Effect of Dexamethasone on Immune Response of Mice with Different Behavioral Types

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Experiments on a model of paired sensory contact showed that dexamethasone effectively suppressing the response to ACTH not only prevented immunosuppression in male C57Bl/6J mice with submissive behavior formed during different periods of confrontation testing (days 10 and 20), but also stimulated the immune response in comparison with the control. Immune response in aggressive animals after 20-day confrontations was higher than in controls and submissive mice and did not change after dexamethasone injection. The authors conclude that the immunosuppressive effect in submissive animals is realized through ACTH, which little contributes into immunomodulation in aggressive mice.

Key Words: *aggression; submission; dexamethasone; immunity*

The neurochemical pattern of the brain is the key moment of immunomodulation in creation of the behavioral status of C57Bl/6J mice in experimental model of paired sensory contact [2,10]. Enhanced immunogenesis characteristic of the aggressive behavioral type is determined by the predominance of dopaminergic (DAergic) system [1,2] producing an immunoactivating effect [3]. By contrast, in submissive mice the key role in immunosuppression is played by the serotoninergic (5-HTergic) system decreasing the immune response. Though peripheral components are different (the thymus for DAergic system and adrenals for 5-HTergic system), the central mechanism of immunomodulation is common and involves the hypothalamic-pituitary complex [3]. The synthesis and secretion of pituitary hormones are regulated by neurotransmitters. Specifically, ACTH regulation, similarly as secretion of the adrenal glucocorticoid hormones, is largely realized by 5-HT [6,9]. It is noteworthy that 5-HT activity in the cerebral subcortical structures depends on the behavioral type [2,11]. On the other hand, it was shown that dexamethasone not only effectively suppressed the production of corticotropin releasing factor, ACTH, and corticosteroids [14], but also blocked the response of the hypothalamic-pituitary-adrenal system to 5-HT. Presumably, injection of dexamethasone differently modulates the immune response in animals differing by behavioral types (aggression, submission), because different neurotransmitter mechanisms underlie these types. The aim of this study was to clear out this question.

MATERIALS AND METHODS

The study was carried out on 2-2.5-month-old male C57Bl/6J mice (20-23 g, Certificate No. 159-87). The mice were kept under standard vivarium conditions under natural illumination on common ration. Experiments were carried out in accordance with the philosophy presented in Directions of the European Community (86/609/EC) approved by Committee for Biomedical Ethics of Institute of Physiology. The model of paired sensory contact was used for the formation and fixation of alternative behavioral types in mice [5]. Each group consisted of at least 10 animals. Control mice (animals without experience of victories and

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defeats) were placed into individual cages for 5 days in order to eliminate group effects.

Synthetic glucocorticoid dexamethasone (Serva) was injected to mice with aggressive and submissive behavior fixed after 10 and 20 days of confrontations. Dexamethasone was injected intraperitoneally in a dose of 2 mg/kg in 0.2 ml saline 2-2.5 h before immunization with sheep erythrocytes (5×10^8). Controls were injected with the solvent in the same volume at the same time.

The immune response was evaluated on day 5 after immunization by the count of IgM antibody-producing cells (APC) in the spleen by local hemolysis in liquid medium and by the number of rosette-forming cells [4].

The results were statistically processed using Statistica 5.1 software and paired comparison using Student's *t* test.

RESULTS

Comparative analysis showed diverse changes in the immune response at the peak of the reaction to sheep erythrocytes, depending on fixed type of behavior of mice and term of confrontation testing. The counts of APC and rosette-forming cells in aggressive mice with 10-day confrontation experience were close to those in controls; these values increased in mice with 20-day experience of confrontations (Table 1, Fig. 1). In contrast to aggressive animals, submissive mice exhibited an appreciable decrease of the immune reaction irrespective of the duration of confrontation testing (Table 1, Fig. 1).

Neurochemical studies on a model of paired sensory contact in C57Bl/6J mice demonstrated that each behavioral type was characterized by specific neurochemical status, which determined the development of the immune process [2,11]. According to these data, increased 5-HTergic activity in cerebral subcortical structures (amygdala, hippocampus, DAergic nuclei A11, A10, A9, caudate nucleus, and the hypothalamus) during immunization promotes suppression of the immune response in submissive animals after 10- and 20-day confrontations. On the contrary, activity of the 5-HT system decreased after 20 days in aggressive mice and hence, the inhibitory effect of this system on DA system decreased [2], due to close anatomical, biological, and functional interactions between these two systems [7]; the increase of 5-HT system activity led to stimulation of the immune response in our experiments.

The formation of alternative behavioral types is paralleled by intricate neuroendocrine changes with increase in blood levels of glucocorticoids in aggressive and submissive animals [13]. It is noteworthy that the initial elevation of the hormone level in aggressive mice dropped to the baseline values much more rapidly than in submissive mice [8]. It cannot be excluded that the decrease of immune function in submissive mice was also associated with increased levels of circulating ACTH and glucocorticoids, which, as the final effector component of the hypothalamic-pituitary-adrenal complex, play an important role in suppression of immunogenesis [12].

It is known that the effect of dexamethasone inhibiting the production of corticotropin releasing factor, ACTH, and glucocorticoids by the negative feedback mechanism [14] depends on many factors, including the dose [10]. Injection of the hormone in a dose of 2 mg/kg 2.0-2.5 h before immunization, when the effect

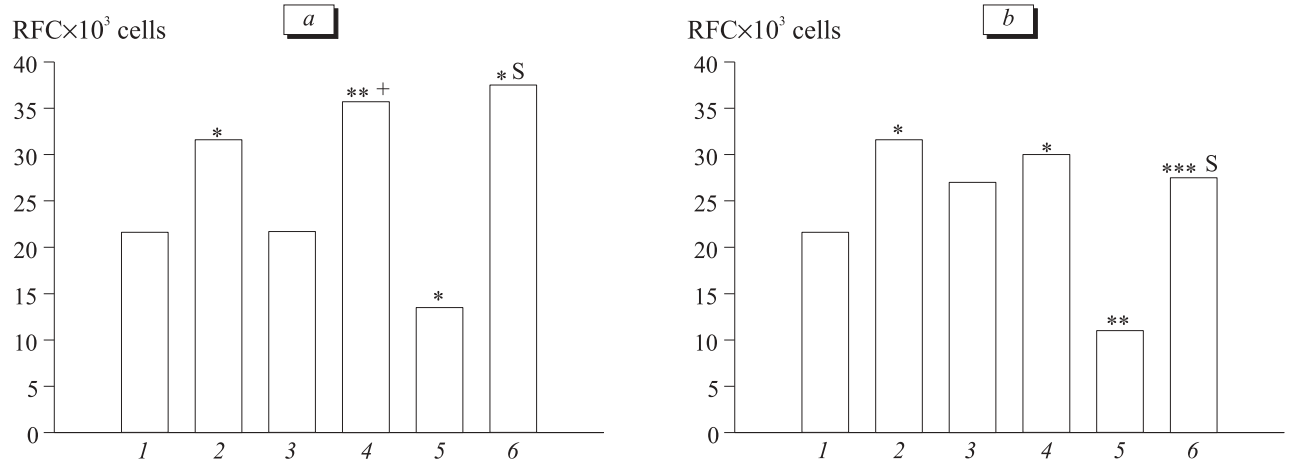

Fig. 1. Immune response in aggressive and submissive C57Bl/6J mice injected with dexamethasone after 10 (*a*) and 20 (*b*) days of confrontations. *1*) control (mice without experience of victories or defeats); *2*) dexamethasone injection; *3*) aggressive mice; *4*) aggressive mice injected with dexamethasone; *5*) submissive mice; *6*) submissive mice injected with dexamethasone. Each group consisted of at least 10 animals. RFC: rosette-forming cells. $^*p<0.01$, $^{**}p<0.001$, $^{***}p<0.0001$ compared to the control; $^+p<0.01$ compared to aggressive mice; $^x p<0.0001$ compared to submissive mice.

TABLE 1. Counts of IgM-APC in Aggressive and Submissive C57Bl/6J Mice after 10 Days of Confrontations and Dexamethasone Treatment

Group	Number of animals	IgM-APC per 10^6 cells
Control (no experience of victories and defeats)	12	323.20±17.50
Dexamethasone injection	12	560.2±48.7*
Aggressive mice	10	345.3±18.25
Submissive mice	10	150.4±15.1**
Aggressive+dexamethasone	11	621.6±37.8***+
Submissive+dexamethasone	10	459.6±6.2***x

Note. *p<0.01, **p<0.001, ***p<0.0001 compared to the control; +p<0.0002 compared to aggressive mice; xp<0.001 compared to submissive mice.

of the drug develops leading to the blockade of the hypothalamic-pituitary-adrenal system in animals, resulted in a similar increase of the immunological activity in aggressive and control mice after 10-day confrontations (Table 1, Fig. 1). However, after 20 days of testing dexamethasone induced no changes in the immune response of aggressors in comparison with the same mice receiving no dexamethasone (Fig. 1).

Dexamethasone prevented suppression of immunogenesis irrespective of the number of confrontations. The direction of the immune response in this case changed towards the increase in the counts of APC and rosette-forming cells in comparison with the control and with animals receiving no dexamethasone (Table 1, Fig. 1). Presumably, dexamethasone appreciably inhibits the ACTH-corticosteroid function essential for the realization of immunosuppression in submissive mice with activated the 5-HT system [11]. On the other hand, the pathway for the realization of immunostimulatory effect of the DAergic system (thymus) reciprocal to the 5-HTergic system is retained. Dopamine dependence of the immune process was proven by previous experiments on CBA mice, when the detected intensification of the immune response to injection of 5-HT precursor after dexamethasone was abolished by haloperidol blocking of DA receptors [3].

Hence, suppression of immune response in C57Bl/6J mice with submissive behavior is realized with participation of ACTH and corticosteroids, not involved in immunostimulation in aggressive mice.

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