulating findings have revealed that immune responses can occur in the brain, especially because of microglial activation. These cells are known to produce proinflammatory cytokines and a relationship with the peripheral system has been suggested. Moreover, it is known that neurovascular functions are altered in aging and neurodegenerative diseases, leading to abnormal states such as increased BBB permeability, decreased nutrient supply, faulty clearance of toxic molecules, and failure of enzymatic function [68]. Moreover, several studies on PD patients and animal models suggest a pathogenic link between BBB disruption and DA neuronal death. Positron emission tomography (PET) and histological studies on PD patients revealed dysfunction of the BBB transport system [69] as well as alteration of blood vessels in the midbrain of PD patients [70]. In addition, levels of vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF) that induce structural changes in blood vessels were higher in PD patients and the MPTP model [71]. Interestingly, injecting VEGF in the SN disrupted BBB and induced DA neuronal death in the ventral mesencephalon [72]. In addition, increased BBB permeability was observed in the MPTP [73] and lipopolysaccharide (LPS) models of PD [74]. These results indicate that BBB disruption is somehow related to neuronal cell death and neuroinflammation in PD. Moreover, the presence of T lymphocytes in the midbrain of PD patients suggests that the potential role of infiltrated peripheral cells is related to the pathogenesis of PD [75]. In the model of LPS-induced inflammation, neutrophils and monocytes infiltrate into the SN region, where they play an important role in neuroinflammation [76]. Brochard et al. [77] reported that many CD4- and CD8-positive cells were detected postmortem in PD patients. In particular, the cytotoxic effects of T cells showed that CD4-deficient mice were resistant to MPTP neurotoxicity in the SN. In addition, the presence of Iba-1 positive cells in disrupted blood vessels indicates that neuroinflammation might contribute to the infiltration of peripheral immune cells and leakage of the BBB in the SN. Taken together, these results suggest that penetration of immune cells plays an important role in the degeneration of DA neurons in PD.

Peripheral inflammation could also have consequences on the degenerative process of DA neurons. There are many pathological circumstances in which peripheral inflammation is a common symptom. Epidemiological studies have shown that incidence of idiopathic PD is about 50% lower in chronic users of NSAIDs or COX inhibitors than in age-matched nonusers [39, 40, 78]. This could be related to inflammation inhibition in the CNS, but also to the inhibition of peripheral inflammation. Moreover, the role of peripheral inflammation in different neurodegenerative diseases has become evident in recent years. The probability of suffering AD doubles in elderly individuals exposed to systemic inflammation [79]. Furthermore, induction of a systemic inflammatory response led to reactivation in animal models of multiple sclerosis [80]. Strang [81] described the increased prevalence of peptic ulcer prodromal to idiopathic Parkinsonism. This was independently produced by Helicobacter or not [82]. Systemic inflammation sensitized microglia to switch to an overactivated proinflammatory state in a model of prion disease [83]. Here, we review the possibility that peripheral inflammation could enhance the degeneration of the nigrostriatal dopaminergic system and thus it would be involved in the incidence of PD.

2. Inflammation Increases the Degeneration of the Dopaminergic System Induced by an Insult

First, we must note that inflammation is capable of increasing the degeneration of the dopaminergic system induced by different insults.

2.1. In Vitro Studies. Gao et al. [84, 85] have shown that low doses of neurotoxin (rotenone or MPTP) and inflammogen LPS synergistically induced a progressive and selective degeneration of dopaminergic neurons in mesencephalic neuron-glia cultures. They showed that glia is required for this effect; in addition, they also showed that the effect was produced by a synergistic increase in NO and the superoxide free radical produced by NADPH oxidase. Similar results were described by Long-Smith et al. [86] using the cytokine-rich conditioned medium (CM) from LPS-treated rat

glial-enriched cortical cultures and 6-OHDA. They also showed that IL-1β in the CM mediated the increase in neuronal death since IL-1 receptor (IL-1R1) was located in dopaminergic neurons and its blockade prevented this effect. However, the authors also pointed out that many other cytotoxic factors such as TNF-α—and indeed some cytoprotective ones, may also be in the medium. Zhang et al. [87] also showed that combinations of $MnCl_2$ and LPS, at minimally effective concentrations when used alone, induced synergistic and preferential damage to DA neurons in rat primary neuron-glia cultures. These authors also showed that this effect could be produced by a significant increase in TNF-α and IL-1β release along with the increased production of reactive oxygen species (ROS) and NO. These effects were attenuated by pretreatment with anti-inflammatory agents, such as minocycline and naloxone.

2.2. In Vivo Studies.. Koprich et al. [88] induced a nontoxic inflammation in the SN, injecting a non-DA-toxic dose of LPS within the SN followed by injecting a low dose of 6-OHDA in the striatum, demonstrating that dopaminergic cell loss increased significantly. The authors identified IL-1β as a potential mediator of the effect, and were able to overcome it by the administing an IL-1R1 antagonist. Similar results have been also described by Godoy et al. [19] who used a similar method, also showing that inflammation induced by a low dose of LPS in SN produced a significant increase in the degeneration of DA neurons in SN induced by 6-OHDA compared with 6-OHDA alone. Moreover, this effect was overcome by dexamethasone (DEX), a well-known anti-inflammatory steroid. These results show that inflammation is able to enhance the degeneration of dopaminergic neurons induced by several insults.

3. Peripheral Inflammation Enhances the Degeneration of the Nigrostriatal Dopaminergic System

It is important to know whether peripheral inflammation, a very common health problem, could affect the degeneration of nigrostriatal dopaminergic neurons. Godoy et al. [19] induced a peripheral-like inflammatory state by injecting an adenoviral vector expressing IL-1β (or β-galactosidase as control) in the tail vein. This model was used to study the effect of peripheral inflammation on the nigrostriatal dopaminergic neurodegeneration induced by injecting 6-OHDA in the striatum. The authors found a statistically significant decrease in the number of TH-positive cells in the SN of the animals treated with 6-OHDA/Ad IL1biv in comparison with the other groups. These data showed that systemic IL-1 expression exacerbates, but does not directly elicit, neurodegeneration in the SN. This effect is produced by an increase in inflammation in SN as has been pointed out by the great reactivation of microglia (stage 4 morphology) found along with other parameter studies. This is the first description of the influence of peripheral inflammation on the degeneration of nigrostriatal dopaminergic system induced by an insult (6-OHDA). Similarly, Mangano and Hayley [89], injecting low amounts of LPS in the SN and the administration of the pesticide paraquat (which has been reported to provoke DA loss) showed a greater loss of TH-positive neurons in SN after two days.

Villarán et al. [90] studied the effect of peripheral inflammation on the degeneration of the nigrostriatal dopaminergic system induced by injecting LPS within the SN. The Ulcerative Colitis model (UC, one of the two major forms of gastrointestinal dysfunction) induced by dextran sodium sulphate (DSS) [91] was used as peripheral inflammation model; dextran sulphate provides an easy and well-characterized model that shares most features of human UC at structural, ultrastructural and clinical levels [92], including peripheral inflammation. The authors found a decrease in the number of TH-positive neurons in the animals with LPS-treated UC, doubling that found in animals treated with LPS. These results are in agreement with those described by Pott Godoy et al. [93], who studied the effect of peripheral inflammation (intravenous injection of adenovirus expressing IL-1β or β-galactosidase) on central inflammation (injecting adenovirus expressing IL-1β into the striatum). They found that chronic, systemic IL-1β expression exacerbated the neurodegeneration induced by IL-1β expression in the SN.

4. Mechanisms by Which Peripheral Inflammation Could Enhance the Degeneration of the Nigrostriatal Dopaminergic System Induced by Insults

As has been described by Gao et al. [84, 85] in studies using primary mesencephalic neuron-glia cultures as *in vitro* model of PD, participation of microglia is required for the induction of the synergistic neurotoxicity induced by inflammation (LPS) on the toxic effect of MPTP or rotenone. This suggests that inflammation (as the reactivation of microglia and secretion of many proinflammatory compounds) could be the cause of the synergistic process. In this context, they described that the release of superoxide free radical and the production of intracellular ROS was synergistic. Since this effect does not occur when NADPH oxidase-deficient (gp91phox$-/-$) mice were used, they also showed that it was catalyzed by NADPH oxidase, an enzyme that seems to be a major source of extracellular superoxide production in microglia. This proposal is in agreement with Godoy et al. [19] who described that stage 4 microglia and MHC II expression were associated with the exacerbation of neurodegeneration and motor symptoms. Similarly to Koprich et al. [88], these authors proposed that microglial activation towards a proinflammatory phenotype that increases IL-1β secretion is responsible for the synergistic effect. They pointed out that IL-1β is the cause of this effect, since glucocorticoid treatment and specific IL-1 inhibition overcome these effects. This proposal is also supported by an *in vitro* study in which IL-1 directly exacerbated 6-OHDA-triggered dopaminergic toxicity. This is also supported by Koprich et al. [88], who showed that the systemic administration of IL-1ra was able to reverse

the vulnerability produced by LPS and therefore eliminate the contribution of 6-OHDA to DA cell death. This is in agreement with Godoy et al. [19], who also proposed that IL-1β is responsible for the synergistic effect seen in the animal model of chronic systemic inflammation produced by injecting an adenoviral vector expressing IL1β (or β-galactosidase as control) in the tail vein. They concluded that IL-1β could be acting directly on neurons, and also indirectly through NO. Furthermore, this is also in agreement with Ferrari et al. [94], who showed that chronic expression of IL-1β in adult rat SNpc using a recombinant adenovirus caused the death of dopaminergic neurons after three weeks. Mangano and Hayley [89] also suggested that the increase in inflammatory cytokines IL-6, IL-2, TNF-α, and IFN-γ was responsible for this effect. Villarán et al. [90] showed that the loss of TH-positive neurons induced in UC-LPS animals was produced along with a significant increase in microglial activation, and almost doubled that produced in animals treated with LPS alone. Moreover, it is worth noting that the authors found a significant increase in many of the cytokines studied in the animals with UC, such as TNF-α, IL-1β, and IL-6. This effect was increased in the UC-LPS animals. These results could be in agreement with Godoy et al. [19], especially regarding the increase in cytokines, in spite of their being unable to distinguish the effect of each in relationship to the degeneration of DAergic neurons. In this work, the authors also found a significant increase in inducible nitric oxide synthase (iNOS), which could also be involved in the effect of peripheral inflammation, which in turn could be in agreement with Cunningham et al. [83] who found that IL-1 could exacerbate disease symptoms in a prion disease model. Moreover, the relationship between these cytokines is worth noting. Koprich et al. [88] described that their model's suppression of LPS sensitivity, produced by the systemic treatment with IL-1ra, also produced a reduction in the levels of the proinflammatory cytokines IFN-γ and TNF-α in the SN, supporting the cross-regulation between them, as had been previously pointed out. TNF-α is also toxic to DA neurons when injected into the rat's brain, but the toxicity is greater when TNF-α and IL-1β were injected together [95]. A study using IL-1β and TNF-α-neutralizing antibodies showed that approximately 50% of the LPS-induced DA neuronal cell death in primary cultures of rat midbrain was mediated by the production of these two cytokines [96]. The implication of TNF-α signalling in the destruction of SN DA neurons in animal models of PD have also been reported [18, 21, 36, 97]. Moreover, TNF-α contributed to the nigrostriatal neurodegeneration provoked by several DA insults [18, 21, 33, 34, 48, 98, 99]. Recently, De Lella Ezcurra et al. [100] have reported that chronic expression of low levels of TNF-α by adenoviral expression in the SN elicits progressive neurodegeneration, delayed motor symptoms and microglia/macrophage activation. All these data suggest that the increase in the inflammatory parameters in the periphery (blood) as a result of peripheral inflammation induced the increase in inflammation in SN and consequently the synergistic effect on the nigrostriatal dopaminergic system. The mechanism is not well known, but it is possible to suggest that at least some of these cytokines could enter. Circulating IL-1β may activate central neurons in a direct manner, especially in regions where BBB is deficient [101]. Furthermore, blood-borne IL-1β seems to stimulate the synthesis of prostaglandin E2 in central vessels, which can then diffuse into brain parenchyma to activate neurons in the SNC [102]. It should be also taken into account that the increase in BBB permeability, as well as its rupture, has been described as consequence of some peripheral inflammatory processes; so, disruption of the BBB has been described during TBS colitis [103, 104]. Reyes et al. [105] have shown that peripheral thermal injuries produced an increase in BBB permeability and rupture. *In vitro*, these effects have been proved to be produced by TNF-α, IL-1β, and IL-6 through the COX pathway [106]. *In vivo*, upregulation of TNF-α and IL-1β, as well as promoted blood-borne inflammatory cell adherence and infiltration, may be responsible [107–109]. Many studies have shown that cytokines may play an important role in the alteration of BBB function [109–112]. There is no agreement in the reports on rupture or increased permeability of the BBB in PD [113–115]. However, PET and histological studies on PD patients have shown dysfunction in the BBB transporter system [69] and alteration of blood vessels [70] in the midbrain of PD patients. Additionally, the levels of VEGF and PEDF that induce structural changes in blood vessels increased in PD patients and the MPTP model [71]. Moreover, Rite et al. [72] showed disrupted BBB and induced DA neuronal death in the ventral mesencephalon after injecting VEGF within the SN. All these data allow us to suggest that these circumstances could favour conditions in which peripheral cytokines were able to enter the CNS.

The degenerative processes of the nigrostriatal DAergic system in PD could be related to McGeer's description of a massive number of cytotoxic T cells (Tc) in the SN of patients with PD [75], along with descriptions that the density of IFN-γ-positive cells was markedly higher in brains of patients with PD [116]. Both facts suggest that the recruitment of T cells to the brain could be associated with the nigrostriatal pathway injury in PD. This possibility has been confirmed by Brochard et al. [77], who described that CD8+ and CD4+ T cells, but not B cells, invaded the brain during the course of neuronal degeneration in the MPTP animal model of PD. The effect of these cells was reinforced by the fact that the MPTP-induced dopaminergic cell death was markedly attenuated in the absence of mature T lymphocytes in two different immunodeficient mice strains (*Rag1*$-/-$ and *Tcrb*$-/-$ mice). However, there was no protection in mice lacking CD8. The implication of these cells was strongly supported, since the authors also found that both CD8+ and CD4+ T cells accumulated markedly in the SNpc of PD patients. These data indicate that T cell-mediated dopaminergic toxicity is almost exclusively mediated by CD4+ T cells. The authors also point out that the lymphocyte infiltration into the brain is not a passive phenomenon, suggesting that the activation of microglia along innate neuroinflammatory processes with the modification of the local microenvironment could be involved. They found the upregulated expression of the intercellular adhesion molecule-1 (ICAM-1) adhesion molecule on both capillaries

and glial cells, which may participate in the attachment of leukocytes to the vascular endothelium and their diapedesis [117]. In this context, Villarán et al. [90] has shown the effect of peripheral inflammation (UC) on the infiltration of circulating monocytes in the SN using flow cytometry analyses. They found that monocytes infiltration into the SN increased in the UC-LPS animals compared with the animals treated with LPS alone. Moreover, they showed the reversion of most of the deleterious effects of peripheral inflammation on microglial activation, BBB disruption, astrocytes loss, and degeneration of nigral dopaminergic neurons induced by LPS after using clodronate encapsulated in liposomes (ClodLip), which produced a peripheral macrophage depletion lasting 5 days in blood, liver, and spleen of normal rats and mice [118–120]. Taken together, these results suggest that peripheral inflammation induced by UC contributes to dopaminergic degeneration; the activation of macrophages seems to play some role, since the destruction of this peripheral leukocyte type by ClodLip abolishes the damaging effects associated with UC in the ventral mesencephalon. Brochard et al. [77] suggested that the overexpression of the ICAM-1 adhesion molecule could be involved in the active migration of these cells. Villarán et al. [90] also found a significant increase in ICAM-1 in the SN of UC-LPS animals. It is also interesting to note that the overexpression of ICAM-1 was also described in the SN of patients with PD and in MPTP-intoxicated monkeys [121], supporting a role for immune regulation outside the CNS and the possible implication of peripheral inflammation.

All these data show that peripheral inflammation could enhance the degeneration of the nigrostriatal system, previously induced by an insult. Therefore, PD symptoms could appear earlier, increasing the incidence of the disease. An increased prevalence of peptic ulcer prodromal to idiopathic Parkinsonism has been described [81, 122]. This prompted some authors to suggest a prominent role of inflammation in the gastrointestinal tract in the aetiology and pathogenesis of idiopathic Parkinsonism, including a possible role for *Helicobacter pylori* infections [82, 123]. This infective process is the most prevalent in the world, affecting approximately 50% of the population [124], and it is considered the causative agent of many gastrointestinal and extradigestive conditions. Colonization of gastric mucosa by *H. pylori* is accompanied by an inflammatory response associated with gastric mucosal damage through the activation of polymorphonuclear neutrophil leukocytes [125] and inflammatory infiltration of lymphocytes, plasma cells, and macrophages in the stomach tissue [126–128]. Also described was the production of proinflammatory factors such as IL-8, IL-1, and TNF-α [115, 129, 130].

It should not be ignored that a high peripheral inflammation could induce the degeneration of the nigrostriatal system alone and also that peripheral inflammation could produce a special sensitivity to other dopaminergic insults. An increase in PD was reported following the Spanish flu in 1918 [131]; however, the viral RNA is reported absent from brain samples of encephalitis lethargica patients from 1916 to 1920 [132]. It is conceivable that the massive immune response or "cytokine storm" [133] created by the virus initiated inflammation in the CNS; also, the great peripheral inflammation could have affected the nigrostriatal dopaminergic neurons. The inflammation produced by a single injection of a large dose of LPS into the periphery has been shown to produce inflammation in the brain, resulting in significant DA neuron loss in the SN [134]. Villarán et al. [90] also reported a decrease in TH positive neurons in the SN in animals with UC after injecting vehicle within this structure. The specific sensitization was reported by Ling et al. [135], who showed that prenatal exposure to LPS increased DA cell loss following adult exposure to 6-OHDA.

5. Conclusions

Our aim was to point out that inflammation is able to enhance the damage to DAergic neurons and, more importantly, that peripheral inflammation is also able to produce a synergistic increase in the effect produced by any insult in the nigrostriatal dopaminergic system, resulting in a greater loss of DA neurons. Consequently, this effect increases the progression rate of PD. The effect of peripheral inflammation seems to be produced through a significantly increased inflammation in the SN. This could be produced by some of the cytokines that increase in blood, such as IL-β and TNF-α, directly or indirectly through its transport to the CNS (SN). Moreover, these effects could be also accompanied by the recruitment of peripheral monocytes that also directly increase the inflammation process in the SN. The implication of peripheral inflammation could explain some epidemiological data on the incidence of PD, and probably also the effect of chronic anti-inflammatory treatments. These effects should be taken into account in the progression of PD.

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References

[1] J. Poirier, S. Kogan, and S. Gauthier, "Environment, genetics and idiopathic Parkinson's disease," *Canadian Journal of Neurological Sciences*, vol. 18, no. 1, pp. 70–76, 1991.

[2] F. Tsang and T. W. Soong, "Interactions between environmental and genetic factors in the pathophysiology of Parkinson's disease," *IUBMB Life*, vol. 55, no. 6, pp. 323–327, 2003.

[3] M. F. Allam, A. S. Del Castillo, and R. F. Navajas, "Parkinson's disease risk factors: genetic, environmental, or both?" *Neurological Research*, vol. 27, no. 2, pp. 206–208, 2005.

[4] Y. C. Chung, H. W. Ko, E. Bok et al., "The role of neuroinflammation on the pathogenesis of Parkinson's disease," *BMB Reports*, vol. 43, no. 4, pp. 225–232, 2010.

[5] P. L. McGeer, S. Itagaki, B. E. Boyes, and E. G. McGeer, "Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains," *Neurology*, vol. 38, no. 8, pp. 1285–1291, 1988.

[6] E. C. Hirsch, S. Hunot, P. Damier, and B. Faucheux, "Glial cells and inflammation in Parkinson's disease: a role in neurodegeneration?" *Annals of Neurology*, vol. 44, no. 3, supplement 1, pp. S115–S120, 1998.

[7] P. S. Whitton, "Inflammation as a causative factor in the aetiology of Parkinson's disease," *British Journal of Pharmacology*, vol. 150, no. 8, pp. 963–976, 2007.

[8] P. L. McGeer and E. G. McGeer, "The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases," *Brain Research Reviews*, vol. 21, no. 2, pp. 195–218, 1995.

[9] E. C. Hirsch, S. Hunot, B. Faucheux et al., "Dopaminergic neurons degenerate by apoptosis in Parkinson's disease," *Movement Disorders*, vol. 14, no. 2, pp. 383–384, 1999.

[10] M. Mogi and T. Nagatsu, "Neurotrophins and cytokines in Parkinson's disease," *Advances in Neurology*, vol. 80, pp. 135–139, 1999.

[11] A. J. Herrera, M. Tomás-Camardiel, J. L. Venero, J. Cano, and A. Machado, "Inflammatory process as a determinant factor for the degeneration of substantia nigra dopaminergic neurons," *Journal of Neural Transmission*, vol. 112, no. 1, pp. 111–119, 2005.

[12] T. Nagatsu and M. Sawada, "Inflammatory process in Parkinson's disease: role for cytokines," *Current Pharmaceutical Design*, vol. 11, no. 8, pp. 999–1016, 2005.

[13] S. Hunot, F. Boissière, B. Faucheux et al., "Nitric oxide synthase and neuronal vulnerability in Parkinson's disease," *Neuroscience*, vol. 72, no. 2, pp. 355–363, 1996.

[14] C. Knott, G. Stern, and G. P. Wilkin, "Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2," *Molecular and Cellular Neuroscience*, vol. 16, no. 6, pp. 724–739, 2000.

[15] W. D. Le, D. B. Rowe, J. Jankovic, W. Xie, and S. H. Appel, "Effects of cerebrospinal fluid from patients with Parkinson disease on dopaminergic cells," *Archives of Neurology*, vol. 56, no. 2, pp. 194–200, 1999.

[16] M. Mogi, M. Harada, P. Riederer, H. Narabayashi, K. Fujita, and T. Nagatsu, "Tumor necrosis factor-α (TNF-α) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients," *Neuroscience Letters*, vol. 165, no. 1-2, pp. 208–210, 1994.

[17] M. Mogi, M. Harada, H. Narabayashi, H. Inagaki, M. Minami, and T. Nagatsu, "Interleukin (IL)-1β, IL-2, IL-4, IL-6 and transforming growth factor-α levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease," *Neuroscience Letters*, vol. 211, no. 1, pp. 13–16, 1996.

[18] B. Ferger, A. Leng, A. Mura, B. Hengerer, and J. Feldon, "Genetic ablation of tumor necrosis factor-alpha (TNF-α) and pharmacological inhibition of TNF-synthesis attenuates MPTP toxicity in mouse striatum," *Journal of Neurochemistry*, vol. 89, no. 4, pp. 822–833, 2004.

[19] M. C. P. Godoy, R. Tarelli, C. C. Ferrari, M. I. Sarchi, and F. J. Pitossi, "Central and systemic IL-1 exacerbates neuro degeneration and motor symptoms in a model of Parkinson's disease," *Brain*, vol. 131, no. 7, pp. 1880–1894, 2008.

[20] P. Klevenyi, O. Andreassen, R. J. Ferrante, J. R. Schleicher, R. M. Friedlander, and M. F. Beal, "Transgenic mice expressing a dominant negative mutant interleukin-1β converting enzyme show resistance to MPTP neurotoxicity," *NeuroReport*, vol. 10, no. 3, pp. 635–638, 1999.

[21] K. Sriram, J. M. Matheson, S. A. Benkovic, D. B. Miller, M. I. Luster, and J. P. O'Callaghan, "Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: implications for Parkinson's disease," *The FASEB Journal*, vol. 16, no. 11, pp. 1474–1476, 2002.

[22] G. Banisadr, W. Rostène, P. Kitabgi, and S. M. Parsadaniantz, "Chemokines and brain functions," *Current Drug Targets: Inflammation and Allergy*, vol. 4, no. 3, pp. 387–399, 2005.

[23] L. Cartier, O. Hartley, M. Dubois-Dauphin, and K. H. Krause, "Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases," *Brain Research Reviews*, vol. 48, no. 1, pp. 16–42, 2005.

[24] K. Biber, E. K. de Jong, H. R. J. van Weering, and H. W. G. M. Boddeke, "Chemokines and their receptors in central nervous system disease," *Current Drug Targets*, vol. 7, no. 1, pp. 29–46, 2006.

[25] M. Minami, T. Katayama, and M. Satoh, "Brain cytokines and chemokines: roles in ischemic injury and pain," *Journal of Pharmacological Sciences*, vol. 100, no. 5, pp. 461–470, 2006.

[26] M. Nishimura, I. Mizuta, E. Mizuta, S. Yamasaki, M. Ohta, and S. Kuno, "Influence of interleukin-1β gene polymorphisms on age-at-onset of sporadic Parkinson's disease," *Neuroscience Letters*, vol. 284, no. 1-2, pp. 73–76, 2000.

[27] T. Schulte, L. Schöls, T. Müller, D. Woitalla, K. Berger, and R. Krüger, "Polymorphisms in the interleukin-1 alpha and beta genes and the risk for Parkinson's disease," *Neuroscience Letters*, vol. 326, no. 1, pp. 70–72, 2002.

[28] M. Nishimura, S. Kuno, I. Mizuta et al., "Influence of monocyte chemoattractant protein 1 gene polymorphism on age at onset of sporadic Parkinson's disease," *Movement Disorders*, vol. 18, no. 8, pp. 953–955, 2003.

[29] C. Huerta, V. Álvarez, I. F. Mata et al., "Chemokines (RANTES and MCP-1) and chemokine-receptors (CCR2 and CCR5) gene polymorphisms in Alzheimer's and Parkinson's disease," *Neuroscience Letters*, vol. 370, no. 2-3, pp. 151–154, 2004.

[30] O. A. Ross, C. O'Neill, I. M. Rea et al., "Functional promoter region polymorphism of the proinflammatory chemokine IL-8 gene associates with Parkinson's disease in the Irish," *Human Immunology*, vol. 65, no. 4, pp. 340–346, 2004.

[31] A. Håkansson, L. Westberg, S. Nilsson et al., "Interaction of polymorphisms in the genes encoding interleukin-6 and estrogen receptor beta on the susceptibility to Parkinson's disease," *American Journal of Medical Genetics*, vol. 133, no. 1, pp. 88–92, 2005.

[32] R. Pattarini, R. J. Smeyne, and J. I. Morgan, "Temporal mRNA profiles of inflammatory mediators in the murine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrimidine model of Parkinson's disease," *Neuroscience*, vol. 145, no. 2, pp. 654–668, 2007.

[33] K. Sriram, J. M. Matheson, S. A. Benkovic, D. B. Miller, M. I. Luster, and J. P. O'Callaghan, "Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: role of TNF-α," *The FASEB Journal*, vol. 20, no. 6, pp. 670–682, 2006.

[34] K. Sriram, D. B. Miller, and J. P. O'Callaghan, "Minocycline attenuates microglial activation but fails to mitigate striatal dopaminergic neurotoxicity: role of tumor necrosis factor-α," *Journal of Neurochemistry*, vol. 96, no. 3, pp. 706–718, 2006.

[35] E. Rousselet, J. Callebert, K. Parain et al., "Role of TNF-α receptors in mice intoxicated with the parkinsonian toxin MPTP," *Experimental Neurology*, vol. 177, no. 1, pp. 183–192, 2002.

[36] A. Leng, A. Mura, J. Feldon, and B. Ferger, "Tumor necrosis factor-alpha receptor ablation in a chronic MPTP mouse model of Parkinson's disease," *Neuroscience Letters*, vol. 375, no. 2, pp. 107–111, 2005.

[37] L. M. Bolin, I. Strycharska-Orczyk, R. Murray, J. W. Langston, and D. Di Monte, "Increased vulnerability of dopaminergic neurons in MPTP-lesioned interleukin-6 deficient mice," *Journal of Neurochemistry*, vol. 83, no. 1, pp. 167–175, 2002.

[38] Z. Liu, Y.-H. Qiu, B. Li, S.-H. Ma, and Y.-P. Peng, "Neuroprotection of interleukin-6 against NMDA-induced apoptosis and its signal-transduction mechanisms," *Neurotoxicity Research*, vol. 19, no. 3, pp. 484–495, 2011.

[39] H. Chen, S. M. Zhang, M. A. Hernán et al., "Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease," *Archives of Neurology*, vol. 60, no. 8, pp. 1059–1064, 2003.

[40] H. Chen, E. Jacobs, M. A. Schwarzschild et al., "Nonsteroidal antiinflammatory drug use and the risk for Parkinson's disease," *Annals of Neurology*, vol. 58, no. 6, pp. 963–967, 2005.

[41] M. Schiess, "Nonsteroidal anti-inflammatory drugs protect against Parkinson neurodegeneration: can an NSAID a day keep Parkinson disease away?" *Archives of Neurology*, vol. 60, no. 8, pp. 1043–1044, 2003.

[42] M. A. Hernán, G. Logroscino, and L. A. García Rodríguez, "Nonsteroidal anti-inflammatory drugs and the incidence of Parkinson disease," *Neurology*, vol. 66, no. 7, pp. 1097–1099, 2006.

[43] D. B. Hancock, E. R. Martin, J. M. Stajich et al., "Smoking, caffeine, and nonsteroidal anti-inflammatory drugs in families with Parkinson disease," *Archives of Neurology*, vol. 64, no. 4, pp. 576–580, 2007.

[44] T. G. Ton, S. R. Heckbert, W. T. Longstreth et al., "Nonsteroidal anti-inflammatory drugs and risk of Parkinson's disease," *Movement Disorders*, vol. 21, no. 7, pp. 964–969, 2006.

[45] B. Ferger, P. Teismann, C. D. Earl, K. Kuschinsky, and W. H. Oertel, "Salicylate protects against MPTP-induced impairments in dopaminergic neurotransmission at the striatal and nigral level in mice," *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 360, no. 3, pp. 256–261, 1999.

[46] K. Sairam, K. S. Saravanan, R. Banerjee, and K. P. Mohanakumar, "Non-steroidal anti-inflammatory drug sodium salicylate, but not diclofenac or celecoxib, protects against 1-methyl-4-phenyl pyridinium-induced dopaminergic neurotoxicity in rats," *Brain Research*, vol. 966, no. 2, pp. 245–252, 2003.

[47] D. S. Maharaj, K. S. Saravanan, H. Maharaj, K. P. Mohanakumar, and S. Daya, "Acetaminophen and aspirin inhibit superoxide anion generation and lipid peroxidation, and protect against 1-methyl-4-phenyl pyridinium-induced dopaminergic neurotoxicity in rats," *Neurochemistry International*, vol. 44, no. 5, pp. 355–360, 2004.

[48] D. C. Wu, V. Jackson-Lewis, M. Vila et al., "Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease," *The Journal of Neuroscience*, vol. 22, no. 5, pp. 1763–1771, 2002.

[49] A. Castaño, A. J. Herrera, J. Cano, and A. Machado, "Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system," *Journal of Neurochemistry*, vol. 70, no. 4, pp. 1584–1592, 1998.

[50] A. Castaño, A. J. Herrera, J. Cano, and A. Machado, "The degenerative effect of a single intranigral injection of LPS on the dopaminergic system is prevented by dexamethasone, and not mimicked by rh-TNF-α IL-1β IFN-γ," *Journal of Neurochemistry*, vol. 81, no. 1, pp. 150–157, 2002.

[51] A. J. Herrera, A. Castaño, J. L. Venero, J. Cano, and A. Machado, "The single intranigral injection of LPS as a new model for studying the selective effects of inflammatory reactions on dopaminergic system," *Neurobiology of Disease*, vol. 7, no. 4, pp. 429–447, 2000.

[52] A. J. Herrera, R. M. De Pablos, E. Carreño-Müller et al., "The intrastriatal injection of thrombin in rat induced a retrograde apoptotic degeneration of nigral dopaminergic neurons through synaptic elimination," *Journal of Neurochemistry*, vol. 105, no. 3, pp. 750–762, 2008.

[53] E. Carreño-Müller, A. J. Herrera, R. M. de Pablos et al., "Thrombin induces in vivo degeneration of nigral dopaminergic neurones along with the activation of microglia," *Journal of Neurochemistry*, vol. 84, no. 5, pp. 1201–1214, 2003.

[54] W. G. Kim, R. P. Mohney, B. Wilson, G. H. Jeohn, B. Liu, and J. S. Hong, "Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia," *The Journal of Neuroscience*, vol. 20, no. 16, pp. 6309–6316, 2000.

[55] R. M. De Pablos, A. J. Herrera, R. F. Villarán, J. Cano, and A. Machado, "Dopamine-dependent neurotoxicity of lipopolysaccharide in substantia nigra," *The FASEB Journal*, vol. 19, no. 3, pp. 407–409, 2005.

[56] R. M. de Pablos, R. F. Villarán, S. Argüelles et al., "Stress increases vulnerability to inflammation in the rat prefrontal cortex," *The Journal of Neuroscience*, vol. 26, no. 21, pp. 5709–5719, 2006.

[57] M. Tomás-Camardiel, I. Rite, A. J. Herrera et al., "Minocycline reduces the lipopolysaccharide-induced inflammatory reaction, peroxynitrite-mediated nitration of proteins, disruption of the blood-brain barrier, and damage in the nigral dopaminergic system," *Neurobiology of Disease*, vol. 16, no. 1, pp. 190–201, 2004.

[58] M. D. C. Hernández-Romero, S. Argüelles, R. F. Villarán et al., "Simvastatin prevents the inflammatory process and the dopaminergic degeneration induced by the intranigral injection of lipopolysaccharide," *Journal of Neurochemistry*, vol. 105, no. 2, pp. 445–459, 2008.

[59] S. Argüelles, A. J. Herrera, E. Carreño-Müller et al., "Degeneration of dopaminergic neurons induced by thrombin injection in the substantia nigra of the rat is enhanced by dexamethasone: role of monoamine oxidase enzyme," *NeuroToxicology*, vol. 31, no. 1, pp. 55–66, 2010.

[60] R. F. Villarán, R. M. de Pablos, S. Argüelles et al., "The intranigral injection of tissue plasminogen activator induced blood-brain barrier disruption, inflammatory process and degeneration of the dopaminergic system of the rat," *NeuroToxicology*, vol. 30, no. 3, pp. 403–413, 2009.

[61] M. Critchley, "Medical aspects of boxing, particularly from a neurological standpoint," *British Medical Journal*, vol. 1, no. 5015, pp. 357–362, 1957.

[62] A. H. Roberts, *Brain Damage in Boxers. A Study of Prevalence of Traumatic Encephalopathy among Ex-Professional Boxers*, Pitman Medical, London, UK, 1969.

[63] I. R. Casson, O. Siegel, and R. Sham, "Brain damage in modern boxers," *Journal of the American Medical Association*, vol. 251, no. 20, pp. 2663–2667, 1984.

[64] A. Guterman and R. W. Smith, "Neurological sequelae of boxing," *Sports Medicine*, vol. 4, no. 3, pp. 194–210, 1987.

[65] J. H. Friedman, "Progressive parkinsonism in boxers," *Southern Medical Journal*, vol. 82, no. 5, pp. 543–546, 1989.

[66] H. Clausen, P. McCrory, and V. Anderson, "The risk of chronic traumatic brain injury in professional boxing: change in exposure variables over the past century," *British Journal of Sports Medicine*, vol. 39, no. 9, pp. 661–664, 2005.

[67] D. J. Loane and K. R. Byrnes, "Role of microglia in neurotrauma," *Neurotherapeutics*, vol. 7, no. 4, pp. 366–377, 2010.

[68] B. O. Popescu, E. C. Toescu, L. M. Popescu et al., "Blood-brain barrier alterations in ageing and dementia," *Journal of the Neurological Sciences*, vol. 283, no. 1-2, pp. 99–106, 2009.

[69] R. Kortekaas, K. L. Leenders, J. C. H. van Oostrom et al., "Blood-brain barrier dysfunction in Parkinsonian midbrain *in vivo*," *Annals of Neurology*, vol. 57, no. 2, pp. 176–179, 2005.

[70] B. A. Faucheux, A. M. Bonnet, Y. Agid, and E. C. Hirsch, "Blood vessels change in the mesencephalon of patients with Parkinson's disease," *The Lancet*, vol. 353, no. 9157, pp. 981–982, 1999.

[71] T. Yasuda, M. Fukuda-Tani, T. Nihira et al., "Correlation between levels of pigment epithelium-derived factor and vascular endothelial growth factor in the striatum of patients with Parkinson's disease," *Experimental Neurology*, vol. 206, no. 2, pp. 308–317, 2007.

[72] I. Rite, A. Machado, J. Cano, and J. L. Venero, "Blood-brain barrier disruption induces *in vivo* degeneration of nigral dopaminergic neurons," *Journal of Neurochemistry*, vol. 101, no. 6, pp. 1567–1582, 2007.

[73] X. Chen, X. Lan, I. Roche, R. Liu, and J. D. Geiger, "Caffeine protects against MPTP-induced blood-brain barrier dysfunction in mouse striatum," *Journal of Neurochemistry*, vol. 107, no. 4, pp. 1147–1157, 2008.

[74] E. Yan, M. Castillo-Meléndez, T. Nicholls, J. Hirst, and D. Walker, "Cerebrovascular responses in the fetal sheep brain to low-dose endotoxin," *Pediatric Research*, vol. 55, no. 5, pp. 855–863, 2004.

[75] P. L. McGeer, S. Itagaki, H. Akiyama, and E. G. McGeer, "Rate of cell death in parkinsonism indicates active neuropathological process," *Annals of Neurology*, vol. 24, no. 4, pp. 574–576, 1988.

[76] K. A. Ji, M. S. Yang, H. K. Jeong et al., "Resident microglia die and infiltrated neutrophils and monocytes become major inflammatory cells in lipopolysaccharide-injected brain," *Glia*, vol. 55, no. 15, pp. 1577–1588, 2007.

[77] V. Brochard, B. Combadière, A. Prigent et al., "Infiltration of $CD4^+$ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease," *The Journal of Clinical Investigation*, vol. 119, no. 1, pp. 182–192, 2009.

[78] E. Esposito, V. Di Matteo, A. Benigno, M. Pierucci, G. Crescimanno, and G. Di Giovanni, "Non-steroidal anti-inflammatory drugs in Parkinson's disease," *Experimental Neurology*, vol. 205, no. 2, pp. 295–312, 2007.

[79] R. S. Tilvis, M. H. Kähönen-Väre, J. Jolkkonen, J. Valvanne, K. H. Pitkala, and T. E. Strandberg, "Predictors of cognitive decline and mortality of aged people over a 10-year period," *The Journals of Gerontology Series A*, vol. 59, no. 3, pp. 268–274, 2004.

[80] S. Serres, D. C. Anthony, Y. Jiang et al., "Systemic inflammatory response reactivates immune-mediated lesions in rat brain," *The Journal of Neuroscience*, vol. 29, no. 15, pp. 4820–4828, 2009.

[81] R. R. Strang, "The association of gastro-duodenal ulceration with Parkinson's disease," *The Medical Journal of Australia*, vol. 310, pp. 842–843, 1965.

[82] R. J. Dobbs, S. M. Dobbs, C. Weller et al., "Role of chronic infection and inflammation in the gastrointestinal tract in the etiology and pathogenesis of idiopathic parkinsonism. Part 1: eradication of Helicobacter in the cachexia of idiopathic parkinsonism," *Helicobacter*, vol. 10, no. 4, pp. 267–275, 2005.

[83] C. Cunningham, D. C. Wilcockson, S. Campion, K. Lunnon, and V. H. Perry, "Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration," *The Journal of Neuroscience*, vol. 25, no. 40, pp. 9275–9284, 2005.

[84] H. M. Gao, J. S. Hong, W. Zhang, and B. Liu, "Synergistic dopaminergic neurotoxicity of the pesticide rotenone and inflammogen lipopolysaccharide: relevance to the etiology of Parkinson's disease," *The Journal of Neuroscience*, vol. 23, no. 4, pp. 1228–1236, 2003.

[85] H.-M. Gao, B. Liu, W. Zhang, and J.-S. Hong, "Synergistic dopaminergic neurotoxicity of MPTP and inflammogen lipopolysaccharide: relevance to the etiology of Parkinson's disease," *The FASEB Journal*, vol. 17, no. 13, pp. 1957–1959, 2003.

[86] C. M. Long-Smith, L. Collins, A. Toulouse, A. M. Sullivan, and Y. M. Nolan, "Interleukin-1β contributes to dopaminergic neuronal death induced by lipopolysaccharide-stimulated rat glia in vitro," *Journal of Neuroimmunology*, vol. 226, no. 1-2, pp. 20–26, 2010.

[87] P. Zhang, K. M. Lokuta, D. E. Turner, and B. Liu, "Synergistic dopaminergic neurotoxicity of manganese and lipopolysaccharide: differential involvement of microglia and astroglia," *Journal of Neurochemistry*, vol. 112, no. 2, pp. 434–443, 2010.

[88] J. B. Koprich, C. Reske-Nielsen, P. Mithal, and O. Isacson, "Neuroinflammation mediated by IL-1β increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease," *Journal of Neuroinflammation*, vol. 5, article 8, 2008.

[89] E. N. Mangano and S. Hayley, "Inflammatory priming of the substantia nigra influences the impact of later paraquat exposure: neuroimmune sensitization of neurodegeneration," *Neurobiology of Aging*, vol. 30, no. 9, pp. 1361–1378, 2009.

[90] R. F. Villarán, A. M. Espinosa-Oliva, M. Sarmiento et al., "Ulcerative colitis exacerbates lipopolysaccharide-induced damage to the nigral dopaminergic system: potential risk factor in Parkinson's disease," *Journal of Neurochemistry*, vol. 114, no. 6, pp. 1687–1700, 2010.

[91] I. Okayasu, S. Hatakeyama, M. Yamada, T. Ohkusa, Y. Inagaki, and R. Nakaya, "A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice," *Gastroenterology*, vol. 98, no. 3, pp. 694–702, 1990.

[92] E. Gaudio, G. Taddei, A. Vetuschi et al., "Dextran sulfate sodium (DSS) colitis in rats: clinical, structural, and ultrastructural aspects," *Digestive Diseases and Sciences*, vol. 44, no. 7, pp. 1458–1475, 1999.

[93] M. C. Pott Godoy, C. C. Ferrari, and F. J. Pitossi, "Nigral neurodegeneration triggered by striatal AdIL-1 administration can be exacerbated by systemic IL-1 expression," *Journal of Neuroimmunology*, vol. 222, no. 1-2, pp. 29–39, 2010.

[94] C. C. Ferrari, M. C. Pott Godoy, R. Tarelli, M. Chertoff, A. M. Depino, and F. J. Pitossi, "Progressive neurodegeneration and motor disabilities induced by chronic expression of IL-1β in the substantia nigra," *Neurobiology of Disease*, vol. 24, no. 1, pp. 183–193, 2006.

[95] P. M. Carvey, E. Y. Chen, J. W. Lipton, C. W. Tong, Q. A. Chang, and Z. D. Ling, "Intra-parenchymal injection of tumor necrosis factor-α and interleukin 1-β produces dopamine neuron loss in the rat," *Journal of Neural Transmission*, vol. 112, no. 5, pp. 601–612, 2005.

[96] D. A. Gayle, Z. Ling, C. Tong, T. Landers, J. W. Lipton, and P. M. Carvey, "Lipopolysaccharide (LPS)-induced dopamine cell loss in culture: roles of tumor necrosis factor-α, interleukin-1β, and nitric oxide," *Brain Research. Developmental Brain Research*, vol. 133, no. 1, pp. 27–35, 2002.

[97] M. K. McCoy, T. N. Martinez, K. A. Ruhn 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," *The Journal of Neuroscience*, vol. 26, no. 37, pp. 9365–9375, 2006.

[98] D. Y. Lee, Y. J. Oh, and B. K. Jin, "Thrombin-activated microglia contribute to death of dopaminergic neurons in rat mesencephalic cultures: dual roles of mitogen-activated protein kinase signaling pathways," *Glia*, vol. 51, no. 2, pp. 98–110, 2005.

[99] H. Takeuchi, S. Jin, J. Wang et al., "Tumor necrosis factor-α induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner," *The Journal of Biological Chemistry*, vol. 281, no. 30, pp. 21362–21368, 2006.

[100] A. L. De Lella Ezcurra, M. Chertoff, C. Ferrari, M. Graciarena, and F. Pitossi, "Chronic expression of low levels of tumor necrosis factor-α in the substantia nigra elicits progressive neurodegeneration, delayed motor symptoms and microglia/macrophage activation," *Neurobiology of Disease*, vol. 37, no. 3, pp. 630–640, 2010.

[101] A. M. van Dam, J. G. J. M. Bol, R. P. A. Gaykema et al., "Vagotomy does not inhibit high dose lipopolysaccharide-induced interleukin-1β immunoreactivity in rat brain and pituitary gland," *Neuroscience Letters*, vol. 285, no. 3, pp. 169–172, 2000.

[102] M. Ek, D. Engblom, S. Saha, A. Blomqvist, P.-J. Jakobsson, and A. Ericsson-Dahlstrand, "Pathway across the blood-brain barrier," *Nature*, vol. 410, no. 6827, pp. 430–431, 2001.

[103] C. A. Hathaway, C. B. Appleyard, W. H. Percy, and J. L. Williams, "Experimental colitis increases blood-brain barrier permeability in rabbits," *American Journal of Physiology*, vol. 276, no. 5, part 1, pp. G1174–G1180, 1999.

[104] S. S. Natah, A. Mouihate, Q. J. Pittman, and K. A. Sharkey, "Disruption of the blood-brain barrier during TNBS colitis," *Neurogastroenterology and Motility*, vol. 17, no. 3, pp. 433–446, 2005.

[105] R. Reyes Jr., Y. Wu, Q. Lai et al., "Early inflammatory response in rat brain after peripheral thermal injury," *Neuroscience Letters*, vol. 407, no. 1, pp. 11–15, 2006.

[106] H. E. De Vries, M. C. M. Blom-Roosemalen, M. van Oosten et al., "The influence of cytokines on the integrity of the blood-brain barrier in vitro," *Journal of Neuroimmunology*, vol. 64, no. 1, pp. 37–43, 1996.

[107] D. Stanimirovic and K. Satoh, "Inflammatory mediators of cerebral endothelium: a role in ischemic brain inflammation," *Brain Pathology*, vol. 10, no. 1, pp. 113–126, 2000.

[108] M. A. Petty and J. G. Wettstein, "Elements of cerebral microvascular ischaemia," *Brain Research Reviews*, vol. 36, no. 1, pp. 23–34, 2001.

[109] J. B. Dietrich, "The adhesion molecule ICAM-1 and its regulation in relation with the blood-brain barrier," *Journal of Neuroimmunology*, vol. 128, no. 1-2, pp. 58–68, 2002.

[110] J. E. Merrill and S. P. Murphy, "Inflammatory events at the blood brain barrier: regulation of adhesion molecules, cytokines, and chemokines by reactive nitrogen and oxygen species," *Brain, Behavior, and Immunity*, vol. 11, no. 4, pp. 245–263, 1997.

[111] N. J. Abbott, "Inflammatory mediators and modulation of blood-brain barrier permeability," *Cellular and Molecular Neurobiology*, vol. 20, no. 2, pp. 131–147, 2000.

[112] P. F. Stahel, E. Shohami, F. M. Younis et al., "Experimental closed head injury: analysis of neurological outcome, blood-brain barrier dysfunction, intracranial neutrophil infiltration, and neuronal cell death in mice deficient in genes for pro-inflammatory cytokines," *Journal of Cerebral Blood Flow and Metabolism*, vol. 20, no. 2, pp. 369–380, 2000.

[113] E. Farkas, G. I. de Jong, R. A. I. de Vos, E. N. H. Jansen Steur, and P. G. M. Luiten, "Pathological features of cerebral cortical capillaries are doubled in Alzheimer's disease and Parkinson's disease," *Acta Neuropathologica*, vol. 100, no. 4, pp. 395–402, 2000.

[114] J. D. Adams Jr., L. K. Klaidman, I. N. Odunze, and J. N. Johannessen, "Effects of MPTP on the cerebrovasculature," *International Journal of Developmental Neuroscience*, vol. 9, no. 2, pp. 155–159, 1991.

[115] C. Zhao, Z. Ling, M. B. Newman, A. Bhatia, and P. M. Carvey, "TNF-α knockout and minocycline treatment attenuates blood-brain barrier leakage in MPTP-treated mice," *Neurobiology of Disease*, vol. 26, no. 1, pp. 36–46, 2007.

[116] S. Hunot, N. Dugas, B. Faucheux et al., "FcεRII/CD23 is expressed in Parkinson's disease and induces, in vitro, production of nitric oxide and tumor necrosis factor-α in glial cells," *The Journal of Neuroscience*, vol. 19, no. 9, pp. 3440–3447, 1999.

[117] T. A. Springer, "Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm," *Cell*, vol. 76, no. 2, pp. 301–314, 1994.

[118] N. van Rooijen, "The liposome-mediated macrophage 'suicide' technique," *Journal of Immunological Methods*, vol. 124, no. 1, pp. 1–6, 1989.

[119] N. van Rooijen, A. Sanders, and T. K. van den Berg, "Apoptosis of macrophages induced by liposome-mediated intracellular delivery of clodronate and propamidine," *Journal of Immunological Methods*, vol. 193, no. 1, pp. 93–99, 1996.

[120] G. A. P. Nieuwenhuijzen, M. F. C. M. Knapen, T. Hendriks, N. van Rooijen, and R. J. A. Goris, "Elimination of various subpopulations of macrophages and the development of multiple-organ dysfunction syndrome in mice," *Archives of Surgery*, vol. 132, no. 5, pp. 533–539, 1997.

[121] J. Miklossy, D. D. Doudet, C. Schwab, S. Yu, E. G. McGeer, and P. L. McGeer, "Role of ICAM-1 in persisting inflammation in Parkinson disease and MPTP monkeys," *Experimental Neurology*, vol. 197, no. 2, pp. 275–283, 2006.

[122] J. R. Warren and B. Marshall, "Unidentified curved bacilli on gastric epithelium in active chronic gastritis," *The Lancet*, vol. 1, no. 8336, pp. 1273–1275, 1983.

[123] C. Weller, N. Oxlade, S. M. Dobbs, R. J. Dobbs, A. Charlett, and I. T. Bjarnason, "Role of inflammation in gastrointestinal tract in aetiology and pathogenesis of idiopathic parkinsonism," *FEMS Immunology and Medical Microbiology*, vol. 44, no. 2, pp. 129–135, 2005.

[124] F. Megraud, "Epidemiology of *Helicobacter pylori* infection," *Gastroenterology Clinics of North America*, vol. 22, no. 1, pp. 73–88, 1993.

[125] P. Ernst, "Review article: the role of inflammation in the pathogenesis of gastric cancer," *Alimentary Pharmacology & Therapeutics*, vol. 13, supplement 1, pp. 13–18, 1999.

[126] T. Shimada and A. Terano, "Chemokine expression in *Helicobacter pylori*-infected gastric mucosa," *Journal of Gastroenterology*, vol. 33, no. 5, pp. 613–617, 1998.

[127] R. M. Peek, "Helicobacter pylori strain-specific activation of signal transduction cascades related to gastric inflammation," *American Journal of Physiology*, vol. 280, no. 4, pp. G525–G530, 2001.

[128] S. Aydemir, I. O. Tekin, G. Numanoglu, A. Borazan, and Y. Ustundag, "Eosinophil infiltration, gastric juice and serum eosinophil cationic protein levels in Helicobacter pylori-associated chronic gastritis and gastric ulcer," *Mediators of Inflammation*, vol. 13, no. 5-6, pp. 369–372, 2004.

[129] V. Supajatura, H. Ushio, A. Wada et al., "Cutting edge: VacA, a vacuolating cytotoxin of Helicobacter pylori, directly activates mast cells for migration and production of proinflammatory cytokines," *Journal of Immunology*, vol. 168, no. 6, pp. 2603–2607, 2002.

[130] S. Brandt, T. Kwok, R. Hartig, W. König, and S. Backert, "NF-κB activation and potentiation of proinflammatory responses by the Helicobacter pylori CagA protein," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 26, pp. 9300–9305, 2005.

[131] R. T. Ravenholt and W. H. Foege, "1918 influenza, encephalitis lethargica, parkinsonism," *The Lancet*, vol. 2, no. 8303, pp. 860–864, 1982.

[132] K. C. Lo, J. F. Geddes, R. S. Daniels, and J. S. Oxford, "Lack of detection of influenza genes in archived formalin-fixed, paraffin wax-embedded brain samples of encephalitis lethargica patients from 1916 to 1920," *Virchows Archiv*, vol. 442, no. 6, pp. 591–596, 2003.

[133] I. A. Clark, "The advent of the cytokine storm," *Immunology and Cell Biology*, vol. 85, no. 4, pp. 271–273, 2007.

[134] L. Qin, X. Wu, M. L. Block et al., "Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration," *Glia*, vol. 55, no. 5, pp. 453–462, 2007.

[135] Z. D. Ling, Q. Chang, J. W. Lipton, C. W. Tong, T. M. Landers, and P. M. Carvey, "Combined toxicity of prenatal bacterial endotoxin exposure and postnatal 6-hydroxydopamine in the adult rat midbrain," *Neuroscience*, vol. 124, no. 3, pp. 619–628, 2004.

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Review Article

Do PPAR-Gamma Agonists Have a Future in Parkinson's Disease Therapy?

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Thiazolidinediones (TZDs) are peroxisome proliferator-activated receptor (PPAR)-γ agonists commonly used as insulin-sensitizing drugs for the treatment of type 2 diabetes. In the last decade, PPAR-γ agonists have received increasing attention for their neuroprotective properties displayed in a variety of neurodegenerative diseases, including Parkinson's disease (PD), likely related to the anti-infammatory activity of these compounds. Recent studies indicate that neuroinflammation, specifically reactive microglia, plays important roles in PD pathogenesis. Moreover, after the discovery of infiltrating activated Limphocytes in the substantia nigra (SN) of PD patients, most recent research supports a role of immune-mediated mechanisms in the pathological process leading to chronic neuroinflammation and dopaminergic degeneration. PPAR-γ are highly expressed in cells of both central and peripheral immune systems, playing a pivotal role in microglial activation as well as in monocytes and T cells differentiation, in which they act as key regulators of immune responses. Here, we review preclinical evidences of PPAR-γ-induced neuroprotection in experimental PD models and highlight relative anti-inflammatory mechanisms involving either central or peripheral immunomodulatory activity. Specific targeting of immune functions contributing to neuroinflammation either directly (central) or indirectly (peripheral) may represent a novel therapeutic approach for disease modifying therapies in PD.

1. Introduction

Thiazolidinediones (TZDs), including rosiglitazone and pioglitazone, are currently in clinical use as insulin-sensitizing agents for the treatment of type 2 diabetes [1]. These drugs were originally designed as agonists of the peroxisome proliferator-activated receptor-γ (PPAR-γ), belonging to the hormone nuclear receptor superfamily. PPAR-γ mediates ligand-dependent transcription and is activated, beside synthetic agonists TZD, by naturally occurring compounds, such as longchain fatty acids and the prostaglandin 15-deoxy Δ, prostaglandin J2 (15d-PGJ2), but also few nonsteroidal anti-inflammatory drugs (NSAIDs), as ibuprofen, fenoprofen, and indomethacin [2–6]. In the last decade, the neuroprotective properties of PPAR-γ agonists have received increasing attention and researchers have provided a multitude of evidences in preclinical models of a variety of acute and chronic neurodegenerative conditions, including PD, Alzheimer's disease, cerebral ischemia, amyotrophic lateral sclerosis, and spinal cord injury. These evidences have led to rosiglitazone evaluation in phase II and III clinical trials in patients with Alzheimer's disease and ischemia [7–15].

2. Safety Concerns of TZDs Therapy

TZDs include troglitazone, which was removed from the market because of hepatotoxicity, and two currently available agents, rosiglitazone (Avandia, GlaxoSmithKline) and pioglitazone (Actos, Takeda). Rosiglitazone was introduced into the market in 1999 and has been widely used as monotherapy or in fixed-dose combinations with either metformin (Avandamet, GlaxoSmithKline) or glimepiride (Avandaryl, GlaxoSmithKline).

TZDs safety has been constantly monitored, mostly for the cardiovascular risks in diabetic patients, since more than 65% of deaths in patients with diabetes are from cardiovascular causes [16]. Multicentre studies aimed at assessing rosiglitazone-associated risks for cardiovascular diseases in diabetes have been recently completed [17–21]. The most reliable and informative studies are the ADOPT [19],

DREAM [21] and the recently completed RECORD studies for rosiglitazone [18], and the PROactive study for pioglitazone [22]. Few meta-analysis have also investigated the cardiovascular risks of TZDs in diabetes, leading however, to controversial conclusion [16, 23, 24]. Both rosiglitazone and pioglitazone have been associated dose-dependently with fluid retention and accumulation, increased body weight, and increased LDL cholesterol concentration, that may indirectly lead to heart failure in diabetic patients [18, 19, 25]. On the other hand, there is no evidence of TZDs direct cardiotoxicity. Moreover, TZDs have been shown to improve some cardiovascular risk markers associated with diabetes, as insulin sensitivity, blood pressure, and coagulation factors [26, 27]. Beside cardiovascular complications, in diabetic patients rosiglitazone has been associated with an increased risk of bone fractures, particularly in woman [18].

Given the intrinsic risk of cardiovascular complications in diabetic patients, concerns on TZDs safety have recently led to rosiglitazone withdrawal from the market in Europe and its inclusion in a restricted access program in the US as hypoglycemic drug (FDA safety Information). (http://www.fda.gov/downloads/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/UCM226959.pdf).

In contrast, no data are available up to date for non-diabetic or PD patients. It should be also noticed that rosiglitazone is currently in phase III clinical trial on Alzheimer disease, as well as several other diseases, including ischemia, cancer, and asthma (NCT00348140, NCT00265148, NCT00405015, NCT00369174, and NCT00119496). Overall, available data on TZDs safety, while not voiding studies for use of these drugs in other chronic diseases as PD, prompt for search of new PPAR-γ agonists with increased CNS permeability, which would likely permit to use lower doses regimens thus reducing peripheral side-effects risks.

3. Cellular Distribution of PPAR-Gamma

PPAR-γ has been demonstrated in a large variety of cells. The highest level of expression is shown by adipose tissue and by cells of the peripheral and central immune systems [6, 28–32]. This distribution pattern reflects the actions of PPAR-γ in regulating glucose and lipid metabolism, in promoting lipid storage and adipocyte differentiation [6, 33, 34]. Moreover, peripheral PPAR-γ is involved in the modulation of inflammatory cytokines production by monocyte/macrophages and endothelial cells, as well as in immune cell differentiation and function [3, 35]. In the central nervous system (CNS), PPAR-γ is expressed in several cell types including microglia, neurons, astrocytes, and oligodendrocytes [2, 36, 37]. Microglial cells constitutively express PPAR-γ, its levels being tightly regulated and dependent on microglial functional state [38]. In neurons, PPAR-γ immunoreactivity appears mainly as a nuclear labeling although sometimes cytoplasmic staining is detectable in some cortical neuron [37, 39]. High levels of PPAR-γ have been found in the piriform cortex and olfactory tubercle, in the basal ganglia, in rhomboid, centromedial, and parafascicular thalamic nuclei, in the reticular formation, and in the stellate cells of cerebellar cortex [37]. The abundance of PPAR-γ in basal ganglia regions, and areas expressing dopamine receptors supports the increasing interest for PPAR-γ agonists in PD management. PPAR-γ expression in astrocytes results in some way inhomogeneous, since in white matter structures PPAR-γ positive and negative astrocytes were found within the same area, albeit they have been found homogeneously expressed in adult cultured cortical astrocytes [36, 37].

4. PPAR-Gamma Agonists in Preclinical Models of PD

PD is a neurodegenerative disorder characterized by the progressive death of dopaminergic neurons of the substantia nigra pars compacta (SNc), resulting in a progressive deficiency of nigrostriatal dopamine transmission. Clinical symptoms of PD generally manifest when striatal dopamine (DA) levels are largely reduced and most nigral neurons are lost. The pathological development underlying neurodegeneration, at the time the diagnosis is made, is characterized by an unbalanced neuronal network due to a complex scenario of malfunctioning cellular components, including oxidative stress, impaired protein disposal systems, and chronic neuroinflammation [40].

Animal models of PD have been fruitfully used for contributing to a better knowledge of major mechanisms involved in this disease and to explore new potential therapies. Animal models of PD should possess the highest number of features of human PD (face validity), underlying neuropathology should evolve as much as possible as PD and should respond to treatments in a manner comparable to human PD (predictive validity). Lastly, they should also reproduce the complex scenario of multiple interaction between neuronal elements and surrounding cells (construct validity). Among cells that play a relevant role in this scenario, microglia, astrocytes, and endothelial cells are major players.

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration has been widely used in animals to reproduce PD symptoms, and, among animals, primates and mice have been used, the latter being easy to handle and affordable. Among the different MPTP-based models used so far, the acute MPTP, the subacute MPTP and the chronic MPTP paradigms have provided compelling results for a role of PPAR-γ in neuroinflammation and neurodegeneration in PD [8, 9, 41]. In this section, evidences for PPAR-γ-mediated neuroprotection in different MPTP-based PD models are summarized.

4.1. Acute MPTP. The acute MPTP model, consisting of male C57BL/6 mice that received four intraperitoneal administration of MPTP-HCl (15 mg/kg) at 2-h intervals in one day has been used by Breidert and colleagues [8]. This treatment determined a significant reduction of tyrosine hydroxylase (TH)-positive cells in the SNc at 2, 5, and 8 days

Table 1: Effect of PPAR-γ agonists on dopamine neuronal loss in the SNc, dopamine, and dopamine metabolites tissue levels in the striatum, in different mouse models of PD.

MPTP/LPS treatment	SNc TH-IM		Striatal TH-IM		Striatal dopamine		Striatal DOPAC		Striatal HVA	
	MPTP		MPTP		MPTP		MPTP		MPTP	
	Saline	Pioglitaone/ rosiglitazone	Saline	Pioglitazone/ rosiglitazone	Saline	Pioglitazone/ rosiglitazone	Saline	Pioglitazone/ rosiglitazone	Saline	Pioglitazone/ rosiglitazone
ACUTE (2 days post MPTP) [8]	−23%	+2%*	−76%	−71%	−85%	−77%*	−75%	−60%	−77%	−42%*
ACUTE (8 days post MPTP) [8]	−18%	−5%*	−56%	−66%	−89%	−79%*	−79%	−74%	−75%	−61%
SUBACUTE (7 days post MPTP) [9]	−50%	−3%*	n.d.	n.d.	−61%	−36%*	−55%	−53%	−26%	−32%
CHRONIC (3 days post MPTP) [41]	−30%	−2%*	n.d.	n.d.	−80%	−60%*	−65%	−32%*	n.d.	n.d.
ACUTE LPS (3 days post LPS) [42]	−21%	0%*	n.d.	n.d.	−61%	−33%*	n.d.	n.d.	n.d.	n.d.

Effect of pioglitazone or rosiglitazone on dopamine neuronal loss in the SNc, dopamine and dopamine metabolites tissue levels in the striatum. Values represent changes expressed as the percentage of control.
* $P < .05$ from MPTP or LPS + saline. Errors standard have not been reported for the search of clearness. TH-IM: TH-immunoreactivity.

after the last neurotoxin injection (Table 1). 20 mg/kg/day of pioglitazone, administered in rodent chows, prevented the dopaminergic cell loss in the SNc and attenuated the MPTP-induced glial activation. Furthermore, whereas TH immunoreactivity in the striatum was decreased in MPTP-treated mice as compared to controls, pioglitazone treatment did not significantly prevent striatal TH immunoreactivity loss, suggesting that the neuroprotective mechanism of this drug at dopamine cell-body level was somehow selective (Table 1). However, MPTP-induced decline of striatal tissue content of DA, DOPAC, and HVA was partially but significantly prevented by pioglitazone (Table 1). Overall, although the authors hypothesized that a higher energy demand of striatal nerve terminals could make the striatum more vulnerable to MPTP toxicity, masking the ability of pioglitazone to show a protective effect, it remains to be investigated why dopamine terminals were less protected than dopamine cell bodies.

4.2. Subacute MPTP. Dehmer and colleagues used the subacute MPTP model in C57BL/6 mice, consisting of 30 mg/kg i.p. MPTP at 24 h intervals for 2 or 5 days [9]. 20 mg/kg/day pioglitazone was administered in rodent chows, starting 4 days before MPTP injection. Animal were killed one week after the last MPTP administration. By using this protocol, the authors showed that subacute MPTP administration caused 50% loss of TH positive neurons in the SNc, whereas pioglitazone administration completely protected the SNc from cell loss. In addition, pioglitazone treatment partially prevented MPTP-induced striatal DA decline, whereas reduction of DOPAC and HVA were not affected (Table 1).

4.3. Chronic MPTP. In the study by Schintu and colleagues, the authors used the chronic MPTP plus probenecid (MPTPp) mouse model of progressive PD to assess the therapeutic efficacy of rosiglitazone on neurodegeneration, neuroinflammation and behavioural impairment [15]. In this study C57Bl/6J mice received 10 doses of MPTP (25 mg/kg i.p.) and probenecid (250 mg/kg i.p.) administered twice a week for 5 weeks. Rosiglitazone (10 mg/kg i.p.) was administered daily until sacrifice, three days after last neurotoxin administration. The efficacy of rosiglitazone in preventing the deleterious effect of chronic MPTPp was assessed in a wide variety of behavioural and biochemical tests. In particular, mice chronically treated with MPTPp displayed typical features of PD, including progressive impairment of motor and olfactory functions (Figure 1) associated with partial loss of TH-positive neurons in the SNc (Table 1), decrease of DA and DOPAC content and dynorphin mRNA levels in the striatum, and intense microglial and astroglial response in the SNc and striatum. Chronic rosiglitazone administered in association with MPTPp, completely prevented motor and olfactory dysfunctions and loss of TH-positive cells in the SNc (Figure 1 and Table 1). In the striatum, MPTPp-induced loss of striatal dopamine was partially prevented by rosiglitazone, whereas decrease in DOPAC content and dynorphin were fully counteracted. Therefore, these results clearly showed that DA neurons preservation by the PPAR-γ agonist was associated with preservation of motor functions. Moreover, while emphasizing the sensibility of striatal DA terminals to MPTPp chronic treatment, this study interrelated rosiglitazone-mediated preservation of motor functions to level of DA damage in the striatum, somehow reproducing a crucial feature of PD, where the appearance of behavioural deficits is strictly correlated with a threshold damage of striatal DA transmission.

4.4. Intrastriatal LPS. Hunter and coworkers tested the neuroprotective potential of the anti-inflammatory drug celecoxib, an inhibitor of cyclo-oxygenase-2 (COX-2) and the PPAR-γ agonist pioglitazone against the neuronal damage induced by intrastriatal injection of lipopolysaccharide (LPS) in rats [42]. Celecoxib (administered twice a day at 10 mg/kg, for four days before LPS injection) and pioglitazone (20 mg/kg daily for four days before LPS injection) prevented the loss of dopaminergic neurons and striatal DA decline, as observed 3 day after LPS injection. In addition, Celecoxib and pioglitazone decreased the neuroinflammatory reaction and restored mitochondrial function, providing a mechanism of neuroprotection [43].

5. PPAR-γ Agonists and Central Inflammation in PD

5.1. Microglia. Recent studies indicate that neuroinflammation and microglia activation play important roles in PD pathogenesis, as suggested by the high levels of reactive microglia found in the SNc of PD patients [44–46]. Microglia are the resident immune-competent cells of the CNS, commonly described as the CNS equivalent of macrophages, having a role in monitoring the brain for immune insults and invading pathogens [47–51]. It has been recently reported that primitive myeloid precursors give rise, before embryonic day 8, to microglia residing in the adult CNS in the steady state [52]. Recent interpretation of this cell population suggests that microglia do not constitute a uniform cell population but rather comprise a family of cells with different phenotypes, some of which are beneficial and others detrimental and toxic for the CNS [53]. In the healthy brain, the majority of microglia are in the resting state, with rod-shaped soma ramified and tiny processes. In this state, microglia show low expression of molecules associated with macrophage functions [53]. Upon activating stimuli from extracellular environment, as damaged neurons, endotoxins, cytokines, and aberrant proteins, microglia become reactive, progressively switching to different stages of activity, characterized by morphological and phenotypic changes. Assumption of macrophage functions allow them to respond to pathological insults [45, 49, 54, 55]. Morphologically, at least three activity stages have been described for reactive microglia: (i) activated ramified microglia with elongated soma, long ticker processes, (ii) ameboid microglia with round-shaped soma and short tick processes, (iii) phagocitic microglia with round-shaped soma and vacuolated cytoplasm, void of processes [49, 54]. Moreover, microglia can

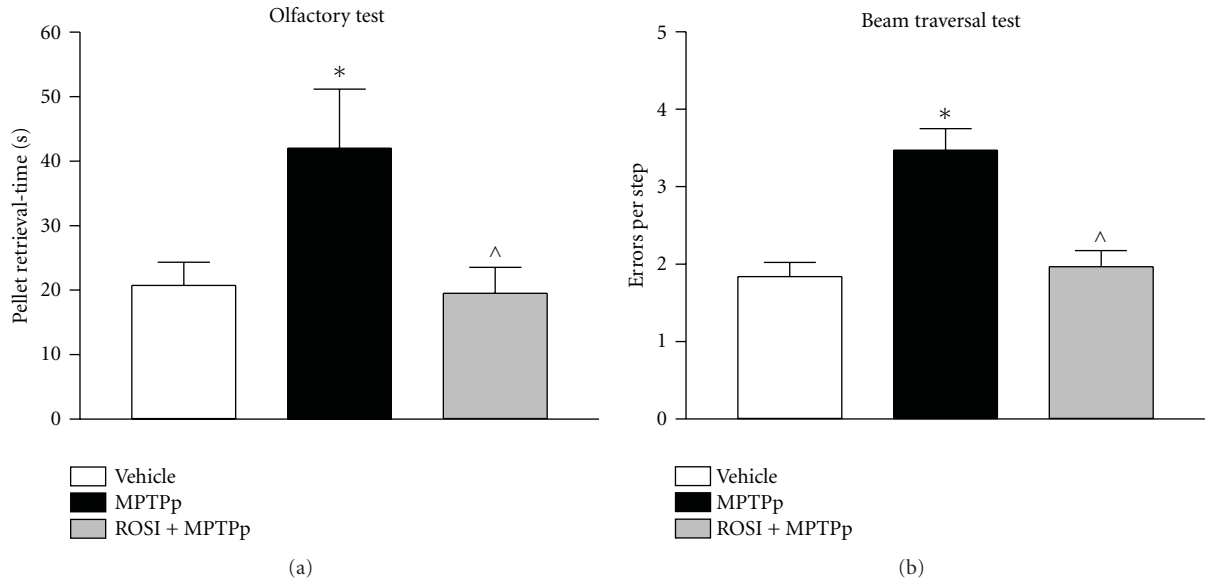

Figure 1: PPAR-γ agonist rosiglitazone prevents the development of behavioural deficits, as olfactory dysfunction and motor impairment, in a chronic model of progressive PD [15].

assume different states of effector functions, likely in relation with the activating stimulus and disease state [55]. Depending on the environment and level of threat for the SNC, microglia can temporarily assume a ctotoxic phenotype with phagocytic functions that is associated with production of pro-inflammatory cytokines as TNF-α, interleukin (IL) -1β, IL-2, IL-6, and nitric oxide (NO). Alternatively, microglia can act as antigen-presenting cells, expressing the major histocompatibility complex (MCH) class II, being therefore able to interact with adaptive immunity cells (T cells). In turn, T cells can stimulate microglia to assume either a cytotoxic phenotype or a phenotype with functions of neuroprotection and cell renewal through the upregulation of beneficial factors as insulin-like growth factor (IGF)-1 and anti-inflammatory cytokines and downregulation of neurodegenerative compounds, as TNF-α [56, 57].

5.2. Reactive Gliosis in PD: Human. In PD, postmortem studies have reported presence of activated microglia agglomerates around degenerating dopaminergic neurons and extracellular melanin in the SNc [46]. Moreover, pro-inflammatory cytokines, as TNF-α, IL-1β, IL-2, and IL-6 have been found in high levels in parkinsonian brains as well as in the serum and cerebrospinal fluid of PD patients [44, 58–62]. Accordingly, increased levels and nuclear translocation of nuclear factor (NF)-kB, a transcription factor controlling cytokines expression, were also observed in the SNc of PD patients [63, 64]. Interestingly, in PD gliosis seems to be limited to microglial activation. Indeed, most reports did not find reactive astrocytosis in the SN of PD patients, suggesting that the inflammatory process in PD is a unique phenomenon diverse from other neurodegenerative disorders (Mirza et al. [65]).

5.3. Reactive Gliosis in PD: Experimental PD. Studies in animal models of PD support the involvement of neuroinflammation and pro-inflammatory cytokines in dopaminergic neurodegeneration. MPTP and 6-OHDA-induced neurotoxicity in rodents are associated with an intense microglial reaction and with elevated levels of pro-inflammatory cytokines in the SNc [41, 66–72]. Moreover, in the LPS inflammatory model of neurodegeneration, systemic LPS induces intense neuroinflammation and increased levels of pro-inflammatory cytokines in the mouse brain, followed by dopaminergic degeneration in the SNc [73]. Accordingly, an atypical production of pro-inflammatory cytokines has been described in a 6-OHDA model of PD, where microglial activation was associated with a selective subset of cytokines increase [67]. Remarkably, 6-OHDA-induced dopaminergic neurodegeneration is exacerbated by the overexpression, and decreased by inhibition, of the pro-inflammatory cytokine IL-1β, whereas chronic TNF-α expression elicits nigral degeneration, which demonstrate a direct involvement of toxic cytokines in DA neurons degeneration [54, 74].

Although most reports of reactive microglia in the PD brain were derived from observations of terminal stage cases, leaving unknown if reactive gliosis is a cause or consequence of the disease, findings from animal models of PD suggest that microglia is chronically activated in a neurotoxic phenotype [44] (Figure 2). Products of degenerating neurons in PD, as aggregated α-synuclein, ATP, and neuromelanin, may act as self-antigen to activate microglia in order to induce a defensive reaction [47, 75–78]. However, in PD, activated microglia is engaged in a vicious cycle of inflammation, where products from dying neurons and inflammatory compounds chronically released by the microglia itself may sustain a condition called reactive microgliosis, in which neuroinflammation propagates and amplifies to destroy

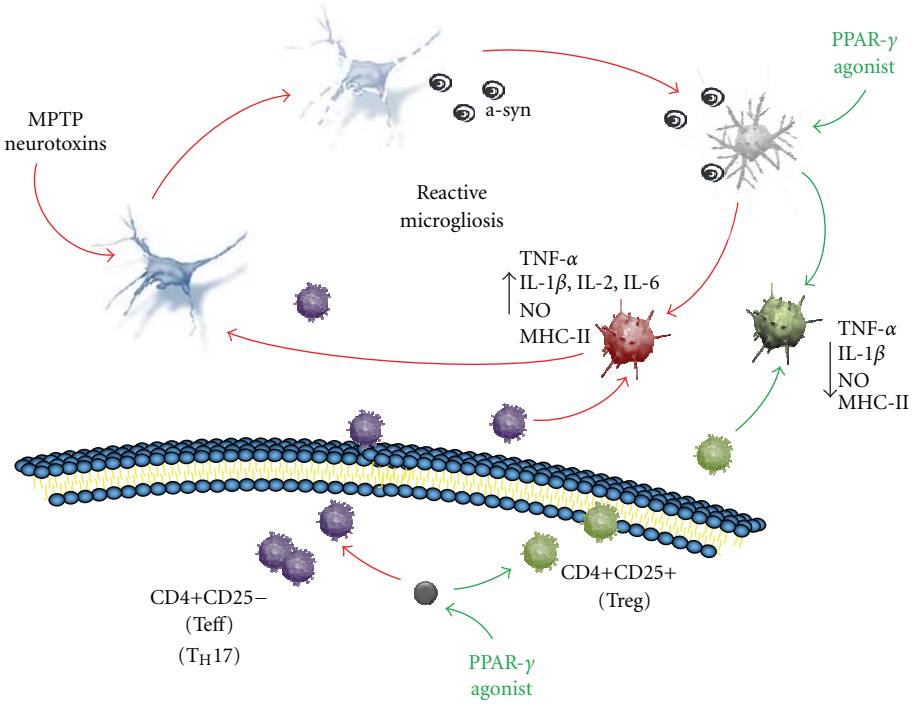

Figure 2: PPAR-γ agonists might achieve neuroprotection in PD by anti-inflammatory activity targeting cells of the central and peripheral immune systems. Products from dying neurons as α-synuclein (α-syn), ATP, and neuromelanin activate microglia to assume a proinflammatory phenotype that will be involved in clearing the environment from dangerous agents through the release of factors such as cytokines endowed with inflammatory and recruitment properties. In PD, microglia might have lost the ability to self-modulate, chronically maintaining a pro-inflammatory phenotype (red microglia) and failing to assume an anti-inflammatory and neuroprotective function (green microglia). Activated microglia becomes, therefore, engaged in a vicious cycle called reactive microgliosis, in which sustained neuroinflammation contributes to neuronal damage. PPAR-γ, through the specific inhibition of pro-inflammatory cytokines production and a stimulatory effect on anti-inflammatory cytokines, may suppress the microglia activation toward a pro-inflammatory/neurotoxic phenotypes, while directing it toward a neuroprotective phenotype (see text for references). In addition, in PD peripheral T lymphocytes activation is altered in that pro-inflammatory phenotypes (violet) exceed anti-inflammatory ones (green). Abnormally activated T cells infiltrate into the CNS, reaching the damaged SNc. Herein, they may drive microglia to acquire the neurotoxic phenotype to the detriment of less toxic or neuroprotective states, actively contributing to the pathological processes. Peripheral PPAR-γ can direct lymphocytes activation, selectively suppressing subsets of activated T cells which sustain tissue inflammation. Counteracting the disease-dysregulated peripheral immune functions by PPAR-γ agonists may, therefore, represent an adjunctive target for neuroprotection (see text for references).

more neurons [44, 67, 79–83] (Figure 2). In such a toxic environment, it has been suggested that the elevated plasticity of microglia and their interaction with adaptive immunity cells (see later in this review) may lead them to assume maladaptive functions, loosing their ability to self-modulate themselves therefore perpetuating a neurotoxic phenotype of activation, while failing to assume a neuroprotective function [53, 76] (Figure 2). This view would prompt for search of therapeutic strategies aimed at finely modulate microglia activation. Optimal anti-inflammatory therapies with neuroprotective target should be directed at selectively suppress dangerous microglia phenotypes while stimulating the neuroprotective ones, rather than generally suppress microglia activation.

5.4. PPAR-γ Agonists and Reactive Gliosis in PD. Studies in PD models *in vivo* have shown that PPAR-γ agonists-mediated neuroprotection is consistently associated with inhibition of microglial reactivity (Table 2). In MPTP-exposed mice, the neuroprotective effect of pioglitazone was associated with the inhibition of microglial reactivity in the SNc [8, 9]. Since pioglitazone exerts an inhibitory effect on monoamine oxidase B (MAO-B), therefore blocking the conversion of MPTP to the toxic metabolite MPP+, it has been claimed that this mechanism might account, at least partly, for the neuroprotective activity displayed by this drug upon MPTP intoxication [84] (Table 2). However, in a different PD model obtained by intrastriatal infusion of lipopolysaccharide (LPS), neuroprotection by pioglitazone was associated with inhibition of microglial reactivity in the SNc and inhibition of LPS-induced increase in mitochondrial proteins uncoupling protein 2 (UPS2) and mitoNEET [42, 43]. Moreover, in a recent study conducted by our group in a mouse model of progressive PD induced by chronic delivery of MPTPp, rosiglitazone prevented microglia activation [15]. Interestingly, while microglial response was fully prevented by rosiglitazone in

Table 2: Effect of PPAR-γ agonists on astroglia or microglia activation in the SNc or in the striatum of mice treated with different MPTP schedules.

MPTP treatment	SNc astroglia		Striatal astroglia		SNc microglia		Striatal microglia	
	MPTP		MPTP		MPTP		MPTP	
	Saline	Pioglitazone/ Rosiglitazone	Saline	Pioglitazone/ Rosiglitazone	Saline	Pioglitazone/ Rosiglitazone	Saline	Pioglitazone/ Rosiglita one
ACUTE (2 days post MPTP) [8]	280%	114%*	↑	↑	1596	185*	↑	↑
ACUTE (5 days post MPTP) [8]	421%	268%*	↑↑	↑↑	711	0*	↑↑	↑↑
ACUTE (8 days post MPTP) [8]	229%	107%*	↑	↑	0	0	↑	↑
SUBACUTE (7 days post MPTP) [9]	290%	105%*	745%	240%*	88.1	40.4*	99.4	34.0*
SUBACUTE (7 days post MPTP) [9]	459%	203%*	711%	510%*	78.1	47.4*	83.3	36.2*
CHRONIC (3 days post MPTP) [41]	684%	260%*	1000%	940%	421	10*	231	121*

Effect of pioglitazone or rosiglitazone on astroglia or microglia activation in the SNc or in the striatum of mice treated with different MPTP administrations. Values represent changes expressed as % of control, that is, saline-treated mice. Arrows indicate a slight (↑) or strong (↑↑) increase in glial immunorectivity, as shown by the pictures, when not quantified. *$P < .05$ from MPTP + saline. Standard errors have not been reported for the search of clearness.

the SNc, in line with a complete preservation of DA cell bodies, in the striatum, a partial microglia inhibition was associated with a partial rescue of DA content decline, further interrelating the anti-inflammatory activity with the neuroprotective effect [15]. Furthermore, when rosiglitazone was introduced late in the chronic MPTP treatment, in presence of an intense microgliosis and partial dopaminergic degeneration, microglial response was partially inhibited (unpublished observation). Importantly, in contrast to pioglitazone, MPP+ levels were not altered by rosiglitazone in mice chronically treated with MPTP, ruling out an effect on MPTP metabolism [15].

Several NSAID, as ibuprofen, fenoprofen, flufenamidic acid, indometacin, display a PPAR-γ agonistic activity [3, 4]. Noteworthy, these drugs have provided neuroprotection in PD models, supporting a role for the anti-inflammatory activity as mechanism of PPAR-γ-mediated neuroprotection [85].

5.5. Modulation of Microglial Response as a Mechanism of PPAR-γ-Mediated Neuroprotection. Although the preclinical studies suggest the anti-inflammatory activity as a mechanism of neuroprotection by PPAR-γ agonists in PD, a direct causal link has not been demonstrated. The *in vivo* studies in PD models have reported the inhibition of iNOS synthesis by pioglitazone, through an inhibition of NF-kB activation, a main regulator of inflammatory genes transcription, both in neurons and glial cells, offering a molecular mechanism for an anti-inflammatory-mediated neuroprotection [9]. Accordingly, in an *in vitro* study conducted in mesencephalic neuron-microglia mixed cultures, pioglitazone inhibited LPS-induced cyclooxygenase-2 (COX-2) activity, iNOS expression, NO production and p38 MAPK activity, achieving neuroprotection [86, 87].

Moreover, a direct evidence for PPAR-γ-mediated modulation of cytokines expression in experimental PD models *in vivo* is lacking although a wealth of evidences supporting this effect as a mechanism of neuroprotection come from *in vitro* studies. In LPS-stimulated microglial cells, natural and synthetic PPAR-γ agonists were shown to inhibit the production of pro-inflammatory and neurotoxic mediators as TNF-α, IL-1β, IL-12, iNOS, as well as IFN-γ-induced expression of major histocompatibility complex (MHC) class II antigen [38, 88–91]. Interestingly, a recent study suggested a role for the anti-inflammatory cytokine IL-4 in the PPAR-γ-mediated inhibitory effect, showing that rosiglitazone attenuated LPS-induced increase of IL-1β and MHC-II in microglia prepared from wild-type mice, but it failed to exert any effect in glia prepared from IL-4-deficient mice [92].

In addition to evidences gained from experimental PD, a number of *in vivo* and *in vitro* studies in models of neurodegenerative and neuroinflammatory conditions other than PD, have demonstrated a cytokine-modulatory activity of PPAR-γ agonists as mechanism of neuroprotection. In experimental cerebral ischemia, different TZDs, as pioglitazone, troglitazone, and rosiglitazone were neuroprotective and reduced protein and mRNA levels for the pro-inflammatory cytokines IL-1β, IL-6, COX-2, and iNOS through an inhibition of NF-kB signaling [93–95]. In an *in vitro* model of Alzheimer disease, PPAR-γ agonists troglitazone and ciglitazone suppressed the expression of the IL-6 and TNF-α genes in Aβ-stimulated microglial cells, improving neuronal survival [96]. Interestingly, in this study neuroprotection was not achieved by direct application of PPAR-γ agonists to the neurons, indicating that PPAR-γ agonists were directly targeting microglial function [96].

All together, evidence in PD models and the knowledge gained from diverse neurodegenerative conditions, suggest that PPAR-γ agonists may achieve neuroprotection in PD by mean of their anti-inflammatory activity and, specifically, by finely modulating cytokines expression in microglia through a main inhibitory effect on NF-kB activity. The specific inhibition of pro-inflammatory cytokines production by microglia, together with a stimulatory effect on anti-inflammatory cytokines, suggest that PPAR-γ agonists may direct activated microglia toward a less toxic or neuroprotective phenotype, while suppressing pro-inflammatory/neurotoxic phenotypes (Figure 2).

BOX. The exact molecular mechanism of the PPAR-γ-mediated anti-inflammatory activity remains controversial. Upon activation by natural and synthetic agonists, PPAR-γ heterodimerize with the retinoid X receptor (RXR) in the cytoplasm, in this form translocating to the nucleus. Herein, it binds to the PPAR-γ responsive elements (PPRE) in the promoter region of PPAR target genes to modulate their expression [97–99]. In the absence of ligands, the PPAR/RXR heterodimer is stabilized by the binding of corepressors to suppress transcription, whereas ligand-binding causes release of corepressors and recruitment of co-activators, to activate transcription [100]. Besides this transactivating activity, a ligand-dependent transcriptional transrepression mechanism has been described, by which activated PPAR-γ represses gene transcription in a DNA-binding independent way through physically sequestering activated transcriptional factors or their coactivators [101]. For instance, it was recently demonstrated that PPAR-γ can inhibit NF-kB by physical interaction with subunit p65, or by increasing inhibitory kappa B alpha (IkBα) expression [102]. In addition, a small, ubiquitin-like modifier (SUMO)ylation of PPAR-γ has been described as a mechanism of transrepression of NF-kB target pro-inflammatory genes, conferring to PPAR-γ a ductile function of activator or repressor of NF-kB target genes [103].

6. PPAR-γ and Peripheral Inflammation in PD

6.1. Peripheral Inflammation in PD: Human. Postmortem as well as *in vivo* studies in PD patients have suggested that the pathological process leading to neurodegeneration may involve cells of the peripheral immune system and immune-mediated mechanisms. First report by McGeer and colleagues demonstrated the presence of activated T lymphocytes (CD8+) in the Parkinsonian SN, together with elements of the complement pathway [104, 105]. Thereafter,

in an elegant study Orr and co-workers demonstrated presence of IgG-immunopositive pigmented neurons in the SN of both idiopathic and genetic forms of PD, associated with an increase of activated microglia expressing high affinity activating IgG receptors (FcγRI) [106]. Microglia contained pigment granules, supporting their involvement in a phagocytic attack on IgG immunopositive pigmented neurons [106]. These authors suggested that IgG binding to DA neurons may result in their selective targeting and subsequent destruction by activated microglia [106]. Lately, Brochard and colleagues reported higher densities of CD8+ and CD4+ T lymphocytes in the brain of patients with PD than in healthy brains [107].

Interestingly, abnormalities in peripheral immune functions have been repeatedly described in the blood of patients with PD, which suggest un imbalance toward pro-inflammatory phenotypes for activated T lymphocytes. Hisanaga and co-workers reported a significantly greater population of circulating CD3+ CD4+ CD8+ T lymphocytes in blood of PD patients than in age-matched control subjects [108]. In a study conducted in Parkinsonian versus normal individuals, Baba and colleagues suggested a shift of activated lymphocytes to a pro-inflammatory phenotype by showing that patients with PD had significantly decreased CD4+: CD8+ T-cell ratio, fewer CD4+CD25+ T regulatory cells (Treg), and increased ratios of IFN-γ-producing to IL-4-producing T cells [109]. Accordingly, other studies have reported higher levels of serum interleukins and pro-inflammatory cytokines [59, 110, 111].

6.2. Peripheral Inflammation in PD: Experimental PD. Studies in experimental PD models support an involvement of peripheral immunity in dopaminergic cell loss. He et al. [112] demonstrated that injection of IgG from serum of PD patients into the mouse SNc leads to microglial activation and subsequent dopaminergic degeneration, suggesting that humoral immune mechanisms can trigger microglial-mediated neuronal injury. Investigating a possible pathological relevance of lymphocytes infiltration in a experimental MPTP model of PD, Brochard et al. [107] showed that MPTP-damaged SNc specifically displayed presence of infiltrating T cells. Moreover, removal of CD4+ T cells in mutant mice resulted in a lower sensitivity to MPTP and lower degree of cell death in the SNc, strongly supporting a deleterious contribution of peripheral lymphocytes to dopaminergic degeneration within the SNc.

Moreover, a role for a dysregulated activation of T lymphocytes in PD neuropathology has been recently suggested in an in vivo PD model [113]. Thus, inoculation of CD4+CD25+ Treg cells, but not CD4+CD25− effector T cells (Teff), reduced microglial reactivity and neurodegeneration in MPTP-treated mice [113]. In addition, a more recent study by the same authors strongly corroborates the relevance of adaptive immunity cells subpopulations in directing microglial response in PD [114]. Hence, the phenotype undertaken by α-synuclein-activated microglia in culture, depended upon the interaction with specific subpopulations of activated T cells, with CD4+CD25+ Treg cells suppressing α-synuclein-induced production of reactive oxygen species and NF-kB activation. In contrast, CD4+CD25- effector T cells exacerbated microglial inflammation and neurotoxic responses [76, 114].

Although it is not clear if abnormalities of the peripheral immune system are secondary to changes in central immune system, data from PD cases and experimental PD consistently report an infiltration of abnormally activated immune cells across the blood brain barrier in PD. Consistent with a T cell function in directing microglia phenotype [56, 57], abnormally activated T cells present in the damaged area may influence the microenvironment by driving microglia to acquire a neurotoxic phenotype to the detriment of less toxic or neuroprotective states, actively contributing to the pathological processes (Figure 2).

6.3. PPAR-Gamma Agonists and Peripheral Inflammation in PD. PPAR-γ agonists exert profound and long-lasting anti-inflammatory effects in peripheral immune cells, mainly directing their differentiation into alternate phenotypes [3, 10, 115]. In the light of the growing relevance that peripheral immunity is gaining in PD pathology, this unique feature of PPAR-γ agonists sues for further attention toward these drugs as disease modifying strategy in PD. Albeit a direct evidence for a contribution of this mechanism in PPAR-γ-mediated neuroprotection in PD is currently missing, a wealth of data indicate that modulation of peripheral immunity is a main target for PPAR-γ-mediated protective therapies in chronic inflammatory diseases, including neuroinflammatory conditions as multiple sclerosis, where a dysregulation of the peripheral immune system is instrumental to the pathology.

PPAR-γ can affect adaptive immune responses by modulating T cells differentiation and activity through mechanisms involving the suppression of pro-inflammatory cytokines, as IL-2, which are known to play an important role in directing T cells phenotype [116–118]. Noteworthy, PPAR-γ-mediated suppression of a particular subset of activated T cells, named T helper 17 (T_H17), provides beneficial effects to multiple sclerosis patients, asserting PPAR-γ as a promising target for specific immunointervention in autoimmune disorders [119]. T_H17 has been recently described, playing an important role in inducing autoimmune tissue inflammations by the preferential release of IL-17. IL-17 in turn promotes inflammation through the production of pro-inflammatory cytokines as IL-6, TNF-α, IL-1β, chemokines, and potentiate tissue pathology by inducing the production of nitric oxide and matrix metalloproteinases [120]. Evidencing the prominent role played by PPAR-γ in the development of autoimmunity, rosiglitazone can affect CD4+ T cells function by specifically suppressing their differentiation into T_H17 [117, 119].

In addition to the anti-inflammatory function in adaptive immune cells, PPAR-γ agonists suppress monocyte elaboration of inflammatory cytokines and can prime monocytes to differentiate into macrophages with an anti-inflammatory phenotype [3, 121, 122]. In human

atherosclerotic lesions, and in cultured human monocytes, PPAR-γ stimulation primed primary human monocytes to be differentiated into the M2 form, the "alternative" anti-inflammatory macrophage phenotype. This in turn affected M1 macrophages, which displayed a more pronounced anti-inflammatory activity (the "classical" proatherogenic phenotype) [122–124].

To summarize, peripheral PPAR-γ holds the double function of selectively suppressing CD4+ activated T cells that sustain tissue inflammation and of inducing macrophages to differentiate into the anti-inflammatory M2 phenotype. Therefore, this receptor contributes to keep in control the inflammatory reactions in the tissue microenvironment and to maintain immune homeostasis, either in the presence of foreign pathogens/antigens or self-peptides insults. Within a view that includes a role for humoral immunity in PD pathogenesis, the restoration of disease-dysregulated peripheral immune functions by PPAR-γ agonists, may represent an adjunctive target for neuroprotection in this neurodegenerative disorder, prompting for further investigation in this field (Figure 2).

7. Conclusions and Future Perspectives

Currently, available drugs for PD therapy only provide symptomatic amelioration, while therapeutic strategies aimed at stopping or modifying disease progression are still strongly sought. Neuroinflammation plays a crucial role in the neurodegenerative processes. Most recent research suggests that both the central and peripheral immune systems are dysregulated in PD, as suggested by a chronic prevalence of a neurotoxic phenotype over anti-inflammatory states of activation, reported for either microglia or T cells. Therefore, therapeutic strategies aimed at finely modulating microglial activation, reinstating the physiological shift toward less neurotoxic phenotypes, may represent a goal in neuroprotection. This goal may be achieved by using PPAR-γ agonists, because their ability in modulating the expression of pro- and anti-inflammatory cytokines at the transcriptional level in both central and peripheral immune cells.

While the ability of PPAR-γ agonists to prevent neurodegeneration has been demonstrated in several experimental models of PD, additional studies are needed to prove PPAR-γ agonists efficacy on disease progression. Moreover, in spite of recent warning on the safety of these drugs in diabetes, to our knowledge no safety records are available in non diabetic individuals or PD patients. Therefore, translation to the clinical trial is warranted to fully evaluate the therapeutic potential in PD although safety could become a critical issue.

References

[1] A. H. Barnett, "Redefining the role of thiazolidinediones in the management of type 2 diabetes," *Vascular Health and Risk Management*, vol. 5, pp. 141–151, 2009.

[2] A. Bernardo and L. Minghetti, "Regulation of glial cell functions by PPAR-γ natural and synthetic agonists," *PPAR Research*, vol. 2008, Article ID 864140, 2008.

[3] C. Jiang, A. T. Ting, and B. Seed, "PPAR-γ agonists inhibit production of monocyte inflammatory cytokines," *Nature*, vol. 391, no. 6662, pp. 82–86, 1998.

[4] J. M. Lehmann, J. M. Lenhard, B. B. Oliver, G. M. Ringold, and S. A. Kliewer, "Peroxisome proliferator-activated receptors α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs," *Journal of Biological Chemistry*, vol. 272, no. 6, pp. 3406–3410, 1997.

[5] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[6] D. S. Straus and C. K. Glass, "Anti-inflammatory actions of PPAR ligands: new insights on cellular and molecular mechanisms," *Trends in Immunology*, vol. 28, no. 12, pp. 551–558, 2007.

[7] E. L. Akuffo, J. B. Davis, S. M. Fox et al., "The discovery and early validation of novel plasma biomarkers in mild-to-moderate Alzheimer's disease patients responding to treatment with rosiglitazone," *Biomarkers*, vol. 13, no. 6, pp. 618–636, 2008.

[8] T. Breidert, J. Callebert, M. T. Heneka, G. Landreth, J. M. Launay, and E. C. Hirsch, "Protective action of the peroxisome proliferator-activated receptor-γ agonist pioglitazone in a mouse model of Parkinson's disease," *Journal of Neurochemistry*, vol. 82, no. 3, pp. 615–624, 2002.

[9] T. Dehmer, M. T. Heneka, M. Sastre, J. Dichgans, and J. B. Schulz, "Protection by pioglitazone in the MPTP model of Parkinson's disease correlates with IκBα induction and block of NFκB and iNOS activation," *Journal of Neurochemistry*, vol. 88, no. 2, pp. 494–501, 2004.

[10] M. T. Heneka, G. E. Landreth, and M. Hüll, "Drug insight: effects mediated by peroxisome proliferator-activated receptor-γ in CNS disorders," *Nature Clinical Practice Neurology*, vol. 3, no. 9, pp. 496–504, 2007.

[11] M. C. Irizarry, D. J. Webb, C. Bains et al., "Predictors of placebo group decline in the Alzheimer's disease assessment scale-cognitive subscale (ADAS-Cog) in 24 week clinical trials of Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 14, no. 3, pp. 301–311, 2008.

[12] M. Kiaei, K. Kipiani, J. Chen, N. Y. Calingasan, and M. F. Beal, "Peroxisome proliferator-activated receptor-gamma agonist extends survival in transgenic mouse model of amyotrophic lateral sclerosis," *Experimental Neurology*, vol. 191, no. 2, pp. 331–336, 2005.

[13] Y. Luo, W. Yin, A. P. Signore et al., "Neuroprotection against focal ischemic brain injury by the peroxisome proliferator-activated receptor-γ agonist rosiglitazone," *Journal of Neurochemistry*, vol. 97, no. 2, pp. 435–448, 2006.

[14] S.-W. Park, J.-H. Yi, G. Miranpuri et al., "Thiazolidinedione class of peroxisome proliferator-activated receptor γ agonists prevents neuronal damage, motor dysfunction, myelin loss, neuropathic pain, and inflammation after spinal cord injury in adult rats," *Journal of Pharmacology and Experimental Therapeutics*, vol. 320, no. 3, pp. 1002–1012, 2007.

[15] N. Schintu, L. Frau, M. Ibba et al., "PPAR-gamma-mediated neuroprotection in a chronic mouse model of Parkinson's disease," *European Journal of Neuroscience*, vol. 29, no. 5, pp. 954–963, 2009.

[16] S. E. Nissen and K. Wolski, "Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes," *The New England Journal of Medicine*, vol. 356, no. 24, pp. 2457–2471, 2007.

[17] H. J. Dargie, P. R. Hildebrandt, G. A. J. Riegger et al., "A randomized, placebo-controlled trial assessing the eff ects of rosiglitazone on echocardiographic function and cardiac status in type 2 diabetic patients with New York heart association functional class I or II heart failure," *Journal of the American College of Cardiology*, vol. 49, no. 16, pp. 1696–1704, 2007.

[18] P. D. Home, S. J. Pocock, H. Beck-Nielsen et al., "Rosiglitazone evaluated for cardiovascular outcomes in oral agent combination therapy for type 2 diabetes (RECORD): a multicentre, randomised, open-label trial," *The Lancet*, vol. 373, no. 9681, pp. 2125–2135, 2009.

[19] S. E. Kahn, S. M. Haffner, M. A. Heise et al., "Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy," *The New England Journal of Medicine*, vol. 355, no. 23, pp. 2427–2443, 2006.

[20] R. W. Nesto, D. Bell, R. O. Bonow et al., "Thiazolidinedione use, fl uid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association," *Circulation*, vol. 108, no. 23, pp. 2941–2948, 2003.

[21] The DREAM (Diabetes REduction Assessment with ramipril and rosiglitazone Medication) Trial Investigators, H. C. Gerstein, S. Yusuf et al., "Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial," *The Lancet*, vol. 368, no. 9541, pp. 1096–1105, 2006.

[22] J. A. Dormandy, B. Charbonnel, D. J. Eckland et al., "Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial in macroVascular Events): a randomised controlled trial," *The Lancet*, vol. 366, no. 9493, pp. 1279–1289, 2005.

[23] S. E. Nissen and K. Wolski, "Rosiglitazone revisited: an updated meta-analysis of risk for myocardial infarction and cardiovascular mortality," *Archives of Internal Medicine*, vol. 170, no. 14, pp. 1191–1201, 2010.

[24] R. A. Ajjan and P. J. Grant, "The cardiovascular safety of rosiglitazone," *Expert Opinion on Drug Safety*, vol. 7, no. 4, pp. 367–376, 2008.

[25] M. I. Freed, R. Ratner, S. M. Marcovina et al., "Effects of rosiglitazone alone and in combination with atorvastatin on the metabolic abnormalities in type 2 diabetes mellitus," *American Journal of Cardiology*, vol. 90, no. 9, pp. 947–952, 2002.

[26] O. Barbier, I. P. Torra, Y. Duguay et al., "Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 22, no. 5, pp. 717–726, 2002.

[27] M. Komajda, P. Curtis, M. Hanefeld et al., "Effect of the addition of rosiglitazone to metformin or sulfonylureas versus metformin/sulfonylurea combination therapy on ambulatory blood pressure in people with type 2 diabetes: a randomized controlled trial (the RECORD study)," *Cardiovascular Diabetology*, vol. 7, article 10, 2008.

[28] A. Amoruso, C. Bardelli, G. Gunella, L. G. Fresu, V. Ferrero, and S. Brunelleschi, "Quantification of PPAR-γ protein in monocyte/macrophages from healthy smokers and non-smokers: a possible direct effect of nicotine," *Life Sciences*, vol. 81, no. 11, pp. 906–915, 2007.

[29] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[30] A. C. Li and W. Palinski, "Peroxisome proliferator-activated receptors: how their effects on macrophages can lead to the development of a new drug therapy against atherosclerosis," *Annual Review of Pharmacology and Toxicology*, vol. 46, pp. 1–39, 2006.

[31] E. Rigamonti, C. Fontaine, B. Lefebvre et al., "Induction of CXCR2 receptor by peroxisome proliferator-activated receptor γ in human macrophages," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 5, pp. 932–939, 2008.

[32] A. Szanto and T. Roszer, "Nuclear receptors in macrophages: a link between metabolism and inflammation," *FEBS Letters*, vol. 582, no. 1, pp. 106–116, 2008.

[33] B. Desvergne, L. Michalik, and W. Wahli, "Be fit or be sick: peroxisome proliferator-activated receptors are down the road," *Molecular Endocrinology*, vol. 18, no. 6, pp. 1321–1332, 2004.

[34] E. D. Rosen, P. Sarraf, A. E. Troy et al., "PPARγ is required for the differentiation of adipose tissue in vivo and in vitro," *Molecular Cell*, vol. 4, no. 4, pp. 611–617, 1999.

[35] R. Cunard, M. Ricote, D. DiCampli et al., "Regulation of cytokine expression by ligands of peroxisome proliferator activated receptors," *Journal of Immunology*, vol. 168, no. 6, pp. 2795–2802, 2002.

[36] T. E. Cullingford, K. Bhakoo, S. Peuchen, C. T. Dolphin, R. Patel, and J. B. Clark, "Distribution of mRNAs encoding the peroxisome proliferator-activated receptor α, β, and γ and the retinoid X receptor α, β, and γ in rat central nervous system," *Journal of Neurochemistry*, vol. 70, no. 4, pp. 1366–1375, 1998.

[37] S. Moreno, S. Farioli-vecchioli, and M. P. Cerù, "Immunolocalization of peroxisome proliferator-activated receptors and retinoid X receptors in the adult rat CNS," *Neuroscience*, vol. 123, no. 1, pp. 131–145, 2004.

[38] A. Bernardo, G. Levi, and L. Minghetti, "Role of the peroxisome proliferator-activated receptor-γ (PPAR-γ) and its natural ligand 15-deoxy-Δ(12,14)-prostaglandin J in the regulation of microglial functions," *European Journal of Neuroscience*, vol. 12, no. 7, pp. 2215–2223, 2000.

[39] A. Cimini, E. Benedetti, L. Cristiano et al., "Expression of peroxisome proliferator-activated receptors (PPARs) and retinoic acid receptors (RXRs) in rat cortical neurons," *Neuroscience*, vol. 130, no. 2, pp. 325–337, 2005.

[40] C. W. Olanow, "The pathogenesis of cell death in Parkinson's disease—2007," *Movement Disorders*, vol. 22, supplement 17, pp. S335–S342, 2007.

[41] N. Schintu, L. Frau, M. Ibba, A. Garau, E. Carboni, and A. R. Carta, "Progressive dopaminergic degeneration in the chronic MPTPp mouse model of parkinson's disease," *Neurotoxicity Research*, vol. 16, no. 2, pp. 127–139, 2009.

[42] R. L. Hunter, N. Dragicevic, K. Seifert et al., "Inflammation induces mitochondrial dysfunction and dopaminergic neurodegeneration in the nigrostriatal system," *Journal of Neurochemistry*, vol. 100, no. 5, pp. 1375–1386, 2007.

[43] R. L. Hunter, D. Y. Choi, S. A. Ross, and G. Bing, "Protective properties afforded by pioglitazone against intrastriatal LPS in Sprague-Dawley rats," *Neuroscience Letters*, vol. 432, no. 3, pp. 198–201, 2008.

[44] E. C. Hirsch and S. Hunot, "Neuroinflammation in Parkinson's disease: a target for neuroprotection?" *The Lancet Neurology*, vol. 8, no. 4, pp. 382–397, 2009.

[45] C. M. Long-Smith, A. M. Sullivan, and Y. M. Nolan, "The influence of microglia on the pathogenesis of Parkinson's

disease," *Progress in Neurobiology*, vol. 89, no. 3, pp. 277–287, 2009.

[46] P. L. McGeer and E. G. McGeer, "Glial reactions in Parkinson's disease," *Movement Disorders*, vol. 23, no. 4, pp. 474–483, 2008.

[47] D. Davalos, J. Grutzendler, G. Yang et al., "ATP mediates rapid microglial response to local brain injury in vivo," *Nature Neuroscience*, vol. 8, no. 6, pp. 752–758, 2005.

[48] M. B. Graeber, "Changing face of microglia," *Science*, vol. 330, no. 6005, pp. 783–788, 2010.

[49] G. W. Kreutzberg, "Microglia: a sensor for pathological events in the CNS," *Trends in Neurosciences*, vol. 19, no. 8, pp. 312–318, 1996.

[50] A. Nimmerjahn, F. Kirchhoff, and F. Helmchen, "Neuroscience: resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo," *Science*, vol. 308, no. 5726, pp. 1314–1318, 2005.

[51] G. Raivich, "Like cops on the beat: the active role of resting microglia," *Trends in Neurosciences*, vol. 28, no. 11, pp. 571–573, 2005.

[52] F. Ginhoux, M. Greter, and M. Leboeuf et al., "Fate mapping analysis reveals that adult microglia derive from primitive macrophages," *Science*, vol. 330, no. 6005, pp. 841–845, 2010.

[53] M. Schwartz, O. Butovsky, W. Brück, and U. K. Hanisch, "Microglial phenotype: is the commitment reversible?" *Trends in Neurosciences*, vol. 29, no. 2, pp. 68–74, 2006.

[54] M. C. P. Godoy, R. Tarelli, C. C. Ferrari, M. I. Sarchi, and F. J. Pitossi, "Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease," *Brain*, vol. 131, no. 7, pp. 1880–1894, 2008.

[55] R. M. Ransohoff and V. H. Perry, "Microglial physiology: unique stimuli, specialized responses," *Annual Review of Immunology*, vol. 27, pp. 119–145, 2009.

[56] O. Butovsky, A. E. Talpalar, K. Ben-Yaakov, and M. Schwartz, "Activation of microglia by aggregated β-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-γ and IL-4 render them protective," *Molecular and Cellular Neuroscience*, vol. 29, no. 3, pp. 381–393, 2005.

[57] I. Shaked, D. Tchoresh, R. Gersner et al., "Protective autoimmunity: interferon-γ enables microglia to remove glutamate without evoking inflammatory mediators," *Journal of Neurochemistry*, vol. 92, no. 5, pp. 997–1009, 2005.

[58] D. Blum-Degena, T. Müller, W. Kuhn, M. Gerlach, H. Przuntek, and P. Riederer, "Interleukin-1β and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients," *Neuroscience Letters*, vol. 202, no. 1-2, pp. 17–20, 1995.

[59] R. J. Dobbs, A. Charlett, A. G. Purkiss, S. M. Dobbs, C. Weller, and D. W. Peterson, "Association of circulating TNF-α and IL-6 with ageing and parkinsonism," *Acta Neurologica Scandinavica*, vol. 100, no. 1, pp. 34–41, 1999.

[60] S. Hunot, N. Dugas, B. Faucheux et al., "FcεRII/CD23 is expressed in Parkinson's disease and induces, in vitro, production of nitric oxide and tumor necrosis factor-α in glial cells," *Journal of Neuroscience*, vol. 19, no. 9, pp. 3440–3447, 1999.

[61] M. Mogi, M. Harada, T. Kondob et al., "Interleukin-1β, interleukin-6, epidermal growth factor and transforming growth factor-α are elevated in the brain from parkinsonian patients," *Neuroscience Letters*, vol. 180, no. 2, pp. 147–150, 1994.

[62] M. Mogi, M. Harada, P. Riederer, H. Narabayashi, K. Fujita, and T. Nagatsu, "Tumor necrosis factor-α (TNF-α) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients," *Neuroscience Letters*, vol. 165, no. 1-2, pp. 208–210, 1994.

[63] S. Hunot, B. Brugg, D. Ricard et al., "Nuclear translocation of NF-κb is increased in dopaminergic neurons of patients with Parkinson disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 14, pp. 7531–7536, 1997.

[64] M. Mogi, T. Kondo, Y. Mizuno, and T. Nagatsu, "p53 protein, interferon-γ, and NF-κB levels are elevated in the parkinsonian brain," *Neuroscience Letters*, vol. 414, no. 1, pp. 94–97, 2007.

[65] B. Mirza, H. Hadberg, P. Thomsen, and T. Moos, "The absence of reactive astrocytosis is indicative of a unique inflammatory process in Parkinson's disease," *Neuroscience*, vol. 95, no. 2, pp. 425–432, 2000.

[66] F. Blandini, M. T. Armentero, and E. Martignoni, "The 6-hydroxydopamine model: news from the past," *Parkinsonism and Related Disorders*, vol. 14, supplement 2, pp. S124–S129, 2008.

[67] A. M. Depino, C. Earl, E. Kaczmarczyk et al., "Microglial activation with atypical proinflammatory cytokine expression in a rat model of Parkinson's disease," *European Journal of Neuroscience*, vol. 18, no. 10, pp. 2731–2742, 2003.

[68] I. 59. Kurkowska-Jastrzebska, A. Wrońska, M. Kohutnicka, A. Członkowski, and A. Członkowska, "MHC class II positive microglia and lymphocytic infiltration are present in the substantia nigra and striatum in mouse model of Parkinson's disease," *Acta Neurobiologiae Experimentalis*, vol. 59, no. 1, pp. 1–8, 1999.

[69] D. D. Lofrumento, C. Saponaro, A. Cianciulli et al., "MPTP-induced neuroinflammation increases the expression of pro-inflammatory cytokines and their receptors in mouse brain," *NeuroImmunoModulation*, vol. 18, no. 2, pp. 79–88, 2010.

[70] D. W. Luchtman, DI. Shao, and C. Song, "Behavior, neurotransmitters and inflammation in three regimens of the MPTP mouse model of Parkinson's disease," *Physiology and Behavior*, vol. 98, no. 1-2, pp. 130–138, 2009.

[71] R. Pattarini, R. J. Smeyne, and J. I. Morgan, "Temporal mRNA profiles of inflammatory mediators in the murine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrimidine model of Parkinson's disease," *Neuroscience*, vol. 145, no. 2, pp. 654–668, 2007.

[72] M. Vila, V. Jackson-Lewis, C. Guégan et al., "The role of glial cells in Parkinson's disease," *Current Opinion in Neurology*, vol. 14, no. 4, pp. 483–489, 2001.

[73] L. Qin, X. Wu, M. L. Block et al., "Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration," *GLIA*, vol. 55, no. 5, pp. 453–462, 2007.

[74] A. L. De Lella Ezcurra, M. Chertoff, C. Ferrari, M. Graciarena, and F. Pitossi, "Chronic expression of low levels of tumor necrosis factor-α in the substantia nigra elicits progressive neurodegeneration, delayed motor symptoms and microglia/macrophage activation," *Neurobiology of Disease*, vol. 37, no. 3, pp. 630–640, 2010.

[75] A. M. Floden, S. Li, and C. K. Combs, "β-Amyloid-stimulated microglia induce neuron death via synergistic stimulation of tumor necrosis factor α and NMDA receptors," *Journal of Neuroscience*, vol. 25, no. 10, pp. 2566–2575, 2005.

[76] A. D. Reynolds, D. K. Stone, J. A. L. Hutter, E. J. Benner, R. L. Mosley, and H. E. Gendelman, "Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic

neurodegeneration in a model of Parkinson's disease," *Journal of Immunology*, vol. 184, no. 5, pp. 2261–2271, 2010.

[77] H. Wilms, P. Rosenstiel, J. Sievers, G. Deuschl, L. Zecca, and R. Lucius, "Activation of microglia by human neuromelanin is NF-kappaB dependent and involves p38 mitogen-activated protein kinase: implications for Parkinson's disease," *The FASEB Journal*, vol. 17, no. 3, pp. 500–502, 2003.

[78] W. Zhang, T. Wang, Z. Pei et al., "Aggregated α-synuclein activates microglia: a process leading to disease progression in Parkinson's disease," *FASEB Journal*, vol. 19, no. 6, pp. 533–542, 2005.

[79] F. Cicchetti, A. L. Brownell, K. Williams, Y. I. Chen, E. Livni, and O. Isacson, "Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging," *European Journal of Neuroscience*, vol. 15, no. 6, pp. 991–998, 2002.

[80] A. Członkowska, M. Kohutnicka, I. Kurkowska-Jastrzebska, and A. Członkowski, "Microglial reaction in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced Parkinson's disease mice model," *Neurodegeneration*, vol. 5, no. 2, pp. 137–143, 1996.

[81] M. Kohutnicka, E. Lewandowska, I. Kurkowska-Jastrzebska, A. Członkowski, and A. Członkowska, "Microglial and astrocytic involvement in a murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)," *Immunopharmacology*, vol. 39, no. 3, pp. 167–180, 1998.

[82] G. T. Liberatore, V. Jackson-Lewis, S. Vukosavic et al., "Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease," *Nature Medicine*, vol. 5, no. 12, pp. 1403–1409, 1999.

[83] K. Sriram, J. M. Matheson, S. A. Benkovic, D. B. Miller, M. I. Luster, and J. P. O'Callaghan, "Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: role of TNF-α," *FASEB Journal*, vol. 20, no. 6, pp. 670–682, 2006.

[84] L. P. Quinn, B. Crook, M. E. Hows et al., "The PPARγ agonist pioglitazone is effective in the MPTP mouse model of Parkinson's disease through inhibition of monoamine oxidase B," *British Journal of Pharmacology*, vol. 154, no. 1, pp. 226–233, 2008.

[85] E. Esposito, V. Di Matteo, A. Benigno, M. Pierucci, G. Crescimanno, and G. Di Giovanni, "Non-steroidal anti-inflammatory drugs in Parkinson's disease," *Experimental Neurology*, vol. 205, no. 2, pp. 295–312, 2007.

[86] B. Xing, M. Liu, and G. Bing, "Neuroprotection with pioglitazone against LPS insult on dopaminergic neurons may be associated with its inhibition of NF-κB and JNK activation and suppression of COX-2 activity," *Journal of Neuroimmunology*, vol. 192, no. 1-2, pp. 89–98, 2007.

[87] B. Xing, T. Xin, R. L. Hunter, and G. Bing, "Pioglitazone inhibition of lipopolysaccharide-induced nitric oxide synthase is associated with altered activity of p38 MAP kinase and PI3K/Akt," *Journal of Neuroinflammation*, vol. 5, article 4, 2008.

[88] A. Bernardo and L. Minghetti, "PPAR-γ agonists as regulators of microglial activation and brain inflammation," *Current Pharmaceutical Design*, vol. 12, no. 1, pp. 93–109, 2006.

[89] T. Koppal, T. V. Petrova, and L. J. Van Eldik, "Cyclopentenone prostaglandin 15-deoxy-Δ-prostaglandin J acts as a general inhibitor of inflammatory responses in activated BV-2 microglial cells," *Brain Research*, vol. 867, no. 1-2, pp. 115–121, 2000.

[90] R. Luna-Medina, M. Cortes-Canteli, M. Alonso, A. Santos, A. Martínez, and A. Perez-Castillo, "Regulation of inflammatory response in neural cells in vitro by thiadiazolidinones derivatives through peroxisome proliferator-activated receptor γ activation," *Journal of Biological Chemistry*, vol. 280, no. 22, pp. 21453–21462, 2005.

[91] J. Xu and P. D. Drew, "Peroxisome proliferator-activated receptor-γ agonists suppress the production of IL-12 family cytokines by activated glia," *Journal of Immunology*, vol. 178, no. 3, pp. 1904–1913, 2007.

[92] D. J. Loane, B. F. Deighan, R. M. Clarke, R. J. Griffin, A. M. Lynch, and M. A. Lynch, "Interleukin-4 mediates the neuroprotective effects of rosiglitazone in the aged brain," *Neurobiology of Aging*, vol. 30, no. 6, pp. 920–931, 2009.

[93] A. Patzer, YI. Zhao, I. Stöck, P. Gohlke, T. Herdegen, and J. Culman, "Peroxisome proliferator-activated receptorsγ (PPARγ) differently modulate the interleukin-6 expression in the peri-infarct cortical tissue in the acute and delayed phases of cerebral ischaemia," *European Journal of Neuroscience*, vol. 28, no. 9, pp. 1786–1794, 2008.

[94] M. P. Pereira, O. Hurtado, A. Cárdenas et al., "Rosiglitazone and 15-deoxy-Δ12,14-prostaglandin J 2 cause potent neuroprotection after experimental stroke through noncompletely overlapping mechanisms," *Journal of Cerebral Blood Flow and Metabolism*, vol. 26, no. 2, pp. 218–229, 2006.

[95] S. Sundararajan, J. L. Gamboa, N. A. Victor, E. W. Wanderi, W. D. Lust, and G. E. Landreth, "Peroxisome proliferator-activated receptor-γ ligands reduce inflammation and infarction size in transient focal ischemia," *Neuroscience*, vol. 130, no. 3, pp. 685–696, 2005.

[96] C. K. Combs, D. E. Johnson, J. C. Karlo, S. B. Cannady, and G. E. Landreth, "Inflammatory mechanisms in Alzheimer's disease: inhibition of beta-amyloid-stimulated proinflammatory responses and neurotoxicity by PPARgamma agonists," *The Journal of Neuroscience*, vol. 20, no. 2, pp. 558–567, 2000.

[97] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[98] C. Qi, Y. Zhu, and J. K. Reddy, "Peroxisome proliferator-activated receptors, coactivators, and downstream targets," *Cell Biochemistry and Biophysics*, vol. 32, pp. 187–204, 2000.

[99] S. van Neerven and J. Mey, "RAR/RXR and PPAR/RXR signaling in spinal cord injury," *PPAR Research*, vol. 2007, Article ID 29275, 2007.

[100] D. S. Straus and C. K. Glass, "Cyclopentenone prostaglandins: new insights on biological activities and cellular targets," *Medicinal Research Reviews*, vol. 21, no. 3, pp. 185–210, 2001.

[101] M. Ricote and C. K. Glass, "PPARs and molecular mechanisms of transrepression," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 926–935, 2007.

[102] H. L. Zhang, Z. L. Gu, S. I. Savitz, F. Han, K. Fukunaga, and Z. H. Qin, "Neuroprotective effects of prostaglandin A in rat models of permanent focal cerebral ischemia are associated with nuclear factor-κB inhibition and peroxisome proliferator-activated receptor-γ up-regulation," *Journal of Neuroscience Research*, vol. 86, no. 5, pp. 1132–1141, 2008.

[103] G. Pascual, A. L. Fong, S. Ogawa et al., "A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-γ," *Nature*, vol. 437, no. 7059, pp. 759–763, 2005.

[104] P. L. McGeer, S. Itagaki, B. E. Boyes, and E. G. McGeer, "Reactive microglia are positive for HLA-DR in the substantia nigra

of Parkinson's and Alzheimer's disease brains," *Neurology*, vol. 38, no. 8, pp. 1285–1291, 1988.

[105] T. Yamada, P. L. McGeer, and E. G. McGeer, "Lewy bodies in Parkinson's disease are recognized by antibodies to complement proteins," *Acta Neuropathologica*, vol. 84, no. 1, pp. 100–104, 1992.

[106] C. F. Orr, D. B. Rowe, Y. Mizuno, H. Mori, and G. M. Halliday, "A possible role for humoral immunity in the pathogenesis of Parkinson's disease," *Brain*, vol. 128, no. 11, pp. 2665–2674, 2005.

[107] V. Brochard, B. Combadière, A. Prigent et al., "Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease," *Journal of Clinical Investigation*, vol. 119, no. 1, pp. 182–192, 2009.

[108] K. Hisanaga, M. Asagi, Y. Itoyama, and Y. Iwasaki, "Increase in peripheral CD4+ bright CD8 dull+ T cells in Parkinson disease," *Archives of Neurology*, vol. 58, no. 10, pp. 1580–1583, 2001.

[109] Y. Baba, A. Kuroiwa, R. J. Uitti, Z. K. Wszolek, and T. Yamada, "Alterations of T-lymphocyte populations in Parkinson disease," *Parkinsonism and Related Disorders*, vol. 11, no. 8, pp. 493–498, 2005.

[110] M. Reale, C. Iarlori, A. Thomas et al., "Peripheral cytokines profile in Parkinson's disease," *Brain, Behavior, and Immunity*, vol. 23, no. 1, pp. 55–63, 2009.

[111] G. Stypuła, J. Kunert-Radek, H. Stepień, K. Zylińska, and M. Pawlikowski, "Evaluation of interleukins, ACTH, cortisol and prolactin concentrations in the blood of patients with Parkinson's disease," *NeuroImmunoModulation*, vol. 3, no. 2-3, pp. 131–134, 1996.

[112] YI. He, W. D. Le, and S. H. Appel, "Role of Fcγ receptors in nigral cell injury induced by Parkinson disease immunoglobulin injection into mouse substantia Nigra," *Experimental Neurology*, vol. 176, no. 2, pp. 322–327, 2002.

[113] A. D. Reynolds, R. Banerjee, J. Liu, H. E. Gendelman, and R. L. Mosley, "Neuroprotective activities of CD4+CD25+ regulatory T cells in an animal model of Parkinson's disease," *Journal of Leukocyte Biology*, vol. 82, no. 5, pp. 1083–1094, 2007.

[114] A. D. Reynolds, D. K. Stone, R. L. Mosley, and H. E. Gendelman, "Nitrated α-synuclein-induced alterations in microglial immunity are regulated by CD4+ T cell subsets," *Journal of Immunology*, vol. 182, no. 7, pp. 4137–4149, 2009.

[115] M. Ricote, J. S. Welch, and C. K. Glass, "Regulation of macrophage gene expression by the peroxisome proliferator-activated receptor-γ," *Hormone Research*, vol. 54, no. 5-6, pp. 275–280, 2000.

[116] R. B. Clark, D. Bishop-Bailey, T. Estrada-Hernandez, T. Hla, L. Puddington, and S. J. Padula, "The nuclear receptor PPARγ and immunoregulation: PPARγ mediates inhibition of helper T cell responses," *Journal of Immunology*, vol. 164, no. 3, pp. 1364–1371, 2000.

[117] C. K. Glass and K. Saijo, "Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells," *Nature Reviews Immunology*, vol. 10, no. 5, pp. 365–376, 2010.

[118] H. Y. Won, H. J. Min, W. H. Lee, S. G. Kim, and E. S. Hwang, "Gα12 is critical for TCR-induced IL-2 production and differentiation of T helper 2 and T helper 17 cells," *Biochemical and Biophysical Research Communications*, vol. 394, no. 3, pp. 811–816, 2010.

[119] L. Klotz, S. Burgdorf, I. Dani et al., "The nuclear receptor PPARγ selectively inhibits Th17 differentiation in a T cell-intrinsic fashion and suppresses CNS autoimmunity," *Journal of Experimental Medicine*, vol. 206, no. 10, pp. 2079–2089, 2009.

[120] A. Awasthi and V. K. Kuchroo, "T17 cells: from precursors to players in inflammation and infection," *International Immunology*, vol. 21, no. 5, pp. 489–498, 2009.

[121] A. P. Woster and C. K. Combs, "Differential ability of a thiazolidinedione PPARγ agonist to attenuate cytokine secretion in primary microglia and macrophage-like cells," *Journal of Neurochemistry*, vol. 103, no. 1, pp. 67–76, 2007.

[122] M. A. Bouhlel, B. Derudas, E. Rigamonti et al., "PPARγ activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties," *Cell Metabolism*, vol. 6, no. 2, pp. 137–143, 2007.

[123] M. A. Bouhlel, J. Brozek, B. Derudas et al., "Unlike PPARγ, PPARα or PPARβ/δ activation does not promote human monocyte differentiation toward alternative macrophages," *Biochemical and Biophysical Research Communications*, vol. 386, no. 3, pp. 459–462, 2009.

[124] F. Lovren, Y. Pan, A. Quan et al., "Adiponectin primes human monocytes into alternative anti-inflammatory M2 macrophages," *American Journal of Physiology*, vol. 299, no. 3, pp. H656–H663, 2010.

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Review Article

Transcriptional Factor NF-κB as a Target for Therapy in Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative condition characterized by chronic inflammation. Nuclear factor κB (NF-κB) is a family of inducible transcription factors that are expressed in a wide variety of cells and tissues, including microglia, astrocytes, and neurons, and the classical NF-κB pathway plays a key role in the activation and regulation of inflammatory mediator production during inflammation. Activation of the classical NF-κB pathway is mediated through the activity of the IKK kinase complex, which consists of a heterotrimer of IKKα, IKKβ, and IKKγ subunits. Targeting NF-κB has been proposed as an approach to the treatment of acute and chronic inflammatory conditions, and the use of inhibitors specific for either IKKβ or IKKγ has now been found to inhibit neurodegeneration of TH+ DA-producing neurons in murine and primate models of Parkinson's disease. These studies suggest that targeting the classical pathway of NF-κB through the inhibition of the IKK complex can serve as a useful therapeutic approach to the treatment of PD.

1. Introduction

Parkinson's disease (PD) is a progressive degenerative disorder of the central nervous system (CNS) that leads to impairment of motor skills and speech, as well as other functions. While the disease mechanisms that ultimately cause PD are still unclear, it is believed that the progressive nature of PD is characterized by chronic inflammation-induced neurodegeneration of dopamine-producing neurons within the substantia nigra (SN) and striatum [1–4]. It is now well documented that microglial activation results in the loss of dopaminergic neurons (DA-neurons). The premise of microglia activation in PD has been supported by analysis of postmortem brains from PD patients, which provides clear evidence of microglia activation in the SN. In the brains of patients with PD, large numbers of human leukocyte antigen (HLA-DR) and CD11b-positive reactive microglia were found in the SN, a region in which the degeneration of DA-neurons was most prominent [5–8]. In addition, levels of proinflammatory mediators, including TNFα, IL-1β, IL-6, and eicosanoids are elevated in the brains and peripheral blood mononuclear cells (PBMCs) of patients with PD [6, 7]. Nitrite in the cerebrospinal fluid as well as increased expression of inducible nitric oxide synthase (iNOS) within the SN have been found in PD patients [9, 10]. All of these findings lend strong support to the association of inflammation and PD. Many of these inflammatory mediators have been demonstrated to have strong neurotoxic effects on DA-neurons [1, 11–14], and the central cell implicated in the initiation and execution of inflammatory responses within the CNS is the microglial cell. The pro-inflammatory response of the microglial cell is primarily mediated by the transcription factor NF-κB, which is activated by pro-inflammatory signals and controls

the gene expression of most of the inflammatory mediators produced by microglial cells. Understanding the role of inflammation in the etiology of PD, developing effective anti-inflammatory therapies directed at NF-κB activity in microglia, and determining the efficacy of these NF-κB inhibitors in protecting DA-neurons from degeneration are of particular interest as therapeutic approaches aimed at stopping and reversing the debilitating effects of PD.

2. Microglial Cells, PD, and Mechanisms of Inflammation

The etiology of PD suggests that chronic production of inflammatory mediators such as TNFα, nitric oxide (NO), and IL-1β mediates a significant amount of neuronal tissue destruction, and the major cell within the CNS that produces these mediators is the microglial cell. Several agents which directly activate microglia have been shown to induce neurotoxicity to DA-producing neurons both *in vitro* and *in vivo*. These include LPS [14], f-Met-Leu-Phe (fMLP), β-amyloid peptides [15], Parkin [16], and aggregated or nitrated-α-synuclein [17]. In addition, several direct neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) can activate microglia through the release of cellular contents, which leads to an exacerbation of the neurotoxicity [18–21]. There is also a strong correlation between environmental factors which lead to the induction of an inflammatory response within the brain, including traumatic brain injury [22, 23] and infection [24], and the ultimate onset of PD. Once activated, microglial cells produce a wide variety of inflammatory mediators which serve to promote an innate immune response, including inflammatory cytokines, chemokines, prostaglandins and leukotrienes, NO, reactive oxygen species (ROS), and glutamate.

Figure 1 proposes our model on the role of inflammation in progressive neurodegeneration like that seen in PD. Pro-inflammatory signals such as infection, trauma, stress, or exposure to environmental factors can directly activate microglial cells. Activated microglial cells, like macrophages, undergo changes that enhance their microbicidal effectiveness and their ability to modulate the inflammatory immune response. These changes include enhanced phagocytic ability, microbial killing, antigen presentation, and inflammatory cytokine production. Activated microglial cells then secrete these inflammatory cytokines, such as TNF-α, IL-1β, MIP-1α, and IL-6 [25], which can both recruit new inflammatory cells as well as lead to direct killing of DA neurons.

Activated microglial cells also release NO, which is produced by inducible nitric oxide synthase (iNOS) [26]. Furthermore, microglial cell activation leads to enhanced respiratory burst activity as well. The microglial respiratory burst is characterized by the release of various reactive oxygen species (ROS) including superoxide radicals and hydrogen peroxide. It is now clear that many of the inflammatory mediators produced by activated microglia can actively trigger apoptosis in neuronal cell cultures. These mediators include tumor necrosis factor-α (TNF-α) [27] nitric oxide (NO), interleukin-1β (IL-1b, cathepsin-B) [7, 28], glutamate

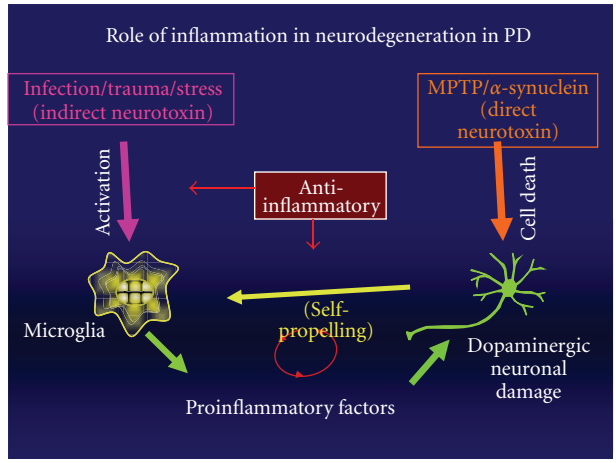

Figure 1

[29, 30], IL-8 [31], and ROS [10]. Highly elevated levels of several of these inflammatory mediators (TNF, NO, PGE2, IL-1β, IL-6) can be found in the cerebrospinal fluid (CSF) of PD patients, as well as in the brain SNpc tissue of PD patients when analyzed in postmortem autopsies [7, 9, 10, 27, 32, 33]. Interestingly, NF-κB activation is required for all of these mediators to be produced by microglial cells. In addition, microglial cells can be activated following the death of DA-neurons from either inflammatory damage or from the direct effects of DA-neurotoxins such as MPTP or 6-OHDA. It has also been found that intranigral and/or plasma TNF levels remained elevated in MPTP-treated rodents or nonhuman primates one year after administration of the neurotoxin [34]. Once microglial cells have been activated to release neurotoxic inflammatory mediators, further DA neuronal damage occurs which results in the generation of reactive microgliosis, a process by which there is a self-perpetuating cycle resulting in sustained chronic neuroinflammation that drives the progressive neurodegeneration in PD. This process leads to the chronic nature of the disease and to sustained neuronal damage over a prolonged period of time. Therefore, intervention with anti-inflammatory reagents such as NF-κB inhibitors could function to inhibit the initial activation of microglial cells or to halt the continual reactivation which occurs during reactive microgliosis, which is the hallmark of this chronic inflammatory condition. Ultimately, reactive microgliosis, if left unchecked, will result in destruction of DA-producing neurons.

3. The Biology of NF-κB

Many of the inflammatory mediators involved in inflammation and DA neurodegeneration in PD have a common feature: their expression in microglial cells is primarily regulated by NF-κB. The transcription factor NF-κB, first described by David Baltimore's group in 1986 as a transcription factor which is essential for the expression of mouse kappa light chain genes [35, 36], has now been found to control gene expression of many of proinflammatory responses. NF-κB is a "master switch" for inflammatory

gene expression [37]. Inflammatory cytokines such as TNF and IL-1α and β, bacterial products such as lipopolysaccharide (LPS), and products of cellular damage strongly activate inflammatory responses through the activation of NF-κB. NFκB subsequently plays an essential role in the inflammatory response through regulation of genes encoding inflammatory cytokines (IL-1β, TNFα, IL-12/23), chemokines (IL-8, MIP-1α, MCP-1 [38–40]), nitric oxide production (iNOS), NADPH oxidase subunits p47 and p67 [41, 42], and adhesion molecules (ICAM-1, VCAM, and E-selectin [43, 44]). Activation of NF-κB is a key event in many chronic inflammatory diseases such as asthma, cardiovascular disease [45], tissue reperfusion injury [46], experimental autoimmune encephalomyelitis (EAE) [47], rheumatoid arthritis [48], and inflammatory bowel disease (IBD) [49]. Many of the standard agents used to treat human inflammatory conditions, including sulfasalazine, 5-aminosalicylates, and corticosteroids, as well as some natural anti-inflammatory compounds such as IL-10, TGFβ1, β2AR agonists, glutamate, and curcumin, among others, have been postulated to exert some of their anti-inflammatory effects through NF-κB inhibition [41, 50–52]. We and others have found that these compounds are potent inhibitors of microglial activation and are neuroprotective to DA neurons *in vitro* and/or *in vivo*. Thus, NF-κB activity emerges as a key target to control the chronic inflammation in humans, and strategies for its use in PD to inhibit NF-κB activity in microglial cells more potently may lead to more effective treatments for PD.

The NF-κB family consists of dimeric transcription factors which include five members: c-Rel, RelA (p65), RelB, NF-κB1 (p50/p105), and NF-κB2 (p52/p100). There are two major pathways of activation: the classical or canonical pathway and the alternate or noncanonical pathway. The classical pathway, which is thought to regulate the production of most pro-inflammatory mediators, is mediated through the activation of a dimer of Rel proteins p50 and p65, complexed within the cytosol to the inhibitory complex IκBα [41]. The activation of the classical NF-κB pathway is dependent on the phosphorylation, ubiquitination, and subsequent proteasome-dependent degradation of IκBα. The phosphorylation of IκBα on serine residues is mediated by IκB kinase (IKK), which is a molecular complex of three proteins consisting of a heterodimer of the two catalytic units IKKα and IKKβ, along with IKKγ (the NF-κB essential modulator, NEMO) [53–55]. Mice null for IKKβ, IKKγ, or p65 but not IKKα are embryonic lethal as a result of massive liver apoptosis. Cells derived from these embryos are unresponsive to classical NF-κB inducers such as TNFα and IL-1β [56–58], demonstrating a signaling link between p65, IKKβ, and IKKγ subunits. Activation of the IKK in response to inflammatory mediators like TNFα, IL-1β, and LPS depends critically on the presence of the IKKγ (NEMO) subunit of the IKK complex [55, 59] and results in the phosphorylation of the IκB by the kinase activity of IKKβ [53, 54]. An N-terminal region of NEMO associates with a hexapeptide sequence within the C-terminus of both IKKα and IKKβ, named the NEMO-binding domain (NBD), and disruption or mutation of this NEMO-NBD interaction site on either IKKβ or IKKγ results in a loss of responsiveness of cells to pro-inflammatory signaling.

On the other hand, the noncanonical pathway of NF-κB consists of heterodimers of Rel proteins p100/RelB that also have transcriptional activity but appear to play more of a regulatory role in cellular activation and differentiation rather than in inflammation. In response to a set of factors that include CD40L, B cell-activating factor, and lymphotoxin-β, NF-κB is activated through an alternative pathway independent of IKK [60–65]. Instead, activation proceeds through the NF-κB-inducing kinase (NIK) that phosphorylates and activates IKK$\alpha\alpha$ homodimers which, in turn, phosphorylate p100 in complex with RelB. This leads to ubiquitin-dependent processing of p100 to p52 and translocation of p52/RelB to the nucleus [63, 64]. Cytokine-induced activation of the noncanonical pathway of NF-κB is accompanied by an increase in the concentration of nuclear IKKα that phosphorylates histone H3 [66, 67]. In cells exposed to cytokines, nuclear IKKα regulates gene expression through promoter-associated histone phosphorylation and binding to promoter regions of NF-κB responsive genes. Mice deficient in IKKα die perinatally, with phenotypical changes of dermal and skeletal development [68–71]. B-cell activating factor, NIK, and p100/p52 knockout mice have similar phenotypes [72, 73], suggesting that these molecules are all part of the same linear nonclassical signaling cascade. In addition, the classical and alternative pathways are thought to regulate distinct genes in response to their various activators [65]. Relevant to PD, it has now been found that the canonical pathway is highly activated within the SN of animals undergoing DA neurodegeneration, and aftermortem in the brains of PD patients [74, 75]. On the other hand, the non-canonical NF-κB pathway is found to be activated in regenerating DA-neurons from rats treated with glial-derived neurotrophic factor (GDNF), while the canonical p65/p50 pathway is concomitantly decreased, suggesting that this non-canonical NF-κB pathway is important in neuron regeneration of DA neurons within the SN [76].

4. Therapeutic Usage of Specific NF-κB Inhibitors in Chronic Inflammation

Due to the central role of the IKKγ and Ikkβ molecules within the IKK complex in activating inflammation, the identification of selective IKKβ and IKKγ inhibitors that do not target IKKα or the P100/p52 pathway as therapeutic agents in treating chronic inflammation is of considerable interest. Two specific inhibitors of NF-κB have emerged that appear to be highly therapeutically active in the treatment of several chronic inflammatory diseases, and which provide possible therapeutic approaches to the treatment of PD. The first is a peptide directed against the N-terminal region of NEMO that associates with a hexapeptide sequence within the C-terminus of both IKKα and IKKβ, named the NEMO-binding domain (NBD). This cell permeable peptide spans the NBD and disrupts the association of NEMO with IKKs *in vitro* and blocks TNFα-induced NF-κB activation *in vivo* [77, 78]. Notably, the NBD peptide does not affect basal activity of the IKK but only suppresses the induction of activity

in response to inflammatory cytokines [77]. Continuous administration of the NBD peptide effectively ameliorates inflammatory responses in animal models without overt signs of toxicity [78]. Additionally, in mouse models of chronic inflammation, including collagen-induced arthritis (CIA) [79, 80], experimental allergic encephalomyelitis (EAE), Duchenne's muscular dystrophy (Peterson et al., manuscript in submission), and inflammatory bowel disease (IBD) [81], *in vivo* treatment with NBD peptides blocked disease activity, inflammatory cytokine expression, and homing of cells to inflammatory sites. Furthermore, mice treated systemically with an NBD peptide for five days after induction of CIA maintained clinical and histological improvement for nearly three weeks following termination of peptide administration [79]. It is important to note that in the therapeutic use of NBD peptide, there was no evidence of undesired off-target effects as the treatment with NBD peptide was shown to be specific for inhibiting NF-κB signaling, exhibiting no inhibitory effects towards JNK or p38 MAPK pathways [78]. The safety profile for NBD is favorable as well. *In vivo*, systemic delivery of NBD is not associated with any described toxicity in mice or rats, and inhibition of NF-κB has been demonstrated to ameliorate an ever-growing list of inflammatory disease conditions [77–79, 82, 83]. Therefore, the therapeutic effect of the short-lived NBD peptide may far outlast its pharmacokinetic properties. Thus, selective IKK inhibition by NBD peptides may (i) be an effective therapeutic intervention in chronic inflammatory diseases; (ii) lead to durable alterations in immune responses that correlate with durable clinical efficacy; (iii) minimize potential toxicity concerns associated as basal NF-κB activity remains intact as does the alternative pathway of NF-κB activation necessary for B-cell development and lymphoid organogenesis.

A second approach to the inhibition of inflammation has been to utilize small molecule inhibitors that specifically block the kinase enzymatic activity of IKKβ. One such specific inhibitor, called Compound A, is a small molecule inhibitor of the kinase activity of IKKβ but not IKKα. Compound A, also known as BAY-65-1942 (7-[2-(cyclopropylmethoxy)-6-hydroxyphenyl]-5-[(3S)-3-piperidinyl]-1,4-dihydro-2Hpyrido [2,3-d] [1, 3]-oxazin-2-one hydrochloride), has been shown to specifically and effectively block the catalytic activity of IKKβ, inhibiting its ability to phosphorylate IκB and activate the cytosolic p50/p65 NF-κB heterodimers [84, 85]. Compound A has been used extensively *in vivo*, and it has now been found to prevent pulmonary inflammation [86], to attenuate myocardial injury and dysfunction after ischemia-reperfusion injury [84], and to prevent graft versus host disease in murine models of GVHD (Serody et al., personal communication). Other IKKβ inhibitors, including PS-1145 [87] and TPCA-1 [88], have been shown to effectively prevent graft versus host disease in a murine bone marrow transplant model [89], to enhance sensitivity of multiple myeloma cells to chemotherapy by inhibiting the protective effects of NF-κB [90], to inhibit melanoma growth in vivo [91–93], and to inhibit the growth of colon cancer [94]. These inhibitors work primarily through the inhibition of IKKβ, and their specific suppression of the canonical NF-κB signaling pathway and consequent decrease in serum levels of TNFα and IL-6 are the main features which mediate their inhibitory activity.

5. NF-κB and DA Neuroinflammation

We have found that a number of anti-inflammatory compounds have been demonstrated to have efficacy in protecting DA-neurons from degeneration mediated by inflammatory damage. These compounds include IL-10 [95, 96], TGFβ1 [97, 98], morphinan derivatives [99], and DPI, an inhibitor of oxidative stress responses [100]. It is well known that while these compounds have numerous regulatory effects on multiple biological targets, one of their primary anti-inflammatory features is the inhibition of NF-κB within macrophages and microglial cells [101]. Recently, it has been demonstrated that several other compounds, including pioglitazone (a PPARγ agonist), curcumin [102], and salmeterol (a β2AR agonist, our reference [103]), function to inhibit DA neurodegeneration by inhibiting NF-κB. However, the most compelling data suggesting a central role for NF-κB as a regulatory target for PD therapy comes from the use of specific NF-κB inhibitors, which have recently been used in murine models of PD to determine if therapeutic administration of these inhibitors could halt the progression of neurodegeneration induced by the neurotoxin MPTP [104] or by activation of CNS inflammation by the intracranial injection of LPS [105]. Compounds that block the activation of NF-κB are capable of inhibiting the two major inflammatory pathways in microglia—activation of oxidative stress and production of inflammatory mediators, including cytokines TNFα, IL-1β, and IL-6, as well as chemokines associated with inflammation [101]. Ghosh et al. have used the NBD peptide and a mutant peptide control to study the efficacy of NF-κB inhibition in stopping or reversing the neurodegenerative effects of MPTP administration in a murine model of PD [104]. Evidence shows that there is a marked increase in NF-κB activation within the midbrain of animals undergoing neurodegeneration as a result of MPTP administration, as well as in the SNpc of PD patients [75, 104], and this activation occurs in the TH+ DA-neurons and in astrocytes and microglia. Administration of NBD peptide but not the mutant control peptide was shown to inhibit MPP+ induced NF-κB activation *in vitro* in microglia, astrocytes, as well as in BV-2 microglial cells, as determined by DNA binding and transcriptional activity. More importantly, administration of NBD peptide but not the mutant peptide prior to the injection of MPTP *in vivo* significantly inhibits the activation of NF-κB within the midbrain region. This inhibition of NF-κB activation is accompanied by a concomitant reduction in inflammatory mediator mRNA expression within the SNpc, as well as the expression of activation markers CD11b and GFAP by microglia and astrocytes, respectively. Mice receiving NBD peptide but not mutant peptide prior to MPTP injection also showed highly significant protection of the nigrostriatum from MPTP-induced neurodegeneration of the TH+ neurons and the loss of dopamine production, as well as improvement in their

locomotor function compared with MPTP-injected mice given mutant peptide. More importantly, administration of NBD peptide 2 days subsequent to the injection of MPTP shows substantial protection of TH+ neurons, suggesting that NBD peptide can be used therapeutically to slow down or halt the progression of DA neurodegeneration in MPTP-treated animals [104]. Infrared analysis of the brains of NBD-treated animals determined that peptide could be found within the brain tissue in significant quantities, suggesting that the NBD peptide could cross the blood-brain barrier (BBB) and reach the site of inflammation. It remains to be determined the mechanism of NF-κB inhibition within the SNpc, but these data suggest that NF-κB is a viable target for therapy for PD patients.

Subsequently, we have used the IKKβ inhibitor compound A in LPS-induced neurodegeneration to determine if, similar to NBD peptide, inhibition of the canonical NF-κB pathway could halt inflammation-induced DA neurodegeneration. In this model, LPS is injected directly into one side of the midbrain of rats, leading to inflammation-induced degeneration of DA-neurons [105]. It was found that IKKβ inhibitor compound A was capable of strongly inhibiting the activation of NF-κB *in vitro* and *in vivo*, as well as the mRNA expression and subsequent release of pro-inflammatory mediators. Compound A also significantly inhibited LPS- and MPTP-induced DA neurotoxicity *in vitro*, and this neuroprotective activity required the presence of microglial cells. Most importantly, administration of compound A to animals injected intranigrally with LPS attenuated LPS injection-induced DA neuronal loss and microglia activation within the SNpc [105]. Taken together, these data provide strong evidence that NF-κB offers an excellent therapeutic target to inhibit DA neurodegeneration, and that significant additional work needs to be performed to determine the optimal approach and agent best suited for the treatment of PD.

6. Conclusion

Strong evidence now exists that inflammation plays a key role in the neurodegeneration seen in PD, and that effective anti-inflammatory therapy can be very useful in slowing down or preventing the chronic destruction of DA-neurons which is the hallmark of the disease. While a number of anti-inflammatories have already been developed and utilized, many of these compounds have multiple off-target effects or do not show strong efficacy in the treatment of PD. In addition, it is not yet clear the best target for anti-inflammatory therapy in PD and other chronic inflammatory conditions. NF-κB, the "master switch" of inflammation, offers an ideal target for therapy because of its key role in the production of the inflammatory mediators known to exhibit DA neurotoxicity in vitro and in vivo. Targeting the activation pathway of NF-κB by inhibiting the IKK complex has now been shown to be a highly effective therapy for the treatment of neurodegeneration in murine models of PD and offers us a new avenue of investigation towards the development of more effective therapies aimed at stopping and reversing DA-neuron loss in this disease.

References

[1] M. L. Block and J. S. Hong, "Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism," *Progress in Neurobiology*, vol. 76, no. 2, pp. 77–98, 2005.

[2] A. L. Bartels and K. L. Leenders, "Neuroinflammation in the pathophysiology of Parkinson's disease: evidence from animal models to human in vivo studies with [C]-PK11195 PET," *Movement Disorders*, vol. 22, no. 13, pp. 1852–1856, 2007.

[3] W. Dauer and S. Przedborski, "Parkinson's disease: mechanisms and models," *Neuron*, vol. 39, no. 6, pp. 889–909, 2003.

[4] H. M. Gao and J. S. Hong, "Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression," *Trends in Immunology*, vol. 29, no. 8, pp. 357–365, 2008.

[5] P. L. McGeer, S. Itagaki, B. E. Boyes, and E. G. McGeer, "Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains," *Neurology*, vol. 38, no. 8, pp. 1285–1291, 1988.

[6] T. Nagatsu, M. Mogi, H. Ichinose, and A. Togari, "Changes in cytokines and neurotrophins in Parkinson's disease," *Journal of Neural Transmission*, no. 60, supplement, pp. 277–290, 2000.

[7] M. Mogi, M. Harada, T. Kondob et al., "Interleukin-1β, interleukin-6, epidermal growth factor and transforming growth factor-α are elevated in the brain from parkinsonian patients," *Neuroscience Letters*, vol. 180, no. 2, pp. 147–150, 1994.

[8] Y. Ouchi, S. Yagi, M. Yokokura, and M. Sakamoto, "Neuroinflammation in the living brain of Parkinson's disease," *Parkinsonism and Related Disorders*, vol. 15, no. 3, pp. S200–S204, 2009.

[9] G. A. Qureshi, S. Baig, I. Bednar, P. Sodersten, G. Forsberg, and A. Siden, "Increased cerebrospinal fluid concentration of nitrite ire Parkinson's disease," *NeuroReport*, vol. 6, no. 12, pp. 1642–1644, 1995.

[10] S. Hunot, F. Boissière, B. Faucheux et al., "Nitric oxide synthase and neuronal vulnerability in Parkinson's disease," *Neuroscience*, vol. 72, no. 2, pp. 355–363, 1996.

[11] L. Qian and P. M. Flood, "Microglial cells and Parkinson's disease," *Immunologic Research*, vol. 41, no. 3, pp. 155–164, 2008.

[12] M. K. McCoy and M. G. Tansey, "TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease," *Journal of Neuroinflammation*, vol. 5, article 45, 2008.

[13] P. Jenner, "Oxidative mechanisms in nigral cell death in Parkinson's disease," *Movement Disorders*, vol. 13, supplement 1, pp. 24–34, 1998.

[14] J. Zielasek and H. P. Hartung, "Molecular mechanisms of microglial activation," *Advances in Neuroimmunology*, vol. 6, no. 2, pp. 191–222, 1996.

[15] J. Rogers and L. F. Lue, "Microglial chemotaxis, activation, and phagocytosis of amyloid β-peptide as linked phenomena in Alzheimer's disease," *Neurochemistry International*, vol. 39, no. 5-6, pp. 333–340, 2001.

[16] T. C. Frank-Cannon, T. Tran, K. A. Ruhn et al., "Parkin deficiency increases vulnerability to inflammation-related nigral degeneration," *Journal of Neuroscience*, vol. 28, no. 43, pp. 10825–10834, 2008.

[17] A. D. Reynolds, J. G. Glanzer, I. Kadiu et al., "Nitrated alpha-synuclein-activated microglial profiling for Parkinson's disease," *Journal of Neurochemistry*, vol. 104, no. 6, pp. 1504–1525, 2008.

[18] E. C. Hirsch, S. Hunot, and A. Hartmann, "Neuroinflammatory processes in Parkinson's disease," *Parkinsonism and Related Disorders*, vol. 11, no. 1, pp. S9–S15, 2005.

[19] H. Wilms, L. Zecca, P. Rosenstiel, J. Sievers, G. Deuschl, and R. Lucius, "Inflammation in Parkinson's diseases and other neurodegenerative diseases: cause and therapeutic implications," *Current Pharmaceutical Design*, vol. 13, no. 18, pp. 1925–1928, 2007.

[20] P. L. McGeer, C. Schwab, A. Parent, and D. Doudet, "Presence of reactive microglia in monkey Substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration," *Annals of Neurology*, vol. 54, no. 5, pp. 599–604, 2003.

[21] F. Cicchetti, A. L. Brownell, K. Williams, Y. I. Chen, E. Livni, and O. Isacson, "Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging," *European Journal of Neuroscience*, vol. 15, no. 6, pp. 991–998, 2002.

[22] W. Meissner, C. Prunier, D. Guilloteau, S. Chalon, C. E. Gross, and E. Bezard, "Time-course of nigrostriatal degeneration in a progressive MPTP-lesioned macaque model of Parkinson's disease," *Molecular Neurobiology*, vol. 28, no. 3, pp. 209–218, 2003.

[23] A. D. Ebert, J. H. Hoo, and M. C. Bohn, "Progressive degeneration of dopamine neurons in 6-hydroxydopamine rat model of Parkinson's disease does not involve activation of caspase-9 and caspase-3," *Journal of Neuroscience Research*, vol. 86, no. 2, pp. 317–325, 2008.

[24] A. R. Simard and S. Rivest, "Do pathogen exposure and innate immunity cause brain diseases?" *Neurological Research*, vol. 27, no. 7, pp. 717–725, 2005.

[25] R. M. Ransohoff and V. H. Perry, "Microglial physiology: unique stimuli, specialized responses," *Annual Review of Immunology*, vol. 27, pp. 119–145, 2009.

[26] G. C. Brown and J. J. Neher, "Inflammatory neurodegeneration and mechanisms of microglial killing of neurons," *Molecular Neurobiology*, vol. 41, no. 2-3, pp. 242–247, 2010.

[27] M. Mogi, M. Harada, P. Riederer, H. Narabayashi, K. Fujita, and T. Nagatsu, "Tumor necrosis factor-α (TNF-α) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients," *Neuroscience Letters*, vol. 165, no. 1-2, pp. 208–210, 1994.

[28] H. Arai, T. Furuya, T. Yasuda, M. Miura, Y. Mizuno, and H. Mochizuki, "Neurotoxic effects of lipopolysaccharide on nigral dopaminergic neurons are mediated by microglial activation, interleukin-1β, and expression of caspase-11 in mice," *Journal of Biological Chemistry*, vol. 279, no. 49, pp. 51647–51653, 2004.

[29] W. M. Caudle and J. Zhang, "Glutamate, excitotoxicity, and programmed cell death in parkinson disease," *Experimental Neurology*, vol. 220, no. 2, pp. 230–233, 2009.

[30] D. W. Choi, "Glutamate neurotoxicity and diseases of the nervous system," *Neuron*, vol. 1, no. 8, pp. 623–634, 1988.

[31] L. Thirumangalakudi, L. Yin, H. V. Rao, and P. Grammas, "IL-8 induces expression of matrix metalloproteinases, cell cycle and pro-apoptotic proteins, and cell death in cultured neurons," *Journal of Alzheimer's Disease*, vol. 11, no. 3, pp. 305–311, 2007.

[32] D. A. Loeffler, A. J. DeMaggio, P. L. Juneau, M. K. Havaich, and P. A. LeWitt, "Effects of enhanced striatal dopamine turnover in vivo on glutathione oxidation," *Clinical Neuropharmacology*, vol. 17, no. 4, pp. 370–379, 1994.

[33] K. Imamura, N. Hishikawa, M. Sawada, T. Nagatsu, M. Yoshida, and Y. Hashizume, "Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains," *Acta Neuropathologica*, vol. 106, no. 6, pp. 518–526, 2003.

[34] C. Barcia, V. De Pablos, V. Bautista-Hernández et al., "Increased plasma levels of TNF-α but not of IL1-β in MPTP-treated monkeys one year after the MPTP administration," *Parkinsonism and Related Disorders*, vol. 11, no. 7, pp. 435–439, 2005.

[35] R. Sen and D. Baltimore, "Multiple nuclear factors interact with the immunoglobulin enhancer sequences," *Cell*, vol. 46, no. 5, pp. 705–716, 1986.

[36] R. Sen and D. Baltimore, "Inducibility of K immunoglobulin enhancer-binding protein NF-κB by a posttranslational mechanism," *Cell*, vol. 47, no. 6, pp. 921–928, 1986.

[37] G. Tsoulfas and D. A. Geller, "NF-κB in transplantation: friend or foe?" *Transplant Infectious Disease*, vol. 3, no. 4, pp. 212–219, 2001.

[38] K. A. Roebuck, "Regulation of interleukin-8 gene expression," *Journal of Interferon and Cytokine Research*, vol. 19, no. 5, pp. 429–438, 1999.

[39] Y. Xia, M. E. Pauza, L. Feng, and D. Lo, "RelB regulation of chemokine expression modulates local inflammation," *American Journal of Pathology*, vol. 151, no. 2, pp. 375–387, 1997.

[40] K. A. Roebuck, L. R. Carpenter, V. Lakshminarayanan, S. M. Page, J. N. Moy, and L. L. Thomas, "Stimulus-specific regulation of chemokine expression involves differential activation of the redox-responsive transcription factors AP-1 and NF-κB," *Journal of Leukocyte Biology*, vol. 65, no. 3, pp. 291–298, 1999.

[41] T. Lawrence, "The nuclear factor NF-kappaB pathway in inflammation," *Cold Spring Harbor Perspectives in Biology*, vol. 1, no. 6, article a001651, 2009.

[42] K. A. Gauss, L. K. Nelson-Overton, D. W. Siemsen, Y. Gao, F. R. DeLeo, and M. T. Quinn, "Role of NF-κB in transcriptional regulation of the phagocyte NADPH oxidase by tumor necrosis factor-α," *Journal of Leukocyte Biology*, vol. 82, no. 3, pp. 729–741, 2007.

[43] C. C. Chen and A. M. Manning, "Transcriptional regulation of endothelial cell adhesion molecules: a dominant role for NF-κB," *Agents and Actions Supplements*, vol. 47, pp. 135–141, 1995.

[44] P. P. Tak and G. S. Firestein, "NF-κB: a key role in inflammatory diseases," *Journal of Clinical Investigation*, vol. 107, no. 1, pp. 7–11, 2001.

[45] K. Van der Heiden, S. Cuhlmann, A. Luong, M. Zakkar, and P. C. Evans, "Role of nuclear factor kappaB in cardiovascular health and disease," *Clinical Science*, vol. 118, no. 10, pp. 593–605, 2010.

[46] C. A. Latanich and L. H. Toledo-Pereyra, "Searching for NF-κB-based treatments of ischemia reperfusion injury," *Journal of Investigative Surgery*, vol. 22, no. 4, pp. 301–315, 2009.

[47] K. Vandenbroeck, I. Alloza, M. Gadina, and P. Matthys, "Inhibiting cytokines of the interleukin-12 family: recent advances and novel challenges," *Journal of Pharmacy and Pharmacology*, vol. 56, no. 2, pp. 145–160, 2004.

[48] L. A. Criswell, "Gene discovery in rheumatoid arthritis highlights the CD40/NF-κB signaling pathway in disease pathogenesis," *Immunological Reviews*, vol. 233, no. 1, pp. 55–61, 2010.

[49] I. Atreya, R. Atreya, and M. F. Neurath, "NF-κB in inflammatory bowel disease," *Journal of Internal Medicine*, vol. 263, no. 6, pp. 591–596, 2008.

[50] T. Lawrence and C. Fong, "The resolution of inflammation: anti-inflammatory roles for NF-κB," *International Journal of Biochemistry and Cell Biology*, vol. 42, no. 4, pp. 519–523, 2010.

[51] S. G. Pereira and F. Oakley, "Nuclear factor-κB1: regulation and function," *International Journal of Biochemistry and Cell Biology*, vol. 40, no. 8, pp. 1425–1430, 2008.

[52] M. S. Wang, S. Boddapati, S. Emadi, and M. R. Sierks, "Curcumin reduces α-synuclein induced cytotoxicity in Parkinson's disease cell model," *BMC Neuroscience*, vol. 11, article 57, 2010.

[53] T. Huxford and G. Ghosh, "A structural guide to proteins of the NF-kappaB signaling module," *Cold Spring Harbor Perspectives in Biology*, vol. 1, no. 3, article a000075, 2009.

[54] A. Oeckinghaus and S. Ghosh, "The NF-kappaB family of transcription factors and its regulation," *Cold Spring Harbor Perspectives in Biology*, vol. 1, no. 4, article a000034, 2009.

[55] M. J. May, R. B. Marienfeld, and S. Ghosh, "Characterization of the IκB-kinase NEMO binding domain," *Journal of Biological Chemistry*, vol. 277, no. 48, pp. 45992–46000, 2002.

[56] N. Sizemore et al., "Distinct roles of the Ikappa B kinase alpha and beta subunits in liberating nuclear factor kappa B (NF-kappa B) from Ikappa B and in phosphorylating the p65 subunit of NF-kappa B," *The Journal of Biological Chemistry*, vol. 277, no. 6, pp. 3863–3869, 2002.

[57] N. Sizemore, A. Agarwal, K. Das et al., "Inhibitor of κB kinase is required to activate a subset of interferon γ-stimulated genes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 21, pp. 7994–7998, 2004.

[58] J. Y. Reuther-Madrid, D. Kashatus, S. Chen et al., "The p65/RelA subunit of NF-κB suppresses the sustained, anti-apoptotic activity of Jun kinase induced by tumor necrosis factor," *Molecular and Cellular Biology*, vol. 22, no. 23, pp. 8175–8183, 2002.

[59] D. Rudolph, W. C. Yeh, A. Wakeham et al., "Severe liver degeneration and lack of NF-κB activation in NEMO/IKK γ-deficient mice," *Genes and Development*, vol. 14, no. 7, pp. 854–862, 2000.

[60] Z. P. Xia and Z. J. Chen, "TRAF2: a double-edged sword?" *Science's STKE*, vol. 2005, no. 272, p. e7, 2005.

[61] M. Neumann and M. Naumann, "Beyond IκBs: alternative regulation of NF-κB activity," *FASEB Journal*, vol. 21, no. 11, pp. 2642–2654, 2007.

[62] K. D. Brown, E. Claudio, and U. Siebenlist, "The roles of the classical and alternative nuclear factor-κB pathways: potential implications for autoimmunity and rheumatoid arthritis," *Arthritis Research and Therapy*, vol. 10, no. 4, article 212, 2008.

[63] A. Israel, "The IKK complex, a central regulator of NF-kappaB activation," *Cold Spring Harbor Perspectives in Biology*, vol. 2, no. 3, article a000158, 2010.

[64] N. Bakkar and D. C. Guttridge, "NF-κB signaling: a tale of two pathways in skeletal myogenesis," *Physiological Reviews*, vol. 90, no. 2, pp. 495–511, 2010.

[65] J. L. Pomerantz and D. Baltimore, "Two pathways to NF-κB," *Molecular Cell*, vol. 10, no. 4, pp. 693–695, 2002.

[66] Y. Yamamoto, U. N. Verma, S. Prajapati, K. Youn-Tae, and R. B. Gaynor, "Histone H3 phosphorylation by ikk-α is critical for cytokine-induced gene expression," *Nature*, vol. 423, no. 6940, pp. 655–659, 2003.

[67] V. Anest, J. L. Hanson, P. C. Cogswell, K. A. Steinbrecher, B. D. Strahl, and A. S. Baldwin, "A nucleosomal function for IκB kinase-α in NF-κB-dependent gene expression," *Nature*, vol. 423, no. 6940, pp. 659–663, 2003.

[68] A. K. Sil, S. Maeda, Y. Sono, D. B. Roop, and M. Karin, "IκB kinase-α acts in the epidermis to control skeletal and craniofacial morphogenesis," *Nature*, vol. 428, no. 6983, pp. 660–664, 2004.

[69] R. Gareus, M. Huth, B. Breiden et al., "Normal epidermal differentiation but impaired skin-barrier formation upon keratinocyte-restricted IKK1 ablation," *Nature Cell Biology*, vol. 9, no. 4, pp. 461–469, 2007.

[70] J. M. Dahlman, N. Bakkar, W. He, and D. C. Guttridge, "NF-κB functions in stromal fibroblasts to regulate early postnatal muscle development," *Journal of Biological Chemistry*, vol. 285, no. 8, pp. 5479–5487, 2010.

[71] N. Bakkar, J. Wang, K. J. Ladner et al., "IKK/NF-κB regulates skeletal myogenesis via a signaling switch to inhibit differentiation and promote mitochondrial biogenesis," *Journal of Cell Biology*, vol. 180, no. 4, pp. 787–802, 2008.

[72] Y. Xue, X. Wang, Z. Li, N. Gotoh, D. Chapman, and E. Y. Skolnik, "Mesodermal patterning defect in mice lacking the Ste20 NCK interacting kinase (NIK)," *Development*, vol. 128, no. 9, pp. 1559–1572, 2001.

[73] N. S. Soysa, N. Alles, D. Weih et al., "The pivotal role of the alternative NF-κB pathway in maintenance of basal bone homeostasis and osteoclastogenesis," *Journal of Bone and Mineral Research*, vol. 25, no. 4, pp. 809–818, 2010.

[74] M. Mogi, T. Kondo, Y. Mizuno, and T. Nagatsu, "p53 protein, interferon-γ, and NF-κB levels are elevated in the parkinsonian brain," *Neuroscience Letters*, vol. 414, no. 1, pp. 94–97, 2007.

[75] S. Hunot, B. Brugg, D. Ricard et al., "Nuclear translocation of NF-κb is increased in dopaminergic neurons of patients with Parkinson disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 14, pp. 7531–7536, 1997.

[76] J. P. Cao, H. J. Wang, J. K. Yu, H. M. Liu, and D. S. Gao, "The involvement of NF-κB p65/p52 in the effects of GDNF on DA neurons in early PD rats," *Brain Research Bulletin*, vol. 76, no. 5, pp. 505–511, 2008.

[77] M. J. May, F. D'Acquisto, L. A. Madge, J. Glockner, J. S. Pober, and S. Ghosh, "Selective inhibition of NF-κB activation by a peptide that blocks the interaction of NEMO with the IκB kinase complex," *Science*, vol. 289, no. 5484, pp. 1550–1554, 2000.

[78] P. Di Meglio, A. Ianaro, and S. Ghosh, "Amelioration of acute inflammation by systemic administration of a cell-permeable peptide inhibitor of NF-κB activation," *Arthritis and Rheumatism*, vol. 52, no. 3, pp. 951–958, 2005.

[79] E. Jimi, K. Aoki, H. Saito et al., "Selective inhibition of NF-κB blocks osteoclastogenesis and prevents inflammatory bone destruction in vivo," *Nature Medicine*, vol. 10, no. 6, pp. 617–624, 2004.

[80] S. Dai, T. Hirayama, S. Abbas, and Y. Abu-Amer, "The IκB kinase (IKK) inhibitor, NEMO-binding domain peptide, blocks osteoclastogenesis and bone erosion in inflammatory arthritis," *Journal of Biological Chemistry*, vol. 279, no. 36, pp. 37219–37222, 2004.

[81] S. H. Davé, J. S. Tilstra, K. Matsuoka et al., "Amelioration of chronic murine colitis by peptide-mediated transduction of the IκB kinase inhibitor NEMO binding domain peptide," *Journal of Immunology*, vol. 179, no. 11, pp. 7852–7859, 2007.

[82] A. Desai, N. Singh, and R. Raghubir, "Neuroprotective potential of the NF-[kappa]B inhibitor peptide IKK-NBD in cerebral ischemia-reperfusion injury," *Neurochemistry International*, vol. 57, no. 8, pp. 876–883, 2010.

[83] G. Grassia et al., "The IkappaB kinase inhibitor nuclear factor-kappaB essential modulator-binding domain peptide for inhibition of balloon injury-induced neointimal formation," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 30, no. 12, pp. 2458–2466, 2010.

[84] N. C. Moss, W. E. Stansfield, M. S. Willis, R. H. Tang, and C. H. Selzman, "IKKβ inhibition attenuates myocardial injury and dysfunction following acute ischemia-reperfusion injury," *American Journal of Physiology*, vol. 293, no. 4, pp. H2248–H2253, 2007.

[85] F. Zhang, L. Qian, P. M. Flood, J. S. Shi, J. S. Hong, and H. M. Gao, "Inhibition of IκB kinase-β protects dopamine neurons against lipopolysaccharide-induced neurotoxicity," *Journal of Pharmacology and Experimental Therapeutics*, vol. 333, no. 3, pp. 822–833, 2010.

[86] K. Ziegelbauer, F. Gantner, N. W. Lukacs et al., "A selective novel low-molecular-weight inhibitor of IκB kinase-β (IKK-β) prevents pulmonary inflammation and shows broad anti-inflammatory activity," *British Journal of Pharmacology*, vol. 145, no. 2, pp. 178–192, 2005.

[87] T. Hideshima, D. Chauhan, P. Richardson et al., "NF-κB as a therapeutic target in multiple myeloma," *Journal of Biological Chemistry*, vol. 277, no. 19, pp. 16639–16647, 2002.

[88] P. L. Podolin, J. F. Callahan, B. J. Bolognese et al., "Attenuation of murine collagen-induced arthritis by a novel, potent, selective small molecule inhibitor of IκB kinase 2, TPCA-1 (2-[(aminocarbonyl)amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide), occurs via reduction of proinflammatory cytokines and antigen-induced T cell proliferation," *Journal of Pharmacology and Experimental Therapeutics*, vol. 312, no. 1, pp. 373–381, 2005.

[89] M. J. O'Shaughnessy, C. Vogtenhuber, K. Sun et al., "Ex vivo inhibition of NF-κB signaling in alloreactive T-cells prevents graft-versus-host disease," *American Journal of Transplantation*, vol. 9, no. 3, pp. 452–462, 2009.

[90] T. Hideshima, T. Hayashi, D. Chauhan, M. Akiyama, P. Richardson, and K. Anderson, "Biologic sequelae of c-Jun NH-terminal kinase (JNK) activation in multiple myeloma cell lines," *Oncogene*, vol. 22, no. 54, pp. 8797–8801, 2003.

[91] J. Yang et al., "Conditional ablation of Ikkb inhibits melanoma tumor development in mice," *The Journal of Clinical Investigation*, vol. 120, no. 7, pp. 2563–2574, 2010.

[92] M. Schön, B. G. Wienrich, S. Kneitz et al., "KINK-1, a novel small-molecule inhibitor of IKKβ, and the susceptibility of melanoma cells to antitumoral treatment," *Journal of the National Cancer Institute*, vol. 100, no. 12, pp. 862–875, 2008.

[93] K. Amschler, M. P. Schön, N. Pletz, K. Wallbrecht, L. Erpenbeck, and M. Schön, "NF-κB inhibition through proteasome inhibition or ikkβ blockade increases the susceptibility of melanoma cells to cytostatic treatment through distinct pathways," *Journal of Investigative Dermatology*, vol. 130, no. 4, pp. 1073–1086, 2010.

[94] A. Yemelyanov, A. Gasparian, P. Lindholm et al., "Effects of IKK inhibitor PS1145 on NF-κB function, proliferation, apoptosis and invasion activity in prostate carcinoma cells," *Oncogene*, vol. 25, no. 3, pp. 387–398, 2006.

[95] K. W. Moore, R. De Waal Malefyt, R. L. Coffman, and A. O'Garra, "Interleukin-10 and the interleukin-10 receptor," *Annual Review of Immunology*, vol. 19, pp. 683–765, 2001.

[96] K. Strle, J. H. Zhou, W. H. Shen et al., "Interleukin-10 in the brain," *Critical Reviews in Immunology*, vol. 21, no. 5, pp. 427–449, 2001.

[97] Y. Zhu, G. Y. Yang, B. Ahlemeyer et al., "Transforming growth factor-β1 increases bad phosphorylation and protects neurons against damage," *Journal of Neuroscience*, vol. 22, no. 10, pp. 3898–3909, 2002.

[98] M. Szczepanik, M. Tutaj, K. Bryniarski, and B. N. Dittel, "Epicutaneously induced TGF-β-dependent tolerance inhibits experimental autoimmune encephalomyelitis," *Journal of Neuroimmunology*, vol. 164, no. 1-2, pp. 105–114, 2005.

[99] W. Zhang, J. S. Hong, H. C. Kim, W. Zhang, and M. L. Block, "Morphinan neuroprotection: new insight into the therapy of neurodegeneration," *Critical Reviews in Neurobiology*, vol. 16, no. 4, pp. 271–302, 2004.

[100] L. Qian et al., "NADPH oxidase inhibitor DPI is neuroprotective at femtomolar concentrations through inhibition of microglia over-activation," *Parkinsonism and Related Disorders*, vol. 13, supplement 3, pp. S316–S320, 2007.

[101] L. Qian, P. M. Flood, and J. S. Hong, "Neuroinflammation is a key player in Parkinson's disease and a prime target for therapy," *Journal of Neural Transmission*, vol. 117, no. 8, pp. 971–979, 2010.

[102] S. Yang, D. Zhang, Z. Yang et al., "Curcumin protects dopaminergic neuron against LPS induced neurotoxicity in primary rat neuron/glia culture," *Neurochemical Research*, vol. 33, no. 10, pp. 2044–2053, 2008.

[103] L. Qian, H. -M. Wu, S. -H. Chen et al., "β2-adrenergic receptor activation prevents rodent dopaminergic neurotoxicity by inhibiting microglia via a novel signaling pathway," *Journal of Immunology*, vol. 186, no. 7, pp. 4443–4454, 2011.

[104] A. Ghosh, A. Roy, X. Liu et al., "Selective inhibition of NF-κB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 47, pp. 18754–18759, 2007.

[105] F. Zhang, L. Qian, P. M. Flood, J. S. Shi, J. S. Hong, and H. M. Gao, "Inhibition of IκB kinase-β protects dopamine neurons against lipopolysaccharide-induced neurotoxicity," *Journal of Pharmacology and Experimental Therapeutics*, vol. 333, no. 3, pp. 822–833, 2010.

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Review Article

The Involvement of Neuroinflammation and Kynurenine Pathway in Parkinson's Disease

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Parkinson's disease (PD) is a common neurodegenerative disorder characterised by loss of dopaminergic neurons and localized neuroinflammation occurring in the midbrain several years before the actual onset of symptoms. Activated microglia themselves release a large number of inflammatory mediators thus perpetuating neuroinflammation and neurotoxicity. The Kynurenine pathway (KP), the main catabolic pathway for tryptophan, is one of the major regulators of the immune response and may also be implicated in the inflammatory response in parkinsonism. The KP generates several neuroactive compounds and therefore has either a neurotoxic or neuroprotective effect. Several of these molecules produced by microglia can activate the N-methyl-D-aspartate (NMDA) receptor-signalling pathway, leading to an excitotoxic response. Previous studies have shown that NMDA antagonists can ease symptoms and exert a neuroprotective effect in PD both *in vivo* and *in vitro*. There are to date several lines of evidence linking some of the KP intermediates and the neuropathogenesis of PD. Moreover, it is likely that pharmacological modulation of the KP will represent a new therapeutic strategy for PD.

1. Introduction

Parkinson's disease (PD) is the most common movement disorder and is the second most common chronic progressive neurodegenerative disorder after Alzheimer's disease. PD is a sporadic and age-dependent disease in 90% of cases and affects more than 1% of the world population over the age of 65 [1]. PD is characterised by motor symptoms including bradykinesia, tremor, rigidity, postural instability as well as nonmotor symptoms such as dementia, sleep disturbance, neurobehavioral, and sensory abnormalities [2].

PD is neuropathologically characterized by the loss of midbrain-pigmented neurons in the *substantia nigra pars compacta* (SNpc). Under normal conditions, these neurons produce dopamine at the striatum and other basal ganglia nuclei [3]. It has been estimated that at the onset of PD symptoms, up to 70% of dopaminergic neurons have been lost. Postmortem examinations have also shown that more than 90% of these neurons have been depleted [4]. Dopaminergic loss leads to an irreversible degeneration of the nigrostriatal pathway, followed by stratial dopaminergic denervation which causes pathological changes in neurotransmission of basal ganglia motor circuit and results in characteristic Parkinsonian symptoms [5]. Another pathological hallmark of the disease is the presence of protein inclusions called Lewy bodies (LBs), which are abnormal intracellular α-synuclein (SYN) aggregates in the cytoplasm and axons of the remaining neurons [6]. Neurons containing LBs undergo neurodegenerative processes and subsequently die.

To date, there is no available cure for PD. However, L-Dopa and dopaminergic agonists are useful in treating PD symptoms. This type of therapy mainly aims to replace dopamine in the striatum but does not slow neurodegenerative processes. Moreover, long-term use is associated with serious side effects such as dyskinesia and motor fluctuations [7] resulting in a diminished effect of treatment [8].

Although the aetiology of PD is relatively unknown, it has been suggested that there is an association with mitochondrial dysfunction in nigral neurons and neurotoxicity from excess glutamate and reactive oxygen species (ROS)

production [9, 10]. Microglia are the prime immune cells of the central nervous system (CNS) and are important producers of neuroactive molecules involved in oxidative stress, excitotoxicity and neuroinflammation. Microglia respond to a wide range of immunologic stimuli or CNS injuries and either initiate protective and/or neuroinflammatory processes [11]. The SN contains the highest concentration of microglia compared with other brain areas [12].

Resting microglia have a characteristic ramified morphology; the small cell body remains stationary whilst the long branches are constantly moving and are sensitive to any minor physiological changes [13, 14]. At the site of inflammation, activated microglia change their morphology becoming amoeboid and may act similarly to macrophages: they possibly perform phagocytosis, express increased levels of major histocompatibility complex (MHC) antigens, and secrete various cytotoxins, which may ultimately activate additional microglia to remove harmful stimuli and even initiate healing processes [15, 16]. The total number of MHC class II microglia has been shown to be significantly increased not only in SN and putamen but also in the hippocampus, transentorhinal cortex, cingulate cortex, and temporal cortex in PD brains [17]. This implies that microglia are activated and are likely to be associated with the neuropathological phenomenon, which ultimately damages neurons [17, 18]. The microglial reaction is a very tightly regulated process which is essential for a precise immune response; excessive microglial activation leads to a continuous release of inflammatory mediators such as cytokines, chemokines, reactive free radicals, and proteases [19]. This process is referred to as "reactive microgliosis" and involves the proliferation, recruitment, and activation of microglia which is then followed by neuronal damage [20], all of which are secondary to actual neuronal injury. Thus, initial, acute damage from microgliosis may provoke a continuous cycle of events, which then develops into chronic, progressive neurodegeneration which is a common characteristic of Parkinson's disease [21].

2. The Role of Neuroinflammation in the Pathogenesis of PD

A large number of studies involving cells, animal models, and human patients indicate the involvement of neuroinflammation in the neuropathogenesis of PD.

2.1. In Vitro/In Vivo. To demonstrate the delayed and progressive nature of neuroinflammation observed in PD, lipopolysaccharide (LPS) was administered to rodents as a single dose or a chronic infusion [22]. While LPS has no direct effect on neurons, it is capable of initiating a chronic inflammation and a delayed, secondary progressive degeneration of dopaminergic neurons in the SN [22, 23]. An *in vitro* study has also shown that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) can initiate direct neuronal injury in neuron-glia cultures which is then followed by the induction of reactive microgliosis [24]. Furthermore, in a microglia free neuronal-astrocytic coculture, MPTP induced only acute, non-progressive neurotoxicity [21]. MPTP is selectively toxic to dopaminergic neurons and is often used to induce an *in vivo* PD-like disease in animal models [25]. Moreover, inhibition of microglial activation results in a strong decrease in neurotoxicity in both MPTP mouse and LPS rat models [26, 27].

2.2. Human Studies. A large epidemiological study on approximately 150,000 men and women has shown that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) can prevent or delay the onset of PD [28]. Chen et al. have also observed a similar effect in chronic users of ibuprofen, a NSAID acting on cyclooxygenase (COX) [29]. A correlation has also been found between high plasma concentrations of interleukin-6, a proinflammatory cytokine, and an increased risk of developing PD [30]. Moreover, *in vivo* imaging studies on patients with idiopathic PD have shown an increase in neuroinflammatory areas in basal ganglia, striatum, and frontal and temporal cortical regions compared with age-matched healthy controls [31]. All of these studies suggest that microglial activation occurs at an early stage of the disease either before (or in parallel with) the important loss of dopaminergic neurons. In postmortem PD tissues, activated microglial cells have been detected around impaired dopaminergic neurons in the SN, thus demonstrating the presence of neuroinflammation [32]. As previously discussed, MPTP causes Parkinsonism in both humans and primates. This leads to the chronic presence of activated microglia around dopaminergic neurons in the SN for up to 10 years after exposure [33, 34], even without L-DOPA treatment [35]. Substantial evidence of microglial activation associated with dopaminergic neuronal damage suggests that degenerating neurons initiate microgliosis, which then leads to further neuronal loss. Microglial activation represents an initiator and/or a secondary responder in this disease process. Therefore, suppressing neuroinflammation by preventing microglial activation could potentially slow down or stop this continuous and deleterious cycle which damages neurons.

However, the initial stimulus driving excessive inflammation is still unknown. There are several compounds released by damaged neurons, which are able to induce microgliosis and ROS production. These include (i) matrix metalloproteinase 3 (released by damaged dopaminergic neurons), which induces superoxide production by microglia leading to neuronal death [36]. (ii) Neuromelanin, a neuronal pigment released in PD by dying neurons which is capable of activating microglia [37]. (iii) SYN, a component of LB neurons, typically found in PD that is toxic to neurons but only in the presence of microglia. (iv) Aggregated SYN-activated microglia are toxic to dopaminergic neurons isolated from embryonic mouse brain. Importantly, its toxicity is dependant on the presence of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase following ROS formation [38]. Another study has shown that neuroinflammation is accompanied by dopaminergic loss and aggregation of oxidized SYN in the cytoplasm of SN neurons when human SYN is present in the mouse brain [39]. Taken together, these studies suggest that there is a link between protein aggregation and the production of ROS by activated microglia.

Over production of ROS by microglia has been directly linked to neuronal toxicity and death via the nitric oxide (NO) mechanism [40, 41]. NO induces oxidative stress, a major cause of neuronal injury, which is strongly linked to the pathogenesis of PD and physiological aging [42, 43]. For example, NO can react with dopamine to generate quinone products, which are known to have a damaging effect on brain mitochondria [44]. Basal level of lipid peroxidation is increased in the SN of PD patients, suggesting a higher sensitivity of this area to free radicals and ROS [45]. Aging also contributes to microglial "priming": activated microglia in healthy aged brains release excessive quantities of proinflammatory cytokines compared to younger individuals [46, 47]. Furthermore, there is an increased probability of developing a neurodegenerative disorder after 60 years of age due to age-related increases in oxidative, metabolic, or inflammatory activation [48].

Inflammatory cytokines (IL-1β, TNF-α, IL-6, and IFN-γ) are also released by activated microglia and amplify the inflammatory response. Excessive production of these cytokines has been reported in the SN of PD patients [49, 50] as well as in cerebrospinal fluid (CSF) and blood compartments [51, 52]. Cytokines can stimulate inactivated microglia and also directly bind to receptors on the cellular surface of dopaminergic neurons thereby promoting apoptotic cell death and subsequent phagocytosis of DA neurons [53]. Neurons in the midbrain, unlike those in the hippocampus or cortex, exhibit a greater sensitivity to proinflammatory cytokines. Moreover, this sensitivity has been directly related to a high degree of oxidative processes [19]. In contrast, activated microglia also produce anti-inflammatory cytokines such as TGF-β1, IL-10, and IL-1. These cytokines play a role in the inhibition of the inflammatory response. Importantly, the balance between pro- and anti-inflammatory cytokine production is impaired during neuroinflammation [54].

On the other hand, the excitatory neurotransmitter glutamate plays a critical role in glutamatergic transmission in basal ganglia functions [55]. The action of glutamate on neurons is mediated by ionotropic and metabotropic glutamate receptors. Ionotropic N-methyl-D-aspartate (NMDA) receptors are known to mediate excitotoxicity caused by high levels of glutamate and can be found on dopaminergic neurons [56]. Activation of NMDA receptors located on DA neurons leads to neurotoxicity both *in vitro* and *in vivo* [57, 58]. The functional organisation of basal ganglia also contributes to the genesis of symptoms observed in movement disorder. The striatum (the input nucleus of the basal ganglia circuit) is the main recipient of dopaminergic fibres from the SN. The reduction in dopaminergic innervations of the striatum and changes in the activity of basal ganglia induces complex changes in the structure and function of basal ganglia NMDA receptor [59]. Glutamatergic excitation is increased and glutamatergic neurons become uninhibited under PD conditions, especially due to the excessive firing from the subthalamic nucleus to the SN [60] (Figure 1). It has been shown that the neurotoxicity of activated microglia is primarily mediated by glutamate released through NMDA receptor signalling [61]. Neuritic beading (a focal bead-like swelling in dendrites and axons) is a neuropathological

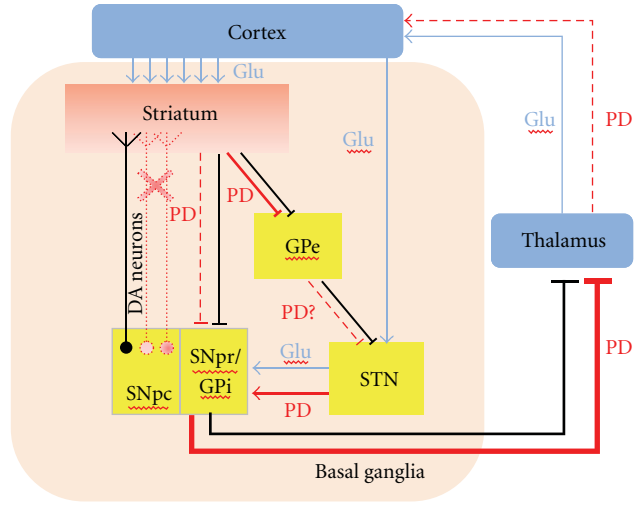

Figure 1: Basal ganglia motor circuit in Parkinson's disease: dopaminergic neurons (DA) create a direct pathway between Substantia Nigra pars compacta (SNpc) and striatum—the input nuclei of the basal ganglia. Another direct pathway connects the striatum to the internal segment of globus pallidus (GPi) and the substantia nigra pars reticulata (SNpr). GPi and SNpr are the output nuclei of the basal ganglia, which projects to the thalamus and from there to the cortex. The indirect pathway connects the striatum to output nuclei through external segment of the globus pallidus (GPe) and then subthalamic nucleus (STN). In Parkinson's disease (PD), the dopaminergic input from SNpc is progressively lost, causing a reduction in the direct pathway signal. Indirect pathway increases its activity through STN in the output nuclei and has inhibitory influence on the thalamus. It leads to a reduction of thalamic glutamateric input on the motor cortex and subsequent reduction in movement, as rigidity and bradykinesia are observed in PD patients.

sign in PD [62]. It can also be induced by microglia activated through the NMDA receptor [61]. NMDA receptors have been linked with disturbed energy metabolism and glutamate transmission leading to neuronal death, and have therefore been investigated as important therapeutic targets in pharmacological PD research [63]. Accordingly, reducing glutamatergic transmission may lead to an "anti-PD activity". Indeed, injections of the NMDA antagonist, MK-801, reverses parkinsonian symptoms in MPTP-treated monkeys [64]. Several studies using rodent PD models have shown that glutamate antagonists have both symptomatic and neuroprotective effects in PD [59]. Recently, PD patients treated with memantine, another NMDA receptor antagonist have shown moderate but significant improvements in terms of cognitive symptoms [65]. The use of amantadine as an adjuvant to levodopa has demonstrated beneficial effects on motor response complications [66]. Additional evidence has been reviewed and has demonstrated the potential of NMDA receptor blockade in reversing parkinsonian symptoms [59].

3. The Kynurenine Pathway

The kynurenine pathway (KP) represents the main catabolic pathway of the essential amino acid tryptophan (TRP),

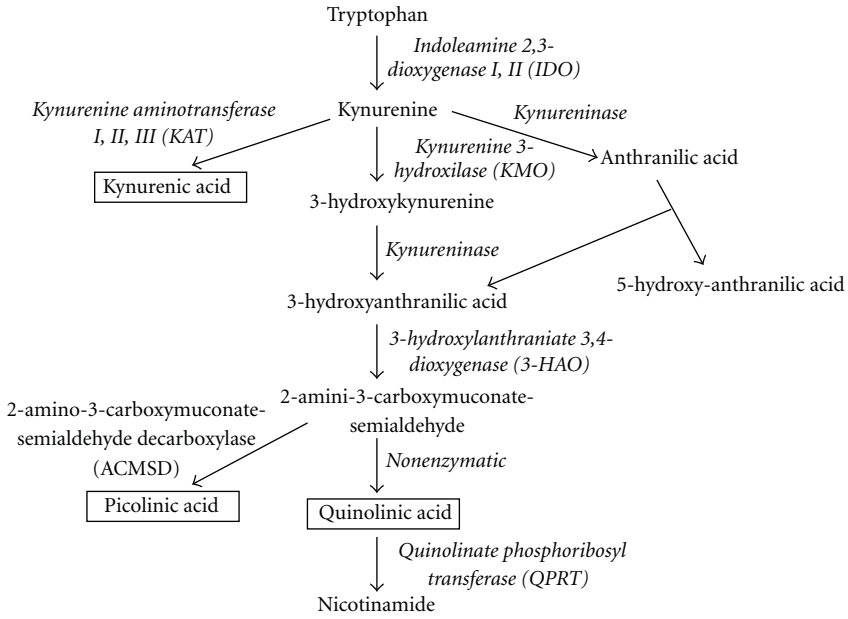

Figure 2: Simplified diagram of Kynurenine pathway: during neuroinflammation, 95% of the dietary tryptophan is metabolized along the KP within the brain. The remaining 5% serves as a precursor to the synthesis of the neurotransmitter serotonin. IDO catalyses the initial and rate-limiting step in the degradation of tryptophan through KP that ultimately leads to the production of nicotinamide.

which ultimately leads to the production of the central metabolic cofactor, nicotinamide adenine dinucleotide (NAD^+) (Figure 2). The KP is also one of the major regulatory mechanisms of the immune response [67]. Two nonmutually exclusive theories have been proposed: (1) that TRP degradation suppresses T-cell proliferation by dramatically depleting the supply of this critical amino acid and (2) that various downstream KP metabolites suppress certain immune cells [67]. Induction of the KP by the rate-limiting enzyme, indoleamine 2,3 dioxygenase (IDO1) in dendritic cells completely inhibits clonal expansion of T cells [68]. Moreover, TRP depletion and IDO1/KP activation have been implicated in the facilitation of immune tolerance associated with pregnancy and tumour persistence [69].

The cellular expression of the KP in the brain is only partially understood. It is complete in cells of monocytic lineage, including macrophages and microglia [70], but only partially present in human astrocytes [71], neurons [72], and endothelial cells [73]. The various KP metabolites can have either neurotoxic or neuroprotective effects and occasionally both depending on their concentration. The neurotoxicity of several KP metabolites has been investigated in relation to oxidative stress generation and neuronal death *in vitro* and *in vivo* in animal models of neurodegenerative disorders [74–77]. 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3HAA) and 5-hydroxyanthranilic acid (5HAA) are known to induce cell death in cultures of rat neurons [78]. 3-HK is toxic to stratial neuronal cultures, mainly due to its ability to generate ROS and initiate apoptosis [79]. Quinolinic acid (QUIN) however, is likely to be the most important in terms of biological activity. QUIN can selectively activate NMDA receptors producing excitation and which ultimately causes selective neuronal lesions in the rat brain [80, 81]. Acute QUIN production can lead to human neuronal death and chronic production causes dysfunction by at least six separate mechanisms [82, 83]. In pathophysiological concentrations, QUIN activates the NMDA receptor [84]. QUIN also increases glutamate release in neurons and inhibits glutamate uptake and catabolism in astrocytes. QUIN can potentiate its own toxicity and that of other excitotoxins, for example, NMDA and glutamate thus producing progressive mitochondrial dysfunction [85]. Finally, QUIN can increase free radical generation by inducing nitric oxide synthase production (NOS) in astrocytes and neurons which in turn leads to oxidative stress [86, 87]. Within the brain, QUIN is produced by activated microglia and infiltrating macrophages [70]. Neurons and astrocytes do not produce QUIN [88, 89]. Recent findings have demonstrated that QUIN excitotoxicity in human astrocytes and neurons is mediated through activation of an NMDA-like receptor [87]. In addition, QUIN-induced damage can be increased in the presence of 3-HK, 6-hydroxidopamine, a specific dopaminergic neuron toxin, or ROS [90–92]. Human glial cells, such as astrocytes and microglia produce most components of the KP [93]. The KP components are also present in macrophages that are capable of penetrating the blood-brain barrier (BBB) in the presence of brain damage or infection [94]. Thus, up-regulation of QUIN production alone or with additional neurotoxic factors during inflammation could easily lead to over activation of the NMDA receptor. This is followed by oxidative stress, which occurs in early PD development.

In contrast to the neurotoxic activity of QUIN, kynurenic acid (KYNA) is a neuroprotective metabolite, antagonising

all ionotropic glutamate receptors (including NMDA) and thus blocks some of the neurotoxic effects of QUIN and other excitotoxins. KYNA is produced from kynurenine by the kynurenine aminotransferase enzymes (KAT) I, KAT II, and KAT III, in astrocytes [71]. Endogenous generation of KYNA in rat brain has been shown to be more effective than KYNA applied exogenously, suggesting the importance of localised KYNA production and physical proximity to NMDA receptors [95]. An increase in endogenous KYNA levels can prevent SN dopaminergic loss caused by focal infusion of QUIN or NMDA [96]. Nanomolar concentrations of KYNA significantly reduce glutamate output from striatal neurons in rat brain, similar to the kynurenine hydroxylase (KMO) inhibitors [97]. Both, KYNA and QUIN are produced in the SN or the adjoining striatum region [98, 99]. Based on previous studies, it can be hypothesised that under normal conditions local concentrations of KYNA and QUIN are low and physiologically regulate NMDA receptor function. However, in disease states, where QUIN production is high, it is thought that there is insufficient KYNA concentration to block QUIN production [100].

Picolinic acid is another endogenous neuroprotective compound [101] and is also the main metal chelator in the brain [102]. Previously, we have shown that it is produced in micromolar concentrations by human primary neurons [72]. PD is associated with neuropathological features such as protein aggregation and oxidative stress associated with the involvement of metal ions [103]. Therefore, use of chelating agents has also been suggested as a form of therapy for PD.

The KP, under normal physiological conditions is well balanced and produces all KP intermediates leading ultimately to NAD^+ production. However, under pathologic conditions, IDO1 is activated and astrocytes produce kynurenine (KYN) and KYNA, [104], neurons produce PIC [88] and activated microglia/infiltrating macrophages produce QUIN [89]. It is important to note that PIC and KYNA can partly antagonise the neurotoxic effects of QUIN [105]. However, astroglial secretion of large quantities of KYN can lead to further synthesis of QUIN by microglia, suggesting that the cerebral synthesis of QUIN largely overtakes the neuroprotective effects of PIC and KYNA [106].

4. Evidence for the Involvement of the KP in PD

Impaired KP metabolism and altered KYNA levels have been previously reported in the brain of PD patients. This occurs when the KYNA/TRP ratio in serum and cerebrospinal fluid (CSF) is significantly increased together with 3-HK levels, a neurotoxic compound that contributes to oxidative damage in the putamen and SNpc [107, 108]. These findings suggest that endogenous KYNA concentrations are decreased and unable to effectively block NMDA receptor and prevent neurotoxicity induced by 3-HK. KAT I expression, the KP enzyme which leads to KYNA formation, is decreased in the SNpc of MPTP-treated mice [109]. KAT-I immunoreactivity in dopaminergic neurons and surrounding microglia has been linked to increased vulnerability of SN neurons to toxicity. Lowered KYNA concentrations have also been found in the frontal cortex, putamen, and SNpc of PD patients [107]. KYNA, but not the highly selective NMDA antagonist 7-chlorokynurenic acid exhibits partial protection against MPP+ toxin on dopaminergic terminals of rat striatum [110].

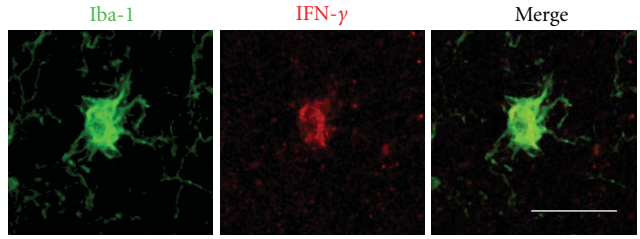

Figure 3: Activated microglial cells express IFN-γ in Parkinsonism: confocal images of the immunofluorescence of IFN-γ (red) combined with microglia cells marker—Iba-1 (green) in the SNpc of a parkinsonian monkey. Scale bar: 35 mm.

However, increased KAT II activity, which is an enzyme responsible for 75% of the KYNA synthesis in the brain, has been found in peripheral red blood cells of PD patients. It is not however found in plasma [111]. The increased KAT II activity correlates with higher blood KYNA concentrations; this elevation may be caused by 3-HK released from the CNS. As KYNA has limited abilities to cross the BBB, it has been suggested that peripheral KYNA is likely to be transported to the brain by large neutral amino acid carriers and there it has neuroprotective effects [112]. Another recent study has shown that KYNA is involved in leukocyte recruitment and the investigators hypothesised that KYNA might therefore have an anti-inflammatory action [113]. Based on preclinical and clinical data, KYNA or its analogues are thought to have neuroprotective effects in PD trough binding as antagonists to the NMDA receptor. This in turn causes slow neuronal excitotoxic damage [114].

Unpublished data from our group shows an increase in the production of IFN-γ by microglia in the SN of MPTP-treated macaques' brain (Figure 3). This is of particular significance, as IFN-γ is also a potent inducer of the KP [115]. In the same study, we have also shown that QUIN is produced and accumulated by activated microglia. These microglia colocalise with dopaminergic neurons in the SN of MPTP-treated macaques. Several other studies have shown extensive evidence of activated microglial cells and $NMDAR^+$ dopaminergic neurons in the SNpc. This suggests that the NMDA receptor is likely to be activated by endogenous QUIN released by microglia and followed closely by glutamate [116, 117] (Figure 4).

5. Recent KP Inhibitors for the Treatment of PD

Several drugs that block the KP are currently under therapeutic investigation both in our laboratory and by other investigators. For example, 4-chlorokynurenine crosses the BBB and blocks QUIN toxicity at the glycine site on NMDA receptors [118]. Kynurenic acid analogues are currently due to enter clinical trials for the treatment of epilepsy,

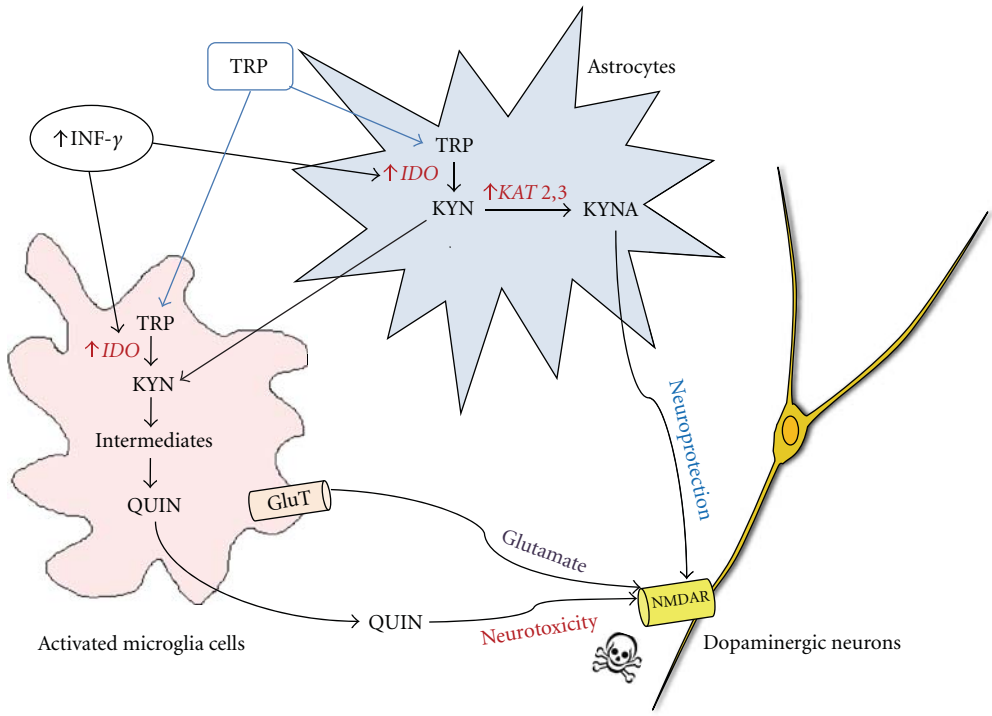

Figure 4: Model for Kynurenine pathway interactions between astrocytes, neurons, and microglia during brain inflammation. Abbreviations: TRP: tryptophan; IDO: Indoleamine 2,3-dioxygenase; KYN: kynurenine: QUIN: quinolinic acid; NMDAR: NMDA receptor; KAT: Kynurenine aminotransferase; GluT: glutamate transporter.

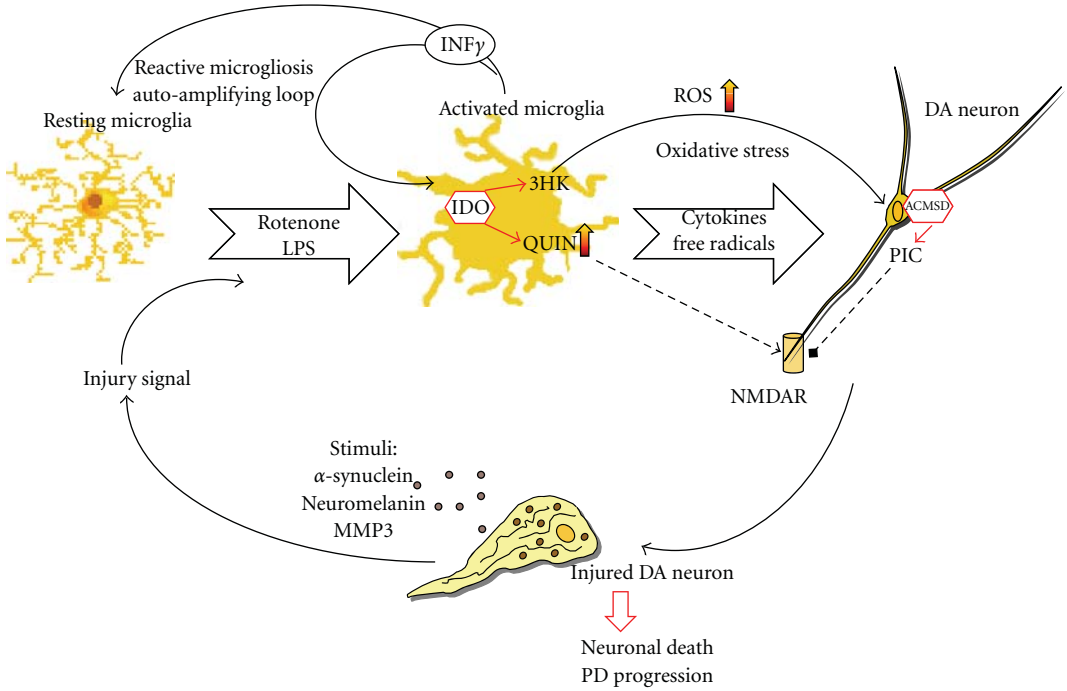

Figure 5: The possible role of Kynurenine pathway involvement in dopaminergic neurodegenerative process through microglia activation: Parkinson's disease is associated with chronic activation of microglia, which also can be induced by LPS or Rotenone treatments. Classic microglia activation release neurotoxic substances including reactive oxygen species (ROS) and proinflammatory cytokines as INF-γ, potent activator of Kynurenine pathway (KP). KP in activated microglia leads to upregulation of 3HK and QUIN. 3HK is toxic primarily as a result of conversion to ROS. The combined effects of ROS and NMDA receptor-mediated excitotoxicity by QUIN contribute to the dysfunction of neurons and their death. However, picolinic acid (PIC) produced through KP activation in neurons, has the ability to protect neurons against QUIN-induced neurotoxicity, being NMDA agonist. Microglia can become overactivated, by proinflammatory mediators and stimuli from dying neurons and cause perpetuating cycle of further microglia activation microgliosis. Excessive microgliosis will cause neurotoxicity to neighbouring neurons and resulting in neuronal death, contributing to progression of Parkinson's disease.

stroke, and possibly PD as potential neuroprotective agents [119]. Two KP analogues are at present under investigation in a phase III clinical trial. These are Teriflunomide (Sanofi-Aventis) and Laquinimod (Teva Neuroscience) [120]. Recently, one KP analogue reached the Japanese market as a potent immunomodulatory drug for the treatment of arthritis, asthma, and dermatitis [120]: Tranilast/Rizaben (Kissei Pharmaceutics) is an anthranilic acid derivative and it has been proposed as a treatment for autoimmune diseases such as Multiple Sclerosis [121]. Finally, 8-OH Quinolinone metal attenuating compounds—Clioquinol and PBT2 (Prana) rapidly decrease soluble brain amyloid-beta and improve cognitive performance [122]. Interestingly, these 2 compounds share close structural similarity and similar biochemical properties with KYNA and QUIN.

Conjugates of KYNA analogues with D-glucose or D-galactose increase its ability to cross the BBB and prevent excitotoxicity and seizures in an animal model [123]. Kynurenine 3-hydroxylase inhibitors significantly reduce the severity of dystonia in hamsters and may therefore be a potential candidate for managing dyskinesia associated with striatal dysfunction [124]. There is also an increasing interest in the use of pharmacological modulation of the KP in treating numerous disorders like AIDS-dementia and many other neurodegenerative diseases, diabetes, depression, infections, tumour development, glaucoma, and cataract formation [116].

6. Conclusions

PD seems to be associated with an imbalance between the two main branches of the KP within the brain. KYNA synthesis by astrocytes is decreased and concomitantly, QUIN production by microglia is increased (Figure 5). There are many therapeutic opportunities for intervention and modification of an impaired KP that may prevent the progression of neurodegenerative disorders such as PD. Using specific KP enzyme inhibitors, it may be possible to reinstate a physiologically normal KP, which is neuroprotective. This neuroprotective state might also be synergistically improved by concomitantly blocking the NMDA receptor using its antagonists, such as memantine or MK801. Additionally, neuroprotection may be achieved by designing KYNA analogues that are able to penetrate the BBB and deliver neuroprotective compounds to brain pools thus reducing hyperactivation of glutamatergic receptors.

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References

[1] C. M. Tanner, "Epidemiology of Parkinson's disease," *Neurologic Clinics*, vol. 10, no. 2, pp. 317–329, 1992.

[2] J. Jankovic, "Parkinson's disease: clinical features and diagnosis," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 79, no. 4, pp. 368–376, 2008.

[3] D. J. Gelb, E. Oliver, and S. Gilman, "Diagnostic criteria for Parkinson disease," *Archives of Neurology*, vol. 56, no. 1, pp. 33–39, 1999.

[4] E. Bezard, S. Dovero, C. Prunier et al., "Relationship between the appearance of symptoms and the level of nigrostriatal degeneration in a progressive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease," *Journal of Neuroscience*, vol. 21, no. 17, pp. 6853–6861, 2001.

[5] T. Wichmann and M. R. DeLong, "Functional neuroanatomy of the basal ganglia in Parkinson's disease," *Advances in neurology*, vol. 91, pp. 9–18, 2003.

[6] M. G. Spillantini, R. A. Crowther, R. Jakes, M. Hasegawa, and M. Goedert, "α-synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 11, pp. 6469–6473, 1998.

[7] J. L. Montastruc, O. Rascol, and J. M. Senard, "Current status of dopamine agonists in Parkinson's disease management," *Drugs*, vol. 46, no. 3, pp. 384–393, 1993.

[8] A. H. V. Schapira, "Molecular and clinical pathways to neuroprotection of dopaminergic drugs in Parkinson disease," *Neurology*, vol. 72, no. 7, pp. S44–S50, 2009.

[9] W. Dauer and S. Przedborski, "Parkinson's disease: mechanisms and models," *Neuron*, vol. 39, no. 6, pp. 889–909, 2003.

[10] S. Przedborski, V. Jackson-Lewis, M. Vila et al., "Free radical and nitric oxide toxicity in Parkinson's disease," *Advances in neurology*, vol. 91, pp. 83–94, 2003.

[11] G. W. Kreutzberg, "Microglia: a sensor for pathological events in the CNS," *Trends in Neurosciences*, vol. 19, no. 8, pp. 312–318, 1996.

[12] P. L. McGeer, S. Itagaki, B. E. Boyes, and E. G. McGeer, "Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains," *Neurology*, vol. 38, no. 8, pp. 1285–1291, 1988.

[13] A. Nimmerjahn, F. Kirchhoff, and F. Helmchen, "Neuroscience: resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo," *Science*, vol. 308, no. 5726, pp. 1314–1318, 2005.

[14] B. Liu and J. S. Hong, "Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention," *Journal of Pharmacology and Experimental Therapeutics*, vol. 304, no. 1, pp. 1–7, 2003.

[15] J. Gehrmann, Y. Matsumoto, and G. W. Kreutzberg, "Microglia: intrinsic immuneffector cell of the brain," *Brain Research Reviews*, vol. 20, no. 3, pp. 269–287, 1995.

[16] G. M. Hayes, M. N. Woodroofe, and M. L. Cuzner, "Microglia express MHC class II in normal and demyelinating human white matter," *Annals of the New York Academy of Sciences*, vol. 540, pp. 501–503, 1988.

[17] K. Imamura, N. Hishikawa, M. Sawada, T. Nagatsu, M. Yoshida, and Y. Hashizume, "Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains," *Acta Neuropathologica*, vol. 106, no. 6, pp. 518–526, 2003.

[18] W. F. Hickey and H. Kimura, "Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo," *Science*, vol. 239, no. 4837, pp. 290–292, 1988.

[19] M. L. Block, L. Zecca, and J. S. Hong, "Microglia-mediated neurotoxicity: uncovering the molecular mechanisms," *Nature Reviews Neuroscience*, vol. 8, no. 1, pp. 57–69, 2007.

[20] W. J. Streit, S. A. Walter, and N. A. Pennell, "Reactive microgliosis," *Progress in Neurobiology*, vol. 57, no. 6, pp. 563–581, 1999.

[21] H. M. Gao and J. S. Hong, "Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression," *Trends in Immunology*, vol. 29, no. 8, pp. 357–365, 2008.

[22] A. Castaño, A. J. Herrera, J. Cano, and A. Machado, "Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system," *Journal of Neurochemistry*, vol. 70, no. 4, pp. 1584–1592, 1998.

[23] H. M. Gao, J. Jiang, B. Wilson, W. Zhang, J. S. Hong, and B. Liu, "Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease," *Journal of Neurochemistry*, vol. 81, no. 6, pp. 1285–1297, 2002.

[24] H. M. Gao, B. Liu, W. Zhang, and J. S. Hong, "Critical role of microglial NADPH oxidase-derived free radicals in the in vitro MPTP model of Parkinson's disease," *The FASEB Journal*, vol. 17, pp. 1954–1956, 2003.

[25] M. Gerlach, P. Riederer, H. Przuntek, and M. B. H. Youdim, "MPTP mechanisms of neurotoxicity and their implications for Parkinson's disease," *European Journal of Pharmacology*, vol. 208, no. 4, pp. 273–286, 1991.

[26] D. C. Wu, V. Jackson-Lewis, M. Vila et al., "Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease," *Journal of Neuroscience*, vol. 22, no. 5, pp. 1763–1771, 2002.

[27] B. Liu, L. Du, and J. S. Hong, "Naloxone protects rat dopaminergic neurons against inflammatory damage through inhibition of microglia activation and superoxide generation," *Journal of Pharmacology and Experimental Therapeutics*, vol. 293, no. 2, pp. 607–617, 2000.

[28] H. Chen, S. M. Zhang, M. A. Hernán et al., "Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease," *Archives of Neurology*, vol. 60, no. 8, pp. 1059–1064, 2003.

[29] H. Chen, E. Jacobs, M. A. Schwarzschild et al., "Nonsteroidal antiinflammatory drug use and the risk for Parkinson's disease," *Annals of Neurology*, vol. 58, no. 6, pp. 963–967, 2005.

[30] H. Chen, E. J. O'Reilly, M. A. Schwarzschild, and A. Ascherio, "Peripheral inflammatory biomarkers and risk of Parkinson's disease," *American Journal of Epidemiology*, vol. 167, no. 1, pp. 90–95, 2008.

[31] A. Gerhard, N. Pavese, G. Hotton et al., "In vivo imaging of microglial activation with [^{11}C](*R*)-PK11195 PET in idiopathic Parkinson's disease," *Neurobiology of Disease*, vol. 21, no. 2, pp. 404–412, 2006.

[32] P. L. McGeer and E. G. McGeer, "Glial reactions in Parkinson's disease," *Movement Disorders*, vol. 23, no. 4, pp. 474–483, 2008.

[33] P. L. McGeer, C. Schwab, A. Parent, and D. Doudet, "Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration," *Annals of Neurology*, vol. 54, no. 5, pp. 599–604, 2003.

[34] J. W. Langston, L. S. Forno, J. Tetrud, A. G. Reeves, J. A. Kaplan, and D. Karluk, "Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure," *Annals of Neurology*, vol. 46, no. 4, pp. 598–605, 1999.

[35] C. Barcia, A. Sánchez Bahillo, E. Fernández-Villalba et al., "Evidence of active microglia in substantia nigra pars compacta of parkinsonian monkeys 1 year after MPTP exposure," *GLIA*, vol. 46, no. 4, pp. 402–409, 2004.

[36] Y. S. Kim, D. H. Choi, M. L. Block et al., "A pivotal role of matrix metalloproteinase-3 activity in dopaminergic neuronal degeneration via microglial activation," *The FASEB Journal*, vol. 21, no. 1, pp. 179–187, 2007.

[37] L. Zecca, F. A. Zucca, H. Wilms, and D. Sulzer, "Neuromelanin of the substantia nigra: a neuronal black hole with protective and toxic characteristics," *Trends in Neurosciences*, vol. 26, no. 11, pp. 578–580, 2003.

[38] W. Zhang, T. Wang, Z. Pei et al., "Aggregated α-synuclein activates microglia: a process leading to disease progression in Parkinson's disease," *The FASEB Journal*, vol. 19, no. 6, pp. 533–542, 2005.

[39] H. M. Gao, P. T. Kotzbauer, K. Uryu, S. Leight, J. Q. Trojanowski, and V. M. Y. Lee, "Neuroinflammation and oxidation/nitration of α-synuclein linked to dopaminergic neurodegeneration," *Journal of Neuroscience*, vol. 28, no. 30, pp. 7687–7698, 2008.

[40] Y. S. Kim and T. H. Joh, "Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease," *Experimental and Molecular Medicine*, vol. 38, no. 4, pp. 333–347, 2006.

[41] C. C. Chao, S. Hu, T. W. Molitor, E. G. Shaskan, and P. K. Peterson, "Activated microglia mediate neuronal cell injury via a nitric oxide mechanism," *Journal of Immunology*, vol. 149, no. 8, pp. 2736–2741, 1992.

[42] P. Jenner, "Oxidative stress in Parkinson's disease," *Annals of Neurology*, vol. 53, no. 3, pp. S26–S38, 2003.

[43] E. Koutsilieri, C. Scheller, E. Grünblatt, K. Nara, J. Li, and P. Riederer, "Free radicals in Parkinson's disease," *Journal of Neurology*, vol. 249, supplement 2, pp. II1–II5, 2002.

[44] S. Jana, A. K. Maiti, M. B. Bagh et al., "Dopamine but not 3,4-dihydroxy phenylacetic acid (DOPAC) inhibits brain respiratory chain activity by autoxidation and mitochondria catalyzed oxidation to quinone products: implications in Parkinson's disease," *Brain Research*, vol. 1139, no. 1, pp. 195–200, 2007.

[45] D. T. Dexter, C. J. Carter, F. R. Wells et al., "Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease," *Journal of Neurochemistry*, vol. 52, no. 2, pp. 381–389, 1989.

[46] R. N. Dilger and R. W. Johnson, "Aging, microglial cell priming, and the discordant central inflammatory response to signals from the peripheral immune system," *Journal of Leukocyte Biology*, vol. 84, no. 4, pp. 932–939, 2008.

[47] Y. Huang, C. J. Henry, R. Dantzer, R. W. Johnson, and J. P. Godbout, "Exaggerated sickness behavior and brain proinflammatory cytokine expression in aged mice in response to intracerebroventricular lipopolysaccharide," *Neurobiology of Aging*, vol. 29, no. 11, pp. 1744–1753, 2008.

[48] R. von Bernhardi, J. E. Tichauer, and J. Eugenín, "Aging-dependent changes of microglial cells and their relevance for neurodegenerative disorders," *Journal of Neurochemistry*, vol. 112, no. 5, pp. 1099–1114, 2010.

[49] J. E. Merrill and E. N. Benveniste, "Cytokines in inflammatory brain lesions: helpful and harmful," *Trends in Neurosciences*, vol. 19, no. 8, pp. 331–338, 1996.

[50] T. Nagatsu, M. Mogi, H. Ichinose, and A. Togari, "Cytokines in Parkinson's disease," *Journal of Neural Transmission. Supplementa*, no. 58, pp. 143–151, 2000.

[51] M. Mogi, M. Harada, P. Riederer, H. Narabayashi, K. Fujita, and T. Nagatsu, "Tumor necrosis factor-α (TNF-α) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients," *Neuroscience Letters*, vol. 165, no. 1-2, pp. 208–210, 1994.

[52] G. Stypuła, J. Kunert-Radek, H. Stepien, K. Zylińska, and M. Pawlikowski, "Evaluation of interleukins, ACTH, cortisol and prolactin concentrations in the blood of patients with Parkinson's disease," *NeuroImmunoModulation*, vol. 3, no. 2-3, pp. 131–134, 1996.

[53] E. C. Hirsch, "Glial cells and Parkinson's disease," *Journal of Neurology*, vol. 247, supplement 2, no. 2, pp. 58–62, 2000.

[54] T. Nagatsu and M. Sawada, "Inflammatory process in Parkinson's disease: role for cytokines," *Current Pharmaceutical Design*, vol. 11, no. 8, pp. 999–1016, 2005.

[55] P. Ravenscroft and J. Brotchie, "NMDA receptors in the basal ganglia," *Journal of Anatomy*, vol. 196, no. 4, pp. 577–585, 2000.

[56] E. A. Waxman and D. R. Lynch, "N-methyl-D-aspartate receptor subtype mediated bidirectional control of p38 mitogen-activated protein kinase," *Journal of Biological Chemistry*, vol. 280, no. 32, pp. 29322–29333, 2005.

[57] S. Kikuchi and S. U. Kim, "Glutamate neurotoxicity in mesencephalic dopaminergic neurons in culture," *Journal of Neuroscience Research*, vol. 36, no. 5, pp. 558–569, 1993.

[58] B. P. Connop, R. J. Boegman, K. Jhamandas, and R. J. Beninger, "Excitotoxic action of NMDA agonists on nigrostriatal dopaminergic neurons: modulation by inhibition of nitric oxide synthesis," *Brain Research*, vol. 676, no. 1, pp. 124–132, 1995.

[59] P. J. Hallett and D. G. Standaert, "Rationale for and use of NMDA receptor antagonists in Parkinson's disease," *Pharmacology & Therapeutics*, vol. 102, no. 2, pp. 155–174, 2004.

[60] W. J. Schmidt, M. Bubser, and W. Hauber, "Behavioural pharmacology of glutamate in the basal ganglia," *Journal of Neural Transmission. Supplementa*, no. 38, pp. 65–89, 1992.

[61] H. Takeuchi, T. Mizuno, G. Zhang et al., "Neuritic beading induced by activated microglia is an early feature of neuronal dysfunction toward neuronal death by inhibition of mitochondrial respiration and axonal transport," *Journal of Biological Chemistry*, vol. 280, no. 11, pp. 10444–10454, 2005.

[62] P. M. Mattila, J. O. Rinne, H. Helenius, and M. Röyttä, "Neuritic degeneration in the hippocampus and amygdala in Parkinson's disease in relation to Alzheimer pathology," *Acta Neuropathologica*, vol. 98, no. 2, pp. 157–164, 1999.

[63] C. Ikonomidou and L. Turski, "Neurodegenerative disorders: clues from glutamate and energy metabolism," *Critical Reviews in Neurobiology*, vol. 10, no. 2, pp. 239–263, 1996.

[64] J. T. Greenamyre and C. F. O'Brien, "N-methyl-D-aspartate antagonists in the treatment of Parkinson's disease," *Archives of Neurology*, vol. 48, no. 9, pp. 977–981, 1991.

[65] D. Aarsland, C. Ballard, Z. Walker et al., "Memantine in patients with Parkinson's disease dementia or dementia with Lewy bodies: a double-blind, placebo-controlled, multicentre trial," *The Lancet Neurology*, vol. 8, no. 7, pp. 613–618, 2009.

[66] L. Verhagen Metman, P. Del Dotto, P. van den Munckhof, J. Fang, M. M. Mouradian, and T. N. Chase, "Amantadine as treatment for dyskinesias and motor fluctuations in Parkinson's disease," *Neurology*, vol. 50, no. 5, pp. 1323–1326, 1998.

[67] J. R. Moffett and M. A. Namboodiri, "Tryptophan and the immune response," *Immunology and Cell Biology*, vol. 81, no. 4, pp. 247–265, 2003.

[68] A. L. Mellor, B. Baban, P. Chandler et al., "Cutting edge: induced indoleamine 2,3 dioxygenase expression in dendritic cell subsets suppresses T cell clonal expansion," *Journal of Immunology*, vol. 171, no. 4, pp. 1652–1655, 2003.

[69] D. H. Munn and A. L. Mellor, "IDO and tolerance to tumors," *Trends in Molecular Medicine*, vol. 10, no. 1, pp. 15–18, 2004.

[70] G. J. Guillemin, D. G. Smith, G. A. Smythe, P. J. Armati, and B. J. Brew, "Expression of the kynurenine pathway enzymes in human microglia and macrophages," *Advances in Experimental Medicine and Biology*, vol. 527, pp. 105–112, 2003.

[71] G. J. Guillemin, S. J. Kerr, G. A. Smythe et al., "Kynurenine pathway metabolism in human astrocytes: a paradox for neuronal protection," *Journal of Neurochemistry*, vol. 78, no. 4, pp. 842–853, 2001.

[72] G. J. Guillemin, K. M. Cullen, C. K. Lim et al., "Characterization of the kynurenine pathway in human neurons," *Journal of Neuroscience*, vol. 27, no. 47, pp. 12884–12892, 2007.

[73] R. Owe-Young, N. L. Webster, M. Mukhtar et al., "Kynurenine pathway metabolism in human blood-brain-barrier cells: implications for immune tolerance and neurotoxicity," *Journal of Neurochemistry*, vol. 105, no. 4, pp. 1346–1357, 2008.

[74] H. Q. Wu, P. Guidetti, J. H. Goodman et al., "Kynurenergic manipulations influence excitatory synaptic function and excitotoxic vulnerability in the rat hippocampus in vivo," *Neuroscience*, vol. 97, no. 2, pp. 243–251, 2000.

[75] A. Chiarugi, E. Meli, and F. Moroni, "Similarities and differences in the neuronal death processes activated by 3OH-kynurenine and quinolinic acid," *Journal of Neurochemistry*, vol. 77, no. 5, pp. 1310–1318, 2001.

[76] R. Schwarcz and R. Pellicciari, "Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities," *Journal of Pharmacology and Experimental Therapeutics*, vol. 303, no. 1, pp. 1–10, 2002.

[77] A. J. Smith, T. W. Stone, and R. A. Smith, "Neurotoxicity of tryptophan metabolites," *Biochemical Society Transactions*, vol. 35, no. 5, pp. 1287–1289, 2007.

[78] A. J. Smith, R. A. Smith, and T. W. Stone, "5-hydroxyanthranilic acid, a tryptophan metabolite, generates oxidative stress and neuronal death via p38 activation in cultured cerebellar granule neurones," *Neurotoxicity Re- search*, vol. 15, no. 4, pp. 303–310, 2009.

[79] S. Okuda, N. Nishiyama, H. Saito, and H. Katsuki, "3-hydroxykynurenine, an endogenous oxidative stress generator, causes neuronal cell death with apoptotic features and region selectivity," *Journal of Neurochemistry*, vol. 70, no. 1, pp. 299–307, 1998.

[80] T. W. Stone and M. N. Perkins, "Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS," *European Journal of Pharmacology*, vol. 72, no. 4, pp. 411–412, 1981.

[81] R. Schwarcz, W. O. Whetsell, and R. M. Mangano, "Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain," *Science*, vol. 219, no. 4582, pp. 316–318, 1983.

[82] G. J. Guillemin and B. J. Brew, "Implications of the kynurenine pathway and quinolinic acid in Alzheimer's disease," *Redox Report*, vol. 7, no. 4, pp. 199–206, 2002.

[83] G. J. Guillemin, L. Wang, and B. J. Brew, "Quinolinic acid selectively induces apoptosis of human astocytes: potential role in AIDS dementia complex," *Journal of Neuroinflammation*, vol. 2, article 16, 2005.

[84] A. Schurr, C. A. West, and B. M. Rigor, "Neurotoxicity of quinolinic acid and its derivatives in hypoxic rat hippocampal slices," *Brain Research*, vol. 568, no. 1-2, pp. 199–204, 1991.

[85] Y. M. Bordelon, M. F. Chesselet, D. Nelson, F. Welsh, and M. Erecińska, "Energetic dysfunction in quinolinic acid-lesioned rat striatum," *Journal of Neurochemistry*, vol. 69, no. 4, pp. 1629–1639, 1997.

[86] H. Baran, B. Kepplinger, M. Herrera-Marschitz, K. Stolze, G. Lubec, and H. Nohl, "Increased kynurenic acid in the brain after neonatal asphyxia," *Life Sciences*, vol. 69, no. 11, pp. 1249–1256, 2001.

[87] N. Braidy, R. Grant, S. Adams, B. J. Brew, and G. J. Guillemin, "Mechanism for quinolinic acid cytotoxicity in human astrocytes and neurons," *Neurotoxicity Research*, vol. 16, no. 1, pp. 77–86, 2009.

[88] G. J. Guillemin, K. M. Cullen, C. K. Lim et al., "Characterization of the kynurenine pathway in human neurons," *Journal of Neuroscience*, vol. 27, no. 47, pp. 12884–12892, 2007.

[89] G. J. Guillemin, G. Smythe, O. Takikawa, and B. J. Brew, "Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons," *GLIA*, vol. 49, no. 1, pp. 15–23, 2005.

[90] P. Guidetti and R. Schwarcz, "3-hydroxykynurenine potentiates quinolinate but not NMDA toxicity in the rat striatum," *European Journal of Neuroscience*, vol. 11, no. 11, pp. 3857–3863, 1999.

[91] W. M. H. Behan and T. W. Stone, "Enhanced neuronal damage by co-administration of quinolinic acid and free radicals, and protection by adenosine A_{2A} receptor antagonists," *British Journal of Pharmacology*, vol. 135, no. 6, pp. 1435–1442, 2002.

[92] I. Ghorayeb, Z. Puschban, P. O. Fernagut et al., "Simultaneous intrastriatal 6-hydroxydopamine and quinolinic acid injection: a model of early-stage striatonigral degeneration," *Experimental Neurology*, vol. 167, no. 1, pp. 133–147, 2001.

[93] M. G. Espey, O. N. Chernyshev, J. F. Reinhard, M. A. A. Namboodiri, and C. A. Colton, "Activated human microglia produce the excitotoxin quinolinic acid," *NeuroReport*, vol. 8, no. 2, pp. 431–434, 1997.

[94] M. P. Heyes, K. Saito, and S. P. Markey, "Human macrophages convert L-tryptophan into the neurotoxin quinolinic acid," *Biochemical Journal*, vol. 283, no. 3, pp. 633–635, 1992.

[95] H. E. Scharfman, P. S. Hodgkins, S. C. Lee, and R. Schwarcz, "Quantitative differences in the effects of de novo produced and exogenous kynurenic acid in rat brain slices," *Neuroscience Letters*, vol. 274, no. 2, pp. 111–114, 1999.

[96] A. F. Miranda, R. J. Boegman, R. J. Beninger, and K. Jhamandas, "Protection against quinolinic acid-mediated excitotoxicity in nigrostriatal dopaminergic neurons by endogenous kynurenic acid," *Neuroscience*, vol. 78, no. 4, pp. 967–975, 1997.

[97] R. Carpenedo, A. Pittaluga, A. Cozzi et al., "Presynaptic kynurenate-sensitive receptors inhibit glutamate release," *European Journal of Neuroscience*, vol. 13, no. 11, pp. 2141–2147, 2001.

[98] R. C. Roberts, K. E. McCarthy, F. Du, E. Okuno, and R. Schwarcz, "Immunocytochemical localization of the quinolinic acid synthesizing enzyme, 3-hydroxyanthranilic acid oxygenase, in the rat substantia nigra," *Brain Research*, vol. 650, no. 2, pp. 229–238, 1994.

[99] R. Schwarcz, F. Du, W. Schmidt et al., "Kynurenic acid: a potential pathogen in brain disorders," *Annals of the New York Academy of Sciences*, vol. 648, pp. 140–153, 1992.

[100] A. C. Foster, A. Vezzani, E. D. French, and R. Schwarcz, "Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid," *Neuroscience Letters*, vol. 48, no. 3, pp. 273–278, 1984.

[101] K. Jhamandas, R. J. Boegman, R. J. Beninger, and M. Bialik, "Quinolinate-induced cortical cholinergic damage: modulation by tryptophan metabolites," *Brain Research*, vol. 529, no. 1-2, pp. 185–191, 1990.

[102] U. Testa, F. Louache, M. Titeux, P. Thomopoulos, and H. Rochant, "The iron-chelating agent picolinic acid enhances transferrin receptors expression in human erythroleukaemic cell lines," *British Journal of Haematology*, vol. 60, no. 3, pp. 491–502, 1985.

[103] F. Molina-Holgado, R. C. Hider, A. Gaeta, R. Williams, and P. Francis, "Metals ions and neurodegeneration," *BioMetals*, vol. 20, no. 3-4, pp. 639–654, 2007.

[104] G. J. Guillemin, S. J. Kerr, L. A. Pemberton et al., "IFN-β induces kynurenine pathway metabolism in human macrophages: potential implications for multiple sclerosis treatment," *Journal of Interferon and Cytokine Research*, vol. 21, no. 12, pp. 1097–1101, 2001.

[105] R. J. Beninger, A. M. Colton, J. L. Ingles, K. Jhamandas, and R. J. Boegman, "Picolinic acid blocks the neurotoxic but not the neuroexcitant properties of quinolinic acid in the rat brain: evidence from turning behaviour and tyrosine hydroxylase immunohistochemistry," *Neuroscience*, vol. 61, no. 3, pp. 603–612, 1994.

[106] R. Owe-Young, N. L. Webster, M. Mukhtar et al., "Kynurenine pathway metabolism in human blood-brain-barrier cells: implications for immune tolerance and neurotoxicity," *Journal of Neurochemistry*, vol. 105, no. 4, pp. 1346–1357, 2008.

[107] T. Ogawa, W. R. Matson, M. F. Beal et al., "Kynurenine pathway abnormalities in Parkinson's disease," *Neurology*, vol. 42, no. 9, pp. 1702–1706, 1992.

[108] M. Flint Beal, W. R. Matson, E. Storey et al., "Kynurenic acid concentrations are reduced in Huntington's disease cerebral cortex," *Journal of the Neurological Sciences*, vol. 108, no. 1, pp. 80–87, 1992.

[109] E. Knyihár-Csillik, B. Csillik, M. Pákáski et al., "Decreased expression of kynurenine aminotransferase-I (KAT-I) in the substantia nigra of mice after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment," *Neuroscience*, vol. 126, no. 4, pp. 899–914, 2004.

[110] M. Merino, M. L. Vizuete, J. Cano, and A. Machado, "The non-NMDA glutamate receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline, but not NMDA antagonists, block the intrastriatal neurotoxic effect of MPP^+," *Journal of Neurochemistry*, vol. 73, no. 2, pp. 750–757, 1999.

[111] Z. Hartai, P. Klivenyi, T. Janaky, B. Penke, L. Dux, and L. Vecsei, "Kynurenine metabolism in plasma and in red blood cells in Parkinson's disease," *Journal of the Neurological Sciences*, vol. 239, no. 1, pp. 31–35, 2005.

[112] S. Fukui, R. Schwarcz, S. I. Rapoport, Y. Takada, and Q. R. Smith, "Blood-brain barrier transport of kynurenines: implications for brain synthesis and metabolism," *Journal of Neurochemistry*, vol. 56, no. 6, pp. 2007–2017, 1991.

[113] M. C. Barth, N. Ahluwalia, T. J. T. Anderson et al., "Kynurenic acid triggers firm arrest of leukocytes to vascular

endothelium under flow conditions," *Journal of Biological Chemistry*, vol. 284, no. 29, pp. 19189–19195, 2009.

[114] H. Németh, J. Toldi, and L. Vécsei, "Kynurenines, Parkinson's disease and other neurodegenerative disorders: preclinical and clinical studies," *Journal of Neural Transmission. Supplementa*, no. 70, pp. 285–304, 2006.

[115] S. Fujigaki, K. Saito, K. Sekikawa et al., "Lipopolysaccharide induction of indoleamine 2,3-dioxygenase is mediated dominantly by an IFN-γ-independent mechanism," *European Journal of Immunology*, vol. 31, no. 8, pp. 2313–2318, 2001.

[116] T. W. Stone and L. G. Darlington, "Endogenous kynurenines as targets for drug discovery and development," *Nature Reviews Drug Discovery*, vol. 1, no. 8, pp. 609–620, 2002.

[117] L. McNally, Z. Bhagwagar, and J. Hannestad, "Inflammation, glutamate, and glia in depression: a literature review," *CNS Spectrums*, vol. 13, no. 6, pp. 501–510, 2008.

[118] H. Q. Wu, S. C. Lee, and R. Schwarcz, "Systemic administration of 4-chlorokynurenine prevents quinolinate neurotoxicity in the rat hippocampus," *European Journal of Pharmacology*, vol. 390, no. 3, pp. 267–274, 2000.

[119] T. W. Stone, "Development and therapeutic potential of kynurenic acid and kynurenine derivatives for neuroprotection," *Trends in Pharmacological Sciences*, vol. 21, no. 4, pp. 149–154, 2000.

[120] M. Platten, P. P. Ho, and L. Steinman, "Anti-inflammatory strategies for the treatment of multiple sclerosis—tryptophan catabolites may hold the key," *Drug Discovery Today: Therapeutic Strategies*, vol. 3, no. 3, pp. 401–408, 2006.

[121] M. Platten, P. P. Ho, S. Youssef et al., "Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite," *Science*, vol. 310, no. 5749, pp. 850–855, 2005.

[122] C. W. Ritchie, A. I. Bush, and C. L. Masters, "Metal-protein attenuating compounds and Alzheimer's disease," *Expert Opinion on Investigational Drugs*, vol. 13, no. 12, pp. 1585–1592, 2004.

[123] J. R. Moffett, T. Els, M. G. Espey, S. A. Walter, W. J. Streit, and M. A. A. Namboodiri, "Quinolinate immunoreactivity in experimental rat brain tumors is present in macrophages but not in astrocytes," *Experimental Neurology*, vol. 144, no. 2, pp. 287–301, 1997.

[124] M. Hamann, S. E. Sander, and A. Richter, "Effects of the kynurenine 3-hydroxylase inhibitor Ro 61-8048 after intrastriatal injections on the severity of dystonia in the dt mutant," *European Journal of Pharmacology*, vol. 586, no. 1–3, pp. 156–159, 2008.