Inhibition of Alcohol Uptake in C57Bl/6 Mice by Splenocytes Extracorporeally Stimulated with Vasopressin

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Experiments on C57Bl/6 mice kept under conditions of free choice between alcohol and water for 4 and 12 months showed that adoptive transfer of splenocytes extracorporeally stimulated with arginine vasopressin suppressed alcohol intake.

Key words: *alcohol; alcohol motivation; splenocytes; lymphocytes; vasopressin*

Regular long-term intake of high alcohol doses, as well as withdrawal of alcohol can result in sustained secondary immune deficiency associated with a decrease in antibody production in response to various antigens, suppression of cell-mediated immune reactions, and low resistance to infections caused by various agents [5]. Experiments with alcohol-preferring C57Bl/6 and nonpreferring CBA mice showed that adoptive transfer of splenocytes from abstinent animals increased alcohol intake in recipients [2,6]. Preincubation of splenocytes from abstinent mice with antiserotonin antibodies modifying the functions of lymphocytes and macrophages [1] abolished immune stimulation of alcohol intake in mice [2]. In light of this, the effects of lymphocytes stimulated *in vitro* with immunomodulators on alcohol motivation in animals are of special interest. Previous studies demonstrated that arginine vasopressin enhanced immune response to sheep erythrocytes in experimental animals, decreased the level of antigen-specific suppressor T-cells, and stimulated phagocytic activity of peritoneal macrophages [3,4].

This work was designed to study the effects of splenocytes *in vitro* treated with arginine vasopressin on alcohol intake in C57Bl/6 mice at various stages of the formation of alcohol dependence.

MATERIALS AND METHODS

Experiments were performed on male C57Bl/6 mice (initial body weight 20-22 g). The mice were caged in groups of five and offered free choice between alcohol (15% ethanol) and water for 4 or 12 months. Mice displaying stable alcohol intake (8-10 g/kg 96° ethanol) were taken for the experiment.

The mice were divided into groups of donors and recipients. Six hours before the experiment, donor mice were deprived of alcohol and then narcotized with ether. Spleens were removed; splenocytes were isolated, and erythrocytes were removed by osmotic shock. Splenocytes were transferred to cell culture flasks with RPMI-1640 medium supplemented with 20% calf serum and antibiotics (penicillin and streptomycin, 100 U/ml each). The cells were incubated with arginine vasopressin (10^{-7} M, Sigma) at 37°C for 1 h and then washed two times by centrifugation at 1000 rpm in RPMI-1640 medium. Cell viability was evaluated by trypan blue exclusion. Suspensions containing no less than 95% viable cells were used for experiments.

Two experimental series were performed. In series I, recipient mice were offered free choice between water and 15% alcohol for 4 months. Before the experiment, the mice were divided into three groups (30 animals per each). Group 1 mice were injected with arginine vasopressin-stimulated splenocytes from alcohol-treated mice in a dose of 2×10^7 cells in 0.4 ml physiological saline; group 2 received intraperitoneal injections of arginine vasopressin-stimulated splenocytes from healthy mice of the same age in a dose of 2×10^7 cells in 0.4 ml physiological saline; group 3 received 0.4 ml physiological saline. Drinking behavior was recorded for 1 month under conditions of free choice between 15% alcohol and water. Series II was

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performed according to the same protocol except for a longer period (12 months) free-choice. In addition, group 4 mice injected intraperitoneally with arginine vasopressin in a dose of 0.1 μg/kg, which is equivalent to 10^{-7} M, served as positive control. The time course of alcohol consumption in the free-choice paradigm was recorded in all 4 groups.

The data were analyzed statistically by Student's *t* test using Statgraphics software.

RESULTS

In series I, the adoptive transfer of arginine vasopressin-stimulated splenocytes strongly inhibited alcohol intake in recipients (Fig 1, *a*). The maximum decrease in alcohol intake was observed on day 3 after lymphocyte transfer; this parameter returned to the control level on day 7, then significantly decreased again and remained at this low level on day 21. On day 30, alcohol intake in test groups did not differ from that in the control group. Similar results were obtained in series II. Adoptive transfer of lymphocytes *in vitro* stimulated with arginine vasopressin strongly inhibited alcohol intake for 21 days (Fig 1, *b*), whereas single intraperitoneal injection of arginine vasopressin did not decreased alcohol consumption in the free choice paradigm (Fig 1, *b*).

These results suggest that injection of *in vitro* stimulated lymphocytes isolated from mice subjected to long-term alcohol treatment suppressed alcohol motivation in recipient mice. Our previous studies showed an increase in alcohol intake after adoptive transfer of lymphocytes from abstinent mice [6]. Taken together, these data suggest that lymphocytes are involved in modulation of alcohol motivation. Long-term alcohol consumption and withdrawal cause functional changes in lymphocytes. They produce cytokines possessing neuromodulatory activity and affecting alcohol motivation [5]. This assumption is consistent with the increases in alcohol intake in mice injected with serum from abstinent mice [6]. Adoptive transfer of lymphocytes stimulating alcohol intake after incubation with immunomodulator arginine vasopressin considerably suppressed strong alcohol motivation in alcohol-dependent mice, probably due to normalization of cell-cell interactions in the immune system and discontinuation of the production of factors potentiating alcohol dependence. This can be associated with transfer of a membrane-associated neuropeptide. Systemic administration of arginine vasopressin is known to decrease alcohol intake in alcoholic monkeys [7]. In our experiments, the dose used for systemic administration was insufficient for modulation of alcohol motivation.

These data can be used in developing adoptive immunotherapy of alcoholism.

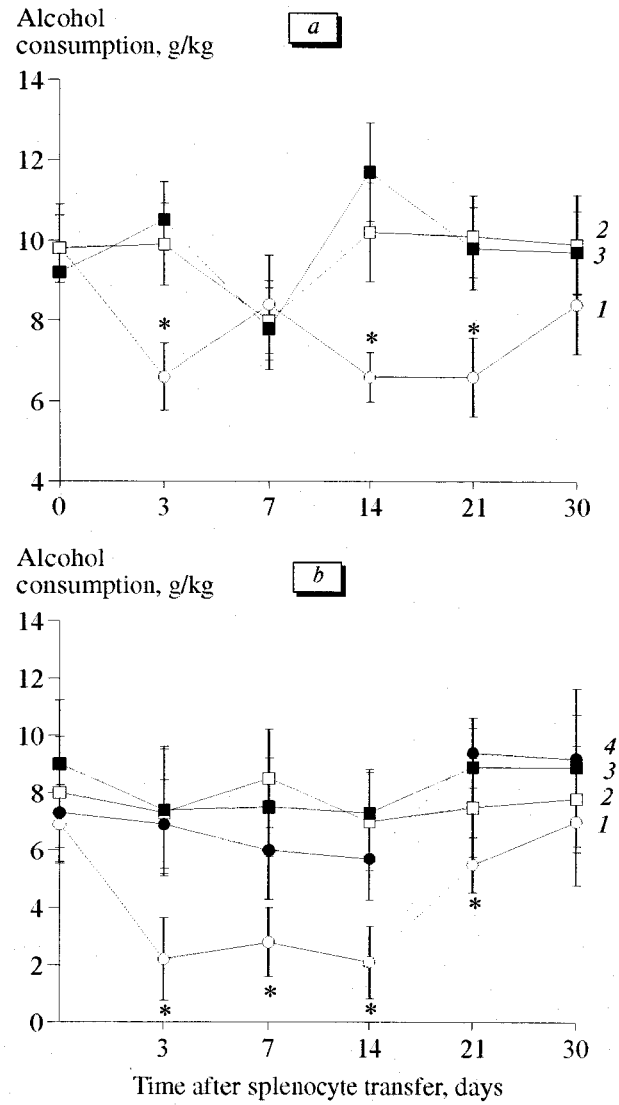

Fig. 1. Effect of adoptive transfer of splenocytes *in vitro* stimulated with arginine vasopressin on alcohol consumption in C57Bl/6 mice subjected to 4- (*a*) and 12- months (*b*) alcoholization. *1*) splenocytes from alcohol-treated mice; *2*) splenocytes from normal mice; *3*) control (0.9% NaCl); *4*) vasopressin. *$p<0.05$ compared to the control.

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