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REVIEW

Inflammation as a causative factor in the aetiology of Parkinson's disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting mainly the elderly, although a small proportion of PD patients develop the illness at a much younger age. In the former group, idiopathic PD patients, the causes of the illness have been the subject of longstanding debate with environmental toxins, mitochondrial dysfunction, abnormal protein handling and oxidative stress being suggested. One problem has been that the epidemiology of PD has offered few clues to provide evidence for a single major causative factor. Comparatively recently it has been found that in both patients and experimental models of PD in animals neuroinflammation appears to be a ubiquitous finding. These cases present with all of the classical features of inflammation including phagocyte activation, increased synthesis and release of proinflammatory cytokines and complement activation. Although this process is vital for normal function and protection in both the CNS, as in the periphery, it is postulated that in the aetiology of PD this process may spiral out of control with over activation of microglia, over production of cytokines and other proinflammatory mediators as well as the release of destructive molecules such as reactive oxygen species. Given that dopaminergic neurons in the substantia nigra are relatively vulnerable to 'stress' and the region has a large population of microglia in comparison to other CNS structures, these events may easily trigger neurodegeneration. These factors are examined in this review along with a consideration of the possible use of anti-inflammatory drugs in PD.
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Abbreviations: BBB, blood brain barrier; BV, bacterial vaginosis; CD, complement receptors; ICAM, intercellular cell adhesion molecule; IFN, interferon; iNOS, inducible nitric oxide synthase; IL, interleukin; LPS, lipopolysaccharide; MHC, major histocompatiability complex; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NADPH, nicotinamide adenine dinuleotide phosphate; NSAID, non-steroidal anti-inflammatory drug; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; PHOX, phagocyte oxidase; ROS, reactive oxygen species; SN, substantia nigra; TNF-α, tumour necrosis factor-α; VCAM, vascular cell adhesion molecule

Introduction

Parkinson's disease (PD), first described by James Parkinson in 1817 as paralysis agitans, or the 'shaking palsy', is the result of a quite specific and progressive neurodegeneration of pigmented nigrostriatal dopaminergic neurons. The symptoms of PD are only apparent when loss of at least 50% of the dopaminergic neurons in the substantia nigra (SN) pars compacta occurs, leading to an over 80% reduction in dopamine (DA) levels in the striatum (Lang and Lozano, 1998; Deumens *et al*., 2002). The disease is today recognized as the second most common neurodegenerative disorder after Alzheimer's disease with a prevalence of 0.1% of the global population. Although PD is an age-related disorder afflicting about 3% of people over 65 years and 4–5% of people over 85 years of age, 5–10% of the patients are under 40 years of age. However, with an ageing population, the result of general improvement in health care, as the average life span increases it must logically follow that the proportion of PD patients will rise. Epidemiological studies and pathological analyses demonstrate that sporadic PD cases with late onset occur in 95% patients (Tanner, 2003), whereas the remaining 5% PD cases are seen in familial clusters with early onset (Mizuno *et al*., 2001). Although the majority of PD cases cannot be linked to a specific causative factor, familial PD has been linked with mutations in several genes such as parkin, ubiquitin C-terminal hydrolase L1 and α-synuclein (Gwinn-Hardy, 2002). Additionally, the presence of widespread levels of Lewy bodies, which are the principal location of α-synuclein, is characteristic of the disease (Gwinn-Hardy, 2002). Lewy bodies are eosinophilic inclusions, which consist of intracytoplasmic aggregates of

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α-synuclein. They are usually rounded with an eosinophilic core and a surrounding pale halo. Whether these structures are symptomatic or causative in PD is not clear.

The clinical features of PD include both motor and non-motor manifestations. The principal motor characteristics of PD are difficulty in initiating voluntary movement (akinesia), slowing and diminished range of movements (bradykinesia), a rhythmic involuntary 5–7 Hz tremor in patients at rest (the characteristic 'pill rolling movement') and the extrapyramidal rigidity in which major muscle groups become stiff. Hypomania and eye blinking, as well as hypophonia, are also early manifestations of the disease (Deumens *et al*., 2002).

As indicated above, although the cause of non-familial PD remains unclear, several theories have been described regarding the possible factors behind the neuronal degeneration that occurs. These include environmental toxins, genetic factors and mitochondrial dysfunction as well as free radical-mediated cell death and oxidative stress (Schapira, 1994; Ben-Shacher *et al*., 1995; Hoehn and Yhar, 1998; Rosenberg, 2002). Recently, however, there is increasing recognition of the possible role of neuroinflammation as a major factor in the pathogenesis of PD (McGeer *et al*., 2001), induced by exposure to either infectious agents or toxicants with proinflammatory characteristics.

Potential causative factors in PD

Some of the main possible contributory factors in PD will be considered briefly as they may work individually or in tandem with neuroinflammation in the aetiology of the illness. Numerous hypotheses have been proposed regarding the loss of dopaminergic neurons in PD. Oxidative stress has consistently been associated with development of PD, due in large part to the highly oxidative conditions that generally prevail in dopaminergic neurons. Other studies have shown severe defects in mitochondrial function (Schapira, 1994; Sherer *et al*., 2002), which would be expected to lead to impaired energy metabolism and cell death. The proteolytic hypothesis describes nigral neuron loss in PD patients as a result of the toxic accumulation of misfolded and aggregated proteins, such as α-synuclein (Lim *et al*., 2003; Hald and Lotharius, 2005).

The role of oxidative stress in the aetiology of PD has received widespread and sustained interest (Halliwell and Gutteridge, 1999). Oxidative stress occurs in the brain when the ability of the endogenous antioxidant systems is overcome by the generation of reactive oxygen species (ROS), which ultimately leads to cellular damage and death as a result of the modification of nucleic acids, lipids and proteins (Hald and Lotharius, 2005). ROS are generally produced in normal physiological conditions at low levels and are scavenged by endogenous antioxidants, such as superoxide dismutase, glutathione peroxidase, catalase and small molecules such as vitamin C and E, but these protective factors appear to be overwhelmed by the disease process (Halliwell, 2001, 2006).

All tissues can be damaged as a result of oxidative stress; however, the brain appears to be particularly susceptible, no doubt due to its heavy oxygen demands (Halliwell, 2001, 2006). Although regional differences are evident, the nigra seems to be highly susceptible to oxidative stress, presumably due, at least in part, to its large population of dopaminergic neurons, which produce abundant quantities of ROS. The presence of oxidative stress in PD has been associated with increased oxidation of lipids, DNA and proteins and the generation of ROS (Zhang *et al*., 1999). Furthermore, oxidative stress has been demonstrated in PD sufferers (Halliwell and Gutteridge, 1999; Waragai *et al*., 2006) and evidence also clearly supports the involvement of impaired mitochondrial function in PD (Keeny *et al*., 2006; Schapira, 2006). For example, inhibition of complex-I, mitochondrial respiratory enzyme, by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) leads to a PD-like state (Beal, 2003). Moreover, the endogenous molecule tetrahydrobiopterin inhibits mitochondrial complexes I and IV, leading to a breakdown in function of the electron transport train, oxidative stress and ultimately PD-like symptoms (Choi *et al*., 2006). Additionally, untreated PD patients have been shown to have decreased activity of complexes I and I/III in platelet mitochondria (Schapira, 1999). It has also been recognized that oxidative stress leading to caspase activation and consequent apoptosis are clearly evident in PD (Friedlander, 2003).

DA metabolism has also been associated with increased levels of ROS: nigrostriatal DA is normally deaminated by monoamine oxidase, which results in the production of dihydroxyphenylacetic acid and H_2O_2 (Gotz *et al*., 1994), but is also metabolized by auto-oxidation leading to the formation of H_2O_2, DA quinones and semi-quinones, which have been shown to contribute to structural protein alterations and decreased levels of glutathione (Tse *et al*., 1976; Graham *et al*., 1978; Stokes *et al*., 1999). The H_2O_2 produced is converted into highly destructive hydroxyl radicals by the Fenton reaction, with Fe^{2+} being liberated as a result (Graham *et al*., 1978; Maker *et al*., 1981). These hydroxyl radicals subsequently react with almost all cellular macromolecules and thus lead to widespread cellular damage (Halliwell, 2006). Furthermore, DA has been demonstrated to inhibit complex-I when injected into the brain ventricles of rats, indicating that it is involved in oxidative stress as well as proteolytic dysfunction and an increased accumulation of protein aggregation, particularly α-synuclein (Saha *et al*., 2003). Evidence also indicates that DA-associated oxidative stress may contribute to an inflammatory reaction seen in PD with a subset of PD patients having been shown to produce antibodies against proteins that were modified by DA oxidation products (Rowe *et al*., 1998). Additionally, microglia exposed to a mixture of antibodies from PD patients and DA-quinone or H_2O_2-modified DA cell membranes are activated and produce ROS as well as cytokines (Le *et al*., 2001). Additionally, neuromelanin, which is normally released by dying DA neurons, has been reported to activate microglia *in vitro* (Wilms *et al*., 2003). It also has been suggested that the presence of high levels of ROS may lead to or potentiate a chronic inflammatory reaction by causing modifications in various biomolecules. This is also seen in diseases such as systemic lupus erythematosus and rheumatoid arthritis in which ROS-modified DNA was shown to be

an important contributory factor in the development of autoimmunity (Ahsan *et al*., 2003).

Microglia as a contributor of proinflammatory and neurotoxic factors

Del Rio Hortega initially described microglia as a separate cell type with differing morphology from other glial cells such as astrocytes and oligodendrocytes. Microglia are resident immunocompetent and phagocytic cells in the central nervous system (CNS), and are thought to mediate the innate defence system and thus serve a critical role in normal CNS function (Kim and de Vellis, 2005). Additionally, although astrocytes provide homoeostatic control of the extracellular environment of the neurons and respond to various stimuli such as disease, chemicals or physical damage, microglia also act as scavenger cells in the event of infection, inflammation, trauma, ischaemia and neurodegeneration in the CNS (Beyer *et al*., 2000).

It has been generally believed that microglial cells are formed during embryonic development when blood monocytes enter the brain and differentiate into resident microglial cells exhibiting the cell surface antigens found on macrophages (Kim and de Vellis, 2005). Microglia also have active roles in late embryonic brain development and early post-natal brain maturation in which they have been shown to carry out the programmed elimination of neural cells (Milligan *et al*., 1991; Barron, 1995).

Resting microglia appear to be highly sensitive to many forms of disturbance within the microenvironment of the brain and can quickly activate when pathological events occur, such as infection and inflammation, with a resulting well-characterized and graded response (Wojtera *et al*., 2005). In their resting state, microglial cells display a downgraded phenotype and in the healthy brain have a ramified morphology and a low expression of membrane receptors, which are necessary for mediating normal macrophage functions, such as leucocyte common antigen (LCA/complement receptor (CD)45), CD14 and mac-1 (CD11b/CD18) (Kreutzberg, 1996). It has been shown that early microglial activation, within 24 h of stimulus exposure, leads to elevated microglial immunoglobulin (Ig)G reactivity, upregulation of CD1 and cell adhesion molecules, such as lymphocyte function-associated antigen 1 (LFA-1) (CD11a/CD18), intercellular adhesion molecule (ICAM)-1 (CD54) and vascular cell adhesion molecule (VCAM)-1(CD106) (Orr *et al*., 2002). When the activating stimulus continues to be present, microglia then proceed to adhere to neurons (Kreutzberg, 1996) directed by chemokines such as monocyte chemoattractant protein-1 and interferon (IFN)-inducible protein-10 that are expressed by the neurons themselves (Aloisi *et al*., 2000; Aloisi, 2001).

Furthermore, once activated, the inner cytoskeleton of the microglia changes, the cell body becomes enlarged, displaying a macrophage-like appearance and an increase in numbers occurs (Raivich *et al*., 1999). Aloisi *et al*. (2000) reported that ultimately, with the continued presence of the inducing stimulus, the microglia maintain this functional transformation with the upregulation of major histocompatibility complex (MHC) class two molecules and inflammatory glycoproteins, such as CD40, B7.1 (CD80) and B7.2 (CD86), which provide a powerful stimulus for immune cell activation (Aloisi *et al*., 2000). Moreover, microglia constitutively express β2-integrins CD11a, CD11b and CD11c. ICAM1, a ligand for the β2-integrins (FA-1/CD11a/CD18) and mac-1 (CD11b/CD18), is an Ig superfamily adhesion molecule that is also upregulated on activated microglia. Furthermore, overactivation of complement, as is thought to occur in neuroinflammation, may provide additional ligands for microglial integrins (Marlin and Springer, 1987; Greenwood *et al*., 2003; Kim and de Vellis, 2005).

It has, however, been suggested that even at this point the numbers of microglia can decrease and lose their activation markers, subsequently returning to the resting state if the activating stimulus disappears. However, if the pathologic stimulus is maintained, substantial increases in activation markers and cell adhesion molecules occur and finally the activated microglia cluster around DA neurons and become phagocytic (Bronstein *et al*., 1995; Banati *et al*., 1998; McGeer, 1998). This combination of released factors and surface adhesion to dopaminergic neurons leads to a progressive and irreversible neuronal cell death, which is worsened by the release of chemoattractants by the dying neurons to induce even greater infiltration of the region by activated microglia (Aloisi, 2001; Kim and De Vellis, 2005; Sriram *et al*., 2006).

Evidence for a neuroinflammatory response in PD patients and PD models

Patient studies

The involvement of inflammation in PD was initially suggested by McGeer *et al*. (1988) who described the upregulation of MHC molecules in PD patients. Furthermore, these authors reported increased number of activated microglia in PD patients. Additionally, increased levels of β2-microglobulin (the light-chain of MHC) in the striatum of patients were reported (Mogi *et al*., 1995), whereas increased levels of antibodies to proteins modified by DA oxidation products in PD patients have been found in other studies (Rowe *et al*., 1998). Elevated levels of proinflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 in the colony-stimulating factor (CSF) and striatum in PD brains have also been demonstrated (Mogi *et al*., 1994; Blum-Degen *et al*., 1995; Muller *et al*., 1998). Upregulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) containing amaoeboid microglia has been observed in the SN of PD patients but not in control subjects (Knott *et al*., 2000). Extensive proliferation of reactive amoeboid macrophages and microglia (HLA-DR positive) was described in the SN of PD patients in post-mortem studies (McGeer *et al*., 1988), indicating that activated microglia may lead to dopaminergic neurodegeneration. Activated glial cells expressing pro-inflammatory cytokines, such as TNF-α, IL-1β and IFN-γ as well as iNOS have been reported in the SN in PD (Hunot *et al*., 1996; Hirsh *et al*., 1998). Furthermore, enhanced expression of IL-1, IL-6 and TNF-α has also been shown in the cerebrospinal fluid as

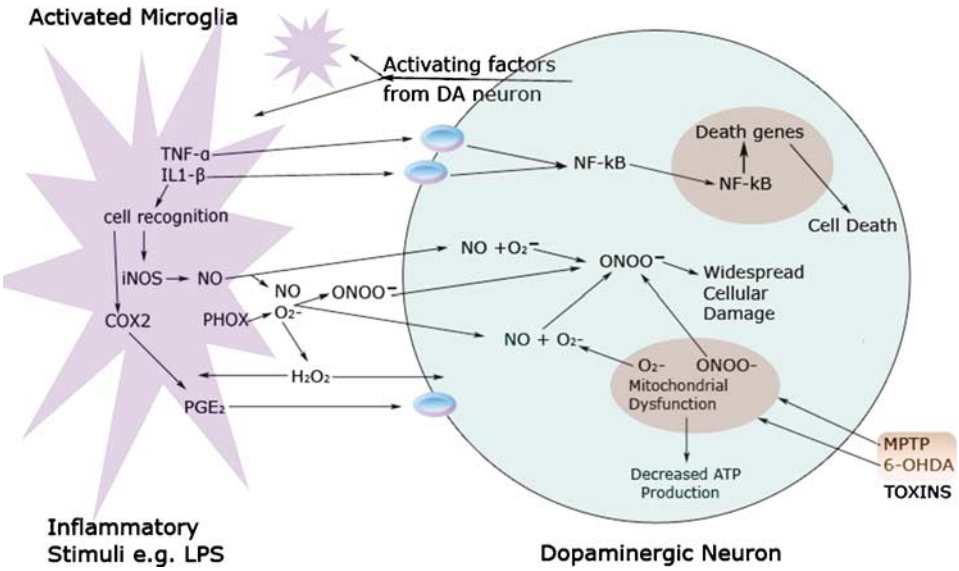

Figure 1 A simplified schematic of the interaction between microglia and dopaminergic neurons leading to nigral cell damage and death. Activated microglia (subsequent to immune activation or neuronal lesion caused by exposure to toxins such as MPTP or 6-OHDA) can contribute to the degeneration of DA neurons by releasing neurotoxic factors such as PHOX-induced superoxide (H_2O_2) and cytokines (e.g. TNF-α, IL-1β). Cytokines can then activate receptor-mediated proapoptotic pathways within the DA neuron as well as further stimulation of the microglia in the form of iNOS and COX2 induction. The former will lead to greatly increased NO generation and resulting elevation of ROS, which damage the cell as a result of DNA damage, protein disruption and lipid peroxidation. The latter causes increased PGE_2 production leading to direct toxicity to the DA neuron. Superoxide further stimulates microglial cytokine production as well as increases the quantities of ROS in the dopaminergic neuron. As signals from damaged DA cells further recruit and also stimulate microglia, the process can readily spiral out of control into full-blown neurodegeneration.

well as in the basal ganglia of PD patients (Nagatsu *et al*., 2000: Nagatsu, 2002). These inflammatory cytokines, along with factors released from the dying dopaminergic cells, seem to amplify and sustain the neuroinflammation as well as further consequent immune responses leading to a potentially lethal descent into irreversible destruction of SN dopaminergic neurons (Orr *et al*., 2002). These processes are summarized in Figure 1.

Langston *et al*. (1999) found that in post-mortem examination of brains from humans exposed to MPTP, activated microglia were present up to 16 years after exposure, indicating a protracted and ongoing inflammatory response. These patients did not have Lewy bodies present, however, revealing a significant difference between MPTP-induced neurotoxicity and PD itself, despite the considerable similarities between the two conditions. Nevertheless, overall these observations are strongly indicative of a process by which an ongoing stimulus could lead to disease progression long after the initial toxic insult. These findings are supported by studies in the SN of primates, which show that activated microglia and dopaminergic cell loss continue to occur years after exposure to MPTP (McGeer *et al*., 2003). ICAM-1, which appears to be important in sustaining neuroinflammation (Collins *et al*., 1994), has been found to be overexpressed in reactive astrocytes in the SN of PD patients (Miklossy *et al*., 2006) as has its counter receptor, LFA-1 (CD11a/CD18), in reactive microglia in the SN tissue matrix (Miklossy *et al*., 2006). In these patients, ICAM-1 expression is particularly intense around residual neurons in areas of extensive cell loss. Similar findings were observed by these authors in MPTP-treated monkeys (up to 14 years post-exposure). This suggests that ICAM-1 and LFA-1 interactions may play an important role in sustaining inflammation in PD patients as well as in MPTP-treated monkeys (Miklossy *et al*., 2006).

Animal models

Several animal paradigms of PD, such as the MPTP (Czlonkowska *et al*., 1996) rotenone (Gao *et al*., 2002a; Sherer *et al*., 2002) and 6-hydroxydopamine (6-OHDA) (Ciccetti *et al*., 2002) models, have been shown to activate microglia. Already substantial, and still accumulating evidence shows that lipopolysaccharide (LPS)-induced microglial activation causes dopaminergic neurodegeneration *in vitro* and *in vivo* (Castrano *et al*., 1998; Hererra *et al*., 2000; Gao *et al*., 2002b; Gayle *et al*., 2002; Arimoto and Bing, 2003; Liu *et al*., 2003). Also, this iNOS is generally found to be upregulated in experimental PD models (Liberatore *et al*., 1999; Iravani *et al*., 2002) and inhibition of iNOS reduces the toxicity of LPS or LPS and IFN-γ-activated microglia on dopaminergic neurons *in vitro* by around 75% (Hemmer *et al*., 2001; Le *et al*., 2001). Evidence now clearly indicates that inflammatory cytokines, such as TNF-α, IL-1, IL-6 and the signalling molecule NO are toxic to neurons (Allan and Rothwell, 2001; Fisher *et al*., 2001; Gayle *et al*., 2002; Liu *et al*., 2002; Ma and Ma, 2002; Sriram *et al*., 2002). Furthermore, inactivation of the genes involved in the synthesis of proinflammatory molecules such as COX-2 (Feng *et al*., 2002), nicotinamide adenine dinuleotide phosphate (NADPH) oxidase (Wu *et al*., 2003) and both TNF-α receptors (Sriram *et al*., 2002) were shown to protect DA neurons against MPTP-induced neurotoxicity, thus indicat-

ing that inflammation plays an important role in MPTP-mediated as well as other types of nigrostriatal neurodegeneration. Interestingly, it has been observed that MPTP and LPS act synergistically to mediate nigral dopaminergic neurotoxicity, probably by stimulating release of the superoxide free radical (Gao *et al*., 2003b).

Triggers of microglial activation and neurodegeneration

It has been shown that there are various microglial activating agents that may ultimately induce neurotoxicity. These agents include immunological insults, such as LPS, environmental toxins, endogenous disease proteins and neuronal injury (e.g. Lima *et al*., 2006). Additionally, injured neurons recruit nearby microglia and astrocytes for 'support' (Neumann, 2001) as well as releasing substances that influence the activation of microglia themselves (e.g. Le *et al*., 2001).

Lipopolysaccharide

LPS is a Gram-negative bacterial endotoxin and is a potent microglial cell activator (Lieberman *et al*., 1989; Kim *et al*., 2000). LPS is now well established as an effective initiator of SN dopaminergic neuronal loss and resulting Parkinsonian-like symptoms in experimental animals (Castrano *et al*., 1998; Hererra *et al*., 2000; Kim *et al*., 2000). *In vitro* LPS decreased the number of tyrosine hydroxylase-positive cells in primary mesencephalic cultures as well as in increasing cytokine output (Gayle *et al*., 2002). LPS binds to an LPS membrane receptor complex on microglial cells and activates nuclear localization of transcription factor nuclear factor κB (NF-κB) and subsequent activation of genes in the proinflammatory pathways (Orr *et al*., 2002). However, Iravani *et al*. (2005) found evidence to suggest that acute microglial activation by LPS does not in itself lead to long-term cell death.

It has been shown that an intranigral injection of LPS causes strong microglial activation and degeneration of DA neurons in both the SN and striatum (Hererra *et al*., 2000). However, and of considerable interest, when LPS at similar doses is injected into other areas of the rat brain such as the hippocampus or cortex, cell death does not seem to take place (Kim *et al*., 2000). Critically, the damage to the dopaminergic neurons is still clearly evident 1 year post-injection, indicating that a transient exposure to a proinflammatory substance may initiate a sequence of events leading to apparently permanent neurodegeneration as occurs in PD itself (Hererra *et al*., 2000). Also of particular relevance in regard to PD is that the neurotoxicity induced by LPS appears selective for dopaminergic neurons, whereas there was no measurable damage to GABAergic or serotonergic neurons at the same LPS doses (Hererra *et al*., 2000; Gao *et al*., 2002b).

Intraperitoneal injection of LPS has been reported to produce an increase in gene expression for Toll-like receptor 2, inerferon κB-α, COX-2, IL-6 and IL-6 receptor in the CNS in rodents (Vallieres and Rivest, 1997; Laflamme *et al*., 2001), which may be significant in the LPS model of PD. LPS also induces expression of caspase-11 and increased IL-1β levels (Arai *et al*., 2006), which are proinflammatory, whereas caspase-11 knockout mice are resistant to LPS-induced increases in IL-1β, microglial activation and DA cell loss (Arai *et al*., 2004, 2006).

It has been shown that caspase-11, which contributes to IL-1β secretion (Kim *et al*., 2003) and apoptosis (Kang *et al*., 2000; Hisahara *et al*., 2003), mediates MPTP-induced nigrostriatal dopaminergic degeneration (Furuya *et al*., 2004), and it is generally believed that caspase-11 is involved in both MPTP and LPS-induced selective dopaminergic neurotoxicity (Furuya *et al*., 2004). Moreover, DA itself seems to play a role in LPS-induced neurodegeneration, as inhibition of tyrosine hydroxylase (TH) by α-methyl-*p*-tyrosine alleviates microglial activation and DA cell loss (De Pablos *et al*., 2004). Significantly, systemic LPS causes a breakdown of the blood brain barrier (BBB) and a consequent infiltration of granulocytes into the CNS (Bohatschek *et al*., 2001).

Rotenone

Rotenone is a common pesticide/herbicide, which is considered as a potential environmental risk factor for the development of PD (Betarbet *et al*., 2000). Chronic administration of rotenone leads to selective damage to nigrostriatal DA neurons, and the formation of cytoplasmic inclusions in nigral neurons, as well as the development of hypokinesia and rigidity in rats (Greenamyre *et al*., 1999; Betarbet *et al*., 2000).

Rotenone inhibits the activity of complex-I of the mitochondrial respiratory enzymes (Greenamyre *et al*., 1999; Jenner, 2001), and recent evidence has shown that this pesticide also activates microglia (Gao *et al*., 2002a; Sherer *et al*., 2002). High concentrations (up to 20 nM) of rotenone cause direct neurotoxicity in neuron-enriched cultures lacking microglia (Gao *et al*., 2002a); however, neuron-glia cultures treated with rotenone at concentrations as low as 1 nM showed selective DA neurotoxicity (Gao *et al*., 2002a), indicating that rotenone causes enhanced neurodegeneration in the presence of microglia.

α-Synuclein

α-Synuclein is a synaptic vesicle protein that is a component of Lewy bodies (Takahashi and Wakabyashi, 2001). Recently, it has been shown that α-synuclein activates microglia, and in microglia-depleted cultures, low concentrations of α-synuclein fail to induce DA toxicity (Zhang *et al*., 2005). It has been reported that α-synuclein activates microglia to generate extracellular superoxide, increase intracellular ROS and further induce morphological changes in microglial cells (Zhang *et al*., 2005). α-Synuclein appears to be phagocytosed by microglia and this has been suggested to be a major mechanism by which α-synuclein induces microglial activation (Zhang *et al*., 2005). It is also been suggested that the release of α-synuclein from damaged neurons could further potentiate neuronal death through the activation of microglia (Block and Hong, 2005). Importantly, α-synuclein has very recently been reported to strongly stimulate human astrocyte and U-373 MG astrocytoma cells to upregulate

both IL-6 and ICAM-1, and this is associated with activation of the major mitogen-activated protein (MAP) kinase pathways (Klegeris *et al*., 2006).

6-Hydroxydopamine

6-OHDA is a neurotoxin, which, when directly injected into the medial forebrain bundle, striatum or SN, induces nigrostriatal DA neuronal degeneration (e.g. see Rodriguez *et al*., 2001). Although 6-OHDA leads to clear apoptosis of nigrostriatal dopaminergic cells, evidence indicates that the toxic effects of 6-OHDA are in part mediated through the activation of microglia. Direct administration of 6-OHDA into the SN of mice activates microglia and increases the number of activated microglia in the SN with the subsequent loss of dopaminergic neurons after 1 week (He *et al*., 2001). Furthermore, 6-OHDA-lesioned rats have been demonstrated to have increased levels of TNF-α in both SN and striatum (Mogi *et al*., 1999). The effects of striatal injection of 6-OHDA in rats was followed using PET scanning of presynaptic DA transporters following administration of transport site-specific ligands (Ciccetti *et al*., 2002). These authors observed progressive striatal neurodegeneration, whereas at the same time, an increase in activated microglia in the striatum and nigra occurred, which was initially focal but by 4 weeks had become widespread (Ciccetti *et al*., 2002). This led the authors to conclude that neuroinflammation is a significant factor in the 6-OHDA neurodegenerative process. Depino *et al*. (2003) reported an atypical cytokine response to subacute 6-OHDA injection into the striatum. An increase in mRNA for both IL-1α and -1β mRNAs was found at 30 days post-injection (2- and 16-fold, respectively), but no induction for IL-1α or β protein expression was seen. As a control, a proinflammatory stimulus, bacterial endotoxin, induced both cytokines at both mRNA and also at the protein level, although TNF-α mRNA was barely detectable in the SN. These authors concluded that neuronal death *per se* does not induce secretion of proinflammatory cytokines but requires an additional stimulus for proinflammatory cytokine production. However, Nagatsu and Sawada (2006) have reported increased levels of a range of proinflamatory cytokines, including TNF-α, IL-1β and IL-6, as well as decreased levels of neurotrophins such as brain-derived neurotrophic factor in the CSF of PD patients and the nigrostriata of 6-OHDA-treated rats.

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

MPTP is a neurotoxin that induces Parkinsonian features in humans, rodents and non-human primates (Langston *et al*., 1999), and has been demonstrated to cause rapid and selective DA neurotoxicity (Langston *et al*., 1999). MPTP is taken up by DA neurons causing oxidative mitochondrial damage, which results in neuronal death (Decker *et al*., 1993). MPTP also leads to a sustained inflammatory response in both humans and primates, which has been shown to continue for many years after MPTP exposure (Langston *et al*., 1999; McGeer and McGeer, 2004). To this end, MPTP-induced degeneration shows many similarities to PD, although the lack of Lewy bodies displayed after MPTP toxicity indicates differences. With regard to neuroinflammation, MPTP-induced neurotoxicity is linked to microglial activation (Gao *et al*., 2002b; McGeer *et al*., 2003). This is clearly demonstrated when microglia are added to neuronal cultures leading to a greatly increased level of MPTP-induced DA toxicity (Gao *et al*., 2003a).

An increased expression of both MHC class 1 and II antigens and upregulation of iNOS have been demonstrated in the striatum and SNc of mice given MPTP (Kukowska-Jatrzebska *et al*., 1999) as well as increases in proinflammatory cytokines such as IL-1β and IL-6 (Nagatsu *et al*., 2000). MPTP also modifies the expression of numerous genes involved in the process of inflammation including IL-1, IL-6, IL-10 and TNF-α (Mandel *et al*., 2003).

Although it appears that MPTP and its active metabolite MPP+ do not activate microglia (Gao *et al*., 2003a), in animal studies, MPTP toxicity is significantly decreased in mice with deficient production of proinflammatory mediators such as superoxide (Wu *et al*., 2003), prostaglandins (Feng *et al*., 2002; Teismann *et al*., 2003a, b) and TNF-α (Sriram *et al*., 2002). This clearly indicates that microglial-derived proinflammatory factors play a key role in the process of dopaminergic MPTP-induced neuronal toxicity. Thus it has been suggested that after the initial toxic insult, activation of microglia occurs and is potentially amplified as the disease progresses and this may, at least in part, underlie the progressive ongoing neurodegeneration produced by MPTP, which may be supported by the observation that MPTP and LPS show clear synergy in mediating dopaminergic cell death (Gao *et al*., 2003b).

Nicotinamide adenine dinucleotide phosphate oxidase

NADPH oxidase, also known as phagocyte oxidase (PHOX), is a membrane-bound enzyme, which leads to the production of superoxide (O_2^-) from oxygen. NADPH oxidase is the major source of ROS following a range of initiating activators and it has been shown that NADPH oxidase is the common mechanism through which microglia are toxic to neurons (Gao *et al*., 2003a; Wu *et al*., 2005) and has been reported to be upregulated in PD models (Wu *et al*., 2003). It is normally inactive in resting phagocytes but is activated when the cell is acted upon by stimuli such as bacterial toxins and some inflammatory peptides (Babior, 2000). Microglia regularly generate ROS when activated by multiple proinflammatory stimuli, including environmental factors (LPS, diesel exhaust particles, rotenone, paraquat), and endogenous proteins (e.g. β-amyloid protein; Block and Hong, 2005). Many studies indicate that superoxide and hydrogen peroxide are critical for both the intensification and induction of microglia-mediated neurotoxicity. Qin *et al*. (2004) observed that LPS-mediated loss of nigral DA neurons *in vivo* and *in vitro* was significantly less in PHOX (NADPH oxidase)-deficient ($PHOX^{-/-}$) mice compared with wild-type ($PHOX^{+/+}$) mice. This illustrates the essential role of PHOX in mediating inflammation-associated neurotoxicity. In the same study, it was demonstrated that $PHOX^{+/+}$ and $PHOX^{-/-}$ neuron and glial cultures that were chemically depleted of microglia did not show any DA cellular neurotoxicity in the presence of LPS (Qin *et al*., 2004). It therefore seems to be the case that

microglia are a major source of PHOX, which is responsible for the initial oxidative insult and subsequent neurodegeneration in these models (Min *et al*., 2004; Wu *et al*., 2005).

Furthermore, it has been shown that the activation of PHOX contributes to over 50% of the LPS-induced increase in intracellular ROS (Qin *et al*., 2004) and that intracellular ROS is significant for the activation of microglia and the production of proinflammatory mediators such as TNF-α (Qin *et al*., 2004) or prostaglandin E_2 (PGE_2) (Block and Hong, 2005). Moreover, although LPS increased the expression of molecules such as IL-1, IL-6, TNF-α, iNOS and MAP kinases, the phosphorylation of NF-κB was inhibited by the addition of NADPH oxidase inhibitors and catalase (Pawate *et al*., 2004). Gao *et al*. (2003a, b) have shown that neuron-glia cultures from mice lacking functional NADPH oxidase have decreased DA toxicity in the presence of MPTP and its active metabolite MPP+. This suggests that the formation of extracellular superoxide contributes to MPP+ and MPTP-induced toxicity (Gao *et al*., 2003a).

Thus, NADPH oxidase is heavily involved in microglia-induced neurotoxicity as a result of either generating extracellular ROS or by increasing microglial intracellular ROS production, which activates the generation of proinflammatory mediators, which are subsequently toxic to neurons.

Reactive microgliosis

Microglial activation following some form of insult to the CNS has been generally regarded as a transient self-regulating phenomenon. However, evidence has shown that activated microglia-induced inflammation can be both sustained and progressive (Gao *et al*., 2002b, 2003a; McGeer *et al*., 2003). This type of cycle consists of microglial activation that leads to neurodegeneration and neuronal injury, which may in turn lead to further reactive microglial activation. Such events may be particularly damaging to the DA neurons in the SN (Liu *et al*., 2003), which contains four to five times more microglial cells compared with other areas of the brain (Kim *et al*., 2000), and consequently has increased susceptibility to immunological insult (Langston *et al*., 1999; McGeer *et al*., 2003).

Furthermore, DA neurons show evidence of decreased antioxidant ability because of having relatively low intracellular glutathione, making them more susceptible to oxidative stress and microglial activation when compared with other neurons in the CNS (Loeffler *et al*., 1994). Activated microglia under the influence of T cells produce ROS (Gao *et al*., 2003a; Wu *et al*., 2003), as well as proinflammatory prostaglandins and cytokines, which cause further disease progression (Arai *et al*., 2004, 2006; Lucas *et al*., 2006).

Effects of developmental exposure to proinflammatory agents

Recent reports indicate that prenatal infections may represent a risk factor for PD, as systemically administered LPS is known to enter the chorioamniotic environment, and Ling *et al*. (2002) have observed that prenatal LPS exposure causes loss of DA neurons in the post-natal rat midbrain. Bacterial vaginosis (BV) is a fairly common condition in humans, occurring in pregnancy and associated with overgrowth of Gram-negative bacteria, a source of LPS (Thorsen *et al*., 1998). Thus, BV has been linked with elevated levels of LPS and IL-1β in the chorioamniotic surroundings, and BV is also implicated in a variety of neurological disorders, such as white matter damage, intraventricular haemorrhage and cerebral palsy (Ando *et al*., 1988; Dammann and Leviton, 1997; Ling *et al*., 2002). As it is known that the SN has a higher sensitivity to LPS than other regions of the brain (Kim *et al*., 2000), and given the relatively unformed state of the foetal BBB, this raises the possibility that prenatal neuroinflammation may predispose to a higher risk of PD in later life. Such a possibility could in part explain the apparently random epidemiology of idiopathic PD, as a prenatal cerebral infection with no symptomology at birth would not be connected to the future development of PD. Indeed, prenatal infection in rats leads to a more prolonged response to inflammatory stimuli in the adult (Ling *et al*., 2006). In contrast, treatment with the antioxidant *N*-acetylcysteine reduced LPS-induced increases in IL-6 and IL-10 in rat amniotic fluid (Beloosesky *et al*., 2006), the latter being protective against inflammation-mediated degeneration of dopaminergic neurons in the SN (Arimoto *et al*., 2006).

Mechanisms and mediators of inflammatory-mediated neurotoxicity in PD

The involvement of inflammation in PD was initially suggested by McGeer *et al*. (1988) when they described the upregulation of major MHC molecules in PD patients. Similarly, increased levels of β2-microglobulin in the striatum of PD patients were reported (Mogi *et al*., 1995). Subsequently, a wide range of other proinflammatory factors has been implicated in the process (Arai *et al*., 2006). An increased density of microglial cells expressing iNOS in the SN of Parkinsonian patients compared with control patients has been observed (Hunot *et al*., 1996; Knott *et al*., 2000). Furthermore, nitrite levels (an index of NO production) have been shown to be elevated in the CSF of patients with PD (Quershi *et al*., 1995). The cytotoxic mechanism that involves the activation of iNOS has been widely described. iNOS is known to mediate NO production, which has been shown to cause neuronal toxicity (Dawson *et al*., 1993). Thus, it is suggested that toxic NO levels may occur in close proximity to dopaminergic neurons. It is believed that NO can react with superoxide radicals forming the highly toxic peroxynitrite, which causes nitration of tyrosine residues (3-nitrotyrosine) on cellular proteins resulting in both structural and functional alterations (Ischiropoulos *et al*., 1992). The significance of this neurotoxic mechanism is supported by studies that demonstrate increased 3-nitrotyrosine immunostaining in Lewy bodies in PD patients (Good *et al*., 1998) and also the presence of increased 3-nitrotyrosine in MPTP-lesioned mice (Schultz *et al*., 1995; Pennathur *et al*., 1999).

NO has also been shown to release iron from its intracellular buffering protein ferritin, thus leading to the build up of free iron (Reif and Simmons, 1990). Free iron

participates in the Fenton reaction to produce highly reactive hydroxyl radicals, which have been shown to extensively damage cells (Hirsh *et al*., 2001). Furthermore, the deletion of inflammatory iNOS using gene targeting has been shown to have a neuroprotective effect in MPTP-treated mice (Liberatore *et al*., 1999; Dehmer *et al*., 2000).

In rodent glial cells, the proinflammatory cytokines TNF-α, IL-1β and IFN-γ cause potent activation of iNOS (Hunot *et al*., 2001). However in humans, some studies have failed to show increased iNOS expression in microglial cells using proinflammatory cytokines (Peterson *et al*., 1994; Ding *et al*., 1997). On the other hand, it has been reported that in other patient groups cytokine-stimulated expression of the low-affinity IgE receptor CD23 parcipitates in the activation of iNOS, with the subsequent release of NO (Hunot *et al*., 1999), and that CD23 is expressed exclusively in glial cells in the SN of PD patients compared with control groups (Hunot *et al*., 1999). Collectively, such studies have indicated that cytokine/CD23-dependent activation of iNOS in microglial cells may be involved in the cascade of events leading to DA cell death (Hirsh *et al*., 2001). However, the principal ligand for CD23-IgE has not been detected in the SN of PD patients and it is thought that other likely ligands such as the α chain CD11b and CD11c of the adhesion molecules CD11b/CD18 and CD11c/CD18 may trigger CD23 activation in PD (Bonnefoy *et al*., 1996). Additionally, PGE_2, produced by COX-2, can induce an intraneuronal toxic effect directly on dopaminergic neurons (Gao *et al*., 2003c). TNF-α is secreted by glial cells surrounding dopaminergic neurons in the SNc of PD patients (Boka *et al*., 1994; Hunot *et al*., 1999), whereas *in vitro* and *in vivo* studies using TNF-R1 and TNF-R2 knockout mice report that activation of TNF-R1 is toxic to neurons, whereas activation of TNF-R2 has a neuroprotective effect (Fontaine *et al*., 2002; Kassiotis and Kollias, 2001). This suggests that TNF-α may act through its receptors to cause dopaminergic loss by activating apoptotic transduction pathways. Increased levels of TNF-R1 receptor have been shown to be present in the SN of PD patients compared with control groups (Mogi *et al*., 2000). In rat primary DA cell cultures, TNF-α-induced cell death takes place at concentrations similar to those produced by microglia in cultures (Clarke and Branton, 2002). Additionally, the toxic effects of LPS are reduced by about 50% after the addition of neutralizing antibodies to TNF-α in rat primary dopaminergic neurons (Gayle *et al*., 2002).

One possible factor in the TNF-α-induced apoptotic pathway may involve the trimerization of TNF-R1 through binding of TNF-α. This trimerizatiuon occurs through the adapter proteins TNF-R1-associating protein with a death domain and Fas-associating protein with a death domain. This leads to the activation of caspase-8 through autoproteolysis, which may subsequently either cleave effector caspases such as caspase-3 directly or intensify the death signal through translocation of Bid, a proapoptotic member of the B-cell lymphoma family, to mitochondria with the resulting release of cytochrome *c* from the mitochondrial intermembrane space into the cytosol (Hengartner, 2000). Cytochrome *c* release ultimately also triggers caspase-3 activation, thus inducing the proteolysis of a large number of crucial nuclear and extranuclear cellular constituents (Hirsh *et al*., 2001).

It has also been suggested that Fas and TNF-R1 receptor may also stimulate the ceramide pathway, as increased ceramide production has been reported following TNF-R1 receptor activation (Obeid *et al*., 1993). Ceramide is a breakdown product of sphingomyelin, a key cellular membrane phospholipid that is hydrolyzed by sphingomyelinases (Testi, 1996). Ceramide has been shown to act as a cellular second messenger and has been reported to induce apoptosis in a range of cell systems (Testi, 1996). FAN is a WD-40 repeat protein, which mediates the activation of ceramide from sphingomyelin hydrolysis, thus enhancing caspase processing and hence TNF-activated apoptosis (Adam-Klages *et al*., 1996). Ceramide has been reported to activate the Ras/MAP kinase pathway, which is associated with proapoptotic signaling (Verheij *et al*., 1996), and increased release of activated caspase-8 was observed in SN of mice that were subchronically administered MPTP (Hirsh *et al*., 2001). These results indicate that caspase-8 activation occurs before dopaminergic cell death, as MPTP-mediated death of DA neurons can be demonstrated at least 1 week after the end of subchronic treatment (Tatton and Kish, 1997).

Peripheral granulocyte infiltration into brain across the BBB

The BBB is composed of complex microvascular endothelial cells that are interconnected by tight junctions, which prevent the movement of most blood-borne cells and molecules (Prat *et al*., 2001). In the healthy CNS, only a few T lymphocytes are normally found circulating in the neuronal parenchyma and thus their passage into the brain is limited by the presence of the BBB (e.g. Carvey *et al*., 2005). It is thought that under conditions of inflammation, microglial release of proinflammatory cytokines acts on the endothelium of BBB cells to stimulate upregulation of VCAM-1 and ICAM-1 (Neumann and Wekele, 1998). Consequently, this upregulation leads to the recruitment of passing T cells and monocytes, which express the counter receptors including CD11a/CD18 (LFA-1) and very late antigen-4 (Neumann and Wekele, 1998), which go on to release more cytokines.

Previously, damage to the BBB has been demonstrated in systemic infections as a result of the activation of various mediators that cause multiple organ failure including the brain (Herrera *et al*., 2005). Significantly, dysfunction of the BBB has been shown in PD patients who had significantly reduced function of the molecular pump *p*-glycoprotein (Kortekaas *et al*., 2005). Additional evidence comes from the systemic injection of LPS, which can cause a functional breakdown of the BBB leading to granulocyte infiltration into the mouse brain (Bohatschek *et al*., 2001), suggesting that systemic infection and raised LPS can have an indirect but profound effect on neurons in the CNS. Other external factors such as infection, stroke and trauma may also disturb the BBB, which can lead to the extravasation of substances and the activation of microglia (Carvey *et al*., 2005; Herrera *et al*., 2005) and microglia of blood origin may also activate

the immune system and thus lead to CNS damage (Simard and Rivest, 2004). For example, it has been reported that bone marrow-derived microglial cells add to the neuroinflammatory response and express iNOS in the MPTP mouse model of PD (Kokovay and Cunningham, 2005). These findings indicate that systemic infection may be a significant risk factor in the genesis of PD resulting from a breakdown of the BBB and passage of proinflammatory factors into the SN (Carvey *et al*., 2005).

Complement activation

The complement system is thought to enhance the effectiveness of both the nonspecific and specific immunological defences. It is designed to destroy invading pathogens, encourage inflammation and support in the phagocytosis of waste materials (Rus *et al*., 2006). There are numerous complement proteins, mostly present in blood and tissue fluids as soluble monomers. Activation leads to a cascade of events culminating in the destruction of the microbial cell surface with three pathways recognized, which converge at the level of the C3 protein (Bonifati and Kishore, 2006).

It has been shown that once the complement cascade is activated, it produces anaphylatoxins that promote further inflammation, opsonising components, which stain material for phagocytosis and producing the membrane attack complex (MAC). MAC is designed to insert into cell membranes, causing organelles to leak and thereby inducing cell death. Furthermore, although the MAC is intended to destroy foreign cells and viruses, it is thought that nearby bystander host cells are also at significant risk of lysis, if they are not protected (McGeer and McGeer, 2005; Bonifati and Kishore, 2006).

Complement proteins, together with all the components of the MAC, have been demonstrated intracellularly on Lewy bodies and on oligodendroglia in the SN in sporadic PD (Yamada *et al*., 1992) and familial PD (Yamada *et al*., 1993). Complement may also promote glial secretion of inflammatory cytokines (O'Barr and Cooper, 2000). Additionally, the presence of MAC has been identified in PD (McGeer and McGeer, 2005), and elevated levels of C-reactive protein, an inflammation marker, has been shown in the SN of PD patients (McGeer and McGeer, 2004). Overall, it seems clear that there is a role for the complement system in inflammation-mediated neurodegeneration and PD (McGeer and McGeer, 2004; Bonifati and Kishore, 2006), an observation strongly supported by the finding that complement activation can be demonstrated on Lewy bodies in the SN of PD patients (Loeffler *et al*., 2006).

The potential role of anti-inflammatory drugs in PD

Inflammation is clearly primarily a beneficial phenomenon and in the CNS it seems to be a time- and site-specific defence mechanism designed to eliminate irreversibly damaged neurons. However, at later stages, it has been shown that inflammation can develop as an uncontrolled chronic process. Promising experimental data suggest that inhibition of the inflammatory response may reduce the degeneration of DA-neurons in numerous models of PD (Gao *et al*., 2003c).

The anti-inflammatory steroid dexamethasone was reported to inhibit microglial reaction, decrease the production of proinflammatory cytokines and NO, thereby reducing the degeneration of DA neurons induced by MPTP, indicating that inhibition of NF-κB could be a therapeutic target in PD (Kukowska-Jatrzebska *et al*., 1999). However, this has not been supported in another study, which reports that dexamethasone failed to protect DA neurons against MPTP-induced toxicity (Aubin *et al*., 1998). In addition, non-steroidal anti-inflammatory drugs (NSAIDs) have been reported to be potential agents in the treatment of PD. Sodium salicylate, a non-selective COX enzyme inhibitor, has been observed to significantly lessen MPP^+-induced striatal DA depletion (Sairam *et al*., 2003). However, although COX-2 inhibitors have been suggested to prevent the degeneration of DA neurons induced by MPTP (Teismann and Ferger, 2001; Sairam *et al*., 2003; Teismann *et al*., 2003a, b), there are differences between these studies perhaps as a result of using different administration protocols, which make it difficult to obtain definitive conclusions. Similarly, meloxicam, a selective COX-2 inhibitor, was shown to decrease significantly dopaminergic cell loss in mice administered MPTP (Teismann and Ferger, 2001).

A large cohort study of patients has shown that the risk of developing PD in regular NSAID users (for cardiovascular protection) was decreased by up to 45% compared with those who take NSAIDs on a non-regular basis (Chen *et al*., 2003). Thus, it is suggested that the use of NSAIDs may lead to neuroprotection in PD (Hald and Lotharius, 2005). However, the neuroprotective effect of COX-2 inhibitors against MPTP *in vivo* may not be due to the reduced microglial activation, but rather has been linked to inhibition of COX-induced DA oxidation (Teismann *et al*., 2003a, b). Indeed, this effect may be of considerable importance, given that DA oxidation itself may trigger neurodegeneration in PD (see above).

It was shown that minocycline, a tetracycline derivative, decreased the production of inflammatory cytokines, such as IL-1β, as well as iNOS and NADPH oxidase, compared with levels in untreated animals (Wu *et al*., 2002). Similarly, minocycline was shown to have neuroprotective effects on dopaminergic neurons in MPTP, LPS and 6-OHDA models of PD (Du *et al*., 2001; He *et al*., 2001; Wu *et al*., 2002; Tomas-Camardiel *et al*., 2004). Collectively, this would seem to lend strong support to the potential use of anti-inflammatory drugs in PD. However, such drugs could only be given once the symptoms present and their effects would be to restrict further neurodegeneration and both steroids and NSAIDs have unpleasant and potentially dangerous side effects when given over a long period of time. It may indeed be the case that patients who are on long-term treatment with NSAIDs for conditions such as cardiovascular disease will be protected, but in these individuals the assessment of an anti-PD-like effect is fortuitous and not planned. Although arresting disease progression in PD would be a laudable achievement, reversing the severity of the lesion would clearly be the desired goal. Given that around 95% of PD sufferers have no way of predicting the disease, there would

probably be marked limits to NSAIDS effectiveness as they would have to be given before lesion development to have a significant impact. Thus, administration of 'protective' substances in animal models of PD usually commences before or at the same time as the toxic insult is given (Di Matteo *et al*., 2006). With no current way of predicting susceptibility to idiopathic PD, there is therefore no obvious way in which this could be successfully achieved in patients. Nevertheless, a significant decrease in the rate of degeneration even after PD is diagnosed would constitute clear progress and afford a greater degree of freedom in prescribing current treatments to better suit the needs of the patient.

Conclusion

PD is a relatively common neurodegenerative disease with a well-characterized group of symptoms. Although a number of different mechanisms have been proposed in the aetiology of PD, none have been considered to have absolute predominance. In part, this has been due to the difficulty in establishing any clear epidemiology for idiopathic PD, which is responsible for some 95% of cases. Indeed, on the face of it, idiopathic PD appears to strike at random with no apparent pattern either geographically or among patient groups. The observations in patients and experimental models of PD that neuroinflammation appears to be one of the, if not the, most consistant pathological change, opens new possibilities in understanding this illness. The CNS had been considered to be a relatively immunologically quiescent organ but this is now undergoing a considerable re-evaluation. It has become apparent from a number of lines of research, including a substantial volume relating to PD, that microglial cells can become activated by a wide range of stimuli. Critically, these include infections of peripheral origin. The findings by Ling *et al*. (2002, 2006) that prenatal infection of female rats leads to progressive neuroinflammatory and degenerative PD-like changes in the offspring, following a subsequent LPS challenge, are of great interest. The implication is that a predisposition to SN neurodegeneration may be established before birth or possibly by infections afterwards. That these could in part underlie a disease that might not emerge until decades later could indeed account for the difficulty in 'predicting' PD, as the events would be highly unlikely to be connected. The potential use of anti-inflammatory drugs in PD is interesting but it is hard to envisage great benefit unless the intervention is made with a very early diagnosis leading to the possibility that calming the neuroinflammatory crisis in the SN might at least slow disease progression.

Conflict of Interest

The author state no conflict of interest.

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