Adoptive Immunotherapy of Alcoholism in C57Bl/6 Mice with Splenocytes Extracorporeally Stimulated with Reaferon

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Experiments on C57Bl/6 mice maintained under conditions of free-choice between ethanol and water for 12 months showed that adoptive transfer of splenocytes extracorporeally stimulated with reaferon suppressed alcohol consumption within 1 month.

Key Words: *alcohol; alcohol preference; splenocytes; lymphocytes; reaferon; adoptive immunotherapy*

Experiments on different mouse strains characterized by high and low alcohol preference showed that adoptive transfer of lymphocytes from abstinent animals stimulated alcohol consumption [2,4]. Preincubation of lymphocytes from abstinent animals with antibodies against serotonin or arginine-vasopressin not only abolished the increase in ethanol consumption caused by "abstinent" lymphocytes, but also decreased this parameter to the baseline level [2,3].

In this context of particular interest is the possibility of using lymphocytes extracorporeally stimulated with immunomodulators for adoptive immunotherapy of alcoholism. Reaferon (preparation of interferon-α with dual neuroimmunomodulatory activity) was used as an immunomodulator for extracorporeal stimulation of lymphocytes. Interferon-α produced by lymphocytes modulates cell-cell interactions in the immune system, stimulates the immune response [8], and suppresses morphine and alcohol dependence by affecting the opiate system in the brain [1,5,7].

Here we studied the effects of adoptive immunotherapy with splenocytes extracorporeally stimulated with reaferon and systemic administration of reaferon on ethanol consumption by C57Bl/6 mice with experimental alcoholism.

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MATERIALS AND METHODS

Experiments were performed on male C57Bl/6 mice weighing 18-20 g. The animals were maintained under conditions of free choice between 15% ethanol and water for 12 months.

Some animals were used as donors, and others served as recipients. Six hours before the experiment the donor mice were placed in cages with free access to water. The animals were euthanized with ether, the spleens were removed, and splenocytes suspension was prepared. Erythrocytes were removed by osmotic shock.

Splenocytes were placed in cell culture flasks with RPMI-1640 medium supplemented with 20% fetal bovine serum, 100 U/ml penicillin, and 100 U/m streptomycin. Reaferon (1000 U, Olaina) was added to the splenocytes suspension. The cells were incubated in a thermostat at 37°C for 1 h. Lymphocytes were washed 2 times by centrifugation in RPMI-1640 medium at 1000 rpm. Lymphocyte viability was estimated by the trypan blue exclusion test. The experiments were carried out with suspensions containing not less than 95% viable cells.

Recipient mice were maintained under conditions of free choice between 15% ethanol and water for 12 months. The animals were divided into 3 groups. Group 1 mice received adoptive immunotherapy with splenocytes extracorporeally stimulated with reaferon (2×10^7 cells/0.4 ml physiological saline). Group 2 mice were

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intraperitoneally injected with reaferon in a dose of 1000 U in 0.4 ml physiological saline. Group 3 mice received 0.4 ml physiological saline. Alcohol consumption was recorded for 1 months in a free choice paradigm (15% ethanol and water).

The data were analyzed by Student's *t* test (Statgraphics).

RESULTS

Over the last 6 months of alcoholization the mice consumed considerable amounts of ethanol (more than 10.5 g 96% ethanol/kg, Fig. 1). Adoptive transfer of reaferon-stimulated splenocytes markedly decreased alcohol consumption in recipient animals (Table 1). Alcohol consumption progressively decreased starting from day 1 and dropped to a minimum on days 15-18. Thirty days after adoptive transfer of reaferon-stimulated splenocytes alcohol consumption in the recipient mice did not differ from that in controls.

After systemic administration of reaferon alcohol consumption decreased for 7 days, but then this parameter did not differ from the control. On days 1-4 and 15-18 after adoptive transfer of lymphocytes extracorporeally stimulated with reaferon alcohol consumption in mice was far lower than in animals injected with reaferon.

Thus, splenocytes extracorporeally stimulated with reaferon reduced alcohol consumption in C57Bl/6 mice with experimental alcoholism. Systemic administration of reaferon was less effective than adoptive immunotherapy with reaferon-stimulated lymphocytes.

Long-term alcoholization followed by alcohol withdrawal leads to sustained disorders in the immune system related to functional disturbances in lymphocytes and production of lymphocytic factors modulating alcohol addiction.

TABLE 1. Consumption of 96% Ethanol (g/kg/day) in C57Bl/6 Mice after 12-Month Alcoholization and Adoptive Immunotherapy with Reaferon ($M\pm m$, n=16)

Days after adoptive immunotherapy	Control	Reaferon	Reaferon-stimulated splenocytes
1-4	13.1±2.4	6.1±0.1**	4.25±0.80**+
5-7	14.3±2.0	7.0±1.1**	6.1±0.3**
8-10	8.5±1.2	6.1±1.5	3.3±0.7**
11-14	6.8±1.5	6.0±1.5	4.8±0.8
15-18	7.25±1.20	6.1±1.6	1.3±0.8*+
19-21	8.3±0.7	6.3±0.3	4.3±2.0
28-30	12.8±1.0	11.8±0.5	9.8±1.2

Note. *p<0.001 and **p<0.05 compared to the control; +p<0.05 compared to reaferon.

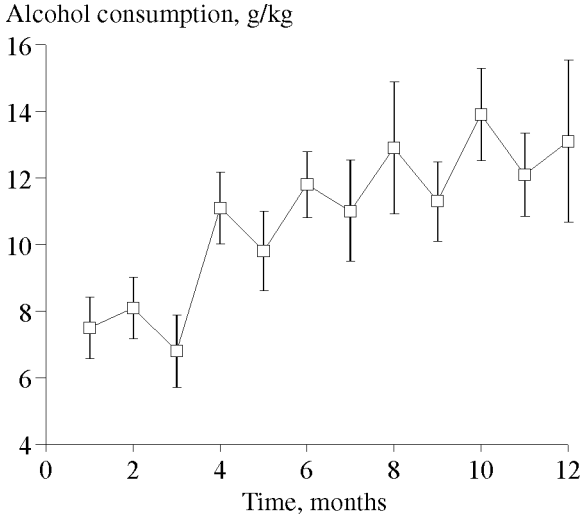

Fig. 1. Consumption of 96% ethanol by C57Bl/6 mice.

Previous studies showed that adoptive transfer of lymphocytes from abstinent animals increased alcohol consumption, which confirmed the involvement of lymphocytes in the formation of alcohol dependence [2,4]. Incubation of lymphocytes with immunomodulator reaferon or arginine-vasopressin [3] probably normalizes cell-cell interactions in the immune system and suppresses production of factors enhancing alcohol dependence.

These findings suggest that reaferon modulates alcohol dependence not only via opiate receptors in the brain [1,5,6], but also by acting on cells of the immune system and normalizing the state of lymphocytes stimulating alcohol consumption.

REFERENCES

1. A. M. Balashov, O. B. Petrichenko, T. N. Alyab'eva, and L. F. Panchenko, *Vopr. Med. Khimii*, No. 1, 34-38 (1993).
2. T. V. Davydova, V. A. Evseev, V. G. Fomina, *et al*., *Byull. Eksp. Biol. Med*., **126**, No. 9, 328-330 (1998).
3. T. V. Davydova, V. A. Evseev, V. G. Fomina, and O. I. Mikovskaya, *Ibid*., **129**, No. 6, 617-619 (2000).
4. K. D. Pletsityi, T. V. Davydova, and V. G. Fomina, *Vopr. Med. Khimii*, No. 6, 48-50 (1995).
5. L. F. Panchenko, N. N. Terebilina, V. V. Malinovskaya, *et al*., *Ibid*., No. 3, 71-73 (1990).
6. J. E. Blalock and E. M. Smith, *Biochem. Biophys. Res. Commun*., **101**, 472-478 (1981).
7. N. Dafny, M. Zielinski, C. Reyes-Vasques, *Neuropeptides*, **3**, 453-463 (1983).
8. P. Lengyel, *Annu. Rev. Biochem*., **51**, 251-282 (1982).