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Glial cell response: A pathogenic factor in Parkinson's disease

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Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). The loss of these neurons is associated with a glial response composed mainly of activated microglial cells and, to a lesser extent, of reactive astrocytes. This glial response may be the source of trophic factors and can protect against reactive oxygen species and glutamate. Alternatively, this glial response can also mediate a variety of deleterious events related to the production of pro-oxidant reactive species, proinflammatory prostaglandin, and cytokines. In this review, the authors discuss the potential protective and deleterious effects of glial cells in the SNpc of PD and examine how these factors may contribute to the pathogenesis of this disease. *Journal of NeuroVirology* (2002) **8,** 551–558.

Keywords: astrocyte; gliosis; IL-1β; iNOS; microglia; MPTP; neurodegeneration; Parkinson's disease

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized mainly by resting tremor, slowness of movement, rigidity, and postural instability (Fahn and Przedborski, 2000) and associated with a dramatic loss of dopamine-containing neurons in the substantia nigra pars compacta (SNpc) (Hornykiewicz and Kish, 1987). Currently, the number of PD patients has been estimated at ~1,000,000 in North America, with ~50,000 newly affected individuals each year (Fahn and Przedborski, 2000). Thus far, the most effective treatment for PD remains the administration of a precursor of dopamine, L-DOPA, which, by replenishing the brain in dopamine, alleviates almost all PD symptoms. However, the chronic administration of L-DOPA often causes motor and psychiatric side effects, which may be as debilitating as PD itself (Fahn, 1989). Also, thus far, there is no supportive evidence that L-DOPA therapy can impede the neurodegenerative process in PD. Given these facts, there is an urgent need to acquire a deeper understanding of both etiologic (i.e., causes) and pathogenic (i.e., mechanisms of cell death) factors implicated in PD, not only to prevent the disease, but also to develop therapeutic strategies aimed at halting its progression. Over the years, it has been increasingly recognized that although etiologic factors (e.g., α-synuclein, parkin, and several others that remain to be identified) are presumably pivotal in the initiation of the demise of SNpc dopaminergic neurons in PD, additional factors underlie the propagation of the neurodegenerative process. To elucidate such factors, and consequently to develop new therapies, the neuropathology of PD has been revisited in search of abnormalities that could shed light on these additional pathogenic culprits. In keeping with this goal, it is important to mention that aside from the dramatic loss of dopaminergic neurons, the SNpc is also the

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site of a glial reaction in both PD and experimental models of PD (McGeer *et al*, 1988; Forno *et al*, 1992; Sheng *et al*, 1993; Kohutnicka *et al*, 1998). Gliosis is a prominent neuropathological feature of many diseases of the brain whose sole and unique function was thought, for many years, to be the removal of cellular debris. Since then, mounting evidence indicates that the role played by gliosis in pathological situations may not be restricted to its "housekeeping" function, but may also include actions that significantly and actively contribute to the demise of neurons, especially in neurodegenerative diseases like PD. Interestingly, several lines of evidence demonstrate that gliosis may actually exert very different effects in the diseased brain, because, depending upon the situation, it may mediate either beneficial or harmful events. In this review, we will summarize the observations regarding gliosis in PD and in experimental models of PD as well as outline recent findings regarding the potential role of gliosis in the overall neurodegenerative process that occurs in PD.

First of all, it is important to remind the reader that glia are composed of macroglia, which includes astrocytes and oligodendrocytes, and of microglia. As mentioned by Wilkin and Knott (1999), so far, oligodendrocytes, which are involved in the process of myelination, have not yet been implicated in PD, whereas both astrocytes and microglial cells have. Accordingly, the focus of this review will be on astrocytes and microglial cells. Astrocytes are crucial, in the normal, undamaged adult brain, to the homeostatic control of the neuronal extracellular environment (Wilkin and Knott, 1999). As for resting microglia in the brain, they may play the role of immune surveillance and the inflammatory response to injury or infection (Gehrmann *et al*, 1993; Brown *et al*, 1998). Following an injury to the brain, astrocytes and microglial cells undergo various phenotypic changes that enable them to both respond to and to play a role in pathological processes (Eddleston and Mucke, 1993; Gehrmann *et al*, 1995). For instance, microglial activation is characterized by: proliferation; increased or *de novo* expression of marker molecules (such as major histocompatibility complex antigens); migration; and eventually transformation into a macrophage-like appearance (Banati *et al*, 1993).

Glial reaction in PD

In normal brains, neither resting astrocytes nor microglial cells are evenly distributed (Lawson *et al*, 1990; Damier *et al*, 1993). The density of microglial cells is remarkably higher in the substantia nigra (SN) compared to other midbrain areas and brain regions such as hippocampus (Kim *et al*, 2000). This observation, combined with the finding that SN neurons are much more susceptible to activated microglial-mediated injury (Kim *et al*, 2000), lends support to the idea that gliosis may play an especially meaningful role in PD.

The nigrostriatal pathway is the most affected dopaminergic system in PD. The neurons that form this pathway have their cell bodies in the SNpc and their nerve terminals in the striatum. Of particular relevance to this review is the finding that the loss of dopaminergic neurons in postmortem parkinsonian brains is associated with a significant glial reaction (McGeer *et al*, 1988; Forno *et al*, 1992; Banati *et al*, 1998; Mirza *et al*, 2000). However, although the damage to dopaminergic elements is consistently more severe in the striatum than in the SNpc, the response of glial cells is consistently more robust in the SNpc than in the striatum (McGeer *et al*, 1988). This discrepancy can be explained by the fact that dopaminergic structures that are degenerating are in dominance in the SNpc, whereas they are in the minority in the striatum (e.g., dopaminergic synapses represent <15% of the entire pool of synapses in the striatum). Aside from this topographical difference, the magnitudes of the astrocytic and microglial responses in parkinsonian brains are also very different. The SNpc of many but not all postmortem PD cases exhibits, at best, a mild increase in the number of astrocytes and in immunoreactivity for glial fibrillary acid proteins (GFAPs) (Forno *et al*, 1992; Mirza *et al*, 2000), and full-blown reactive astrocytes have been observed only in a few instances (Forno *et al*, 1992). The density of GFAP-positive astrocytes appears to be inversely related to the magnitude of dopaminergic neuronal loss across the different main dopaminergic areas of the brain in PD postmortem samples (Damier *et al*, 1993). This suggests that dopaminergic neurons within areas poorly populated with astrocytes are more prone to degenerate. Among the astrocytic pathological features seen in PD, what does correlate positively with the severity of SNpc dopaminergic neuronal loss is the number of α-synuclein–positive inclusions within SNpc astrocytes (Wakabayashi *et al*, 2000); whether these inclusions have any pathogenic significance is still unknown. Unlike the astrocytic response, the activation of microglial cells in PD is consistently dramatic (McGeer *et al*, 1988; Banati *et al*, 1998; Mirza *et al*, 2000). Microscopically, this microglial response in the SNpc culminates in those subregions most affected by the neurodegenerative process (McGeer *et al*, 1988; Banati *et al*, 1998; Mirza *et al*, 2000). Moreover, activated microglial cells are predominantly found in close proximity to free neuromelanin in the neuropil and to remaining neurons, onto which they sometimes agglomerate to produce an image of neuronophagia (McGeer *et al*, 1988).

Glial reaction in experimental models of PD

The neuropathological picture found in experimental models of PD is very similar to that found in PD itself. Among these models (Beal, 2001), the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model is the one that has been the most extensively used and, thus, not surprisingly the one for which we have

the largest amount of neuropathological data. As a preamble to our discussion, it is worth providing a brief review of the MPTP model (Przedborski and Vila, 2001). The fact that MPTP causes a parkinsonian syndrome was discovered in 1982 when a group of drug addicts in California were rushed to the emergency room with a severe bradykinetic and rigid syndrome (Langston *et al*, 1983). Subsequently, it was discovered that this syndrome was induced by the self-administration of street batches of a synthetic meperidine analogue whose synthesis was heavily contaminated with a by-product, MPTP (Langston and Irwin, 1986). In the period of a few days following the administration of MPTP, these patients exhibited a severe akinetic rigid syndrome reminiscent of PD, and L-DOPA was tried with great success, relieving the symptoms of these patients. Since the discovery that MPTP causes parkinsonism in human and nonhuman primates as well as in various other mammalian species, this neurotoxin has been used extensively as a model of PD (Kopin and Markey, 1988; Heikkila *et al*, 1989; Przedborski and Vila, 2001). For a technical review of MPTP utility and safety, refer to Przedborski *et al* (2001b).

In human and nonhuman primates, MPTP produces an irreversible and severe parkinsonian syndrome that replicates all of the cardinal clinical features of PD. However, although it is believed that the neurodegenerative process in PD occurs over several years, the most active phase of neuronal death following MPTP administration is presumably completed over a short period of time, producing a clinical condition consistent with "end-stage PD" in a few days. Still, brain imaging and neuropathological data suggest that, following the acute phase of neuronal death, nigrostriatal dopaminergic neurons continue to succumb at a much lower rate for many years after MPTP exposure (Vingerhoets *et al*, 1994; Langston *et al*, 1999). From a neuropathological standpoint, MPTP administration causes damage to the dopaminergic pathways identical to that seen in PD, with a resemblance that goes beyond the degeneration of nigrostriatal dopaminergic neurons. For instance, as in PD, MPTP causes a greater loss of dopaminergic neurons in the SNpc than in the ventral tegmental area (Seniuk *et al*, 1990; Muthane *et al*, 1994) and a greater degeneration of dopaminergic nerve terminals in the putamen than in the caudate nucleus, at least in monkeys treated with low dose of MPTP (Moratalla *et al*, 1992), but apparently not in acutely intoxicated humans (Snow *et al*, 2000). On the other hand, two typical neuropathological features of PD have, until now, been lacking in the MPTP model. First, except for the SNpc, other pigmented nuclei such as the locus coeruleus have been spared, according to most published reports. Second, the eosinophilic intraneuronal inclusions called Lewy bodies, which are so characteristic of PD, have thus far not been convincingly observed in MPTP-induced parkinsonism (Forno *et al*, 1986). More relevant to the topic of this review is the fact that in the few MPTP-intoxicated individuals who came to autopsy, postmortem examination reveals a marked glial reaction in the SNpc whose magnitude seems to parallel that of dopaminergic neuronal loss (Langston *et al*, 1999). In all three autopsy cases, reactive astrocytes, activated microglial cells, and images of neuronophagia are abundantly seen in the SNpc (Langston *et al*, 1999).

The aforementioned studies indicate that the glial response in the SNpc is fairly similar between humans with PD and those intoxicated by MPTP, although a more significant astrocytic reaction is seen in the latter (Langston *et al*, 1999). From a neuropathological standpoint, microglial activation and especially neuronophagia is indicative of an active, ongoing process of cell death. Although this contention is consistent with the fact that PD is a progressive condition, it challenges the notion that MPTP produces a "hit-and-run" kind of damage and rather suggests that a single acute insult in the SNpc could set in motion a self-sustaining cascade of events with long-lasting deleterious effects. Yet, neither postmortem studies in PD nor in MPTP-intoxicated individuals can provide information about the temporal relationship between the loss of dopaminergic neurons and the glial reaction in the SNpc. The situation is quite different in rodents. For instance, looking at mice injected with MPTP and killed at different time points thereafter, it appears that the time course of reactive astrocyte formation parallels that of dopaminergic structure destruction in both the striatum and the SNpc, and that GFAP expression remains up-regulated even after the main wave of neuronal death has passed (Czlonkowska *et al*, 1996; Kohutnicka *et al*, 1998; Liberatore *et al*, 1999). These findings suggest that, in the MPTP mouse model (Przedborski *et al*, 2000a), the astrocytic reaction is consecutive to the death of neurons and not the reverse. This is supported by the demonstration that blockade of 1-methyl-4-phenylperydinium (MPP^+)—the active metabolite of MPTP (Przedborski *et al*, 2000a)—uptake into dopaminergic neurons not only completely prevents SNpc dopaminergic neuronal death but also GFAP up-regulation (O'Callaghan *et al*, 1990). Remarkably, activation of microglial cells, which is also quite strong in the MPTP mouse model (Czlonkowska *et al*, 1996; Kohutnicka *et al*, 1998; Liberatore *et al*, 1999; Dehmer *et al*, 2000), occurs much earlier than that of astrocytes and, more importantly, reaches a maximum before the peak of dopaminergic neurodegeneration (Liberatore *et al*, 1999). In light of the MPTP data presented above, it can be surmised that the response of both astrocytes and microglial cells in the SNpc clearly occurs within a timeframe that allows these glial cells to participate in the demise of dopaminergic neurons in the MPTP mouse model and possibly in PD. In the following sections, we will examine through which beneficial or detrimental mechanisms the glial response in PD can possibly play out in the neurodegenerative process.

The protective effect of glial cells in PD

As mentioned above, glial response to injury may in fact have beneficial effects that, in the case of PD, could attenuate neurodegeneration. Among the different mechanisms by which glial-derived neuroprotection could be mediated, the first that come to mind involve the production of trophic factors.

To date, it is well recognized that many mature and, even more so, immature tissues and cell types, including glial cells, possess trophic properties that are essential for the survival of dopaminergic neurons. Relevant to this is the observation that striatal oligodendrocyte type 2 astrocytes greatly improve the survival and phenotype expression of mesencephalic dopaminergic neurons in culture, while simultaneously decreasing the apoptotic demise of these neurons (Sortwell *et al*, 2000). Although the actual identity of this glial-related trophic factor remains to be established, several others have already been well characterized. Among these, glial-derived neurotrophic factor (GDNF), which can be released by activated microglia, seems to be the most potent factor in supporting SNpc dopaminergic neurons during their period of natural, developmental death in postnatal ventral midbrain cultures (Burke *et al*, 1998). It is also worth emphasizing that GDNF induces dopaminergic nerve fiber sprouting in the injured rodent striatum (Batchelor *et al*, 1999), and that this effect is markedly decreased when GDNF expression is inhibited by intrastriatal infusion of antisense oligonucleotides (Batchelor *et al*, 2000). Furthermore, GDNF, delivered either by infusion of the recombinant protein or by viral vectors, has been shown to markedly attenuate dopaminergic neuronal death and to significantly boost dopaminergic function within injured neurons in both MPTP-treated monkeys and mice (Gash *et al*, 1996; Kordower *et al*, 2000; Eberhardt *et al*, 2000). Unfortunately, in humans with PD, much less enthusiastic results have been obtained thus far, in that repetitive intraventricular injections of recombinant GDNF to one advanced parkinsonian patient was poorly tolerated and failed to halt the progression of the disease (Kordower *et al*, 1999).

Glial cells may also protect dopaminergic neurons against degeneration by scavenging toxic compounds released by the dying neurons. Dopamine can produce reactive oxygen species (ROS) through different routes (Przedborski and Jackson-Lewis, 2000). Along this line, glial cells may protect remaining neurons against the resulting oxidative stress by metabolizing dopamine via monoamine oxidase-B and catechol-*O*-methyl transferase present in astrocytes, and by detoxifying ROS through the enzyme glutathione peroxidase, which is detected almost exclusively in glial cells (Hirsch *et al*, 1999). Glia, which can avidly take up extracellular glutamate, may mitigate the presumed harmful effects of the subthalamic excitotoxic input to the SN (Benazzouz *et al*, 2000), which, due to the nigrostriatal denervation, becomes hyperactive in PD (DeLong, 1990). Taken together, the data reviewed here support the contention that glial cells could have neuroprotective roles in PD. However, whether any of those actually dampen the neurodegenerative process in parkinsonian patients remains to be demonstrated.

The deleterious role of glial cells in PD

As we will see now, there are also many compelling findings that support the contention that glial cells could be harmful in PD. In this context, the spotlight appears to be more on activated microglial cells and less on reactive astrocytes. If astrocytes and microglia normally play supportive roles, then their shift to activated states in neurodegenerative diseasees such as PD could be doubly damaging to neurons. That is, pathology may result not only from direct glial attack but also from the loss of normal support by resting glia (which have now become activated). The importance of activated microglial cells in the neurodegenerative process is underscored by the demonstrations in rats (Liu *et al*, 2000; Herrera *et al*, 2000) (i) that the stereotaxic injection of bacterial endotoxin lipopolysaccharide (LPS) into the SNpc causes a strong activation of microglia throughout the SN, followed by a marked degeneration of dopaminergic neurons; and (ii) that the pharmacological inhibition of microglial activation prevents LPS-induced SNpc neuronal death.

Activated microglial cells can produce a variety of noxious compounds, including ROS, reactive nitrogen species (RNS), proinflammatory prostaglandins, and cytokines. Among the array of reactive species, lately the lion's share of attention has been given to RNS due to the prevalent idea that nitric oxide (NO)-mediated nitrating stress could be pivotal in the pathogenesis of PD (Przedborski *et al*, 1996; Ara *et al*, 1998; Pennathur *et al*, 1999; Giasson *et al*, 2000; Przedborski *et al*, 2001a). So far, however, none of the characterized isoforms of NO synthase (NOS) have been identified within SNpc dopaminergic neurons; hence, NO involved in the nitrating stress of PD most likely originates from other neurons and/or glial cells, as we hypothesized previously (Przedborski *et al*, 1996). It is thus particularly relevant to mention that numerous glial cells in the SNpc of both PD patients (Hunot *et al*, 1996) and MPTP-treated mice (Liberatore *et al*, 1999; Dehmer *et al*, 2000), but not of controls, express high levels of inducible NOS (iNOS). This NOS isoform, upon its induction, produces high amounts of NO for a prolonged period of time (Nathan and Xie, 1994), as well as superoxide radicals (Xia and Zweier, 1997)—two reactive species that can either directly or indirectly promote neuronal death.

Prostaglandins and their synthesizing enzymes, such as cyclooxygenase type 2 (Cox-2), constitute a second group of potential culprits. Indeed, Cox-2 has emerged as an important determinant of cytotoxicity associated with inflammation (Seibert *et al*, 1995;

O'Banion, 1999). In the normal brain, Cox-2 is significantly expressed only in specific subsets of forebrain neurons that are primarily glutamatergic in nature (Kaufmann *et al*, 1996), which suggests a role for Cox-2 in the postsynaptic signaling of excitatory neurons. However, under pathological conditions, especially those associated with a glial response, Cox-2 expression in the brain can increase significantly, as does the level of its products (e.g., prostaglandin E_2), which are responsible for many of the cytotoxic effects of inflammation. Interestingly, Cox-2 promoter shares many features with iNOS promoter (Nathan and Xie, 1994) and thus, these two enzymes are often coexpressed in disease states associated with gliosis. Therefore, it is not surprising to find Cox-2 and iNOS expressed in SNpc glial cells of postmortem PD samples (Knott *et al*, 2000); PGE_2 content is also elevated in SNpc from PD patients (Mattammal *et al*, 1995). Of relevance to the potential role of prostaglandin in the pathogenesis of PD is the demonstration that the pharmacological inhibition of both Cox-2 and Cox-1 attenuates MPTP toxicity in mice (Teismann and Ferger, 2001).

A third group of glial-derived compounds that can inflict damage in PD is the proinflammatory cytokines. Several among these, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), are increased in both SNpc tissues and cerebrospinal fluids of PD patients (Mogi *et al*, 1994, 1996, 2000), although some of the reported alterations may be related to the chronic use of the anti-PD therapy L-DOPA (Bessler *et al*, 1999). Nevertheless, at autopsy, convincing immunostaining for TNF-α, IL-1β, and interferon-γ (IFN-γ) is observed in SNpc glial cells from PD patients (Hunot *et al*, 1999). These cytokines may act in PD on at least two levels. First, although they are produced by glial cells, they can stimulate other glial cells not yet activated, thereby amplifying and propagating the glial response and consequently the glial-related injury to neurons. Relevant to this scenario are the following demonstrations (Hunot *et al*, 1999): First, glial-derived TNF-α, IL-1β, and IFN-γ activate other microglial cells, which start to express the macrophage cell surface antigen FcϵR11 (CD23). Activation of CD-23 on these newly activated microglial cells induces iNOS expression and the subsequent production of NO, which, in turn, can amplify the production of cytokines within glial cells (e.g., TNF-α) and can diffuse to neighboring neurons. Second, glial-derived cytokines may also act directly on dopaminergic neurons by binding to specific cell surface cytokine receptors (e.g., TNF-α receptor). Once activated, these cytokine receptors trigger intracellular death-related signaling pathways, whose molecular correlates include translocation of the transcription nuclear factor-κB (NF-κB) from the cytoplasm to the nucleus and activation of the apoptotic machinery. In connection with this, PD patients exhibit a 70-fold increase in the proportion of dopaminergic neurons with NF-κB immunoreactivity in their nuclei compared to control subjects (Hunot *et al*, 1997). In relation to apoptosis, Bax, a potent proapoptotic protein, is up-regulated after MPTP administration and

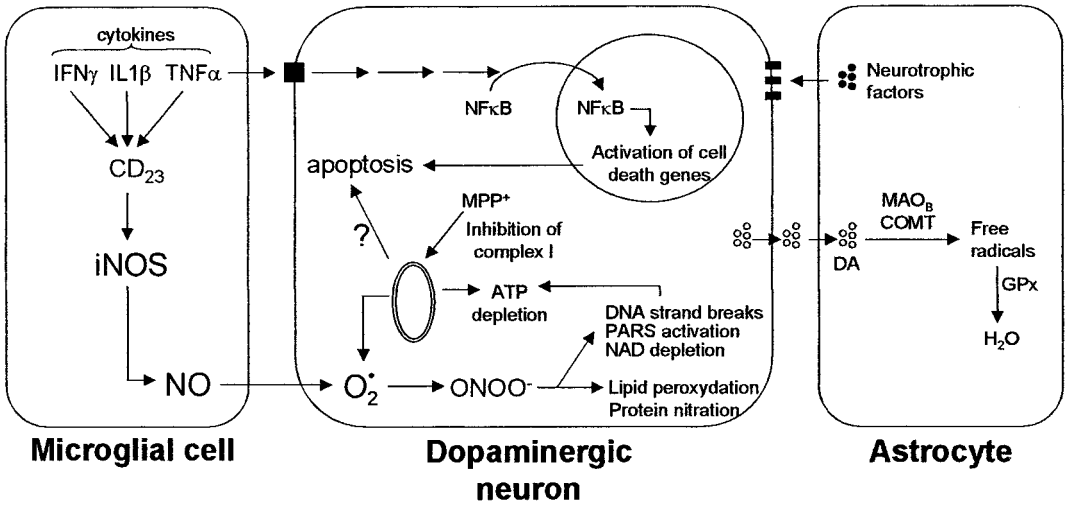

Figure 1 Potential involvement of glial cells in the pathogenesis of Parkinson's disease. Activated microglial cells may contribute to dopaminergic neurodegeneration by releasing cytotoxic compounds such as cytokines. Cytokines may exert a direct effect on dopaminergic neurons by activating transduction pathways that lead to apoptosis or, alternatively, by inducing the expression of iNOS within glial cells and the subsequent formation of NO. NO is membrane permeable and can diffuse to neighboring dopaminergic neurons. If the neighboring cell has elevated levels of superoxide ($O_2^{\cdot}$), there is an increased probability that superoxide will react with NO to form peroxynitrite ($ONOO^-$), which can damage lipids, proteins, and DNA. Damaged DNA stimulates poly(ADP-ribose) synthase (PARS) activity, which further contributes to the ATP depletion induced by the MPP^+-mediated inhibition of the mitochondrial complex I. Other glial cells, such as astrocytes, may have a neuroprotective effect on dopaminergic neurons by producing neurotrophic factors, such as GDNF, or by metabolizing dopamine by monoamine oxidase-B (MAO_B) or catechol-*O*-methyl transferase (COMT), then eliminating free radicals using glutathione peroxidase (GPx).

its ablation prevents the loss of SNpc dopaminergic neurons in this experimental model (Vila *et al*, 2001). Furthermore, caspase-3, a key agent of apoptosis, is also activated in postmortem PD samples (Hartmann *et al*, 2000).

Conclusion

In this review, we have tried to succinctly discuss the issue of glial response in PD and how this cellular component of PD neuropathology, which has been neglected far too long, plays out in the overall neurodegenerative process (Figure 1). Accordingly, key findings, and, as often as possible, recent studies were included in our discussion to give an up-to-date look at this question. Although we have tried to provide the reader with a balanced view on the issue, it is our opinion that, given the available evidence to date, data supporting a detrimental role for the glial response in PD outweigh those supporting a beneficial role. We also believe that, should the glial response in PD indeed be implicated in the neurodegenerative process, it is unlikely that any aspect of the glial response initiates the death of SNpc dopaminergic neurons, but quite possibly propagates the neurodegenerative process. This view, if confirmed, could have far-reaching therapeutic implications, because targeting a specific aspect of the glial-related cascade of deleterious events may prove successful in slowing or even halting further neurodegeneration in PD (Przedborski *et al*, 2000b).

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