EXPERIMENTAL BIOLOGY

Relationship between Behavioral Selection and Primary and Secondary Immune Response in Wild Gray Rats

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The main characteristics of primary and secondary immune response (number of antibody producing cells and the amount of produced antibodies) are reduced in rats selected by elimination of aggressive behavior in comparison with animals selected by stimulation of this behavior. In parallel, the reaction of the pituitary-adrenal system during immune response was modified in these rats. Presumably, the differences in immune reactions of rats selected by contrast behavior are determined by changes in reactivity of the pituitary-adrenal system to stress and immune stimulation during selection.

Key Words: *gray rats; behavior; glucocorticoids; humoral immune response*

The nervous, endocrine, and immune systems are anatomically and functionally related. These systems express and respond to common regulatory molecules, including steroid hormones, neuropeptides, mediators, and cytokines providing the molecular basis for coordinated reactions of these systems to changes in homeostasis in response to stress, inflammation, or infection [5,14,15]. The pituitary-adrenal system (PAS) occupies a special place, because in many cases the regulatory effects of the neuroendocrine systems on the function of immune cells are mediated by the hormones, primarily glucocorticoids [9].

Until recently glucocorticoids were regarded as immunosuppressors. This viewpoint was a result of studies with pharmacological doses of glucocorticoids and synthetic hormones, such as dexamethasone. However, the effects of pharmacological and physiological doses can be opposite [3,13].

Autobred strains of gray rats with different activity of the central and peripheral components of PAS at rest and during stress were created at Institute of Cytology and Genetics after long selection for elimination and stimulation of aggressive behavior [1].

Here we investigated primary and secondary immune response and PAS reactions to injection of an antigen in wild gray rats selected by opposite behavior.

MATERIALS AND METHODS

Experiments were carried out on adult wild male gray rats of generations 56-58, selected for aggressive or nonaggressive behavior towards humans. The rats were immunized with sheep erythrocytes (SE) in a dose of 2×10^9 cells (a single intraperitoneal injection). Each experimental group consisted of 12 animals. Blood for corticosterone measurements was collected from the caudal vein [6] before immunization, on the next day and on day 5 after immunization from the same animal. For evaluation of secondary immune response, the rats were immunized with SE in the same dose twice with 14-day interval. Blood was collected before and on days 5, 14, and 19 after the start of the experiment. The intensity of immune response was evaluated on day 5 after immunization by the number of antibody-producing cells (APC) in the spleen. In

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addition, blood antibody titers during secondary immune response were determined by the direct agglutination test with SE on days 5, 14, and 19 of the experiment.

The number of APC was estimated by local hemolysis in liquid medium. The number of APC in secondary immune response was evaluated using rabbit antiserum to rat Ig (ICN Biomedicals, Inc.). Plasma corticosterone level was measured by competitive protein binding [11]. The results were statistically processed using analysis of dispersions (ANOVA-MANOVA) followed by comparison of differences between the groups using Newman-Keuls test for repeated measurements.

RESULTS

Humoral immune response was markedly reduced in nonaggressive rats compared to aggressive animals. The number of APC in the spleen after a single injection of the antigen was almost 20-fold lower in nonaggressive rats compared to aggressive animals (Fig. 1, *a*). Differences in immune response were also observed after repeated injection of the antigen (Fig. 1, *b*). The number of APC in the spleen in secondary immune response was significantly higher than in primary immune response. It is known that memory cells are retained after the end of primary immune response; these cells provide more intensive and specific production of antibodies after repeated challenge with the same antigen.

Antibody titers on days 5 and 14 after a single injection of SE in nonaggressive rats were almost 2-fold lower than in aggressive animals (Fig. 2). Antibody titer in secondary immune response in aggressive animals on day 19 of the experiment virtually did not change in comparison with the previous terms. In nonaggressive rats it significantly increased ($p<0.001$ compared to day 5, $p<0.05$ compared to day 14), but remained significantly lower than in aggressive animals ($p<0.01$). Bifactorial analysis of dispersions also showed that the dynamics of antibody titers in general differed significantly in animals with opposite behavior ($F_{3.80}=8.46$, $p<0.001$). It should be noted that higher antibody titer in the blood of aggressive animals (in comparison with nonaggressive rats) was associated with a greater number of APC in the spleen (Fig. 1).

Hence, the main parameters of humoral immune response (number of antibody producers and of antibodies produced by them) are decreased in gray rats selected by nonaggressive behavior in comparison with aggressive animals. The parameters of secondary immune response suggest that the number of immunological memory cells is also lower in nonaggressive rats.

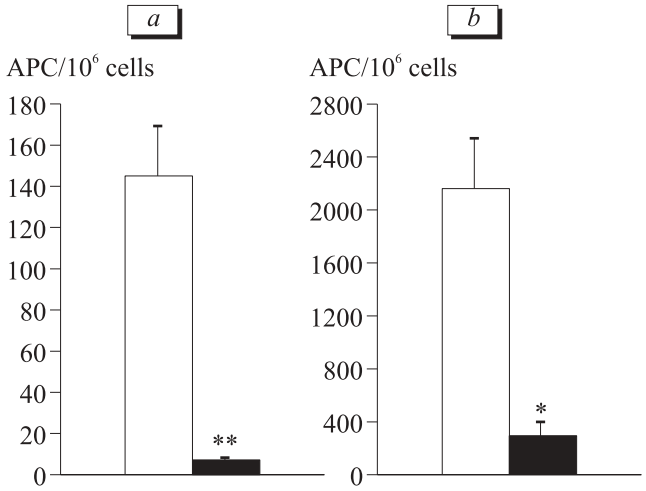

Fig. 1. Primary (*a*) and secondary (*b*) immune response in gray rats of opposite behavior. Here and in Figs. 2 and 3: light bars: aggressive rats; dark bars: nonaggressive rats. *$p<0.001$, **$p<0.01$ compared to aggressive animals. APC: antibody producing cells.

Activity of PAS during the development of primary and secondary immune response was studied. Bifactorial analysis of dispersions showed that the dynamics of corticosterone content during both primary and secondary immune response was different in rats with different behavior ($F_{2.126}=3.36$, $p<0.01$; $F_{3.83}=3.13$, $p<0.05$, respectively). Blood corticosterone level in nonaggressive rats was significantly lower than in aggressive animals (Fig. 3). A single injection of the antigen significantly increased blood corticosterone level in both groups as soon as 24 h after immunization ($p<0.001$; during the initial (inductive) phase of humoral immune response, when T and B

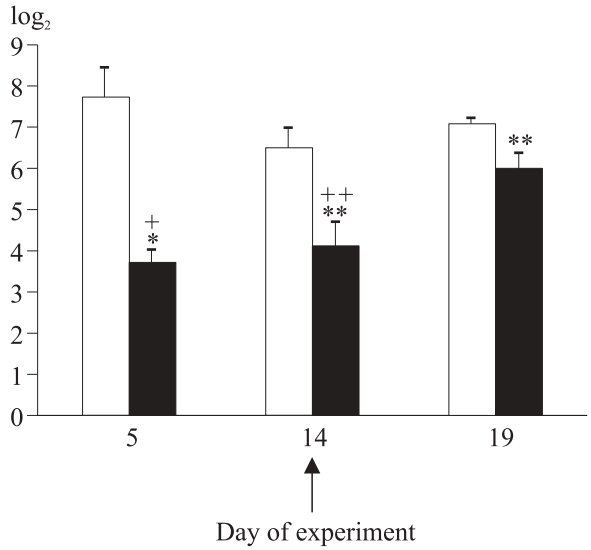

Fig. 2. Antibody titers in the plasma in immune response of rats with different behavior. Abscissa: day of experiment. Here and in Fig. 3: arrow shows the moment of repeated injection of the antigen. $^{+}p<0.001$, $^{++}p<0.05$ compared to day 19.

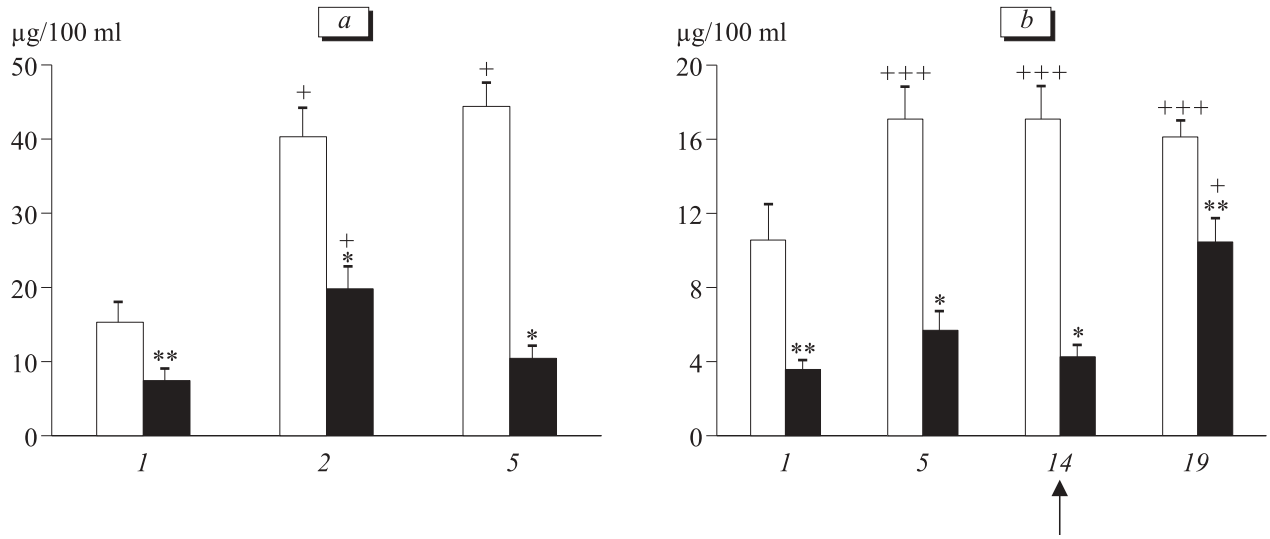

Fig. 3. Plasma corticosterone level in rats with different behavior in primary (*a*) and secondary (*b*) immune response. *1*) basal hormone level; *2*, *5*, *14*, *19*: hormone levels on the corresponding days of experiment. $^{+}p<0.001$, $^{++}p<0.01$, $^{+++}p<0.05$ compared to basal level.

lymphocytes proliferate and differentiate). On day 5 after immunization the concentration of corticosterone in aggressive animals did not change in comparison with the previous term, while in nonaggressive rats it decreased significantly to the baseline values ($p<0.01$). The same regularity was observed after the first challenge with the antigen in secondary immune response (Fig. 3, *b*). The concentration of corticosterone on day 14 of the experiment remained unchanged in all groups in comparison with day 5, surpassed the baseline level in aggressive rats, and corresponded to levels observed during stress [1,2]. According to published reports, stress-associated level of corticosterone after injection of the antigen stimulates the development of immune response [12]. Presumably, increased level of corticosterone in the blood before repeated immunization was also essential for the development of secondary immune response in aggressive animals in comparison with nonaggressive ones (Fig. 1, *b*). No further changes in the hormone level were detected in aggressive rats on day 19 of the experiment, while in nonaggressive rats it significantly increased ($p<0.001$). This increase in corticosterone level during secondary immune response can be explained by changes in the dynamics of the immune response to SE. We previously showed that these behavioral groups of rats differ by the dynamics of response to such immunological stimuli as interleukin-2 and LPS [2].

The mechanisms of immunomodulating effect of glucocorticoids on humoral immune response are not yet quite clear. Presumably, the increase in hormone level improves specificity of the immune response, because corticosterone suppresses poorly differentiated lymphocytes with low affinity for antigen [4]. The basal level of corticosterone is essential for the start of IgM production, while the increase in hormone level after injection of the antigen is needed for transition to IgG production [7]. T-helpers type 2 are involved in humoral immune response [10]. Glucocorticoids stimulate differentiation and secretory activity of T-helpers type 2 and suppress these processes in T-helpers type 1 [8]. All these factors to a great extent determine higher parameters of humoral immune response and antibody titers in aggressive rats in comparison with nonaggressive animals.

The number of antibody-producing cells increased 40-fold in nonaggressive rats and only 15-fold in aggressive animals (Fig. 1, *a*, *b*). Antibody titer in nonaggressive rats was also higher in secondary immune response in comparison with primary response. Presumably, this was due to increased PAS activity after repeated injection of the antigen to nonaggressive animals, which was seen from increased blood corticosterone content on day 19 of the experiment. On the other hand, T-helpers type 1 inducing secretion of IgG_2 can be involved (along with T-helpers type 2) in humoral immune response in animals with low stress reactivity of PAS [12]. It is also possible that the increase in antibody titer after repeated injection of the antigen in nonaggressive animals is caused by increased secretion of these Ig.

Hence, differences in primary and secondary immune response of gray rats selected by opposite behavior were revealed. The number of APC and antibodies produced by them are low in rats selected for elimination of aggressive behavior, in comparison with aggressive rats. Moreover, these animals exhibited low reaction of PAS to a single and repeated challenge with the antigen. Taking into account the immunomodulating role of PAS in many immune processes we

conclude that selection of wild gray rats for elimination of aggressive behavior, which is simultaneously selection for stress reactivity, reduces functional activity of PAS and changes immune reactivity. However the possibility of parallel changes in these systems during selection by behavior cannot be ruled out.

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REFERENCES

1. I. N. Os'kina and I. Z. Plyusnina, *Modern Concepts of Evolution Genetics* [in Russian], Novosibirsk (2000), pp. 327-333.
2. S. G. Shikhevich, I. N. Os'kina, and I. Z. Plyusnina, *Ros. Fiziol. Zh.*, **88**, No. 6, 781-789 (2002).
3. J. D. Ashwell, F. W. Lu, and M. S. Vacchio, *Annu. Rev. Immunol.*, **18**, 309-345 (2000).
4. H. O. Besedovsky and A. Del Rey, *Endocr. Rev.*, **17**, 64-106 (1996).
5. J. E. Blalock, *Immunol. Today*, **15**, 504-511 (1994).
6. F. S. Dhabhar, B. S. McEven, and R. L. Spencer, *Neuroendocrinology*, **65**, 360-368 (1997).
7. M. Fleshner, T. Deak, K. T. Nguen, *et al.*, *J. Immunol.*, **166**, 3813-3819 (2001).
8. D. Kovalovsky, D. Refojo, F. Holsboer, and E. Arzt, *J. Neuroimmunol.*, **109**, 23-29 (2000).
9. B. S. McEwen, C. A. Biron, K. W. Brunson, *et al.*, *Brain Res. Rev.*, **23**, 79-133 (1997).
10. T. R. Mosmann and S. Sad, *Immunol. Today*, **17**, 138-146 (1996).
11. I. Plyusnina and I. Oskina, *Physiol. Behav.*, **61**, 381-385 (1997).
12. N. Shanks and A. W. Kusnecov, *Ibid.*, **65**, 95-103 (1998).
13. E. M. Sternberg, *J. Endocrinology*, **169**, 429-435 (2001).
14. A. V. Tumbull and C. L. Rivier, *Physiol. Rev.*, **79**, 1-71 (1999).
15. R. L. Wilder, *Annu. Rev. Immunol.*, **13**, 307-429 (1995).