INDUCTION OF IMMUNODEFICIENCY BY BENZENE AND ITS CORRECTION BY ANABOL

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Immunomodulating therapy is a promising method of treatment of many internal diseases and of rehabilitation of patients. The development of new and effective immunomodulators is dependent on thoroughly studied experimental models of disturbances of immunity, appropriate for the conditions of human pathology, being available. Nevertheless, few such models yet exist, and these have been inadequately studied in relation to the concrete mechanisms of disturbed immune functions and the biochemical changes lying at the basis of the observed immunologic disturbances.

In this paper we examine the possibility of using chronic benzene poisoning (CBP) in mice as a model of immunodeficiency and of correcting this immunodeficiency by a new immunomodulating agent, anabol (lactostimulin), produced by the Soviet microbiological indsutry.

EXPERIMENTAL METHODS

Experiments were carried out on C57Bl/10 mice. CBP was induced by daily subcutaneous injections of benzene (5 times a week) in a dose of 2 ml/kg body weight for 6 months. Some of the mice with CBP, 2 weeks before the end of benzene administration, were given 25 mg/kg of anabol daily via gastric tube. The effect of anabol on normal animals also was studied. At the end of the experiment the animals were killed and the state of their cellular immunity (CI) and activity of enzymes of xenobiotic metabolism (EXM) in their lymphocytes were determined. The number of lymphocytes in the blood, the number and composition of the bone marrow, spleen, and thymus cells, and the numbers of T and B lymphocytes also were studied. Activity of T-cell subpopulations induced in mixed lymphocyte cultures (MLC) and immune to H-2 transplantation antigens was estimated by the cytotoxic index (CTI, in %) (killer T cells [1]), by inhibition of ^{3}H-thymidine incorporation into MLC – the inhibition index – (II, in %) (suppressor T cells [1]), and by production of macrophage migration inhibition factors (MIF producers), expressed as II (in %) [10]. Activity of natural killer (NK) cells relative to IAC-1 tumor cells [11] and the number of antibody-forming cells (AFC) [13] also were determined. Activity of MLC was judged as cytochrome P-450 [14], cytochrome c-reductase, and glutathione-S-transferase [15].

EXPERIMENTAL RESULTS

The development of CBP was accompanied by marked inhibition of hematopoiesis (the number of nucleated cells in the bone marrow was reduced by more than half, that in the spleen by 2.8 times, and in the thymus by more than 10 times; the blood leukocyte count was reduced by 3.2 times, mainly due to lymphocytes). Besides hypoplasia of hematopoiesis, persistent inhibition of immunogenesis also developed. Most of the parameters of CI studied were reduced in value (Table 1), but the degree of this reduction varied: the number of AFC fell by almost 80%, whereas activity of killer T cells, suppressor T cells, and NK cells was inhibited by a lesser degree, and activity of MIF-producers was virtually unchanged.

CBP was accompanied not only by inhibition of CI, but also by disturbances of activity of EXM in the lymphocytes, and in particular, activity of enzymes involved in the first phase

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TABLE 1. Effect of Anabol on Parameters of Immunity of Mice with CBP (M ± m)

Experimental conditions	Killer T cells (CTI, %)		MIF products (II, %)	Suppressor T cells (II, %)		NK cells (CTI, %)			AFC
	dose of lymphocytes incubated, mg								
	$3 \cdot 10^5$	10^5	$5 \cdot 10^6$	1:5	1:50	10^6	$5 \cdot 10^5$	$2{,}5 \cdot 10^5$	10^6
Control mice (n = 32)	62,9±8,1	28,3±5,2	47,8±5,3	89,6±5,7	78,9±4,6	29,4±4,1	23,1±2,2	16,4±1,5	120,0±3,0
Mice with CBP	32,1±2,6*	14,6±8,3	32,5±5,7	44,1±3,5*	66,3±4,6*	23,7±4,0	11,6±3,2*	7,5±2,6	24,3±4,8*
Mice with CBP treated with anabol (n = 31)	52,6±4,7**	20,3±2,0	31,4±4,8*	73,4±6,2**	64,2±7,4	26,3±4,2	25,7±1,9**	8,7±3,0*	82,6±5,2*, **

Legend. Duration of culture for killer T cells 5 days, for MIF-producers 1 day, for suppressor T cells 3 days. Here and in Table 2: *$p \leq 0.05$ compared with control, **$p \leq 0.05$ compared with CBP.

TABLE 2. Effect of Anabol on Activity of EXM in Lymphocytes (M ± m)

Experimental conditions	Cytochrome P-450, nmoles/(mg·min)	Cytochrome c-reductase, nmoles/mg·min	Glutathione-S-transferase, nmoles/mg·min
	Dose of lymphocytes incubated, mg		
Control mice ($n = 30$)	3,5±0,8	3,1±0,7	23,2±5,3
Mice with CBP ($n = 30$)	1,1±0,4*	1,2±0,3*	14,6±3,9
Mice with CBP treated with anabol (n = 30)	2,6±0,6**	2,9±0,5**	26,2±3,9**

Legend. Duration of culture for cytochrome P-450 was 3 days.

of xenobiotic metabolism, namely cytochrome P-450 and cytochrome c-reductase, whereas inhibition of activity of glutathione-S-transferase, a second phase enzyme, was weaker and was not statistically significant (Table 2).

In animals receiving anabol together with benzene, inhibition of hematopoiesis and changes in CI and activity of EXM were much less marked. For instance, activity of killer T cells, suppressor T cells, and NK cells was higher in mice treated with anabol than in the animals receiving benzene alone, and was virtually indistinguishable from the corresponding values for normal animals (Table 1). Administration of anabol also increased the number of AFC, when sharply depressed in CBP, although this parameter in treated mice was significantly lower at the end of the experiment than in normal mice.

Anabol had an even stronger effect on activity of EXM of the lymphocytes in CBP (Table 2). In the treated mice, activity of all the enzymes studied was virtually the same as in the control.

Anabol thus had a marked immunomodulating effect on activity of EXM and on the state of CI in CBP. This mechanism of its action may probably lie at the basis of another recuperative effect observed in the treated mice: their hematologic parameters were restored virtually to normal (except the number of cells in the thymus, which remained depressed by 30%; mild lymphocytopenia also persisted – 87% of the normal number). Administration of anabol to normal mice did not lead to any significant deviations of CI, EXM activity in the lymphocytes, or hematologic parameters, from normal.

The experiments are evidence that benzene induces an immunodespressive effect. Data in the literature indicate that this effect may be connected both with its cytotoxic action on the thymus and on T lymphocytes and with disturbances of regulatory character [3, 5]. Disturbances of immunity in CBP are considered to be closely linked with the hematotropic,

oncogenic, and other aspects of the action of benzene on the body [9]. Administration of thymus homogenate has been shown to lead to normalization of both immunologic and hematologic parameters [4].

The present investigation showed that disturbances caused by benzene in the immune system have a number of special features that distinguish the secondary immunodeficiency associated with CBP from immunodeficiencies caused by other xenobiotics [12]. These differences may be due to differences in the sensitivity of precursors of the T-cell subpopulations studied. We know, for example, that precursors of suppressor T cells and of MIF-producers are more sensitive to the action of cyclophosphamide and hydrocortisone than killer T cells [10]. In the case of benzene, killer T cells and NK cells were found to be more sensitive (Table 1). The higher resistance of the suppressors may be due to heterogeneity of this subpopulation induced in MLC; indeed, some of the suppressors contained in it are not T cells [1]. Meanwhile similarity can be observed between immunologic and enzymic disturbances in CBP and the secondary immunodeficiencies associated with diseases of the internal organs [2, 8], thereby confirming the clinical importance of the use of CBP as a model with which to study the immunomodualting properties of various preparations.

The authors previously showed that anabol can be successfully used for the immunologic rehabilitation of patients with long-standing pneumonias [6], when restoration of the immune status was accompanied by marked improvement in the clinical picture of the disease. Administration of anabol in CBP also restored the immune status with respect to all parameters studied. Restoration of activity of EXM in the lymphocytes was observed at the same time, confirming that activity of EXM in lymphoid cells is linked with their function [7]. Administration of anabol to normal animals was not followed by any significant changes in CI or in activity of EXM, which is further confirmation of the immunomodulating character of the action of anabol.

Thus anabol possesses the properties of an immunomodulator mainly in relation to the effector stage of the T-system of immunity and it has a normalizing action on activity of EXM involved in both the 1st and 2nd phases of xenobiotic transformation in lymphocytes.

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