Changes in the Cytokine Profile and Reduced Function of Lymphocyte Subpopulations in Subacute Tetrachloromethane Poisoning

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Experiments on outbred albino rats showed that subacute poisoning with tetrachloromethane in a total dose of 1.0 LD_{50} appreciably decreased blood concentrations of cytokines (IFN-γ, IL-2, IL-4, IL-6, IL-10), reduced the IFN-γ/IL-4 ratio in comparison with the control, and suppressed the immune reactions, which attests to greater damage to Th1 compared to Th2 lymphocytes.

Key Words: *tetrachloromethane; immunotoxicity; cytokines; Th1, Th2 lymphocytes*

Tetrachloromethane (TCM, carbon tetrachloride, perchloromethane, Freon-10, chladon-10) is widely used in industry as the solvent for oil, fat, rubber, bitumen, and resin, as an agent for cloth cleaning and degreasing under industrial conditions and at home. Intoxication is often caused by oral TCM intake. Inhalation poisoning can be a result of neglected safety regulations and cleaning of clothes in small poorly aired rooms. In clinical toxicology, TCM intoxication is responsible for up to 60% cases of toxic liver damage [1]. Mortality (30% in oral intoxication and 15-20% in inhalations) can be related to secondary immunodeficiency [2,3]. Damage inflicted to the antigen-presenting cells, T-lymphocyte populations, and B cells by TCM determine the pattern of secondary immunodeficiency formation and hence, the methods for correction of immune disorders [2,3]. It is known that T-helpers are heterogeneous and include Th0, Th1, Th2, and Th3 lymphocytes [6]. Th0 lymphocytes produce IL-2 stimulating proliferation of B cells. In addition, numerous cytokines are produced by Th1, Th2, and other cells [4]. The Th1 cells produce IFN-γ, participating in the realization of cellular immune reactions [4,6,10]. They also participate in the production of IgM and IgG2a by B-lymphocytes (plasmacytes) [10]. By synthesizing IL-4, IL-5, IL-6, and IL-13, Th2 lymphocytes promote activation, proliferation, and differentiation of B cells, synthesis of the major immunoglobulin classes and subclasses (IgG1-4, IgA1, IgA2, IgE, and IgD) by plasmacytes. In addition, IL-4 and IL-13 inhibit the production of proinflammatory cytokines by macrophages, while IL-10, produced by Th0, Th2, and macrophages, reduces cytokine synthesis by Th1 lymphocytes [4,6,10].

The Th1 and Th2 lymphocytes are involved in the formation of allergic reactions. 3-ntact (skin) allergic reactions are linked with the Th1 lymphocyte function, while the respiratory ones are associated with activity of Th2 lymphocytes (IgE synthesis) [3,4,14]. The Th1/Th2 lymphocyte ratio (Th1/Th2 paradigm of two helper types [13]) determines the probability of viral or bacterial infections, respectively [7], and the formation of contact or respiratory hypersensitivity [9,11].

We studied cytokine profiles (blood levels of IFN-γ, IL-2, IL-4, IL-6, and IL-10) and immune

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reactions reflecting the function of Th1 and Th2 lymphocytes in subacute TCM intoxication.

MATERIALS AND METHODS

Experiments were carried out on male and female outbred albino rats (180-200 g). The toxin (TCM) in olive oil was administered orally (TCM LD_{50}= 6.5±0.6 g/kg). In order to evaluate the functions of Th1 and Th2 lymphocytes, TCM was used in a dose of $^1/_4$ LD_{50} for 4 days and $^1/_{13}$ LD_{50} for 13 days, respectively. Plasma concentration of IFN-γ was measured 4 days after the first dose of TCM, IL-2, IL-4, IL-6, and IL-10 cytokines were measured 13 days after the first TCM dose using ELISA kits (BioSource Int.). The periods of blood cytokine measurements corresponded to parameters of immunogenesis after immunization with sheep erythrocytes (SE). The immunity parameters were evaluated by routine immunotoxicological immunological methods [3,4]. Humoral immune reaction to thymus-dependent antigen (SE) characterizing Th1 capacity to participate in the production of IgM by B-lymphocytes (plasma cells), was evaluated by the count of antibody-producing cells in the spleen 4 days after immunization. The function of Th1 lymphocytes was evaluated by delayed-type hypersensitivity (DTH) reaction. The formation of DTH was evaluated by the hind paw weight increment (in %). The resolving SE dose (5×10^8 cells) was injected under the hind paw aponeurosis on day 4 after immunization. The DTH reaction was evaluated after 24 h. The function of Th2 lymphocytes was evaluated by the number of antibody-producing cells producing IgG to SE in the spleen at the peak of this immunoglobulin production (day 14) by indirect local hemolysis in gel [4]. The rats were immunized intravenously with SE in a dose of 2×10^8 cells simultaneously with the first TCM dose. Hence, all immune reactions were evaluated after the animals received a summary equivalent TCM dose (1.0 LD_{50}). The data were statistically processed using Student's *t* test.

RESULTS

Measurements of plasma cytokines in rats revealed reduced level of IFN-γ on day 5 and of IL-4 on day 14 after TCM intoxication (2.50 and 1.58 times, respectively; $p<0.05$; Table 1). Obviously, the decrease in the IFN-γ/IL-4 ratio (4.2 *vs*. 6.6 in the control) under the effect of TCM times indicates greater suppression of Th1 activity in comparison with Th2 function [5].

The concentrations of IL-2, IL-6, and IL-10 after subacute TCM poisoning decreased by 2.49, 1.70, and 1.78 times, respectively ($p<0.05$).

Reduction of plasma concentration of IL-2 under the effect of TCM indicates its suppressed production by T-lymphocytes (by $CD4^+$ cells, belonging to the Th0 lymphocytes, and by some $CD8^+$ cells), reduced proliferation of T and B cells (synthesis of the immunoglobulin molecule J chains), and reduced activity of natural killer cells [4,6].

Low blood concentration of IL-6 (proinflammatory cytokine) characterizes reduction of its production by Th2 lymphocytes (and Th0 cells), by macrophages and lymphoid dendritic cells of surface tissue in the entry of injection, reduction and suppression of B cell activation [4,6,10].

The concentration of IL-10 (antiinflammatory cytokine inhibiting secretion of IFN-γ by Th0, Th1, and natural killer cells) [10,12] reduced under the effect of TCM, similarly as IL-6 concentration. This effect is characteristic of heavy metals [8], dinitrochlorobenzene, formaldehyde, and other toxicants [15]. Lesser suppression of IL-6 and IL-10 synthesis in comparison with IFN-γ confirms a greater toxic effect of TCM towards Th1 lymphocytes, shown in our study.

Humoral immune response (characterizing IgM production by B cells and the function of Th1) to T-dependent antigen (evaluated by the number of antibody-producing cells in the spleen) decreased 2.57 times ($p<0.05$; Table 2) 4 days after TCM poisoning. On day 5 after subacute TCM poisoning, the DTH reaction (Th1 function) was significantly inhibited (by 2.27 times; $p<0.05$). The production of IgG (evaluated by the number of antibody-producing cells in the spleen) decreased by 1.42 times ($p<0.05$) at the peak of immune response (evaluated by IgG; 13 days after immunization), which attested to reduced function of Th2 lymphocytes.

The parameters characterizing the immune reactions and the function of Th1 lymphocytes involved in them decreased by on average 2.42 times

TABLE 1. Effect of Subacute TCM Intoxication on Plasma Cytokine Concentrations in Rats (pg/ml; n=7; $M\pm m$)

Cytokines		Control	TCM
Day 5	IFN-γ	987±78	395±37*
Day 14	IL-4	150±22	95±10*
	IFN-γ/IL-4	6.6	4.2
	IL-2	1446±95	580±34*
	IL-6	51±6	30±5*
	IL-10	342±41	192±20*

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

TABLE 2. Effects of Subacute TCM Intoxication on Th1 and Th2 Functions in Rats (n=9-11; $M \pm m$)

Group	Th1 function		Th2 function
	APC to SE (IgM), 10^3	DTH, %	APC to SE (IgG), 10^3
Control	43.5±4.0	38.8±3.3	52.8±5.4
TCM	16.9±2.1*	17.1±2.2*	37.2±3.8*

Note. APC: antibody-producing cells.

under the effect of TCM. The suppression of immune response maintained by Th2 lymphocytes (and B cells) in TCM intoxication was significantly less pronounced (as shown by reduction in the number of IgG-producing cells). These data confirm greater damage inflicted by TCM to Th1 in comparison with Th2 lymphocytes.

The decrease in activity of Th1 cells caused by TCM can be due to reduction of blood corticosterone concentration as a result of subacute intoxication [3]; Th1 lymphocytes are more sensitive to this factor than Th2 [4]. Other possible causes of this reduction are the anticholinesterase effect of TCM and its metabolites [2,3] and, presumably, higher content of esterases on the outer membrane and in the cytosol of Th1 cells.

Hence, subacute TCM intoxication leads to a significant reduction of blood cytokine levels (IFN-γ, IL-2, IL-4, IL-6, IL-10) and IFN-γ/IL-4 ratio in comparison with the control. The Th1 cells are more sensitive to TCM intoxication than Th2 lymphocytes.

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