Effect of Benzo[a]pyrene on the Immune Status of Mice with Anxious-Depressive Syndrome

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We studied the effect of benzo[a]pyrene on cells of lymphoid organs, energy metabolism of blood lymphocytes, and immunological reactivity in mice with anxious depressive syndrome produced by social stress. Benzo[a]pyrene exhibited a more pronounced immunotoxic activity in anxious-depressive animals, which was more than a simple sum of the effects produced by adverse ecological and psychic factors.

Key Words: *benzo[a]pyrene; social stress; immune status*

Much attention is now paid to the mechanism of integration of the nervous and immune system. The influence of brain functions controlling psychic activity on the immune system is extensively studied. Published data show that neurochemical properties of the brain modulate immune resistance and energy metabolism in lymphocytes [3]. Psychoemotional disorders produced by social stress (anxiety, depression, and phobia) have severe symptoms and are hardly curable [1]. A strong correlation was revealed between epidemiological indexes and contamination of the atmospheric air with chemical pollutants. The presence of suspended substances, oxides, and polycyclic aromatic carbohydrates (PAC) is the major risk factor for ecopathology. PAC exist in a free state or are presented by suspension of particles [4]. According to WHO reports benzo[a]pyrene (BP) is the most hazardous PAC with immunotoxic activity [7,13]. The effect of simultaneous exposure to adverse ecological and social factors remains unknown.

Here we studied the effect of BP on the immune status of animals with anxious-depressive syndrome (ADS).

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MATERIALS AND METHODS

Experiments were performed with adult male C57Bl/6J mice aging 2.5-3 months and weighing 26-28 g. The study was conducted on the model of sensory contact [12]. The animals were exposed to unavoidable social stress. Successive defeats in 20 confrontations with an aggressive partner resulted in the development of submissive behavior and ADS [1,3,12]. The mice with ADS were divided into 3 groups (6-7 animals per group). Group 1 animals (ADS+BP) intraperitoneally received BP in a minimum volume of olive oil (0.02 ml) for 3 days (total dose 60 mg/kg). Group 2 animals (ADS+olive oil) received the minimum volume of olive oil (solvent of BP). Group 3 animals (ADS) were not injected. The mice of 3 control groups (5 mice per group) were housed individually for 5 days and remained intact (control) or received BP and olive oil. The animals were decapitated under light ether anesthesia on the next day after the 3rd injection of BP or olive oil. The cell suspension was obtained from lymphoid organs (thymus, spleen, and mesenteric lymph nodes) by soft crushing in a glass homogenizer. The total number of cells was estimated in a Goryaev chamber. Smears were prepared on cover glasses by the dried-drop method and subjected to Romanowsky—Giemsa staining. The percentage of small lymphocytes and blast cells was de-

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termined under a light microscope (oil immersion, ×1350). The total number of blood leukocytes was estimated in a Goryaev chamber. Differential leukocyte count was determined in blood smears stained by the method of Romanovsky—Giemsa [8]. The humoral immune response to T-dependent antigen (sheep erythrocytes) was studied by calculating antibody-producing cells in the spleen 4 days after intraperitoneal injection of the antigen (Cunningham method) [6]. Activities of succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), and α-glycerophosphate dehydrogenase in lymphocytes were measured in blood smears by the cytochemical method [9].

The results were analyzed by Statistica 5 software. The significance of differences was evaluated by nonparametric Mann—Whitney test (multifactor analysis of variance, 95% significance level).

RESULTS

Total cell count and absolute number of small lymphocytes and blast cells in the spleen decreased in mice with ADS exposed to social stress (compared to the control). The total cell count and absolute number of small lymphocytes in mesenteric lymph nodes decreased. The percentage of peripheral blood lymphocytes also decreased in these animals (Fig. 1).

BP produced various effects on the humoral immune response and cell composition of lymphoid organs and blood in animals with ADS and control mice.

BP decreased the relative number of antibody-producing cells in animals with ADS. BP inhibited the humoral immune response to sheep erythrocytes in ADS animals, but had no effect in control mice. Previous studies showed that the immune response is suppressed in depressive mice [3,11]. These data indicate that anxious-depressive state is not accompanied by impaired immunological reactivity. By contrast, the immune system is characterized by high sensitivity to toxic factors under these conditions. After administration of BP the percentage of blast cells in the spleen increased in ADS mice, but decreased in control animals.

BP decreased the total number of cells in mesenteric lymph nodes of mice with ADS. The absolute number of lymphocytes decreased after administration of BP and, to a lesser extent, of olive oil. BP had no effect on the cell composition of lymph nodes in control animals.

BP decreased the absolute number of leukocytes and lymphocytes in the blood from ADS mice. Injection of BP decreased blood lymphocyte count in control animals (Figs. 2 and 3).

Chronic social stress and variations in the immune status are accompanied by changes in lymphocyte energy metabolism [2,3,10]. We compared the effects of BP on activity of oxidation-reduction enzymes in peripheral blood lymphocytes. LDH activity in ADS mice was lower than in control animals, which reflects decreased compensatory capacity and/or suppression of lymphocyte activation [2,10]. BP produced different effects on enzyme activities in ADS and control

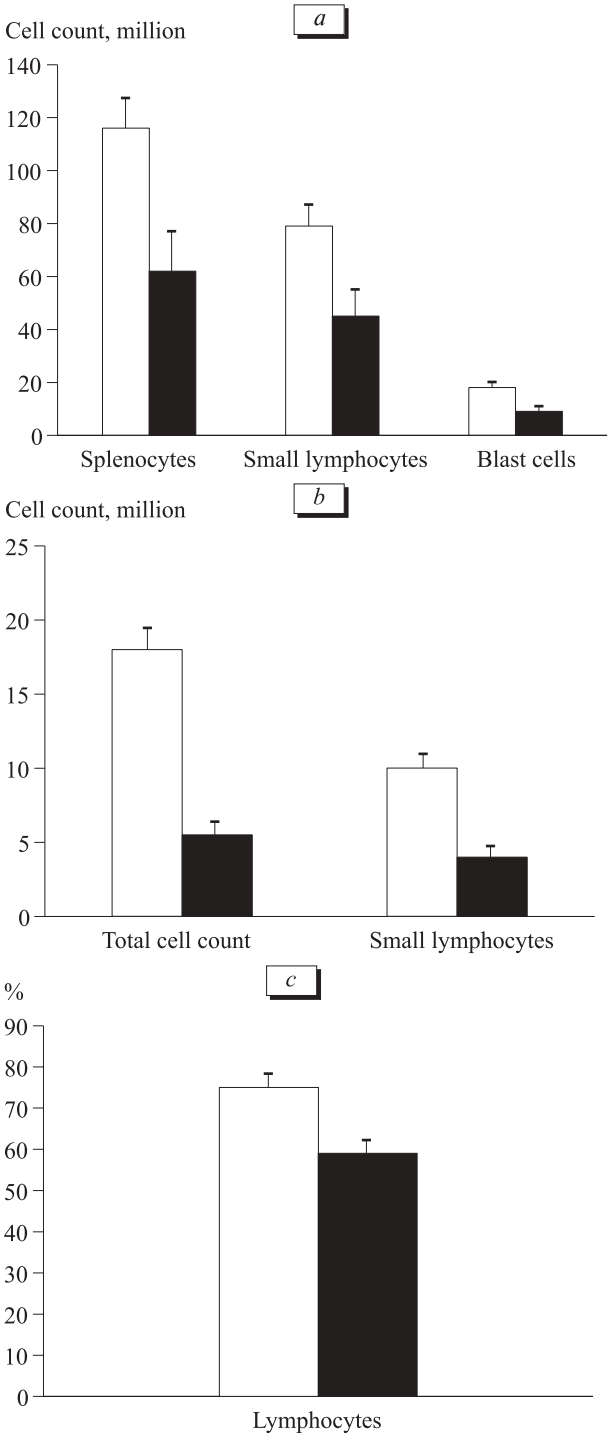

Fig. 1. Cell composition of lymphoid organs and blood in C57Bl/6J mice with anxious-depressive syndrome (ADS): spleen (*a*), mesenteric lymph node (*b*), and blood (*c*). Light bars: control. Dark bars: ADS.

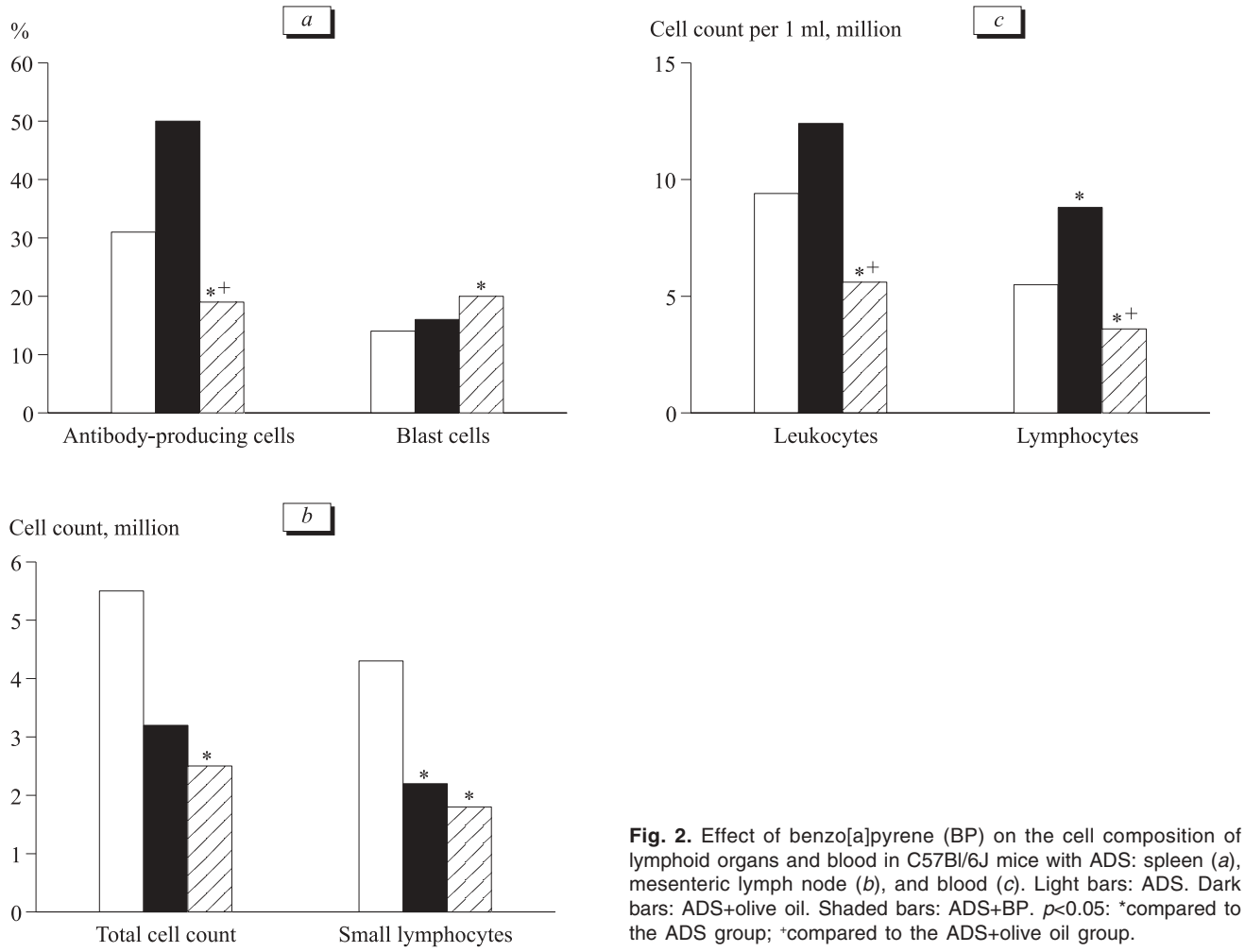

Fig. 2. Effect of benzo[a]pyrene (BP) on the cell composition of lymphoid organs and blood in C57Bl/6J mice with ADS: spleen (*a*), mesenteric lymph node (*b*), and blood (*c*). Light bars: ADS. Dark bars: ADS+olive oil. Shaded bars: ADS+BP. $p<0.05$: *compared to the ADS group; +compared to the ADS+olive oil group.

mice. Activities of SDH and LDH decreased in mice with ADS and, to a greater extent, in control animals. These changes were probably associated with the adaptive response of depressive animals after 20 confrontations [3]. By contrast, the decrease in α-glycerophosphate dehydrogenase activity produced by BP was

TABLE 1. Effect of BP on Dehydrogenase Activities in Blood Lymphocytes from Control Mice ($M\pm m$)

Group	SDH activity	LDH activity	α-Glycerophosphate dehydrogenase activity
Control	12.40±1.07	22.15±0.99	12.35±0.74
Olive oil (active control)	14.09±0.89	22.54±1.11	14.55±0.52
BP	11.47±0.32^{+++}	15.66±0.51^{*+}	12.82±0.51^{++}

Note. $^{*}p<0.001$ compared to the control; $^{+}p<0.001$, $^{++}p<0.05$, and $^{+++}p=0.02$ compared to olive oil.

TABLE 2. Effect of BP on Dehydrogenase Activities in Blood Lymphocytes from ADS Mice ($M\pm m$)

Group	SDH activity	LDH activity	α-Glycerophosphate dehydrogenase activity
ADS	12.05±0.53	14.23±0.18	13.62±1.59
ADS+olive oil (active control)	14.46±0.84	17.86±0.80*	15.96±0.61
ADS+BP	12.44±0.54^{+}	14.60±0.41^{+}	12.62±0.32^{+}

Note. $p<0.05$: *compared to the ADS control; +compared to the ADS+olive oil group.

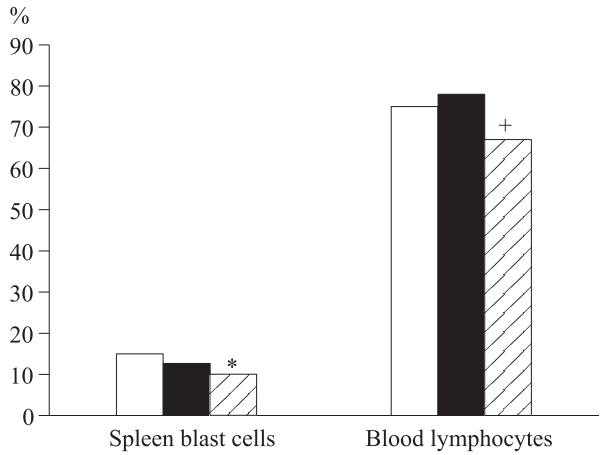

Fig. 3. Effect of benzo[a]pyrene (BP) on cell composition of lymphoid organs and blood in control C57Bl/6J mice. Light bars: control group. Dark bars: olive oil. Shaded bars: BP. $p<0.05$: *compared to the control; +compared to the olive oil group.

more pronounced in animals with depression. BP inhibited the immune response in these mice (Tables 1 and 2). These findings are consistent with the relationship between α-glycerophosphate dehydrogenase activity and the type of the immune response [2,9].

Our results show that BP produces different effects on intact and depressive mice. The immunosuppressive effect of BP is more pronounced in depressive animals. The effects of BP on activity of enzymes for energy metabolism differ in intact and depressive mice. It can be hypothesized that simultaneous exposure to adverse social and toxic factors determines a qualitatively new response of the organism [5]. These data should be taken into account in the development of hygienic recommendations for various groups of people exposed to social and ecological pressing.

REFERENCES

1. Yu. I. Borodin, N. N. Kudryavtseva, M. V. Tenditnik, *et al.*, *Byull. Sib. Otd. Ros. Akad. Med. Nauk*, No. 4, 45-56 (2001).
2. A. S. Bykov, A. E. Kondakov, and V. V. Sarzhevskii, *Ibid*., No. 3, 47-50 (1992).
3. N. I. Gryazeva, A. V. Shurlygina, L. V. Verbitskaya, *et al*., *Ros. Fiziol. Zh*., **85**, No. 8, 1035-1042 (1999).
4. S. V. Zykov, A. P. Sadovskii, S. E. Ol'kin, and V. F. Raputa, *Byull. Sib. Otd. Ros. Akad. Med. Nauk*, No. 3-4, 30-40 (2000).
5. V. P. Kaznacheev and G. I. Nepomnyashchikh, *Ideas about Problems of General Pathology at the Boundary of the XXI Century* [in Russian], Novosibirsk (2000).
6. V. A. Kozlov, O. T. Kudaeva, E. N. Naumova, and T. V. Eliseeva, *Methodical Manual on the Method of Local Hemolysis and Statistical Analysis of Data* [in Russian], Novosibirsk (1988).
7. S. I. Kolesnikov, S. V. Michurina, A. V. Semenyuk, and G. M. Vakulin, *Liver and Regional Lymph Nodes upon Treatment with 3,4-Benzo[a]pyrene* [in Russian], Novosibirsk (1995).
8. V. V. Men'shikov, L. N. Delektorskaya, R. P. Zolotnitskaya, *et al*., *Laboratory Methods of Study in Clinical Practice* [in Russian], Moscow (1987).
9. R. P Nartsissov, *Arkh. Anat*., **56**, No. 5, 85-91 (1969).
10. A. V. Shurlygina, G. I. Litvinenko, T. I. Dergacheva, and V. A. Trufakin, *Byull. Eksp. Biol. Med*., **125**, No. 5, 576-578 (1998).
11. L. V. Devoino, E. L. Alperina, N. N. Kudryavtseva, *et al*., *Brain Behav. Immun*., **7**, 91-96 (1993).
12. N. N. Kudryavtseva, *Aggress. Behav*., **17**, No. 5, 285-291 (1991).
13. J. W. Rodriguez, W. G. Kirlin, Y. G. Wirsiy, *et al*., *Immunopharmacol. Immunotoxicol*., **21**, No. 2, 379-396 (1999).