Inhibition of Function of T Cell Subpopulations and Decrease in Cytokine Production during Subacute Poisoning with Various Toxicants

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Experiments on outbred albino rats showed that subacute intoxication with organophosphorus compounds dimethyldichlorovinyl phosphate and malathion primarily decreased functional activity of Th1 lymphocytes, immune reactions associated with these cells, and interferon-γ production compared to that of Th2 lymphocytes and interleukin-4 synthesis. Acrylic acid nitrile and methanol produced the opposite effect. Sulfur mustard and sodium arsenite were equally potent in reducing the function of Th1 and Th2 lymphocytes and production of cytokines.

Key Words: *toxicants; immunotoxicity; Th1 and Th2 lymphocytes; cytokines*

Toxic chemicals produce different effects on functional activity of Th1 and Th2 lymphocytes and inducing impairment of primarily cellular or humoral immunity. Th1 cells are involved into cellular immune response (production of interferon-γ, IFN-γ) and provide the synthesis of IgM and IgG2a by B lymphocytes. Th2 lymphocytes synthesize interleukin-4 (IL-4) and, therefore, play a role in the humoral immune response. These cells provide production of major immunoglobulins (*e.g.*, IgG1, IgA, IgE, and IgD) by B lymphocytes [4,6]. The Th1/Th2 functional ratio determines the probability of viral and microbial infections, respectively [7], as well as the development of cutaneous or respiratory hypersensitivity [8,10]. The relationship exists between activity of Th2 lymphocytes and IgE synthesis, which contributes to manifestations of respiratory allergy [4,12].

Studying the specific features of damage to Th1 and Th2 lymphocytes caused by toxic chemicals will allow us to predict the risk of cellular or humoral immune dysfunction leading to infectious complications and diseases and to select immunomodulators for adequate treatment of immune disorders [1,2,6].

This work was designed to study peculiarities of Th1 and Th2 lymphocyte dysfunction and changes in cytokine production (IFN-γ and IL-4) during suppression of humoral and cellular immune response after acute poisoning with various toxicants.

MATERIALS AND METHODS

Experiments were performed on male and female outbred rats weighing 180-240 g. Immune parameters were estimated by standard immunotoxicological and immunological methods [1,4]. The humoral immune response to thymus-dependent antigen (sheep erythrocytes) characterizing the capacity of Th1 lymphocytes to participate in IgM production by B lymphocytes (plasma cells) [6], was estimated from the number of antibody-producing cells (APC) in the spleen. Th1 lymphocyte function was determined from the delayed-type hypersensitivity response (increase in the weight of the hindlimb pad 24 h after provocation, in percents). Aqueous

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solutions of organophosphorus compounds dimethyldichlorovinyl phosphate (DDVP) and malathion, sodium arsenite, and acrylic acid nitrile (AAN) were injected subcutaneously. The solution of sulfur mustard in dimethylsulfoxide was injected subcutaneously. Methanol (40% solution) was given perorally. Toxicants were administered in a dose of $^1/_4$ LD_{50} for 4 days. LD_{50} for DDVP, malathion, sulfur mustard, sodium arsenite, AAN, and methanol are 64.5±2.3, 815.4±28.0, 5.5±0.3, 38.5±4.2 and 45.4±2.9 mg/kg and 9.1±1.2 g/kg, respectively. The reactions were studied after 5 days. Th2 lymphocyte function was estimated by the number of APC synthesizing IgG to sheep erythrocytes in the spleen at the peak of production of this immunoglobulin (day 14) by indirect local hemolysis in gel [4]. Toxicants in a dose of $^1/_{13}$ LD_{50} were administered for 13 days. The rats were intravenously immunized with sheep erythrocytes in a dose of 2×10^8 cells (simultaneously with the 1st administration of toxicants). Sheep erythrocytes in a resolving dose of 5×10^8 cells were injected under hindlimb pad aponeurosis on day 4 of immunization (after administration of the toxicant and immunizing dose). The immune response was studied in animals receiving an equivalent dose of the toxicant (1.0 LD_{50}). The concentration of cytokines IFN-γ and IL-4 reflects the function of Th1 and Th2 lymphocytes, respectively [4,5,6]. Blood cytokine concentration in rats was measured on days 5 and 14 after the 1st injection of AAN, respectively. The measurements were performed by ELISA using ELISA Kits (BioSource Int.). Intact animals receiving no toxicants served as the control. The results were analyzed by Student's *t* test.

RESULTS

The humoral immune response to T cell-dependent antigen (number of APC in the spleen) characterizes IgM synthesis and Th1 lymphocyte function. The response decreased 4 days after administration of DDVP, malathion, sulfur mustard, sodium arsenite, AAN, and methanol by 2.31, 1.94, 4.06, 2.56, 2.02, and 1.75 times, respectively, compared to the control ($p<0.05$; Table 1). On day 13 after immunization (peak of immune response evaluated by IgG production), production of IgG (number of APC in the spleen) decreased under the effect of DDVP, malathion, sulfur mustard, sodium arsenite, AAN, and methanol by 1.43, 1.26, 3.85, 2.26, 3.17, and 1.90 times, respectively ($p<0.05$), which attested to suppression of Th2 lymphocyte function (Table 1). The delayed-type hypersensitivity response (Th1 cell function) was significantly reduced on day 5 after subacute poisoning with DDVP, malathion, sulfur mustard, sodium arsenite, AAN, and methanol by 2.20, 1.78, 3.75, 2.09, 2.04, and 1.69 times, respectively ($p<0.05$).

The parameters characterizing different immune reactions and related functions of Th1 and Th2 lymphocytes decreased after intoxication with DDVP and malathion by 2.06 and 1.34 times, respectively, with sulfur mustard by 3.90 and 3.85 times, respectively, with sodium arsenite by 2.32 and 2.26 times, respectively, with AAN by 2.03 and 3.17 times, respectively, and with methanol by 1.72 and 2.43 times, respectively. Hence, intoxication with organophosphorus compounds primarily decreased functional activity of Th1 lymphocytes. Sulfur mustard and sodium arsenite are equally potent in reducing the function of Th1 and Th2 lymphocytes. By contrast, AAN and methanol primarily suppressed the function of Th2 lymphocytes.

Published data show that the immunotoxic effect of methanol is associated with not only IgG production, but also dysfunction of B lymphocytes due to disorders in folic acid metabolism [3,11].

Various toxicants produce specific effects on the functions of Th1 and Th2 lymphocytes, which is confirmed by variations in blood cytokine concentration (Table 2). After DDVP poisoning, the concentrations of IFN-γ and IL-4 decreased on days 5 and 14, respectively (by 1.88 and 1.47 times, $p<0.05$). The concentrations of IFN-γ and IL-4 also decreased after intoxication with malathion (by 1.80 and 1.44 times, respectively, $p<0.05$), sulfur mustard (by 2.82 and 2.98 times, respectively, $p<0.05$), sodium arsenite (by 2.35 and 2.39 times, respectively, $p<0.05$), AAN (by 1.89 and 2.63 times, respectively, $p<0.05$), and methanol (by 1.27 and 1.84 times, respectively, $p<0.05$).

The suppressor effect of toxicants on IFN-γ synthesis increased in the following order: methanol<organophosphorus compounds<AAN<sodium arsenite<sulfur mustard. The ability of these toxicants to decrease IL-4 concentration in the blood increased in the following order: organophosphorus compounds< methanol<sodium arsenite<AAN< sulfur mustard.

The increase in the IFN-γ/IL-4 ratio reflects reduction of Th2 lymphocyte function compared to that of Th1 cells, while the decrease in this ratio indicates predominant suppression of Th1 lymphocytes compared to Th2 cells [5]. During poisoning with organophosphorus compounds, sulfur mustard, sodium arsenite, AAN, and methanol, the IFN-γ/IL-4 ratio was 4.8, 6.4, 6.3, 8.6, and 9.0, respectively (*vs*. 6.2 in the control). These data confirm the specificity of toxicant-induced injury to Th1 and Th2 cells.

TABLE 1. Effect of Subacute Intoxication with Various Toxic Chemicals on Function of Th1 and Th2 Lymphocytes in Rats (*M*±*m*, *n*=8-11)

Substance	Th1 lymphocyte function		Th2 lymphocyte function
	APC to SE (IgM), 10^3	DTH, %	APC to SE (IgG), 10^3
Control	45.5±3.9	39.8±2.8	60.8±4.1
DDVP	19.7±2.0*	18.1±1.9*	42.5±3.5*
Malathion	23.5±2.1*	22.3±2.3*	48.3±3.7*
Sulfur mustard	11.2±1.5*	10.6±1.4*	15.8±1.7*
Sodium arsenite	17.8±1.7*	19.0±2.0*	26.9±2.5*
AAN	22.5±2.4*	19.5±1.9*	19.2±1.8*
Methanol	26.0±2.5*	23.5±2.2*	25.0±2.2*

Note. DTH, delayed-type hypersensitivity; SE, sheep erythrocytes. Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. Effect of Subacute Intoxication with Various Toxic Chemicals on Blood Cytokine Concentration in Rats (pg/ml, *M*±*m*, *n*=6)

Substance	IFN-γ	IL-4	IFN-γ/IL-4
Control	835±78	134±15	6.2
DDVP	445±57*	91±10*	4.7
Malathion	464±59*	93±11*	5.0
Sulfur mustard	268±45*	42±7*	6.4
Sodium arsenite	355±41*	56±11*	6.3
AAN	441±47*	51±7*	8.6
Methanol	657±66*	73±8*	9.0

The suppressor effect of organophosphorus compounds on Th1 lymphocytes is related to a significant increase in blood corticosterone concentration [1]. Th1 lymphocytes are more sensitive to corticosterone than Th2 lymphocytes [4]. Sulfur mustard-induced injury to Th1 and Th2 lymphocytes is associated with its alkylating effect on cell DNA [9].

The immunotoxic effect of sodium arsenite is related to the inhibition of monothiol and dithiol enzymes (*e.g.*, dehydrolipoic acid of the pyruvate oxidase system) in immune cells, including Th1 and Th2 cells [3].

AAN and methanol caused more pronounced reduction of Th2 lymphocyte function compared to Th1 cells. Cyan ion (most toxic product of AAN metabolism) inhibits component a_3 in the enzyme system of tissue respiration of lymphocyte subpopulations [3]. Moreover, Th2 lymphocytes are extremely sensitive to high-toxicity products of methanol biotransformation (formaldehyde and formic acid) [3,11].

Our results indicate that subacute poisoning with various toxic chemical in equivalent doses is accompanied by severe damage to Th1 and Th2 lymphocytes. Similar dysfunction of cells is observed after intoxication with the test compounds. Poisoning with DDVP and malathion primarily decreased functional activity of Th1 lymphocytes, associated immune reactions, and IFN-γ production (as compared to Th2 lymphocytes and IL-4 synthesis). AAN and methanol produce the opposite effect. Sulfur mustard and sodium arsenite are equally potent in reducing the function of Th1 and Th2 lymphocytes and production of cytokines.

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