Minireview

Parkinson's disease and enhanced inflammatory response

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Abstract

Parkinson's disease (PD) is the first and second most prevalent motor and neurodegenerative disease, respectively. The clinical symptoms of PD result from a loss of midbrain dopaminergic (DA) neurons. However, the molecular cause of DA neuron loss remains elusive. Mounting evidence implicates enhanced inflammatory response in the development and progression of PD pathology. This review examines current research connecting PD and inflammatory response.

Keywords: Parkinson's disease, neuroinflammation, inflammation, disease models

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Introduction

In the USA, with a prevalence of 1% in people over 60 and over 4% by age 85, Parkinson's disease (PD) is the most prevalent motor disease.[1] PD motor features are the result of decreased dopamine levels in the striatum due to progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SN) region of the midbrain. Currently, there are only symptomatic treatments for PD and no therapies exist for addressing the underlying neurodegeneration.[2]

Over 90% of PD cases do not exhibit a heritable pattern of transmittance suggesting a substantial environmental contribution to this disease.[1,3] Therefore, great effort has been made to identify environmental factors involved in PD. In this review, we will explore how inappropriate inflammatory response by the immune system, which can be stimulated by environmental toxins such as paraquat, bacterial infection, head trauma, or other insults such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), may manifest in DA neuron loss. In addition, rare monogenic causes of PD that may enhance inflammatory response or damage are discussed.

Inflammation and PD

There exists substantial evidence indicating a close association between enhanced inflammatory response and PD. A link between inflammation and PD was first provided in a postmortem study by McGeer and colleagues in 1988 where the SN of PD patients displayed activated microglia.[4] Subsequently, numerous clinical studies have confirmed this association by reporting increased microglial activation and elevated pro-inflammatory cytokines in postmortem brains and CSF.[5,6] Moreover, CD4+ and CD8+ T cell extravasation into the SN has been observed.[6] These findings are backed by epidemiological evidence indicating a reduced occurrence of PD with persistent use of non-steroidal anti-inflammatory drugs (NSAIDs)[7–10] although this contention remains controversial.[11,12] Yet, these reports indicate that early anti-inflammatory intervention might be a useful strategy to prevent PD. Additionally, head injury, which may result in a neuroinflammatory response, has also been described to boost PD risk.[13] Similarly, allergic rhinitis, commonly associated with nasal airway inflammation, exhibits linkage to an increased incidence of PD.[14] There also exist case studies that appear to exhibit linkage of PD and inflammatory response.[15,16] For example, Mark Hallet's group described a case of Parkinsonism in a middle-aged man following an insect sting.[15] Treatment with the immunosuppressant azathioprine, plasmapheresis, and immunoglobulin therapy appeared to halt and perhaps partially reverse the course of the disease. Preclinical PD models also suggest that inflammation is a driving force in DA neuron loss. For example, chronic intraperitoneal or intranasal injection of bacterial lipopolysaccharide (LPS) elicits a systemic immune response and leads to DA neuron loss and PD pathology in mice.[17–19] These purely inflammatory models of PD suggest peripheral/systemic inflammatory stress can manifest in DA neuron loss likely through infiltration of peripheral leukocytes. Evidence also suggests that PD modeling neurotoxins such as MPTP facilitate DA neuron loss at least in part by induction of inflammatory response.[20] Additionally, in mice, enhanced inflammation has been shown to recapitulate α-synuclein aggregation and oxidation in affected neurons.[18,21] A large clinical study also reported that discontinuation of lipophilic statins correlated with an elevated incidence of PD.[76] Another clinical study reports a

23% reduction in the incidence of PD among statin users suggesting that this class of drugs might not only be beneficial for coronary artery disease but also against PD.[77] Thus, intensive efforts have been focused on elucidating inflammatory contributions and mechanisms of PD pathology.

Inflammation, oxidative damage, and PD

Inflammation is a widely encompassing term referring to the complex biological response of the immune system to tissue damage, toxic proteins, infection, or abnormal molecular signals. Central nervous system (CNS) immune response is thought to be orchestrated principally by microglia with lesser contributions by neurons, oligodendrocytes, and astrocytes.[22] However, it should be noted that leukocytes have been reported to cross from the periphery to initiate or participate in CNS immune response activities.[23] In healthy brains, microglia generally exhibit a resting phenotype and perform a scavenging role by removing debris and waste material from the parenchyma yet, following infection, tissue damage, accumulation of toxic protein, or other triggering signal, microglia become activated. Activated microglia are the major source of reactive oxygen species (ROS) during neuroinflammatory reaction through intracellular peroxidases, cell surface NADPH oxidase activity, and oxidative processes in mitochondria.[24] Of importance to PD, DA neurons are particularly susceptible to ROS-mediated death. This is believed in part due to an elevated level of oxidative stress produced from reactive quinone by auto-oxidation of dopamine.[25] Additionally, it has been reported that DA neurons possess depressed amounts of antioxidant enzymes.[24] At high levels, ROS can damage or inactivate proteins leading to aberrant intracellular signaling, cellular degeneration, and death. Supporting this notion are reports citing protective effects of several classes of antioxidants against rodent models of PD.[26] Activated microglia also produce pro-inflammatory cytokines which can cause neurotoxicity as well as chemokines that recruit leukocytes to the CNS exacerbating inflammatory response.[27] Transient activation is necessary for local tissue repair while sustained activation of microglia can lead to neurodegenerative pathology. It has been proposed that a self-perpetuating cycle of microglial activation results from discharge of chemoattractants by DA neurons undergoing death leading to further activation of microglia and neuroinflammation.[28] If true, then inflammation need not be the trigger for the initial neuronal loss but may serve to perpetuate and enhance neurodegeneration once underway.

Bacterial LPS-based inflammatory response modeling of PD

To date, it is unknown whether inflammatory response is causal for or a consequence of human PD. Mounting epidemiological evidence indicates traumatic head injury increases risk for PD suggesting an inflammatory event may precede neuronal loss.[13] Perhaps the most compelling evidence for inflammatory response-triggered PD was gained through the use of preclinical animal models. In order to specifically address inflammatory response-driven DA neuron loss, investigators have utilized bacterial LPS to mimic bacterial infection in rodents using various methods of delivery. LPS engagement of Toll-Like Receptor 4 on immune cells elicits pro-inflammatory cytokine and chemokine secretion that initiates an innate inflammatory response.[29,30]

Intranigral, intrastriatal, and intraventricular LPS

In 1998, Castaño *et al.* reported that intranigral injection of LPS led to SN DA neuron loss at 15 and 21 days postinjection.[31] Subsequent studies found that intranigral LPS injection resulted in microglial activation, proinflammatory cytokine production that include IL-6, IL-1 β, TNF-α, and NO.[32–34] Interestingly, a solitary injection of LPS intranigrally caused a specific DA neuron loss with no observed effect on serotoninergic or GABAergic neurons and this loss was detected one year following injection.[32] The specificity to DA neuron loss could be attributed to an increased susceptibility of these neurons to oxidative damage. Iravani *et al.* reported that 24 h following intranigral LPS injection astrogliosis and heightened expression of proinflammatory cytokines that was correlated with elevated levels of glial derived neurotrophic factor (GDNF) was observed.[35] The authors propose that GDNF may serve as a protective factor in a proinflammatory environment. This notion has been supported by subsequent studies showing protective effects of GDNF on DA neurons in oxidizing environments.[36,37] These results suggest that robust inflammatory response induces specific loss of SN DA neurons and that intrinsic mechanisms exist to mitigate neuronal loss during these events.

The axons of SN DA neurons terminate into the striatum where these neurons release dopamine as a neurotransmitter. Several groups have injected this region with LPS and observed decreases in SN DA neuron soma, reduced striatal dopamine, and production of proinflammatory cytokines.[38–40] Furthermore, this procedure is reported to result in buildup of alpha-synuclein in the soma of DA neurons and deficits in motor performance.[38–40] This indicates that inflammatory response in the striatum causes insult that is transmitted to the neuronal soma in the SN or that the inflammatory reaction is not confined to the striatum and encompasses the SN.

A single intraventricular LPS injection was shown to induce an inflammatory response that depletes 22 and 40% of SN DA neurons at 24 and 48 weeks following injection, respectively.[41] The authors also show that activated microglia persist in LPS-injected mice at 48 weeks postinjection. This suggests that a single neuroinflammatory event in a region distant to the SN can transmit insult likely through cerebrospinal fluid and have long-lasting effects.

Systemic LPS

A report by Qin *et al.* reported that intraperitoneal LPS administration resulted in microglial activation and caused SN DA neuron loss at seven and nine months postinjection.[19] Evidence was presented that increased TNF-α

production was required for this neurotoxicity. This effect is likely not mediated by LPS entering the CNS since work has shown that LPS is not transported across and also does not affect blood–brain barrier (BBB) permeability.[42] One possibility is that LPS induces cytokine production in the periphery that mediates neuroinflammation. Supporting this notion are studies showing that IL-1 and TNF-α can traverse the BBB.[43,44] Frank-Cannon *et al.* performed an extensive examination of intraperitoneal injection of LPS and found that wild-type mice, and more robustly mice lacking a PD-gene (*parkin*), showed specific loss of SN DA neurons after six months (trending but not significant at three months) of twice/week injections.[17] Increased production of proinflammatory cytokine (TNF-α) and antioxidant gene mRNA (*nrf2* and *inos*) in the midbrain was reported at six months posttreatment. This paradigm has been utilized by other groups reporting similar loss of DA neurons in the SN.[45] Current data indicate that the relationship between peripheral and CNS inflammatory response and DA neuron loss is closely linked.

Intranasal LPS

He *et al.* describe an intriguing PD model that involves unilateral intranasal administration of LPS every other day for five months.[18] The authors report that this regimen induces SN DA neuron loss, striatal dopamine depletion, and α-synuclein aggregation in the SN. Since the loss of smell has been reported to precede motor symptoms of PD and the reported observation that synucleinopathy occurs in the olfactory bulb before the SN, it has been hypothesized that PD may be a primary disorder of olfaction.[18,46] Consistent with this idea, a clinical case–control study found an increased coincidence of PD and allergic rhinitis.[14]

Intraventricular cytokines

LPS treatment requires the production of cytokines to affect an inflammatory response. It is perhaps not surprising that administration of proinflammatory cytokines can elicit similar effects to LPS on midbrain DA neurons. Chakrabarty *et al.* performed intraventricular injection of mouse pups with rAAV2/1 carrying an IFN-gamma overexpression construct.[47] Degeneration of the nigrostriatal system was not evident at three months of age. However, by five months of age virtually all DA neuron labeling had disappeared. This is further evidence that generalized inflammation within the brain leads to specific loss of DA neurons in the SN.

Neuroinflammation in PD neurotoxin models

Epidemiological evidence suggests there is a significant environmental component to sporadic PD. As a result, efforts to identify environmental PD toxins have yielded several chemicals that cause Parkinsonism in rodents and primates. Considerable evidence suggests that these compounds work by inducing oxidative damage in target cells. Research has also found that these PD toxins exert a robust inflammatory response and that anti-inflammatory therapies are protective in animal models.[20] This supports the theory that DA neurons are lost in PD due to an especially high susceptibility to oxidative damage which may be the result of, at least in part, inflammatory response. We will now discuss three of these toxins in the context of neuroinflammation.

MPTP and neuroinflammation

The neurotoxin MPTP was identified as the causative agent in cases of human Parkinsonism.[48] MPTP is a lipophilic agent that can traverse the BBB.[20,49] Once inside the brain parenchyma, MPTP may be processed to MPP+, the active toxin, by monoamine oxidase-B (MAOB) activity in glial cells. MPP+ utilizes the dopamine transporter to selectively target DA neurons where MPP+ inhibits mitochondrial complex I causing increased ROS and neuron demise.[20,50] However, the mechanism of death may be more complicated in light of evidence implicating inflammatory response in MPTP toxicity. MPTP treatment of mice leads to loss of DA neuron cell bodies in the SN and axon terminals in the striatum as well as reduced dopamine levels. It should be noted that some key features of human PD are not observed in this model such as α-synuclein aggregation and characteristic motor symptoms.[51] MPTP also exhibits a high degree of variability of sensitivity among strains of mice and is ineffective in rats.[52]

MPTP induces an inflammatory response that facilitates neurodegeneration. The height of glial response occurs before DA neuron loss and includes microglial activation as well as IL-6, IFN-γ, and TNF-α proinflammatory cytokine production.[53,54] Supporting these findings are reports citing resistance to MPTP-induced DA neuron loss by mice lacking TNF or IFN-γ signaling.[55,56] Similar findings were reported in primates where TNF-α and IFN-γ expression in the brain was increased upon MPTP treatment.[57] Additional evidence implicates the adaptive arm of immune response in MPTP-mediated neurodegeneration. Brochard *et al.* reported that T-cells (CD4+ as well as CD8+ lymphocytes) infiltrate the SN of human PD patients.[23] The authors also found T cell infiltration in the SN of mice treated with MPTP and that DA neuron loss was attenuated in T cell deficient mice suggesting a proinflammatory role for T cells (CD 4+) in MPTP toxicity.

Success of anti-inflammatory therapy in MPTP laboratory models is perhaps the strongest evidence for involvement of inflammatory processes in MPTP toxicity. Nomura *et al.* reported that pharmacological or genetic (monoacylglycerol lipase −/−) inhibition of inflammatory prostaglandin synthesis resulted in complete protection against MPTP neurodegeneration.[58] Minocycline is an antibiotic that also exerts anti-inflammatory effects on microglia likely through blockage of TNF-α signaling.[59] This compound has exhibited protective effects against MPTP for DA neurons in mice.[60] NSAIDs such as Ibuprofen have been shown to be protective of neurons undergoing the stress of MPTP.[61] The flavonoid pycnogenol, in mice models, created a marked decrease in neuroinflammation, neurodegeneration, and behavioral impairments caused by MPTP.[62] Silymarin, an extract of

the herb milk thistle, has demonstrated protection against inflammation by blocking the loss of dopamine and shows protection against MPTP neurotoxicity.[63] Carnosine, a peptide produced in the brain, has been shown to reduce inflammatory cytokine production and oxidative stress caused by MPTP.[64]

Paraquat and neuroinflammation

Paraquat was originally assessed as a PD toxin due to its structural similarity to MPP+.[65] However, while both toxins induce oxidative stress, the mechanisms for ROS generation vary.[65] Unlike MPP+, the mechanism for the selectivity of SN DA neuron loss from paraquat is unknown.[65]

Despite the strong oxidizing power of paraquat, evidence presented by Purisai *et al.* indicates that microglial inhibitor, minocycline, protects mice against paraquat.[66] This suggests that the resulting inflammatory response is the chief contributor to neurodegeneration in paraquat-treated mice. Interestingly, the authors also showed that pretreating mice with a systemic bolus of LPS preceding a lone dose of paraquat resulted in a neurodegeneration not produced by a single paraquat dose alone suggesting that primed microglia may mediate this pathology. Proinflammatory cytokines are also associated with paraquat use in mice. A group reported that midbrain TNF-α levels were increased following paraquat administration.[67] This group also provided evidence that paraquat mediates DA neuron loss through an oxidative mechanism because anti-oxidant therapy protects mice. These findings suggest that an initial oxidative insult by paraquat triggers an inflammatory response that facilitates DA neuron loss.

6-hydroxydopamine (6-OHDA) and neuroinflammation

An early model of PD utilized 6-OHDA.[68] The structure of 6-OHDA is identical to dopamine with the exception of a hydroxyl group on the six prime carbon. It is believed that 6-OHDA is imported into DA neurons by dopamine reuptake transporters.[20] Unlike MPTP, 6-OHDA cannot traverse the BBB and therefore must be administered through stereotactic injection into the striatum or SN. Upon entry into neurons, 6-OHDA auto-oxidizes to generate ROS and quinones leading to oxidative damage and neuronal death.[20] Nigral or striatal 6-OHDA injection results in loss of DA neurons in a manner that causes sizable and reproducible lesions.[20]

Numerous studies provide evidence for a substantial inflammatory component to 6-OHDA neurotoxicity. Sadeghian *et al.* reported that peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists protect against 6-OHDA in rats while reducing OX-6 and CD68 positive microglia (activated microglia).[69] Blocking microglial activation with minocycline or the COX-2 inhibitor celecoxib was shown to mitigate 6-OHDA-mediated DA neuron loss in the SN.[70,71] Additionally, 6-OHDA treatment increases TNF-α and blocking TNF-α protected DA neurons from 6-OHDA-mediated death.[72,73] Shrivastava *et al.* found that piperine, a bioactive compound from the *Piper nigrum* plant, is protective in a 6-OHDA rat model of PD.[74] This group reported that piperine reduced proinflammatory cytokine production, decreased lipid peroxidation, and increased glutathione in the striatum of 6-OHDA-treated mice. A group has also reported that two statin drugs, atorvastatin and simvastatin, reduced proinflammatory cytokines and bettered motor function using a 6-OHDA model in rats.[75]

PD genes and inflammatory response

Roughly 90% of PD cases exhibit a sporadic mode of incidence leaving approximately 10% attributed to genetic forms of the disease. Considerable effort has focused on rare monogenic causes of PD with particular emphasis on the role of alpha-synuclein, LRRK2, Parkin, DJ-1, and PINK1 in inflammatory response with the hope that understanding the underlying disease-causing mechanisms would shed light on all forms of the disease. Mutation of Parkin, DJ-1, and PINK1 has been reported to give rise to autosomal recessive forms of PD by modulating neuroinflammation.[78] PD has been associated with dysfunctional mitochondria, impaired autophagy, oxidative stress, and dysfunctional protein homeostasis. Parkin, DJ-1, and PINK1 are thought to exhibit neuroprotective properties by lessening oxidative stress, enhancing mitochondrial function, and mediating protein homeostasis.[79] It should be noted that genetic forms of PD may have some phenotypic differences from idiopathic PD.

SNCA (PARK1)

The first gene attributed to familial PD was alpha-synuclein (*SNCA*).[80] The function of alpha-synuclein is still unknown but evidence indicates it localizes to presynaptic terminals suggesting a possible role in neurotransmitter release.[81] Misfolded alpha-synuclein aggregates to form insoluble intracellular fibrils termed Lewy bodies in familial and sporadic PD.[5,82] Mutations in *SNCA* (A53T, A30P, and E46K) result in autosomal dominant PD.[5] Additionally, expressing heightened levels of unaltered alpha-synuclein leads to PD.[83] In fact, the level of alpha-synuclein expression is closely correlated with disease severity and inversely proportional to age of onset.[84]

Couch *et al.* suggest that the presence of extracellular alpha-synuclein protein causes a robust microglial response that includes increased nuclear factor kappa binding (NF-κB) to proinflammatory genes as well as the production of proinflammatory cytokines.[85] When mice were injected with alpha-synuclein in the SN and subsequently challenged with systemic LPS, SN levels IL-1β were similar to those produced by SN injection of LPS.[85] The injection of bovine serum albumin and subsequent challenge with systemic LPS did not result in a marked induction of inflammatory response suggesting that alpha-synuclein enhanced neuroinflammatory reaction triggered by systemic LPS.[85]

Gao *et al.* showed that intraperitoneal injection of LPS into control and mice overexpressing human A53T mutant alpha-synuclein led both groups to exhibit similar levels of acute inflammation.[86] Yet, only transgenic mice displayed chronic CNS inflammation. This was accompanied by loss of DA neurons and alpha-synuclein aggregation.[86] In support of a further link between alpha-synuclein and a neuroinflammatory response, Martin *et al.* showed

that mutant A53T overexpressing mice exhibit mitochondrial dysfunction and loss of DA neurons.[87] Oxidized mitochondrial proteins were found in the SN of transgenic mice overexpressing alpha-synuclein carrying the human A30P mutation.[88] Lee *et al.* showed that forced expression of alpha-synuclein harboring A30P results in increased oxidation of cellular constituents and enhanced neuronal sensitivity to oxidative stress.[89] The A30P mutation of alpha-synuclein accelerated synuclein aggregation and stimulated oxidative stress that was proposed to occur through reduced protection by soluble forms of alpha-synuclein.[89] Thus, the aggregation increased susceptibility to oxidative damage.

Watson *et al.* showed microglial activation in mouse models of PD that overexpress wild-type human alpha-synuclein.[90] The proinflammatory cytokine TNF-alpha had increased levels and activation of microglia in the striatum and SN at one month and 5–6 months of age, respectively. The cerebral cortex and cerebellum showed no such increase.[90]

Evidence suggests that extracellular alpha-synuclein may elicit an immunogenic response. Papachroni *et al.* found that approximately 90% of patients with familial PD were positive for alpha-synuclein antibodies and 65% for all PD patients.[91] In spite of the convention that the BBB restricts antibody access, preclinical alpha-synuclein antibody therapy has shown great promise in mouse models of PD.[92] This suggests that antibodies may cross the BBB and that extracellular alpha-synuclein may function as an autoimmunogen within the CNS. It is well documented that alpha-synuclein is secreted by affected neurons and taken up by healthy neurons in cell culture and in mice.[93] This extracellular alpha-synuclein is potentially available for reaction with the innate and adaptive immune systems.

LRRK2 (PARK8)

Discovered in 2004, leucine-rich repeat kinase 2 (LRRK2) has emerged as the leading known genetic contributor to PD.[94,95] However, how mutation of LRRK2 facilitates neurodegeneration and the characteristic symptoms of PD remains unclear. LRRK2 is a protein that possesses a functional kinase and GTPase domain in addition to a WD40 protein interaction domain.[94,95] Deposits of aggregated protein facilitated by LRRK2 mutations could be a potential factor. There are now 16 identified LRRK2 mutations. The mutation G2019S in LRRK2 is the most prevalent genetic cause of PD, resulting in an autosomal dominant familial form of PD, including both early and late onset.[96] LRRK2 has been examined extensively for a role in mediating cell autonomous death of neurons. For example, Smith *et al.* showed that expression of this mutant in primary mouse cortical neurons and SH-SY5Y human neuroblastoma cells induces apoptosis.[97] Evidence is mounting that suggests an increasing role for LRRK2 in cell non-autonomous death mechanisms.

Studies have shown that LRRK2 functions in neuronal cells include vesicular trafficking, cytoskeletal dynamics, mitochondrial function, apoptosis, and regulation of the autophagy pathway.[98] Genome-wide association studies link LRRK2 with diseases possessing a robust inflammatory element, leprosy,[99] and Crohn's disease.[100] It has been shown that LRRK2 may make microglia more prone to inflammatory response. LRRK2 expression is also reported to play a role in the physiology of lymphocytes and monocytic cells, indicating a possible immune function.[101] Gillardon *et al.* observed that microglia obtained from mouse brain express LRRK2.[102] Mutations in LRRK2 may give rise to aberrant cytoskeletal phosphorylation and polymerization of actin and β-tubulin affecting microglial activation and phagocytosis.[98] Additionally, LRRK2 may modulate cytokine production by regulation of transcription factors and vesicular proteins. This aberrant LRRK2 activity could predispose microglia toward a proinflammatory phenotype and heighten response to inflammatory stimuli.[98] Moreover, transgenic mice expressing mutant LRRK2 (R1441G) exhibited enhanced production of proinflammatory cytokines such as IL-6, IL-1β, and TNF-α in LPS-stimulated microglial cells.[102]

Parkin (PARK2)

Parkin mutations have been identified as a causal factor in the development of some cases of young onset PD (without alpha-synuclein aggregates). Parkin is an E3 ubiquitin ligase responsible for targeting cellular components that include proteins and organelles for degradation.[5] Research suggests that Parkin may trigger, in conjunction with PINK1, the destruction of dysfunctional mitochondria thereby reducing ROS generation within the cell.[103,104] More than 200 PD-causing *PARK2* gene mutations have been identified, yet it is uncertain how these mutations facilitate PD. However, considerable evidence indicates that Parkin is an important defense against oxidative damage to cellular molecules and organelles. A number of Parkin mutations identified appear to decrease or abolish E3 ubiquitin ligase activity. MPTP treatment of mice may also inactivate Parkin E3 ubiquitin ligase activity through s-nitrosylation.[105] This finding suggests that, in addition to inhibition of mitochondrial complex I, oxidative damage from MPTP treatment might occur through inhibition of Parkin E3 ligase activity. Additionally, forced expression of Parkin repressed mitochondrial ROS generation while mutant Parkin augmented it in SH-SY5Y and L6 cells.[106]

In addition to serving as a general defense against oxidative stress, Parkin modifies multiple proteins that are involved in regulating immune function. Tran *et al.* showed that IL-1β, TNF-α, and iNOS transcript expression is elevated in macrophages obtained from Parkin null mice.[107] Another group found that Parkin knockout mice were more susceptible than control mice to SN DA neuron loss following systemic inflammatory challenge by LPS. Following LPS challenge, Parkin null mice also displayed deficits in fine motor skills. Parkin deficient (−/−) mice alone did not exhibit DA neuron loss indicating that loss of Parkin is not sufficient to produce PD pathology.[17]

DJ-1 (PARK7)

DJ-1 is a molecular chaperone found in the cytosol and mitochondria.[108] Mutations that cause DJ-1 deficiency, for

which there is great heterogeneity, can lead to an autosomal recessive early onset PD. Analysis of human tissue indicates that DJ-1 is abundantly expressed by astrocytes.[109] This protein is a chaperone that becomes activated by oxidative stress.[110] Evidence suggests that DJ-1 elicits neuroprotective activity by serving as an antioxidant. Due to the role of DJ-1 in antioxidant activity, groups are actively investigating in DJ-1 protection against neuroinflammation-induced oxidative stress and resulting nigral degeneration. Waak *et al.* showed that astrocytes from DJ-1 null mice produced over 10-fold greater levels of nitric oxide than controls following LPS treatment. Additionally, astrocytes from these mice induce production of proinflammatory factors compared to controls.[111] Co-cultures of wild-type neurons with DJ-1 knockout astrocytes displayed a greater level of neuron death following LPS stimulation than wild-type astrocytes.[111] This suggests that DJ-1 represses inflammatory response-mediated neuronal loss and loss of DJ-1 activity augments a proinflammatory environment. The effect of DJ-1 *in vivo* models of PD is controversial. A group has reported that DJ-1 null mice exhibit greater loss of striatal dopamine following MPTP treatment.[112] Conversely, Nguyen *et al.* reported that mice deficient in DJ-1 are not more susceptible to PD pathology following inflammatory insult.[113] These conflicting results suggest that further investigation is warranted.

PINK1 (PARK6)

PTEN-induced putative kinase 1 (PINK1) is a kinase that affects mitochondrial turnover. PINK1 mutations cause an early onset autosomal recessive form of PD. Evidence indicates that PINK1 functions in concert with Parkin to target defective mitochondria for destruction by autophagy thereby reducing the ROS load within cells.[103,104] PINK1 knockout mice display repressed striatal dopamine signaling as well as reduced striatal mitochondrial respiration. Haque *et al.* showed that PINK1 knockout mice have increased susceptibility to the ROS-generating PD toxin MPTP.[114] This group rescued PINK1 deficient mice by overexpressing Parkin or DJ-1 suggesting PINK1 lies upstream of these factors in a common pathway. Additionally, Akundi *et al.* showed repression of PINK1 expression results in heightened susceptibility to inflammatory response-mediated DA neuron loss.[115] Elevated striatal levels of IL-1, IL-10, and IL-12 were detected in PINK1 null mice following LPS treatment. Furthermore, embryonic fibroblasts derived from PINK1 knockout mice exhibited repressed NF-kB activity in response to an inflammatory environment suggesting that PINK1 deficient neurons might have increased susceptibility to inflammatory response-mediated death.[115]

Conclusion

It is becoming increasingly evident that the study of inflammation is of prime importance to elucidate the mechanisms of DA neuron loss in PD and develop suitable strategies to curtail neuronal damage. Oxidative stress appears to be an important and not easily separated part of inflammatory response. For instance, inflammatory response creates an environment of increased oxidative stress and experimental evidence suggests that enhanced susceptibility to oxidative damage leads to heightened inflammatory response. This review has presented and discussed several lines of evidence implicating both inflammatory response and oxidative damage to PD pathology. Mounting evidence obtained from investigations utilizing pro-inflammatory agents as well as genetic studies of the monogenic causes of PD suggest that inflammatory response contributes toward the manifestation of PD and may not be simply a consequence of it. Despite the encouraging results involving NSAID use in clinical and preclinical studies, this class of drugs harbors serious side effects which preclude their effectiveness as PD therapies. In order to pursue very promising avenues of anti-inflammatory therapy, new strategies must be developed. Additionally, understanding the inflammatory contributions to PD may facilitate the development of other strategies such as stem cell therapy whereby existence of an inflammatory environment could curtail the effectiveness of grafted material.

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