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PERSPECTIVE

Neural and Immune Mechanisms in the Pathogenesis of Parkinson's Disease

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Abstract Although almost 50 years have passed since impaired dopaminergic transmission was identified as the main neurochemical defect in Parkinson's disease (PD), the cause of the disease remains unknown. A restricted number of biological mechanisms are likely to contribute to the process of cell death in the nigrostriatal pathway. These mechanisms include mitochondrial defects and enhanced formation of reactive oxygen species—leading to oxidative damage—and abnormal protein aggregation. In addition to or, possibly, intermingled with these mechanisms of neuronal damage there is another crucial factor: neuroinflammation. The inflammatory response associated with cell loss in the dopaminergic nigrostriatal tract and, more in general, the role of immune mechanisms are increasingly recognized in PD pathogenesis. Neuroinflammatory changes have been repeatedly demonstrated, in both neurotoxic and transgenic animal models of PD, as well as in PD patients. Transgenic models based on α-synuclein overexpression, in particular, have provided crucial insights into the correlation between this protein and the dichotomous response that microglia can activate, with the polarization toward a cytotoxic (M1) or cytoprotective (M2) phenotype. Full understanding of such mechanisms may set the ground for a fine tuning of the neuroinflammatory process that accompanies and sustains neurodegeneration, thereby opening new therapeutic perspectives for PD.

Keywords Neuroinflammation · Microglia · Substantia nigra pars compacta · Striatum · Synuclein

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Premise

Parkinson's disease (PD) is the second most common neurodegenerative disease in the general population, after Alzheimer's disease. Age is the main risk factor for PD: lifetime prevalence of the disease in the general population is about 0.2 %; PD prevalence raises to 1–2 % at 65 years and keeps raising with aging, reaching 3–5 % at the age of 85 (de Rijk et al. 1997). As an age-related disorder, PD incidence is destined to increase in the future, due to the progressive aging of the population. This will further enhance the social impact and costs of this condition, thereby justifying the pressing need for a full comprehension of the pathogenic mechanisms. Understanding the pathogenesis of PD is, in fact, indispensable for the development of new therapeutic strategies that may efficiently counteract the disease.

PD pathology and pathophysiology

Two classic pathognomonic markers characterize PD: a) degeneration of melanised, dopaminergic neurons of the substantia nigra pars compacta (SNc) projecting to the corpus striatum; b) the presence, in surviving neurons, of intracytoplasmic, proteinaceous inclusions termed Lewy bodies (LBs); LBs present as spherical, eosinophilic structures with a central, granular core surrounded by a fibrillary halo and positive for protein α-synuclein (Jellinger 2002). The striatal dopaminergic denervation, resulting from SNc cell loss, triggers complex functional modifications within the basal ganglia circuitry. This results in an impairment of voluntary movement execution, which translates into the classic triad of PD motor symptoms: tremor, rigidity and bradykinesia (Blandini et al. 2000). PD is therefore considered the *prototype of movement disorders*.

Other neurotransmitter systems are involved in the disease, including noradrenergic, serotonergic and cholinergic systems

(Jellinger 1991). PD patients are also affected by various *nonmotor symptoms*, including autonomic dysfunctions, sleep disorders, psychiatric symptoms, gastrointestinal dysfunction and cognitive deficits. These symptoms further worsen the quality of life of PD patients and, in some cases, they can precede the onset of the motor manifestations (Gallagher et al. 2010; Lim and Lang 2010; O'Sullivan et al. 2008; Zesiewicz et al. 2006). Autonomic dysfunctions (particularly urinary) seem to be the more frequent nonmotor symptom reported by PD patients (Cheon et al. 2008; Gallagher et al. 2010). Although the impairment of voluntary movement execution remains the crucial feature of PD, nonmotor symptoms are now considered an integral part of the clinical presentation and their potential link with specific aspects of PD pathogenesis is being increasingly recognized. The clinical heterogeneity of PD has been further pointed out by a recent study, conducted in two wide cohorts of patients, which proposed the existence of different clinical subtypes of PD, according to the relative weight of dopaminergic and nondopaminergic symptoms in the clinical presentation (van Rooden et al. 2011).

Treatment of PD is still based on the use of L-3,4-dihydroxyphenylalanine (L-DOPA), the direct precursor of deficient neurotransmitter dopamine. Although almost five decades have passed since L-DOPA was introduced into the clinical practice, this drugs still represents the cornerstone of PD treatment. To avoid peripheral transformation into dopamine, which does not cross the blood–brain barrier, L-DOPA is administered in combination with peripheral dopa decarboxylase inhibitors benserazide or carbidopa. Once in the brain, L-DOPA is taken up by surviving nigrostriatal neurons, transformed into dopamine and released by pre-synaptic terminals at the striatal level. Long-term treatment with L-DOPA, however, is associated with the occurrence of numerous complications, extremely discomforting for the patient. With time, drug's efficacy begins to fade, leading to insufficient control of PD motor symptoms; this translates into progressive shortening (*wearing off*) or complete lack of motor response (*off periods*) to L-DOPA. On the other hand, the peculiar pulsatile nature of L-DOPA kinetics induces molecular changes that impact on the mechanisms regulating synaptic plasticity, at the cortico-striatal level. These changes cause the paradoxical onset of involuntary movements called *dyskinesias*; dyskinesia can become extremely discomforting for PD patients, thereby causing further worsening of their quality of life (Poewe et al. 2010).

PD pathogenesis

Although almost 50 years have passed since the dopaminergic defect was identified as the main neurochemical alteration of PD, the exact cause of the disease remains unknown. An impressive bulk of data has been produced by preclinical and clinical studies in the past decades, but the primary cause of the degenerative process underlying PD remains elusive. The recent recognition that 10–15 % of PD cases are linked to mutations in selected genes has generated formidable insights into PD pathogenesis, while further complicating the general picture.

To date, no single causative factor has yet been identified for sporadic PD, which represents the vast majority of diagnosed cases. Indeed, a multi-factorial nature of the disease has clearly emerged, with a restricted number of biological mechanisms contributing to the process of nigral cell death. These mechanisms include mitochondrial defects and enhanced formation of reactive oxygen species (ROS)—two conditions which are intimately connected—and abnormal protein aggregation (Greenamyre and Hastings 2004), with α-synuclein as the main culprit. These mechanisms will be briefly outlined in this review. A substantial body of evidence also suggests that, in addition to or, possibly, intermingled with these mechanisms, another factor may contribute to the neuronal loss underlying PD: neuroinflammation. The inflammatory response associated with the onset and progression of cell loss in the dopaminergic nigrostriatal tract and, more in general, the role of immune mechanisms are being increasingly recognized as crucial issues in the pathogenesis of PD.

Mitochondrial defects and oxidative stress

The role of mitochondrial defects in PD pathogenesis was first proposed when the mechanism of action of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was clarified. In the early 1980s, Langston et al. (1983) reported the occurrence of marked parkinsonism in young drug users from Northern California; the syndrome was caused by the intravenous injection of a street preparation of an analog of narcotic meperidine containing, in fact, MPTP. It was demonstrated that, after crossing the blood–brain barrier, MPTP is transformed by monoamine oxidase B into its active metabolite, 1-methyl-4-phenylpyridinium ion (MPP+); MPP+ is then carried by the dopamine transporter into dopaminergic neurons of the SNc, where it blocks mitochondrial complex I activity (Heikkila et al. 1985; Javitch et al. 1985). This discovery pointed to the mitochondria of dopaminergic neurons as a preferential target of toxicity, paving the way to myriads of studies that explored mitochondrial function in PD, in the following decades. Postmortem studies showed reduced complex I activity in the SNc of PD patients (Parker et al. 1989; Schapira et al. 1990). This reduction was also observed, with a lesser magnitude, in peripheral blood cells of PD patients, as well as in cytoplasmic hybrid ("cybrid") models. In this latter case, human neuroblastoma cells deprived of mitochondrial DNA

were re-populated with mitochondria derived from the platelets of PD or control subjects. PD cybrids showed stable reduction in complex I activity, increased oxygen radical production, and increased susceptibility to MPP+-induced programmed cell death (Swerdlow et al. 1996).

The concept of mitochondrial impairment in PD has evolved with the evolution of our understanding of mitochondrial functions. A crucial step in this process was the recognition that mitochondria are dynamic organelles undergoing a complex quality control, in which mechanisms known as *fission* and *fusion* play a central role (Cho et al. 2010). *Fission*, which is usually required in dividing cells to ensure inheritance of mitochondria by daughter cells, is also a normal process in post-mitotic cells, such as neurons. *Fusion* is crucial to maintain a tubular network of mitochondria and optimal mitochondrial function, since a mitochondrial network can facilitate the transfer of mitochondrial membrane potential from oxygen-rich to oxygen-poor cellular regions. Neurons are cells with high energy demands and an efficient mitochondrial network is crucial for satisfying such necessity. Mitochondria play a central role, in particular, at the terminus of dopaminergic neurons. At this level, mitochondria are critical for calcium buffering and for providing the energy required for maintaining the vesicular pool of dopamine, as well as for granting the energy necessary for the release and re-uptake of the transmitter (Van Laar and Berman 2009). Any condition that hampers fission/fusion dynamics limits mitochondrial motility and decreases mitochondrial energy production. This causes oxidative damage—also to mitochondrial DNA—and impairs calcium buffering. All these conditions can lead to neuronal death (Van Laar and Berman 2009).

The SNc is exposed to high rates of ROS formation. This is mostly dependent on the abundance of cytoplasmic dopamine, which is prone to autoxidation, and the high iron content typical of this nucleus (Jenner and Olanow 1996). These conditions create an unfavorable environment for mitochondria, whose density and mass are lower in SNc neurons (Liang et al. 2007). The massive oxidative catabolism of dopamine can impair mitochondrial respiration and membrane permeability (Berman and Hastings 1999; Premkumar and Simantov 2002). Moreover, dopamine can modify several chaperones involved in mitochondrial protein quality control (Van Laar and Berman 2009).

Investigation of mutant proteins in familial PD has further refined the understanding of the mitochondrial involvement in PD. In fact, virtually all proteins involved in genetic PD are associated with mitochondria, although with different functional roles. Leucine-rich repeat serine/threonine kinase 2 (LRRK2), which is mutated in the most frequent form of autosomic dominant PD, PARK8, and α-synuclein (PARK 1/4) have been found associated with mitochondria outer membrane. DJ-1 (PARK7) is a cytosolic protein that functions as an oxidative stress sensor and translocates to mitochondria under oxidative conditions. PTEN-induced putative kinase 1 (PINK1), a mitochondrial kinase that mutates in the PARK6 form of genetic PD, can localize to both cytoplasm and mitochondria. Parkin, which is mutated in the most frequent recessive form of genetic PD, PARK2, is a cytosolic protein (a ubiquitin ligase), which can translocate to mitochondria after phosphorylation by PINK1. PINK 1 and parkin, in particular, play a central role in the mitochondria quality control, as their interaction regulates mitochondrial morphology by promoting mitochondrial fission (Yu et al. 2011). More recently, it has been demonstrated that also α-synuclein can interfere with the fusion/fission processes, causing a decline in mitochondrial respiration and neuronal death (Gui et al. 2012; Xie and Chung 2012).

Mitochondrial defects and oxidative stress are strictly correlated. In fact, every condition that impairs mitochondrial function enhances ROS formation. Therefore, it is not surprising that numerous post-mortem studies have shown increased oxidation of proteins, lipids, and DNA in PD brains (Alam et al. 1997; Jenner et al. 1992; Jenner and Olanow 1996). Moreover, all major neurotoxins used to replicate PD pathological features in animals, such as MPTP/MPP+, 6-hydroxydopamine, rotenone or paraquat, are mitochondrial toxins and cause massive oxidative damage in the nigrostriatal tract.

Protein aggregation

The presence of LBs in PD brains is the direct consequence of the aggregation of α-synuclein, the most important protein linked to PD (Spillantini et al. 1997; Spillantini et al. 1998). The normal function of α-synuclein is only partially understood. The synaptic location of the protein and electrophysiological findings indicate that α-synuclein may be involved in neurotransmission and plasticity mechanisms. The protein interacts, in particular, with dopaminergic neurotransmission. In fact, α-synuclein seems to regulate a) activity of enzymes involved in dopamine synthesis, such as tyrosine hydroxylase and aromatic amino acid decarboxylase; b) synaptic vesicle function and dopamine release into the synaptic cleft; c) trafficking of dopamine transporter to the cell surface (Venda et al. 2010). Furthermore, α-synuclein has recently been found to promote assembly of the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complex, which plays a crucial role in the mechanisms of vesicle release by pre-synaptic terminals (Burre et al. 2010).

Various mechanisms, acting at different levels, are likely to intervene in triggering the pathological aggregation of α-synuclein. As a detailed description of these mechanisms goes beyond the purpose of this review, they will be briefly

summarized as follows: a) *gene mutations* (applying only to PD familial forms PARK1 and 4); b) *post-translational modifications*; c) *defects in protein degradation pathways*.

Gene mutations Autosomal dominant mutations (A53T and A30P) in the SNCA gene encoding for α-synuclein were the first mutations ever described in patients with a familial form of PD (Kruger et al. 1998; Polymeropoulos et al. 1997); another mutation can affect the SNCA gene (E46K), causing a form of PD with LB dementia (Zarranz et al. 2004). These mutations are incorporated in the PARK1 form of genetic PD. Duplication and triplication of the wild type gene, leading to overproduction of the protein, have also been described in familial forms of PD and classified as PARK4 (Ibanez et al. 2009; Singleton et al. 2003). All these conditions are associated with an increased propensity of α-synuclein to aggregate within the cell. More recently, genome-wide association studies (GWAS), an increasingly popular approach for identifying genetic factors influencing complex diseases, have explored the potential role of common variants of the SNCA gene in sporadic PD. Multiple GWAS conducted in large cohorts of patients, from different ethnic groups and multiple geographic areas, have independently identified various polymorphisms in the SNCA gene that are associated with an increased risk of developing sporadic PD. Similar associations have also been demonstrated for other genes, such as the *microtubule associated protein tau* (MAPT) or LRRK2. These findings suggest that the influence of genetic factors may extend well beyond the confined borders of mendelian forms (Chang et al. 2011; Liu et al. 2011; Satake et al. 2009; Simon-Sanchez et al. 2009; Simon-Sanchez et al. 2011).

Post-translational modifications Phosphorylation, oxidation or nitration represent other conditions favoring α-synuclein aggregation (Beyer 2006). Phosphorylation of α-synuclein at serine 129 is a typical feature of LBs in PD patients, which enhances protein-mediated neurotoxicity (Chen et al. 2009; Chen and Feany 2005). Given the role of oxidative stress in PD pathogenesis, it is not surprising that LBs have also been shown to contain oxidatively modified α-synuclein, which tends to aggregate far more easily that the non-oxidized form (Giasson et al. 2000). Analogously, several environmental toxins—which act mostly causing ROS overproduction at the mitochondrial level—can induce misfolding or aggregation of α-synuclein (Manning-Bog et al. 2002; Uversky et al. 2001).

Defects in protein degradation pathways The third condition that may favor α-synuclein aggregation is represented by defects in the two major cellular degradation pathways, the ubiquitin-proteasome and autophagy-lysosome pathways. Defects in these systems, which have been proposed for PD, would cause decreased α-synuclein clearance, followed by accumulation and aggregation of the protein. For a detailed description of these mechanisms, the reader can refer to three review articles recently published on this topic (Cook et al. 2012; Cuervo et al. 2010; Xilouri and Stefanis 2011).

Neuroinflammation and PD

The immune response can be of two types: *innate* or *adaptive*. *Innate immunity* represents an immediate, non-specific, first line of defense against any potentially harmful cytotoxic stimulus; *adaptive immunity* relies on delayed cell-mediated, highly specialized responses that aim at removing the threat (Stone et al. 2009). Once believed to be an isolated, "immune privileged" system, the central nervous system (CNS) is in fact capable of activating both types of responses. This may play a central role in PD pathogenesis.

Innate and adaptive immunity in PD

In the CNS, innate immune responses are mainly mediated by microglia, the CNS resident macrophage population, and astrocytes. According to the classic view, in normal brain, deactivated (or resting) microglia are silent elements that become activated in response to pathological events. However, it has recently become apparent that microglia exert an active role of surveillance of the brain microenvironment, even in normal conditions. Microglia show a dynamic and active phenotype, with processes that continuously extend and retract to scan the brain parenchyma. During this patrolling activity, microglial cells are engaged in a permanent cross-talk with neurons, which is important for the development and maintenance of neuronal functions, including plasticity. On the other hand, healthy neurons keep microglia in a quiescent state, both through membrane-bound signals, such as CD200 and CX3CL1, and secreted agents that include neurotransmitters and neurotrophins (for reviews on this topic, see Hanisch and Kettenmann 2007; Raivich 2005). Myriads of danger signals can be sensed by microglia, which are equipped with a wide range of receptors. These receptors include *pattern recognition receptors*, such as toll-like receptors (TLRs), the receptor for advanced glycation end products (RAGE), and scavenger receptors, but also receptors binding neurotransmitters (glutamate, GABA, dopamine, adrenaline), cytokines, chemokines and purines (such as ATP released upon cell death). Once activated, microglia undergo a rapid shift in their phenotype; this cause morphological changes toward a phagocytic phenotype, migration and release of a large number of bioactive substances, including pro- and

anti-inflammatory cytokines, chemokines, growth factors and neurotrophins (Kraft and Harry 2011).

The involvement of innate immunity in PD was first proposed in 1988, when McGeer et al. (1988) reported up-regulation of HLA-DR antigens and the presence of HLA-DR-positive (activated) microglia in the SN of PD patients. Since then, the presence of activated microglia in the areas primarily involved in PD has been repeatedly confirmed (see below). The multi-faceted involvement of innate immunity in PD pathogenesis has been recently reviewed by Huang and Halliday (2012), who also pointed out potential links with genetic PD. Some of the proteins mutated in familial PD, such as parkin (PARK2) or LRRK2 (PARK8), have significant influence on the innate immune signaling. In fact, the cellular mechanisms affected by these mutations (ubiquitination, mitochondrial functioning, endocytosis, synaptic vesicles trafficking) are required for an adequate innate immune response. Moreover, as proposed by Beraud and Maguire-Zeiss (2012), misfolded α-synuclein can directly activate microglia, enhancing the expression of TLRs, which in turn leads to up-regulation of proinflammatory cytokines.

Adaptive immunity, based on delayed cell-mediated responses, is also involved in PD pathogenesis (German et al. 2011). It is now clear that communication between the CNS and peripheral immune system - with passage of immune cells from the periphery to the brain - is a rather common phenomenon. In PD, this has been repeatedly shown by observations conducted in experimental models or PD patients, which showed infiltration of CD4+ and CD8+ lymphocytes at the sites of neuronal damage (see below).

As recently reviewed by Colton (2012), a key link between the innate and adaptive arms of the immune system, in the brain, may be represented by dendritic cells (DCs). DCs are a heterogeneous cell population, deriving from a common hematopoietic progenitor in the bone marrow, which during development enter lymphoid and/or non-lymphoid tissues via the circulation, becoming resident in that tissue. DCs act as antigen presenting cells; once activated, they migrate to the lymph nodes, where they interact with naïve T cells to initiate a T-cell response (Geissmann et al. 2010). In the brain, dendritic cells play an active surveillance role by constantly sampling their local environment, similarly to microglia. The precise function of DCs in the context of neuroinflammation is still undefined; it is also unclear whether DCs in the brain parenchyma come from the periphery or derive from resident microglia. DCs are predominantly located around blood vessels in the brain parenchyma; this "juxtavascular" location supports the hypothesis that brain DCs come from a vascular source, as also suggested by recent experimental findings (Anandasabapathy et al. 2011). The peculiar localization of these cells, between the brain parenchyma and the perivascular space, may allow DCs to present antigens from the brain parenchyma to T-cells within the perivascular space, thereby bridging signals generated by activated microglia to the adaptive arm of peripheral immunity (Colton 2012).

The importance of neuroinflammation and its mediators in PD has strongly emerged in the past decade, as it became evident that a sustained neuroinflammatory response accompanies the disease from its early phases (Glass et al. 2010; Hirsch and Hunot 2009). Inflammatory processes, which normally prompt tissue protection and repair, might become deleterious and amplify cell death in a stressed or intoxicated microenvironment, such as the PD brain. They may contribute, in particular, to the oxidative damage to the nigrostriatal system; this would be related to the massive release of nitric oxide, and subsequent formation of reactive nitrogen species, due to activation of inducible nitric oxide synthase, which is a typical phenomenon associated with microglia activation (Mosley et al. 2012). Therefore, neuroinflammation and the underlying immune mechanisms have been proposed to participate in PD pathogenesis, based on several studies conducted either in patients or in animal models of PD (Fig. 1). The major findings of these studies are summarized below.

Neuroimmune involvement in experimental models of PD

Neuroinflammatory changes have been repeatedly demonstrated in animal models of PD. Virtually all neurotoxins used to replicate the nigrostriatal degeneration that characterizes PD trigger signs of neuroinflammation at the sites of neurodegeneration. The vast majority of findings have been obtained in animals treated with 6-hydroxydopamine (6-OHDA) or MPTP (Hald and Lotharius 2005). Further insights have been provided with the advent of PD transgenic models, particularly of those based on the expression of mutant α-synuclein or overexpression of the wild type protein.

Neurotoxic models

Six-OHDA, a hydroxylated analogue of dopamine with high affinity for the dopamine transporter, is the oldest neurotoxin used to induce nigrostriatal degeneration in rodents (mostly rats). Being unable to cross the blood–brain barrier, 6-OHDA must be injected stereotaxically into the brain. Six-OHDA can be injected at two sites, within the nigrostriatal pathway: 1) at the origin of the medial forebrain bundle, which conveys the dopaminergic fibers originating from SNc cell bodies to the striatum; 2) at the far end of the nigrostriatal pathway, that is, into the striatal terminal fields. The two modalities of injection yields two types of SNc degeneration: massive and extremely rapid in the first case; less marked and more progressive in the second case, with a delay of 1–2 weeks with respect to the striatal damage

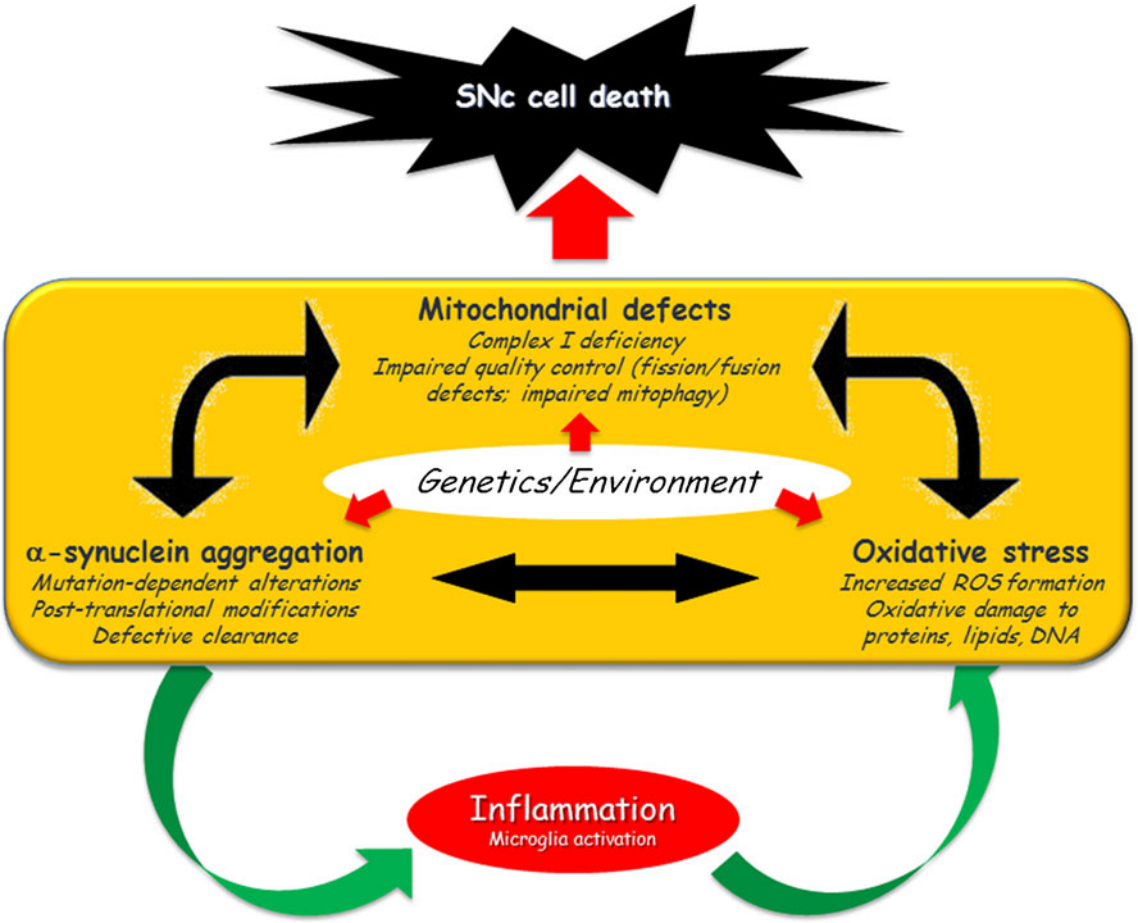

Fig. 1 Schematic representation of the main pathogenic mechanisms of PD. Mitochondrial defects, protein (α-synuclein) aggregation and oxidative stress are intimately linked to each other, in a vicious circle that triggers the neurodegenerative process in the substantia nigra pars compacta (*SNc*). These mechanisms interact over a background where genetic factors (i.e., common variants of specific genes) and/or chronic exposure to environmental agents create predisposing conditions for the development of the disease. Inflammation plays a crucial role, possibly immediately downstream of this vicious circle. In fact, α-synuclein may trigger microglia activation; this, in turn, would fuel the oxidative damage, due to the pro-oxidant conditions associated with microglia activation (nitric oxide release and resulting formation of nitrogen reactive species, in particular). A secondary vicious cycle may, therefore, ensue, which would contribute to cell death in the *SNc*

(Blandini et al. 2008). Regardless of the site of injection, 6-OHDA induced neuronal degeneration is associated with astrocyte and microglia activation in the rat brain, at both nigral and striatal levels (Akiyama and McGeer 1989; Armentero et al. 2006; Cicchetti et al. 2002; Rodrigues et al. 2001; Rodrigues et al. 2004). Microglia activation, in particular, seems to be a very early event; in rats that received intrastriatal 6-OHDA, activated microglia can be detected in the SNc well before the dopaminergic cell loss begins to manifest (Armentero et al. 2006; Marinova-Mutafchieva et al. 2009). Similarly, extensive microglial activation has been reported in the striatum and SNc of rats treated systemically with pesticide rotenone, a potent inhibitor of complex I, before anatomical evidence of dopaminergic lesions was detected (Sherer et al. 2003).

Stereotaxic injection of 6-OHDA can also induce moderate damage of the blood–brain barrier, which under normal conditions exerts a selective control over the passage of blood-borne compounds to the brain. Carvey et al. reported leakage of FITC-labeled albumin or horseradish peroxidase from the vasculature into the nigral or striatal parenchyma, in animals exposed to 6-OHDA (Carvey et al. 2005). Indeed, one of the cardinal signs of inflammation is increased vascular permeability; this largely relies on adhesion molecules, which allow communication amongst adjacent endothelial cells and between endothelium and leukocytes. In line with this concept, we observed complex changes in the expression of intercellular (ICAM-1), vascular (VCAM-1), platelet endothelial (PECAM-1) and neural (NCAM-1) cell adhesion molecules, following striatal 6-OHDA administration; in particular, we observed increased striatal ICAM-1 and PECAM-1 expression coupled with decreased VCAM-1 and NCAM-1 expression at the nigral level. Nigrostriatal degeneration also affected peripheral immune function, with lesioned animals showing increased PECAM serum levels (Armentero et al. 2011).

As for MPTP, systemic administration of the toxin to mice triggers an immune reaction in the SNc and striatum. This reaction includes activation of astrocytes and microglia and infiltration of CD4+ and CD8+ T cells (Czlonkowska et al. 1996; Francis et al. 1995; Kurkowska-Jastrzebska et al. 1999). Infiltration of CD4+ cells, in particular, was proposed to contribute to the neurodegenerative process, as mice lacking CD4+ cells were more resistant to MPTP-induced dopaminergic cell death (Brochard et al. 2009). Interestingly, the role of these cells may vary in different neurodegenerative diseases; for example, CD4+ cells were shown to exert neuroprotection in mice overexpressing mutant Cu(2+)/Zn(2+) superoxide dismutase - a model of familial amyotrophic lateral sclerosis - by stimulating glia trophic properties at sites of motoneuron injury (Beers et al. 2008; Beers et al. 2011). This may be due to the different roles exerted by the different CD4+ T cell subsets, which can contribute either to neuroprotection or to neurotoxicity. In fact, Gendelman's group showed that CD4+CD25+ regulatory T cells (Tregs), which acts as suppressors of immune activation and regulators of immune homeostasis and tolerance, provide neuroprotection when transferred to MPTP-intoxicated mice (Reynolds et al. 2007). The same result is obtained when transferring CD4+ cells from Copolymer 1 immunized mice (Laurie et al. 2007), thereby pointing to the neuroprotective potential of Tregs (Gendelman and Appel 2011).

In MPTP-treated monkeys, reactive gliosis and ICAM-1 over-expression were still detectable, at the site of neurodegeneration, years after the toxin administration (Barcia et al. 2004; McGeer et al. 2003; Miklossy et al. 2006). This proved that a brief exposure to MPTP is sufficient to prompt a prolonged inflammatory response. These results also confirmed previous findings of Langston et al. (1999), who had reported gliosis and clustering of microglia in the SNc of patients intoxicated with MPTP many years before. Age may also be an influencing factor, as older mice show enhanced susceptibility to the toxin and stronger microglia activation, compared to younger mice (Sugama et al. 2003).

Further supporting the role of microglia activation as a favoring agent in the neurodegenerative process, Wu et al. (2002) showed that inhibition of microglial activation by minocyclin can protect against MPTP neurotoxicity. This finding, however, was contradicted a year later by Yang et al. (2003), who reported that the minocyclin-induced blockade of microglia activation was, in fact, enhancing the MPTP-induced damage. The recent findings on the complex mechanisms regulating microglia activation may help explain this apparent discrepancy. In particular, the recognition that specific stimuli can polarize microglia toward two different phenotypes - with neurotoxic or neuroprotective features – is opening new scenarios (see below).

Transgenic models

A recent concept, generated by studies in transgenic PD models, is that misfolded α-synuclein prompts glia activation and production of a wide set of proinflammatory molecules. In vitro, extracellular α-synuclein induces inflammation in human microglial and human THP-1 (human monocytic leukemia) cells; α-synuclein also promotes release of cytotoxic soluble factors (Klegeris et al. 2008; Zhang et al. 2005), this property being enhanced for the A30P, E46K and A53T mutant forms (Klegeris et al. 2008). Aggregated and nitrated α-synuclein, typically found in LBs, also stimulate microglia activation by modifying microglia proteome and triggering innate and adaptive immune responses (Reynolds et al. 2008; Reynolds et al. 2009). In vivo, evidence of microglia activation is a typical finding in mice that over-express wild-type α-synuclein, along with infiltration of B and T lymphocytes in the SNc (Su et al. 2008; Theodore et al. 2008). As mentioned above, Beraud et al. showed that microglia are directly activated by α-synuclein, which is able to alter the microglial expression of TLRs (Beraud et al. 2011; Beraud and Maguire-Zeiss 2012). Furthermore, in a very recent study conducted in mice overexpressing human α-synuclein, Beraud et al. showed that misfolded α-synuclein directly activates microglia, inducing production and release of TNF-α and increasing expression of Nrf2-dependent antioxidant enzymes (Beraud et al. 2012). They also showed that glial activation pattern and antioxidant responses are dependent on the specific structure of misfolded α-synuclein; when BV2 cells, a murine microglial cell line, were exposed to mixtures of different forms of misfolded α-synuclein, massive proinflammatory responses were observed only in the presence of mixtures containing higher levels of low molecular weight α-synuclein conformers (monomers) (Beraud et al. 2012).

Studies conducted in transgenic α-synuclein models have also provided crucial insights into the dichotomous response that microglia can activate. Analogously to macrophages, microglia can undergo *polarization* toward a frankly cytotoxic (M1) or cytoprotective (M2) phenotype (Durafourt et al. 2012; Gordon 2003). This is a fairly recent concept in the field of neurodegeneration, which may help interpret the complexity of the neuroimmune mechanisms associated with PD, also in terms of therapeutic perspectives. It has been reported, for example, that the dynamics of microglia activation and polarization are strongly influenced by the extent of the neuronal death associated with α-synuclein alterations. In this frame, Sanchez-Guajardo et al. (2010) used a recombinant viral vector to express human α-synuclein in rat midbrain, at levels sufficient to induce microglia activation without SNc cell death, or at higher levels that were also causing cell death. They observed fast, transient increases in microglia cell numbers in animals expressing lower levels of α-synuclein; this was followed

by long-term induction of microglia positive for major histocompatibility complex (MHC) class II antigen, which denotes antigen-presenting ability. On the other hand, when α-synuclein expression was high enough to trigger SNc cell death, there was a delayed increase in microglia cell numbers, which correlated with long-lasting expression of phagocytic marker CD68 and a morphology reminiscent of peripheral macrophages. These data have been further confirmed by a very recent study of Barkholt et al. (2012), who analyzed microglia in the midbrain of monkeys overexpressing either A53T or wild type α-synuclein. By stereological quantification of Iba1+ cells, they found a robust increase of microglia population, in both cases, but with distinct phenotypes, depending on the neuronal damage. In fact, overexpression of A53T α-synuclein caused SNc cell death and was associated with long-term increase in macrophagic microglia; conversely, wild type α-synuclein induced moderate neurodegeneration, limited to striatal dopaminergic terminals, and was associated with robust increase of a pluripotent microglia population. Taken together, these data indicate that microglia reacts to the neuronal damage associated with α-synuclein deposition in a multi-modal (at least bi-modal) fashion, with neuroprotective and neurotoxic mechanisms interacting in a complex balance. Such balance could be disrupted by the prolongation of the neurotoxic insult, which triggers microglia-mediated, additional cytotoxicity, resulting in a substantial contribution to the process of neuronal death (Fig. 2).

Neuroimmune involvement in PD patients

Data provided by experimental models of PD are in keeping with the numerous observations reported in PD patients during the past two decades. An increasing body of evidence has been accumulating on the presence of complex inflammatory signs in PD patients. Post-mortem analyses have shown that reactive microgliosis accompanies SNc degeneration in patients with sporadic PD (McGeer et al. 1988; Mirza et al. 2000). As mentioned above, similar findings have been reported in subjects exposed to MPTP many years before (Langston et al. 1999). Positive correlations were observed between disease duration and expression of marker of phagocytic microglia CD68, as well as between the degree of MHC class II expression and α-synuclein deposition in the SNc of PD patients (Croisier et al. 2005). These findings have been confirmed by neuroimaging studies using positron emission tomography (PET) with a radiotracer, isoquinoline derivative [(11)C](R)-PK11195, which binds to activated microglia (Banati 2002). These studies have shown enhanced [(11)C](R)-PK11195 binding in the brain of PD patients, since the early stages of the disease (Bartels and Leenders 2007; Gerhard et al. 2006; Ouchi et al. 2005). More recently, however, Bartels et al. (2010) used [(11)C]-PK11195 PET to investigate microglia activation in PD patients treated with COX-2 inhibitor celecoxib. They failed to report consistent modifications of [(11)C]-PK11195 binding following treatment with celecoxib, which raised concerns on the reliability of this tracer as an accurate means to quantify microglia activation. PK11195 binds to translocator protein 18 kDa (TSPO, formerly known as the *peripheral benzodiazepine receptor*), which is expressed by activated microglia. TSPO, however, may also be present on immunocompetent cells infiltrating the brain areas involved in PD; moreover, affinity of [(11)C]-PK11195 for TSPO, which is high in vitro, seems to be lower in vivo. An intensive search for new ligands, provided with higher affinity for TSPO or directed toward different molecular targets expressed by activated microglia, is currently ongoing (Venneti et al. 2013).

ICAM-1-positive reactive astrocytes have been found in the SNc of PD patients, particularly in areas of heavy neuronal loss and extracellular melanin accumulation (Miklossy et al. 2006). Infiltration of CD8+ and CD4+ T cells, but not B cells, has also been reported (Brochard et al. 2009). These latter findings confirmed previous observations conducted in experimental PD models, indicating alterations of the blood–brain barrier following either local or systemic administration of toxins used in experimental PD models. This may further favor the passage of immune cells from the peripheral circulation into the brain parenchyma. Further supporting a role for innate immunity in PD pathogenesis, in a recent GWAS, Hamza et al. (2010) found that common genetic variation in the HLA region is associated with sporadic (late-onset) PD, with peak significance at the rs3129882 polymorphism in intron 1 of the major histocompatibility complex cell surface receptor (HLA-DRA) gene on chromosome 6.

While confirming the presence of activated microglia in the SNc, Mirza et al. failed to demonstrate clear signs of astroglial activation, which is present in virtually any other neurological disorder, as well as in animal PD models; therefore, they proposed that the progressive SNc loss of dopaminergic neurons in PD may be associated with a peculiar inflammatory process, characterized by a minimum involvement of the surrounding nervous tissue (Mirza et al. 2000).

Increased levels of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL−6 and interferon (IFN)-γ, have been detected at the sites of microglia activation, as well as in the cerebrospinal fluid (CSF) and serum of PD patients (Boka et al. 1994; Brodacki et al. 2008; Mogi et al. 1994a, b; Mogi et al. 1995). Concomitantly, increased levels of anti-inflammatory/cytoprotective cytokines, such as IL-10 and transforming growth factor (TGF)β, have been reported in the serum (Brodacki et al. 2008; Rentzos et al. 2009) and CSF (Mogi et al. 1995; Vawter et al. 1996) of PD patients. This apparent discrepancy further points to the

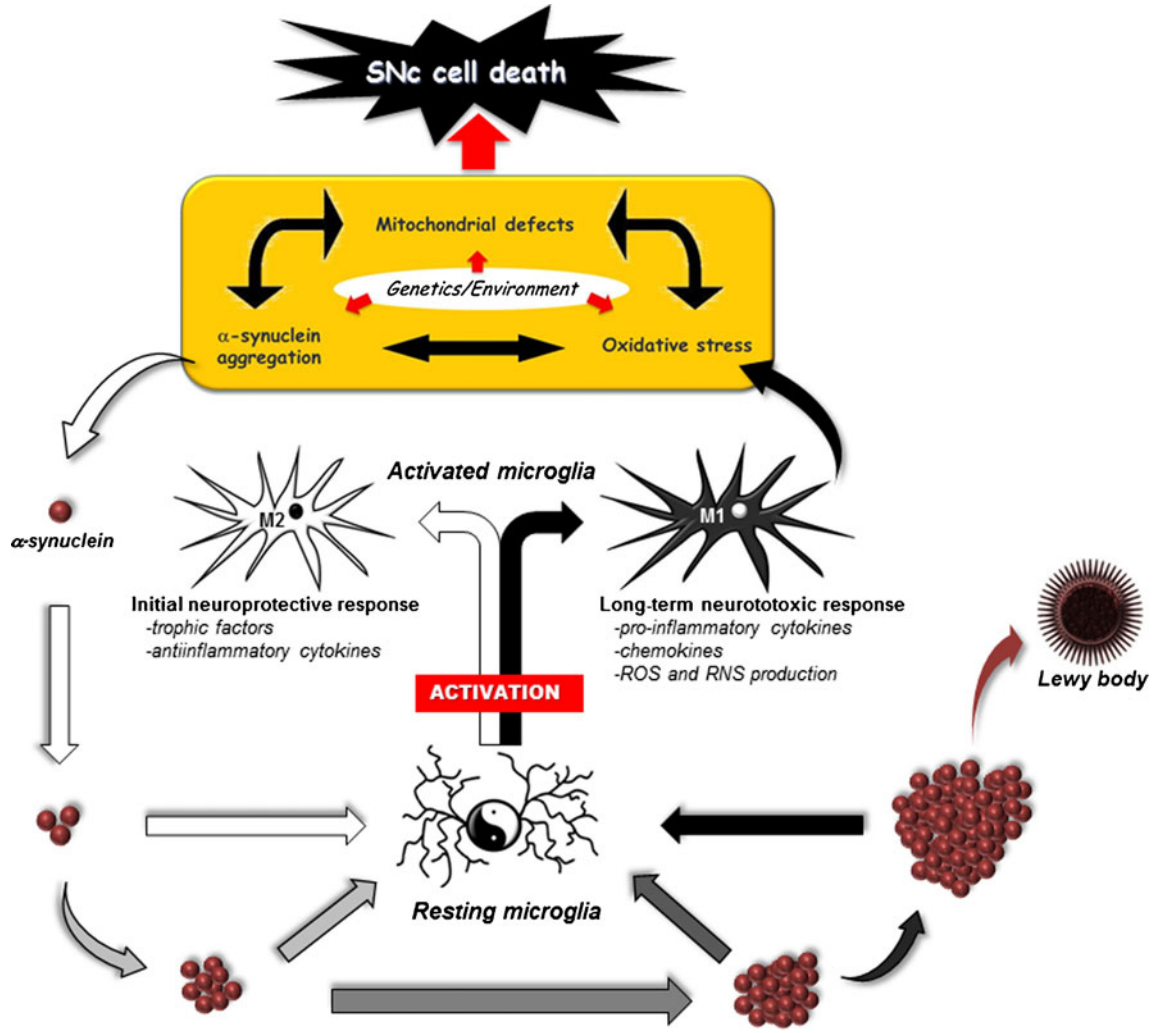

Fig. 2 Role of microglia activation in the pathological cascade of PD. Progressive accumulation of α-synuclein may trigger a dichotomous response in microglia. At an early stage of the disease, α-synuclein aggregates may polarize microglia activation toward a neuroprotective phenotype (M2); pro-survival factors may be released, as an initial protective response against cellular stress. However, the relentless accumulation of insoluble α-synuclein (eventually leading to the formation of Lewy bodies) would maintain a state of chronic activation of microglia, which would progressively evolve toward a neurotoxic phenotype (M1). This would be associated with the release of pro-inflammatory cytokines, chemokines and reactive oxygen (ROS) and nitrogen species (RNS). The resulting oxidative stress would substantially contribute to the pathological cascade of events leading to nigral cell death

complexity of the inflammatory response associated with the disease, where neurotoxic and neuroprotective mechanisms interact in a delicate balance.

A less direct support to the role of inflammation in PD has been provided by earlier epidemiological studies, which reported reduced risk of developing PD among chronic users of nonsteroidal anti-inflammatory drugs (NSAIDs) (Chen et al. 2003; McGeer and McGeer 2007). Other observations have lent further (Wahner et al. 2007) or limited support (Ton et al. 2006) to such correlation. More recent studies, however, tend to deny this association (Becker et al. 2011; Bornebroek et al. 2007; Driver et al. 2011; Etminan et al. 2008; Rugbjerg et al. 2010), suggesting that a number of confounding factors may have influenced earlier results. In a recent overview, Rees et al. (2011) conducted an analysis of electronic databases - including trial registers - complemented with a search of conference proceedings and citations. They concluded that exposure to any NSAIDs or aspirin does not affect the risk of developing PD; when taking into account only non-aspirin NSAIDs, a slightly reduced risk of developing PD (−13 %) was present, which, however, was not statistically significant. When analyzing the few epidemiological reports considering individual drugs, use of ibuprofen in isolation emerged as the only one being associated with significant reduction of PD risk (−27 %). This confirmed the results of a previous meta-analysis (Samii et al. 2009). Finally, no conclusive results have been provided by clinical trials designed to evaluate the capacity of anti-inflammatory compounds to modify PD progression.

Concluding remarks

The role of neuroimmune mechanisms is emerging as a crucial component of the complex pathogenic puzzle of PD. Failure of clinical trials with NSAIDs, which act downstream of the neuroinflammatory cascade, has forced researchers to re-think their strategies. A better understanding of the neuroinflammatory dynamics associated with PD may lead to the development of tailored and targeted therapeutic approaches. Specific attention should be devoted, in particular, to the mechanisms that govern microglia polarization. Full understanding of such mechanisms may set the ground for a fine tuning of the neuroinflammatory process that accompanies and sustains neurodegeneration in PD. This, with its inherent complexity, is the challenge that is ahead of us.

Conflict of interest The author declares that he has no conflict of interest.

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