MICROBIOLOGY AND IMMUNOLOGY

Suppression of Increased Alcohol Consumption Caused by Adoptive Transfer of Splenocytes from Animals with Abstinent Syndrome by Antibodies to Serotonin

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Experiments on C57Bl/6 mice prone to experimental alcoholism demonstrated that adoptive transfer of splenocytes from animals with the abstinent syndrome stimulates alcohol consumption. Incubation of splenocytes with antibodies to serotonin in a dose of 10^{-7} M for 1 or 24 h completely suppressed this effect.

Key Words: *alcohol; alcohol motivation; splenocytes; lymphocytes; antibodies to serotonin*

Antibodies to serotonin (ABS) attenuate the development of alcohol dependence by decreasing alcohol consumption and suppressing the abstinent syndrome (AS), which was confirmed by animal experiments [5,6]. The mechanisms underlying the effect of ABS in alcohol dependence are unknown. One of them may be mediated by the immune system. By directly affecting the immune cells, ABS may induce or suppress the production of neuromodulating factor(s) entering the central nervous system. Addition of 10^{-7} M ABS to a cell culture stimulates functional activity of peritoneal macrophages and attenuates the proliferative response of lymphocytes stimulated by Phytolacca mitogen [4].

We studied the effect of ABS, which is probably mediated by the immune system, on alcohol motivation in C57Bl/6 mice prone to alcohol.

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MATERIALS AND METHODS

Male C57Bl/6 mice weighing 20-22 g were used. ABS were obtained from rabbits routinely immunized [2] with the serotonin-bovine albumin conjugate synthesized as described previously [10]. The ABS titer in enzyme immunoassay [3] was 1:12,000. The specificity of resultant ABS was assessed in competitive inhibition by serotonin. γ-Globulins were isolated from sera of immunized and intact rabbits by reprecipitation with ammonium sulfate [8], purified by dialysis, lyophilized, and stored at 4°C.

The effect of ABS on alcohol dependence mediated by the immune system was studied on a model of increased alcohol consumption after adoptive transfer of splenocytes from animals with AS [7]. The mice were divided into donors and recipients. Donor mice were alcoholized by ethanol aerosol in a special chamber [1] with an ethanol concentration of 14 mg/liter. The animals were left in the chamber for 10 days and drank 15% ethyl alcohol solution instead of water. After alcoholiza-

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tion the mice were transferred into cages with water and after 6 h the degree of AS was assessed [9]. The following clinical symptoms were evaluated: muscle convulsions, tremor, starting at knocking, tail rigidity (Straub's syndrome), and convulsions if raised by the tail. Only mice with pronounced AS were lymphocyte donors. Donor mice were sacrificed by ether vapor, the spleens were removed, and splenocyte suspensions purified from erythrocytes by osmotic shock. For studies of the direct effect of ABS on splenocytes, the cells were transferred to cell culture flasks with RPMI-1640, 20% calf serum, and antibiotics. ABS in a dose of 10^{-7} M were added to the culture medium. Normal rabbit γ-globulin in the same dose was the control. Splenocyte suspension was incubated at 37°C for 1 or 24 h. After incubation, cell viability was assessed in the Trypan Blue test. Suspensions with at least 95% viable cells were selected for experiment.

The recipient mice were offered free choice between water and 15% ethyl alcohol for 2 weeks. They were then divided into 6 groups, 15 animals each. The first 4 groups were the control for the efficacy of adoptive transfer of lymphocytes and specificity of the ABS effect: 1) 0.4 ml normal saline intraperitoneally; 2) native lymphocytes from animals with pronounced AS in a dose of 2×10^7 cells in 0.4 ml; 3) the same lymphocytes incubated *in vitro* with γ-globulin for 1 h; and 4) the same, after a 24-h incubation. Experimental animals were injected with lymphocytes from animals with AS incubated *in vitro* with ABS for 1 h (group 5) and for 24 h (group 6).

After injection of lymphocytes, the animals were observed for month under conditions of free choice between water and 15% ethanol solution.

Results were statistically processed using unpaired parametrical Student's *t* test.

RESULTS

Adoptive transfer of splenocytes from mice with AS markedly increased alcohol consumption by recipient animals, which persisted for 21 days (Fig. 1). Ethanol consumption was the greatest on day 10 after lymphocyte transfer. Transfer of "abstinized" splenocytes after 1- or 24-h preincubation with normal γ-globulin (groups 3 and 4) also increased in alcohol consumption which persisted for only 7 days. On day 10, alcohol consumption in these mice decreased in comparison with recipient mice in the group of positive control for stimulation of consumption by "abstinized" lymphocytes; by day 21 alcohol consumption in these groups did not differ from that in control group 1. Unlike in the above-listed controls for efficacy of immunostimulation and immunosuppression of alcohol motivation, transfer of "abstinized" lymphocytes preincubated with ABS for 1 or 24 h (groups 5 and 6) sharply suppressed alcohol consumption in the recipient mice during the entire period of observation. Alcohol consumption in these recipient mice was much lower in comparison with mice with immunologically stimulated alcohol consumption and with controls injected with normal saline (Fig. 1). Immunosuppression of alcohol motivation in groups 5 and 6 was significantly higher on days 10 and 21 in comparison with groups injected with abstinized lymphocytes pretreated with normal γ-globulin.

Thus, ABS suppress alcohol motivation upon systemic administration to mice with physical alcohol dependence [5,6] and abolish the immunostimulation of increased alcohol consumption by lymphocytes from patients with AS after preincubation of cell suspension with ABS. A similar, although weaker, effect of normal γ-globulin may result from the presence of small amounts of natural ABS in the γ-globulin fraction of "old" rabbits.

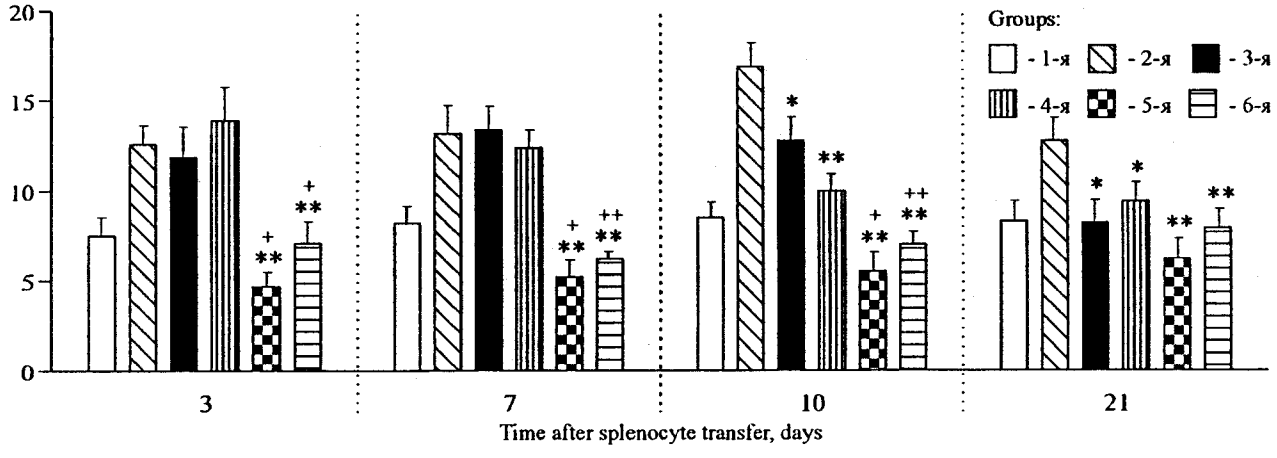

Fig. 1. Alcohol consumption by C57Bl/6 mice after adoptive transfer of splenocytes from animals with the abstinent syndrome, incubated with antibodies to serotonin. Ordinate: consumption of 96° alcohol, g/kg. *$p<0.05$, **$p<0.001$ *vs.* group 2; $p<0.05$: +*vs.* group 3, ++*vs.* group 4.

Our experiments indicate that one of the mechanisms of ABS effect on alcohol dependence can be realized through splenocytes (lymphocytes and macrophages). ABS effect can be due to, at least partially, splenocyte suppression of production of factor(s), for example, lymphocytic cytokine with neuromodulating activity affecting the level of alcohol motivation. Increased alcohol consumption after serum transfer from patients with AS [7] indicates such a possibility.

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