

## The role of neuroinflammation on the pathogenesis of Parkinson's disease

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**Parkinson's Disease (PD) is a common neurodegenerative disease characterized by the progressive degeneration of nigrostriatal dopaminergic (DA) neurons. Although the causative factors of PD remain elusive, many studies on PD animal models or humans suggest that glial activation along with neuroinflammatory processes contribute to the initiation or progression of PD. Additionally, several groups have proposed that dysfunction of the blood-brain barrier (BBB) combined with infiltration of peripheral immune cells play important roles in the degeneration of DA neurons. However, these neuroinflammatory events have only been investigated separately, and the issue of whether these phenomena are neuroprotective or neurotoxic remains controversial. We here review the current knowledge regarding the functions of these neuroinflammatory processes in the brain. Finally, we describe therapeutic strategies for the regulation of neuroinflammation with the goal of improving the symptoms of PD. [BMB reports 2010; 43(4): 225-232]**

### INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by abnormal motor symptoms such as resting tremor, slowness of movement, rigidity and bradykinesia (1). The neuropathological features of PD are progressive death of dopaminergic (DA) neurons in the substantia nigra pars compact (SNpc) and depletion of dopamine in the striatum (STR), which is the site at which these nerve terminals project (2). Along with damage in the SN, eosinophilic inclusions (Lewy bodies) were identified in the brain of PD patients, thus becoming a pathological marker of the disease (3). Nowadays, Lewy bodies and neurites stained with antibodies to ubiquitin,  $\alpha$ -synuclein and other biochemical markers are

detectable in various brain regions; not only the SN, but also the locus coeruleus, raphe, thalamus, amygdala and cerebral cortex (4). These phenomena indicate that PD is involved in multiple neuronal systems in addition to dopamine.

Based on these main neuropathological features, several neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>), 6-hydroxydopamine (6-OHDA), 1,1'-dimethyl-4,4'-bipyridinium (paraquat) and rotenone are currently used for the induction of nigrostriatal DA neuronal degeneration (5). Although these toxin-based models of PD have some limitations in exactly reproducing neurotoxic mechanisms in a genetic mutation model (6), the specific features of PD pathogenesis such as neuronal inclusions (7), mitochondrial dysfunction (8), oxidative stress and inflammation (9) are consistently reported.

Among them, glial activation-derived oxidative stress and inflammatory molecules play important roles in DA neuronal death in PD patients and animal models (10, 11). These mechanisms are comprised of microglial activation, reactive astrocytes, damaged blood-brain barrier (BBB) and infiltrated peripheral immune cells (Fig. 1, 2). However, these molecular and cellular changes are not specific to PD, since neuroinflammation is also implicated in the development of Alzheimer's disease (AD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) as well as other neurodegenerative diseases (12). In this review, we describe the evidence for neuroinflammation as a consequence of nigrostriatal DA neuronal degeneration in the brains of PD patients and animal models of PD. Finally, we highlight possible therapeutic targets associated with inflammation that might help to slow down the progression of PD.

### Involvement of glial activation in PD

#### Microglial activation in PD

Microglia are the resident immune cells in the CNS and constitute about 5-20% of all glial cells. They were first identified as a distinct cell entity by Pio del Rio-Hortega (13). The origin of microglia is derived from circulating blood monocytes, which are the progenitors of microglia (14). In the mature healthy brain, resting microglia adopt a ramified morphology

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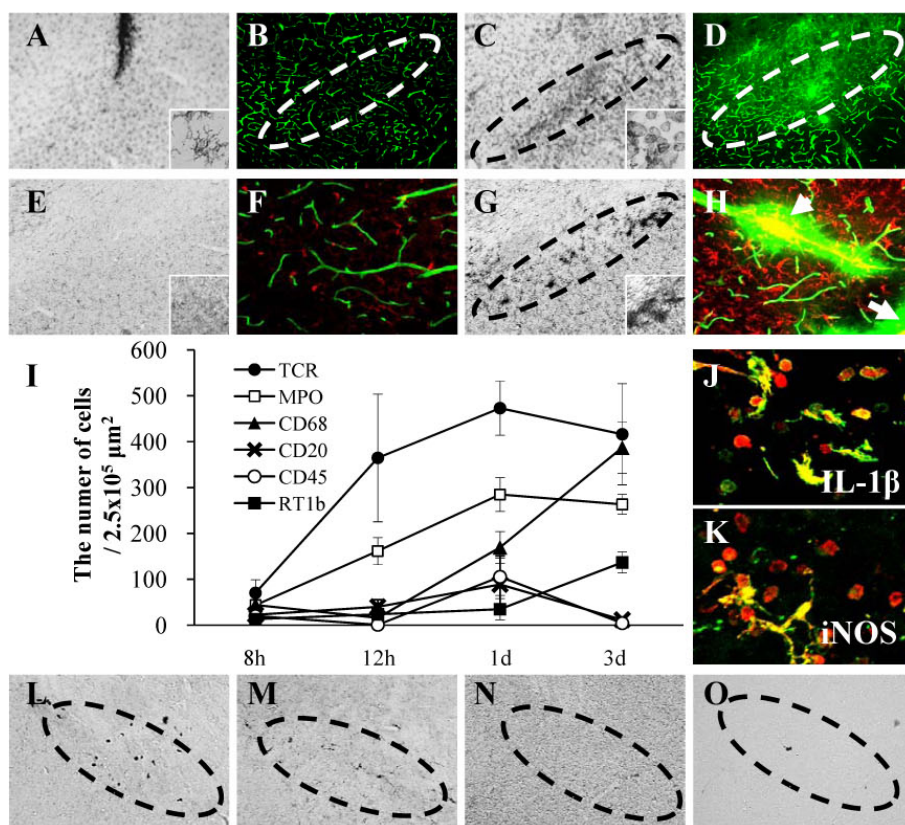
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consisting of small cell bodies. Little is known about the function of resting microglia, but they are suggested to function in immune surveillance and host defense by releasing low levels of growth factors (15).

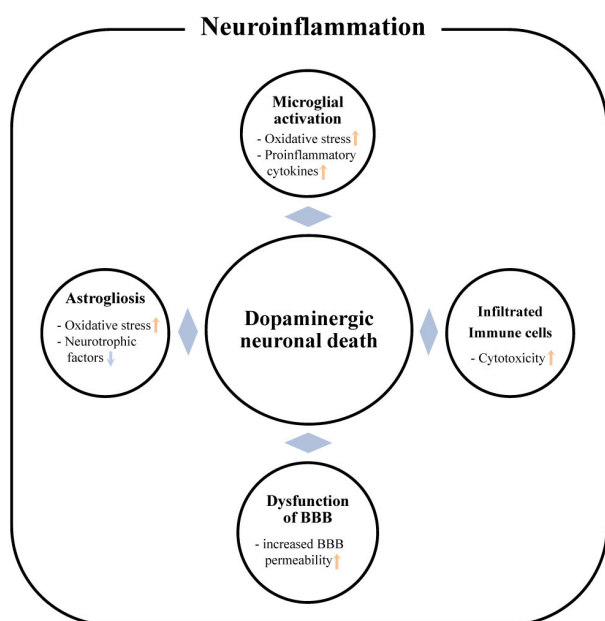
Microglial activation by various stimuli is generally characterized by a gradual change in morphology from a quiescent ramified form (resting state) to an amoeboid form (activated state) (16-19). Several studies have revealed that activated microglia express diverse cell-surface receptors, including the major histocompatibility complex and complement receptors (17, 20) as well as inducible potentially neurotoxic factors (21) that impose chronic inflammation of the brain, leading to neu-

ronal dysfunction and death in the form of neurodegenerative disorders such as PD (22).

In a postmortem analysis of PD patients, activated microglia and reactive human leukocyte antigen-DR (HLA-DR)-positive microglia were found in the SNpc (23, 24). Immunohistochemical studies have shown that numerous activated microglia are present in neurotoxin-treated SNpc in various animal models of PD (25). Consistent with these results, we reported that microglial activation involving the degeneration of DA neurons was observed in the SNpc (26, 27). Several results have suggested that these activated microglia could contribute to nigral DA neurons through oxidative stress and production of



**Fig. 1.** Various neuroinflammatory processes in the animal model of PD. (A-H) CD11b immunostaining and FITC-linked albumin (FITC-LA) assay showing microglial activation and disrupted BBB in the LPS-induced inflammation model (A-D) and MPTP mouse model (E-H). In PBS-treated control groups (A, B, E, F), ramified microglia and intact BBB FITC-LA were observed. At 3 days after LPS (5  $\mu$ g/3  $\mu$ l; C, D) and MPTP (four intraperitoneal injections of 20 mg/Kg; G, H) injection, there was a high amount of activated microglia and FITC-LA leakage in the SN. Colocalization of FITC-LA vessels (green) and CD11b positive cells (red) in the SN treated with PBS (F) or MPTP (H). Two images are merged (yellow). (I-K) Animals receiving intranigral LPS injection were sacrificed at various time points, and tissues were immunostained with each antibody to detect immune cells. The stereological counting results revealed that a number of peripheral immune cells infiltrated into the SN in a time-dependent manner (I). Additionally, colocalization of innate microglia (Iba-1; green) and proinflammatory cytokines (IL-1 $\beta$  and iNOS; red) revealed that innate microglia might contribute to the production of proinflammatory molecules (J, K). Two images are merged (yellow). (L-O) In the MPTP model of PD, immunohistochemical studies revealed that penetration of peripheral immune cells followed different patterns in the SN, compared to LPS-injected SN. The results of stereological counting (data not contain) showed that the majority of infiltrated immune cells were CD3 (L) and CD68 positive (M). Even though a few B cell receptor (N) and MPO-positive cells (O) were detectable in MPTP-treated mice, the total amounts were marginal portions of the total number of immune cells. Dotted lines indicate substantia nigra pars compacta (SNpc) where dopaminergic neurons were degenerated.



**Fig. 2.** Proposed schematic diagram showing neuroinflammatory processes in PD. The lozenges indicate that DA neuronal death propagates microglial activation, reactive astrocytes, BBB dysfunction and infiltration of peripheral immune cells. Increased neuroinflammation then exacerbates DA neuronal death through glial cell-derived oxidative stress and production of proinflammatory cytokines, decreased levels of neurotrophic factors, immune cell-mediated cytotoxicity and penetration of neurotoxic molecules by disrupted BBB.

proinflammatory cytokines (28-31).

Oxidative stress is caused by an imbalance between the production and destruction of ROS and reactive nitrogen species (RNS) (32). ROS/RNS production could be modulated by microglial-NADPH oxidase and nitric oxide synthase (NOS) in a neuropathological condition. In the MPTP model of PD, NADPH oxidase and inducible nitric oxide synthase (iNOS) are major sources of ROS/RNS production where they mediate DA neuronal death in the SN (29, 31). Other *in vivo* and *in vitro* studies have revealed that microglia-derived NADPH oxidase/iNOS play critical roles in the generation of ROS/RNS and contribute to DA neuronal death via oxidative stress (28, 33-35). This is consistent with our recent results that minocycline inhibits NADPH oxidase/iNOS-derived ROS production through the activation of microglia, which prevents oxidative damage to neurons and leads to increased neuronal survival in thrombin-injected SN (36).

Recent reviews have suggested that excessive inflammation may result in DA neuronal loss during the progression of PD (37). Proinflammatory substances such as cyclooxygenase 2 (COX2) and proinflammatory cytokines, including interleukine-1 beta (IL-1 $\beta$ ), interferon-gamma (IFN- $\gamma$ ) and tumor necrosis factor-alpha (TNF- $\alpha$ ), have been elevated in the postmortem

PD brain (38-42). Similarly to PD patients, a 6-OHDA and MPTP model of PD experienced increased levels of these inflammatory mediators as well as deteriorated DA neuronal death (43-46). In agreement with these findings, our recent results report that mitogen-activated protein kinase (MAPK) inhibitors spare nigral DA neurons through inhibition of thrombin-(26) or prothrombin kringle-2 (pKr-2)-induced microglial activation as well as through the overexpression of proinflammatory cytokines in microglia (27). Taken together, these findings indicate that microglial activation-derived oxidative stress and proinflammatory molecules are deleterious to DA neurons in the SN.

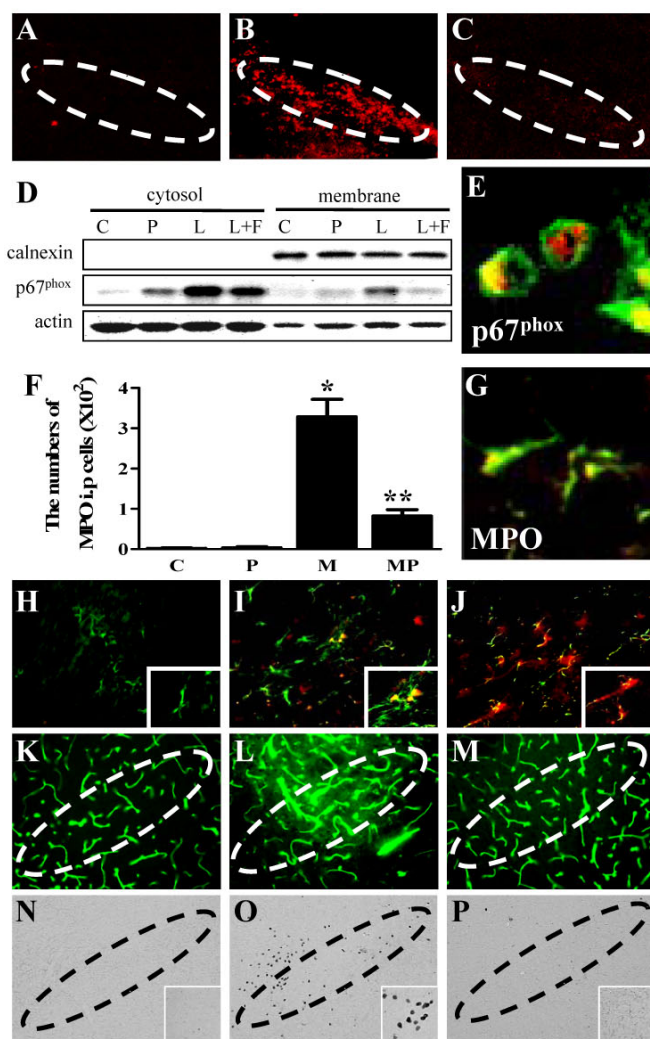
### Reactive astrocytes in PD

Astrocytes, major glial components of the central nervous system (CNS), constitute up to 20-50% of brain volume (47). Astrocytes provide the optimal microenvironment for neuronal function by exerting active control on the cerebral blood flow (48, 49) and by controlling the extracellular concentration of synaptically-released neurotransmitters (50).

Similar to microglial activation, star-shaped astrocytes transformed to reactive form have enlarged bodies and thick dendrites in response to various stimuli (51, 52). Originally, reactive astrocytes was described as a mechanical barrier to neurite outgrowth following injury that consists of scar tissue. Although the presence of reactive astrocytes in the SNpc of PD patients is involved in the progression of PD (53), the role of reactive astrocytes in the development of PD is still unknown and controversial.

Generally, astrocytes promote the survival and maintenance of DA neurons through secretion of various neurotrophic factors in the SN (54). In postmortem PD patients, biochemical analysis and immunostaining results showed that the levels of neurotrophic factors such as glial-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) were decreased (55). Interestingly, supplementation with glial-derived growth factors such as GDNF (56, 57), BDNF (58, 59) and mesencephalic astrocyte-derived neurotrophic factor (MANF) (60) protected DA neurons from neurotoxins (6-OHDA, MPP<sup>+</sup> and H<sub>2</sub>O<sub>2</sub>). Although the relationship between reactive astrocytes and the expression of neurotrophic factors is unknown, these results suggest that decreased levels of astrocyte-derived neurotrophic factors are at least somewhat responsible for DA neuronal death in PD.

Additionally, astrocytes are known to play an important role in antioxidant defense in the brain (61). Recently, astrocytes were found to regulate excessive inflammation via induction of microglial hemoxygenase-1 (HO-1) expression *in vitro* (62). Additionally, Min and colleagues demonstrated that astrocyte-conditioned medium induced nuclear translocation and binding of nuclear factor E2-related factor 2 (Nrf-2) to antioxidant response element (ARE), which reduces IFN- $\gamma$ -induced ROS production. In particular, overexpression of Nrf-2 and



**Fig. 3.** Therapeutic strategies for regulating neuroinflammation in PD. (A-C) Animals receiving MPTP with or without fluoxetine were sacrificed 3 days after MPTP injections, followed by hydroethidine staining to detect ROS production. The results of hydroethidine staining showed that fluoxetine reduced superoxide generation in MPTP-treated mice. PBS as a control (A), MPTP (B) or MPTP and fluoxetine (C). (D, E) Accompanying this, fluoxetine inhibited the activation of NADPH oxidase as a major source of ROS generation at 2 days after nigral LPS injection (D). Colocalization of microglia (OX-42; green) and the subunit of NADPH oxidase (p67<sup>phox</sup>; red) revealed that microglia-derived NADPH oxidase plays an important role in ROS production (E). Two images are merged (yellow). (F, G) Immunostaining and stereological counting showed that paroxetine, another anti-depressant, decreased MPO-positive cells related to RNS production at 3 days after the last MPTP injection (F). The statistical significance of differences was assessed using one-way ANOVA, followed by Student-Newman-Keuls analyses. One asterisk marks  $P < 0.01$ , significant from control (C) and paroxetine (P). Two asterisks mark  $P < 0.05$ , significant from MPTP (M). Localization of MPO immunoreactivity (red) in activated astrocytes (green) in SN treated with MPTP (G). (H-J) Double immunostaining with GFAP and CNTF revealed that capsaicin, a TRPV1 agonist, upregulated MPP<sup>+</sup>-induced CNTF expression in the ipsilateral side of the brain. PBS as a control (H), MPP<sup>+</sup> (I) or MPP<sup>+</sup> and capsaicin (J). (K-P) FITC-LA assay and CD3 immunostaining indicated that WIN55,212-2 prevented BBB leakage and leukocyte infiltration at 3 days after MPTP injection. MPTP induced severe penetration of FITC-LA (L) and increased the number of CD3-positive cells in the SN (O) compared to PBS-treated SN (K, N). Treatment with WIN55,212-2 prevented BBB disruption (M) and infiltration of immune cells (P). Dotted lines indicate SNpc.

Nrf-2 activators protect DA neurons from the neurotoxicity of 6-OHDA *in vivo* (63) and in rat organotypic nigrostriatal cocultures (64). These results collectively suggest that astrocytes are involved in antioxidant signaling both *in vivo* and *in vitro*.

By contrast, myeloperoxidase (MPO), a key enzyme in the generation of RNS, was upregulated in the midbrains of PD patients and MPTP-treated mice (11). Interestingly, this enzyme was localized within reactive astrocytes in MPTP-treated mice, and MPTP neurotoxicity was attenuated by ablation of MPO in the nigrostriatal pathway. This is in accordance with our unpublished observation that numerous MPO-positive cells are present in the MPTP-treated SN whereupon they colocalize within reactive astrocytes (Fig. 3; unpublished data). Although the reason for this apparent functional discrepancy remains unclear, reactive astrocytes do serve an important role in the initiation and progression of PD.

## Microenvironmental changes in PD

### Dysfunction of the blood-brain barrier in PD

The brain demands an adequate blood supply for the regulation of neuronal and synaptic function. To maintain concentrations of ions ( $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$ ) within narrow ranges as well as adequate levels of metabolic substrates in various brain regions, neural milieu are strictly separated from circulatory spaces through BBB formation (65). These unique biological structures are comprised of neurovascular units such as endothelial cells, pericytes, neurons and astrocyte endfeet (66). Endothelial cells tightly connect at junctional complexes such as adherens junctions (AJ), tight junctions (TJ) and gap junctions, thereby conferring low paracellular permeability (67). Pericytes and astrocytes regulate hemodynamic neurovascular coupling, microvascular permeability, matrix interactions, neurotransmitter inactivation, neurotrophic coupling, and angio-



genic and neurogenic coupling through close proximity with each other or with neurons (67).

In aging and neurodegenerative disease, neurovascular functions are altered to abnormal states, such as increased BBB permeability, decreased nutrient supply, faulty clearance of toxic molecules, and failure of enzymatic function (65). Although there is no clear evidence as to whether these altered neurovascular circumstances are responsible for the loss of DA neurons in PD, several studies on PD patients and animal models suggest a pathogenic linkage between BBB disruption and DA neuronal death. Positron emission tomography (PET) and histological studies on PD patients revealed dysfunction in the BBB transporter system (68) as well as alteration of blood vessels (69) in the midbrain of PD patients. Additionally, the levels of vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF) that induce structural changes in blood vessels were increased in PD patients and the MPTP model (70). Interestingly, nigral injection of VEGF disrupted BBB and induced DA neuronal death in the ventral mesencephalon (71). In addition, increased BBB permeability was observed in the MPTP (72) and LPS models of PD (73). Along with previously described results, our unpublished data suggest that increased permeability is revealed by leakage of FITC-linked albumin in MPTP- and LPS-treated SN (Fig. 1). These results collectively suggest that BBB disruption has some relationship with neuronal cell death and neuroinflammation in PD.

### Infiltration of peripheral immune cells in PD

CNS is considered an immunologically-privileged region due to the presence of innate microglia. However, the presence of T lymphocytes in the midbrain of PD patients suggests that the potential role of infiltrated peripheral cells is related to PD pathogenesis (24). In the model of LPS-induced inflammation, neutrophils and monocytes infiltrated into SN regions where they played important roles in neuroinflammation (74). Recently, Brochard and colleagues reported that numerous CD4 and CD8 positive cells were detectable in postmortem PD patients (75). In particular, the cytotoxic effects of T cells provided evidence that CD4 deficient mice were resistant to MPTP neurotoxicity in the SN. Accompanying this finding, our immunohistochemistry results showed that various peripheral immune cells, such as T cells, B cells, microphages and leukocytes, infiltrated into the SN region in the LPS and MPTP models (Fig. 1; BK Jin, unpublished data). Additionally, the presence of Iba-1 positive cells in disrupted blood vessels indicates that neuroinflammation might contribute to the infiltration of peripheral immune cells and leakage of the BBB in the SN. Collectively, these results suggest that penetration of immune cells plays an important role in the degeneration of DA neurons in PD.

### Neuroprotective strategies for the regulation of neuroinflammation in PD

Previous evidence has demonstrated that the regulation of neuroinflammation (Fig. 2; glial activation-derived oxidative damage and proinflammatory cytokines, astrocyte-derived neurotrophic factors, BBB disruption and infiltrated immune cells) could attenuate DA neuronal loss both in *in vitro* and *in vivo* models of PD. Wu and colleagues reported that minocycline attenuated DA neuronal death in MPTP mouse model through inhibition of NADPH oxidase and/or iNOS-derived oxidative stress as well as IL-1 $\beta$  expression (30). The femtomolar concentration of dexamethasone protected mesencephalic DA neurons in LPS-treated microglia-neuron cocultures via reduction of superoxide and proinflammatory cytokines (76). Recently, simvastatin rescued DA neurons from MPTP neurotoxicity through inhibition of nigral p21ras activation, which is related to the production of proinflammatory cytokines and glial activation (77). Accompanying these results, our unpublished data showed that fluoxetine, a common anti-depressant, contributes to the survival of DA neurons by inhibiting the transient expression of iNOS and by attenuating microglial NADPH oxidase activation, ROS/RNS production, and consequent oxidative damage in LPS and MPTP models (Fig. 3; unpublished observation). Interestingly, another anti-depressant, paroxetine, had similar neuroprotective effects on DA neurons (data not shown) as well as inhibitory effects on astroglial MPO-derived RNS production (Fig. 3; unpublished observation). In addition, our other unpublished data showed that capsaicin, the agonist of transient receptor potential vanilloid subtype 1 (TRPV1), prevented neuronal death in MPTP, MPP<sup>+</sup> and LPS models by inhibiting microglial activation and production of proinflammatory cytokines (data not shown). Especially, treatment with capsaicin increased the expression of CNTF in reactive astrocytes, which then increased a neurotrophic factor that plays a neuroprotective role in MPP<sup>+</sup> middle forebrain bundle (MFB) model (Fig. 3; unpublished observation).

In addition to these strategies for regulating neuroinflammation, several therapeutic targets related to the prevention of BBB leakage and infiltration of immune cells have been reported. Chen and colleagues reported that caffeine restrained the MPTP-induced loss of DA neurons, leakage of Evan's blue and FITC-LA while decreasing expression of the TJ proteins ZO-1 and occludin in the striatum (72). Dexamethasone and indomethacin exerted neuroprotective effects on DA neurons by reducing the amount of infiltrated leukocytes in MPTP-treated SN as well as inhibition of glial activation (78, 79). Our unpublished data also demonstrated that WIN55,212-2, a synthetic cannabinoid, inhibited not only microglial activation and ROS production (data not shown) but also disruption of BBB and leukocyte infiltration in the MPTP model (Fig. 3; unpublished observation). Collectively, these results propose that the regulation of neuroinflammation might arrest or improve

the progression of PD.

## Summary and Conclusion

Accumulating evidence indicates that neuroinflammation plays multi-faceted roles in PD, such as neuroprotection or neurotoxicity. Although this apparent discrepancy remains unclear, neuroinflammation is recognized as an important neurophathological feature of PD. Better understanding of these complex mechanisms may provide some clues to the development of interventional therapeutic strategies for PD patients.

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