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Research Article

The Effect of Ethylene Trichloride Chronic Intoxication on Immune Responses, Function of Th1 and Th2 Lymphocytes and Blood Cytokine Concentrations

Pavel Franzevich Zabrodskii*

Saratov Medical University "REAVIZ", Saratov, Russia

***Corresponding Author:** Pavel Franzevich Zabrodskii, Saratov Medical University "REAVIZ", Saratov, Russia.

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Abstract

Experiments on random-bred albino rats showed that that ethylene trichloride chronic intoxication (30 days daily subcutaneous 0,05 LD_{50}) largely decreased Th2 cell activity in comparison with Th1 lymphocytes as well as humoral immune responses compared to the cellular immune response. Ethylene trichloride decreased blood levels of immunoregulatory cytokines IFN-γ, IL-2, IL-4 and increased the concentration of the proinflammatory cytokine IL-6, raised the blood levels of anti-inflammatory cytokines IL-10, IL-13 insignificantly.

Keywords: Ethylene Trichloride; Th1, Th2 Lymphocytes; Immunotoxicity; Cytokines

Introduction

Ethylene trichloride (ETC, trichlorethylene, 1,1,2-trichlorethylene, 1-chloro-2,2-dichloroethylene, 1-chloro-2,2-dichloroethylene, ethylene trichloride, trilene) is a clear oily and volatile liquid with an aromatic odor (chloroform odor), used in the production of refrigerants, various acids, herbicides. ETC is used in industry as a solvent for fats, resins, and rubber for cleaning metal parts and products, for dry cleaning clothes. ETC is a powerful drug [1-4]. ETC can enter the body through the digestive tract, the respiratory tract, is allergic [2,5], mutagenic, carcinogenic [2,3,5,7,8], affects the kidneys [2,4-6] and the liver [2,9,10], causes autoimmune diseases [2,9,11], has an immunotoxic [1,2,11,12] and psychotropic action [1]. Particular danger of ETC may be in production during emergency situations, when, due to its high volatility, a large number of people may be exposed to inhalation poisoning [1]. Dysfunctions of the immune system, in particular, the functions of Th1 and Th2 lymphocytes and the synthesis of cytokines by them and other blood cells, are poisoned with ETC for the purpose of their targeted correction for the prevention of allergic, infectious, oncological and other diseases that have not been studied [1,2,9,11-13].

Aim of the study

The aim of the study was to evaluate the ethylene trichloride chronic intoxication (daily administration of ETC in a dose of 0.05 DL_{50} for 30 days) on immune responses, Th1 and Th2 lymphocyte functions, as well as on blood levels of immunoregulatory, proinflammatory and anti-inflammatory cytokines (γ - interferon - IFN-γ, interleukin-2 - IL-2, IL-4, IL-6, IL-10 and IL-13).

Materials and Methods

Experiments were performed on random-bred albino rats of both sexes weighing 180-240 g. ETC (Sigma-Aldrich) was administered subcutaneously daily for 30 days at a dose of 0.05 LD_{50} (total dose 1.5 LD_{50}). LD_{50} ETC for rats after subcutaneous administration was 4.9 ± 0.4 g/kg. Parameters of the immunity system were assessed by generally accepted methods in experimental immunotoxicology and immunology [1,2,14] after chronic intoxication with ETC 30 days after the first injection of the toxicant.

The humoral immune response to the T-dependent antigen (red sheep blood cells- RSBC), was determined by the number of antibody-forming cells (AFC) in the spleen 4 days after immunization (peak IgM production), which was administered intraperitoneally at a dose of 2×10^8 for 26 days after the first injection of ETC. Similarly, we evaluated the humoral immune response to a T-independent typhoid vi-antigen (Vi-Ag), reflecting the function of B cells and the synthesis of IgM by plasma cells of the rat spleen. In this case, Vi-Ag rats were immunized at a dose of 8 μg/kg [1,2].

The function of Th1 lymphocytes was determined by a delayed-type hypersensitivity (DTH) reaction. DTH was studied in animals by weight gain of the hind paw foot in %. The resolving dose of RSBC (5×10^8) was administered under the aponeurosis of the foot of the hind paw 4 days after immunization, which was performed intraperitoneally for 26 days after the first injection of ETC. The reaction of DTH was evaluated after 1 day [1,2]. The function of Th2 lymphocytes was investigated by the number of AFC, synthesizing IgG to RSBC, in the spleen at the peak of production of this immunoglobulin (14 days after immunization) by indirect local hemolysis in the gel [14]. At the same time, rats were immunized intraperitoneally with RSBC at a dose of 2×10^8 cells for 16 days after the first administration of ETC.

Evaluation of the activity of natural killer cells (NK) and antibody-dependent cellular cytotoxicity (ADCC), was performed by the spectrophotometric method 30 days after the first injection of ETC [1,2]. At the same time, to determine the ADCC, the RSBC immunization (10^8 cells) was carried out 4 days before the last ETC injection (at 26 days).

The function of Th1 and Th2 lymphocytes was also assessed by the concentration of immunoregulatory cytokines IFN-γ (#MBS824935) and IL-4 (#MBS2883072), respectively, in the blood plasma of rats 30 days after the first injection of ETC by enzyme immunosorbent assay (ELISA) using kits (ELISA Kits MyBioSoure) in accordance with the manufacturer's instructions. Similarly, the concentration of immunoregulatory cytokine IL-2 (#MBS2885949), pro-inflammatory cytokine IL-6 (#MBS2885203) and anti-inflammatory cytokines IL-10 (#MBS2087187), IL-13 (#MBS495243) [1,2,15] in the rat blood plasma 30 days after the first injection of ETC was determined. Blood for research was taken from the retroorbital venous sinus. The data obtained were processed statistically using the Student's t-test. Differences between the parameters were considered reliable at $p<0.05$.

Results

ETC chronic intoxication with the daily administration of toxicant at dose of 0.05 LD_{50} caused a decrease in the humoral immune response to T-dependent (AFC to RSBC, IgM), T-independent (AFC to Vi-Ag, IgM) antigens, NK and ADCC activities, respectively, in 1.52; 1.65; 1.33 and 1.41 times ($p<0.05$). The data obtained suggest that cellular immune responses to chronic exposure to ETC are less affected than the humoral immune response (table 1).

Parameters	Control	Ethylene trichloride
AFC to RSBC (IgM), 10^3	44,3±4,5	29,1±3,1*
AFC to Vi-Ag (IgM), 10^3	31,3±3,2	18,8±2,0*
Activity NK, %	25,5±2,7	19,2±1,8*
ADCC, %	13,0±1,4	9,2±1,0*

Table 1: Effect of chronic ethylene trichloride intoxication for 30 days (daily dose - 0.05 LD_{50}) on humoral and cellular immune responses of rats (M ± m, n = 7-11)

* -p <0,05 as compared to control

After ETC chronic intoxication (table 2), there was a reduction in the function of Th1 and Th2 lymphocytes, assessed, respectively, by the DTH reaction and the humoral immune response (the number of AFC to RSBC, IgG) in 1.47 and 1.79 times ($p<0,05$). This suggests that under the influence of ETC, the function of Th2 lymphocytes (as well as the production of IgG of plasma B cells associated with it) is affected to a greater extent than the effect of ETC on Th1 lymphocytes.

Series of experiments	Th1 lymphocyte function	Th2 lymphocyte function
	DTH reaction %	AFC to RSBC, (IgG), 10^3
Control	37,0 ± 3,5	54,3 ± 5,6
Ethylene trichloride	25,1 ± 2,6*	30,3 ± 3,3

Table 2: Effect ethylene trichloride chronic intoxication for 30 days (daily dose - 0.05 DL_{50}) on the function of Th1 and Th2 lymphocytes of rats (M ± m, n = 7-11)

* -p <0,05 as compared to control.

ETC chronic intoxication caused a significant decrease ($p<0.05$) in the blood of rats, the concentrations of IFN-γ (immunoregulatory cytokine) and IL-4 (immunoregulatory and, in certain cases, anti-inflammatory cytokine) [15] respectively in 1.95 and 2,74 times ($p<0.05$) times (Table 3). The ratio of IFNγ/IL-4 in the control was 7.8 ± 0.6, and after the action of ETC - 10.9 ± 0.7. An increase in this ratio [1,2] confirms the suppression of the activity of Th2 lymphocytes to a greater extent than Th2 cells when exposed to ETC.

Cytokines	Control	Ethylene trichloride
IFN-γ	917 ± 98	470 ± 50*
IL-2	1100 ± 115	582 ± 65*
IL-4	118 ± 12	43 ± 6*
IFN-γ /IL-4	7,8 ± 0,6	10,9 ± 0,7*
IL-6	74 ± 8	102 ± 10*
IL-10	468 ± 48	603 ± 62
IL-13	125 ± 13	167 ± 17

Table 3: Effect of ethylene trichloride chronic intoxication for 30 days (daily dose - 0.05 DL_{50}) on the content of cytokines in rats blood, pg / ml (M ± m, n = 8)

* -p <0,05 as compared to control

After ETC chronic intoxication (table 3), the concentration in the blood of IL-2 (immunoregulatory cytokine) decreased in 1.89 times ($p<0.05$), the content of IL-6 (pro-inflammatory cytokine), IL-10 and IL-13 increased (anti-inflammatory cytokines), respectively, in 1.38 ($p <0.05$), 1.29 ($p>0.05$) and 1.34 times ($p>0.05$).

Discussion

The data obtained suggest that a decrease in the activity of Th2 cells compared to Th1 lymphocytes may lead to the development of a microbial infection (the main protective role is played by Th2 lymphocytes and associated IgG-synthesizing of plasma B cells) with a higher probability than a viral infection (the main protective the role along with other cells, NK and ADCC belongs to Th1 lymphocytes) [1,2,14,16].

The reduction in blood concentrations of IFN-γ is due to ETC damage of Th1-lymphocytes, as well as EKK, cells carrying out ASCC, cytotoxic T-lymphocytes [17]. A decrease in blood after chronic intoxication with ETC IL-2 indicates suppression of its production by T-cells, including Th0 and Th1-type lymphocytes, a decrease in T-cell and B-cell proliferation, and NK and ADCC activity [18]. The decrease in IL-4 in the blood is due to the defeat of ETC mainly by Th2 lymphocytes [1,2,14,15], and an increase in IL-6 characterizes the activation of its synthesis by macrophages, monocytes [19] associated with inflammatory changes in the liver and kidneys due to their damage ETC and its metabolic products [5,6,9,10], many of which are more toxic than ETC (dichloroacetyl chloride, dichloroacetic acid, trichloroacetaldehyde, trichloroacetic acid, trichloroethanol, N- (hydroxyacetyl) ethanolamine, trichloroethanediol, oxalic acid) [1,2,7].

Statistically insignificant increase in the blood of the anti-inflammatory cytokine IL-10 and IL-13 under the influence of ETC is apparently due to the compensatory response of CD4+CD25+Foxp3+ regulatory T-cells to the defeat of ETC of lymphocytes, monocytes and macrophages [20,21,22]. Increase of IL-13 can disrupt the modulation of allergic reactions by this cytokine, as well as apoptosis or growth of tumor cells [22].

Thus, the immunotoxic effect of ETC is accompanied by a decrease in humoral and cellular immune responses, impaired cytokine production by lymphocytes and other blood cells. These changes are due to the immunotoxic effect of the poison and its metabolites. The mechanisms of dysfunction of the cells of the immune system after chronic intoxication with ETC may include initiation of lipid peroxidation of immunocyte membranes, damage to the genome and inhibition of their various enzymes, proton-form separation of tissue respiration and oxidative phosphorylation, etc. [1,2,11].

Conclusion

1. Chronic ETC intoxication for 30 days with a daily dose of 0.05 LD_{50} reduces the activity of Th2 cells to a greater extent than Th1 lymphocytes, as well as humoral immune responses as compared to the cellular immune response.
2. After chronic ETC intoxication the content of cytokines IFN-γ, IL-2, IL-4 decreased, the concentration of IL-6 increased, the content of IL-10, IL-13 increased slightly in the blood.

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