Effect of Transplantation of Splenic Lymphoid Cells on Functional Activity of the Immune and Nervous System in Experimental Animals

E. V. Markova, V. V. Abramov, T. G. Ryabicheva, and V. A. Kozlov*

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We studied the effect of transplantation of splenic lymphoid cells on functional activity of the nervous (orientation and exploratory behavior and expression of genes for interleukin-1β, type 1 interleukin-1 receptor, and erythropoietin in the brain) and immune system (cellular and humoral immune response, proliferative activity of immunocompetent cells, and expression of cytokine genes in splenocytes) in syngeneic animals with different behavioral characteristics. Intravenous injection of the lymphoid fraction of splenocytes from donor mice with a certain behavioral pattern in the open-field test had a modulatory effect on vertical locomotor activity of syngeneic recipient mice. The increase or decrease in vertical locomotor activity due to transplantation of immunocompetent cells was accompanied by specific changes in mRNA level for erythropoietin receptor and type 1 interleukin-1 receptor in brain cells of recipient mice. The regulation of orientation and exploratory behavior was also accompanied by changes in functional activity of the immune system in recipient animals. It was manifested in modulation of proliferative activity of thymic and splenic cells and cytokine gene expression in splenocytes.

Key Words: *transplantation of immunocompetent cells; orientation and exploratory behavior; humoral and cellular immune response; cytokines*

Our previous studies showed that intravenous injection of splenocytes from mice with a certain behavioral pattern in the open-filed test is followed by specific changes in behavioral characteristics of syngeneic recipients [1,2,9]. Studying the mechanisms for this effect showed that the monocyte—macrophage fraction of splenocytes plays an important role in the regulation of orientation and exploratory behavior after transplantation of immunocompetent cells. Transplantation of these cells to syngeneic recipients with other psychophysiological parameters has a modulatory effect on the immune function. They affect proliferative activity of cells, cytokine gene expression in splenocytes, and intensity of cellular immune reactions. The observed changes contribute to a regulatory effect of cells on functional activity of the central nervous system (CNS) in recipient animals. This conclusion is derived from variations in orientation and exploratory behavior and cytokine gene expression in brain cells [6].

Here we studied the role of transplantation of splenic lymphoid cells in the regulation of functional activity of the nervous and immune system in experimental animals.

Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk; *Vector-Best Company, Novosibirsk oblast, Kol'tsovo, Russia. ***Address for correspondence:*** evgeniya_markova@mail.ru. E. V. Markova

MATERIALS AND METHODS

Experiments were performed on 304 male (CBA×C57Bl/6)F_1 mice aging 3 months and obtained from the nursery of laboratory animals (Institute of Pharmacology, Siberian Division of the Russian Academy of Medical Sciences). The animals were kept in cages (10 specimens per cage) in a vivarium under normal light/dark conditions and *ad libitum* food (standard diet) and water supply for 2 weeks before the start of the experiments. All manipulations were conducted at 10.00-15.00.

Orientation and exploratory behavior of mice was studied in the open-field test [3]. The open field was a large rectangular chamber (100×100 cm, 100 squares) surrounded by plastic walls (40 cm in height) and illuminated with a 100-W shadow-free lamp (100 cm above the center of the area). The animal was placed into the corner of the chamber. Behavioral activity was registered every minute for 5 min. We recorded the number of crossed central and peripheral squares, count of central (free) and peripheral (leaning on the wall) rearing postures, and total locomotor activity. The degree of emotional strain was estimated from the number of fecal boluses. Depending on the open-field behavior, (CBA×C57Bl/6)F_1 mice were divided into 3 groups: animals with high, intermediate, and low levels of orientation and exploratory behavior (LEB) [7,13].

Immunocompetent cells for transplantation were isolated from the splenocyte suspension of donor mice with high or low LEB. The monocyte—macrophage fraction of cells was removed by adhesion to plastic at 37°C for 2 h. The suspension consisted of 88-93% lymphoid cells. Cell viability was estimated by trypan blue exclusion (93-95%).

The cells (8×10^6 cells in 0.2 ml RPMI-1640 medium) from donors with high LEB were injected intravenously to recipients with low LEB, or vice versa. Orientation and exploratory behavior, cytokine gene expression in brain cells, number of antibody-producing cells (APC) in the spleen, delayed-type hypersensitivity (DTH) reaction, and proliferative response of immunocompetent cells in recipient mice were evaluated on day 5 after transplantation.

Control mice (relative to each treatment group) were subjected to transplantation under similar conditions, except that donors and recipients did not differ in LEB [2].

The levels of mRNA for interleukin-1β (IL-1β), IL-1 receptor type 1, and erythropoietin in the brain and spleen of recipient mice and transplanted fraction of splenocytes from donor animals were measured by the reverse transcriptase-polymerase chain reaction (PCR). Total RNA was isolated as described elsewhere [11]. The reactions of reversion and amplification were performed as described previously [10]. PCR primers for IL-1β, IL-1 receptor type 1, and β-actin (standardization and equalization of data for cDNA samples) were synthesized as described elsewhere [10]. PCR products were visualized using a Pharmacia-LKB densitometer. Semiquantitative analysis of the results was performed by means of Image Master VDS Software. The results were expressed in relative units of optical density (optical density units of cytokine cDNA per optical density units of β-actin cDNA, ×100).

Cytokine concentration in samples of culture supernatants from transplanted (donor) cells was measured by enzyme immunoassay (EIA, ELISA) with specific components for mouse cytokines (R&D Systems). "Sandwich" solid-phase three-step EIA was performed on plates (monoclonal antibodies on the sublayer, conjugate of polyclonal antibodies and biotin). The method for cytokine evaluation was developed at the Vector-Best Company. Kit sensitivity for IL-1β and IL-4 did not exceed 5 pg/ml, for IL-6 and interferon-γ (IFN-γ) 2 pg/ml, and tumor necrosis factor-α (TNF-α) 1 pg/ml.

APC were counted as described previously [12]. The mice were immunized intraperitoneally with sheep erythrocytes (5%, 0.5 ml). The humoral immune response was evaluated from the number of APC in the spleen on day 5 after immunization.

The mice were immunized intraperitoneally with sheep erythrocytes (0.5%, 0.5 ml) to study the DTH reaction. The antigen in a challenge dose (50%, 0.05 ml) was administered subaponeurotically into the hindlimb after 96 h. The DTH reaction was evaluated from hindlimb swelling 24 h after injection. We recorded changes in the thickness of the treated limb compared to thickness of the positive control hindlimb (injection of PRMI-1640 medium). The reaction index of each mouse was calculated as follows:

$$RI=(R_T-R_C)/R_C,$$

where R_T and R_C are the reactions in the treated and control limbs, respectively (thickness of the limb, mm). The index was expressed in percent [14].

The proliferative response of immunocompetent cells in recipient mice was studied by the standard method of lymphocyte blast transformation [8]. *E. coli* O111:B4 lipopolysaccharide (LPS, Sigma) and concanavalin A (Pharmacia) in suboptimal concentrations of 25 and 1 μg/ml, respectively, were used as mitogens.

The results were analyzed by Student's *t* test and pairwise Mann—Whitney test (Jandel Sigma

Plot and Statistica). The results were expressed as means and standard deviations. The differences were significant at $p<0.05$.

RESULTS

Transplantation of splenic lymphoid cells from donors with high LEB to recipients with low LEB was accompanied by a significant increase in vertical activity of recipient animals in the open-field test. Opposite changes were observed after transplantation of cells from donors with low LEB to recipient with high LEB (decrease in behavioral activity of recipient animals, Table 1). Transplantation of splenic lymphoid cells had no effect on orientation and exploratory behavior of control mice (donors and recipient with similar LEB).

Variations in vertical locomotor activity of animals due to transplantation of the lymphoid fraction of splenocytes were accompanied by various changes in the level of erythropoietin receptor mRNA in brain cells of recipient mice. The increase in vertical activity of recipient animals was accompanied by increased expression of this gene. However, the level of mRNA for interleukin-1β receptor type 1 was shown to decrease under these conditions (Fig. 1, *a*). The decrease in behavioral activity of recipient animals was accompanied by reduced expression of erythropoietin receptor mRNA (Fig. 1, *b*).

Transplantation of splenic lymphoid cells had a modulatory effect on the humoral immune response in recipient mice. An increase in the relative and absolute number of APC was observed after transplantation of cells from donor animals with high LEB (Table 2). No differences were found between the control and treatment groups after transplantation of cells from donor mice with low LEB.

Transplantation of the lymphoid fraction of splenocytes had little effect on the hypersensitivity reaction in recipient animals (Table 2). Opposite changes were observed in proliferative activity of immunocompetent cells. Spontaneous proliferative activity of thymocytes and concanavalin A-induced activity of splenocytes were reduced in recipient animals with low LEB. However, we revealed an increase in LPS-induced activity of splenocytes. Transplantation of splenic lymphoid cells to recipient animals with high LEB was accompanied by an increase in spontaneous proliferative activity of thymocytes and decrease in concanavalin A-induced proliferative activity of splenocytes (Table 3). Cytokine gene expression was modified in splenocytes of recipient mice with various behavioral characteristics. The level of erythropoietin receptor mRNA in splenocytes decreased after transplantation of cells from donor animals with high LEB (Fig. 2, *a*). Gene expression for IL-1 receptor type 1 in splenocytes of recipient animals decreased after transplantation of cells from donor mice with low LEB (Fig. 2, *b*).

Splenic lymphoid cells from animals with high and low indexes of LEB differed in cytokine production. Cytokines probably play a role of trigger factors that modulate functional activity of the nervous and immune system in recipient mice after transplantation of these cells. Culture supernatants of splenic lymphoid cells in mice with high LEB differed from those in animals with low LEB in the reduced level of erythropoietin receptor mRNA (57.2±14.2 and 95.2±9.8 relative optical density units, respectively; $p<0.01$) and high concentration of IL-6 (81.8±9.3 and 65.8±3.6 pg/ml, respectively; $p<0.05$). IL-6 is B cell differentiation factor, which promotes maturation of B lymphocytes to antibody-producing cells. The above mentioned changes in the humoral immune response of recipient animals are probably related to transplantation of cells with high-intensity production of IL-6. These changes can be related to the reduced expression of the

TABLE 1. Orientation and Exploratory Behavior of Recipient Mice after Transplantation of Splenic Lymphoid Cells (*M*±*SD*)

Group	Horizontal locomotor activity			Vertical locomotor activity		
	peripheral	central	total	free	leaning on the wall	total
Control 1 (*n*=72)	18.1±9.1	0.6±0.1	18.7±9.2	0.0±0.0	0.4±0.2	0.4±0.2
Treatment 1 (*n*=81)	23.5±16.1	2.6±1.6	26.2±17.4	1.2±0.6**	2.1±0.7**	3.2±1.2**
Control 2 (*n*=61)	188.1±33.7	24.3±6.4	212.4±35.3	2.7±2.0	4.3±1.8	6.9±3.8
Treatment 2 (*n*=90)	93.9±26.5	6.6±5.7	100.6±29.9	0.6±0.8*	1.9±2.0*	2.5±2.6*

Note. Control 1, donors and recipients with low LEB; treatment 1, donors with high LEB and recipients with low LEB; control 2, donors and recipients with high LEB; treatment 2, donors with low LEB and recipients with high LEB. Here and in Table 2 and 3: *$p<0.05$ and **$p<0.01$, differences between animals of the control and treatment groups.

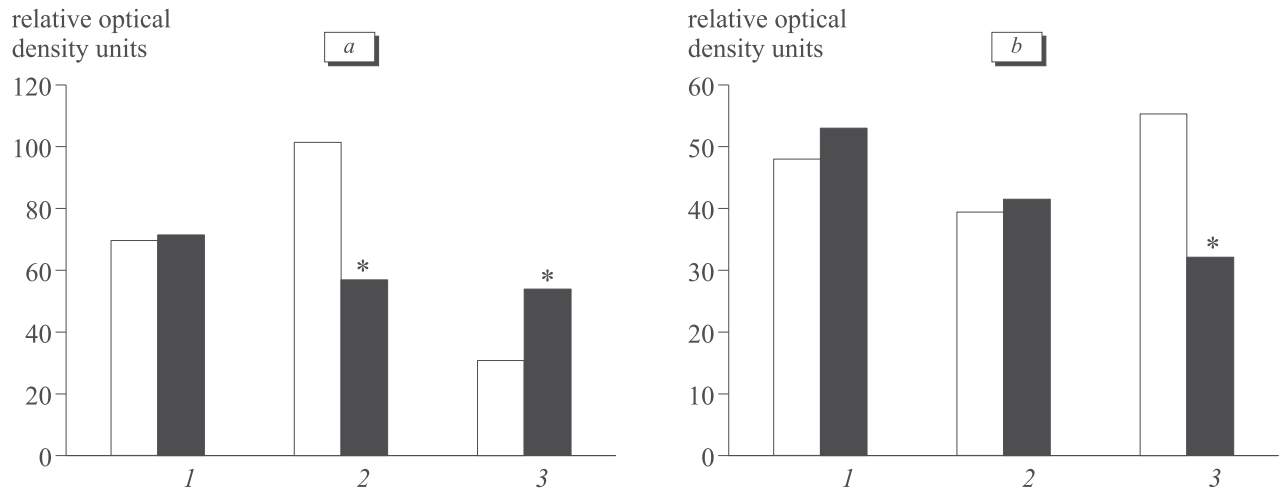

Fig. 1. Level of mRNA for IL-1β, IL-1 receptor type 1, and erythropoietin in brain cells of recipient mice with various LEB in the open-filed test after transplantation of the lymphoid fraction of splenocytes. Here and in Fig. 2: donors with high LEB and recipients with low LEB (*a*); donors with low LEB and recipients with high LEB (*b*). IL-1β gene (*1*); IL-1 receptor type 1 gene (*2*); erythropoietin receptor gene (*3*). Light bars, control group of animals (donors and recipients with similar LEB, no differences from LEB in recipients). Dark bars, treatment group of animals (donors and recipients with various LEB). **p*<0.01, differences between animals of the control and treatment groups.

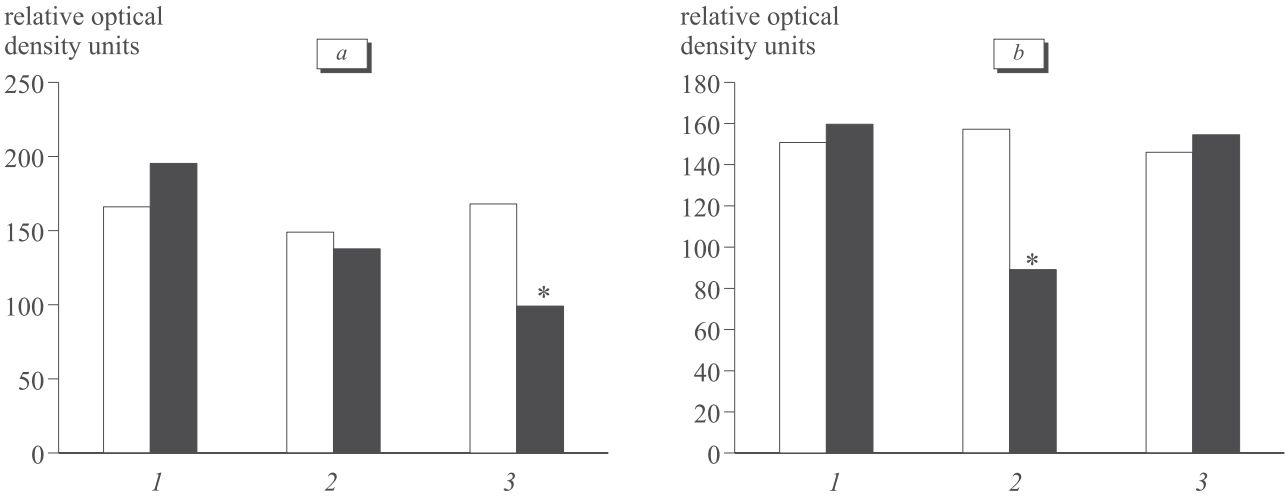

Fig. 2. Level of mRNA for IL-1β, IL-1β receptor type 1, and erythropoietin receptor in splenocytes of recipient mice with various LEB in the open-filed test after transplantation of the lymphoid fraction of splenocytes.

TABLE 2. Humoral and Cellular Immune Response in Recipient Mice with Various LEB after Transplantation of Splenic Lymphoid Cells (*M*±*SD*, *n*=24-30)

Parameter	Type of transplantation			
	I		II	
	control	treatment	control	treatment
Relative number of APC (APC/10^6)	263.7±131.5	604.3±176.3**	420.7±154.8	319.4±113.7
Number of splenocytes	196.4±61.6	240.2±71.2*	175.6±62.3	180.1±68.8
Absolute number of APC	47 655.0±27 033.9	125 017.9±27 763.0**	78 700.6±50 336.2	56 201.1±25 369.1
DTH reaction (RI, %)	20.8±18.0	24.3±14.0	38.4±22.2	35.4±18.5

Note. Here and in Table 3: I, donors with high LEB and recipients with low LEB; II, donors with low LEB and recipients with high LEB. Control, donors and recipients with similar LEB (no differences from LEB in recipients).

TABLE 3. Proliferative Activity of Immunocompetent Cells from Recipient Mice with Various LEB after Transplantation of Splenic Lymphoid Cells (*M*±*SD*)

Group		Type of transplantation			
		I		II	
		thymocytes, cpm	splenocytes, cpm	thymocytes, cpm	splenocytes, cpm
Control					
	spontaneous	1782.7±604.6	2154.2±381.4	499.4±92.5	2579.3±210.4
	concanavalin A	95 012.2±14 311.5	25 666.2±6839.9	28 565.7±3821.4	28 041.1±1309.5
	LPS		11 821.2±1254.9		9286.4±777.5
Treatment					
	spontaneous	562.3±303.8**	4105.9±736.6**	1022.1±340.8*	2702.9±358.9
	concanavalin A	88 094.2±12 976.7	12 666.7±154.7**	30 241.2±5141.3	17 895.7±1454.7**
	LPS		17 107.9±660.2**		8591.7±1087.3

erythropoietin receptor gene in the spleen and subsequent decrease in the inhibitory effect of erythropoietin on the humoral immune response after transplantation. Changes in erythropoietin receptor gene expression in the brain of recipient mice probably contribute to variations in vertical locomotor activity of animals. Our previous studies showed that erythropoietin has a stimulatory effect on these behavioral characteristics [8].

We conclude that transplantation of splenic lymphoid cells to syngeneic recipients with other behavioral characteristics has a modulatory effect on functional activity of the immune system (change in proliferative activity of cells, cytokine gene expression in splenocytes, and humoral immune response) and CNS in recipient animals (change in orientation and exploratory behavior and cytokine gene expression in brain cells).

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