Role of Th1 and Th2 Lymphocytes and Cytokines Produced by These Cells in Suppression of Immune Reactions during Subacute Poisoning with Anticholinesterase Toxicants

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Experiments on Wistar rats showed that subacute poisoning with anticholinesterase toxicants zarin and agent VX (daily subcutaneous injections in $^1/_7$ LD_{50} for 6 days) led to suppression of cellular and humoral immune reactions and to a decrease in blood concentrations of cytokines (IL-2, IL-4, IFN-γ) with a reduction of the IFN-γ/IL-4 and IL-2/IL-4 ratios, which attests to more pronounced decrease in Th1 lymphocyte function in comparison with Th2 cells.

Key Words: *anticholinesterase toxicants; immunotoxicity; Th1, Th2 lymphocytes; cytokines*

Numerous organophosphorus compounds with anticholinesterase effect are used in industry, agriculture, and in house keeping. Drugs inhibiting acetylcholinesterase are widely used in medicine for the treatment of various diseases [2,3]. Despite the fact that, in accordance with international agreements, chemical weapons, specifically, weapons with anticholinesterase toxic chemicals (TC) as the main element, are to be destroyed at special industrial objects, the possibility of their use with terrorist and criminal purposes did not decrease [1,2,4,11]. The accidents with release of TC or products of their destruction into the environment and intoxication of humans can also be excluded [4]. The development of highly effective antidotes to zarin and agent VX (organophosphorus toxicants with nervous paralytic effects, irreversible inhibitors of acetylcholinesterase) is in progress in foreign countries; delayed effects of these substances are analyzed [8,10,12,13].

Poisoning with organophosphorus compounds, anticholinesterase TC, and drugs can be accompanied by infectious complications and diseases associated with the formation of postintoxication immunodeficiency. The development, along with antidotes, of methods for reduction of damage inflicted by these substances to the immune system suggests further study of their immunotropic effects [2,3].

We evaluated the role of Th1 and Th2 lymphocytes and cytokines produced by these cells (IL-2, IL-4, IFN-γ) in suppression of humoral and cellular immune reactions during subacute poisoning with anticholinesterase substances.

MATERIALS AND METHODS

Experiments were carried out on Wistar rats of both sexes (180-240 g). Toxic chemicals were injected in $^1/_7$ LD_{50} daily subcutaneously for 6 days (LD_{50} for zarin and agent VX injected subcutaneously are

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0.21±0.02 and 0.018±0.004 mg/kg, respectively). The immune system parameters were evaluated by common methods of experimental immunotoxicology [2]. Humoral immune reaction to thymus-dependent antigen (sheep erythrocytes; SE) was evaluated by the number of antibody-producing cells (APC) in the spleen on day 5 after acute intoxication with TC with simultaneous intraperitoneal immunization of rats with these antigens in doses of 2×10^8 cells. Humoral immune reaction to SE in this test characterizes the capacity of Th1 lymphocytes to participate in the production of IgM by B cells (plasma cells) [5]. The number of cells producing IgG to SE was evaluated in the spleen by indirect local hemolysis in gel on day 8 [5,14]. According to published data, this method characterizes predominantly the function of Th2 lymphocytes, because Th1 lymphocytes during this period provide the formation of antibody production (except IgM and IgG_{2a}, constituting no more than 20% of all IgG subclasses) [5,9].

Activity of natural killer cells was evaluated spectrophotometrically by the parameters of natural cytotoxicity on day 4 after the first injection of TC. The formation of delayed-type hypersensitivity reaction characterizing the function of Th1 lymphocytes [5] was evaluated in animals by the increment of hind paw weight in percents. To this end, the rats were intraperitoneally immunized with SE (10^8) 30 min after injection of TC. The resolving dose of SE (5×10^8) was injected under the hind paw aponeurosis after 4 days. Delayed-type hypersensitivity reaction was evaluated after 24 h.

The concentrations of cytokines (IL-2, IL-4, IFN-γ) in the peripheral blood were measured 4 and 7 days after the first injection of TC by enzyme immunoassay according to the manufacturer's instruction (BioSource Int. ELISA Kits).

The data were processed using Student's *t* test.

RESULTS

Injection of agent VX and zarin led (after 4 days) to a reduction of humoral immune response to T-dependent antigen (evaluated by APC count in the spleen) characterizing the synthesis of IgM and function of Th1 lymphocytes, by 2.66 and 2.32 times, respectively, in comparison with the control level ($p<0.05$; Table 1). Treatment with agent VX and zarin led to suppression of IgG production (shown by APC count in the spleen after 7 days) reflecting mainly the function of Th2 lymphocytes, by 1.72 and 1.44 times, respectively ($p<0.05$), significant reduction of natural killer cell activity (by 2.56 and 2.23 times) and delayed-type hypersensitivity reaction (by 2.24 and 2.04 times, respectively; $p<0.05$).

According to published data, the count of cells producing antibodies to SE after 4 days characterizes the synthesis of IgM by B cells and function of Th1 cells. Activity of natural killers and formation of delayed-type hypersensitivity also indicate the capacity of Th1 lymphocytes to modulate the above reactions, while the count of cells producing antibodies to SE in the reaction of indirect local hemolysis in gel after 7 days reflects the synthesis of IgG and function of Th2 lymphocytes [5,9,14]. The values characterizing different immune reactions and the functions of Th1 and Th2 lymphocytes related to them decreased by 2.34 and 1.58 times, respectively, under conditions of subacute poisoning with TC. Th1 lymphocytes were sensitive to TC injected to rats in the summary dose of $^4/_7$ LD_{50}, Th2 lymphocytes were sensitive to $^6/_7$ LD_{50}. This suggests that the function of Th1 lymphocytes was more markedly suppressed under the effect of anticholinesterase TC.

This conclusion was confirmed by the results of cytokine measurements in rat peripheral blood (Table 2). The concentrations of IL-2, IL-4, IFN-γ decreased by 2.08, 1.33, and 2.11 times on day 5 of subacute intoxication with agent VX, and by 2.24, 1.49, and 2.58 times on day 8, respectively ($p<0.05$). Zarin caused similar reduction of the studied parameters. These data attest to more pronounced decrease in IL-2 and IFN-γ concentrations in the blood in comparison with IL-4 concentration.

Cytokines IL-2 and IFN-γ are produced by Th1 lymphocytes, IL-4 by Th2 lymphocytes, while increased IFN-γ/IL-4 and IL-2/IL-4 ratios characterize a decrease in functional activity of Th2 lymphocytes in comparison with the function of Th1 cells [5,6,9]. On days 5 and 8 of treatment with agent VX, the IFN-γ/IL-4 ratio was below the control (4.2

TABLE 1. Effect of Subacute Intoxication with Anticholinesterase TC on Immune System Parameters in Rats (*M*±*m*; *n*=7-9)

Group	APC to SE (IgM), $\times10^3$	APC to SE (IgG), $\times10^3$	NC, %	DTH, %
Control	38.3±3.6	15.3±1.6	31.5±3.2	35.7±2.5
Agent VX	14.4±1.5*	8.9±1.1*	12.3±2.2*	15.9±2.1*
Zarin	16.5±1.7*	10.6±1.0*	14.1±2.0*	17.5±1.9*

Note. NC: natural cytotoxicity index; DTH: delayed-type hypersensitivity. Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. Effects of Subacute Intoxication with Anticholinesterase TC on Cytokine Concentrations in Peripheral Blood of Rats (pg/ml; *M*±*m*; *n*=6)

Parameter	Control	5 days after immunization		8 days after immunization	
		agent VX	zarin	agent VX	zarin
IL-2	1321±92	635±32*	556±35*	590±30*	519±28*
IL-4	116±12	87±6*	80±7*	78±5*	69±5*
IFN-γ	778±60	368±30*	402±42*	301±27*	350±33*
IFN-γ/IL-4	6.7	4.2	5.0	3.8	5.1
IL-2/IL-4	11.4	7.3	7.0	7.6	7.5

and 3.8, respectively, *vs*. 6.7). The IL-2/IL-4 ratio in animals with agent VX intoxication on days 5 and 8 was 7.3 and 7.6, respectively, *vs.* 11.4 in the control. Similar data were obtained in experiments with zarin. This indicates more pronounced suppression of Th1 lymphocyte function under the effect of anticholinesterase TC. This effect can be explained by higher inhibitory capacity of TC against acetylcholinesterase on T lymphocyte membranes and α-naphthyl-AS-acetatesterase and α-naphthylbutyratesterase in their cytosol, and by greater role of esterases in the realization of Th1 lymphocyte functions [2,3,7].

Suppression of natural killer cell under the effect of TC is presumably due to reduced production of IL-2 and IFN-γ by Th1 cells. It is known that these cytokines activate natural killer cells [5,9].

Hence, subacute intoxication with anticholinesterase substances (zarin and agent VX) more intensely suppresses the immune reactions linked with Th1 lymphocyte function than the immune response mediated by activation of Th2 lymphocytes. This is confirmed by more pronounced reduction of peripheral blood concentrations of IL-2 and IFN-γ in comparison with IL-4 (reduction of IFN-γ/IL-4 and IL-2/IL-4 ratios) under the effect of anticholinesterase TC.

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