

Original article

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Changes in Th1- and Th2-lymphocytes function and cytokine profile in a chronic ethanol intoxication

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Abstract: In experiments in non-inbred rats, it has been established that chronic intoxication of ethanol (30 days, total dose – 6.0 LD₅₀) essentially reduces concentration of cytokines IFN γ , IL-2, IL-4, IL-10 in blood, increases concentration of IL-6, reduces an interrelation IFN γ /IL-4 in comparison with the control, suppresses of immune responses, which displays the greater lesion of Th1-cells in comparison with Th2-lymphocytes.

Keywords: ethanol, immunotoxicity, cytokines, Th1, Th2, lymphocytes.

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Introduction

Ethanol is used in medicine as an antiseptic and preservative, it is used in the preparation of tinctures and extracts in the pharmaceutical industry and at home. For technical purposes ethanol is used as a de-icer in aviation, a solvent for stains, varnishes, adhesives, etc. Analysis of the causes of the demographic crisis in Russia shows that among the leading factors responsible for this phenomenon is the growth in alcohol consumption, which has increased by 32-38% over the last 5 years, while deaths from accidental poisoning with ethanol increased by 25% and has been steadily progressing in recent years. Mortality after ethanol intoxication may be associated with infection and disease caused by reduction of the immune status. Knowledge of the immunopathogenesis of the ethanol intoxication is necessary for justification of pharmacological correction of postintoxication disorders of immune homeostasis in order to prevent various diseases, and infectious complications [1, 2].

The nature of the formation of secondary immunodeficiency states after ethanol poisoning and, therefore, the methods of correction of immune status depend on the characteristics of antigen-presenting cells lesions, populations of T-lymphocytes, B-cells [1, 3]. It is known that helper T-lymphocytes are heterogeneous and consist of Th0-, Th1-, Th2-, Th3-types [2]. Th0-lymphocytes synthesize IL-2, which stimulates proliferation of B-cells. Besides, they secrete plenty of cytokines produced by Th1-, Th2-cells (and other cells), except for IL-12. Only Th2-lymphocytes produce IL-12 [3]. Th1-cells produce γ -interferon (IFN- γ), participating in the implementation of cellular immune responses [2-4]. In addition, they provide synthesis of B-lymphocytes (plasmacytes) IgM and IgG2a [4]. Th2-lymphocytes synthesize IL-4, IL-5, IL-6, and IL-13, promote activation, proliferation and

differentiation of B-cells, plasmacytes synthesis of major classes and subclasses of immunoglobulins (IgG1, IgA1, IgA2, IgE, and IgD). In addition, IL-4 and IL-13 inhibit the production of proinflammatory cytokines by macrophages, and IL-10 produced by Th0-, Th2-cells and macrophages reduces the synthesis of cytokines, Th1-lymphocytes [2-4].

In the formation of allergic and anaphylactic reactions are also involved lymphocytes of Th1-and Th2-type. Contact (skin) allergic reactions associated with Th1-lymphocyte function, and respiratory allergic reactions associated with the activity of Th2-lymphocytes (the synthesis of IgE) [1, 3, 4]. The ratio of the activity of Th1-, Th2-type lymphocytes (the paradigm of two types of helpers - Th1/Th2 [5]) may depend on the probability of occurrence, respectively, viral or bacterial infections [6], and the formation of contact or respiratory hypersensitivity [7].

The study aimed to evaluate the immune responses that reflect the function of Th1- and Th2-lymphocytes, and cytokine profile (concentration of IFN- γ , IL-2, IL-4, IL-6 and IL-10 in blood) in the chronic ethanol intoxication.

Material and methods

Experiments were performed in non-inbred white rats of both sexes (body weight 180-240 g). The ethanol was administered per os with 40% aqueous solution at a dose of 0.2 DL₅₀ for 30 days (DL₅₀ of the ethanol was 12.3 \pm 1.3 g/kg). Control group of animals received orally an appropriate volume of water. Indicators of the immune system were evaluated by conventional methods in experimental immunology and immunotoxicology [1, 3]. Humoral immune response to thymus-dependent antigen (sheep red blood cells - SRBC), which characterizes the ability of Th1-lymphocytes

involved in the production of plasma cells IgM, determined by the number of antibody-forming cells (AFC) in the spleen at 4 days after immunization (the peak of production IgM), which was carried out abdominally on 26th day at a dose of $2 \cdot 10^8$ after the first injection of ethanol. Th1-lymphocyte function was evaluated by delayed skin reaction (DSR). Formation of DSR was examined in animals in the growth of mass of the foot of the hind paw in percentage (%). Resolving dose of SRBC ($5 \cdot 10^8$) were injected under the aponeurosis of the foot of the hind paw in 4 days after immunization, which was performed abdominally on the 26th day after the first injection of ethanol. The reaction of DSR was assessed after 24 h. Th2-lymphocyte function was studied by the number of AFC synthesizing IgG to SRBC in the spleen at the peak of production of immunoglobulin (within 14 days after immunization) by the method of indirect local hemolysis in gel [3]. When rats were immunized abdominally with this SRBC at a dose of $2 \cdot 10^8$ cells on the 17th day after the first injection of ethanol. Thus, in assessing the immune responses of all the animals received a total equilethal dose (6.0 LD₅₀) of ethanol.

The concentration of cytokine IFN- γ , IL-2, IL-4, IL-6 and IL-10 have been studied in blood plasma of rats in 30 days after the first injection of ethanol by enzyme-linked immunosorbent assay (ELISA) using kits (ELISA Kits) produced by BioSource Int. The data obtained were processed statistically using the Student's t-test.

Results

Under the influence of the ethanol within 30 days (Table 1), reduction of the humoral immune response to T-dependent antigen (the number of AFC in a spleen), which characterizes the synthesis of IgM B-cells and Th1-lymphocyte function. In comparison with the control level reduction was up to 3.04 times ($p < 0.05$). In chronic toxicity a significant suppression of DSR has been observed (Th1-cell function) to 2.96 times ($p < 0.05$), a Th2-lymphocyte function (measured by the number of AFC synthesizing IgG to SRBC) - in 2.10 times ($p < 0.05$).

The parameters of the immune response and related Th1-lymphocyte function under the action of ethanol decreased up to 3.00 times on the average, and indicators related to the function of Th2-cells – up to 2.10 times. A smaller reduction of the immune response has been determined, which provides a function of Th2-lymphocytes (and B-cells) in ethanol poisoning (the corresponding decrease in the number of AFC synthesizing IgG). These data suggest that the function of Th1-lymphocytes under the influence of chronic ethanol intoxication decreases to a greater extent compared to the suppression of the activity of Th2-lymphocytes.

The reduction of Th1-cell activity in the chronic ethanol intoxication may be due to an increase of corticosterone concentration in blood (due to toxicity of ethanol) [1]. Th1-type cells are more susceptible to corticosterone in comparison with Th2-lymphocytes [3].

During the study of cytokine concentrations in plasma of rats the decrease in IFN- γ and IL-4 after 30 days of chronic effect of ethanol up to 3.82 and 2.85 times ($p < 0.05$) has been established (Table 2). We can see that the decrease in the IFN- γ /IL-4 proportion under the influence of ethanol (5.6) is comparable with control (7.5) indicates a greater suppression ($p < 0.05$) activity of Th1-type lymphocytes compared with the function of Th2 cells [1].

The concentrations of IL-2, IL-10 after the chronic intoxication of the ethanol decreased up to 3.1 and 2.05 times respectively ($p < 0.05$), and IL-6 to 1.53 times ($p < 0.05$).

Table 1. Chronic ethanol intoxication effect on the function of Th1-and Th2-lymphocytes in rats (M \pm m, n=9-11)

Set of experiments	Function of Th1-lymphocyte		Function of Th2-lymphocyte
	AFC to SRBC (IgM), 10^3	DSR, %	AFC to SRBC (IgG), 10^3
Control	43.5 \pm 3.1	38.8 \pm 3.3	52.8 \pm 5.4
Ethanol	14.3 \pm 1.5*	13.1 \pm 1.4*	25.1 \pm 2.4*

* – $p < 0.05$ compared with control.

Table 2. Chronic ethanol intoxication effect on the content of cytokines in rat plasma, pg/ml (M \pm m, n=7)

Cytokines	Control	Ethanol
IFN- γ	1032 \pm 98	270 \pm 25*
IL-4	137 \pm 14	48 \pm 5*
IFN- γ / IL-4	7.5 \pm 0.7	5.6 \pm 0.6
IL-2	1369 \pm 98	442 \pm 32*
IL-6	58 \pm 7	89 \pm 8*
IL-10	383 \pm 39	205 \pm 19*

* – $p < 0.05$ compared with control.

Discussion

Reduction of IL-2 in blood plasma under the influence of ethanol indicates the suppression of its production by T-lymphocytes (both CD4, lymphocytes belonging to the Th0-like) and some CD8, reduction of proliferation of T-and B-cells (J-chain synthesis of molecules of the immunoglobulin), the activity of natural killer cells (NK cells) [2, 3].

The decrease of IL-6 in blood (proinflammatory cytokine) represents a decrease of its production by macrophages and lymphoid dendritic cells in the pathogen insertion focus as well as a suppression of B-cells activation [2-4].

The concentration of IL-10 (this anti-inflammatory cytokine is produced by Th0-, Th2-lymphocytes, monocytes, macrophages and B-cells, it also decreases IFN- γ and Th1-lymphocytes secretion [4, 8]) decreased with chronic ethanol intoxication. This effect is typical for heavy metals [9], dinitrochlorobenzene, formaldehyde and other toxins [10]. Reduced synthesis of IL-10 is less than IFN- γ . It confirms the established injurious ethanol effect on Th1-lymphocytes. A relatively small reduction of IL-10 is probably due to a significant decrease in the synthesis of IFN- γ caused by ethanol. This probably is to exclude the regulatory increase Th0-, Th2-lymphocytes, monocytes, macrophages and B-cell production of IL-10, which can enhance the suppression of Th1-lymphocytes function much.

Conclusion

Chronic ethanol effect causes more Th1-cells damage compared with Th2-lymphocytes and reduces the ratio IFN- γ /IL-4 compared with controls.

Chronic ethanol intoxication significantly reduces the blood concentration of cytokines IFN- γ , IL-2, IL-4, IL-10 in blood and less level of IL-6 increases.

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