pression of AFP and AG A2/3 will be established in further investigations.

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REFERENCES

1. G. I. Abelev, in: *Tumors as a Problem of Developmental Biology* [in Russian], Moscow (1979), pp. 148-169.
2. G. I. Abelev, *Immunologiya*, No. 3, 4-9 (1994).
3. G. I. Abelev, D. A. El'gort, and T. L. Eraizer, *Byull. Eksp. Biol. Med.*, **92**, No. 9, 333-335 (1981).
4. G. A. Belitskii, L. M. Fonshtein, V. V. Khudolei, *et al.*, *Eksper. Onkol.*, **9**, No. 3, 20-23 (1987).
5. A. Yu. Kolyada, *Biol. Membrany*, **4**, No. 4, 408-414 (1987).
6. E. V. Lekhttsind and A. E. Gurvich, *Byull. Eksp. Biol. Med.*, **92**, No. 12, 752-754 (1981).
7. I. N. Shvemberger, in: *Cellular Heredity and Malignant Growth* [in Russian], Moscow-Leningrad (1966), pp. 154-167.
8. J. Becker, B. de Nechaud, and V. R. Potter, *Onco-Developmental Gene Expression*, W. H. Fishman and S. Sell (Eds.), New York (1976), pp. 259-270.
9. K. L. Davidson and P. S. Gerald, *Methods Cell Biol.*, **15**, 325-338 (1977).
10. T. L. Eraiser and L. S. Khamzina, *Int. J. Cancer*, **42**, 633-637 (1988).
11. M. W. Roomi and A. Columbano, *Carcinogenesis*, 7, No. 10, 1643-1646 (1986).

Effects of Zoosocial Conflict on Immunological Reactivity of C57Bl/6 Mice

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In aggressive C57Bl/6 mice, the immune response is shown to be enhanced after 20 confrontations with submissive mice. In submissive mice, the response is inhibited after 10-20 confrontations with aggressive partners. It is concluded that stimulation and inhibition of the immune response are associated with the formation of a neurochemical set which is dopaminergic in aggressive mice and serotoninergic in submissive ones.

Key Words: *zoosocial conflict; aggressiveness; submissiveness; immune response*

There is evidence that experimental stressful situations affect immune reactions [4,6]. Changes in the immunoreactivity of animals manifesting natural forms of behavior such as aggressiveness and submissiveness as a result of zoosocial conflicts have not been investigated in sufficient detail. During aggressive confrontations associated with protection of territory, the titers of IgE antibodies in submissive rats are lowered in response to a protein antigen [10], while in submissive C57Bl/6 and DBA mice the production of IgM antibodies is suppressed [12]. It was demonstrated that "high social rank" rats are resistant to infection [11]. Using a model of sensory contact, we showed that the experience of a 10-day-long confrontation stimulates the immune response in aggressive but not in submissive CBA mice in comparison with that in mice which did not participate in conflicts. The immune response was inhibited in submissive C57Bl/6 males after 10 defeats and was not stimulated in aggressive males after 10 victories [1]. With the use of various models it was shown that aggressive behavior is accompanied by activation of

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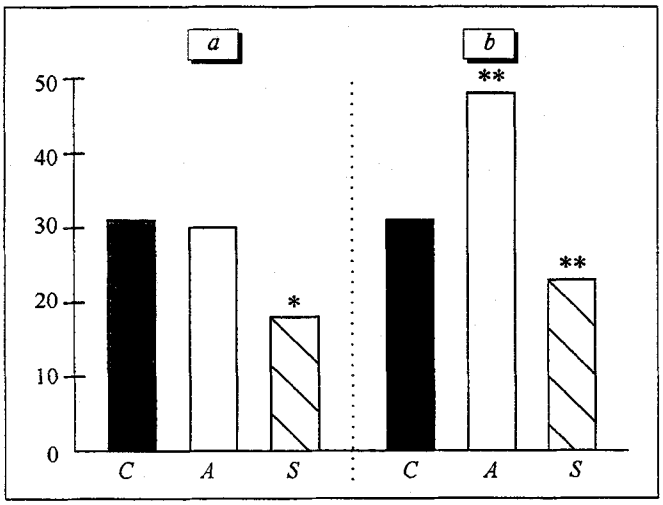

Fig. 1. Changes in the number of rosette-forming cells 5 days after immunization of C57Bl/6 mice with sheep erythrocytes (5×10^8 cells/animal) after 10 (*a*) and 20 (*b*) confrontations. *C* = control mice; *A* = aggressive mice; *S* = submissive mice. *$p<0.01$, **$p<0.001$ in comparison with the control group.

the dopaminergic system in the brain [7,13], whereas the formation and consolidation of submissive behavior are associated with changes in the content and metabolism of serotonin [5]. An immunomodulating effect exerted by the dopaminergic and serotoninergic systems upon their interaction was demonstrated. This interaction leads to the predominance of one system, implying that a particular neurochemical set has been established in the brain [2].

It can be hypothesized that a neurochemical set sufficiently strong to stimulate the immune response is not established in aggressive C57Bl/6 mice after 10 confrontations with submissive partners. Therefore, in order to enhance the neurochemical set the number of confrontations was increased.

MATERIALS AND METHODS

A total of 80 male C57Bl/6 mice aged 2.5-3 months and weighing 23-24 g were used. They were maintained under standard conditions in cages for 8-10 animals. Before experiment, the mice were placed in individual cages for 3 days to remove the group influences. Then two mice of equal body weight were placed in a metal cage divided into two equal compartments by a transparent partition with holes in it so that they could see each other and perceive each other's smell. After 2 days, the partition was removed, which started the confrontation. Confrontations (10 min long, once daily) were continued for a 20-day period. Victory or defeat was consolidated during the following 3 days through contacts with the same adversary. The submissive partner was replaced every day by another "loser," while the aggressive mouse was left in its compartment. Mice kept in individual cages for 7 days to remove the group influences served as controls. Five groups were formed: control males without any experience of confrontations (group 1), aggressive males with the experience of victories in 10 or 20 confrontations (group 2 and group 3, respectively), and submissive males with the experience of defeats in 10 or 20 confrontations (group 4 and group 5, respectively). On day 20 after daily confrontations, mice were immunized intravenously with a suspension of sheep erythrocytes (5×10^8 cells per mouse). The intensity of immune response was evaluated on day 5 after immunization by counting rosette-forming cells (RFC) [3] and plaque-forming cells (PFC) [8] in the spleen.

In order to maintain the established type of behavior, the mice were subjected to daily confrontations until the last day before sacrifice.

The results were analyzed by Student's *t* test.

RESULTS

On day 5 after immunization, the number of RFC in the spleen of aggressive C57Bl/6 males who had experienced 20 victories was significantly higher than that in the controls (Fig. 1). The level of rosette formation in these mice was much higher than that in aggressive mice with the experience of 10 victories, which agrees with our previous findings [1]. In submissive mice with the experience of 20 defeats, the immune response was significantly below the control level but differed little from that in submissive mice with the experience of 10 defeats. Similar changes were revealed in immune response evaluated by the number of PFC (Table 1).

TABLE 1. Numbers of Plaque-Forming Cells (PFC) in Aggressive and Submissive C57Bl/6 Mice Immunized with Sheep Erythrocytes in a Dose of 5×10^8 Cells (*M±m*)

Group	Number of PFC, $\times10^6$	Number of PFC per spleen
Control mice (*n*=17)	189.05±13.4	19407.5±1079.08
Aggressive mice (*n*=15)	245.7±21.3*	27348.5±3361.8*
Submissive mice (*n*=10)	107.9±13.1**	12195.4±1970.5**

Note. *$p<0.05$, **$p<0.01$ in comparison with the control group.

From these findings it can be concluded that the experience of victories or defeats led to the formation of opposite behavioral stereotypes (aggressiveness and submissiveness) in C57Bl/6 mice and stimulation of immune reactions in aggressive mice and inhibition in submissive mice.

Immune response was stimulated in C57Bl/6 males with the experience of 20 but not 10 victories. Since the activity of the dopaminergic system is enhanced in animals with an aggressive type of behavior [7,13] and this system is involved in the stimulation of immunogenesis [9], the enhanced immune response in C57Bl/6 males with the experience of 20 victories may be associated with the brain dopaminergic mechanisms. Aggressive C57Bl/6 mice with the experience of only 10 victories did not develop a dopaminergic set sufficiently strong to stimulate the immune response. On the other hand, inhibition of immune response in submissive C57Bl/6 males with the experience of 20 defeats was similar to that in submissive mice with the experience of 10 defeats. This suggests an earlier development of the serotoninergic set in submissive animals with activated serotoninergic mechanisms [5]. Due to earlier formation of this set they showed a reduced immune response after both 10 and 20 confrontations with an aggressive partner. This agrees with the higher levels of serotonin in the hypothalamus and brain stem in C57Bl/6 mice compared with mice of other strains.

REFERENCES

1. L. V. Devoino, E. A. Al'perina, N. N. Kudryavtseva, and N. K. Popova, *Fiziol. Zh. SSSR*, No. 12, 62-67 (1991).
2. L. V. Devoino and R. Yu. Il'yuchenok, *Neurotransmitter Systems in Psychoneuroimmunomodulation* [in Russian], Novosibirsk (1993).
3. G. V. Idova, M. A. Cheido, and L. V. Devoino, *Zh. Mikrobiol.*, No. 2, 57-60 (1976).
4. E. A. Korneva and E. K. Shkhinek, *Hormones and the Immune System* [in Russian], Leningrad (1988).
5. N. N. Kudryavtseva, I. V. Bakshtanovskaya, and N. K. Popova, *Zh. Vyssh. Nervn. Deyat.*, No. 6, 1134-1141 (1989).
6. A. Amcraut and G. F. Solomon, *Cancer Res.*, **32**, 1428-1433 (1972).
7. R. Bell and P. G. Hepper, *Behav. Brain Res.*, **23**, 1-21 (1987).
8. A. J. Cunningham, *Nature*, **5001**, 1106-1107 (1965).
9. L. Devoino, E. Alperina, and G. Idova, *Int. J. Neurosci.*, **40**, 271-288 (1988).
10. M. Fleshner, M. L. Landenslager, L. Simons, and S. F. Maier, *Physiol. Behav.*, **45**, 1183-1187 (1989).
11. K. Gartner, H. Kirchhoff, K. Mensing, and R. Velleuer, *J. Behav. Med.*, **12**, 487-502 (1989).
12. M. Lyte, S. G. Nelson, and M. L. Tompson, *Clin. Immunol. Immunopathol.*, **57**, 137-147 (1990).
13. J. Tani, J. Kataoka, J. Sakurai, *et al.*, *Pharmacol. Biochem. Behav.*, **26**, 725-729 (1987).