EFFECT OF DIUCIPHON ON NATURAL CYTOTOXIC ACTIVITY OF SPLEEN CELLS OF OLD MICE

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The increase in the frequency of tumors during aging is associated with weakening of the effectiveness of the immune system. In recent years an important role in antitumor protection has been ascribed to the system of natural resistance and, in particular, to natural, normal killers (NK) cells. The level of activity of NK cells in old mice is considerably depressed [10, 13]. Previously, in a study of the number of NK cells present in the spleen of old mice and the ability of these cells to respond by increased activity to interferon, the writers showed that the ability of the NK cell population to respond to leukocytic interferon and to interferon inducer (synthetic polyribonucleotide) is modified in old animals [2]. In the search for ways of regulating function of the immune system, the attention of many investigators has been drawn to interleukin-2 (IL-2), one of the central mediators of the immune system, maintaining proliferation and increasing the cytotoxic activity of NK cells. There is evidence that IL-2 production is depressed in old animals [8, 12, 14]. Among the substances inducing IL-2 synthesis, a special place is occupied by diuciphon, which can stimulate production of T-cell growth factor, without any direct influence on the process of cell proliferation [4-6]. Diuciphon has been shown to raise the level of natural cytotoxic activity of human blood cells and of mouse splenocytes [1, 7]. The writers previously reported that diuciphon can modify the functional activity of spleen cells not only of young, but also of old mice, increasing the ability of these cells to respond by proliferation to mitogens and to alloantigens 1-3 days after injection of the preparation [3].

The aim of the present investigation was a comparative study of the effect of diuciphon on the level of natural cytotoxic activity of spleen cells of young and old mice.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice aged 2-3 and 24-36 months, obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR. Diuciphon was injected intraperitoneally in doses of 5 or 25 mg/kg body weight. The mice were decapitated 1, 3, and 5 days after injection of the preparation. Cell suspensions were prepared from the spleens. The natural cytotoxic activity of the cells was determined by the test of release of ^{51}Cr from labeled target cells, as which T-lymphoma cells from YAC-1 mice, transplanted in vitro, were used. The target cells $(5\text{-}10)\cdot10^6$ in number, were incubated with 100 μCi $Na_2{}^{51}CrO_4$ (specific radioactivity 1 mCi/mmole, from Amersham International, England) for 1 h at 37°C in medium RPMI-1640 with 10% fetal serum and 1% glutamine. Labeled target cells ($2\cdot10^4$) were placed in wells of round-bottomed panels with effector cells in the ratio of 1:100 1:50, and 1:25 (total volume 0.2 ml). The cells were incubated for 4 h at 37°C. The panels were then centrifuged at 200 g for 3 min. Radioactivity of 0.1 ml of the supernatant was measured by means of a Rack Gamma gamma-counter. The cytotoxic index (CI) was calculated in percent by the formula:

$$CI = \frac{\text{Number of counts (experiment} - \text{spontaneous yield)}}{\text{Number of counts (maximal yield} - \text{spontaneous yield)}} \times 100.$$

In one series of experiments splenocytes of intact mice were treated with diuciphon (5. 1, and 0.5 μg/ml) for 1-3 h at 37°C before testing of their cytotoxic activity, and the preparation was then washed or left in the test system.

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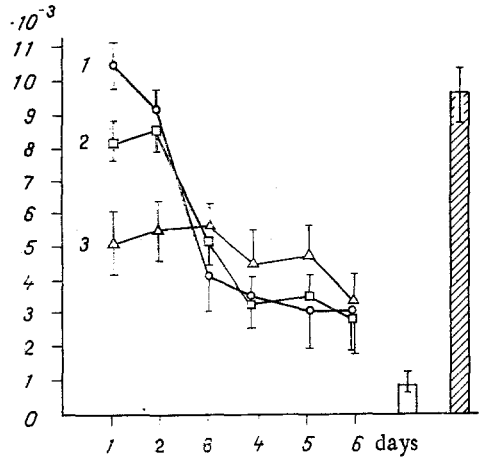

Fig. 1. Incorporation of ^{3}H-thymidine by IL-2-dependent cells during culture for 24 h with supernatants from con-A-stimulated cultures of spleen cells of CBA mice aged 2.5 (I), 10 (II), and 30 (III) months. Unshaded column — medium, shaded column — IL-2.

To obtain supernatants containing IL-2, splenocytes of intact young or old CBA mice, in a concentration of $5 \cdot 10^{6}$ cells/ml, were incubated with concanavalin A (con A) in a concentration of 5 μg/ml for 1, 2, 3, 4, 5, and 6 days at 37°C. The cell suspension was centrifuged at 300 g for 10 min. The supernatant was filtered through a millipore filter (0.22 μ) and kept at —40°C.

Activity of IL-2 was tested by means of 96-h T blast cells [9], activated by con A under the same conditions as to obtain supernatants. Before testing for IL-2 the blast cells were washed twice or three times with medium. Into the wells of the panels 100 μl of a suspension of blast cells ($0.75 \cdot 10^{5}$) was added, followed by 100 μl of the test supernatants. After incubation for 20 h, 20 μl of ^{3}H-thymidine (1 μCi, specific activity 5-6 Ci/mmole) was added to each well. The cells were harvested on a filter by means of a 12-channel harvester. Radioactivity of the acid-insoluble fraction was measured on a Packard scintillation counter.

EXPERIMENTAL RESULTS

The study of the ability of spleen cells from CBA mice to produce IL-2 in response to stimulation by con A yielded results (Fig. 1) showing that splenocytes of old mice produce much less IL-2 than those of young mice. The rate of decrease of the IL-2 concentration in the supernatants during culture of mitogen-stimulated cells for a few days characterizes the intensity of ultilization of IL-2 by the cells, which was clearly higher in cell cultures from young animals.

Spleen cells from old mice had a much lower level of natural cytotoxic activity than splenocytes of young animals (Table 1). A single injection of diuciphon, inducing synthesis of T-cell growth-maintaining factor, led to some increase in NK-cell activity after 1 and 3 days in the young animals, and to a more marked increase in old mice.

Preliminary treatment of the splenocytes with diuciphon in vitro followed by rinsing or leaving the preparation in the test system caused no increase in natural cytotoxic activity of cells of either young or old animals (Table 2). Diuciphon thus had no stimulating effect on natural cytotoxic activity of splenocytes during short-term incubation with the cells in vitro for 5-7 h. It has been shown that this preparation does not affect cell proliferation and has no interferon-producing action [4, 6]. The supernatant obtained after 24 h from cell cultures treated with diuciphon was able to maintain growth of mitogen-stimulated T cells [4]. This factor was close to IL-2 in its biological action and in some of its biochemical characteristics.

It was shown previously that NK cell activity is increased in young mice 24 h after receiving diuciphon [1, 7]. The results of the present investigation demonstrated that diuciphon can cause the level of natural cytotoxic activity of the splenocytes of old mice to be raised 24 h after administration of the preparation. This effect lasted a further 2-3 days. Although the increase in NK cell activity in old mice was higher relative to its initial level than in young mice, the parameters of diuciphon-stimulated NK-cell activity were far lower in these animals than in young mice. The low level of NK cell activity in old mice is due, at least in part, to a decrease in the number of effector cells in the splenocyte population [2]. It has been shown that during aging the number of NK-cell precursors also falls considerably [8, 11]. Previously the writers attempted to correct the level of NK cell

TABLE 1. Changes in Level of Natural Cytotoxic Activity of Spleen Cells from Old and Young CBA Mice after Treatment with Diuciphon

Time after addition of diuciphon, days	Ratio of effector cells:target cells		
	100:1	50:1	25:1
	Young mice		
0	18,2±1,8	15,8±2,7	10,6±2,4
1	19,0±2,7 (+4,4)	19,5±3,2 (+23,4)	14,2±3,3 (+34,0)
3	22,9±2,8 (+25,3)	20,2±2,5 (+27,9)	12,5±2,1 (+17,9)
5	15,6±6,5 (−14,3)	13,0±4,3 (−17,3)	9,1±2,4 (−14,3)
	Old mice		
0	3,4±0,7	4,5±0,8	2,8±1,0
1	5,4±1,2 (+58,8)	6,9±1,9 (+53,3)	5,5±1,8 (+100,0)
3	5,8±0,7 (+70,6)	5,8±0,7 (+128,9)	4,2±0,7 (+150,0)
5	5,2±1,1 (+52,9)	4,9±1,1 (+8,9)	2,9±0,7 (+3,6)

Legend. Values of CI (M ± m) are given, with percentage of increase or decrease of CI in parentheses.

TABLE 2. Effect of Preliminary Treatment of Mouse Spleen Cells with Diuciphon on NK Cell Activity

Concentration of diuciphon, μg/ml	Ratio of effector cells:target cells		
	100:1	50:1	25:1
	Young mice		
—	25,7±1,5	19,4±1,7	11,6±1,0
5	26,0±1,6	18,5±1,3	11,3±1,2
1	22,5±1,3	18,6±1,2	11,6±1,1
0,5	26,0±1,5	20,6±1,3	11,9±0,9
	Old mice		
—	2,9±0,3	3,9±0,3	2,9±0,5
5	2,8±0,3	3,4±0,4	2,0±0,3
1	1,8±0,2	2,8±0,3	1,9±0,2
0,5	1,9±0,3	3,0±0,4	2,5±0,3

Legend. Values of CI (M ± m) given.

activity in old mice with the aid of α-interferon [2]. Neither intraperitoneal injection of α-interferon nor treatment of cells in vitro gave any significant increase in natural cytotoxic activity of the splenocytes of old animals.

The results of experiments to study the possibility of modifying NK cell activity in old mice with the aid of a substance (diuciphon) stimulating cells to produce IL-2 also proved to be not sufficiently successful. Thus the low level of natural cytotoxic activity in old mice, despite a definite increase after administration of diuciphon, cannot be substantially enhanced (brought close to its level in young animals) by administration of a lymphokine inducer with the ability to regulate natural killer cell activity positively.

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