GENETICS

Cytokine Gene Expression in Cerebral Hemispheres and Behavioral Reactions of (CBA×C57BL)F_1 Mice

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Interleukin-1β mRNA, interleukin-1β receptor mRNA, and erythropoietin receptor mRNA are expressed in the brain of (CBA×C57Bl)F_1 mice. Immunization with sheep erythrocytes stimulated the expression of these cytokines in the cerebral hemispheres. Injection of recombinant erythropoietin reduced expression of interleukin-1β, interleukin-1β receptor, and erythropoietin receptor genes in the brain of experimental animals and considerably increased their motor activity in the open field test.

Key Words: *interleukin-I, central nervous system, erythropoietin receptor, immune response, behavior*

Cytokines were initially characterized as factors secreted by immunocompetent cells and intended for functional regulation within the immune system. At present, there is evidence that these factors regulate physiological functions in the brain and mediate the interactions between the immune and nervous systems [7,13,16]. Cytokine genes are expressed in the CNS [11,13]. The effects of cytokines (particularly, interleukin-1 and 6) on the nervous system were studied [8,10,13]. However, the relationship between animal behavior and cytokine gene expression in the brain remains little studied. It was interesting to study the relationship between changes in the expression of interleukin-1β (IL-1β) mRNA, IL-1β receptor (IL-1β-R) mRNA, and erythropoietin receptor (EP-R) mRNA in the cerebral hemispheres and behavioral reactions of (CBA×C57Bl)F_1 mice. Despite numerous reports about the role of IL-1 in the regulation of CNS functions, the majority of these studies deal with protein products, but not with mRNA. Injection of *E. coli* LPS induced a pronounced increase in the content of IL-1β mRNA in the brain [8]. However, there are no reports about the effect of immunization on EP-R mRNA, and the data on EP-R mRNA expression in the brain and neuroregulatory and neuroprotective role of EP appeared just recently. Therefore, injection of exogenous EP was chosen as the challenge, which can modulate expression of EP-R mRNA. The aim of the present study was to analyze changes in the expression of IL-1β, IL-1β-R, and EP-R genes in cerebral hemispheres and animal behavioral.

MATERIALS AND METHODS

Male (CBA×C57Bl)F_1 mice aged 3 months were obtained from Breeding Center of Research Laboratory of Experimental Biomodelling, Russian Academy of Medical Sciences (Tomsk). Recombinant EP (Recormon, Boehringer Mannheim GmbH) was injected subcutaneously in a dose of 10 U/animal three times before immunization [4]. Preliminary experiments showed that this EP dose stimulated the formation of colony-forming units in the spleen of (CBA×C57Bl)F_1 mice on day 8.

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Published data on cytokine gene expression in the brain are scanty and contradictory [11], and therefore we evaluated cytokine mRNA expression in mouse cerebral hemispheres and then studied the effect of immunization with sheep erythrocytes (SE) on mRNA expression and mouse behavior in the open field test.

The animals were immunized intravenously with SE in a dose of 2×10^8. Controls were injected with the same volume of 0.9% NaCl. Orientation and exploratory behavior and total motor activity were evaluated in the open field test [1]. Total RNA was isolated as described previously [9], reverse transcriptase/polymerase chain reaction was carried out as described elsewhere [6]. Primers to EP-R, IL-1β-R, IL-1β, and β-actin for PCR were synthesized in accordance with the structure described previously [6,12]. PCR products were visualized in UV light on a Pharmacia-LKB densitometer, semiquantitative evaluation was carried out using ImageMaster VDS Software.

The results were statistically processed using Student's *t* test and paired Mann—Whitney test. Arithmetic means are presented, the differences were considered significant at $p<0.05$.

RESULTS

Specific IL-1β, IL-1β-R, and EP-R mRNA are expressed in the brain hemispheres of (CBA×C57Bl)F_1 mice. Immunization with SE increased the expression of all studied mRNA (Fig. 1). Injection of exogenous EP suppressed the expression of all studied genes (Fig. 1).

Open field testing showed that vertical motor activity slightly increased 24 h after immunization with SE, while after 96 h no changes in the orientation and exploratory activity were seen (Table 1). EP significantly increased horizontal motor activity (peripheral, central, total) 24 h postinjection and vertical motor activity (free, cage, and total) 96 h after the last injection (Table 1).

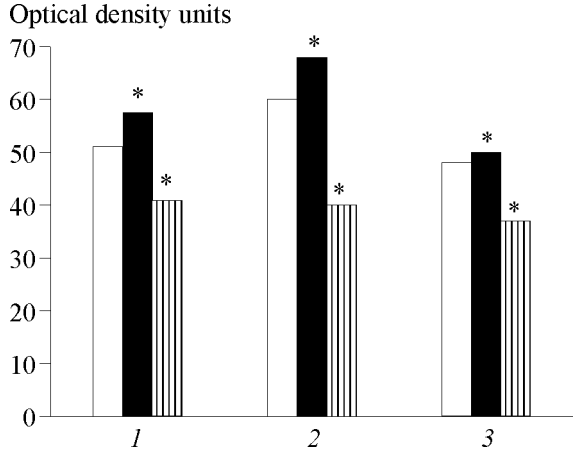

Fig. 1. Effects of immunization and injection of erythropoietin on expression of interleukin-1β (*1*), interleukin-1β receptor (*2*), and erythropoietin receptor genes (*3*) in the brain of (CBA×C57Bl)F_1 mice. Light bars: control; dark bars: immunization with sheep erythrocytes; cross-hatched bars: injection of erythropoietin. **p*<0.05 compared to the control.

It can be hypothesized that more pronounced activation of open field behavior after injection of EP can be due to low expression of IL-1β gene and production of this cytokine in the brain. It is known that injection of IL-1β into cerebral ventricles induces slow-wave sleep, loss of appetite, and morbid behavior (fever and sleepiness) [10,13,14]. We found no pronounced effects of immunization with SE on

TABLE 1. Effect of Immunization with SE and Exogenous EP on Open Field Behavior of (CBA×C57Bl)F_1 Mice 24 h (Numerator) and 96 h (Denominator) after the Last Injection (*M*±*m*, *n*=20)

Motor activity		Control for EP	EP	Control for SE	SE
Horizontal	peripheral	77.9±10.4 / 128.1±16.7	123.9±12.6* / 158.7±12.1	118.4±17.6 / 89.3±15.7	148.5±14.3 / 106.6±16.0
	central	3.25±1.60 / 8.9±2.2	10.1±2.3** / 14.9±3.2	12.3±3.1 / 15.2±5.1	14.6±3.2 / 13.5±3.5
	total	81.1±10.0 / 137.0±18.4	133.9±14.1** / 173.6±14.1	130.7±19.0 / 100.1±18.6	161.3±16.0 / 120.1±19.4
Vertical	free	0.5±2 / 0.4±0.2	1.1±0.5 / 2.1±0.7**	0.9±0.4 / 0.3±0.1	1.4±0.4 / 0.7±0.3
	wall support	8.3±1.5 / 7.0±1.5	11.2±2.2 / 12.9±1.9**	8.4±1.9 / 5.0±1.2	14.3±1.9** / 5.1±1.4
	total	8.8±1.6 / 7.3±1.6	12.2±2.6 / 15.0±2.2**	9.3±2.2 / 5.1±1.2	15.6±2.1** / 5.6±1.5

Note. **p*<0.01, ***p*<0.05 compared to the control.

animal behavior in the open field. This is in line with published data that the formation of immune response did not modify animal behavior in the open field test [15] or slightly increased exploratory and motor activities [2]. It can be hypothesized that the increase in vertical motor activity 24 h after immunization was due to changes in the neurochemical structure of the brain, specifically, activation of the cerebral dopaminergic system [3,5].

Hence, genes of all studied cytokines (IL-1β, IL-1βb-R, EP-R) are expressed in the brain of (CBA× C57Bl)F_1 mice. Immunization and injection of EP modifies expression of cytokine genes in the brain and parameters of animal behavior. Our findings and published data attest to a relationship between cytokine gene expression in the brain of (CBA× C57Bl)F_1 mice and their open field behavior. Hence, regulation of social functions in animals can be realized via molecular mechanisms.

REFERENCES

1. Ya. Buresh, O. Bureshova, and D. P. Houston, *Methods and Main Experiments for Studies of the Brain and Behavior* [in Russian], Moscow (1991), p. 399.
2. Yu. V. Burov, L. E. Davydov, and T. N. Robakidze, *Byull. Eksp. Biol. Med.,* **118,** No. 11, 474-476 (1994).
3. E. L. Dovedova and M. Yu. Monakov, *Ibid.,* **130,** No. 9, 289-291 (2000).
4. I. N. Zhuravkin, V. P. Lozovoi, and V. A. Kozlov, *Ibid.,* **85,** No. 5, 565-567 (1978).
5. N. A. Trekova, L. A. Vetrile, L. A. Basharova, *et al., Zh. Vyssh. Nervn. Deyat.,* **49,** No. 5, 799-807 (1999).
6. R. D. Allen, T. A. Staley, and C. L. Sidman, *Eur. J. Immunol.,* **23,** 333-337 (1993).
7. E. Ban, F. Haour, and R. Lenstra, *Cytokine,* **4,** 48-54 (1992).
8. C. M. Blatteis, *Yale J. Biol. Med.,* **63,** 133-146 (1990).
9. P. Chomczynsky and N. Sacchi, *Analyt. Biochem.,* **162,** 156-159 (1987).
10. R. Dantzer, A. Aubert, R. M. Bluthe, *et al., Adv. Exp. Med. Biol.,* **461,** 83-105 (1999).
11. M. M. Gabellec, R. Griffais, G. Fillion, and F. Haour, *Brain Res. Mol. Brain Res.,* **31,** 122-130 (1995).
12. J. Gui-Quan and J. C. Gutierrez-Ramos, *Eur. Cytokine Netw.,* **6,** 253-255 (1995).
13. B. Schobitz, E. R. De Kloet, and F. Holsboer, *Prog. Neurobiol.,* **44,** No. 4, 397-432 (1994).
14. A. M. Van Dam, M. Brouns, S. Louisse, and F. Berkenbosch, *Brain Res.,* **588,** 291-296 (1991).
15. J. Vidal, *Psychol. Rep.,* **85,** No. 3, Pt. 1, 845-846 (1999).
16. J. M. Weiss and S. K. Sundar, *Neurobiology of Cytokines,* Ed. B. De Sousa, New York (1993), Pt. B, pp. 185-208.