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# Effect of Antiserotonin Antibodies on Functional Activity of T and B Lymphocytes and Peritoneal Macrophages

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Experiments on C57Bl/6 mice showed that antiserotonin antibodies injected intraperitoneally in a dose of 25 mg/kg or added to cell culture in a dose of $10^{-7}$ mol/ml suppress lymphocyte proliferative response to pokeweed mitogen and stimulate functional activity of macrophages.

**Key Words:** *antiserotonin antibodies; T lymphocytes; B lymphocytes; peritoneal macrophages*

Both neurotransmitters and neuropeptides are involved in neuroimmune interrelationships and interactions. Neuroimmunomodulatory function of serotonin (5-HT) has been demonstrated [3,4]. It was shown that activation of serotoninergic mechanisms in the central nervous system is accompanied by suppression of the immune system mediated through pituitary-adrenal regulatory mechanisms. The possibility of direct effect of 5-HT or antiserotonin antibodies (ASAB) on immunocytes has not yet been explored. Previously, we showed that 5-HT and ASAB stimulate functional activity of macrophages [5]. The aim of the present study was to compare the immunomodulating effect of ASAB on functional activity of macrophages and lymphocytes.

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## MATERIALS AND METHODS

Experiments were carried out on C57Bl/6 male mice weighing 23-24 g. In series I the effect of systemic injection of ASAB on functional activity of T and B lymphocytes and peritoneal macrophages was studied. Antiserotonin antibodies were injected intraperitoneally in a single dose of 25 mg/kg (protein concentration). In series II we studied the effect of ASAB ($10^{-7}$mol/ml) on the same immunological parameters in cell culture.

Antiserotonin antibodies were isolated from rabbits immunized according to the standard scheme [1]

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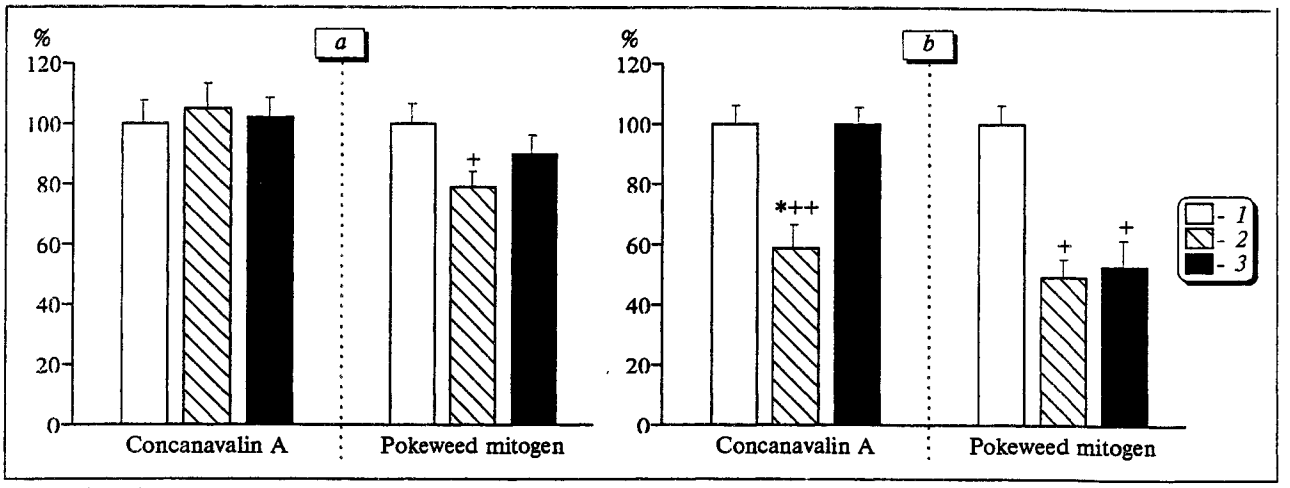


Fig. 1. Effect of systemic injection of antiserotonin antibodies on lymphocyte blastogenic response to concanavalin A and pokeweed mitogen on days 1 (*a*) and 5 (*b*) postinjection. Here and on Fig. 2, *1*) control group (physiological saline) taken as 100%; *2*) antiserotonin antibodies; *3*) γ-globulin (control II). *$p<0.05$ compared with control I, +$p<0.05$, ++$p<0.01$, compared with control II.

with 5-HT-bovine serum albumin conjugate prepared as described previously [9]. Titer of ASAB measured by enzyme linked immunoassay [2] was 1:12,000. The specificity of obtained antibodies was assessed by the reaction of competitive inhibition with 5-HT. γ-Globulin serum fractions were isolated from serum of immune and intact animals by ammonium sulfate precipitation [7], dialyzed, lyophilized, and stored at 4°C.

Functional activity of T and B lymphocytes was evaluated by blastogenic response to concanavalin A (ConA) and pokeweed mitogen. The effect was assessed by the intensity of $^3$H-thymidine incorporation into cell DNA. To this end, $10^7$ lymphocytes were incubated for 72 h at 37°C in RPMI-1640 medium supplemented with 10% fetal calf serum, antibiotics and mitogens (10 μg each). $^3$H-thymidine (1 μCi) was added to each sample 6 h prior to the end of incubation. The samples were washed three times with RPMI-1640 medium, the pellet was lysed with 1 ml 10% Triton X-100, 0.2 ml lysate was transferred into a scintillation vial, and specific activity measured in an Intertechnique counter. Phagocytic activity of mouse peritoneal macrophages with respect to *Staphylococcus aureus* (Zhaev strain) was evaluated in cell culture [6] by determining phagocytic number (% of phagocytizing macrophages) and phagocytic index (the number of bacteria per phagocyte).

The data were processed statistically using the Student *t* test.

## RESULTS

In series I systemic injection of ASAB induced pronounced shifts in functional activity of lymphocytes and macrophages. As seen from Fig. 1, blastogenic response to pokeweed mitogen (B lymphocyte—specific mitogen) was lowered as soon as 1 day after injection of ASAB. On day 5, blastogenic response of both T and B lymphocytes to mitogens decreased. In contrast, ASAB markedly stimulated phagocytic activity of peritoneal macrophages (Table 1): on days

TABLE 1. Effect of Systemic Injection of ASAB on Phagocytic Activity of Peritoneal Macrophages in C57Bl/6 Mice ($M\pm m$, $n$=6)

| Group | Parameters of phagocytosis | | | |
|---|---|---|---|---|
| | phagocytic number, % | | phagocytic index | |
| | days postinjection | | | |
| | 1 | 5 | 1 | 5 |
| Control I, γ-globulin, 25 mg/kg | 72.5±0.9** | 65.0±2.4 | 5.3±0.3 | 4.9±0.5 |
| Control II, physiological saline | 59.0±1.4 | 59.2±1.2 | 5.0±0.3 | 5.1±0.4 |
| Experimental group, ASAB, 25 mg/kg | 79.0±1.1*++ | 68.0±1.2+ | 5.6±0.2 | 5.2±0.6 |

Note. Here and in Table 2: *$p<0.05$ compared with control I, +$p<0.05$, ++$p<0.01$, compared with control II.

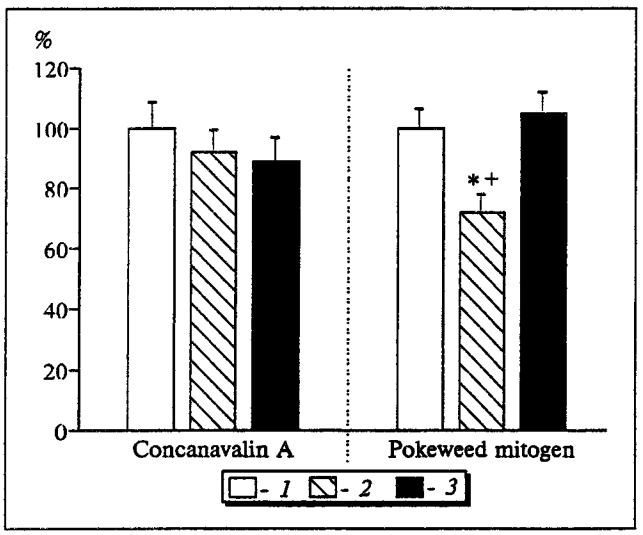


Fig. 2. Effect of antiserotonin antibodies on lymphocyte blastogenic response to concanavalin A and pokeweed mitogen added to lymphocyte culture.

TABLE 2. Effect of ASAB Added to Cultured Peritoneal Macrophages from C57Bl/6 Mice on Their Phagocytic Activity ($M \pm m$, $n$=8)

| Group | Parameters of phagocytosis | |
|---|---|---|
| | phagocytic number, % | phagocytic index |
| Control I, γ-globulin, $10^{-7}$ mol/ml | 69.0±1.2** | 5.0±0.1 |
| Control II, physiological Saline | 59.0±1.4 | 5.0±0.3 |
| Experimental group, ASAB, $10^{-7}$ mol/ml | 77.0±1.0*** | 5.3±0.2 |

1 and 5 postinjection, phagocytic number in ASAB-treated mice was significantly higher than in mice injected with γ-globulin and physiological saline. Phagocytic index in ASAB-treated mice did not differ from that in both control groups.

In series II, the effect of ASAB on functional activity of B lymphocytes and phagocytic activity of peritoneal macrophages in culture did not differ from the effect of systemic injection. As seen from Fig. 2, addition of ASAB to lymphocyte culture considerably suppressed blastogenesis induced by pokeweed mitogen and stimulated phagocytic activity of peritoneal macrophages (Table 2). These results point to a direct effect of ASAB on serotonin receptors on lymphocytes and macrophages, which is confirmed by the fact that 5-HT exerts similar effects on macrophages [5] and lymphocytes. It was found that 5-HT inhibits mitogen-induced proliferative response in lymphocytes [8]. The observed effect can be attributed to the presence of anti-idiotypic antibodies in the ASAB pool, which affect with specific serotonin receptors on immunocytes.

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