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Review Article

The Endotoxin-Induced Neuroinflammation Model of Parkinson's Disease

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Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the progressive loss of dopaminergic (DA) neurons in the substantia nigra. Although the exact cause of the dopaminergic neurodegeneration remains elusive, recent postmortem and experimental studies have revealed an essential role for neuroinflammation that is initiated and driven by activated microglial and infiltrated peripheral immune cells and their neurotoxic products (such as proinflammatory cytokines, reactive oxygen species, and nitric oxide) in the pathogenesis of PD. A bacterial endotoxin-based experimental model of PD has been established, representing a purely inflammation-driven animal model for the induction of nigrostriatal dopaminergic neurodegeneration. This model, by itself or together with genetic and toxin-based animal models, provides an important tool to delineate the precise mechanisms of neuroinflammation-mediated dopaminergic neuron loss. Here, we review the characteristics of this model and the contribution of neuroinflammatory processes, induced by the *in vivo* administration of bacterial endotoxin, to neurodegeneration. Furthermore, we summarize the recent experimental therapeutic strategies targeting endotoxin-induced neuroinflammation to elicit neuroprotection in the nigrostriatal dopaminergic system. The potential of the endotoxin-based PD model in the development of an early-stage specific diagnostic biomarker is also emphasized.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by tremor, rigidity, bradykinesia, and postural instability, which result from the progressive loss of dopaminergic (DA) neurons in the substantia nigra [1]. The primary cause of PD is still unknown although aging seems to be a major risk factor.

Parkinson's disease displays racial differences as can be seen from recent studies which have shown that incidence of PD in African-Americans is lower than in Caucasian whites and Asians [2, 3]. Both environmental and genetic factor contribute to PD pathogenesis. Pesticides exposure (paraquat, organophosphates, and rotenone), rural living, farming, well water drinking, metals (manganese, copper, mercury, lead, iron, zinc, and aluminum), diet, head trauma, and infections have been proposed as potential risk factors [4–6]. Caffeine intake and smoking reduces the risk of PD [4, 5]. 10%–15% of all PD cases have a genetic component [7]. Fifteen chromosomal loci have been linked to PD [8]. Genes associated with PD are α-synuclein, parkin, ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), PTEN-induced putative kinase 1 (PINK1), DJ-1, and leucine-rich repeat kinase 2 (LRRK2 or dardarin) [6]. Recent data has shown the involvement of mitochondrial dysfunction in molecular cell death pathways in PD [9]. Moreover, some studies revealed that several PD-associated genes impact on mitochondrial integrity directly or indirectly, which provides a specific link between mitochondrial dysfunction observed in sporadic PD [10, 11]. α-syn, Parkin, PTEN-induced kinase 1 (PINK1), DJ-1, leucine rich repeat kinase 2 (LRRK2), and HTR2A were found to be localized in the mitochondria under certain conditions where they maintain mitochondrial integrity and morphology [11, 12]. Although mitochondria produce energy for cellular events, during catabolism, this organelle also produces reactive oxygen

species (ROS) that can cause oxidative damage, directly on mitochondrial enzymes, mitochondrial genome, and mitochondrial membrane permeability resulting in apoptosis. For neurodegenerative diseases, mitochondrial dysfunction is one of the hallmarks of pathogenesis caused by ROS inducing cell death [13]. Mitochondrial dysfunction and neuroinflammation may simultaneously induce neuronal cell death. Because mitochondria is the major source of ROS, and mitochondria can be easily affected by ROS [14, 15]. The α-synuclein mutation is autosomal dominant whereas the *parkin*, *DJ-1,* and *PINK1* gene mutations are autosomal recessive during inheritance. *LRRK2* is frequently mutated in late onset PD [16]. PD diagnosis is based on clinical findings, but there is no conclusive test for diagnosis yet [17]. The pathological hallmark of PD is selective loss of dopaminergic, neuromelanin-containing neurons in the pars compacta of the substantia nigra and presence of intraneuronal inclusions called the Lewy body [6]. Mechanisms involved in neurodegeneration in PD are protein misfolding, mitochondrial and ubiquitin-proteasome dysfunction, oxidative stress, inflammation, and apoptosis [18]. There is no current treatment in PD, but replacement of L-DOPA- is a viable therapeutic approach for arresting PD [19].

The current knowledge about pathogenesis of PD is still limited; therefore, the development of animal models is essential for better understanding of PD pathogenesis and the testing of new drugs [20]. An ideal animal model should mimic clinical and pathological features of the disease. Available animal models of PD can be divided into two categories: toxin-based and genetic [21]. 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) selectively destroy catecholaminergic neurons. Recent studies have shown that environmental toxins such as rotenone and paraquat induce progressive loss of DA neurons through inhibition of mitochondrial respiratory chain complex I [21]. Toxin-based animal models for PD are limited in that they do not model the slow and progressive loss of dopaminergic neurons and the decrease in generation of Lewy bodies [8].

Like toxin-induced models, genetic animal models of PD have contributed to the understanding of the disease. Knockout mice with deletion of *parkin*, *DJ-1* or *PINK1* genes have been generated [22–24]. Several transgenic mouse models of *α-synuclein* gene have been developed, including mice overexpressing α-synuclein [25], carrying the point mutations of α-synuclein [26] or knockout mice for α-synuclein [27]. Recently, conditional knockout models of PD have been generated. In MitoPark mice, the mitochondrial transcription factor A (TFAM) has been selectively deleted in dopaminergic neurons [21]. Loss of TFAM activity in MitoPark mice leads to impaired oxidative phosphorylation specifically in dopaminergic neurons [21].

There is some evidence that inflammation plays a major role in the pathogenesis of PD. Activated microglia were found in the striatum and the substantia nigra in PD [28, 29] and proinflammatory cytokine such as tumor necrosis factor (TNF), interleukin-1beta (IL-1β), interleukin-6 (IL-6), and inducible nitric oxide synthase (iNOS) are increased in cerebrospinal fluid of patients with PD [30, 31]. Epidemiological studies also support the role of inflammation in PD disease. A study found that the risk of PD was lower in persons who regularly took nonsteroidal anti-inflammatory drugs (NSAIDs) than in persons who did not take these drugs [32]. In addition, inflammation has a major impact on pathogenesis toxin-induced and even genetic models for PD [33, 34]. Due to the role of inflammation in PD, the need for purely inflammation-driven animal models has emerged. Firstly, an in vitro model developed by (lipopolysaccharide) LPS-induced neurotoxicity in mixed cortical neuron/glia cultures [35]. Later, an in vivo LPS-induced PD model was devoloped by Castaño et al. [36]. Since then, LPS-induced PD model has been widely accepted and used for understanding pathogenesis of PD and testing new drugs in the treatment of PD. In this paper, we will summarize the various in vivo LPS-induced PD models. Furthermore, we will highlight the combined models of LPS with toxin-induced or genetic models and pathogenesis of LPS-induced PD models. We have mentioned the contribution of LPS-induced PD models to studies of PD pathogenesis and to new drug development for the treatment of PD.

2. Neuroinflammation in Parkinson's Disease (Epidemiological Data, Toxin-Based Animal Models, Genetic Models, PET Imaging, and Peripheral Immune System in PD)

The process of neuroinflammation has been shown to be involved in PD by McGeer et al. in 1988. They have found that activated microglia and T-lymphocytes are present around the Substantia Nigra pars compacta (SNpc) of postmortem PD patients [28, 37, 38]. Followup studies have confirmed the presence of inflammation related enzymes iNOS and cyclooxygenase-2 (COX2) in SNpc.; Mogi et al. reported the increased levels of TNFα, β2-microglobulin, epidermal growth factor (EGF), transforming growth factor α (TGFα), TGFβ1, and interleukins 1β, 6, and 2 in striatum of PD brain at the molecular level [39–42]. When the cerebrospinal fluid and serum of PD patients were analyzed, IL-2, TNFα, IL-6, and RANTES levels were found to be increased [42–45]. Immunological studies have also shown the presence of activated (CD4+ CD45RO+) T-cells in serum of PD patients [46, 47]. In order to monitor activated microglia in the PD brain, [^{11}C](*R*)-PK11195, which is a marker of peripheral benzodiazepine binding sites that is selectively expressed by activated microglia, is used in PET studies [48, 49]. It has been found that the density of activated microglia is highest in clinically affected regions of the brain, supporting the fact that inflammatory responses by intrinsic microglia contribute to the progression of PD. All these studies show that activated microglia take part in PD pathogenesis; however, in most of the studies, late stages of PD brains were examined and involvement of microglia to the inflammation at early or late stages was mere speculations. Recent data from tissue culture studies, however, supports the notion that microglia contribution occurs in early stage PD [50, 51]. In addition to etiologic studies, the determination of risk factors for developing PD has been

tried. For genetic analyses, polymorphisms of TNFα, IL-1β, IL-1α, IL-6, and CD14 genes were analyzed, and association studies demonstrated that the polymorphisms are common among patients [52–57].

In l-Methyl-4-Phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of PD, mitochondria complex I is inhibited and ATP levels decrease resulting in cell death. In this model, activated microglia and infiltration of activated T-lymphocytes were detected in brains of MPTP-treated animals [58–61]. In another model for PD, 6-hydroxydopamine (6-OHDA), cells are selectively killed by generation of free radicals and oxidative stress. Crotty et al. have recently shown a significant increase in number of activated microglia in 6-OHDA lesioned rats [62]. A study by Depino et al. concerning 6-OHDA lesioned rats did not find an increase in TNFα both on the mRNA and protein levels. An increase in IL-1β protein levels has not been detected whereas significant increase in mRNA levels of IL-1β has been detected [63].

3. Experimental Considerations

LPS is now well established as an effective initiator of DA neurodegeneration. The neurotoxic effect of LPS has been first demonstrated in cell culture-based *in vitro* models. The *in vitro* cell culture model of LPS-mediated neuroinflammation and neurotoxicity is based on the mesencephalic mixed neuron-glia culture system [64]. In vitro studies on rat mesencephalic cultures suggest that dopaminergic neurons are twice as sensitive to LPS as nondopaminergic neurons and that the toxicity of LPS occurs via microglial activation [65, 66]. As an economical and efficient system, *in vitro* studies are still valuable to explore the molecular mechanisms of LPS-mediated neurotoxicity and for screening candidate therapeutic compounds.

3.1. Characteristics and Versions of the Model. To extend the observations made in the *in vitro* LPS-mediated neuroinflammation model to a physiologically more relevant setting, the single intranigral LPS injection model has been developed in 1998 [36]. Compared with the *in vitro* LPS model, a single injection of low microgram quantities of LPS to the SN enables the comparison of the relative vulnerability to inflammatory damage of dopaminergic neurons in the SN versus those in the VTA, dopaminergic versus nondopaminergic neurons in the SN, and dopaminergic versus nondopaminergic neuronal projections in the corpus striatum [36, 51, 64]. Consistent with previous *in vitro* findings, an *in vivo* endotoxin model has shown that LPS-induced neurodegeneration is primarily observed in dopaminergic neurons and nondopaminergic neurons such as GABAergic neurons. SN are mostly spared by this process; microglial activation precedes dopaminergic neurodegeneration indicating a temporal relationship between glial activation and neurodegeneration, and finally LPS-induced microglial activation plays a more prominent role than astroglial activation in the release of various neurotoxic mediators that lead to dopaminergic neurodegeneration [64]. Acute intranigral or supranigral LPS injections (2 μg) produce a rapid activation of microglia (within 24 h) and loss of striatal dopamine (by day 4) accompanied by loss of SN DA neurons (within 21 days) [67, 68]. Injection of LPS to the SN results in an irreversible, but not progressive loss of the dopaminergic neurons in SNpc. While striatal DA is rapidly reduced, no further decline is seen during 1 year, indicating a permanent lesion but a lack of progression [69]. This model does not induce DA neuron death directly by activating microglia/monocytes. Although acute intranigral LPS administration produces rapid and intense microglial activation, microglia morphology reverts to normal form within 30 days, indicating a short-lived response and not a prolonged or progressive state of activation [70]. The successful demonstration of single intracerebral LPS injection induced dopaminergic neurodegeneration prompted further examination on whether a less intense and chronic period of inflammation in the SN would lead to a delayed and progressive nigrostriatal dopaminergic neurodegeneration. Indeed, chronic infusion of nanogram quantities of LPS to the SN via an osmotic minipump for two weeks induces significant glial activation accompanied by delayed, progressive, and preferential degeneration of SNpc dopaminergic neurons [71]. Although the SN is far more sensitive than the striatum to the inflammatory stimulus [69], intrastriatal or intrapallidal injection of LPS also induces neuroinflammation and dopaminergic neurodegeneration in rodents [72–76]. The Globus pallidus is an integral component of the basal ganglia that is important in regulation of movement. The intranigral LPS model has recently been established in mice [77]. Future research can be performed using knockout mice to study other potential mechanisms of neuroinflammation-induced neurodegeneration [77, 78]. Systemic inflammation has been suspected to influence the activities of the immune cells in the brain and consequently contributes to the chronic neurodegenerative process for diseases such as PD [79]. Systemic administration of LPS has been found to induce progressive degeneration of nigral dopaminergic neurons in male C57BL/6 mice [80]. Systemic LPS injection also induces apoptotic cell death in SN [81]. Interestingly, progressive dopaminergic cell loss occurs in mice given a single systemic exposure to LPS, which contrasts with the lack of progressive dopaminergic neuron loss in rats provided with a single, acute, intranigral LPS infusion [67, 69, 70].

Several experimental considerations including LPS strain, administration route and dosing of LPS, strain, gender, and age differences of experimental animals should be taken into account for the design of experimental setting in LPS-based PD model. As discussed above, administration route and location of LPS determine the characteristics of the LPS-based PD model. While a single intranigral or supranigral injection of LPS does not cause progressive dopaminergic cell loss, chronic infusion of endotoxin to SN or systemic LPS administration leads to a time-dependent progression in dopaminergic neurodegeneration. The degree of dopaminergic neurodegeneration is also concentration-dependent [51]. 14 days after injection of 0.1 μg to 10 μg LPS into the rat SN, TH-positive (TH+) neurons in the SN were decreased by 5%, 15%, 20%, 45%, 96%, and 99%, respectively [82]. The possible effect of the differences between LPS strains has not been evaluated to date.

3.2. Strain, Gender, and Age Differences. Although different mouse strains present striking differences in the extent of dopaminergic neurodegeneration induced by neurotoxin MPTP, injection of LPS to the SN region of Wistar, Fisher, or Sprague-Dawley rats have a similar loss of SNpc DA neurons [36, 51, 83]. Differences between rat strains have not been reported for the acute intracerebral LPS model.

Gender differences seem to be an important factor in the sensitivity to the LPS-induced dopaminergic neurodegeneration. C57BL/6 female mice are more resistant to systemic LPS than male mice [80, 81]. Repeated monthly LPS injections are required to cause both motor behavioral deficits and dopaminergic neuronal loss in female mice.

Several studies compared the detrimental effects of LPS on the nigrostriatal pathway and its behavioral consequences between young and aged animals. Four weeks after bilateral intrapallidal injection of LPS (10 μg), a greater loss of SNpc DA neurons in the older (16 months old) than the younger Fisher F344 rats (3 months old) with alpha-synuclein-positive intracellular inclusions in the SN dopaminergic neurons of the LPS-injected middle-aged rats could be observed [76]. While young rats recovered from LPS-induced locomotor deficits four weeks after intrapallidal LPS injection, aged rats failed to improve on measures of speed and total distance moved, which may be caused by microglial activation and proinflammatory cytokine expression [74]. In addition, greater nitration of proteins like alpha-synuclein occurred in the SN of elderly rats versus young rats, accompanied by higher expression level of iNOS. The Lewy body, a pathological hallmark of PD, contains nitrated alpha-synuclein, which is prone to oligomerization. These results imply that an exaggerated neuroinflammatory response that occurs with aging might be involved in the increase in prevalence of neurodegenerative diseases like PD [74]. One month after intrastriatal injection of LPS microglial activation, lipid peroxidation, ferritin expression, and total nigral iron content in aged rats significantly increased. In addition, LPS significantly altered the turnover ratio of HVA to DA [74]. Injection of LPS into the globus pallidus of young and middle-aged rats substantially decreased TH as can be evidenced by immunostaining in SNpc one month after injection [76]. Loss of TH expression was accompanied by increase iron and iron-storage protein ferritin levels in glial cells of the SN pars reticulata. Despite great increase in nigral iron levels, ferritin induction was less pronounced in older rats, suggesting the regulation of ferritin is compromised with age. Intrapallidal LPS injection also increased expression of alpha-synuclein and ubiquitin in TH(+) neurons of the SNpc. These findings suggest that pallidal inflammation significantly increases stress on dopaminergic neurons in the SNpc. Alterations in nigral iron levels may increase the vulnerability of nigral neurons to degenerative processes. Thus, an age-related increase in iron as well as susceptibility to inflammation may play an important role in PD-related neurodegeneration, as free radicals produced from the inflammatory response can become more toxic through increased ferrous iron catalyzed Fenton chemistry. This may enhance oxidative stress, exacerbate microglia activation, and drive the progression of PD [76].

3.3. Assessment of the Neuroinflammation, Neurodegeneration, and Their Effect. Several immunohistochemical, histological, biochemical, and behavioral parameters are used to evaluate the neuroinflammation and neurodegeneration in LPS-based PD models. Reduction of TH immunoreactivity is used as an index for dopaminergic cell death. The preferential degeneration of SNpc DA neurons was further corroborated by studies that employ fluorogold retrograde labeling of the striatonigral DA pathway prior to LPS injection.

Similar reduction of TH immunoreactivity and fluorogold-labelled neurons in the SN following LPS administration suggests dopaminergic cell death rather than downregulation of TH [68]. The number of TH(+) cells is determined using stereological analysis.

TH enzyme activity from striatal tissue can be measured as an indirect index of dopaminergic neurodegeneration. A single intranigral injection of LPS causes reduction in TH enzyme activity [36]. In vivo microdialysis can be used to measure changes in extracellular concentrations of dopamine and its metabolites in freely moving rats in response to administration of an endotoxin. In a recent study, dopamine metabolites in the dialysate obtained from the rat brain were measured by high-performance liquid chromatography (HPLC) using electrochemical detection [84]. Results showed that intrastriatal perfusion of different concentrations of LPS produced a dose-dependent decrease in the extracellular DOPAC output.

Intracerebral injections of LPS (5 or 10 μg) into the cortex, hippocampus, striatum, or SN of rats enhances the death of only SN DA neurons, possibly because microglial cell density in the SN is 4-5 times higher than in other regions [69, 71, 85]. LPS administration induces a rapid activation of microglia within hours as demonstrated by morphological transformation of OX-42-positive microglia. SN microglia became fully activated exhibiting the characteristic amoeboid morphology [71]. This is accompanied by intense expression of glial fibrillary acidic protein- (GFAP-) immunoreactive astrocytosis in the SN [68]. Double immunostaining of the tissue slices shows that iNOS and 3-nitrotyrosine (3-NT)-immunoreactive cells are predominantly microglia [70]. Activated microglia can even be found in the basal ganglia and brainstem of PD cases or in rodents using positron emission tomography (PET) with [^{11}C](R)-PK11195 [86–89]. To the best of our knowledge, in vivo PET imaging for the evaluation of microglial activation has only been used in intraperitoneally LPS-treated rats in a recent study by Ito et al. [90]. For this model, the authors have concluded that the intensity of peripheral benzodiazepine receptor signals in [(11)C]PK11195 PET may be related to the level of microglial activation rather than the number of activated microglia.

Neuroinflammation-mediated dopaminergic neuronal loss induced by LPS may also have functional significance as demonstrated by behavioral tests. Thirty days following supranigral LPS injection, rats show unilateral behavioral deficits as evidenced by ipsilateral circling following amphetamine administration [70]. Intrapallidal LPS injection causes permanently slowed locomotor activity in aged rats [76]. Automated movement tracking analyses has shown that young rats (3 months old) recovered from

LPS-induced locomotor deficits four weeks after intrapallidal LPS injection, yet older rats (16 months old) failed to improve on measures of speed and total distance moved. In contrast to MPTP and 6-OHDA, intranigral LPS administration does not produce behavioral dysfunction in early periods (1, 3, and 7 days after the lesions); however, LPS drastically increases HVA at the first time point, simulating features of the premotor phase of PD [91]. The combination of both systemic LPS and MPTP causes striatal DA and gait instability as revealed by reduced stride length in male C57Bl/6J mice at 4 months after injection [92].

3.4. Combined Models. In most environmental models for PD, a single neurodegenerative agent is introduced to cause nigrostriatal dopamine depletion. However, cell loss in human PD may often be caused synergistically by multiple toxins or vulnerabilities. Recent studies have also focused on the effects of LPS challenge in toxin-based and genetic models of PD. As discussed in Section 2, the findings of neuroinflammation are also observed in toxin-based and genetic models of PD. Increased mRNA and protein expression of both CD14 and TLR4 in the SN, but not in the caudate-putamen nuclei of mice treated with MPTP, in comparison to untreated animals, suggests that the endotoxin receptors are overexpressed in specific areas of the CNS during experimental PD [93]. Thus, the neurotoxin challenge may also cause a predisposition for the exacerbation of chronic neuroinflammation.

A recent study by Koprich et al. has shown that injection of a nontoxic dose of LPS into adult rat SNpc leads to microglial activation and increased levels of IL-1β, without causing death of dopaminergic neurons *in vivo*, but causing increased vulnerability for DA neurons to a subsequent low dose of 6-OHDA [94]. This exacerbation of 6-OHDA-induced neuronal loss by LPS appears to be partly mediated by IL-1β, since treatment with both LPS and IL-1 receptor antagonist rescued some of the dopaminergic neurons from 6-OHDA-induced death following LPS-induced sensitization to dopaminergic degeneration. Another recent study has shown that 6-OHDA injection into the adult rat striatum and subsequent nontoxic LPS injection into the SNpc cause an increased level of dopaminergic neuronal death and motor deficits compared with the administration of either toxin alone [95]. Thus, the initial insult causes priming of microglia, while the second insult shifts microglial activation towards a proinflammatory phenotype with increased IL-1β secretion. Specific IL-1β inhibition reversed these effects and nitric oxide (NO), a downstream molecule of IL-1β action, is partially responsible for the exacerbation of the neurodegeneration that has been observed [95]. The combination of systemic LPS and MPTP, but not either alone, causes striatal DA and gait instability in male C57Bl/6J mice about 4 months after injection [92]. MPTP alone acutely reduced striatal DA levels, but this effect was transient as striatal DA recovered to normal levels after 4 months. The nigrostriatal dopaminergic neurons can succumb to multiple toxic agents that independently may have only a transient adverse effect. The effect of methamphetamine (MA) dopaminergic toxicity, like MPTP toxicity, frequently cited as a model of PD, is potentiated by intrastriatal LPS administration [96]. This combined model leads to behavioral impairment and striatal dopaminergic deficits, but not to alteration in other monoaminergic systems including serotonin, norepinephrine, and histamine. The combination of striatal LPS and MA results in microglial activation limited to the nigrostriatal region. Furthermore, neuroinflammation, oxidative stress, and proapoptotic changes in the striatum are more accentuated with combined treatment of LPS and MA compared to individual treatments. In addition, cytoplasmic accumulation of alpha synuclein has been observed in the SN of mice treated with LPS and MA. L-Dopa treatment, also, significantly attenuates behavioral changes, and dopaminergic deficits can be induced by LPS and MA [96].

Inflammatory priming of the SN by LPS influences the impact of later neurotoxin exposure, and this process was called as neuroimmune sensitization of neurodegeneration [97].

Repeated injection with the herbicide paraquat causes oxidative stress and a selective loss of dopaminergic neurons in mice. In this model, the first paraquat exposure, though not sufficient to induce any neurodegeneration, predisposes neurons to damage by subsequent insults. Multiple toxin exposure may synergistically influence microglial-dependent DA neuronal loss and, in fact, pretreatment with one toxin may sensitize DA neurons to the impact of subsequent insults. Priming the SNpc neurons with LPS influences the impact of later exposure to paraquat [97]. LPS infusion into the SN-sensitized DA neurons to the neurodegenerative effects of a series of paraquat injections commencing 2 days later. In contrast, LPS pretreatment protects against some of neurodegenerative effects of paraquat when the pesticide is administered 7 days after the endotoxin, suggesting the importance of the time of exposure. These results suggest that inflammatory priming may influence DA neuronal sensitivity to subsequent environmental toxins by modulating the state of glial and immune factors, and these findings may be important for neurodegenerative conditions, such as PD [97]. Microglial activation acts as a priming event leading to paraquat-induced dopaminergic cell degeneration. A study by Purisai et al. elucidated the mechanism underlying this priming event. They found that a single paraquat exposure is followed by an increase in the number of cells with immunohistochemical, morphological, and biochemical characteristics of activated microglia, including induction of NADPH oxidase [98]. When initial microglial response was inhibited by the anti-inflammatory drug minocycline, subsequent exposures to the paraquat fail to cause oxidative stress and neurodegeneration. If microglial activation was induced by pretreatment with LPS, a single paraquat exposure suffices to trigger a loss of dopaminergic neurons. Moreover, mutant mice lacking functional NADPH oxidase are spared from neurodegeneration caused by repeated paraquat exposure [98].

The LPS-based model has also been combined with a genetic model of PD [77]. In mutant alpha synuclein (αSYN) transgenic mice, but not synuclein knockout mice, intranigral LPS administration led to neuroinflammation

associated with dopaminergic neuronal death and the accumulation of insoluble SYN aggregates as cytoplasmic inclusions in nigral neurons. Nitrated/oxidized SYN has also been detected in these inclusions. These results suggest that NO and superoxide release by activated microglia may be the mediator that links inflammation and abnormal αSYN in PD neurodegeneration [77]. Although loss-of-function mutations in the parkin gene cause early-onset familial PD, Parkin-deficient (parkin$-/-$) mice do not display the nigrostriatal degeneration pathway, suggesting that a genetic factor is not sufficient, and an environmental trigger may be needed to cause dopaminergic neuron loss. Upon administration of low-dose systemic LPS for prolonged periods, parkin$-/-$ mice display subtle fine-motor deficits and selective loss of dopaminergic neurons in the SN, suggesting that the loss of the Parkin function increases the vulnerability of the nigral DA neurons to inflammation-related degeneration [99].

4. Neuroinflammation Model of Parkinson's Disease Induced by Prenatal Exposure to Lipopolysaccharide

Parkinson's disease symptoms' typically manifest in late adulthood, after loss of dopaminergic neurons in the nigrostriatal system. Lack of heritability for idiopathic PD has implicated adulthood environmental factors in the etiology of the disease. However, compelling evidence from recent experimental studies has shown that exposure to a wide variety of environmental factors during the perinatal period (environmental toxins such as pesticides) and during the prenatal period (bacterial endotoxin LPS) can either directly cause a reduction in the number of dopamine neurons or cause an increased susceptibility to degeneration of these neurons with subsequent environmental insults or with aging alone [100] (Figure 1). A fraction of pregnant women suffer from vaginal or cervical bacterial infections, and there may be a risk for bacterial toxins including LPS to impact the fetal development. One of the potential targets for an endotoxin assault may be the developing nigrostriatal DA pathway [64]. The endotoxin model implies a role of proinflammatory cytokines, which may relate to epidemiological studies of early-life infectious agents and intrauterine infections.

The proinflammatory cytokine TNFα kills DA neurons and is elevated in the brains of patients with PD (Figure 1). LPS is a potent inducer of TNFα and both are increased in the chorioamniotic environment of women who have bacterial vaginosis during pregnancy. This suggests that prenatal maternal infection might interfere with the normal development of fetal DA neurons [101]. In utero exposure to LPS following a single injection of the endotoxin intraperitoneally (10 000 endotoxin units) into gravid Sprague-Dawley rats at embryonic day 10.5, a critical time point during embryonic dopaminergic neuron development, causes a significant reduction in the striatal DA and nigral dopaminergic cell number, accompanied by elevated levels of striatal and nigral TNFα in offspring sacrificed at 21 days, indicating that prenatal exposure to LPS not only creates a neuroinflammatory response but also disrupts the normal development of dopaminergic neurons [101]. Dopaminergic neuron loss is apparently permanent as it is still present in 16 months old animals [102]. In utero LPS exposure does not appear to affect dopaminergic neurons in the ventral tegmental area (VTA) or nondopaminergic neurons in the substantia nigra [101]. In contrast to TNFα, levels of IL-1β are not affected by prenatal LPS treatment [101]. LPS administration results in significant microglial activation and sustained elevation of TNFα in both the SN and the corpus striatum, even several weeks after the sole initial exposure, suggesting a persisting effect [103].

However, endotoxin-induced dopaminergic cell loss does not seem to progress as prenatal LPS reduces the baseline number of dopaminergic neurons in offspring, but the baseline remains stable once it has been established even beyond 16 months of age (similarly 20%–30% reduction in the number of SNpc dopaminergic neurons across studies and across ages) [100, 101, 103, 104].

In utero LPS exposure may predispose the nigrostriatal dopaminergic system of the pups to enhanced susceptibility to neurotoxins such as rotenone and 6-OHDA [103, 104]. Using male offspring at 3 months of age, Ling et al. has not been able to find any synergistic toxic effects of prenatal LPS and postnatal 6-hydroxydopamine (6OHDA) exposures [104]. In contrast, a subtoxic dose of neurotoxin rotenone (1.25 mg/kg/day, 14 days, intrajugular) injected at 18 months of age to female rats exposed prenatally to LPS, exerted a synergistic effect on dopaminergic cell loss, suggesting that a preexisting proinflammatory state can be a risk factor for environmental toxins [103]. One subtoxic rotenone dose did not directly lead to cell loss in these aged female rats. However, against the background of prenatal LPS exposure, cell loss was significant in the SNpc, displaying an interaction of prenatal exposure and adulthood challenges, which suggests that age and multiple environmental hits play a role. Dopaminergic cell loss was associated with decreased striatal DA and increased striatal dopaminergic activity ([HVA]/[DA]). Animals prenatally exposed to LPS exhibited a marked increase in the number of reactive microglia that was further increased by rotenone exposure. Prenatal LPS exposure also led to increased levels of oxidized proteins and the formation of α-Syn and eosin positive inclusions resembling Lewy bodies. These results suggest that exposure to low doses of an environmental neurotoxin like rotenone can produce synergistic dopaminergic neuron losses in animals with a preexisting proinflammatory state [103]. This supports the notion that PD may be caused by multiple factors and the result of multiple hits from environmental toxins. Yet, despite neuroinflammation, the progressive loss of dopaminergic neurons that characterizes PD is rarely seen in animals. In a recent study, 7-month-old male rats prenatally exposed to LPS were subjected to supranigral infusion of LPS and sacrificed after 2 or 12 weeks [105]. LPS infusion into animals prenatally exposed to LPS produced a neuroinflammatory response during the 14 days of LPS infusion that subsequently reverted to normal state over the next 70 days. In animals with preexisting inflammation (i.e., prenatal LPS); however, the acute changes seen were attenuated but the return to normal

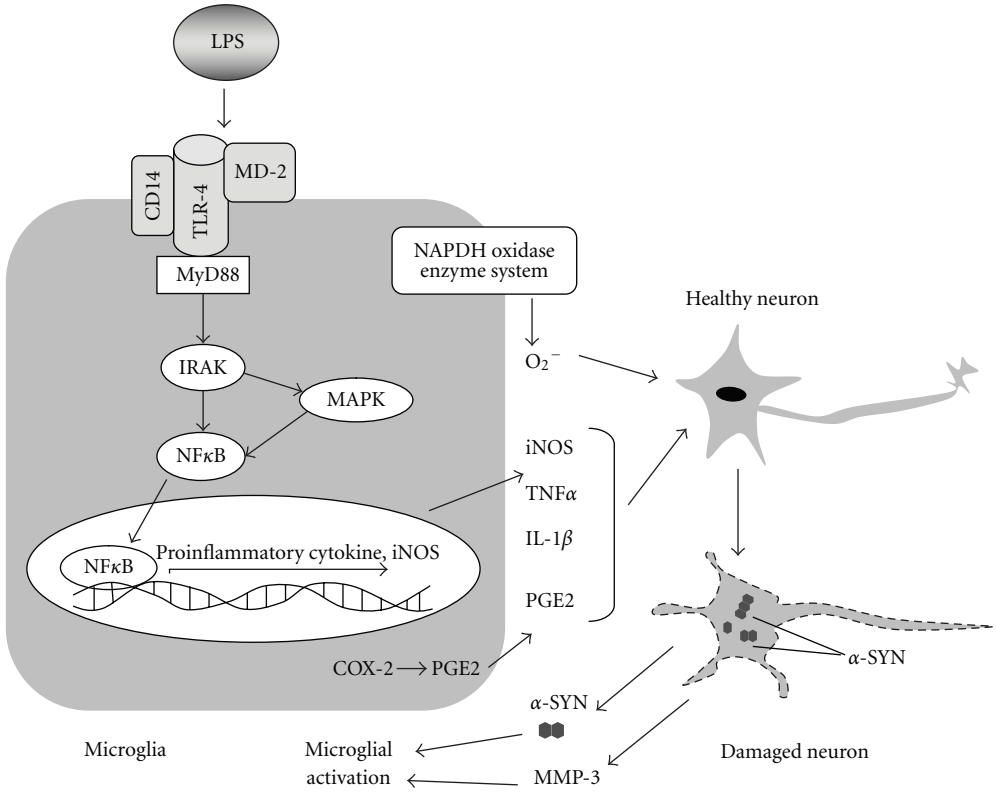

Figure 1: Simplified schematic representation of the link between LPS-induced microglial activation, inflammatory mediators, and dopaminergic neurodegeneration. Microglia respond to pathogens, proinflammatory cytokines, neuronal dysfunction, and cellular debris after injury or necrosis. These cells are at the forefront of the defence mechanisms that could set the conditions for repair or contribute to neuronal damage. Such equilibrium might depend on the expression and function of specific TLRs and how they are activated by endogenous and exogenous ligands and signals. Recognition of such signals lead to transcriptional activation of innate immune genes. Bacterial endotoxin LPS is a potent stimulator of macrophages, monocytes, microglia, and astrocytes causing release of various immunoregulatory and proinflammatory cytokines and free radicals. Neurons do not express functional TLR-4. Thus, LPS does not appear to have a direct effect on neurons, making it an ideal activator to study indirect neuronal injury mediated by microglial activation [64]. LPS binds to its intermediate receptor CD14 and in concert with TLR4 and accessory adaptor protein MD2 triggers the activation of kinases of various intracellular signaling pathways. The MyD88-dependent cascade initiates NFκB activation through the IKKs and/or the MAPK pathway, leading to the upregulated expression of proinflammatory cytokines (TNFα, IL-1β) and increased production of other inflammatory mediators (NO and PGE2, synthesized by iNOS and COX-2, resp.). These soluble mediators collectively damage nigral dopaminergic neuron. MMP-3 and αSYN released by stressed neurons aggravate microglial activation. Astrocyte, different activation states of microglia, peripheral immune cells, many molecules involved in intracellular signaling pathways, and crosstalk between TLR signaling pathway and NADPH oxidase enzyme system are not shown for the simplicity. Please see text for the abbreviations and the details of TLR signaling pathway.

state took much longer. Prenatal LPS exposure also causes a disturbance in the glutathione homeostasis in offspring brain, which renders dopaminergic neurons susceptible to secondary endotoxin insults in adulthood [106].

When rats, prenatally exposed to LPS, were evaluated at 4, 14, and 17 months, the progressive dopaminergic neuron loss was parallel to that of the controls suggesting that prenatal LPS exposure does not produce an accelerated rate of dopaminergic neuron loss [107]. Prenatal LPS exposure disrupted the dopaminergic system involving motor function, but this neurochemical effect was not accompanied by behavioral impairment, which is probably due to adaptive plasticity processes [108]. Prenatal LPS administration (100 μg/kg, i.p.) on gestational day 9.5 impairs the male offspring's general activity and decreases the striatal dopamine and metabolite levels in adulthood after an additional immune challenge [108]. Following prenatal LPS exposure, significant reductions in DA and 5-hydroxytryptamine (5-HT) levels were found in the frontal cortex, nucleus accumbens, striatum, amygdala, hippocampus, and hypothalamus of male offspring at 4 months of age [109]. The loss of DA and 5-HT were accompanied by a significant increase in homovanillic acid over DA and 5-hydroxyindoleacetic acid over 5-HT ratios in most tested areas. These data further validate prenatal LPS exposure as a model of PD, since DA and 5-HT changes are similar to those seen in PD patients.

The neonatal period is developmentally distinct from the gestational period, and exposures to endotoxin in either may lead to different consequences. In an *in vivo* study using a mouse model with nigrostriatal lesions, produced by the administration of MPTP, microglia activated by systemic LPS

were neurotoxic toward dopamine neurons in aged mice but unexpectedly neuroprotective in neonatal mice [110]. The inflammatory process in the brain, which is accompanied by changes in the levels of proinflammatory cytokines and neurotrophins, along with the presence of activated microglia, has recently gained much attention in the area of neurodegenerative diseases. Activated microglia produce either neuroprotective or neurotoxic factors. Many reports indicate that activated microglia promote degeneration of dopaminergic neurons in PD. On the other hand, there are several lines of evidence that microglia also have a neuroprotective function [111]. Microglia activated with LPS in the nigrostriatum of neonatal mice protect dopaminergic neurons against the neurotoxin MPTP whereas activated microglia in aged mice promote death of dopaminergic cells by MPTP. Recent findings suggest that the function of activated microglia may change *in vivo* from neuroprotective to neurotoxic during aging as neurodegeneration progresses in the PD brain [111]. These results suggest that the activated microglia in neonatal mice are different from those in aged mice, with the former having neurotrophic potential toward the dopamine neurons in the SN in contrast to the neurotoxic effect of the latter [112].

As discussed above, recent studies have begun to identify specific factors occurring as part of the in utero or perinatal environment that may predispose or even cause damage to the nigrostriatal system, suggesting that environmental factors early in life of an individual cause a predisposition to develop symptoms of PD. Interactions of prenatal environment, adulthood environment, gender, age, and genetic background may also modify this risk [100]. Recently, animal studies have been described that specifically consider the role of gestational exposures in disrupting the nigrostriatal system and each has implications for elaborating on our current understanding of the etiology of PD.

5. Cellular and Molecular Mediators of Endotoxin-Mediated Dopaminergic Neurodegeneration

Unlike the direct death of dopaminergic neurons caused by neurotoxins such as MPP^+ or 6-OHDA, endotoxin-mediated dopaminergic neurodegeneration seems to result from indirect neuronal death due to inflammatory reactions. Bacterial endotoxin LPS is capable of activating glial cells, predominantly microglia, to release a wide variety of proinflammatory and neurotoxic factors that include reactive oxygen and nitrogen species, proinflammatory cytokines, and lipid mediators [113]. A number of mechanisms by which inflammatory-activated microglia and astrocytes kill neurons have been identified in cell-culture studies [114]. Results from studies employing enzyme inhibitors, neutralizing antibodies, specific inhibitors of inflammatory signaling pathways, and knockout animals have identified these soluble factors and signaling molecules involved in microglial activation as major contributors to the endotoxin-mediated dopaminergic neurodegeneration [64].

The toll encoding gene has first been identified in Drosophila embryos, where it has a role in dorsoventral axis determination [115, 116]. Many organisms have multiple homologues of the Drosophila toll gene, which is very conserved among species [117]. In vertebrates, TLR (Toll-like receptors) recognize pathogen associated molecular patterns of bacteria, fungi, and viruses and play roles in host defense mechanism. TLR4 takes part in recognition of strongly conserved patterns of gram-negative cell wall components, LPS and discriminates indigenous from foreign molecules [118]. In TLR4 signaling, TLR4 must first associate with its extracellular binding partner, myeloid differentiation factor 2 (MD-2), before ligands can bind to the TLR4-MD-2 complex [119, 120]. The TLR4-MD-2-Ligand complex forms a heterodimer with another TLR4-MD-2 ligand complex and the signal is transferred to the TLR4's Toll/interleukin-1 receptor (TIR) domain. The signal is than further transduced via an unknown mechanism [118, 121]. The signal is then transmitted to two separate pathways which are the MyD88 path activating Nf-κB and Toll/IL-1 receptor also containing adaptor inducing IFN-β (TRIF) path. In the MyD88 path, MyD88 adaptor-like protein (Mal or TIRAP) mediates the TIR-TIR association between TLR4 and MyD88 [122]. Next, an interaction occurs between IL-1 receptor-associated kinase (IRAK) and MyD88. That interaction results in the activation of a cascade leading to the phosphorylation of Nf-κB transcription factors. This path results in activation of Activator Protein-1, RelA and p50 heterodimers and regulates expression of proinflammatory cytokines [123, 124]. In the other pathway, TRIF and TLR4 require an adaptor molecule called TRAM for signal transduction, which mediates endocytosis of the TLR4 receptor complex [125, 126]. TRIF forwards the signal after incorporation of TRAF3- or TRAF6-mediated adaptor molecules to either TRIF-binding kinase- (TBK-) IKK or RIP, respectively [127]. TBK-IKK terminates Interferon regulatory factor-3 dimerization and translocation into nucleus to induce IFN-β synthesis; in this way, TBK-IKK regulates cellular response to inflammation [128]. On the other hand, TRAF6 interacts with RIP and activates Nf-κB through TAK1, which operates the same as in the MyD88 pathway, causing late phase Nf-κB activation [127].

5.1. Nitric Oxide. Nitric oxide (NO) is an important messenger molecule in a variety of physiological systems. NO, a gas, is produced from L-arginine by different isoforms of NOS and takes part in many normal physiological functions, such as promoting vasodilation of blood vessels and mediating communication between cells of the nervous system. In addition to its physiological actions, free radical activity of NO can cause cellular damage through a phenomenon known as nitrosative stress [129]. Although many *in vitro* and *in vivo* studies support an involvement of NO in microglial-mediated dopaminergic neuronal death due to LPS-treatment, some studies suggested that NO is not involved [113]. For instance, the first in vivo study of the endotoxin-based PD model reported that the neurotoxic effect of LPS was not mediated by NO [36]. However, increasing evidence from recent studies supports for the notion that excessive production and accumulation of NO in the LPS-induced DA lead to neurodegeneration [64].

Intracerebral administration of LPS causes increase in the iNOS enzyme activity and NO production [130, 131]. Immunofluorescence and immunohistochemical analyses have revealed that iNOS is located in fully activated microglia having a characteristic amoeboid morphology [70, 132]. After intranigral LPS injection, iNOS mRNA levels and protein expression increase [132]. In Western blot analysis, iNOS has been shown to be induced in the SN after injection of LPS in a time- and dose-dependent manner [133]. The increase in iNOS expression inversely correlates with the TH immunolabeling and animals pretreated with a selective inhibitor of iNOS, N(G)-nitro-L-arginine methyl ester (L-NAME), exhibited complete protection against behavioral deficits induced by intrastriatal LPS injection [130]. Furthermore, LPS-induced loss of dopaminergic neurons is significantly inhibited by the administration of L-NAME [133]. Decrease in DA level and increase in cytochrome-c release and caspase-3 activation were significantly reversed with treatment of L-NAME [131]. Thus, increased NO availability subsequent to iNOS induction seems to play an important role in the initial phase of neurodegeneration. Hunter et al. have suggested that permanent expression of the iNOS plays a role in the progressive loss of dopaminergic neurons but not the initial loss induced by LPS [75]. Although the mechanism of NO-mediated neurodegeneration remains uncertain, it has been suggested that NO contributes to LPS-induced dopaminergic neurodegeneration through several mechanisms. NO has been shown to modify protein function by nitrosylation and nitrotyrosination, contribute to glutamate excitotoxicity, inhibit mitochondrial respiratory complexes, participate in organelle fragmentation, and mobilize zinc from internal stores [129, 134]. NO can react with superoxide radicals to form peroxynitrite radicals that are short-lived oxidants and highly damaging to neurons [64, 135]. Mitochondrial injury is prevented by treatment with L-N(6)-(l-iminoethyl)-lysine, an iNOS inhibitor, suggesting that iNOS-derived NO is also associated with the mitochondrial impairment [72]. NO inhibits cytochrome oxidase in competition with oxygen, resulting in glutamate release and excitotoxicity [114].

The main cellular source of NO in the CNS are microglia whereas astroglia constitute the main defense system against oxidative stress. However, under pathological or chronic inflammatory conditions, astroglial cells may also release neurotoxic mediators. Although the PD-associated gene DJ-1 mediates direct neuroprotection, the upregulation of DJ-1 in reactive astrocytes also suggests a role in glia [136]. The intracerebral LPS-based PD model is associated with a moderate reactive astrogliosis [70]. DJ-1 acts as a regulator of proinflammatory responses, and its loss contributes to PD pathogenesis by deregulation of astrocytic neuroinflammatory damage [137]. When treated with LPS, DJ-1-knockout astrocytes generate significantly more NO than littermate controls. The enhanced NO production in DJ-1(−/−) astrocytes is mediated by a signaling pathway involving reactive oxygen species (ROS) leading to specific hyperinduction of iNOS. These effects coincide with significantly increased phosphorylation of the p38 mitogen-activated protein kinase (MAPK), p38 inhibition, suppressed NO production, and iNOS mRNA as well as protein induction. DJ-1(−/−) astrocytes also induce the proinflammatory mediators COX-2 and IL-6 in high levels. Primary neuron cultures grown on DJ-1(−/−) astrocytes became apoptotic in response to LPS in an iNOS-dependent manner suggesting the neurotoxic potential of astrocytic DJ-1 deficiency [137]. These findings warrant *in vivo* confirmation.

5.2. Reactive Oxygen Species. A large body of evidence supports the involvement of oxidative stress in the pathogenesis of PD [134]. Besides NO, ROS generated by activated glia, especially microglia are major mediators of the DA neurodegeneration cause by inflammation [64]. ROS can cause lipid peroxidation, protein oxidation, DNA damage, and mitochondrial dysfunction. LPS-induced ROS production in microglia is mediated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a multisubunit enzyme [114]. This complex is responsible for the production of both extracellular and intracellular ROS by microglia. Importantly, NADPH oxidase expression is upregulated in PD and is an essential component of microglia-mediated dopaminergic neurotoxicity. Activation of microglial NADPH oxidase causes neurotoxicity through two mechanisms. Firstly, extracellular ROS released from activated microglia are directly toxic to neurons. Secondly, intracellular ROS amplifies the production of several proinflammatory and neurotoxic cytokines and compounds such as TNFα, prostaglandin E2 (PGE2), COX-2, and IL-1β [138]. The activation of the phagocyte NADPH oxidase (PHOX) by cytokines, LPS, or arachidonic acid metabolites causes microglial proliferation and inflammatory activation; thus, PHOX is a key regulator of inflammation. Pharmacologic inhibition of NADPH oxidase provides protection against LPS-induced neurotoxicity and PHOX knockout mice are resistant to LPS-induced loss of SNpc dopaminergic neurons [139, 140]. Gene expression and release of tumor necrosis factor alpha was much lower in PHOX−/− mice than in control PHOX+/+ mice [140]. By injecting LPS into the striatum of wild type and Nox1 knockout mice, it has been shown that Nox1, a subunit of NADPH oxidase, also enhances microglial production of cytotoxic nitrite species and promotes loss of presynaptic proteins in striatal neurons [141]. Activation of PHOX alone causes no cell death, but when combined with expressed iNOS, it results in extensive neuronal cell death via the production of peroxynitrite [114]. The relationship between the signaling pathway downstream of TLR4, after LPS stimulation, and the activation of the oxidase remains elusive. Using mice lacking a functional TLR4, it has been demonstrated that TLR4 and ROS work in concert to mediate microglia activation [142]. Both TLR4(−/−) and TLR4(+/+) microglia display a similar increase in extracellular superoxide production when exposed to LPS. These data indicate that LPS-induced superoxide production in microglia is independent of TLR4 and that ROS derived from the production of extracellular superoxide in microglia mediates the LPS-induced TNF-α response of both the TLR4-dependent and independent pathway [142].

The integrin CD11b/CD18 (MAC1, macrophage antigen complex-1) pattern recognition receptor mediates LPS-induced production of superoxide by microglia [143]. MAC1 is a TLR4-independent receptor for the endotoxin LPS. MAC1 is essential for LPS-induction of superoxide in microglia, implicating that MAC1 acts as a critical trigger in microglial-derived oxidative stress during inflammation-mediated neurodegeneration. Interestingly, MAC1 mediates reactive microgliosis and progressive dopaminergic neurodegeneration in the MPTP model of PD, suggesting a role for this receptor in neurodegeneration [144]. Activated matrix metalloproteinase-3 (MMP-3) released from stressed dopaminergic neurons is also responsible for microglial activation and generation of NADPH oxidase-derived superoxide and eventually enhances nigrostriatal DA neuronal degeneration [145].

5.3. Proinflammatory Cytokines. Of the variety of cytokines that are released by LPS-activated glia, the proinflammatory IL-1β and TNFα may be the major cytokines involved in the LPS-induced dopaminergic neurodegeneration [64]. The contribution of these cytokines to neurodegeneration is supported by studies showing that neutralizing antibodies against TNFα or IL-1 markedly reduce the LPS-induced loss of nigral dopaminergic neurons [64]. Activated microglial cells in the SN are found in all animal models of PD and patients with the illness. Compared with astroglia or microglia, they appear to possess a larger repertoire of cytokine production [64, 113]. Elevated levels of TNFα in the cerebrospinal fluid (CSF) and the postmortem brains of PD patients as well as in animal models of PD implicate that proinflammatory cytokines significantly influence the pathophysiology of the disease [146]. TNFα has a pivotal role in mediating the loss of DA neurons in PD, which has been demonstrated using the endotoxin-based model. A sustained elevation of TNFα has been observed in the striatum and the mesencephalon of rats prenatally exposed to LPS [104]. Furthermore, in the chronic LPS nigral infusion model of PD, the loss of SNpc dopaminergic neurons, and the activation of microglia are significantly reduced by blockade of the soluble form of the TNFα receptor [146]. Systemic LPS administration results in rapid increase of TNFα in the brain, which remains elevated for 10 months [80]. Furthermore, LPS leads to microglial activation, to an increase in the expression of proinflammatory factors such as IL-1β, and NFκB p65, and to a progressive loss of nigral TH-immunoreactive neurons in wild-type mice, but not in mice lacking TNFα receptors [80]. Nontoxic doses of LPS also induce secretion of cytokines and predispose dopaminergic neurons to be more vulnerable to a subsequent low dose of neurotoxins such as 6-OHDA. Alterations in cytokines, prominently an increase in IL-1β, have been identified as being potential mediators of this effect that is associated with the activation of microglia [94, 95]. Administration of an IL-1 receptor antagonist results in significant reductions in TNFα and interferon gamma and attenuates the augmented loss of dopaminergic neurons caused by the LPS-induced sensitization to dopaminergic degeneration. Nigral injection of LPS in a degenerating SN exacerbates neurodegeneration and accelerates and increases motor signs and shifts microglial activation towards a proinflammatory phenotype with increased IL-1β secretion [95]. Importantly, chronic systemic expression of IL-1 also exacerbates neurodegeneration and causes microglial activation in the SN. It has been found by *in vivo* studies that NO is a downstream molecule of IL-1 action and partially responsible for the exacerbation of dopaminergic neurodegeneration, suggesting that IL-1 exerts its exacerbating effect on degenerating dopaminergic neurons by direct and indirect mechanisms [95].

Part of the challenge to sort out the contributions of individual cytokines to neurodegeneration may be a result of the complex interplay by various positive or negative feedback and feedforward loops among various cytokines, pro- and anti-inflammatory cytokines [64]. Microglial TNFα not only upregulates its own production in an autocrine fashion but also can further increase the surface expression of the neuronal TNFα cell death receptor (TNF p55 receptor) in a paracrine manner, thus exacerbating the LPS-induced neurotoxicity [64]. On the other hand, anti-inflammatory cytokines have been shown to reduce LPS-induced microglial activation and loss of SNpc dopaminergic neurons [147, 148]. The transforming growth factor beta 1 (TGF-β1), one of the most potent endogenous immune modulators of inflammation, exerts significant neuroprotection against LPS induction via its anti-inflammatory properties [147]. TGF-β1 inhibits the translocation of the cytosolic subunit p47phox of the LPS-induced PHOX from the cytosol to the membrane in cultured microglia. The molecular mechanisms of TGF-β1-mediated anti-inflammatory properties works via the inhibition of PHOX activity by preventing the ERK-dependent phosphorylation of Ser345 on PHOX's cytosolic subunit p47phox in microglia, thus reducing oxidase activities induced by LPS [147]. Using the terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) assay and electron microscopy, Arimoto et al. have shown that intranigral injection of LPS causes marked microglial activation and a dose-dependent selective loss of dopaminergic neurons, which is mediated by apoptosis [148]. LPS injection leads to an increase in the mRNA expression of the proapoptotic proteins Bax, Fas, and the proinflammatory cytokines IL-1β, IL-6, and TNFα, while expression of the antiapoptotic gene Bcl-2 is decreased. Infusion of interleukin-10 (IL-10) by osmotic minipump protects against LPS-induced cell death of dopaminergic neurons. A corresponding decrease in the number of activated microglia suggests that the reduction in microglia-mediated release of anti-inflammatory mediators may contribute to the anti-inflammatory effect of IL-10 [148].

NFκB plays a key role in regulating neuroinflammation. Activation of NFκB depends on the phosphorylation of its inhibitor, IkappaB, by the specific IkappaB kinase (IKK) subunit IKK-beta. Compound A, a potent and selective inhibitor of IKK-beta, inhibits the activation of microglia, induced by nigral injection of LPS, and significantly attenuates LPS-induced loss of dopaminergic neurons in the SN [149]. Selective inhibition of NFκB activation affords neuroprotection by suppressing the activity of microglial NADPH oxidase

and by decreasing the production of ROS, and by inhibiting gene transcription of various proinflammatory mediators in microglia via IKK-beta suppression. Microglial activation may involve kinase pathways controlled by mixed lineage kinases (MLKs), a distinct family of mitogen-activated protein kinases, which might contribute to the pathology of PD. A potent MLK inhibitor, CEP-1347, inhibits brain TNFα production induced by intracerebroventricular injection of LPS in mice [150]. Coinjections of LPS with a p38 MAP kinase inhibitor to SN reduces iNOS and caspase-11 mRNA expression and rescues dopaminergic neurons in the SN [132]. Thus, LPS-induced dopaminergic cellular death in SN could be mediated, at least in part, by the p38 signal pathway leading to activation of inducible nitric oxide synthase and caspase-11.

5.4. Cyclo-Oxygenase-2 and Prostaglandin E2. Prostaglandins are potent autocrine and paracrine oxygenated lipid molecules that contribute appreciably to physiologic and pathophysiologic responses in brain and other organs [151]. Emerging data indicate that PGE2 plays a central role in neurodegenerative diseases. PGE2 signaling is mediated by interactions with four distinct G protein-coupled receptors, EP1-4, which are differentially expressed on neuronal and glial cells throughout the CNS, (here something is missing to make a sentence) [151]. EP2 activation has been shown to mediate microglial-induced paracrine neurotoxicity as well as to suppress the internalization of aggregated neurotoxic peptides in microglia [152]. PGE2 is produced at high levels in the injured CNS, where it is generally considered a cytotoxic mediator of inflammation. LPS upregulates the expression of COX-2 and increase the release of PGE2 in cultured microglia [64]. Intracerebral injections of LPS result in a significant upregulation of the striatal and nigral protein expression of COX-2 as well as the activation of microglia [153, 154]. Double labeling using immunohistochemistry identified that activated microglia rather than intact resting microglia are the main intracellular locations of COX-2 expression [64, 155]. *In vivo* pharmacological inhibition of COX-2 activity protects nigral dopaminergic neuronal loss and decreases microglial activation induced by intracerebral LPS injection, supporting the role of COX-2 in the pathogenesis of neuroinflammation-mediated neurodegeneration [153, 155, 156].

A local injection of LPS into the rat SN led to the induction of microsomal prostaglandin E2 synthase (mPGES)-1 in activated microglia [157]. Further *in vitro* and *in vivo* experiments with mPGES-1 knockout mice indicate the necessity of mPGES-1 for microglial PGE2 production. This study has shown that the activation of microglia contributes to PGE2 production through the concerted *de novo* synthesis of mPGES-1 and COX-2 at the sites of inflammation in the brain parenchyma. In contrast to that, a recent *in vitro* study suggests that mPGES-1 expression is not strictly coupled to the expression of COX-2 [158]. Activation of cultured spinal microglia via TLR4 produces PGE2 and causes NO release from these cells, showing that COX-PGE2 pathway is regulated by p38 and iNOS [159]. These findings emphasize that p38 in spinal microglia is a key player among inflammatory mediators, such as PGE2 and NO. *In vitro* experiments also indicate that microglial PGE2 plays an important role in astrocyte proliferation, identifying PGE2 as a key neuroinflammatory molecule that triggers the pathological response related to uncontrollable astrocyte proliferation [160].

5.5. Matrix Metalloproteinase-3. As discussed above (Section 5.2), the release of MMP-3 from apoptotic neurons may play a major role in degenerative human brain disorders, such as PD. The catalytic domain of recombinant MMP-3 induces the generation of TNFα, IL-6, IL-1β, and IL-1 receptor antagonist but not of IL-12 and iNOS, which are readily induced by LPS, in cultured microglia, suggesting that there is a characteristic pattern of microglial cytokine induction by apoptotic neurons [145]. MMP-3 activates the nuclear factor-kappaB (NFκB) pathway, and these microglial responses were totally abolished by preincubation with an MMP-3 inhibitor. MMP-3-mediated microglial activation mostly depends on ERK (extracellular signal-regulated kinase) phosphorylation but not on either JNK (c-Jun N-terminal protein kinase) or p38 activation. MMP-3-activated microglial cells caused apoptosis of neuronal cells in *in vitro* experiments. These results suggest that the distinctive signal of neuronal apoptosis is the release of the active form of MMP-3 that activates microglia and subsequently exacerbates neuronal degeneration [145]. The released active form of MMP-3, as well as the catalytically active recombinant from of MMP-3 leads to superoxide generation in cultured microglia [161]. MMP-3 causes dopaminergic cell death in mesencephalic neuron-glia mixed cultures of wild-type mice, but this is attenuated in the culture of NADPH oxidase subunit null mice (gp91(phox$-/-$)), suggesting that NADPH oxidase mediates the MMP-3-induced microglial production of superoxide and the following dopaminergic cell death. Moreover, in the MPTP model of PD, the nigrostriatal dopaminergic neuronal degeneration, microglial activation, and superoxide generation are largely attenuated in MMP-3$-/-$ mice. These results indicate that MMP-3 released from stressed dopaminergic neurons is responsible for microglial activation and generation of NADPH oxidase-derived superoxide and in turn exacerbates the nigrostriatal dopaminergic neuronal degeneration [161].

αSYN also induces the expression of MMP-3 in cultured microglia from rat [162]. The inhibition of MMP-3 significantly reduces NO and ROS levels and suppresses the expression of TNFα and IL-1β. Inhibition of MMP-3 also suppresses the activities of MAPK and transcription factors, NFκB and AP-1. The specific inhibitor of the protease-activated receptor-1 (PAR-1) and a PAR-1 antagonist significantly suppress cytokine levels, NO, and ROS production in αSYN-treated microglia, indicating that MMP-3 secreted by αSYN-stimulated microglia activate PAR-1 and amplify microglial inflammatory signals in an autocrine or paracrine manner [162]. *In vivo*, LPS injection into the SN of rats increases MMP-3 expression and activation suggesting that MMP-3 may participate in neuroinflammation-induced dopaminergic neurotoxicity [163]. These studies propose that the *in vivo* modulation of MMP-3 expression and

activity may provide the neuroprotection for dopaminergic neurons. Indeed, an antibiotic, doxycycline, shows neuroprotection for the dopaminergic system in a toxin-based model of PD and this appears to derive from antiapoptotic and anti-inflammatory mechanisms involving downregulation of MMP-3 [164].

5.6. Microenvironmental Changes and Intercellular Interactions. The CNS microenvironment plays a significant role in determining the phenotypes of both CNS-resident microglia and CNS-infiltrating macrophages. In this section, we summarize the microenvironmental changes such as astroglial responses, BBB alterations, and a wide range of intercellular interactions in the context of the endotoxin-based PD model.

5.6.1. Reactive Astrocytes and Parkinson's Disease. Astrocytes are the most abundant cell types in the CNS and participate in the local innate immune response triggered by a variety of insults. The role of astrocytes in the pathogenesis of PD is even less well understood than the one of microglia but they are known to secrete both inflammatory and anti-inflammatory molecules [165]. It has been proposed that astrocytes may play dual roles in PD [166]. Similar to microglial activation, star-shaped astrocytes transformed to reactive form have enlarged and thick bodies and respond to various stimuli, which coined the term reactive astrocytes [167]. Reactive astrogliosis is generally mild or moderate and rarely severely pronounced in autopsy specimens from the SN of PD patients [166]. Classic reactive astrocytes are observed in multiple system atrophy, progressive supranuclear palsy, and corticobasal degeneration, but not in PD cases; the extent of reactivity correlates with indices of neurodegeneration and disease stage [168]. Different subpopulations of astrocytes express disease-related proteins such as αSYN, parkin, and p-tau at different levels and in different combinations in different Parkinsonian syndromes but the roles of astrocytes in these conditions are not yet well defined [167, 168].

The role of astrocytes in the development of PD is still unknown and controversial. Astrocytes provide the optimal microenvironment for neuronal function by exerting active control over the cerebral blood flow and by controlling the extracellular concentration of synaptically released neurotransmitters [167]. Generally, astrocytes promote the survival and maintenance of dopaminergic neurons through secretion of various neurotrophic factors in the SN. The decreased levels of astrocyte-derived neurotrophic factors are at least in part responsible for DA neuronal death in PD [167]. Astrocytes become activated and synthesize pro- and anti-inflammatory cytokines, chemokines, antioxidants, neurotrophic factors, and prostanoids during neuroinflammation and neurodegeneration and interact with other immune competent cells. These mediators act as double-edged swords, exerting both detrimental and neuroprotective effects. For example, myeloperoxidase (MPO), a key enzyme in the generation of reactive nitrogen species (RNS), is upregulated in the midbrains of PD patients and MPTP treated mice [169]. This enzyme is localized within reactive astrocytes in MPTP-treated mice, and MPTP neurotoxicity is attenuated by ablation of MPO from the nigrostriatal pathway [167, 169].

5.6.2. Region-Specific Astroglial Responses in the Brain. Degenerative disorders of the brain often occur in a region specific fashion, suggesting differences in the activity and reactivity of innate immune cells. This may make astrocytes likely candidates to be responsible for region-specific incidence rates of neurological and neurodegenerative disorders. Cultured astrocytes from the cortex and midbrain already differ in their capacity and profile of cytokine expression under unstimulated conditions [170]. In response to LPS, both a region specific pattern of upregulation of distinct cytokines, and differences in the extent and time course of activation are observed. Thus, astrocytes reveal a region-specific basal profile of cytokine expression and a selective area specific regulation of cytokines upon LPS-induced inflammation [170]. The densities of astrocytes are much lower in the intact SNpc, compared with the cortex [171]. Furthermore, after LPS injection, damage to endothelial cells and astrocytes and the blood-brain barrier (BBB) permeability are more pronounced in the SNpc [171]. The *in vitro* responses of microglia and astroglia to inflammatory stimuli or environmental toxins also differ. Manganese significantly potentiates LPS-induced release of TNF-α and IL-1β in microglia, but not in astroglia [172]. These agents are more effective in inducing the formation of ROS and NO in microglia than in astroglia.

5.6.3. DJ-1, Oxidative Stress and Astrocytes. Recent findings support the developing view that astrocytic dysfunction, in addition to neuronal dysfunction, may contribute to the progression of a variety of neurodegenerative disorders. Thus, the treatments that support the beneficial aspects of astrocyte function may represent novel approaches targeting astrocytes to promote dopaminergic neurorescue. Although aging enhances the neuroinflammatory response and the alpha-synuclein nitration [73], the antioxidant capacity and glutathione metabolism of astrocytes are preserved from mature adulthood into senescence [173]. Thus, the oxidative stress seen in aging brains is likely due to factors extrinsic to astrocytes, rather than being caused by an impairment of the antioxidative functions of astrocytes. The PARK7 (DJ-1) gene, which has been implicated in some forms of early-onset, autosomal recessive PD, is apparently expressed mainly by the astrocytes in the human brain. Loss-of-function mutations lead to the characteristic selective neurodegeneration of nigrostriatal dopaminergic neurons. In addition to cell-autonomous neuroprotective roles, DJ-1 may act in a transcellular manner, being upregulated in reactive astrocytes in chronic neurodegenerative diseases, for example. In sporadic PD, and many other neurodegenerative diseases, reactive astrocytes overexpress DJ-1 whereas neurons maintain the expression at normal levels [136]. Since DJ-1 has neuroprotective properties and since astrocytes are known to support and protect neurons, DJ-1 overexpression in reactive astrocytes may reflect an attempt to protect themselves and the surrounding neurons against disease progression. Knocking down DJ-1 in astrocytes impairs

astrocyte mediated neuroprotection against rotenone [174]. DJ-1 is a ubiquitous redox responsive and cytoprotective protein with diverse functions. DJ-1 regulates redox signaling kinase pathways and acts as a transcriptional regulator of antioxidative genes. DJ-1 scavenges H_2O_2 by cysteine oxidation in response to oxidative stress and, thus, confers neuroprotection. Therefore, DJ-1 is an important redox-reactive signaling intermediate, controlling oxidative stress upon neuroinflammation and during age-related neurodegenerative processes such as PD [136]. However, the functional basis of neuroprotection elicited by DJ-1 has remained vague. DJ-1 stabilizes erythroid 2-related factor (Nrf2), a master regulator of antioxidant transcriptional responses, by preventing its association with the inhibitor protein Keap1 and by blocking Nrf2's subsequent ubiquitination [175]. Without intact DJ-1, Nrf2 protein is unstable, and transcriptional responses are thereby decreased both basally and after induction [175] though a recent study suggests that activation of the Nrf2 is independent of DJ-1 [176].

5.6.4. Nrf2/ARE Pathway and Parkinson's Disease. The expression of phase II detoxification and antioxidant enzymes is governed by a cis-acting regulatory element named the antioxidant response element (ARE). Nrf2 regulates genes containing the ARE element and is a member of the Cap'n'Collar basic-leucine-zipper family of transcription factors. Following activation, Nrf2 dissociates from Keap1, translocates to the nucleus, and binds to the ARE promoter sequences, as a part of the coordinated induction of a battery of cytoprotective genes including antioxidants and anti-inflammatory genes [177]. ARE-regulated genes are preferentially activated in astrocytes, which consequently have more efficient detoxification and antioxidant defense mechanisms than neurons. Astrocytes closely interact with neurons to provide structural, metabolic, and trophic support, as well as actively participating in the modulation of neuronal excitability and neurotransmission [177]. Therefore, alterations in astroglial function can modulate the interaction with surrounding cells such as neurons and microglia. Activation of Nrf2 in astrocytes protects neurons from a wide array of insults in different *in vitro* and *in vivo* paradigms, confirming the role of astrocytes in determining the vulnerability of neurons to deleterious stimuli [177]. Nrf2 has been shown to be important for protection against oxidative stress and cell death in toxin-based models of PD [177–181]. These findings remain to be confirmed in endotoxin-based models. Genetic data suggest that variation in Nrf2 gene NFE2L2 modifies the PD process, which provides another link between oxidative stress and neurodegeneration [182]. Nrf2 activating agents such as synthetic triterpenoids and sulforaphane are potential therapeutic targets for the prevention of neurodegeneration in PD [183–185].

5.6.5. Nrf2/ARE Pathway and Microglial Activation. The deficiency of Nrf2 results in an exacerbated inflammatory response and in microglial activation of the expression of the neurotoxin MPTP whereas inducers of Nrf2 downmodulate neuroinflammation [181]. Nrf2-deficient mice exhibit more astrogliosis and microgliosis, as determined by an increase in mRNA and protein expression levels for GFAP and F4/80, respectively, than wild-type mice. Inflammation markers, characteristic of classical microglial activation like COX-2, iNOS, IL-6, and TNF-alpha, are also increased. At the same time, anti-inflammatory markers, attributable to alternative microglial activation, such as FIZZ-1, YM-1, Arginase-1, and IL-4 are decreased [181]. These results demonstrate a role of Nrf2 in tuning the balance between classical and alternative microglial activation. The restoration of the redox balance may be a determinant in driving microglia back to the resting state. ROS generated by microglia could help to eliminate pathogens in the extracellular milieu and also to act on the microglia itself, altering the intracellular redox balance and functioning as a second messengers in the induction of proinflammatory genes. The modulation of microglial activation is a matter closely correlated with control of oxidative stress in this cell type and is crucial to restore its inactive state and modulate the inflammation in neurologic diseases [186]. Nrf2 is essential for the regulation of NADPH oxidase-dependent ROS-mediated TLR4 activation in macrophages [187]. Nrf2 activation by sulforaphane inhibits the inflammatory response to LPS in cultured rodent microglia [185]. These findings remain to be tested in the context of *in vivo* endotoxin-based PD models. Interestingly, LPS by itself is able to activate the cell's defense against oxidative and electrophilic stress, activating Nrf2 [185]. This mechanism may be a mediator of LPS preconditioning or endotoxin tolerance, a phenomenon which by prior exposure of innate immune cells like monocytes/macrophages to minute amounts of endotoxin causes them to become refractory to subsequent endotoxin challenges [188]. In contrast to the well-known protective effect of this phenomenon, in acute ischemic conditions, only one *in vitro* study has reported this benefit in dopaminergic neurotoxicity [189]. Further understanding the underlying mechanism of LPS preconditioning may open a new window for the treatment of PD.

Astroglial cells are also involved in the microglial modulation by Nrf2 [177]. These cells are known to play an important role in antioxidant defense and in modulating microglial activity in the CNS [165, 166]. Recently, astrocytes have been found to regulate excessive inflammation via induction of the microglial hemooxygenase-1 (HO-1) expression *in vitro* [190]. While pharmacological or genetic intervention on Nrf2 may provide a neuroprotective benefit, HO-1 does not protect or enhance the sensitivity to neuronal death in the MPTP model [191]. These results support the idea that the modulation of a master transcription factor may be a better strategy than targeting individual genes.

5.6.6. Blood-Brain Barrier Dysfunction and Peripheral Immune Cell Infiltration. The brain demands an adequate blood supply for the regulation of neuronal and synaptic function. To maintain concentrations of ions within narrow ranges as well as the adequate levels of metabolic substrates in various brain regions, neural milieu are strictly separated from circulatory spaces through BBB formation [167]. These unique biological structures are comprised of neurovascular

units such as brain capillary endothelial cells, pericytes, neurons, and astrocyte end-feet. Endothelial cells tightly connect at junctional complexes such as adherens junctions, tight junctions, and gap junctions confer low paracellular permeability. Pericytes and astrocytes regulate hemodynamic neurovascular coupling, microvascular permeability, matrix interactions, neurotransmitter inactivation, neurotrophic coupling, and angiogenic as well as neurogenic coupling through close proximity with neurons [167, 192]. Although there is no clear evidence as to whether these altered neurovascular circumstances are responsible for the loss of dopaminergic neurons in PD, several studies on PD patients and animal models suggest a pathogenic linkage between BBB disruption and dopaminergic neuronal death [167]. PET and histological studies on PD patients revealed BBB dysfunction in the midbrain of PD patients [193]. In addition, increased BBB permeability has been observed in the MPTP and the LPS models for PD [194]. These studies suggest that the disruption of the BBB has a relationship with neuronal cell death and neuroinflammation in PD [167]. There is also a direct correlation between the location of IgG immunoreactivity-a, a marker for disruption of neurodegenerative processes, including the death of nigral dopaminergic cells and reactive astrocytes. A precise spatial correlation also exists between disruption of the BBB and 3-nitrotyrosine immunoreactivity [194]. LPS-activated microglia can induce the dysfunction of the BBB in an *in vitro* coculture system with rat brain microvascular endothelial cells and microglia [195]. In the presence of LPS-activated microglia, tight junction proteins are fragmented, and barrier disintegrity and dysfunction induced by LPS-activated microglia are blocked by an NADPH oxidase inhibition, suggesting that LPS activates microglia to induce dysfunction of the BBB by producing ROS through NADPH oxidase.

Recent studies have shown that the dysfunction of the BBB combined with the infiltration of peripheral immune cells plays an important role in the degeneration of dopaminergic neurons [167]. However, these molecular and cellular changes are not specific to the PD, since they are also implicated in the pathogenesis of other neurodegenerative diseases [196]. The neuroinflammation may contribute to the infiltration of peripheral immune cells and leakage of the BBB into the SN. Various peripheral immune cells, such as T-cells, B-cells, microphages, and leukocytes infiltrate into the SN region in the LPS and MPTP models [167, 171, 197]. CD11b and MPO double-positive neutrophils infiltrate the SNpc following LPS injection [197]. MPO(+) neutrophils observed in SNpc express iNOS, IL-1β, COX-2, and monocyte chemoattractant protein-1 (MCP-1). In intact rodent brain, the densities of microglia are similar in SNpc and cortex [197]. In addition, the densities of astrocytes are much lower in the intact SNpc, compared with the cortex. However, LPS injection induces microgliosis and causes neutrophil infiltration into the SNpc, but not into the cortex [171]. The extent of neutrophil infiltration appears to be correlated with neuronal damage. The loss of neurons in the SNpc is significantly reduced in neutropenic rats versus normal rats following LPS injection. Furthermore, after LPS injection, damage to endothelial cells and astrocytes and increased BBB permeability are more pronounced in the SNpc. Excessive neutrophil infiltration, lower astrocyte density, and higher BBB permeability following LPS exposure contributes to severe inflammation and neuronal death in the SNpc compared with the cortex [171].

The links between T-cell immunity and the nigrostriatal neurodegeneration are supported by laboratory, animal model, and human pathologic investigations [198]. The presence of T-lymphocytes in the midbrain of PD patients suggests that the potential role of infiltrated peripheral cells is a factor of the PD pathogenesis [199]. Recently, Brochard et al. have reported that numerous CD4 and CD8 positive cells are detectable in postmortem PD patients [200]. The infiltration of CD4+ lymphocytes into the brain also contributes to the neurodegeneration in the MPTP model for PD [200]. Specifically, invading T-lymphocytes contribute to neuronal cell death via the Fas/FasL cell death pathway, implicating the emerging role of the adaptive immune system in the pathogenesis of PD [201].

The adoptive transfer of CD3-activated CD4+CD25+ regulatory T-cells (Tregs) is known to suppress immune activation and maintain immune homeostasis and tolerance. In MPTP-treated mice, it protects the nigrostriatal system from degeneration through suppression of microglial oxidative stress and inflammation [202]. Tregs also attenuates Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in the MPTP model [203]. In addition, these cells suppress nitrated αSYN-induced microglial ROS production and NFκB activation supporting the importance of adaptive immunity in the regulation of PD-associated microglial inflammation [204]. Taken together, these studies provide a rationale for future immunization strategies in PD [198].

Accumulating evidence suggests that the penetration of immune cells into the brain plays an important role in the degeneration of dopaminergic neurons in PD. Further understanding of the cellular and molecular mechanisms responsible for trafficking of immune cells from the periphery into the diseased CNS may be the key to targeting these cells for therapeutic intervention in PD [196].

In addition to glia-neuron crosstalk, multiple cell-to-cell interactions and immune regulations, critical for neuronal homeostasis, also influence immune responses [198, 205]. Microglia can be activated by MCP-1, which is expressed by dopaminergic neurons and can interact with its receptor CCR2 on microglial cells. The neuroimmune regulatory proteins CD47 and CD200 inhibit macrophage and microglia activation through binding to their receptors SIRPalpha and CD200R, expressed on phagocytes [206]. Upon stress, nigral dopaminergic neurons secrete MMP-3 and α-SYN, which activates microglial and astroglial cells [145, 207]. As disease progresses, secretions from α-SYN-activated microglia can engage neighboring glia cells in a cycle of autocrine and paracrine amplification of neurotoxic immune products. Astrocytes differentially regulate neutrophil functions through direct or indirect interactions between the two cell types [208]. Many of these established interactions between different cell types involved in neuroinflammation have been demonstrated *in vitro* and remain to be confirmed *in vivo*.

Dissecting the molecular determinants of complex interplay between CNS cells and immune cells in the context of the endotoxin-based PD model will give the possibility to test novel therapeutic strategies to promote restoration of injured nigrostriatal dopaminergic neurons.

6. Therapeutic Approaches

The endotoxin-induced neuroinflammation model for PD is a purely inflammation-driven model. However, all clinical and pathological features of PD can be observed in this model. Therefore, the LPS-induced model can be used to search for novel treatment strategies for the therapy of PD. In this section, we summarize known neuroprotective molecules, which have been tested using the LPS-induced PD models.

COX-2 is a rate-limiting enzyme in prostaglandin synthesis. Experimental and epidemiological evidence supports the protective role of COX-2 inhibition in PD. COX-2 is upregulated in SN both in the PD and in the MPTP model [209]. Pharmacological inhibition of COX-2 or the knockout of the COX-2 gene provides resistance to MPTP *in vivo* [209, 210] and to 6-OHDA-induced dopaminergic toxicity *in vitro* [211]. There is epidemiological evidence that the use of some NSAIDs lowers the incidence of PD [212]. On the other hand, according to meta-analyses of NSAID studies in PD, ibuprofen shows a slight protection against PD whereas aspirin and acetaminophen did not show any protective effects [213, 214]. Hunter et al. used the COX-2 inhibitor Celecoxib (Celebrex) in LPS-induced PD animal model for the first time. They were able to show that Celecoxib protects dopaminergic neurons by decreasing inflammation and by restoring mitochondrial function in the intrastriatal LPS-induced PD model [153]. Using the intranigral LPS rat model, Sui et al. [155] have shown that another COX-2 inhibitor, meloxicam, diminishes the activation of OX-42 positive microglia and reduces the loss of dopaminergic neurons in the SNpc. Clinical studies suggest that inhibition of COX-2 may cause side effects such as trombogenic cardiovascular diseases [156, 215]. In order to avoid potential side effects of COX-2 inhibition, new drugs have been targeted for dual inhibition of COX-2 and lipoxygenase (LOX) [156]. Dual inhibitor of COX-2 and 5-LOX has been shown to lower gastrointestinal side effects. Moreover, combination of the two inhibitors achieves a more potent neuroprotection than usage of single inhibitors [216]. Li et al. tested the dopaminergic neuroprotective effect of COX, LOX, and the combination of COX and LOX inhibitors in the intrastriatal LPS-induced animal model for PD. They found that the dual COX and LOX inhibitor, phenidone, is better than COX or LOX inhibitors alone for suppressing LPS-induced neurotoxicity [156].

Dexamethasone is a potent anti-inflammatory drug that has been tested in the intranigral LPS-induced PD model [67, 133]. These studies have shown that dopaminergic degeneration and microglial activation induced by LPS can be prevented by administration of dexamethasone [67, 133]. Dexamethasone also decreases the exacerbating effect of LPS during neurodegeneration induced by 6-OHDA [95].

Experimental and epidemiological evidence supports the protective role of nicotine in PD. Epidemiological studies have confirmed that there is an inverse correlation between cigarette smoking and the incidence of PD [217]. *In vitro* nicotine pretreatment inhibits LPS-induced TNF-α release in murine-derived microglial cells via the α-7 nicotinic receptor [218]. These results suggest that nicotine could protect dopaminergic neurons in the animal model of PD. Indeed, Park et al. have shown that nicotine significantly decreases the release of TNFα and the dopaminergic neuronal loss induced by LPS stimulation. Both effects were blocked by α7-nicotinic acetylcholine receptor blockers [219].

Peroxisome proliferators activated receptor (PPAR-γ) is a nuclear receptor that regulates transcription of various genes. It has been shown that the PPAR-γ agonist inhibits cytokine secretion in microglia and macrophage-like cells [220]. Hunter et al. have shown that a PPAR-γ agonist, pioglitazone, provides neuroprotection by decreasing inflammation and restoring mitochondrial function. Pioglitazone administration partially reduces the LPS-induced striatal dopamine loss and the TH-positive cell loss in the SN [153].

Minocycline is a semisynthetic tetracycline that exerts anti-inflammatory activities [221]. Minocycline significantly reduces the SN microglial activation induced by intranigral LPS administration [194]. Minocycline prevents the LPS-induced increase of mRNA levels of proinflammatory cytokines and diminishes the production of peroxynitrites [194].

Naloxone, an opioid receptor competitive antagonist, has been found to reduce microglial activation-mediated DA neurodegeneration in mouse cortical neuron-glia cocultures [64]. Systemic infusion of naloxone protects dopaminergic neurons against inflammation-mediated degeneration and decreases microglial activation *in vivo* through inactivation of NADPH oxidase [139, 222].

The neuroprotective effects of statins in CNS disorders such as experimental autoimmune encephalomyelitis, stroke, and Alzheimer's disease have been previously described [223–225]. Selley has shown that oral administration of simvastatin attenuates the depletion of dopamine DOPAC and HVA inhibits the formation of 3-nitrotyrosine and the production of TNFα in mice treated with MPTP [226]. Simvastatin has also been tested in the intranigral LPS-induced PD [227] and the LPS perfusion model [228]. Simvastatin prevents the loss of dopaminergic neurons and astrocytes induced by LPS in both models [227, 228]. Simvastatin increases BDNF expression [228], which may support neuronal and astroglial survival.

Osteopontin (OPN) is a glycosylated phosphoprotein that has first been identified in 1986 in osteoblasts [229]. OPN is constitutively expressed in most tissues, including the brain [208]. Iczkiewicz et al. have shown that OPN is constitutively present in dopaminergic neurons, in the SN, and that its expression is decreased in the MPTP model of PD and in patients with PD [230]. It has been reported that the intranigral injection of LPS enhances expression of OPN [231]. These results suggest that OPN may have a regulatory role in neuroinflammation. One peptide fragment of OPN contains the arginine-glycine-aspartic acid (RGD) domain

that has been associated with the neuroprotective effects of OPN [232]. Iczkiewicz et al. have tested RGD containing peptide fragments of OPN in the LPS-induced PD model. They found that the RGD containing peptide fragment of OPN protects against LPS-induced TH positive cell loss and alters gliosis in the rat SN [233].

Urocortin is a neuroprotective agent that is structurally related to the corticotrophin releasing factor (CRF) [234–236]. Abuirmeileh et al. have used urocortin for the treatment of the LPS-induced PD model. They have shown that urocortin reduces nigrostriatal damage induced by LPS and that this effect of urocortin is mediated by CRF_1 receptors [237–239].

7. Conclusion

Parkinson's disease (PD) is the second most common neurodegenerative disease with increasing incidence worldwide. Although the pathogenesis of PD remains elusive, accumulating evidence from many studies on animal models and patients shows that the pivotal role of microglial activation along with neuroinflammatory processes contribute to the initiation and progression of the nigrostriatal dopaminergic neurodegeneration in PD. In addition to that, recent studies have proposed that the BBB dysfunction combined with the infiltration of peripheral immune cells into the CNS plays an essential role in the degeneration of nigral dopaminergic neurons. Thus, using a purely inflammatory experimental model induced by the administration of the bacterial endotoxin, LPS, provides a valuable tool for the *in vivo* modeling of the characteristics of progressive dopaminergic neurodegeneration associated with neuroinflammation. Except for the acute direct administration of LPS to the nigral region, other modified forms of the model, including the prenatal one, realistically simulate the slow and progressive dopaminergic neuronal loss and permanent neuroinflammation. Furthermore, the combination of endotoxin-based PD models with genetic and toxin-based models is fruitful for the delineation of the complex interactions among the environmental and genetic factors and inflammatory processes involved in PD. Many experimental variables including sex, age, and strain of the animals have the potential to significantly perturb the functional and pathologic outcomes. These methodological issues should be considered in respect to the studies.

Several novel techniques, such as *in vivo* imaging of microglial activation, are waiting to be applied in the endotoxin-based model of PD. Molecular studies from the domains of transcriptomics, proteomics, and microRNomics will be valuable to gain in potential diagnostic markers for the disease [240]. Since the inflammatory responses precede the neurodegeneration and the motor dysfunctions, alterations of the immune parameters, both in CSF and blood, are likely to be useful as early diagnostic markers. The major challenge in this area is the enhancement of the specificity and sensitivity of the potential markers. Despite intensive research, the mechanisms of neuroinflammation-mediated nigral neurodegeneration are poorly understood. Whether neuroinflammation is a consequence or a cause of nigral neuronal loss is still unknown. Neuroinflammation seems to be a trigger of the initiation of neurodegeneration and progressive neurodegeneration continuously aggravates chronic neuroinflammatory processes. In this context, the stimulation of TLR4 by endogenous ligands released by injured dopaminergic neurons may contribute to this vicious circle [241].

In vivo imaging and molecular studies will also extend our understanding of the complex interplay between CNS and immune cells. Especially, the novel links between neuroinflammatory processes, oxidative stress, and Nrf2/ARE pathways that are mainly based on data from toxin-based models of PD should be confirmed by the endotoxin based model.

Based on the recent data, adaptive immune responses along with innate immunity are important mediators of neuroinflammation-mediated dopaminergic neurodegeneration. Recent evidence suggests that the importance of nonautonomous pathological mechanisms are involved in PD, which are mostly mediated by activated microglia and peripheral immune cells. Thus, the harnessing of the immune system by immunomodulating drugs or by immunisation aiming at the downregulation of immune responses remains promising future therapeutic options. Immune parameters will also be indispensable for the monitoring of therapeutic responses.

Abbreviations

MPTP:	1-Methyl-4-Phenyl-1,2,3,6-tetrahydropyridine
DOPAC:	3,4-dihydroxyphenylacetic acid
6-OHDA:	6-hydroxydopamine
ARE:	Antioxidant response element
BBB:	Blood-brain barrier
CNS:	Central nervous system
CSF:	Cerebrospinal fluid
COX-2:	Cyclo-oxygenase-2
EGF:	Epidermal growth factor
ERK:	Extracellular signal-regulated kinase
DA:	Dopamine
GFAP:	Glial fibrillary acidic protein
HO-1:	Hemooxygenase-1
HPLC:	High-performance liquid chromatography
HVA:	Homovanillic acid
5-HT:	5-hydroxytryptamine
IKK:	IkappaB kinase
IRAK:	IL-1 receptor-associated kinase
iNOS:	Inducible nitric oxide synthase
IFN-β:	Interferon-beta
IL-2:	Interleukin-2
IL-6:	Interleukin-6
IL-10:	Interleukin-10
IL-1β:	Interleukin 1β
JNK:	c-Jun N-terminal protein kinase
LPS:	Lipopolysaccharide
MMP-3:	Matrix metalloproteinase-3
MPP^+:	1-methyl-4-phenylpyridinium
MA:	Methamphetamine

MLKs: Mixed lineage kinases
TFAM: Mitochondrial transcription factor A
MD-2: Myeloid differentiation factor 2
Mal or TIRAP: MyD88 adaptor-like protein
PHOX: NADPH oxidase
L-NAME: N(G)-nitro-L-arginine methyl ester
NADPH: Nicotinamide adenine dinucleotide phosphate
NO: Nitric oxide
Nrf2: Nuclear factor erythroid 2-related factor
NFκB: Nuclear factor-kappaB
PD: Parkinson's disease
PPAR-γ: Peroxisome proliferator-activated receptor
PGE2: Prostaglandin E2
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
Tregs: Regulatory T cells
SN: Substantia nigra
SNpc: Substantia nigra pars compacta
SNpr: Substantia nigra pars reticulata
TUNEL: Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling
TH+: Tyrosine hydroxylase-positive
TLRs: Toll-like receptors
TIR: Toll/interleukin-1 receptor
TRIF: Toll/IL-1 receptor containing adaptor inducing IFN-β
TGFα: Transforming growth factor-alpha
TGF-β1: Transforming growth factor-beta 1
TBK: TRIF-binding kinase
TNFα: Tumor necrosis factor-alpha
TH: Tyrosine hydroxylase
VTA: Ventral tegmental area.

Conflict of Interest Disclosure

The authors declare no competing financial interests.

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