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Post intoxicative therapeutic immunization with myelin oligodendrocyte glycoproteine (MOG 35-55) suppresses spontaneous regeneration of dopaminergic neurons injured with 1-methyl-4 phenyl-1,2,3,6-tetrahydropiridine (MPTP)

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Abstract. The pathological process of neurodegeneration is accompanied by an inflammatory reaction that is believed to contribute to the pathogenesis of neurodegenerative diseases. The aim of our study was to evaluate the influence of autoimmune reaction induced by post-traumatic vaccination with myelin self-antigen on spontaneous regeneration of dopaminergic neurons, injured with MPTP. C57BL mice were intoxicated with 40 mg/kg MPTP and seven days later immunized with MOG 35-55 peptide in CFA. On the 7th day following intoxication, the MPTP treated mice showed decrease of the dopamine level by 63% as compared to the control mice. However, starting from the 14th day following intoxication, a spectacular increase of dopamine content was observed. Immunization with MOG resulted in a statistically significant reduction of the increase in striatum as compared to non-immunized animals, and was lower by 23%, 17% and 15% on days 14, 28 and 50, respectively. Our results show suppressive influence of autoimmune reaction induced after injury on regeneration of dopamine cells intoxicated with MPTP.

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INTRODUCTION

Propagation of damage is a common occurrence after any central nervous system (CNS) insult. Consequently, the outcome of injury is far more severe than might be expected from the effect of the insult. Secondary degeneration plays the main role in this process. The term "secondary degeneration" is defined as the tendency of the damage in CNS to spread from the area where neurons are injured by the primary insult to adjacent neurons that were initially spared. Secondary degeneration arises, because injured CNS cells produce high levels of molecules that are toxic to neighboring cell bodies and cell processes (Cohen and Schwartz 1999).

The CNS is a privileged immune site, where influx of the immune cells is limited in the physiological state (Schwartz and Cohen 2000). However, T lymphocytes and macrophages have been shown to enter CNS and in the case of injury to gather in the place of the insult (Schwartz and Kipnis 2001). Although the presence of immune cells in the CNS may induce development of an autoimmune disease, in some situations the autoimmunity may be protective (Schwartz and Cohen 2000). Recently, autoreactive anti-myelin T lymphocytes have been shown to protect CNS after the insult of the spinal cord and partial injury of the optic nerve (Fisher et al. 2001, Hauben et al. 2001, Moalem et al. 2000a, Moalem et al. 2000b). Pre-injury injection of either autoreactive T lymphocytes or one of the myelin-specific proteins resulted in significantly promoted recovery after spinal cord contusion injury and a reduced number of injured retinal ganglion cells after the optic nerve injury (Hauben et al. 2001). Active induction of autoimmune reaction (by myelin protein treatment) has been shown also to be protective on time of first signs of experimental autoimmune encephalomyelitis (EAE) (5-14 days, dependent on the route of induction and animal strain). It was also shown on the model of injured mouse optic nerve, that active immunization with the encephalitogenic peptide leads to neuroprotection (Schori et al. 2001).

We have reported previously, that immunization with myelin oligodendrocyte glycoprotein 35-55 (MOG 35-55) prior to 1-methyl-4phenyl-1,2,3,6-tetrahydropiridine (MPTP) intoxication leads to protection of the dopaminergic neurons in substantia nigra (Kurkowska-Jastrzębska et al. 2002). Here we demonstrate that induction of the autoimmune reaction after the injury of CNS causes the opposite effect. We also show the route of spontaneous regeneration of the dopaminergic neurons, injured with MPTP.

METHODS

Animals

We used C57BL male mice, 8-14 weeks old, 20-25 g of weight. Animals were housed in standard conditions with free access to food and water. Mice were divided into 4 groups, 6-8 animals in each: the control group receiving 0.9% NaCl; MPTP group receiving MPTP-HCl (Sigma), MOG group receiving MOG 35-55 peptide in complete Freund adjuvant (CFA) and MPTP+MOG group receiving MPTP and seven days later immunized with MOG 35-55 in CFA. Animals were sacrificed on the day 7 after MPTP administration (groups MPTP and control), 14, 28 and 50 days after MPTP administration (groups MPTP, MOG, MPTP+MOG, control).

MPTP administration

MPTP-HCl (Sigma) was dissolved in 0.9% NaCl (2 mg/ml) and given in four intraperitoneal i.p. injections of 10 mg/kg, at 1 h intervals, to achieve a total dose of 40 mg/kg. The control group received 4 i.p. injections of 0.9% NaCl in the same pattern as for MPTP.

Active induction of experimental autoimmune encephalomyelitis (EAE)

EAE was induced by s.c. injections of 150 μg of MOG 35-55 peptide (Neosystem, France) in complete Freund adjuvant (CFA) (Difco, Detroit, MI) with 500 μg of *Mycobacterium Tuberculosis* on day 7th after MPTP administration, supplemented by i.v. injections of 300 ng of pertusis toxin (Sigma, Poland), as described previously (Fisher et al. 2001).

Clinical assessment of animals

A clinical score was assigned daily for 43 days. The clinical score was graded on scale of 0 to 5 with graduations 0.5 for intermediate scores: 0, no clinical signs; 1, flaccid tail; 2, hind limb weakness and abnormal gait; 3, complete hind limb paralysis; 4, complete hind limb paralysis with forelimb weakness or paralysis; 5, moribund or deceased. Supplementary food and water were provided on the cage floor for disabled animals.

HPLC analysis of dopamine content

HPLC evaluation of the dopamine content in striatum was performed on the 7th, 14th, 28th and 50th day following MPTP intoxication. Striata were rapidly dissected from the brain tissue, weighed, and homogenized in 1000 µl ice cold 0.1 N HClO4 and centrifuged at 13,000 x g for 15 min. The supernatant was removed, filtered (0.2 µm pore size; Whatman, USA) and examined for its contents of DA. Dopamine (supplied by RBI), its metabolite DOPAC (3.4 dihydroxyphenylaceticacid; RBI), HVA (homovanilic acid; Sigma), 5HT (5-hydroxytryptamine; Sigma) and 5-HIAA (5-hydroxyindolacetic acid; Sigma) were measured using high-performance liquid chromatography (HPLC) with electrochemical detection and glassy carbon electrode. The electrochemical potential was set at 0.8 V with respect to an Ag/Ag Cl reference electrode. The chromatograph system consisted of an autosampler automatic injector (Knauer Basic Marathon), a pump (Mini-Star K- 500; Knauer) and an electochemical detector (L-3500A; Merck). The mobile phase comprised 32 mM sodium phosphate (Sigma), 39 mM citric acid (Sigma), 1 mM octan sulfonic acid (Aldrich), 54 µM ethylenediaminetetraacetic acid (EDTA, Sigma) in deionized water containing 0.15% acetonitrile (Merck) and 6.5% methanol (Merck). Separation of monoamines was achieved with a C-18 column (250 mm x 4 mm reverse phase, Nucleosil, 5 µm particle size; Macherey- Nagel, Germany) and mobile phase flow ratio maintained at 0.8 ml/min.

Samples were quantified by comparison with standard solutions of known concentration. Area under peaks was quantified with the HPLC software. Data were collected and analyzed by Eurochrom 2000 for Windows (Knauer).

Statistical analysis

All data were analyzed using Statistica for Windows. Groups were compared by the Mann-Whitney U-test. $P<0.01$ was considered to be statistically significant.

RESULTS

Active immunization with the MOG 35-55 peptide in C57BL mice with injured dopaminergic system results in relapsing-remitting EAE course (Fig. 1). EAE had a relapsing-remitting course. The first attack started on the day 2nd after immunization (9th after MPTP intoxication), with a peak (mean clinical score 4) on the day 8th (15th after MPTP intoxication) and lasted till the 14th day (21st day after MPTP intoxication). The sec-

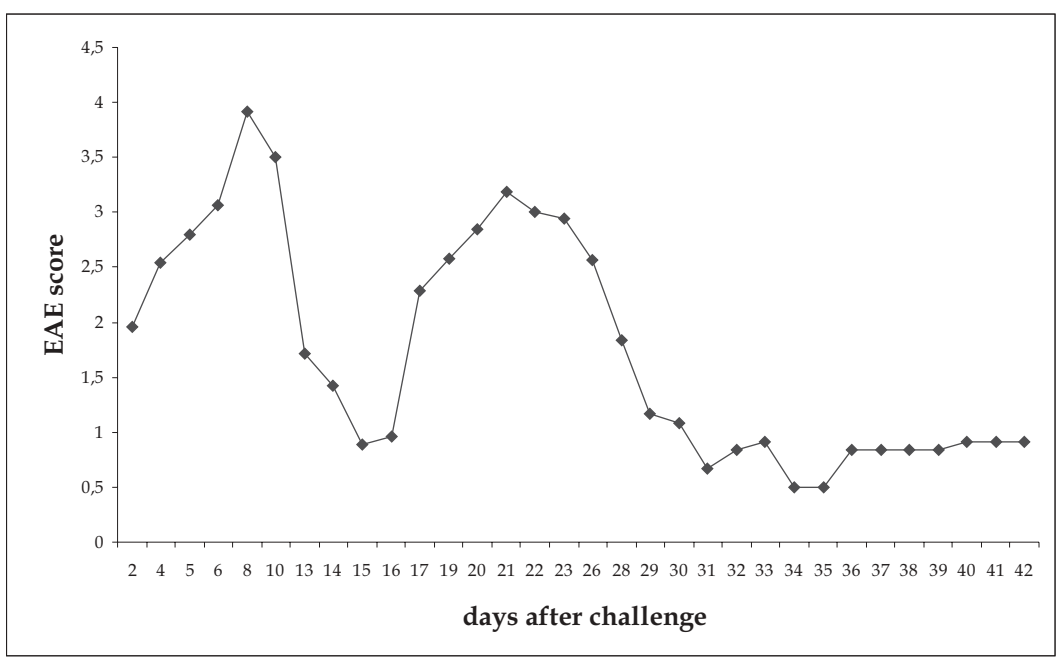

Fig. 1. Clinical course of EAE induced by MOG 35-55 immunization in mice intoxicated 7 days earlier with MPTP. Immunization of mice with 150 mg of MOG 35-55 peptide resulted in relapsing-remitting EAE course, with two relapses (with mean clinical scores of 4 and 3). From the 31st day EAE had chronic course.

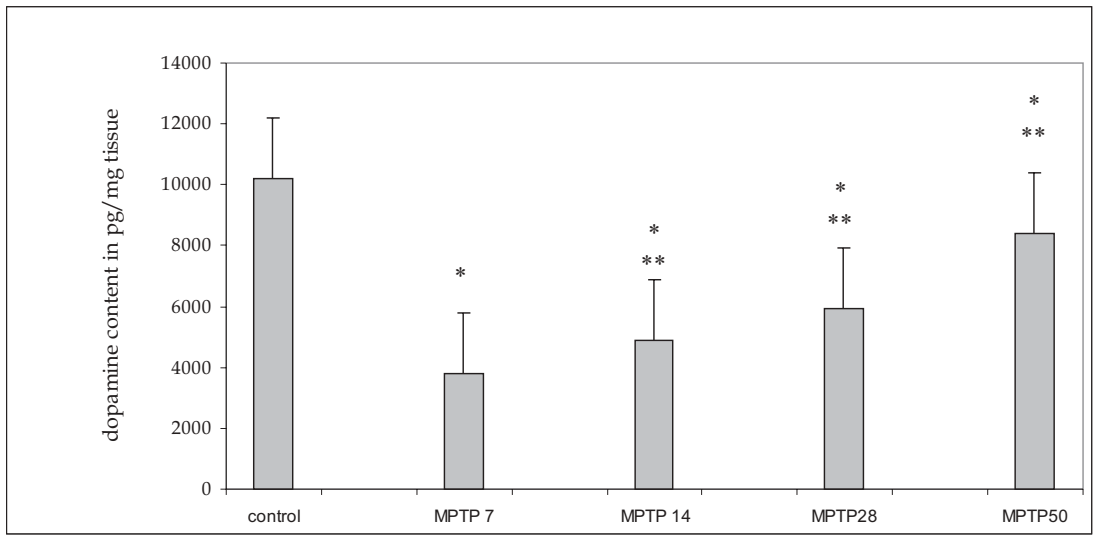

Fig. 2. Dopamine content in the striata of the MPTP treated mice. From the 14th day of the experiment constant increase in dopamine content was observed, indicating regeneration of the dopaminergic neurons injured with MPTP. Significant difference: *, from control; **, from MPTP 7.

ond attack started on the day 16th after immunization (23rd after MPTP intoxication), with a peak (mean clinical score 3) on the day 21st (28th after MPTP intoxication) and lasted till the 29th day (36th day after MPTP intoxication). Starting from the 31st day till the end of the experiment (50th day after the MPTP administration) EAE had chronic course, with the mean clinical score 1.

Spontaneous regeneration of the dopaminergic neurons after MPTP intoxication

Dopamine levels were measured on the days 7, 14, 28 and 50 after intoxication. MPTP intoxication caused 63% dopamine depletion on the day 7, 52% on the day 14, 42% on the day 28 and 18% on the day 50, as compared to the control group. Thus starting from the 14th day, a continuous increase in dopamine content was observed. The dopamine level on the 14th day was higher by 11%, on the 28th day by 21%, and on the 50th day by 45%, as compared with the 7th day level. However, on the 50th day dopamine content was still lower from the control one (Fig. 2).

Influence of autoimmune reaction on dopamine neuron regeneration after MPTP intoxication

We observed a larger decrease of the dopamine levels at all time points in groups of mice vaccinated with the MOG 35-55 on the 7th day after MPTP administration, than in the groups treated only with MPTP on respective days. However, percentage of decrease was strongest in the acute phase of EAE (14th day, 75%), middle on the 28th day (second phase of EAE, 59%) and the weakest on the 50th day (recovery phase, 33%). Administration of the MOG 35-55 alone did not change dopamine level as compared to the control (Fig. 3).

DISCUSSION

The results presented herein demonstrate that the regeneration of 1-methyl-4phenyl-1,2,3,6-tetrahydropiridine (MPTP) injured dopaminergic neurons is significantly decreased due to subsequent active immunization with an encephalitogenic MOG 35-55 peptide. The initial purpose of the study was to determine how intense is the spontaneous regeneration of dopaminergic neurons after MPTP administration. We have previously reported, that for the first 7 days after intoxication mainly necrotic dopaminergic neurons are present in the substantia nigra, but starting from the 14th day regenerating neurons are also visible (Bieganowska et al. 1993, Kohutnicka et al. 1998, Kurkowska-Jastrzebska et al. 1999). However, the exact extent of regeneration of the dopaminergic neurons after MPTP injury has never been established before. In this study we showed a significant increase in the dopamine level, indicating regeneration of injured neurons that started 14 days and

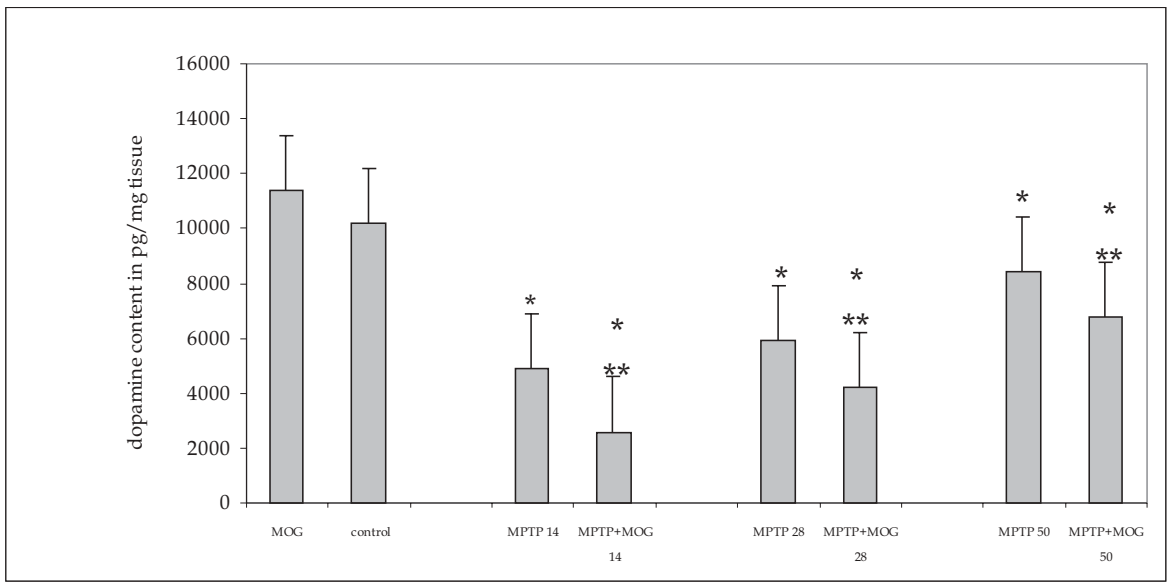

Fig. 3. Autoimmune reaction induced after MPTP intoxication suppresses regeneration of dopaminergic neurons. Post-injury immunization resulted in the inhibition of regeneration of the dopaminergic neurons. Inhibition was strongest during the first, strongest attack of EAE, but the chronic autoimmune reaction (from the 31st day of experiment- mean clinical score 1) also influenced regeneration of the dopaminergic neurons. Significant difference: *, from control, **, from MPTP in the respective day.

achieved 82% of the control level 50 days after intoxication.

Inflammation present in the area of injury was proved to play a detrimental role for the neurodegeneration processes in the CNS (Lotan and Schwartz 1995). Immunologic reaction has been long claimed to be harmful for the central nervous system. Inflammatory reaction observed during pathological processes of neurodegeneration, such as in the Alzheimer's and Parkinson's diseases was believed to contribute to the pathogenesis and accelerate degeneration of the nervous tissue (Akiyama et al. 2000). Moreover, in AD the retrospective and prospective studies have strongly suggested that an anti-inflammatory treatment with conventional anti-inflammatory drugs may delay the onset or slow the progression of the disease (Mackenzie 2000, McGeer et al. 1996, Veld et al. 2001).

On the other side, it was shown, that immune cells, due to their properties and function can support regeneration and suppress degeneration of the CNS cells (Prewitt et al. 1997, Rapalino et al. 1998). Microglia can phagocyte cellular debris and secrete cytokines that stimulate regeneration of injured cells (Rapalino et al. 1998). Similarly, T lymphocytes have been shown to stimulate regeneration mainly by secretion of the neurotrophic factors (Hammarberg et al. 2000).

It is suggested that the increased production of the neutrophins (NT-3, NT-4/5, brain derived neurotrophic factor -BDNF) after the antigen stimulation may be responsible for the neuroprotective properties of the T lymphocytes (Kerschensteiner 1999).

Moreover, autoreactive anti-myelin T lymphocytes have been proved to play a detrimental role in the CNS neuroprotection after injury of the myelinated CNS axons in the spinal cord or optic nerve (Hammarberg et al. 2000, Moalem et al. 1999). Systemic injection of the activated T cells after the CNS injury leads to an increase in the number of accumulated T cells in the damaged area, regardless of their antigen specificity (Cohen and Schwartz 1999). However, only T cells that encounter their specific antigen at the lesion site are capable of ameliorating the consequences of the insult. T cells specific to myelin-associated self-antigens or to peptides derived from them are able to partially counteract the secondary degeneration, thereby improving recovery (Cohen and Schwartz 1999).

An animal model of multiple sclerosis- experimental autoimmune encephalomyelitis (EAE) is employed in the studies on autoreactive reaction influence on neurodegeneration. EAE may be induced by either myelin protein or autoreactive anti-myelin T lymphocytes administration (Devaux et al. 1997, Rapalino et al. 1998). Both ways of induction may contribute to neurodegeneration after CNS injury (Fisher et al. 2001, Hauben et al. 2001).

The MPTP model is considered suitable for CNS inflammation research, because besides of causing depletion of the dopamine content in the striatum and a decrease in the number of dopaminergic cells in the substantia nigra, it provokes an abundant reaction of microglia and astrocytes (Czlonkowska et al. 1996, Kohutnicka et al. 1998). Additionally, an increased expression of IL-6, MHC class I and II, ICAM-1 and lymphocyte infiltration characterises pathological changes after MPTP intoxication (Kurkowska-Jastrzebska et al. 1999). Modulation of inflammatory process after MPTP intoxication by such anti-inflammatory drugs as dexamethasone and indomethacine has been shown to protect dopaminergic neurons from degeneration (Kurkowska-Jastrzebska et al. 2002, Kurkowska--Jastrzębska et al. (in press)). Similarly, autoimmune reaction after the MOG 35-55 immunization was shown to be protective, if induced 6 days before the MPTP administration. This result supports the hypothesis about the crucial role of the extent of inflammation in defining the area of injury (Kurkowska-Jastrzebska et al. 2002). The therapeutic window for the passive transfer of T cells was found to be at least 1 week, but T the lymphocytes have been detected in the area of the injury for 3 days after a passive transfer (Moalem et al. 1999). Autoimmune response has been shown to be protective only in cases, in which the induced EAE is mild, the immunization has no beneficial effect in mice that develop severe EAE (Schwartz 2000).

In our present study, vaccination with MOG 35-55 seven days after MPTP administration caused an inhibition of regeneration. It is posible, that seven days after MPTP intoxication, inflammatory reaction in the area of substantia nigra is becoming balanced and starts supporting regeneration of the dopaminergic neurons (Kurkowska-Jastrzebska et al. 1999). Disregulation of this balance may cause immune reaction to become destructive for the regenerating neurons. An observation that suppression of spontaneous regeneration was strongest in acute phase of EAE, and became weaker along with the regression of the disease, supports that conclusion. However, full explanation of this phenomenon needs further studies.

In summary, immunization with the CNS myelin antigen MOG 35-55 seven days after the MPTP intoxication was shown here to suppress spontaneous regeneration of the dopaminergic neurons. The therapeutic window is considered to be an important factor in all studies on the vaccination-induced neuroprotection. It is possible, that in our model the inflammatory reaction in the area of substantia nigra was too robust to support regeneration of dopaminergic neurons. Thus, inflammatory reaction might be considered as a protective factor for the CNS only if a balanced, concerted action of different immune cells is present in the area of the injury.

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