GENETICS

Effects of Interleukin-1β and Indomethacin on Expression of Interleukin-1β Gene in Brain Hemispheres

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 127, No. 5, pp. 557-559, May, 1999
Original article submitted June 3, 1998

Quantitative dot-hybridization showed that recombinant interleukin-1β stimulates the expression of interleukin-1β gene in the right and left brain hemispheres in a dose-dependent manner. Endogenous prostaglandins inhibit the expression of this gene in the right hemisphere.

Key Words: *interleukin-1β; indomethacin; central nervous system; asymmetry; immune response*

Recent studies showed that cytokines of the immune system are produced in intact CNS and exert immunoregulating effects. Interleukin-1 (IL-1) is the first and best studied cytokine, whose central effect on the immunity was described [12,14]. The levels of IL-1 and its receptors in the CNS change upon activation of the immune system [13].

Functional asymmetry of the brain hemispheres is well known [2]. Previously we demonstrated asymmetrical expression of the IL-1β gene in contralateral hemispheres of mouse brain [1]. In this study we investigated the effect of IL-1β and indomethacin, an inhibitor of prostaglandin synthesis, on the content of IL-1β mRNA in contralateral brain hemispheres.

MATERIALS AND METHODS

Male (CBA×C57Bl/6) F_1 mice aged 3 months weighing 20-22 g were used. For studies of the effects of peripheral prostaglandins on the expression of IL-1β mRNA in brain hemispheres, prostaglandin synthesis was blocked with indomethacin. Indomethacin (Sigma) was dissolved in ethanol and then in medium 199. Final solution containing 5% ethanol and 1 mg/ml drug was injected intraperitoneally in a dose of 0.25 mg/mouse [3] 30, 8, and 6 h before analysis. To controls, equivalent volumes of 5% ethanol were injected. Recombinant rat IL-1β (graciously given by Dr. R. Dantzer within the framework of Research Foundation of INSERM Program) was injected in single doses of 10, 100, and 1000 ng/mouse (0.5 ml) into the caudal vein 2 and 24 h before sacrifice. The same volume of normal saline was used as a negative control. The hemispheres and spleen from 5 animals were pooled and frozen in liquid nitrogen. Two independent series of each experiment were carried out (a total of 8 observations).

Total RNA was isolated by a guanidine thiocyanate method [5]. The concentration and content of RNA were measured spectrophotometrically (1 opt. unit=40 μg RNA), its intactness was evaluated by electrophoresis in 1% agarose in Tris-acetate buffer. Quantitative dot-hybridization was carried out with total RNA samples denatured with formaldehyde and layered (diluted by half) onto nitrocellulose [4]. The filters were dried in vacuum at 80°C for 2 h. Prehybridization and hybridization were carried out as described previously [1]. The probe was a fragment of mouse IL-1β cDNA (275-1329 bp.) cloned in the

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0007-4888/99/0005-0504$22.00

EcoR I site of pBS plasmid (a gift from Dr. T. A. Hamilton) [9]. After hybridization and washings, the filters were exposed with an RM-B x-ray film for 48 h using amplifying screens. The results of hybridization were quantitatively analyzed on an Ultroscan-XL laser densitometer (LKB). The data from different filters were standardized using internal controls [4]. The results were statistically processed using the nonparametric Wilcoxon—Mann—Whitney test.

RESULTS

Expression of IL-1β in the left hemisphere considerably increased 2 and 24 h after intravenous injection of recombinant IL-1β in a dose of 100 ng (Fig. 1, *a*). Neither lower (10 ng) nor higher (1000 ng) doses of recombinant IL-1β modified the level of IL-1β mRNA in the left hemisphere. A different effect of IL-1β was observed in the right hemisphere (Fig. 1, *b*). In a low dose (10 ng) recombinant IL-1β did not affect the content of IL-1β mRNA, while a higher dose (100 ng) stimulated expression of IL-1β 2 and 24 h postinjection. Further increase of the cytokine dose to 1000 ng did not potentiate the stimulating effect on IL-1β mRNA in the right hemisphere. In the spleen recombinant IL-1β increased the level of IL-1β mRNA both 2 and 24 h postinjection (Fig. 1, *c*). In a dose of 10 ng, IL-1β did not increase the expression of IL-1β mRNA during the studied period. Further increase of the dose to 100 ng increased the content of IL-1β mRNA in the spleen, the level of expression decreasing after 24 h in comparison with 2 h postinjection. In a dose of 1000 ng, recombinant IL-1β had a more pronounced stimulating effect, and the level of IL-1β mRNA little varied during the studied period.

In other words, the expression of IL-1β gene was increased in the left and right hemispheres of the brain in response to various doses of recombinant IL-1β after peripheral injection. The stimulating effect on the left hemisphere manifested only after administration of a medium dose and disappeared as the dose was increased. Similar reaction of the right hemisphere and spleen to intravenous injection of recombinant IL-1β manifested by a higher expression of this gene after administration of medium and high doses of IL-1β. It means that IL-1 can regulate asymmetric expression of IL-1β gene in the brain hemispheres. However, recombinant IL-1 modified the expression of IL-1β gene in the brain at high doses. We believe that apart from this cytokine, other immune system factors are involved in the regulation of IL-1β gene expression in the brain hemispheres.

Peripheral injection of indomethacin (inhibitor of prostaglandin synthesis) increased the content of IL-1β mRNA in the left hemisphere (Fig. 2). However, this

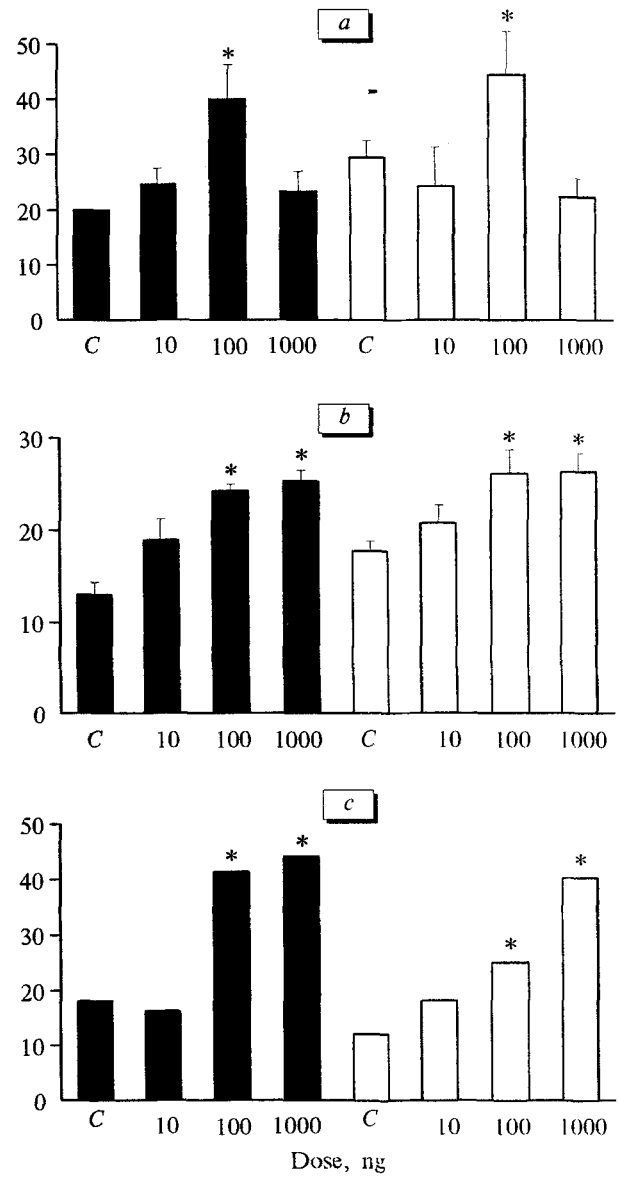

Fig. 1. Expression of interleukin-1β (IL-1β) gene mRNA in the left (*a*) and right (*b*) hemispheres of the brain and spleen (*c*) of male (CBAxC57Bl/6) F_1 mice 2 h (dark bars) and 24 h (light bars) after intravenous injection of recombinant rat IL-1β. Ordinate: expression, arb. units. Here and in Fig. 2: *$p<0.05$ *vs.* the control (C).

increase was statistically insignificant in comparison with the control. After indomethacin injection, the level of IL-1β mRNA significantly increased in the right hemisphere and insignificantly in the spleen (Fig. 2).

Therefore, blocking of prostaglandin synthesis by peripheral injection of indomethacin significantly stimulates the expression of IL-1β in the right hemisphere, which indicates a possible inhibitory effect of prostaglandins on the expression of IL-1β gene in the right hemisphere.

We observed a relationship between the expression of IL-1β in the right hemisphere and the spleen. However, IL-1β stimulated the expression of specific

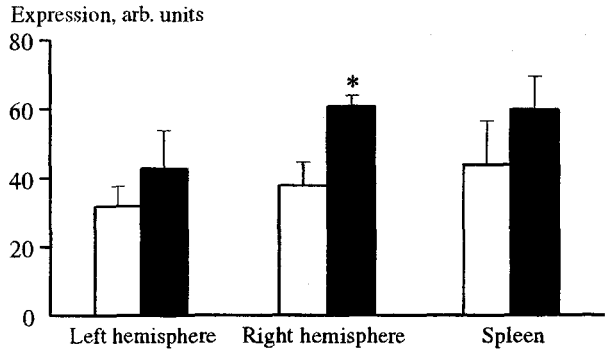

Fig. 2. Effect of indomethacin on expression of interleukin-1β gene in brain hemispheres and spleen of male (CBAxC57Bl/6) F_1 mice in the control (light bars) and experiment (dark bars).

IL-1β mRNA in the left hemisphere as well. It means that IL-1 can regulate asymmetric expression of IL-1β gene in the brain hemispheres. The effects of recombinant IL-1β on the expression of IL-1β are similarly directed in the left and right hemispheres; only higher doses exert opposite effects on the left and right hemispheres. Presumably, IL-1 in systemic circulation is not the leading factor of the formation and maintenance of asymmetric IL-1β gene expression in the brain hemispheres. Other factors of the immune system, *e.g.* prostaglandins, are probably involved in the regulation of IL-1β gene expression. It was previously shown that prostaglandin E_2 stimulates the production of IL-1 in the CNS [8]. In our experiments, inhibition of the prostaglandin synthesis increases the content of IL-1β mRNA in both hemispheres, but to a greater extent in the right hemisphere. Our disagreement with the previous report [8] can be explained by difference in the studied objects: these authors used an *in vitro* model of cultured hypothalamus slices, while we investigated brain hemispheres *in vivo*. Our findings indicate that the prostaglandin-dependent mechanism of suppression of IL-1β expressin affects mainly the right brain hemisphere.

Therefore, there are good grounds to assert that some factors of the immune system (IL-1 and prostaglandins) are involved in the regulation of asymmetric expression of IL-1β gene in the brain hemispheres.

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