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INTERLEUKIN 2-INDUCED RECOVERY OF NATURAL KILLER ACTIVITY IN STRESS

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Interleukin 2 (IL-2), or T-cell growth factor, promotes the maintenance and proliferation of functionally active populations of T lymphocytes and natural killer cells (NKC) [5-7, 9]. This lymphokine is produced mainly by T helper/amplifier cells and it acts on cytotoxic lymphocytes [9]. Besides its distant action, which characterizes it as a hormone-like factor of the immune system, IL-2 can effect autocrine stimulation, as has been shown for some cytotoxic T lymphocytes and NKC [4, 10].

The writers previously showed inhibition of concanavalin A (con A) -induced IL-2 production by the spleen cells of mice exposed to stress [2]. On the basis of these findings it was postulated that disturbance of IL-2 production may lie at the basis of the pathogenesis of stress-induced disturbances of the immune system. A deficiency of this lymphokine is evidently one of the main links in the mechanism of stress-induced depression of NKC activity.

On the basis of the above remarks, and considering the inducing action of IL-2 on NKC, in the investigation described below IL-2 was studied as one of the pathogenetic agents in the experimental treatment of the immunodepressive state of the system of natural antitumor resistance, arising as a result of exposure to stress.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice aged 12 weeks and exposed to immobilization stress for 6 h [2]. NKC activity was determined 24 and 48 h after the end of exposure to stress and injection of IL-2 in the microcytotoxicity test, with release of radioactive chromium from YAC-1 and K 562 target cells [1, 3]. IL-2 (titer (1:512), obtained from the supernatant of a culture of BALB/c mouse spleen cells (5×10^6 cells/ml), stimulated by con A (5 μg/ml), was used in experiments *in vivo* and *in vitro* [2]. The residual leukin in the IL-2 preparations was neutralized with α-methyl-D-mannoside (20 mg/kg) or was removed by adsorption on Sephadex G-150 [8].

EXPERIMENTAL RESULTS

There were two stages to the investigation. In the first stage the action of IL-2 was assessed *in vitro* on NKC of intact mice and of animals exposed to immobilization stress. IL-2 (10 μl) was added to the wells in culture panels containing effector and target cells (T lymphoma, YAC-1) in a total volume of 200 μl and cultured for 4 h at 37°C in a CO_2 incubator. During this time no changes were found in NKC activity in the presence of IL-2 in either of the test groups.

In the next experiments the effector cells were incubated with IL-2 for a longer period — 18 h. At the end of this the effector cells were washed to remove IL-2, after which ^{51}Cr-labeled YAC-1 target cells were added to it and incubation was continued for a further 4 h. Preliminary incubation of the spleen cells with IL-2 for 18 h led to an increase in NKC ac-

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TABLE 1. Effect of IL-2 on Stress-Induced Depression of NKC Activity 48 h After Injection of IL-2

Group of animals	Activity of NKC (in %) with effector and target cells in the ratio given below			
	100:1	50:1	25:1	10:1
1. Control	23,1±2,5	13,8±1,5	9,0±1,1	2,5±0,3
2. Control + IL-2	21,2±2,2	17,6±1,6	9,5±1,2	3,5±0,2
3. Stress	15,2±1,6	11,3±1,4	5,7±0,6	1,9±0,3
4. Stress + IL-2	20,2±2,1	18,5±1,6	10,8±0,8	4,3±0,3
P_1—P_3	<0,05	>0,05	<0,05	>0,05
P_3—P_4	<0,05	<0,05	<0,05	<0,05

Legend. At least 10 mice were used for each determination.

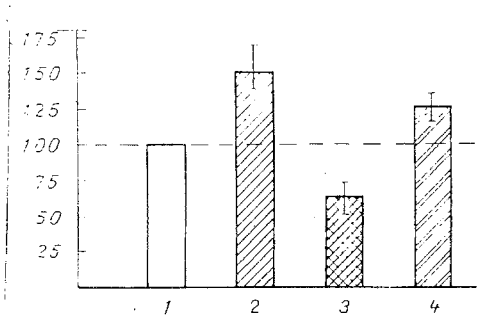

Fig. 1. Effect of interleukin 2 *in vitro* on activity of splenic NKC of control mice and of mice exposed to stress. Ordinate, activity of NKC (%). 1) Control; 2) control + IL-2; 3) stress; 4) stress + IL-2.

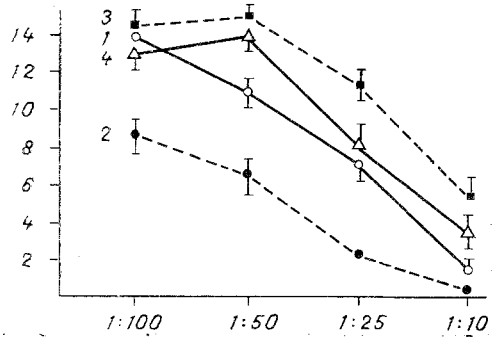

Fig. 2. Recovery of NKC activity, after stress-induced depression, in a splenocyte population from CBA mice with the aid of IL-2. Abscissa, ratio of effectors to target cells; ordinate, cytotoxic index (%). 1) Control; 2) stress; 3) control + IL-2; 4) stress + IL-2.

tivity of the intact animals by about 50% (Fig. 1). IL-2 doubled the lytic activity of NKC of animals exposed to stress (Fig. 1).

The successful use of IL-2 *in vitro* to repair stress-induced disturbances of NKC activity meant that it was possible to move on to experiments to study immunotherapy with this lymphokine *in vivo*.

The second stage of the work was to study the action of IL-2 *in vivo* on activity of mouse NKC. The IL-2 preparation was injected intraperitoneally into mice in a single dose of 500 units. Mice of the control group were given an intraperitoneal injection of culture medium not containing IL-2 or con A. IL-2 was injected into the mice immediately after the

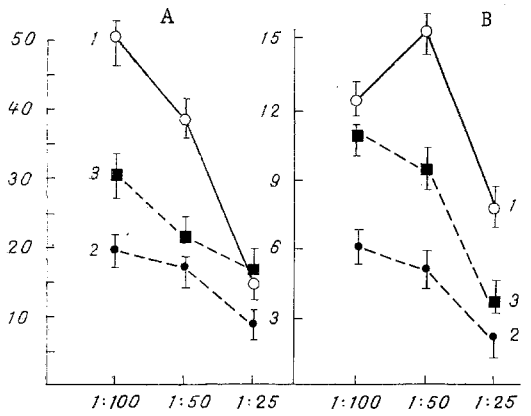

Fig. 3. Effect of IL-2 on restoration of depressed NKC activity and of effectors of lectin-induced cellular cytotoxicity in CBA mice exposed to immobilization stress. A) Cytotoxity induced by agglutinin from *Helix pomatia*; B) activity of NKC (target cells K 562). Abscissa, ratio of effectors to target cells; ordinate, cytotoxic index (%). 1) Control, 2) stress, 3) stress + IL-2.

end of exposure to stress, and NCK activity was estimated 24 h and 48 h after injection of IL-2.

The data given in Fig. 2 reflect the results of experimental treatment of depression of NKC activity 24 h after injection of IL-2. By this time IL-2 had significantly increased the NKC activity of intact animals and had restored the activity of these cells when depressed by stress. Activation of the cytolytic function of mouse NKC by IL-2 after exposure to stress was so marked that the value of the cytotoxic index, with certain dilutions of effector cells, exceeded the corresponding values in animals of the control group.

Data on the therapeutic action of IL-2 48 h after its injection on NKC activity are given in Table 1. They show a reduction in stress-induced depression of the cytolytic activity of NKC, characteristic of this period of testing, and they demonstrate complete restoration of NKC function by means of IL-2.

This effect of IL-2 was verified also in a natural cytotoxicity system with the use of different target cells (K-562), and also in the lectin-induced cellular cytotoxicity test (LICC). The results of these experiments are given in Fig. 3. Activity of NKC against the xenogeneic target K 562 (human erythromyeloleukemia) was increased in mice exposed to stress, receiving IL-2. The same pattern was clearly revealed in the LICC test also.

These experiments confirmed yet again the high efficacy of IL-2 when used in nonspecific immunotherapy to correct the function of cells of natural resistance, when disturbed by exposure to stress.

The IL-containing supernatant was tested for the presence of interferons, which, as we know, are modulators of NKC function. The IL-2 preparations used did not contain gamma-interferon. Thus the immunotherapeutic effect of these preparations can be attributed entirely to IL-2.

The results are evidence that IL-2 can be used as a promising pathogenetic agent for the correction of established disturbances of natural killer cell activity in an organism exposed to extremal external environmental factors.

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