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Effect of Antidepressants on Immunological Reactivity in ASC Mice with Genetically Determined Depression-Like State

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The effect of chronic treatment with antidepressant drugs fluoxetine (20 mg/kg) and imipramine (25 mg/kg) on the number of antibody-producing cells and the main T cell subpopulations in ASC mice characterized by genetic predisposition to depression-like states was studied at the peak of the SE-induced immune response (5×10^8). Fluoxetine produced an immunostimulatory effect manifested in an increase in the relative and absolute number of IgM antibody-producing cells in the spleen and index of immunoreactivity (CD4/CD8). Administration of fluoxetine to parental mouse strains without depression (CBA and AKR) had no effect (CBA) or reduced the immune response. The CD4/CD8 ratio did not increase under these conditions. Imipramine was ineffective in the correction of immune reactions in a depression-like state.

Key Words: *antidepressants; fluoxetine; imipramine; IgM antibody-producing cells; T cell subpopulations*

Depressive disorders, the most prevalent mental disorders, are associated with not only neurophysiological disturbances, but also immune dysfunction. According to the results of clinical trials, the efficiency of antidepressants is only 50-70%. The development of a differential (personalized) approach to the therapy of depressive states is an urgent problem. Imipramine is the first tricyclic antidepressant that modulates the uptake of serotonin and other neurotransmitters. More than 35 drugs for the therapy of depressive disorders were synthesized from the moment of imipramine appearance. New-generation antidepressants, selective serotonin reuptake inhibitors (SSRI), are more efficient than tricyclic antidepressants and cause less side effects.

Genetic predisposition has an important role in the development of genetic disorders [9]. This characteristic is also important for the immune function [14]. ASC (antidepressant-sensitive catalepsy) mice genetically predisposed to depression-like states are characterized by not only high predisposition to catalepsy and depressive behavior [2], but also low antibody production [1,12], abnormal $CD4^+$ T cell content in the blood and spleen, and variations in the $CD4^+/CD8^+$ T cell ratio (CD4/CD8) [5] (in comparison with non-depressive parental CBA and AKR strains).

This work was designed to study the effect of chronic treatment with antidepressant drugs fluoxetine and imipramine on the immune response and major subpopulations of T cells in ASC mice.

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MATERIALS AND METHODS

Experiments were performed on 170 male CBA/Lac, AKR/J, and ASC/lcg mice weighing 24-28 g and aging 2.5-3.0 months. The animals were obtained from the vivarium of the Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences. The study was conducted according to the requirements of the European Community Directive (86/609/EC) and Biomedical Ethics Committee (Institute of Cytology and Genetics).

Experiments were conducted with fluoxetine (Sigma; one of the most common antidepressants of the SSRI group) and tricyclic antidepressant imipramine (Sigma). Imipramine inhibits reuptake of not only serotonin, but also of other neurotransmitters. Fluoxetine (20 mg/kg) and imipramine (25 mg/kg) were dissolved in physiological saline and injected intraperitoneally (0.2 ml) for 10 days before immunization. CBA/Lac and AKR/J mice were divided into 2 groups receiving the solvent (control) or fluoxetine. Three groups of ASC/lcg mice received the solvent (control), fluoxetine, or imipramine.

During immunization, the animals received single injection of SE (5×10^8 cells in 0.5 ml physiological saline) into the caudal vein. The immune response was evaluated from the number of IgM antibody-producing cells (IgM-APC) per 10^6 spleen lymphocytes (relative content) and spleen (absolute content) and percentage of T cell subpopulations in the blood on day 4 after immunization. The number of $CD4^+$ T cells (T helper cells) and $CD8^+$ T cells (T suppressor cells) was estimated by flow cytofluorometry. FITC-labeled monoclonal antibodies against CD4 and PE-labeled anybodies against CD8 were used for immunophenotyping of cells. The study was performed on a FACSCalibur cytofluorometer (BD) with CellQuest Pro software. At least 3000 cells were analyzed in each sample. Immunoreactivity index (IRI) was calculated from the $CD4^+/CD8^+$ T cell ratio.

The results were analyzed by one-way analysis of variance (ANOVA) with Statistica 10.0 software. The data were compared by Student's *t* test for independent samples.

RESULTS

Evaluation of the immune response showed that on day 4 after antigen administration, the number of IgM-APC per 10^6 spleen cells ($p<0.001$) and spleen ($p<0.01$) in ASC mice was lower than in the parental cataleptic CBA strain (not demonstrating depressive behavior; Table 1). However, the relative ($p<0.001$) and absolute number ($p<0.001$) of IgM-APC in ASC mice was higher than in the AKR parental strain without catalepsy and depressive signs (Table 1). Similar interstrain differences in the immune response on day 4 after SE administration were reported previously [1]. At the same time, the immune reaction in ASC mice on day 5 was much lower than in animals of both parental strains.

Chronic administration of fluoxetine produced different effects on the immune response in ASC, CBA, and AKR mice. The drug had no effect on the content of APC in CBA mice (Table 1), but decreased the count of these cells in AKR mice (reduction of the

TABLE 1. Effect of Chronic Treatment with Fluoxetine (20 mg/kg) on the Number of IgM-APC in the spleen of CBA, AKR, and ASC Mice on day 4 after Immunization with SE ($M\pm m$)

Mouse strain	Substance	Group number	IgM-APC per 10^6 cells	IgM-APC per spleen
CBA	Solvent	1	274.90±31.78 (n=15)	1715.6±165.13 (n=15)
	Fluoxetine	2	264.10±34.36 (n=14)	1603.3±197.25 (n=12)
AKR	Solvent	3	90.00±8.59 (n=54)	674.20±72.77 (n=47)
	Fluoxetine	4	55.90±9.12 (n=18)	390.4±67.08 (n=17)
			$p_3<0.05$	$p_3<0.05$
ASC	Solvent	5	182.80±10.55 (n=39)	1134.10±66.16 (n=43)
			$p_1<0.001$	$p_1<0.01$
			$p_3<0.00001$	$p_3<0.00001$
	Fluoxetine	6	244.30±27.97 (n=11)	1477.11±134.75 (n=9)
			$p_4<0.001$	$p_4<0.01$
			$p_5<0.05$	$p_5<0.05$

Note. Index at the certainty factor: number of the reference group.

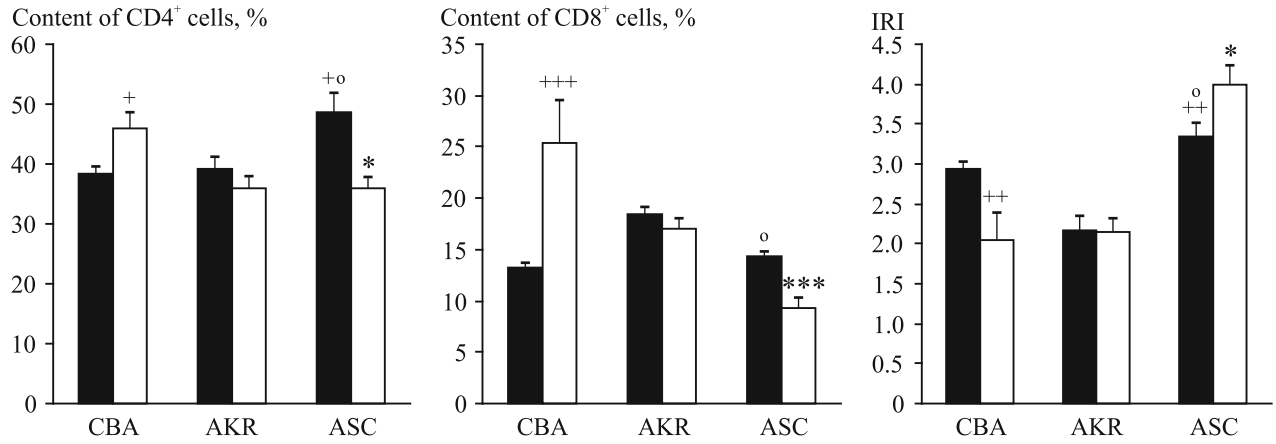

Fig. 1. Effect of fluoxetine (20 mg/kg, light bars) on subpopulations of $CD4^+$ and $CD8^+$ cells and their ratio in CBA, AKR, and ASC mice (in comparison with the control). $^+p<0.05$, $^{++}p<0.001$, and $^{+++}p<0.0001$ in comparison with control CBA mice; $^op<0.0001$ in comparison with control AKR mice; $^*p<0.05$ and $^{**}p<0.001$ in comparison with control ASC mice.

relative and absolute number of IgM-APC; $p<0.05$). However, fluoxetine increase the relative ($p<0.05$) and absolute number ($p<0.05$) of IgM-APC in ASC mice in comparison with the control (Table 1). Previous studies showed that chronic treatment with fluoxetine is followed by an increase in the reduced number of rosette-forming cells in ASC mice, but has no effect on the count of these cells in CBA mice [15].

In contrast to fluoxetine, chronic administration of imipramine (25 mg/kg) did not modulate the immune response in ASC mice. The number of APC under these conditions did not differ from the control. Our results are consistent with published data that a long-term treatment with imipramine in this dose has no effect on behavioral indexes in the Porsolt test and open field (typical of a depressive state in ASC mice) [7].

IRI in non-immunized ASC mice was lower than in the CBA parental strain [5]. However, on day 4 after immunization this index in ASC mice was higher than in CBA and AKR mice (Fig. 1). The content of T cell subpopulations and effect of fluoxetine on this parameter depended on the strain of mice (Fig. 1).

Chronic administration of fluoxetine (20 mg/kg) increased the content of $CD8^+$ and $CD4^+$ T cells, but decreased the CD4/CD8 ratio in CBA mice. The number of $CD8^+$ and $CD4^+$ T cells and their ratio in the blood after fluoxetine treatment remained unchanged in another parental strain (AKR; Fig. 1). The content of $CD8^+$ and $CD4^+$ T cells was reduced in fluoxetine-receiving ASC mice. As differentiated from parental strains, IRI in these mice was elevated ($p<0.05$; Fig. 1). Chronic administration of imipramine (25 mg/kg) was followed by a tendency toward the increase in $CD8^+$ T cell number, but produced a significant decrease in the count of $CD4^+$ T cells ($p<0.001$) and IRI ($p<0.001$; Fig. 1). These data are consistent with the results of clinical trials [4].

Fluoxetine inhibits serotonin reuptake, which is accompanied by an increase in activity of the serotoninergic system. The serotoninergic system plays an immunosuppressive role [6,11,13]. Therefore, the observed stimulatory effect of study antidepressant seems to be surprising. Moreover, single administration of fluoxetine or sertraline (another antidepressant that inhibits serotonin reuptake) diminishes the immune response to SE [6], mitogen-stimulated lymphocyte proliferation, and cytotoxic activity of natural killer cells [13].

It can be hypothesized that the positive effect of chronic treatment with fluoxetine is related to its influence on other neurotransmitter systems of the brain [3]. One of these systems is the dopaminergic system [8], which has an important role in immunostimulation [6,10,11]. Moreover, long-term administration of SSRI can be followed by stimulation of inhibitory presynaptic serotonin receptors and subsequent decrease in serotonin synthesis in the brain. We showed that imipramine does not cause immunostimulation. It should be emphasized that this drug (as differentiated from fluoxetine) modulates several neurotransmitter systems, which can produce various effects on the immune function.

We conclude that a widely used antidepressant fluoxetine has the immunocorrecting effect in ASC mice with a depression-like state. This effect is manifested in an increase of the immune response and IRI at the peak of immune reactions. However, administration of fluoxetine to mice without depressive symptoms does not change the number of APC (CBA mice) or even inhibits the immune system (AKR mice). As differentiated from fluoxetine, chronic treatment with imipramine (25 mg/kg) was ineffective in the correction of immune reactivity in animals with a genetically determined depression-like state.

REFERENCES

1. E. L. Alperina, A. V. Kulikov, N. K. Popova, and G. V. Idova, Immune response in mice of a new strain ASC (antidepressants sensitive catalepsy). *Bull. Exp. Biol. Med*., **144**, No. 2, 221-223 (2007).
2. D. V. Bazovkina, A. V. Kulikov, E. M. Kondaurova, and N. K. Popova, Selection for the predisposition to catalepsy enhances depressive-like traits in mice. *Russ. J. Genetics*, **41**, No. 9, 1002-1007 (2005).
3. A. S. Bazyan and G. A. Grigoryan, Molecular-Chemical Basis of Emotional States and Reinforcement. *Uspekhi Fiziol. Nauk*, **37**, No. 1, 68-83 (2006).
4. T. P. Vetlugina, T. I. Nevidimova, O. A. Lobacheva, and V. B. Nikitina, *Technology of Immunocorrection in Mental Disorders* [in Russian], Tomsk (2010).
5. M. M. Gevorgyan, E. L. Alperina, A. V. Kulikov, and G. V. Idova, Changes in the content of $CD4^+$ and $CD8^+$ T-lymphocytes in peripheral blood and spleen in mice of the ASC strain (antidepressant sensitive catalepsy) with predisposition to depressive-like behavior. *Immunologiya*, **34**, No. 5, 262-264 (2013).
6. L. V. Devoino, G. V. Idova, E. L. Alperina, M. A. Cheido, S. M. Davydova, and M. M. Gevorgyan, Neuromediator brain system in the immune reaction modulation (dopamin, serotonin. GABA). *Neiroimmunologiya*, **3**, No. 1, 11-18 (2005).
7. M. A. Tikhonova, V. V. Lebedeva, A. V. Kulikov, D. V. Bazovkina, and N. K. Popova, Effect of imipramine on the behavior and cerebral 5-HT1A serotonin receptors in mice genetically predisposed to catalepsy. *Bull. Exp. Biol. Med.*, **141**, No. 1, 5348-50 (2006).
8. G. T. Shishkina, N. N. Dygalo, A. M. Yudina, T. S. Kalinina, T. G. Tolstikova, I. V. Sorokina, I. L. Kovalenko, and L. V. Anikina, The effects of fluoxetine and its complexes with glycerrhizic acid on behavior in rats and brain monoamine levels. *Neurosci. Behav. Physiol*., **36**, No. 4, 329-333 (2006).
9. L. Capuron, A. Miller, and M. R. Irwin, Psychoneuroimmunology of depressive disorder: mechanisms and clinical implications, in: *Psychoneuroimmunology*, Ed. R. Ader, San Diego (2007), Vol. 1, pp. 509-530.
10. L. V. Devoino, E. L. Al'perina, M. M. Gevorgyan, and M. A. Cheido, Involvement of dopamine D_1 and D_2 receptors in the rat nucleus accumbens in immunostimulation. *Neurosci. Behav. Physiol*., **37**, No. 2, 147-151 (2007).
11. G. V. Idova, E. L. Alperina, and M. A. Cheido, Contribution of brain dopamine, serotonin and opioid receptors in the mechanisms of neuroimmunomodulation: evidence from pharmacological analysis. *Int. Immunopharmacol*., **12**, No. 4, 618-625 (2012).
12. G. V. Idova, E. L. Al'Perina, M. M. Gevorgyan, E. N. Zhukova, D. V. Yur'Ev, and A. V. Kulikov, T-lymphocyte subpopulation composition and the immune response in depression-like behavior in ASC mice. *Neurosci. Behav. Physiol*., **43**, No. 8, 946-950 (2013).
13. T. C. Pellegrino and B. M. Bayer, Role of central 5-HT(2) receptors in fluoxetine-induced decreases in T lymphocyte activity. *Brain Behav. Immun*., **16**, No. 2, 87-103 (2002).
14. J. M. Petitto, Behavioral genetics and immunity, in: *Psychoneuroimmunology*, Eds. R. Ader, D. L. Felten, and N. Cohen, San Diego (2001), Vol. 2, pp. 173-186.
15. M. A. Tikhonova, E. L. Alperina, T. G. Tolstikova, D. V. Bazovkina, V. Y. Di, G. V Idova., A. V. Kulikov, and N. K. Popova, Effects of chronic fluoxetine treatment on catalepsy and the immune response in mice with a genetic predisposition to freezing reactions: the roles of types 1A and 2A serotonin receptors and the tph2 and SERT genes. *Neurosci. Behav. Physiol*., **40**, No. 5, 521-527 (2010).