PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Lymphocytes from Mice with a 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine(MPTP)-Induced Parkinsonian Syndrome Reduce Motor Activity in C57Bl/6 Mice

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Motor activity of C57Bl/6 mice is found to be decreased following syngeneic transfer to them of splenocytes from mice with an MPTP-induced parkinsonian syndrome. The decrease in motor activity does not result from the transfer of MPTP and is apparently associated with the transfer of B lymphocytes and with antibody production by these cells.

Key Words: *motor activity; MPTP; Parkinson's syndrome; splenocytes*

The mechanisms responsible for degenerative damage to CNS structures (substantia nigra, corpus striatum) in Parkinson's disease remain largely unknown. It has been shown recently that an important part in the pathogenesis of this disease is played by immunological processes. Blood sera from human patients and animals with Parkinson's syndrome have been found to contain antibodies that attack selectively dopaminergic neurons of the substantia nigra [6], as well as antibodies to neurotransmitters such as dopamine and norepinephrine [1,2]. In rats, intracaudate injection of anti-dopamine antibodies obtained by immunizing rabbits with dopamine-protein conjugates or of anti-dopamine-containing sera from parkinsonian patients led to an experimental parkinsonism manifested in akinesia, tremor, and rigidity [2]. The question of what contribution cell-mediated immunity might make to the development of Parkinson's and other neuropathological syndromes has been given little study, but there is some scattered evidence in the literature that cellular mechanisms of immunity are implicated. For example, B lymphocytes from C57Bl/6 mice were shown to be capable of transferring the analgesic effect induced in them by intraventricular injection of morphine [5], while adoptive transfer of splenocytes from rats with morphine abstinence to rats in which the naloxone-induced abstinence syndrome had been suppressed by X radiation was found to result in the reappearance of a full-blown abstinence syndrome in the latter animals [4].

In this study, we explored whether parkinsonian symptoms could arise in C57Bl/6 mice following adoptive transfer to them of lymphocytes from mice of the same strain with the characteristic parkinsonian symptoms of akinesia and rigidity induced by systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

MATERIALS AND METHODS

Male C57Bl/6 mice aged 10 months and weighing 25-30 g before the tests were used. One model of

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damage to the dopamine system in Parkinson's disease is the parkinsonian syndrome produced in mice by injecting them with the neurotoxin MPTP [7]. MPTP was injected into mice twice daily in a dose of 20 mg/kg per injection at 12-h intervals for 10 days. Their motor activity was evaluated automatically in an Opto-Varimex system (Columbus Instruments, USA) using the Auto Track software package run on an Apple IIe microcomputer. For these tests, mice were placed in a 37×37 cm plastic chamber equipped with infrared sensors, and the distance they covered, their resting time, and the time of their movement were then measured during 3 min at room temperature, after which the speed of movement was calculated from the data obtained. The motor activity of MPTP-treated mice was evaluated before and after the MPTP treatment. The results were processed statistically using Student's t test.

Lymphocyte donors were MPTP-treated mice killed on day 10, when Parkinson's syndrome was at its height, as judged by their display of strongly marked oligokinesia progressing to akinesia (the distance covered decreased from 903.4±37 to 70.8±31.1 cm while the resting time increased from 34±2.9 to 173.4±2.3 sec; $p<0.001$), as well as by the rigidity of the trunk (kyphosis). Their spleens were removed and splenocyte suspensions consisting of T and B lymphocytes and macrophages were prepared. From the suspensions a B-lymphocyte population that also contained macrophages was obtained by the conventional cytolytic procedure [3] using a monoclonal antibody against murine T lymphocytes (an anti-Thy1 serum obtained at the Laboratory of Clinical Radioimmunology of the Russian Cancer Research Center). In these experiments, cells that had shown 95% viability in the dye (trypan blue) exclusion test were used.

There were two test and three control groups of C57Bl/6 mice. Mice of test group 1 were injected i.p. with 2×10^7 splenocytes from MPTP-treated mice, while mice of test group 2 received the same number of B lymphocytes from MPTP-treated mice by the same route. Mice of control group 1 were injected i.p. with splenocytes isolated from intact mice and then cultured for 60 min at 37°C with 2 mg MPTP/ml in RPMI 1640 medium containing 10% calf serum (Serva); mice of control group 2 were injected i.p., with splenocytes from intact mice, and those of control group 3, also i.p., with physiological saline. The splenocyte and B-lymphocyte suspensions and saline were injected in a volume of 0.5 ml. Motor activity in all groups was measured three times - just before the injection of splenocytes, B lymphocytes, or saline and then on days 7 and 14 after their injection.

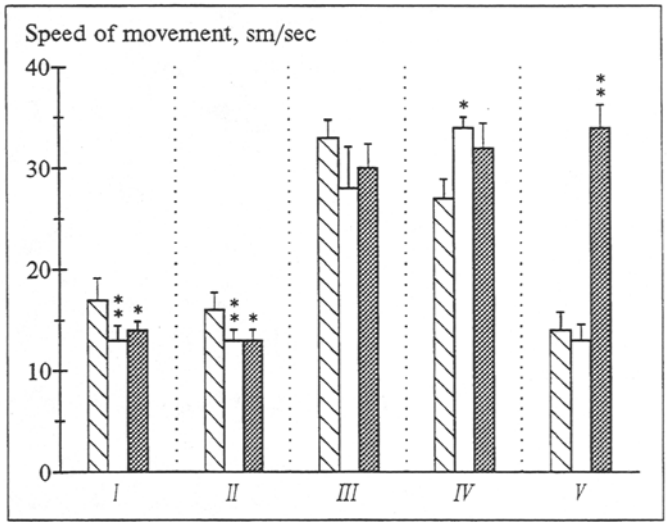

Fig. 1. Effect of syngeneic splenocyte transfer from MPTP-treated mice to intact C57Bl/6 mice on the speed of their movement. *I*) splenocytes transferred from MPTP-treated mice; *II*) B lymphocytes transferred from MPTP-treated mice; *III*) transfer of MPTP-treated splenocytes; *IV*) transfer of intact splenocytes; *V*) saline-treated controls. Hatched bars: baseline speed; white bars: day 7 after transfer; black bars: day 14 after transfer. Asterisks indicate significant differences from baseline: $^*p<0.05$, $^{**}p<0.001$.

RESULTS

The results are presented in Table 1. It can be seen that the distance covered by mice and the time of their movement were decreased while their resting time was increased on days 7 and 14 in both test groups: in group 1 after the adoptive syngeneic transfer of the entire pool of immunocompetent cells, i.e., of all splenocytes, from MPTP-treated mice and in test group 2 after the transfer of B lymphocytes from such mice. In control group 2, which had received splenocytes from intact mice, the time of movement decreased and the resting time increased significantly on day 7, whereas no significant change in the distance covered occurred either on day 7 or on day 14. No significant changes in motor activity were recorded in control group 1, which had received splenocytes recovered from intact mice and then cultured with MPTP. These findings indicate that the reduction in motor activity observed in mice transferred with splenocytes or B lymphocytes from animals with the experimental parkinsonian syndrome did not result from the transfer of MPTP toxin by immunocomponent cells.

It should be noted that the mice administered splenocytes or B lymphocytes from animals with

TABLE 1. Effect of Syngeneic Splenocyte Transfer from MPTP–Treated Mice on Motor Activity of C57Bl/6 Mice

Materials given to recipients	Distance covered (cm):			Movement time (sec):			Resting time (sec):		
	before transfer	after transfer		before transfer	after transfer		before transfer	after transfer	
		day 7	day 14		day 7	day 14		day 7	day 14
Splenocytes from MPTP–treated mice ($n=12$)	854.2±40.9	340.0±41.2**	284.3±34.5**	48.6±3.26	26.9±2.4**	21.5±1.7**	73.2±7.2	96.5±6.4	102.8±4.4*
B lymphocytes from MPTP–treated mice ($n=10$)	726.6±31.3	365.5±22.3**	400.5±35.5**	46.3±4.2	29.2±2.8**	30.1±4.6**	70.8±4.1	80.1±6.4	78.4±7.7
MPTP–treated splenocytes ($n=8$)	826.5±76.2	709.7±113.6	680.4±95.3	26.2±2.7	25.6±4.9	22.9±4.1	119.3±8.6	121.3±8.3	128.1±11.9
Intact splenocytes ($n=8$)	726.6±93.3	477.5±115.7	639.0±122.6	27.2±2.9	13.4±3.4*	21.0±3.8	118.3±6.9	145.3±6.2**	133.3±6.4
Saline (control) ($n=10$)	768.0±42.4	802.4±47.5	786.1±95.2	58.6±3.8	62.0±0.9	23.5±2.4**	42.5±4.2	35.6±3.4	132.1±12.6**

Note. n – number of animals. Asterisks indicate the significance of differences from baseline motor activity: *$p<0.05$; **$p<0.01$.

the parkinsonian syndrome moved at a reduced speed in the chamber (Fig. 1), which may be taken as evidence that they had developed the bradykinesia characteristic of this syndrome. The control mice given saline or lymphocytes from intact mice moved at a somewhat accelerated speed.

The findings of this study suggest that the decrease in motor activity shown by C57Bl/6 mice following the transfer of splenocytes from mice with the MPTP-induced parkinsonian syndrome resulted from the transfer of B lymphocytes. There are reasons to believe that this effect was due in part to the production of antibodies by the latter. Such antibodies could evidently be antibodies to dopamine, which cause an experimental parkinsonian syndrome to develop [1,2]. However, macrophages might also be involved in this process, since the B-lymphocyte suspensions contained these cells.

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