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MICROBIOLOGY AND IMMUNOLOGY

Mechanisms of Immunotropic Effects of Organophosphorus Compounds

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In connection with the wide application of organophosphorus compounds (OPC) in agriculture and the chemical industry and the destruction of OPC classified as toxic substances, with the concomitant risk of acute and chronic intoxications (in particular, during possible accidents), there is unflagging interest in investigations of the mechanisms of action of the above toxicants on the immune system. Such studies are aimed at the development of methods of preventing and treating postintoxication immunodeficient states accompanied by various infectious complications and diseases [5]. However, analysis of recent publications on the immunosuppressive effects of OPC [2,4,11] indicates that much remains unclear regarding the significance of nonspecific (associated with the stress response) and specific mechanisms in the development of disturbances of the immune status during exposure to OPC.

The aim of the present study was to assess the role of nonspecific and specific mechanisms in the formation of the principal immune responses to OPC.

MATERIALS AND METHODS

The experiments were carried out on male CBA mice weighing 18-22 g. The anti-cholinesterase insecticide dimethylchlorvinylphosphate (DDVP) in doses of 0.25, 0.5, and 1.0 of LD_{50} (40±1.2 mg/kg, subcutaneously) was used as the OPC. The immune responses to DDVP were compared with the

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TABLE 1. Effect of DDVP, Immobilization Stress (IS), Hydrocortisone, and Acetylcholine on the Major Immune Responses, Concentration of Corticosterone in the Blood, and Activity of α-naphthylbutyratesterase in the Splenocytes ($M \pm m$)

Parameter	Control	DDVP, LD_{50}			IS	Hydrocortisone, 100 mg/kg	Acetylcholine, 5 mg/kg
		0.25	0.5	1.0			
Number of CFU in spleen	9.2±1.5 (15)	13.3±2.4 (10)	15.4±2.6* (10)	18.1±2.8* (10)	5.1±1.0* (10)	5.7±1.1* (10)	14.2±1.7* (10)
T-cell count in thymus, 10^6	82±7 (10)	68±9 (7)	54±6* (7)	30±4* (7)	52±5* (7)	55±6* (7)	60±6* (7)
DTH response (increase in weight of paw), mg	40.5±1.9 (9)	33.4±1.2* (9)	30.1±1.3 (9)	25.1±1.0* (9)	28.7±1.4* (9)	31.4±1.5* (9)	44.6±1.6 (9)
NC, %	32±4 (15)	25±4 (10)	15±3* (10)	11±2* (10)	21±3* (10)	17±5* (10)	27±6 (10)
ADCC, %	9.3±1.3 (10)	6.1±1.0* (10)	5.2±1.1* (10)	3.3±0.7* (10)	7.9±1.2 (10)	8.8±1.6 (10)	12.5±1.4 (10)
APC, 10^3	31.2±3.0 (9)	21.3±3.3* (7)	18.1±3.1* (7)	15.3±2.7* (7)	17.2±2.9* (7)	20.3±3.1* (7)	43.3±4.2* (7)
Corticosterone, ng/ml	35±4 (9)	51±6* (9)	68±6* (9)	106±9* (9)	112±10* (9)	— (9)	41±5*
Activity of α-naphthylbutyratesterase in splenocytes (% of positively stained cells)	42±4 (10)	33±3 (10)	27±3* (10)	16±2* (10)	37±4 (10)	46±5 (10)	35±4 (10)

Note. Asterisk indicates reliability of differences ($p<0.05$) vs. control; figures in parentheses show the number of animals.

effects of immobilization stress (6 h) and of hydrocortisone and acetylcholine administered subcutaneously in doses of 100 and 5 mg/kg, respectively.

The migration of hemopoietic stem cells (HSC) from the bone marrow (BM) was assessed judging by the number of colony-forming units (CFU) in the spleen [15] after the mice were exposed to irradiation in a dose of 8 Gy, half of the crus being shielded according to a described method [7]. One day after exposure to the factors studied, the T-cell count in the thymus was determined according to the number of lymphocytes in the organ, which was calculated to include the number of nucleus-containing cells in the thymus and the relative lymphocyte count in the thymogram and also took into account that lymphocytes in the thymus are represented virtually only by the T population. The reaction of hypersensitivity of the delayed type (DTH) was assessed by the increase in the weight of the hind paw. Immunization of the animals was performed using sheep erythrocytes (SE) injected in a dose of 10^8, intravenously. The resolving dose of SE (5×10^8) was injected under the aponeurosis on the hind paw, 4 days after immunization. The test preparations were administered 1 hour before injection of SE in the resolving dose. Exposure to stress was also finished during this period. The response was assessed after 24 hours. The activity of natural killer cells (NKC) was spectrophotometrically determined 24 hours after exposure to stress or after injections of test preparations after Gordienko [3]. Antibody-dependent cell cytotoxicity (ADCC) was assessed 5 days after immunization of the mice with 10^8 SE [6], which was performed along with injections of test preparations or directly after exposure to stress. The number of antibody-producing cells (APC) in the spleen was determined by a routine method 5 days after immunization with 5×10^8 SE, which was performed after exposure to stress or simultaneously with the administration of the test preparations. Corticosterone was fluorometrically determined [14] 2 h after administration of the test preparations or directly after immobilization stress. The activity of α-naphthylbutyratesterase was studied in the splenocytes after Higgi et al. [12]. The data obtained were statistically processed using Student's t test.

RESULTS

It was established that under the influence of DDVP an enhancement of migration of HSC from

the BM into the spleen occurs, this enhancement directly depending on the dose. A similar effect was also characteristic of acetylcholine, whereas exposure to stress and hydrocortisone produced the opposite effect. The data obtained provide evidence that the increased release of HSC from the BM is associated with the effect of acetylcholine, this specific effect prevailing over the inhibition of HSC migration caused by an increased concentration of corticosterone in the blood (non-specific mechanism) during the reversible inhibition of acetylcholinesterase by DDVP. Inhibited migration of HSC from the BM during stimulation of the adrenal cortex is a known fact [8]. Probably, the mechanism of acetylcholine-induced migration of HSC is the same as that described in a study of the mobility of B lymphocytes induced by this transmitter [1].

When the dose of DDVP was increased, a directly dependent decrease of the T-cell count was observed in the thymus. Stress, hydrocortisone, and acetylcholine acted likewise. Probably, during the exposure to OPC thymus involution occurs due primarily to the outflow of thymocytes from the organ under the influence of corticosteroids (nonspecific mechanism), due to the acetylcholine-induced activation of M-cholinoreceptors of thymocytes (specific effect) [13], and, to a lesser extent, due to the cytotoxic effect of hormones of the adrenal cortex.

Intoxication with DDVP and other factors studied, except for acetylcholine, markedly reduced the DTH response. As is demonstrated by the analysis of the changes of the corticosterone content in the blood plasma under the influence of DDVP and during exposure to stress, the above phenomenon is associated with suppression of the given response by corticosteroids (nonspecific mechanism), and, probably, with inactivation of esterases in the monocytes and T cells which are crucial to the formation of DTH (specific effect).

In our investigations, a reduction of the natural cytotoxicity (NC) was found to depend directly on the dose of DDVP. Similar effects are produced by exposure to stress and hydrocortisone (nonspecific mechanism). Suppression of the activity of NKC during stress and the role of interleukin-2 and interferon have been studied in details [9,10]. It should be mentioned that conclusions drawn from these studies suggest that during various kinds of stress, as well as during intoxication with OPC, recovery of the activity of NKC occurs due to the presence of general mechanisms underlying the formation of an immunodeficient state. The interrelationship between the suppression of NC and inhibition of α-naphthylbutyratesterase in the splenocytes attests to the existence of a specific mechanism of NC decline during intoxication with OPC.

A marked reduction of ADCC ensued from exposure to DDVP. The degree of suppression of cytotoxicity depended directly on the dose of DDVP and on the concentration of corticosterone in the blood, and inversely depended on the esterase activity of the splenocytes (specific effect). No effect of stress and hydrocortisone on the ADCC was encountered. Acetylcholine raised this parameter insignificantly. Analysis of the above data attests to a reduced ADCC due to inactivation of esterases in the killer cells.

The number of APC in the spleen inversely depended on the dose of DDVP and on the concentration of corticosterone in the blood plasma (nonspecific effect) and directly depended on esterase activity in the splenocytes (specific effect). Acetylcholine enhanced the humoral immune response, this probably being due to mechanisms described elsewhere [1,13].

Thus, the changes of the immune status under the influence of OPC are associated both with the nonspecific stress effect of corticosteroids on certain immune responses and with specific mechanisms, which must include inhibition of esterases in the immunocompetent cells and the effect of acetylcholine. The significance of nonspecific and specific effects in the formation of the immunodeficient state after intoxication with OPC varies depending on the immune response studied. The presence of a number of general mechanisms of immunosuppression during stress [9,10] and OPC intoxication suggests the possibility of employing similar methods of pharmacological correction of the disturbances of immune homeostasis caused by these factors.

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The Effect of Interleukin-2 on Rosette Formation and Its Dependence on the Level of Serotonin

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It is well known that the interaction between systems is realized by direct cell contacts and transmitters setting up a two-way stream of information. In the present work serotonin, an immunomodulator with a dose-dependent opposite action, was used as a regulator of physiological system. The large group of regulatory peptide factors called "interleukins" [14,16] has generated interest because of their effects on the immune system. Interleukin-2 (IL-2) was preferred in the present study due to its wide use in therapy, as well as the following considerations: 1) IL-2 is a growth factor for T lymphocytes of various functional orientation and may have a direct effect on quiescent T cells [17], B lymphocytes [4], and monocytes [11]; 2) some of the above-mentioned cells containing serotonin-sensitive structures of the receptive type are controlled by the serotoninergic system [7]; 3) there is a functional relationship between IL-2 and the neuroendocrine system, as is attested by its effect on the activity of pituitary cells [1], on nerve growth factor function [3], on the level of some neurotransmitters [13], and on the expression of the IL-2 receptors in the brain [12]. All these facts enable us to consider the possibility of a relationship between serotonin and IL-2 in the process of immunomodulation.

The aim of the present investigation was to explore the modulation of rosette formation (RF) by IL-2 and the interaction between serotonin and IL-2 at the earliest stage of development of the organism's reaction to antigen. A minimal action with practically no side effects was probably related to the simultaneous administration of the drugs and antigen, to the extremely rapid manifestation of the serotonin [4] and IL-2 effects, and to the use of doses, substantially lower than accepted. The models used simulate likely physiological situations and permit assessment of the time required for the engagement of non-specific regulators in the modulation of a specific immune process.

MATERIALS AND METHODS

Experiments were carried out on 320 CBA mice (no less than 7 animals in one group) 3-4.5 months old. Sheep erythrocytes (SE) were used as antigen in doses of 5×10^6 or 5×10^7 per mouse. The reaction to the antigen was assessed on the 5th day after immunization. For this purpose a

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