CHARACTERISTICS OF NORMAL KILLER (NK) CELLS AND THE CYTOTOXIC TEST IN SYRIAN HAMSTERS

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The natural cytotoxicity of lymphoid cells of Syrian hamsters has received little study. In the only paper on this subject [2] normal killer (NK) cells of Syrian hamsters were investigated and some of their characteristics obtained. However, there are reports [6] which cast some doubt on whether this important effector system is present in hamsters. Nevertheless, together with macrophages, NK cells are possibly the first barrier along the pathway of tumor development and effective in the early stages of carcinogenesis [1, 4].

Investigations on other species of animals (rats) and man have led to NK cells being classed as large granular lymphocytes [8, 9].

In the investigation described below the natural cytotoxicity of lymphoid cells of Syrian hamsters was studied. Data are given on the characteristics of NK cells and the trend of their cytolytic activity as revealed by the cytotoxic test *in vitro* with ^{51}Cr-labeled human K-562 myeloma target cells.

EXPERIMENTAL METHOD

The *in vitro* cytotoxic test suggested by Herberman et al. [3] was used. Effector cells were prepared from the spleen, bone marrow, thymus, and peripheral lymph nodes. Peripheral blood lymphocytes (PBL) were isolated on lymphocyte isolation medium (Flow Laboratories) after centrifugation for 30 min at 1500 rpm. In some experiments, to remove adherent lymphoid cells they were adsorbed on plastic petri dishes for 2 h at 37°C or on columns with nylon wadding, and by treatment with iron carbonyl [1]. To isolate NK cells from a suspension of nonadherent lymphoid cells, the suspension was centrifuged in a Ficoll density (1.078) gradient. The target cells in all experiments were cells of a 2-3-day culture of human myeloma strain K-562, labeled with ^{51}Cr in a dose of 50-60 μCi. The cytotoxic test was set up in 96-well conical plates, using three wells for each variant of the experiment. The reaction was read after incubation for 18-20 h in an atmosphere of CO_2 at 37°C. The results were estimated by the equation:

$$\% \text{ lysis of target cells} = \frac{\text{Mean outflow of chromium in experiment} - \text{spontaneous outflow of chromium}}{\text{Maximal outflow of chromium} - \text{spontaneous outflow of chromium}} \cdot 100.$$

For competitive inhibition of the cytotoxic test, according to the method of Herberman et al. [3], competitive unlabeled "cold" K-562 cells were added to the working dose of ^{51}Cr-labeled K-562 target cells in the ratios of 1:1, 5:1, and 10:1 to the labeled K-562 cells. Next, effector lymphoid cells were added to the mixture of labeled and "cold" target cells in a rato of 100:1 to labeled target cells only. The resulting samples were poured in a volume of 0.2 ml into the wells in the plate (three wells for each variant of the experiment). The results were read, just as in the cytotoxic test, after incubation for 18 h at 37°C in an atmosphere of CO_2.

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TABLE 1. Cytotoxic Activity of Spleen Cells of Individual Syrian Hamsters with K-562 Target Cells

No. of animal	Percent lysis of K-562 target cells with undefmentioned ratio of effector to target	
	300:1	75:1
1	18,8	9,7
2	1,6	0
3	1,5	0
4	29,0	12,5
5	8,8	5,1
6	13,6	4,8
7	11,0	7,1
8	17,3	8,1
9	9,4	3,2
10	24,6	7,8
Mean	13,56±2,9	5,82±0,94

TABLE 2. Cytotoxic Activity of Syrian Hamster Spleen Cells after Removal of Adherent Cells and Isolation in Ficoll Density Gradient

Treatment of spleen cells *	Percent lysis of ^{51}Cr-labeled K-562 target cells in cytotoxic test			
	ratio of effector to target			
	200:1	200:1	250:1	100:1 †
Control	18,4	23,0	15,7	12
Adsorption on plastic	21,2	—	—	—
Adsorption on nylon wadding	—	26,7	—	13,7
With iron carbonyl	—	—	13,6	—
Centrifugation in Ficoll density gradient 1,078	—	—	—	62

*In each experiment a mixture of cells from three hamsters aged 3-5 months were used.
†A suspension of spleen cells adsorbed beforehand on nylon wadding was used.

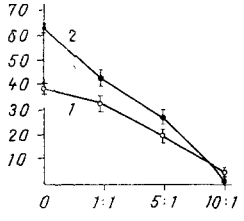

Fig. 1

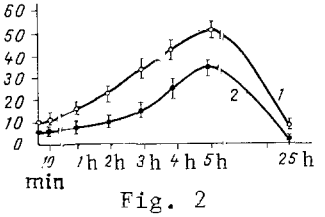

Fig. 2

Fig. 1. Competitive inhibition of cytotoxicity. Abscissa, ratio of unlabeled "cold" cells and ^{51}Cr-labeled target cells; ordinate, % lysis of ^{51}Cr-labeled target cells.

Fig. 2. Determination of optimal times for detecting cytotoxic activity of Syrian hamster NK cells in cytotoxic test *in vitro* with K-562 target cells (results of three experiments). 1) PBL, 2) spleen cells. Abscissa, duration of contact of NK cells with target cells (in h); ordinate, % lysis of K-562 target cells.

TABLE 3. Cytotoxic Activity of Syrian Hamster NK Cells in Various Organs (data of cytotoxic test with ^{51}Cr-labeled K-562 target cells)

No. of experiment	Number of animals	Results of cytotoxic test with NK cells				
		from blood	from spleen	from bone marrow	from thymus	from mesenteric lymph nodes
1	3	22,1 (12—42)	11,6 (0—19)	9,1 (0—15,7)	1,0 (0—3)	0,6 (0—2)
2	3	13,5 (10—15,7)	21,6 (11—40)	5,3 (2,9—8,1)	4,0 (1—7)	0,3 (0—1)
3	2	24,5 (24—25)	9,2 (9—9,4)	1,5 (1,5)	1,75 (1—2,5)	0,5 (0—1)
4	2	17 (17)	8,2 (6,0—10,4)	6 (3—9)	3,7 (3,2—4,2)	0,5 (0—1)
5	3	13,4 (13—14)	15,6 (6—21)	4,9 (2,9—8)	3,6 (0—6,3)	2,3 (1,2—3,4)
6	2	22,0 (15,4—28,6)	22,4 (17,8—27)	5,5 (4—7)	1,4 (0—2,8)	1,4 (0—2,8)
7	3	16,5 (14,2—19)	23,5 (12—41)	7,9 (5,8—10)	1,0 (0—3)	0 (0)
Mean values for 18 hamsters		18,4±2,0	16±2,36	5,7±0,83	2,3±0,5	0,8±0,32

Legend. Ratio of effector to target was 100:1 in all experiments.

EXPERIMENTAL RESULTS

In the first experiment the natural cytotoxicity of splenic lymphoid cells on noninbred 4-month-old Syrian hamsters was investigated. The natural cytotoxicity of splenic effector cells of individual animals relative to K-562 cells was found to vary considerably and on average for 10 hamsters, with a ratio of effector to target of 300:1, it was 13.56 ± 2.0% (Table 1). The lytic activity of the hamsters' splenic lymphocytes was proportional to their dose in the experiment. The optimal ratios of effector to target were 200:1 and 100:1. The specificity of the cytotoxic test was confirmed in competitive inhibition experiments with unlabeled, "cold" K-562 target cells (Fig. 1).

NK cells are known to belong to the nonadherent subpopulation of lymphoid cells. Natural cytotoxicity of hamsters' spleen cells was studied after adsorption on plastic and on nylon wadding, after treatment with iron carbonyl, and also after isolation of NK cells in a Ficoll density gradient (Table 2). It will be clear from Table 2 that the natural cytotoxicity of a spleen cell in the present experiments was associated with nonadherent cells, and these cells could be isolated by centrifugation in a Ficoll density gradient of 1.078, which is selective for large granular lymphocytes [9].

Another important property of NK cells, as has been shown for man, mice, and rats [3, 5, 7], but not for Syrian hamsters, is their rapid activity, which is exhibited in the cytotoxic test after 3-4 h of contact with sensitive target cells. The pooled results of three experiments (Fig. 2) show that the cytotoxic activity of Syrian hamster spleen cells is exhibited only after contact for 8 h with labeled K-562 target cells and reaches maximal values after 10-14 h. An investigation of the natural cytotoxicity of PBL showed that their lytic activity was higher than that of spleen cells, and it was detectable after only 4 h. However, maximal values of cytotoxicity of PBL were obtained after contact with target cells for 12-16 h.

Unlike NK cells of mice, rats, and man, in order to detect maximal cytotoxic activity of Syrian hamster NK cells their contact for at least 10-12 h with sensitive target cells is thus required.

When studying natural cytotoxicity of the nonadherent lymphoid cells their distribution among the organs (spleen, bone marrow, blood, thymus, peripheral lymph nodes) was investigated in 18 Syrian hamsters aged 5-8 months (Table 3). The results showed that the highest natural cytotoxicity occurred in blood and spleen cells, it was low in bone marrow and thymus, and virtually absent in peripheral lymph nodes.

Lymphoid cells of Syrian hamsters, like those of animals of other species, thus possess natural cytotoxicity, which is effected by nonadherent NK cells; cytotoxic NK cells can be isolated from Syrian hamsters by centrifugation in a Ficoll density gradient that is selected for large granular lymphocytes. Unlike NK cells from mice, rats, and man, maximal lytic activity of Syrian hamster NK cells is exhibited after contact for 10-12 h between them and sensitive target cells. The highest natural cytotoxicity in hamsters is possessed by blood

and spleen cells, it is weak in bone marrow and the thymus, and virtually absent in peripheral lymph nodes.

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TUMORS INDUCED BY 1,2-DIMETHYLHYDRAZINE IN HYBRID MICE WITH PITUITARY GRAFTS IN THE KIDNEY

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Development of tumors of the large intestine and the anal region in mice, and also a high percentage of tumors of the uterus or ovaries in some strains of mice can be induced by 1,2-dimethylhydrazine (DMH) [3, 4, 8, 11]. Hormonal factors play an important role in the development of DMH-induced sarcomas of the uterus and vascular tumors of the ovaries in CBA mice [2, 3, 5].

The object of this investigation was to study the effect of prolactin, secreted by a pituitary graft beneath the capsule of the kidney, on carcinogenesis in hybrid mice treated with DMH.

EXPERIMENTAL METHOD

Experiments were carried out on 112 female (CBA × C57BL/6)F_1 mice aged 3 months (Table 1). The pituitary gland from 2-month-old female CBA mice was grafted into mice of groups 1 and 3 beneath the capsule of the left kidney, and a mock operation on the kidney was performed on the mice of group 2. DMH in aqueous solution was injected subcutaneously in a dose of 8 mg/kg once a week into mice of groups 2 and 3 for 30 weeks, starting with the 8th week after the operation on the kidney. The animals were kept under observation until their natural death, but some mice with large tumors were killed in an agonal state; these last mice were killed 60 weeks after the beginning of DMH administration. The material was subjected to a histological analysis and sections were stained with hematoxylin and eosin. The frequency of tumors was determined relative to the number of mice surviving up to 20 weeks — the time of discovery of the first tumor. Differences in frequency were assessed by the χ^2 method.

EXPERIMENTAL RESULTS

The frequency of the most commonly encountered tumors in the different groups of mice is shown in Table 1. DMH induced the development of polyps and adenocarcinomas of the large intestine as well as ademomas and carcinomas of the sebaceous glands of the anal region, in agreement with data in the literature. An increase in the frequency of vascular tumors of the liver was observed in the groups of mice receiving DMH, compared with group 1 ($P < 0.001$). In group 1, for instance, one angioma of the liver and three hepatomas were found, in group 2 there were eight angiomas, three hepatomas, one hepatocellular carcinoma, and one cholangi-

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