Effect of Transplantation of Immunocompetent Cell on Orientation and Exploratory Behavior and Cytokine Gene Expression in the Brain of Experimental Animals

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 9, pp. 309-312, September, 2006
Original article submitted January 30, 2006

Intravenous injection of adherent splenocyte fraction from donor (CBA×C57Bl/6)F_1 mice characterized by specific open-field behavior modified this behavior in syngeneic recipient mice. This was paralleled by appropriate changes in the levels of IL-1β and type 1 IL-1 receptor mRNA in the brain cells of recipient mice. Hence, we demonstrated the possibility of directed regulation of orientation and exploratory behavior in mice by transplantation of immunocompetent cells. Mononuclear phagocytes play an important role in this phenomenon.

Key Words: *immunocompetent cell transplantation; orientation and exploratory behavior; brain; cytokines*

Detection of the functional interactions between the immune and nervous systems is one of the major achievements of modern biology and medicine. Pronounced phenotypical and functional similarity of their cell elements implies the involvement of immunocompetent cells into the regulation of higher nervous activity processes. Our previous studies showed that functional activity of the immune system, specifically of immune cells, correlates with the level of orientation and exploratory behavior (LEB), one of the major behavioral types providing the animals information about the environment and serving as an important psychological mechanism of adaptation in higher vertebrates [1,5-8,14]. We previously demonstrated the capacity of intravenously injected splenic cells isolated from mice characterized by specific behavioral patterns in the open field test to modify the behavior of syngeneic recipients [1,2]. Further studies were aimed at deciphering of the mechanisms of this phenomenon.

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Here we studied the effect of transplantation of adherent splenocyte fraction on the orientation and exploratory behavior and expression of cytokine genes in the brain of recipients with initially different behavioral status.

MATERIALS AND METHODS

The study was carried out on 3-month-old (n=160) male (CBA×C57Bl/6)F_1 mice from Breeding Center of Institute of Pharmacology, Siberian Division of Russian Academy of Medical Sciences. The animals were kept in cages (10 mice per cage) and received standard diet with free access to water and normal day/night regime for at least 2 weeks before the experiment. All experiments were carried out from 10.00 to 15.00. The orientation and exploratory behavior of mice was evaluated in the open field test [3]. The study was carried out in a square (100×100 cm, 100 squares) chamber with 40-cm plastic walls illuminated with a shadowless 100 W lamp positioned at the height of 100 cm above the center of the field. The animal was placed into the corner of the chamber and its minute-by-minute

0007-4888/06/1423-0338

activity was recorded for 5 min. The number of crossed central and peripheral squares, rearings (with and without support upon the wall), and summary motor activity were recorded. In order to evaluate the emotional stress, fecal boluses were counted.

By open-field behavior (CBA×C57Bl/6)F_1 mice were divided into 3 groups: with high, medium, and low LEB [5,8,14].

Immunocompetent cells for transplantation were isolated from splenocyte suspension of donor mice with high and low LEB by adhesion to plastic for 2 h at 37°C after removal of erythrocytes by hemolytic shock. After double washout, the remaining cells were removed from plastic with a cell scraper (Becton Dickinson) and collected into a tube. The resultant suspension contained 86-92% mononuclear phagocytes. Cell viability (evaluated by Trypan blue exclusion) was 93-95%.

The cells (8×10^6 in 0.2 ml RPMI-1640) from donors with high LEB were intravenously injected to recipients with low LEB, while cells from donors with low LEB were injected to recipients with high LEB. The parameters of orientation and exploratory behavior and expression of the cytokine genes in recipient brain were evaluated on day 5 after transplantation. The mice receiving cells from donors with identical LEB served as controls for each experimental group [2].

The levels of IL-1β and type 1 IL-1 receptor mRNA in mouse brain were evaluated by reverse transcription—polymerase chain reaction (PCR). Summary RNA was isolated as described previously [11]; reversion and amplification reactions were carried out as described previously [9]. Primers to IL-1β, type 1 IL-1 receptor, and β-actin were synthesized for PCR (β-actin was used for standardization and leveling of the results of analysis of the studied DNA samples) [9]. PCR products were visualized in a Pharmacia-LKB densitometer; semiquantitative evaluation of the results was carried out using Image Master VDS Software. The results were expressed in arbitrary optical density units.

The results were statistically processed using Student's *t* test and paired Mann—Whitney test (Jandel Sigma Plot, Statistica software). The results were presented as *M*±*SD*, the differences were considered significant at $p<0.05$.

RESULTS

Transplantation of adherent splenocyte fraction consisting mainly of mononuclear phagocytes from donors with high LEB to recipients with low LEB was associated with a significant increase in all behavioral parameters of recipients in the open field test. An opposite picture was observed in pairs, where donors were mice with low LEB and recipients were mice with high LEB: LEB parameters in recipients decreased (Table 1). Opposite changes in the degree of emotional strain of recipient mice (number of boluses) were observed: a decrease of mental stress in recipients with initially low LEB (4.7±1.4 in control and 2.5±1.6 in experiment, $p<0.05$) and its increase in recipients with initially high LEB (0.5±0.8 and 3.6±1.4, respectively, $p<0.05$). In controls, the parameters of orientation and exploratory behavior virtually did not change after transplantation. The fact that similar changes in the behavioral reaction parameters were observed after transplantation of nonfractionated splenocyte suspension [2] attest to an important role of the mononuclear phagocyte system in the mechanisms of directed regulation of orientation and exploratory behavior. IL-1 is one of the main regulatory cytokines of macrophages; it is produced, apart from other cells, in the CNS [4,12,15]. IL-1 after peripheral and central (at the level of the cerebral ventricles) administration decreases locomotor activity of ani-

TABLE 1. Parameters of Orientation and Exploratory Behavior of Recipient Mice after Transplantation of Splenocyte Fraction Adherent to Plastic (*M*±*SD*)

Series, group		Horizontal motor activity			Vertical motor activity		
		peripheral	central	summary	free	with support on the wall	summary
Series I	control (*n*=50)	188.1±33.7	24.3±6.4	212.4±35.3	4.4±3.4	11.4±6.6	15.9±9.2
	experiment (*n*=50)	93.9±26.5**	6.6±5.7**	100.6±29.9**	2.4±1.0*	6.1±1.8*	8.4±1.8*
Series II	control (*n*=30)	12.7±5.8	0	12.7±5.8	0	0.27±0.70	0.27±0.70
	experiment (*n*=30)	67.7±27.9**	3.2±2.8**	70.9±29.9**	0.9±0.8**	4.1±4.0**	4.96±5.00**

Note. Series I) control: donors and recipients with high LEB; experiment: donors with low LEB, recipients with high LEB. Series II) control: donors and recipients with low LEB; experiment: donors with high LEB, recipients with low LEB. *$p<0.05$, **$p<0.01$ compared to the control.

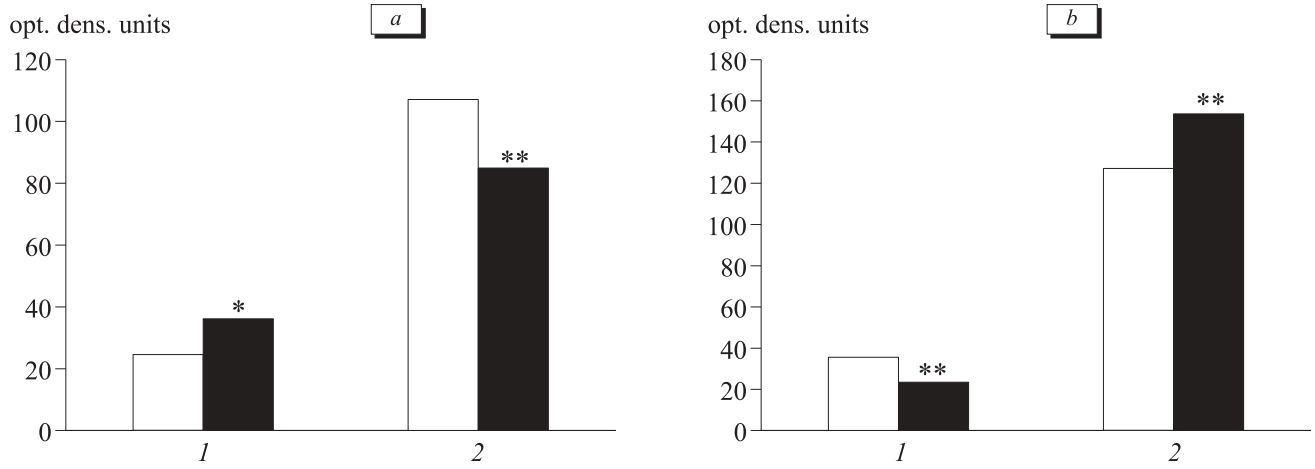

Fig. 1. Levels of IL-1β and type 1 IL-1 receptor mRNA in brain cells of recipient mice with different initial LEB in the open field test after transplantation of adherent splenocyte fraction. *a*) donors with low LEB, recipients with high LEB; *b*) donors with high LEB, recipients with low LEB. *1*) IL-1β gene; *2*) type 1 IL-1 receptor gene. Light bars: control group, dark bars: experimental group. *$p<0.05$, **$p<0.01$ compared to the control

mals [10,12,13]. Our studies also showed that (CBA×C57Bl/6)F_1 mice with different LEB differed significantly by the expression of IL-1β and type 1 IL-1 receptor genes in brain cells [1,8]. Moreover, these changes in animal behavior after transplantation of the splenocyte fraction adherent to plastic were paralleled by opposite changes in mRNA levels for these cytokines in brain cells of recipient mice. The decrease in recipient LEB was associated with an increase in IL-1β mRNA level and a decrease in type 1 IL-1 receptor mRNA level (Fig. 1, *a*), while the increase in recipient LEB was paralleled by a decrease in IL-1β mRNA level and an increase in type 1 IL-1 receptor mRNA (Fig. 1, *b*).

Hence, we showed the possibility of directed regulation of orientation and exploratory behavior and expression of IL-1β and type 1 IL-1 receptor genes in brain cells of animals by means of transplantation of adherent splenocyte fraction consisting mainly of mononuclear phagocytes.

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