Reactivity of Lymphoid Cells in Stressed Mice against the Background of Adrenoceptor Blockade

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Supplement 1, pp. 14-18, January, 2007
Original article submitted November 1, 2006

Injection of α-adrenoblocker dihydroergotamine to (CBA×C57Bl/6)F_1 mice against the background of stress and subsequent immunization considerably suppressed production of Th1 (IFN-γ and IL-2) and Th2 (IL-10) cytokines and IL-6 and stimulated TNF-α production. Injection of β-adrenoceptor blocker propranolol in stressed and immunized mice shifted the cytokine balance towards Th1 by stimulating IFN-γ and IL-2 production and inhibiting IL-10 production at early stages of humoral immune response; the production of TNF-α and IL-6 increased under these conditions.

Key Words: *cytokines; immobilization stress; humoral immune response; α- and β-adrenoceptor blockers*

Injection of antigens, *e.g.* sheep erythrocytes (SE) induces Th2-type immune response by triggering B cell differentiation processes and by shifting cytokine production towards type 2 cytokines [3]. Immobilization stress can suppress the development of specific humoral immune response and reduce phagocytic activity of peritoneal macrophages [2]. Stress reaction is accompanied by a considerable rise of blood levels glucocorticoids and catecholamines [14]. Epinephrine and norepinephrine are potent inhibitors of the production of IL-12 (the main inductor of Th1 reactions), TNF-α, and IL-1 [8]. On the whole, catecholamines suppressing the production of Th1 cytokines do not directly modulate the production of Th2 cytokines by cells and their functions [11]. However, catecholamines can produce different effects when the stress and antigen influences are combined under conditions of specific environment. Here we studied the effects of α- and β-adrenoceptor blockers (AB) on cytokine production by splenocytes from stressed mice against the background of developing humoral immune response.

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MATERIALS AND METHODS

Experiments were carried out on 160 certified (CBA×C57Bl/6)F_1 female mice weighing 18-20 g (age 2-2.5 months) obtained from the Laboratory of Experimental Biomodeling, Institute of Pharmacology (Tomsk Research Center). The animals were immunized with SE, corpuscular thymus-dependent antigen (Microgen Research-and-Production Complex). SE were 3 times washed with sterile physiological saline, 0.2 ml 15% erythrocyte suspension was injected intraperitoneally. For evaluation of the effect of stress on functional activity of lymphoid cells, some mice before immunization were subjected to immobilization in closed hollow cylinders for 24 h. Three-five minutes before the experiment and 6 h after the start of exposure one group of mice (n=40) received α-AB dihydroergotamine (Galena) in a dose of 3.9 mg/kg, another group (n=40) received β-AB propranolol (Isis Pharma) in a dose 5 mg/kg. The preparations were dissolved in physiological saline immediately before

use. Control animals (n=40) were subjected to immobilization and subsequent immunization and received an equivalent volume of physiological saline under the same experimental conditions. Immunized mice (n=40) not subjected to immobilization comprised the reference group. The mice were decapitated under ether narcosis. The material for the study was collected on days 1, 4, 7, and 10 after immunization with SE.

The content of cytokines TNF-α, IL-1β, IFN-γ, IL-2, IL-4, IL-6, and IL-10 was measured in supernatants of splenocyte suspension from experimental animals. For isolation of splenocytes, the spleens were homogenized in a glass homogenizer in medium 199 (Vektor) containing 40 μg/ml gentamicin and 5% FCS (BioClot) [1]. The cells were filtered through a capron filter, centrifuged, and twice washed with medium 199 with 5% FCS. The pellet was resuspended in complete culture medium containing 90% RPMI (Vektor), 10% heat-inactivated FCS (56°C, 30 min), 2 mM L-glutamine (Sigma), 10 mM HEPES (Flow), 40 mg/liter gentamicin, 25 μM 2-mercaptoethanol (Sigma). The number of viable cells in the suspension was evaluated under a microscope using a Goryaev chamber and trypan blue dye. The concentration of viable splenocytes in suspension was adjusted to 2×10^6 cells/ml and incubated in complete nutrient medium at 37°C, 5% CO_2, and 100% humidity for 20 h in the presence of 5 μg/ml concanavalin A (Con A, ICN) or 10 μg/ml LPS from *Escherichia coli* Serotype 055:B5 (Sigma). After incubation, conditioned media were collected and stored at -50°C for no more than 1 month.

TNF-α, IL-1β, and IL-6 were measured in supernatants of splenocytes stimulated with LPS, while IFN-γ, IL-2, IL-4, and IL-10 were assayed after stimulation with Con A by immunoenzyme assay using Amersham Pharmacia Biotech kits according to manufacturer's instructions on a Uniplan AIFR-01 automatic analyzer (Pikon company).

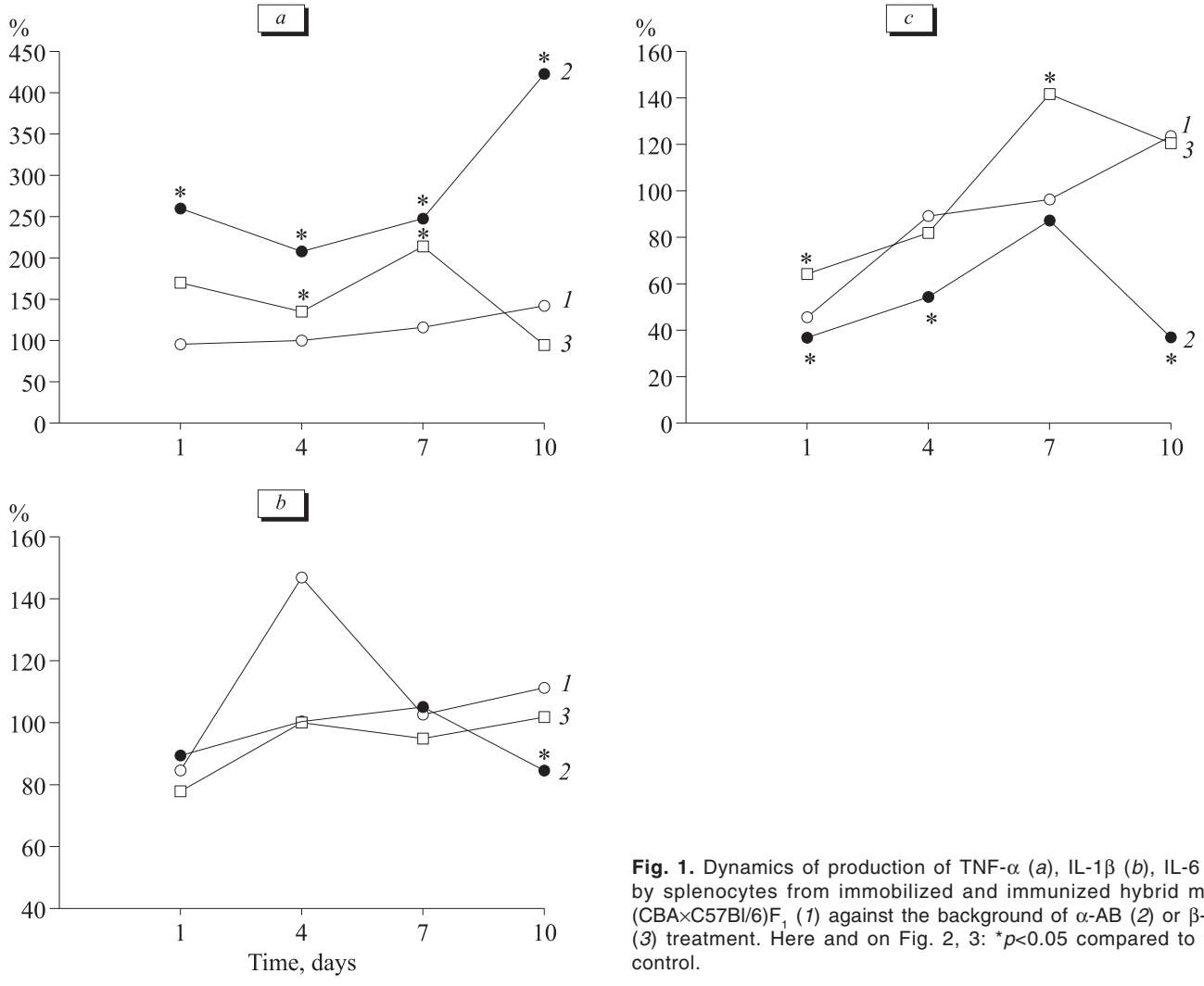

Fig. 1. Dynamics of production of TNF-α (*a*), IL-1β (*b*), IL-6 (*c*) by splenocytes from immobilized and immunized hybrid mice $(CBA\times C57Bl/6)F_1$ (*1*) against the background of α-AB (*2*) or β-AB (*3*) treatment. Here and on Fig. 2, 3: *p<0.05 compared to the control.

The data were processed statistically by Student's *t* test using Statistica 5.0 software (normal distribution was preliminary confirmed).

RESULTS

Immobilization stress before immunization had no effect on TNF-α production by mouse splenocytes compared to mice subjected to immunization only. Injection of α-AB against the background of stress and subsequent immunization enhanced THF-α production throughout the observation period. The use of β-AB under these conditions also increased TNF-α production on days 4 and 7 after immunization (Fig. 1, *a*).

The dynamics of IL-1β production by splenocytes from mice subjected to immobilization and immunization with SE did not significantly differ from that in the immunized group throughout the experiment. Treatment with either α-AB, or β-AB did not affect IL-1β production by splenocytes in stressed animals. Only on day 10 of the experiment we observed a decrease in the production of IL-β in the group of mice receiving α-AB compared to the control (Fig. 1, *b*).

Experiments showed that IL-6 production by splenocytes from stressed mice was significantly lower than in mice subjected to immunization alone on day 1 after administration of SE. Then, the production of this cytokine increased and on day 10 of the experiment we observed a significant increase in IL-6 level in supernatants of splenocytes from stressed animals. Injection of α-AB suppressed IL-6 production by splenocytes from mice subjected to immobilization throughout the entire observation period except day 7 of the experiment. In the contrary, administration of β-AB enhanced production of IL-6 by splenocytes from stressed animals on days 1 and 7 of the experiment (Fig. 1, *c*).

On day 4 of the experiment, the content of IFN-γ in supernatants of splenocytes from mice subjected to immobilization significantly increased compared mice subjected to immunization alone (by 1.55 times). In mice receiving α-AB, the production of IFN-γ decreased on days 4 and 10 of the experiment (compared to the control). Treatment with β-AB did not affect IFN-γ production by splenocytes from stressed animals (Fig. 2, *a*).

Immobilization stress suppressed the production of IL-2 by mouse splenocytes on day 1 after immunization, but then this parameter increased and on day 7 of the experiment the content of IL-2 in supernatants of splenocytes from stressed animals considerably surpassed the corresponding parameter in mice immunized with SE. Injection of α-AB suppressed IL-2 production compared to the control throughout the entire experiment. Administration of β-AB enhanced production of IL-2 by splenocytes from stressed animals on the next day after immunization, but decreased this parameter on days 4 and 7 of the experiment (Fig. 2, *b*).

At early terms after immunization (days 1-4), stress did not affect IL-6 production by splenocytes compared to immunized mice. A reliable decrease in IL-4 production in stressed mice was noted only on day 7 of the experiment. Treatment with either α-AB, or β-AB suppressed IL-4 production by splenocytes from immunized animals on day 7 of the experiment (Fig. 3, *a*).

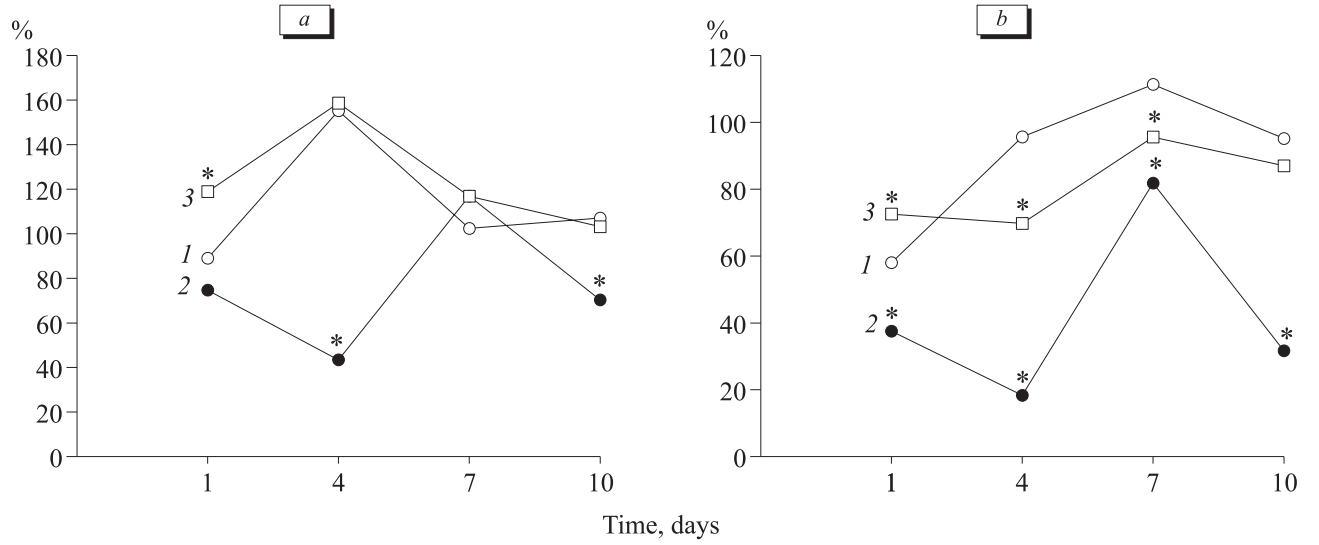

Fig. 2. Dynamics of production of IFN-γ (*a*) and IL-2 (*b*) by splenocytes from immobilized and immunized hybrid mice (CBA×C57Bl/6)F_1 (*1*) against the background of α-AB (*2*) or β-AB (*3*) treatment.

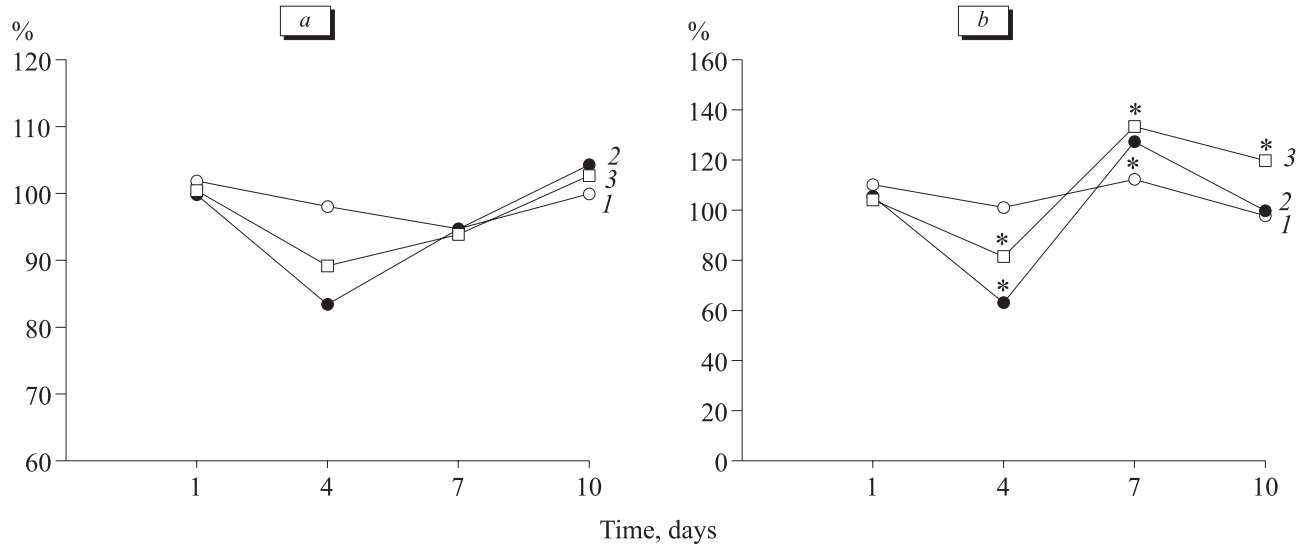

Fig. 3. Dynamics of production of IL-4 (*a*) and IL-10 (*b*) by splenocytes from immobilized and immunized hybrid mice (CBA×C57Bl/6)F_1 (*1*) against the background of α-AB (*2*) or β-AB (*3*) treatment.

The content of IL-10 in culture supernatants from stressed animals did not significantly differ from the corresponding parameter in mice subjected to immunization only throughout the observation period. The use of α-AB and β-AB suppressed IL-10 production by splenocytes from stressed mice on day 4 of the experiment, but enhanced production of this cytokine at later terms (α-AB on day 7 and β-AB on days 7 and 10).

Various stress factors can reduce the reactivity of immunocompetent cells [5,6]. However, the increased levels of catecholamines during stress suppressed primarily Th1 cytokine production and had little effect on Th2-cytokine production [11]. Thus, immobilization before immunization decreased production of Th1 cytokines (IFN-γ and IL-2 at early terms after injection of SE. The production of IL-6, the cytokine acting as anti- and proinflammatory agent, also decreased under these conditions. However, stress had no effect no the production of Th2 cytokines IL-4 and IL-10. Injection of α-AB dihydroergotamine against the background of stress and subsequent immunization led to more pronounced suppression of production of Th1 (IFN-γ and IL-2) and Th2 (IL-10) cytokines and IL-6, but stimulated the production of proinflammatory cytokine TNF-α. Although α-adrenergic receptors are expressed only on some cells (peritoneal and alveolar macrophages, hemopoietic cells, *etc.*, modulation of α_1- and α_2-adrenoceptors can affect the parameters of the immune response [9,12]. Injection of β-AB propranolol in stressed and immunized mice shifted the cytokine balance towards Th1 cytokines by stimulating IFN-γ and IL-2 production and inhibiting IL-10 production at early stages of humoral immune response. The production of TNF-α and IL-6 increased under these conditions, which agrees with previous data that propranolol blocks the inhibitory effect of catecholamines on cytokine-producing cells and markedly potentiated the LPS-stimulated synthesis of TNF-α and IL-12 in mice [9,13]. Thus, the use of α-AB and β-AB can modulate the reactivity of lymphoid cells in stressed mice against the background of developing immune response, in particular, on cytokine production by these cells. On the whole, the suppressing or stimulatory effects of AB on the immune reaction, except experimental conditions, can be related to specificity of the effects of this preparation and with its side effects, the presence or absence of antigen, the presence of proinflammatory cytokines in the microenvironment, and the state of activation or differentiation of macrophages [4,7,10].

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