# Mechanisms of Immune Status Disorders in Chronic Ethanol Intoxication

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Experiments of outbred albino rats showed that chronic ethanol intoxication (20 days, summary dose 5 $LD_{50}$) inhibited immune reactions mainly mediated by Th1-cells, increased blood corticosterone concentration, reduced T-lymphocyte acetylcholinesterase activity, blood concentrations of IFN-γ, IL-2, IL-4, IL-10, and increased IL-6 level.

**Key Words:** *ethanol; cytokines; Th1-, Th2-lymphocytes; corticosterone; acetylcholinesterase*

The consumption of ethyl alcohol in Russia is constantly increasing [1,2,8]. The factors determining the severity and unique status of ethanol situation in Russia are high level of ethanol consumption, consumption of ethanol in the form of strong beverages (primarily vodka) in high single doses, and highly toxic false products and surrogate alcohol [3]. The increase of ethanol consumption is the leading factor responsible for the demographic crisis in Russia. By the incidence of lethal outcomes ethanol intoxication ranks first among intoxications by other chemicals and is characterized by great severity and high mortality [1-3]. Acute ethanol intoxication takes place, as a rule, after its consumption throughout several days or in the presence of chronic alcoholism. The mortality after acute and chronic ethanol intoxication (CEI) can be caused by infectious complications resultant from reduction of the immune status [2]. Ethanol effect on the immunity system is poorly studied [9,13-15]. The knowledge of the immunopathogenesis of ethanol effects in CEI is essential for effective drug correction of the immune status in order to prevent infectious complications and diseases [2,11].

We studied the functions of Th1- and Th2-lymphocytes, blood levels of corticosterone (CS), IFN-γ, IL-2, IL-4, IL-6, IL-10 cytokines, and T-lymphocyte acetylcholinesterase (ACE) in CEI.

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## MATERIALS AND METHODS

Experiments were carried out on outbred albino rats of both genders (180-240 g). The animals received ethanol daily orally in 40% water solution in a dose of 0.25 $LD_{50}$ for 20 days (total dose 5 $LD_{50}$), ethanol $LD_{50}$ being 12.3±1.3 g/kg. Controls received the same volume of water orally. Immunity parameters were evaluated by common methods used in experimental immunotoxicology and immunology [2,4]. Humoral immune reaction to thymus-dependent antigen (sheep erythrocytes), characterizing the capacity of Th1-lymphocytes to participate in the production of IgM by plasma cells [10], was evaluated by the count of antibody-producing cells in the spleen 4 days after immunization (peak of IgM production), which was carried out intraperitoneally in a dose of $2\times10^8$ on day 16 after the first dose of ethanol. The function of Th1-lymphocytes was evaluated by the delayed-type hypersensitivity reaction, evaluated by the increment of the hind paw weight (in %). The resolving dose of sheep erythrocytes ($5\times10^8$) was injected under the hind paw aponeurosis 4 days after intraperitoneal immunization on day 16 after the first dose of ethanol. Delayed-type hypersensitivity reaction was evaluated after 24 h. The function of Th2-lymphocytes was evaluated by the count of antibody-producing cells, synthesizing IgG to sheep erythrocytes, in the spleen 7 days after immunization by indirect local hemolysis in gel [4]. The rats were immunized intraperitone-

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**TABLE 1.** Effects of CEI on the Functions of Th1- and Th2-Lymphocytes in Rats ($M\pm m$; $n$=10-12)

| Parameter | | Control | CEI |
|---|---|---|---|
| Function of Th1-lymphocytes | count of cells producing IgM to sheep erythrocytes, $\times 10^3$ | 40.3±3.8 | 12.7±1.4* |
| | delayed-type hypersensitivity reaction, % | 35.9±3.5 | 15.0±1.6* |
| Function of Th2 lymphocytes | count of cells producing IgM to sheep erythrocytes, $\times 10^3$ | 22.4±2.3 | 11.4±1.4* |

**Note.** Here and in Table 2 and 3: *$p$<0.05 compared to the control.

ally in a dose of $2\times10^8$ cells on day 14 after the first ethanol dose.

Plasma concentrations of cytokines IFN-γ, IL-2, IL-4, IL-6, and IL-10 were measured 20 days after the first ethanol dose by ELISA using ELISA Kits (BioSource Int.).

Activity of ACE in T-lymphocytes and blood level of CS were measured 20 days after intoxication [2]. A μmol of acetylcholine, hydrolyzed within 1 min in 1 ml suspension, containing $10^9$ T-lymphocytes, was taken for a unit of ACE activity.

The data were statistically processed using Student's $t$ test.

## RESULTS

Chronic ethanol intoxication for 20 days impaired humoral immune response to T-dependent antigen (judging from the count of antibody-producing cells in the spleen), characterizing the synthesis of IgM by B-cells and the function of Th1-lymphocytes, 3.17 times in comparison with the control level (Table 1). The formation of delayed-type hypersensitivity reaction (Th1-cell function) decreased 2.39 times and the function of Th2-lymphocytes (evaluated by the count of cells producing IgG to sheep erythrocytes) decreased 1.96 times in CEI in comparison with the control.

Chronic ethanol intoxication reduced the parameters characterizing the immune reactions and the function of Th1-lymphocytes, linked with them, 2.78 times, while the parameter characterizing the function of Th2-cells decreased 1.96 times. These data indicated that CEI inhibited the function of Th1-lymphocytes greater than of Th2 cells.

Chronic ethanol intoxication led to an increase of plasma CS level (2.58 times; $p$<0.05). Activity of ACE in T-lymphocytes isolated from rat spleens after CEI decreased 1.3 times ($p$<0.05; Table 2). The reduction of ACE level in T-lymphocytes was presumably caused by reduction of cholinacetylase activity by ethanol and its metabolite acetaldehyde [7].

The results indicated that activity of ACE in T-cells in CEI was directly related to the immune reaction values, while the relationship between these reactions and blood CS concentration was inverse.

Increase of blood CS level under the effect of ethanol was caused by realization of the common adaptation syndrome (increase of ACTH production by the pituitary and of blood concentration of corticosteroids during the resistance stage) [12]. A more pronounced reduction of Th1 activities in comparison with Th2-lymphocytes in CEI was presumably due to a lasting elevation of CS concentration in the blood, to which Th1-lymphocytes were more sensitive under conditions of 20-day ethanol effect [4].

Inhibition of T-cell ACE with various toxicants and elevation of blood CS concentration are factors causing reduction of immune reactions [2]. Ethanol damage to T-lymphocytes and other cells participating in the immune response seemed to be also a result of disorders in their functions because of reactions of a highly toxic product of ethanol biotransformation – acetaldehyde – with the enzymes' sulfhydryl and amino groups, membranotoxic effect of ethanol and its metabolite, LPO initiation, inhibition of tissue respiration and oxidative phosphorylation [2,7].

Plasma levels of IFN-γ and IL-4 decreased in CEI 3.51 and 2.46 times, respectively (Table 3). Reduction of the IFN-γ/IL-4 proportion in CEI in comparison with the control indicated greater suppression of Th1-lymphocyte activity in comparison with Th2-cell function [5] and confirmed the data obtained in studies of the immune reactions (Table 1).

Plasma levels of IL-2 and IL-10 decreased 2.94 and 1.87 times, respectively, in CEI, while the concentration of IL-6 increased 1.64 times (Table 3). Reduction of IL-2 level in the plasma under the effect of eth-

**TABLE 2.** Effects of CEI on Splenic T-Lymphocyte ACE Activity and Plasma CS Concentration 20 Days after Intoxication ($M\pm m$; $n$=12)

| Parameter | Control | CEI |
|---|---|---|
| CS, ng/ml | 19.5±2.0 | 50.3±5.6* |
| Activity of ACE, U/$10^9$ T-cells | 57.1±4.8 | 44.0±4.0* |

**TABLE 3.** Effects of CEI on Plasma Cytokine Levels, pg/ml (*M*±*m*; *n*=7)

| Cytokines | Control | CEI |
|---|---|---|
| IFN-γ | 931±94 | 265±27* |
| IL-4 | 121±13 | 49±6* |
| IFN-γ/IL-4 | 7.7±0.8 | 5.4±0.6* |
| IL-2 | 1267±118 | 431±44* |
| IL-6 | 52±6 | 85±9* |
| IL-10 | 342±37 | 183±20* |

anol indicated suppression of this cytokine production by T-lymphocytes (Th0-type, cytotoxic T-cells), reduction of T- and B-cell proliferation, and of natural killer cell activity [4,6,10]. Increase of blood level of IL-6 (proinflammatory cytokine) presumably indicated its high production by macrophages, monocytes, and lymphoid dendritic cells in the liver as a result of ethanol damage to the liver (realization of the inflammatory process) [2,4,6,9,10]. Reduction of plasma concentration of IL-10 (anti-inflammatory cytokine) indicated ethanol reduction of the functions of Th0-, Th2- lymphocytes, monocytes, macrophages, and B-cells. This cytokine reduced IFN-γ release by Th1-lymphocytes [4,10]. A less pronounced reduction of IL-10 production in CEI in comparison with IFN-γ production confirmed a greater destructive effect of ethanol, lasting throughout 20 days, towards Th1-lymphocytes. A lesser reduction of IL-10 level in CEI was presumably due to a significant reduction of IFN-γ production by ethanol, this, presumably, ruling out the regulatory elevation of the production of IL-10 (stimulating the suppression of Th1-lympohocyte function) by Th0-, Th2-lymphocytes, monocytes, macrophages, and B-cells.

Hence, CEI caused more pronounced suppression of Th1-lymphocyte activity in comparison with Th2-cell function reduction, increased blood concentrations of CS, reduced ACE activity of T-lymphocytes, blood concentrations of cytokines IFN-γ, IL-2, IL-4, and IL-10, and increased blood level of IL-6.

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