ACTIVATION OF NATURAL KILLER (NK) CELLS OF NORMAL AND TUMOR-BEARING SYRIAN HAMSTERS BY NEWCASTLE DISEASE VIRUS

T. E. Klyuchareva and V. A. Matveeva

UDC 612.112.94.017.4

KEY WORDS: interferon inducer; NK cells; irradiation; Syrian hamsters.

Interferon (IF) and its inducers considerably potentiate the toxicity of mouse [1], rat [3], and human [7] lymphoid cells *in vivo* and *in vitro*. Investigation of the mechanism of activation of natural killer (NK) cells in the cytotoxic test has shown that IF influences oxidation–reduction processes in the cell [2] and activates cellular enzyme systems [8]. Under the influence of IF inactive NK precursors differentiate into mature cytotoxic NK cells [4, 5].

The object of this investigation was to study the possibility of activation of NK cells obtained from various organs of normal Syrian hamsters and also of hamsters with tumors, by means of Newcastle disease virus (NDV), an IF inducer. This paper gives the results of the cytotoxic test on irradiated Syrian hamster NK cells.

EXPERIMENTAL METHOD

The natural cytotoxicity of hamster lymphocytes *in vivo* was activated in random-bred Syrian hamsters (from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, aged 4-5 months) by intraperitonal injection of NDV in a titer of 10^9 ID_{50}. The virus was obtained by infection of the allantoic vesicle of 10-day-old chick embryos. At various periods after introduction of the virus, the hamsters were ether anesthetized, the spleens were sterilely extracted, and a cellular suspension was prepared. In addition to the spleen cells, suspensions of bone marrow cells and peripheral blood lymphocytes were also studied. The obtained cell suspension, freed from adhering cells by passage through a nylon filter, was used as effectors in a cytotoxic test with ^{51}Cr-tracer K-562 target cells. The reactions were followed over the course of 18 h.

Activation of NK lymphoid cells by NDV *in vitro* produced the following: Upon precipitation of the studied effector cells, taken in aliqouts of $1 \cdot 10^7$-$3 \cdot 10^7$ cells, NDV was added in a titer of 10^9 ID_{50}. After contact for 10 min at room temperature the cells were washed free from virus and used for the cytotoxic test. To estimate activation of natural cytotoxicity of the test lymphocytes, the statistical significance of differences between spontaneous and NDV-activated natural cytotoxicity was determined and the activation index was calculated as the quotient obtained by dividing the results of the cytotoxic test in the experiment and control. An index of 2.0 or higher characterized a high degree of activation of NK cells, and index of 1.0 indicated absence of activation.

The experimental hamsters were irradiated once, 2 h before removal of the lymphocytes, in special transparent plastic containers in a "Stebel'-1" γ-apparatus for 1 min (750 rads). Suspensions of effector cells for the cytotoxic test were prepared from the organs of the irradiated hamsters. In some experiments suspensions of lymphocytes from unirradiated animals were irradiated *in vitro* before or after adsorption of NDV in a dose of 750 rads.

EXPERIMENTAL RESULTS

Intraperitoneal injection of NDV into noninbred Syrian hamsters potentiated the natural cytotoxicity of nonadherent spleen cells when investigated in the cytotoxic test *in vitro*

Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 9, pp. 86-89, September, 1983. Original article submitted December 29, 1982.

 0007-4888/83/9609-1294$07.50 © 1984 Plenum Publishing Corporation

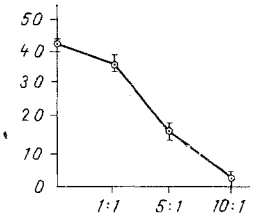

Fig. 1. Competitive inhibition of cytotoxicity: 1) without activation; 2) activation by NDV. Abscissa, ratio between "cold" and ^{51}Cr-labeled target cells; ordinate, % lysis of K-562 target cells.

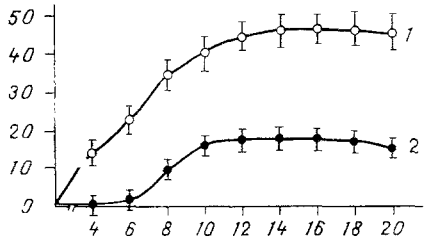

Fig. 2. Cytotoxicity of hamster splenic karyocytes depending on time of activation by NDV *in vivo*. 1) Ratio of effector cells and target cells 300:1; 2) ratio of effector cells and target cells 75:1. Abscissa, time after injection of NDV into hamsters (disregarding reaction time); ordinate, % release of ^{51}Cr.

TABLE 1. Spontaneous and NDV-Activated Cytotoxic Activity of NK Cells of Syrian Hamsters Irradiated *in Vivo* (data of cytotoxic test *in vitro*)

Experimental conditions	Activation by NDV	% lysis of K-562 target cells with NK cells		
		from blood *	from spleen *	from bone marrow †
Control	—	24,7	17,0	2,1
	+	45,2	33,1	28,5
Irradiation with 750 rads	—	27,1	20,5	6,2
	+	48,4	41,4	26,9

*Peripheral blood lympohyctes isolated from blood of three hamsters on Ficoll with density 1.078 were studied.
†Mixture of cells from three hamsters, freed from adherent cells by adsorption on plastic beforehand was studied.

with ^{51}Cr-labeled K-562 target cells. The results of two experiments are summarized in Fig. 1; they show that the level of natural cytotoxicity of hamster splenic NI cells depends on the time of their treatment with NDV. NK activity of the experimental animals was increased two- to sixfold 4-5 h after infection with NDV. However, the activation was of short duration, and by 25 h after addition of NDV the level of cytotoxicity of the NK cells had fallen to its initial values.

It was also shown that contact between NDV and nonadherent spleen cells *in vitro* for 10 min at any temperature was sufficient for subsequent exhibition of maximal cytotoxic activity of the NK cells tested. It must be pointed out that potentiation of cytotoxicity of the lymphocytes under the influence of NDV was not reflected in the specificity of the cytotoxic test, as shown by the results of competitive inhibition of cytotoxicity of K-562 target cells not labeled with ^{51}Cr (Fig. 2).

The next step was to study the cytotoxicity of irradiated normal and NDV-activated Syrian hamster NK cells. NK cells from the mouse spleen are known to be resistant to x-ray

TABLE 2. Cytotoxic Activity of NK Cells of Syrian Hamsters Irradiated *in Vitro* (750 rads) and Activated by NDV (results of cytotoxic test *in vitro*)

Character of treatment of effector cells	Activation by NDV	% lysis of K-562 target cells with cells		
		from spleen*	from bone marrow*	from blood†
Normal (control)	—	7,0	2,1	22,7
	+	22,0	28,5	35,2
Irradiation with 750 rads	—	5,3	1,7	20,7
	+	20,4	25,6	32,4
	(before irradiation)			
	+	24,0	27,7	31,7
	(after irradiation)			

*Mixture of cells from three hamsters, freed from adherent cells by adsorption on plastic beforehand was studied.
†NK cells isolated by centrifugation in Ficoll density gradient.

TABLE 3. Activation by NDV of Cytotoxicity of NK Cells from Normal Syrian Hamsters, Taken from Different Organs (combined data of cytotoxic test with cells from 18 animals)

Organ	Treatment with NDV	% of lysis of K-562 target cells with ratio of effector to target cells of 100:1	Activation index	P*
Spleen	—	16±2,36		<0,01
	+	33,1±3,31	2,0	
Bone marrow	—	5,7±0,83		<0,001
	+	30,2±3,81	5,2	
Blood	—	18,4±2,0		<0,001
	+	38,1±4,1	2,0	
Thymus	—	2,3±0,5		>0,1
	+	3,2±0,42	1,3	
Mesenteric lymph nodes	—	0,8±0,56		>0,1
	+	1,3±0,72	1,6	

*Levels of cytotoxicity of NDV-activated and unactivated cells compared.

TABLE 4. Activation of NDV of Cytotoxicity of NK Cells Taken from Various Organs of Hamsters with Transplanted Tumors (combined results of cytotoxic test with cells from 19 animals)

Organ	Treatment with NDV	% of lysis of K-562 target cells with ratio of effector to target cells of 100:1	Activation index	P
Spleen	—	9,7±2,68		
	+	16,4±3,8	1,6	>0,1
Bone marrow	—	4,1±0,69		
	+	4,0±0,65	0,9	>0,9
Blood	—	17,2±2,21		
	+	24,8±3,29	1,4	<0,05
Thymus	—	6,8±1,57		
	+	10,3±1,3	1,5	⩽0,1
Mesenteric lymph nodes	—	4,7±1,0		
	+	9,4±1,4	1,9	>0,1

irradiation and their cytotoxicity is not reduced by doses of irradiation up to 1000 rads [6]. Experiments were carried out in which hamsters were irradiated in a dose of 750 rads on the "Stebel'-1" γ-apparatus 2 h before the effector cells were obtained. The results of the cytotoxic test with original and NDV-activated NK cells from the spleen, bone marrow, and blood of normal and irradiated animals are given in Table 1.

The results indicate that irradiation of hamsters in a dose of 750 rads did not affect spontaneous or NDV-activated cytotoxicity of NK cells in irradiated animals compared with normal. Similar results were obtained by γ-irradiation (750 rads) of a suspension of splenic NK cells (Table 2). The cytotoxicity of the NK cells was not reduced after irradiation. Treatment of the test cells with NDV before and after irradiation potentiated their cytotoxicity about equally.

NK activity is known to vary considerably in the different lymphoid organs of the hamster. It was strongest in the blood and spleen, but extremely weak in the bone marrow, thymus, and mesenteric lymph nodes. It was deemed important to discover if natural cytotoxicity of lymphocytes in the different organs could be activated by the IF inducer NDV.

Data on spontaneous and NDV-activated natural cytotoxicity of nonadherent cells from the spleen, bone marrow, blood, thymus, and mesenteric lymph nodes of 18 normal Syrian hamsters aged 5-6 months are given in Table 3. It will be clear from Table 3 that treatment with NDV caused a statistically significant increase in the cytotoxicity both of spleen cells and peripheral blood lymphocytes, which have high spontaneous natural cytotoxicity, and of bone marrow cells, with low initial NK activity. A more than fivefold increase in cytotoxicity of the activated bone-marrow lymphocytes was observed. The results can be taken as evidence that the bone marrow of Syrian hamsters contains many inactive NK precursors, whose differentiation into mature cytotoxic NK cells was stimulated by NDV-induced endogenous IF. The low values of spontaneous and activated cytotoxicity of thymus cells and mesenteric lymphocytes were probably connected with the absence of NK cells and their precursors in these organs.

There is evidence in the literature of a decrease in NK activity in animals with transplanted and induced tumors [6, 9]. It was accordingly decided to study to what degree NK cells from tumor-bearing animals could be activated. To study this problem a series of experiments was carried out to determine spontaneous and NDV-induced cytotoxic activity of NK cells from hamsters with transplanted sarcoma induced by SV_{40} virus.

At the time of the investigation each hamster had two to four subcutaneous tumor nodules, which were transplanted in one stage into animals 2 months before investigation. Table 4 summarizes data on natural cytotoxicity (spontaneous and NDV-activated) in 19 hamsters with tumors.

It will be clear from Table 4 that both spontaneous natural cytotoxicity of NK cells in the spleen, blood and, in particular, in the bone marrow of the tumor-bearing animals studied, and their ability to be activated by NDV were much less than in normal hamsters of the same age. Extremely low cytotoxic activity of nonadherent bone marrow lymphocytes was observed in all the animals studied and it was unchanged after treatment of the cells with NDV.

The results are thus evidence of marked inhibition of NK activity in the lymphoid organs of tumor-bearing hamsters and the practically complete exhaustion of the natural cytotoxicity of their bone marrow.

LITERATURE CITED

1. G. I. Deichman, in: Tumor Growth as a Problem in Biological Development [in Russian], Moscow (1979), pp. 208-230.
2. S. Datta, M. Gallagher, and S. Trentin, Int. J. Cancer, 23, 728 (1979).
3. R. B. Herbermann, T. Aoki, M. Nunn, et al., J. Natl. Cancer Inst., 53, 1103 (1974).
4. R. B. Herbermann and H. Holden, J. Natl. Cancer Inst., 62, 441 (1979).
5. M. Nunn, I. Djeu, G. Cannon, et al., J. Natl. Cancer Inst., 56, 393 (1976).
6. R. Rees, L. Hassan, and C. Potter, Br. J. Cancer, 41, 485 (1980).
7. M. Takasugi, M. Mickey, and P. Terasaki, Cancer Res., 33, 2898 (1973).
8. T. Timonen, Cell. Immunol., 48, 133 (1979).
9. T. Timonen, I. Ortaldo, and R. Herbermann, J. Exp. Med., 153, 569 (1981).