Progressive neurodegeneration and motor disabilities induced by chronic expression of IL-1β in the substantia nigra

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The functional role of the long-lasting inflammation found in the substantia nigra (SN) of Parkinson’s disease (PD) patients and animal models is unclear. Proinflammatory cytokines such as interleukin-1β (IL-1β) could be involved in mediating neuronal demise. However, it is unknown whether the chronic expression of cytokines such as IL-1β in the SN can alter neuronal vitality. The aim of this study was to investigate the effects of the chronic expression of IL-1β in the adult rat SN using a recombinant adenovirus expressing IL-1β. The chronic expression of IL-1β for 60 days induced dopaminergic cell death in the SN and unilateral akinesia starting only at 21 days post-injection. Microglial cell activation and inflammatory cell infiltrate were associated with dopaminergic cell death and motor disabilities. Astrocytic activation was delayed and associated with scar formation. The chronic expression of a single proinflammatory cytokine as IL-1β in the SN elicited most of the characteristics of PD, including progressive dopaminergic cell death, akinesia and glial activation. Our data suggest that IL-1β per se is able to mediate inflammatory-mediated toxic effects in the SN if its expression is sustained. This model will be helpful to identify possible therapeutic targets related to inflammation-derived neurodegeneration in the SN.

Keywords: Parkinson; Microglia; Akinesia; Inflammation; Adenovector; Cytokine

Introduction

Progressive loss of dopaminergic neurons in the substantia nigra (SN) is the main characteristic of Parkinson’s disease (PD). Currently approved and experimental treatments against PD aim at restoring this dopaminergic deficit or compensate the imbalanced communication among structures of the basal ganglia (Hagan et al., 1997; Hald and Lotharius, 2005; Lang and Lozano, 1998; Langston et al., 1999; Winkler et al., 2005). Importantly, the pathophysiology of PD is poorly understood and although genetic components have been linked to familial PD, the etiology of sporadic PD remains unknown. One main pathophysiological feature of PD is the presence of brain inflammation. Robust microglial activation was consistently found in animal models and PD patients (Barcia et al., 2004; Depino et al., 2003; Hirsch et al., 2003; Hunot et al., 1999; Hurley et al., 2003; Langston et al., 1999; McGeer et al., 1988; McGeer et al., 2003; Mirza et al., 2000; Mogi and Nagatsu, 1999; Vila et al., 2001) and its blockade has shown to be neuroprotective in rodent models of PD (He et al., 2001; Sanchez-Pernaute et al., 2004; Wu et al., 2002) suggesting that inflammatory processes and microglial activation might contribute to the degeneration of the SN neurons. In animal models, inflammation was observed as long as the neurodegenerative process was active, suggesting that inflammation is present for long periods of time in the SN (Cicchetti et al., 2002; Depino et al., 2003; Hald and Lotharius, 2005; He et al., 2001; Orr et al., 2002; Sugama et al., 2003; Vila et al., 2001). Indeed, in PD patients, microglial activation was observed as long as 16 years after self-administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Langston et al., 1999). However, still the functional role of the inflammatory response associated to the neurodegeneration of the SN is not totally clear (Gao et al., 2003; Hald and Lotharius, 2005; Teismann and Schulz, 2004; Vila et al., 2001).

Cytokines are main mediators of inflammation in the brain and the periphery. IL-1β, a key proinflammatory cytokine, has been shown to play a crucial role in the exacerbation of acute neurodegeneration caused by ischemia, head trauma and stroke and has been implicated in the pathology of multiple sclerosis, Alzheimer’s disease and other chronic diseases of the CNS (Allan and Rothwell, 2001; Boutin et al., 2001; Griffin and Mrak, 2002; Huitinga et al., 2000; Pearson et al., 1999; Perry et al., 2003). Importantly, higher levels of proinflammatory cytokines such as IL-1β have been found in the ventricular cerebrospinal fluid and in postmortem striata of PD patients, compared with control patients (Mogi et al., 1994; Mogi and Nagatsu, 1999). In addition, endotoxin, a cytokine inducer, has been shown to be toxic for dopaminergic neurons in vitro and in vivo (Castano et al., 1998; Kim et al., 2000; Iravani et al., 2002). However, this effect of endotoxin cannot be
mimicked by the acute intranigral injection of TNF-α or IL-1β alone (Castano et al., 2002; Depino et al., 2005). On the other hand, administration of IL-1β has been also shown to increase sprouting (Parish et al., 2002) and ameliorate the neurotoxic effect in the SN of a subsequent intrastriatal injection of 6-OHDA (Saura et al., 2003). Thus, although a toxic effect is often observed, the role of IL-1β on dopaminergic vitality remains unclear. Especially, it is not known whether the chronic expression of a given cytokine, a condition most likely resembling the chronic characteristic of inflammation in PD, has any effect on the vitality of dopaminergic neurons of the SNpc.

The aim of this study was to investigate the effects of the chronic expression of IL-1β in the SN. We characterized the effect of this cytokine on motor behavior, dopaminergic cell death, glial activation and cytokine pattern expression.

Materials and methods

Vectors

Adenoviral vectors were generated, quality controlled and used as already described (Ferrari et al., 2004; Kolb et al., 2001). Briefly, for construction of AdIL-1, human IL-1β cDNA was cloned into a shuttle vector under the control of a human cytomegalovirus promoter and cotransfected on 293 cells with a plasmid containing E1- to E3-deleted type 5 adenoviral genome. The correct recombination was verified with restriction digestions of the purified viral DNA obtained by HIRT, and Southern blot. Transgene expression was checked by Western Blot. The adenoviral vectors were purified by plaque formation under agar. Stocks were obtained from large-scale preparations in HEK293 cells by double cesium chloride gradients and were quantified by plaque assay (final titers: Adβgal=1.5×10¹⁰ pfu/μl, AdIL-1=1×10¹⁰ pfu/μl). Stocks had less than 1 ng/ml of endotoxin, assayed with E-TOXATE® Reagents (Sigma, St. Louis, Missouri). Viral stocks were free of auto-

Animals and injections

Adult male Wistar rats (250 g–300 g), housed in groups of five animals, under controlled temperature (22°C±2°C), artificially lit under a 12-h cycle period and with water and food ad libitum. All animal procedures were performed according to the rules and standards of German animal law and the regulations for the use of laboratory animals of the National Institute of Health, USA. Animal experiments were approved by the Ethical Committee of the Institute Leloir Foundation.

For central injections, the animals were anesthetized with ketamine chlorhydrate (80 mg/kg) and xylazine (8 mg/kg). The adenoviruses were administered with a 50 μm tipped finely drawn glass capillary, stereotactically implanted in the left SN (bregma, −5.3 mm; lateral, +2 mm; ventral, −7.2 mm) (Paxinos and Watson, 1986). Intranasal injections of 1 μl of adenoviral vectors or vehicle were infused over 5 min and kept in place for additional 2 min before removal. All surgery procedures took place during the morning to avoid effects of circadian variations in cytokine expression. Human IL-1β and β-galactosidase expressing adenoviral vectors were diluted in sterile 10 mM Tris–HCl, 1 mM MgCl₂ (pH 7.8) and administered at a dose of 5.10⁶ pfu/rat. The animals were killed at 7, 21, 40, or 60 days post-surgery.

Behavioral tests

Adjusting steps

This test was performed as previously described (Depino et al., 2003) for assessment of akinesia in the unilateral Parkinson model (n=8 per group). Briefly, the experimenter held the rat with one hand fixing the hind limbs and slightly raising the hind part. The rat was moved slowly sideways by the experimenter from right–left direction in first place. The number of adjusting steps was counted for both paws in the backhand and the forehead direction of the movement. The sequence of testing was right (contralateral) paw freehand and backhand and then the left paw. The experimenter handled the rats at least three times before testing to familiarize the animal with the experimenter’s grip. The last assay before surgery was considered as the pre-lesion stepping test.

Cylinder test

Forelimb akinesia was assessed using the test previously described (Schallert and Jones, 1993) (n=8 per group). This test evaluates the use of the forelimb to support the body against the walls of a cylinder. The rats were put individually in an acrylic cylinder (20 cm×30 cm). The test was performed between 16:00 h and 19:00 h. The number of wall contacts performed independently with the left and the right forepaw was counted.

Histology

The animals (n=6 per group) were deeply anesthetized and transcardially perfused with heparinized saline followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB) (pH 7.2). After dissecting the brains, they were placed in the same fixative overnight at 4°C. Then, the tissues were cryoprotected by immersion in 30% sucrose, frozen in isopentane and serially sectioned in a cryostat (40 μm). The 40-μm sections were used either for cresyl violet staining or for free floating immunohistochemistry.

Semithin sections

Anesthetized animals were intracardially perfused 2% glutaraldehyde, 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2) and processed for electron microscopy as previously described (Ferrari et al., 2004) (n=3 per group).

Immunohistochemistry

Free-floating sections were incubated in blocking buffer (1% donkey serum, 0.1% Triton in 0.1 M PB) and incubated overnight with primary antibodies. The antibodies used were anti-tyrosine hydroxylase (TH) for dopaminergic neurons (diluted 1:1000; Chemicon, Temecula, CA), anti-ratIL-1β (1:300; NIBSC, Potters Bar, UK), ED1 (1:200; for macrophages with phagocytic activity; Serotec, Raleigh, NC), MBS II (1:100; specific for neutrophils) (Anthony et al., 1997), anti-glial fibrillary acidic protein (GFAP) (1:700; for astrocytes; Dako, Carpinteria, CA). Alternatively, we handled the rats at least three times before testing to familiarize the animal with the experimenter’s grip. The last assay before surgery was considered as the pre-lesion stepping test.

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Grove, PA) followed by Vectastain standard ABC kit (Vector Laboratories, Burlingame, CA) and developed with 3,3′-diaminobenzidine (Sigma, Saint Louis, Missouri). For double-labeling immunohistochemistry, the sections were incubated with either indocarbocyanine Cy3 (Cy3) conjugated donkey anti-mouse antibody (1:250; Jackson ImmunoResearch Laboratories Inc., West Grove, PA), cyanine Cy2 (Cy2) conjugated donkey anti-rabbit antibody (1:250; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) or Cy2 conjugated streptavidin (1:250; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Digital images were collected in a Zeiss LSM 510 laser scanning confocal microscope equipped with a krypton–argon laser.

**Quantification of TH-positive cells**

Total number of TH-positive cells was counted through the SN pars compacta at 20× magnification. Every sixth 40-μm-thick section was counted. The graph shows the ratio between the TH+ cells in the ipsilateral side related to contralateral side. The sections were counted twice using double-blind procedure.

**Classification of microglial activation**

We classified the microglia activation according to Kreutzberg (1996).

- **Stage 1:** Resting microglia stage. Rod-shaped soma with fine and ramified processes.
- **Stage 2:** Activated ramified microglia. Elongated-shaped cell body with long and thicker processes.
- **Stage 3:** Amoeboid microglia. Round-shaped body with short, thick and stout processes.
- **Stage 4:** Phagocytes cells. Round-shaped cells with vacuolated cytoplasm, no processes can be observed at light microscopy level.

All these cellular types are GSA-positive (GSA+), but the stage 4 can also be ED1-positive (ED1+). MHC-II stained all the activation stages but not resting microglia.

**Measurement of IL-1β and other proinflammatory cytokines**

The animals (n=8 per group) were decapitated and their brains were quickly removed and both the injected and non-injected SN were dissected, snap-frozen in liquid nitrogen and stored at −80°C. Tissue was processed as described previously (Ferrari et al., 2004). Commercially available human IL-1β (hIL-1β), rat IL-1β (rIL-1β) and rat TNF-α (rTNF-α) kits (R&D, Minneapolis, MN) were used according to the manufacture instructions. The sensitivity of these kits was 2 pg/ml for hIL-1β, 15 pg/ml for rIL-1β and 7.8 pg/ml for rTNF-α. To estimate protein recovery, rIL-1β standard was added exogenously to brain homogenates and the ELISA was performed as described before. A recovery of 85–90% of the added rIL-1β standard was found.

**Statistical analysis**

Results are expressed as mean ± SEM of different animals in the different treatment groups. Statistically differences among treatments were determined using one-way analysis of variance test followed by Newman–Keuls multiple comparison test.

For statistical evaluation of the adjusting step test, the data were subject to a one-factor analysis of variance (ANOVA) and Bonferroni’s multiple comparison test. The level of statistical significance was set at p<0.05.

**Results**

**Chronic expression of IL-1β in the SN**

We achieved chronic expression of hIL-1β in the rat SN with the administration of a low dose (5×10⁶ pfu) of a replication-deficient, recombinant adenoaviral vector (AdIL-1) to that region. By X-gal staining of AdILgal-injected SN, we could detect transgene expression all along the SN; around 1 mm along the rostro-caudal axis (data not shown). The expression of hIL-1β was higher at 7 days post-injection (p.i.) (9.95±2.02 ng/mg total protein) and decreased from 21 to 60 days p.i. (1.4±0.7 ng/mg prot, at 60 days) (Fig. 1A). This amount of IL-1β was previously shown to be sufficient to exacerbate excitotoxic damage (Lawrence et al., 1998) and cause demyelination and PMN recruitment in the striatum but not neuronal toxicity when acutely injected in the SN (Depino et al., 2005). hIL-1β was under the detection limit of the ELISA used in the non-injected hemisphere of AdIL-1 rats and in the injected SN of AdILgal rats (data not shown).

We have also measured the endogenous rIL-1β expression at 7, 21, 40 and 60 days p.i. At 7 days posterior to the injection of AdIL-1, there is an induction of rIL-1β (52.18 pg/mg protein±13.24 pg/mg protein) in the AdIL-1-injected hemisphere that was still detected 21 days post-injection. The expression of low levels of rIL-1β can still be detected 40 and 60 days after the vector injection but no statistical differences with the contralateral hemisphere were found at these time points (Fig. 1B). No rIL-1β induction was detected in AdILgal-injected animals (Fig. 1B). Immunohistochemical analysis confirmed the presence of ramified rIL-1β-positive cells (rIL-1β+) in the SN of injected animals surrounding the injection site at 7, 21, 40 and 60 days (Figs. 2K–O).

No induction of TNF-α was detected at any time point studied. TNF-α expression was close to the detection threshold in the SN of either AdIL-1-injected or AdILgal-injected animals (Fig. 1C).

**Partial and progressive dopaminergic cell loss after chronic IL-1β expression in the SN**

The chronic expression of IL-1β in the adult SN caused a progressive loss of dopaminergic tyrosine hydroxylase-positive (TH+) cells in the ipsilateral hemisphere in comparison with its contralateral side (Fig. 2A). No statistical differences in TH cell number were detected at 7 days respect to animals injected with AdILgal. On the contrary, the number of TH+ cells decreased by 23% with respect to the contralateral hemisphere starting at 21 days (p<0.05 vs. AdIL-1-injected animals 7 days p.i. and p<0.01 vs. AdILgal-treated rats at 21 days p.i.). The neurodegenerative effect of IL-1β expression was maintained until 60 days post-AdIL-1 injection (Figs. 2A and B).

**Motor impairments after chronic IL-1β expression in the SN**

We further characterized this model using two different tests in order to evaluated forelimb akinesia: the adjusting steps test and the cylinder test.
The adjusting steps test showed a significant impairment in the right (contralateral) paw performance in both forehand (ANOVA $F_{8,53} = 21.07$ $p < 0.0001$) and in the backhand direction (ANOVA $F_{8,53} = 14.45$ $p < 0.0001$) of the AdIL-1-injected animals at 21, 40 and 60 days compared with the left paw, which resulted in a dragging paw when the rat was moved by the experimenter (Fig. 3A). This behavior showed no recovery at any time point studied. This effect was not observed at 7 days after the injection with the adenovector expressing IL-1β, where no statistically significant differences with the control animals or with the basal measurements were found. The rats injected with the Adβgal showed no statistically significant differences in the number of steps with both paws at any time point studied (Fig. 3A).

The cylinder test was also performed to assess forelimb akinesia as it has been described previously (Schallert and Jones, 1993). The chronic expression of IL-1β decreased wall contacts 21, 40 and 60 days after the AdIL-1 injection. Although a tendency to show motor abnormalities was detected at 7 days post-injection of any adenoviral vector there are no statistical differences at 7 days post-injection in AdIL-1-injected rats or in animals injected with Adβgal at any time point studied (Fig. 3B).

Evaluation of the inflammatory response after chronic IL-1β expression in the SN

Little or no recruitment of any type of leukocytes or vasodilation was observed in the uninjected hemispheres or in animals injected with Adβgal at any time point studied (Fig. 4A).

7 days after AdIL-1 injection, the SN exhibited a great amount of inflammatory infiltrate mostly composed of polymorphonuclear (PMN) neutrophils and some macrophages. Blood vessels were
vasodilated and filled with marginated PMN neutrophils and macrophages at this time point (Fig. 4B). At 21 days post-AdIL-1 injection, the SN showed similar characteristics to 7 days, but the lesion exhibited a much more extensive area with edematized tissue (Figs. 4C, D, E). Analysis of semithin sections corroborated the light microscopic level description, where the necrotic zone is occupied by large amounts of cellular debris immersed in a translucent edematous liquid at this time point (Fig. 4G). The presence of macrophages with vacuolated cytoplasm, neutrophils and activated microglia is also evident. Interestingly, many myelinated axons and some unmyelinated axons are observed immersed in the translucent liquid (Fig. 4G). Only few degenerate axons were observed. Most of the neurons exhibited a degenerative aspect, where the cytoplasm showed a hyaloplasmic matrix filled with dense material (Figs. 4G, H). None of these characteristics were observed in the contralateral hemisphere (Fig. 4F). At 40 days post-injection, the leukocyte population was composed predominantly of monocytes/macrophages with scarce PMN widespread throughout the whole SN (Fig. 4J). Marginated macrophages filled most of the vessels, but no vasodilatation was seen (Figs. 4 I, J). At 60 days, the inflammatory infiltrate is composed exclusively of monocytes/macrophages located within the SN with no PMN. Monocytes/Macrophages can be observed within the vessels which show no vasodilatation (Figs. 4K, L).

**Macrophages/Microglia and astroglia reaction to chronic expression of IL-1β**

Rats with Adβgal, showed microglial activation (stages 2, 3 and 4) 7 days post-injection as observed by ED1 staining and morphological changes detected by GSA labeling (Fig. 5A, F, see Materials and methods for description of stages of activation of microglial cells). This activation was observed at the injection site reflecting the tissue response to surgery and/or the adenoviral load. At 21 days, microglial activation was still present as assessed by morphological changes (GSA) and ED-1 staining and no MHC-II or IL-1β expression can be observed in Adβgal-injected animals (data not shown). No GSA-positive (GSA+), ED1-positive (ED1+) or MHC-II+ cells were seen at 40 and 60 days p.i. in these control animals (data not shown).

On the contrary, at 7 and 21 days, the animals injected with AdIL-1 exhibited mostly stage 4 activated microglial widespread through the whole SN as assessed by morphology and ED1+ staining (Figs. 5B, C, G, H). MHC class II expression showed similar morphology and pattern to GSA labeling (Figs. 5L, M). At 40 days post-injection, the GSA+ and MHC-II+ cells located within the scar exhibited a typical stage 4 appearance of phagocytic cells, i.e., round shape with vacuolated cytoplasm, while the GSA+ and MHC-II+ cells surrounded the scar showed ramified morphology of microglial cells with either elongated soma with ramified processes or ameboidal shape with thick and stout short processes (stages 2 and 3, respectively) (Figs. 5D, I, N). At this time point, most of the ED1+ cells were located within the scar (Fig. 5I). After 60 days, the animals injected with the AdIL-1 had ED1+, GSA+ and MHC-II+ cells only within the scar (Figs. 5E, J, O). However, the GSA+ and MHC-II+ cells around the lesion site were not fully activated with rod shape, thin long and ramified processes and elongated shape body with long and thicker processes (stages 1 and 2, respectively) (Figs. 5E, O).
As expected, GFAP expression was observed under basal conditions and after Adβgal inoculation in the SN pars reticulata (SNpr) (Fig 5P). Astrogliosis as determined by increased GFAP staining was evident after the injection of AdIL-1 in the whole SN (SNpc and SNpr). Increased GFAP+ cells could be observed 7 days post-injection and this activation remained until 60 days in the AdIL-1-injected animals but not in the Adβgal rats (Figs. 5P–T). This expression increased with time, reaching its peak at 60 days p.i. (Figs. 5P–T).

**Discussion**

The present study demonstrates for the first time that the chronic expression of IL-1β, a major proinflammatory cytokine, in the SN induced progressive dopaminergic cell death with motor impairments in parallel with microglia and astroglial activation and inflammatory infiltrate, mainly composed of PMN neutrophils and macrophages. Dopaminergic cell death and motor symptoms were not observed at early time points (7 days post-treatment). They were first detected 21 days after AdIL-1 injection, suggesting that only sustained IL-1β expression could affect dopaminergic neuronal viability and motor behavior.

We have previously shown that the Adβgal injected in the striatum produced minimal microglial activation at the injection site at earlier time points (Ferrari et al., 2004). In this report, using again a low dose of viral vector and fine capillaries for the injection, we also achieved long-term transgene expression with no inflammation due to the vector itself. The levels of expression achieved are within the range that produces an inflammatory response when injected in the periphery in rodents and humans (Dinarello, 1997).

In the SN, we detected microglial activation at the injection site that remains for 21 days after the injection of the control adenovector but no inflammatory infiltrate at any time point studied. Chronic human IL-1β expression was achieved during 60 days in the SN with declining IL-1 amounts detected during this period. IL-1β did not induce TNF-α, confirming that the cytokine network in the brain does not necessarily function as expected from data obtained from the periphery (Blond et al., 2002; Depino et al., 2003). Endogenous rIL-1β was induced but only until day 21 after AdIL-1 injection, showing a degree of tachyphylaxis previously described after long-term IL-1β expression in the striatum (Ferrari et al., 2004). Also similarly to the observations in the striatum, PMN neutrophils were the main immune cell recruited to the site of IL-1 expression. However, contrary to our previous report,
demonstrating a demyelinating effect of the chronic expression of IL-1β in the striatum, no overt demyelination in the SN was detected at any time point studied. Inversely, similar amounts of IL-1β expressed in the striatum for the same amount of days did not cause neurodegeneration but reversible demyelination (Ferrari et al., 2004). Thus, the chronic expression of IL-1β seems to have relevant but differential effects depending on the region of the brain studied, although the cellular profile of the IL-1-dependent recruitment of cells remains similar.

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The chronic expression of IL-1β induced dopaminergic cell death, which was evident only after 21 days of IL-1β expression and remained at least for 60 days. Importantly, signs of motor impairments followed a similar time course as dopaminergic degeneration in the SN. The acute injection of similar amounts of IL-1 as the one expressed chronically in our work was not toxic for dopaminergic neurons in the SN. This was the case when the cytokine was injected alone (10 ng or 1000 U) or in combination with 1000 U of TNF-α and 100 U of IFN-γ in the SN (Castano et al., 2002; Depino et al., 2005). To the best of our knowledge, only one report showed that the acute injection of this amount of IL-1β in the medial forebrain bundle produced a 12.2% decrease in the cell counts in the SN after 14 days, but this decrease was not statistically different from the cell counts of control animals (Carvey et al., 2005). We have not seen any IL-1β-mediated neurodegenerative effects during the first 7 days of treatment, even if IL-1 expression was the highest at this time point. These data support the notion that acute injection or expression of IL-1 in the SN does not result in alterations in dopaminergic cell numbers, while long-term expression has neurodegenerative effects in the SN. Thus, we are providing novel evidence that IL-1β can trigger dopaminergic cell death by itself provided that its expression is sustained for more than 7 days in the SN.

In other neurodegenerative paradigms, IL-1β has been mainly described as an exacerbating agent of on-going neuronal damage, although some neuroprotective effects have been also exceptionally observed in vitro and in vivo (Allan and Rothwell, 2001; Perry et al., 2003). Recently, it has been shown that neurodegeneration can “prime” microglial cells without a typical inflammatory response in prion disease and PD models (Depino et al., 2003; Perry et al., 2002). Interestingly, a second, proinflammatory trigger...
Fig. 5. Activation of glial cells after the injection of the AdIL-1 in the SN. (A–E) Activation of microglial cells as demonstrated by GSA (green)/TH (red). A. 7 days after the injection with the Adβ-gal. GSA+ cells (stages 2, 3 and 4) are observed surrounding the injection site. (B) The AdIL-1 after 7 days cause an stage 4 microglial activation. (C–E) The AdIL-1 injection after either 21 (C) 40 (D) or 60 days (E). (F–J) Activated microglia with macrophage characteristics confirmed by ED1 immunofluorescence. (F) ED1+ cells are observed only within the injection site. 7 days p.i. of the control adenovector (G). The AdIL-1 after 7 days induces the presence of round ED1+ cells within the SN. (H) After 21 days, ED1+ cells are evident within the edematous region. (I–J) 40 days (I) or 60 days (J) after the injection of the AdIL-1 the presence of ED1+ cells are still evident. Ramified ED1+ cells are also seen in the parenchyma surrounding the scar. (K–O) Expression of the major histocompatibility complex class two (MHC-II) (red) and rIL-1β (green) after the injection with the AdIL-1. (K) The control animals show label with MHC-II and rIL-1β at the injection site. 7 days (L) or 21 days (M) after the injection of the AdIL-1. Stage 4 MHC-II+ cells are observed within the SN but ramified MHC-II+ cells are located surrounding the lesion. Ramified rIL-1β+ cells are also observed within the lesion. (N) Round MHC-II+ cells are mostly located within the scar and numerous processes of rIL-1β+ cells are located around the scar. (O) The AdIL-1 after 60 days exhibited scarce MHC-II+ cells within the scar. (P–T) Astroglial activation after the injection of AdIL-1 or Adβ-gal in the SN. (P) Scarce GFAP+ cells are observed in the SN pars reticulate (pr) 7 days after the injection with the Adβ-gal. The injection of the AdIL-1 induces a strong GFAP reaction in both SN pars compacta and SN pars reticulata 7 days (Q) or 21 days (R) p.i. A strong GFAP reaction is observed in the SN after 40 (S) or 60 days (T). pc: pars compacta, pr: pars reticulata. Scale bar: 50 μm.
can produce a full-blown inflammatory response and exacerbate neuronal demise and disease symptoms in prion disease (Combrink et al., 2002; Cunningham et al., 2005). This fully activated microglia is the one found in brain samples in end-stage PD patients (Langston et al., 1999). Fully activated microglia has been proposed to be involved in a self-perpetuating vicious and deleterious cycle for dopaminergic neurons in vivo (Hald and Lotharius, 2005; Hirsch et al., 2005; Nagatsu, 2002; Orr et al., 2002; Vila et al., 2001). Would this be the case in PD, activated microglial-derived IL-1 per se could be mediating neuronal demise in the SN of patients.

In addition, several anti-inflammatory agents such as minocycline and naloxone that have been shown to ameliorate neurodegeneration in PD models also diminished IL-1β expression in those models (reviewed in Hald and Lotharius, 2005; Liu et al., 2000a,b; Sanchez-Pernaute et al., 2004; Wu et al., 2002). Taken together, these and our data may point to IL-1β inhibition as a possible therapeutic target against PD. However, taking into account the many dual effects of single cytokines in the CNS and the existence of reports describing neuroprotective effects of IL-1β, we believe that more exhaustive studies are needed before promoting anti-IL-1 therapies against PD.

At the same time period when neuronal death in the SN and the motor symptoms were observed, we have detected a strong activation of microglial cells and an inflammatory infiltrate composed mainly of PMN neutrophils. Microglial activation was characterized by end-stage morphological changes (stage 4), expression of MHC-II and markedly phagocytic activity. Interestingly, this type of activation was described in the first report describing the inflammatory response in PD brains (McGeer et al., 1988). Although it is clear from our experimental set-up that long-term IL-1β expression can trigger dopaminergic cell death in the SN, it cannot be discarded that products derived from activated microglial cells or neutrophils are mediating or contributing to this toxic effect.

At later time points, neutrophils were absent from the inflammatory infiltrate and the SN exhibited a strong and late astrocytic reaction. Astrocytic activation has been observed in many but not all cases of PD and PD models (Forno, 1992; Hirsch et al., 2003; Mirza et al., 2000; Liberatore et al., 1999; Langston et al., 1999; Przedborski et al., 2000; Sheng et al., 1993; Rodrigues et al., 2001; Depino et al., 2003). In our particular model, where no neurotoxic insult is involved, the astrocytic reaction could be temporally associated with the recovery phase of the tissue.

Here we describe that the chronic expression of a unique cytokine such as IL-1β is involved in the progression of a neurodegenerative process in the SN. The chronic expression of IL-1β exhibited most of the characteristics of the experimental models of PD, including dopaminergic cell death, behavioral response and glial activation. Our model will be helpful to study the mechanisms of inflammatory effects on PD and the possible deleterious events induced by glial activation and cytokine production during the progression of PD.

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