ACTIVITY OF HUMAN NATURAL KILLER CELLS AGAINST TARGET CELLS DIFFERING IN SENSITIVITY TO INTERFERON

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UDC 612.112.94.017.4.014.467:[
616-006.092.19:578.254

KEY WORDS: natural killer cells, target cells, interferon.

An important factor in the assessment of natural cytoltoxicity (NCT) of human lymphocytes is the choice of target cells (TC) used to obtain information about the mechanisms of realization of the cytotoxic potential of natural killer (NK) cells. Most investigations devoted to the study of active human NK cells in various physiological and pathological states have been conducted on a model culture of human K-562 erythromyeloblasts. Several investigators have used lines of T lymphoblasts (MOLT 4) and B lymphoblasts (Raji, Daudi, RPMI-1788) as TC or NK cells. Human lines of TC – BT-20 (mammary gland carcinoma), MA-160 (prostatic adenoma), HHMS (melanoma), etc. have been used in separate investigations.

Considering the key role of interferon (IFN) in the regulation of NK cell activity, including through its action on TC, it was decided to study correlation between the sensitivity of TC to the action of IFN and their resistance to NK cell-induced lysis. The aim of the present investigation was to study the cytotoxic activity of human NK cells against TC differing in their sensitivity to IFN.

EXPERIMENTAL METHOD

Mononuclear cells (MNC) used as effectors of NCT were isolated from peripheral venous blood of 14 healthy Group 1 (0) blood donors in a one-step Ficoll–Paque density gradient (Pharmacia Fine Chemicals, Sweden), d = 1.077 g/cm^3, by the method in [5]. Cells of the following lines maintained over a long period in culture, were used as TC.

1. K-562 (control), a line of human erythromyeloblasts obtained from the pleural exudate of patients with chronic myeloid leukemia in the blast crisis stage, and adapted to culture in vitro [8]. Highly sensitive to the action of NK cells.

2. I-96, a line obtained from the blood of a patient with subacute monocytic leukemia [9]. Highly sensitive to enteroviruses, sensitive to human IFN.

3. I-41, a subline of cells obtained by repeated exposure of I-96 cells to massive doses of Coxsackie B virus [4]. Highly and specifically resistant to Coxsackie B virus, possesses reduced sensitivity to human a/β-IFN.

4. L-929, a transplantable line of mouse fibroblasts [6]. Highly sensitive to murine IFN.

5. MCB, an embryonic cell line obtained from C57B mice [1]. Insensitive to the action of IFN.

Cytotoxic activity of NK cells was determined by a radiometric method [7] in the modification [2] against TC labeled with ^{3}H-uridine in a dose of 3 μCi/ml. The initial suspension of TC contained $10 \cdot 10^4$ cells in 1 ml of medium and the initial suspension of MNC contained $10 \cdot 10^6$ cells in 1 ml. For resuspension of the cells of the monolayer cultures, they were treated beforehand with heated Versene solution for 20 min at 37°C.

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Department of Interferons, N. F. Gameleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 406-409, October, 1990. Original article submitted October 10, 1989.

0007-4888/90/0010-1397$12.50

TABLE 1. Incorporation of ^{3}H-Uridine into TC Differing in Sensitivity to IFN, during the Cytotoxic Test (M ± m)

TC	^{3}H-uridine, cpm
Human model	
K-562 (n=54)	2580±141
I-96 (n=24)	378±48*
I-41 (n=26)	590±96*
Murine model	
K-562 (n=30)	1875±165
L=929 (n=12)	2029±233
MCB (n=14)	500±81*

Legend. *p < 0.001 compared with K-562 TC.

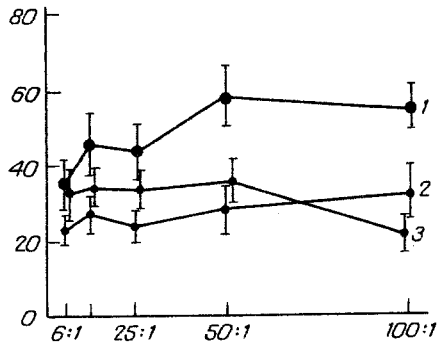

Fig. 1. Cytotoxic activity of NK cells from normal blood donors against TC of human lines I-96 and I-41, differing in their sensitivity to IFN. Here and in Fig. 2: abscissa, ratio E:T; ordinate, CTI (in percent), TC lines: 1) K-562 (n = 6), 2) I-96 (n = 10), 3) I-41 (n = 9).

Combined incubation of MNC and TC was carried out in complete nutrient medium based on RPMI-1640 (Amimed, Switzerland) 88 ml, bovine embryonic serum (N. F. Gamaleya Research Institute of Immunology, Epidemiology, and Microbiology, Academy of Medical Sciences of the USSR) 12 ml, Hepes (Serva, West Germany) 10 mM, glutamine 2 mM, and gentamicin 40 μg/ml, for 14 h at 37°C in a humid atmosphere containing 5% CO_2, and using 96-well round-bottomed microplanchets. The range of ratios of effectors to target cells (E:T) used varied from 100:1 to 6:1 [3].

At the end of incubation, 0.1 ml of supernatant was withdrawn from each well of the planchet with TC of monolayer cultures, 0.1 ml of heated Versene solution was added, and the planchets were incubated for 20 min at 37°C, after which the contents of the wells were transferred to glass fiber filters with a pore diameter of 2.5 μ (Whatman, England), by means of a Dynatech 12-channel biological fraction harvester (England). Residual radioactivity was determined with the aid of a Mark II scintillation β-counter (USA).

The cytotoxic index (CTI), a measure of NK-cell activity, was calculated by the formula:

$$CTI = \left(1 - \frac{\text{number of counts in experimental well}}{\text{number of counts in control}}\right) \times 100\%$$

The control for the cytotoxic test consisted of TC, incubated under the same conditions as the experimental cells, but without MNC.

The variability index (VI) of parameters of NK-cell activity was calculated as the ratio of the maximum deviation to the mean value at the given E:T ratio. The significance of the difference between the mean values was determined by Student's t-test.

TABLE 2. Variability of NK-Cell Activity in Cytotoxic Test Against TC Differing in Sensitivity to IFN (VI)

TC	Ratio E:T				
	100:1	50:1	25:1	12:1	6:1
Human model					
K-562	0,43	0,59	0,57	0,67	0,7
J-96	1,28	1,06	0,8	0,85.	0,74
J-41	1,3	0,86	0,47	0,7	0,84
Murine model					
K-562	0,72	0,48	0,67	0,37	0,5
L-929	0,96	0,94	0,92	0,31	1,15
MCB	0,15	0,79	0,91	1,03	1,93

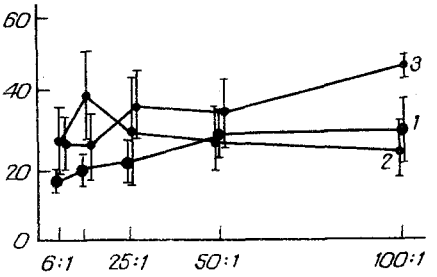

Fig. 2. Cytotoxic activity of healthy human NK cells against TC of murine lines L-929 and MCB, differing in their sensitivity to IFN. TC: 1) K-562 (n = 4), 2) L-929 (n = 6), 3) MCB (n = 6).

EXPERIMENTAL RESULTS

The results showed that human TC of lines I-96 and I-41 were more resistant to the cytotoxic action of NK cells than cells of the model K-562 culture (Fig. 1). Values of CTI for I-96 TC ranged from 32.8 ± 6.5% at a ratio E:T of 100:1 to 23.0 ± 3.6% at a ratio of E:T of 6:1. For I-41 TC, CTI varied from 22.2 ± 4.9% at a ratio E:T of 100:1 to 32.6 ± 5.9% at a ratio E:T of 6:1. Values of NK cell activity against TC of the control K-562 line varied from 55.8 ± 5.75% at a ratio E:T of 100:1 to 35.3 ± 5.9% at a ratio of E:T of 6:1.

Significant differences between the values of CTI for TC of lines I-96 and I-41 compared with parameters of lysis of K-562 cells were observed only for line I-41 with a ratio of E:T of 100:1 ($p < 0.01$). In the remaining cases, a level of significance higher than $p < 0.01$ could not be recorded. Thus TC of lines I-96 and I-41, differing in sensitivity to IFN, did not differ from one another in their resistance to lysis by NK cells.

The tendency for values of NK-cell activity against TC of human monocytic leukemia I-96 and I-41 to fall compared with K-562 TC could be attributed to technical differences during working with these TC and, in particular, to the fact that unlike the K-562 TC, maintained in suspension culture, the I-96 and I-41 TC formed a monolayer during culture. This last fact may interfere with resuspension and labeling of these TC. It will be clear from Table 1 that the intensity of incorporation of ^{3}H-uridine into I-96 and I-41 TC was lower than that for K-562 TC. First, however, parameters of isotope incorporation were four to five times higher than values accepted as background levels for the scintillator (not more than 100 cpm), so that the results can be considered to be technically reliable, and second, the possibility of working at these levels of incorporation is shown by the difference observed in the resistance of I-96 and I-41 TC to the action of NK cells compared with K-562 TC, at a ratio of E:T of 100:1.

Consequently, human cell lines I-96 and I-41, differing in their sensitivity to IFN, do not differ in resistance to the action of NK cells. It can thus be concluded that receptor systems (which have been characterized to a certain degree for IFN but virtually not identified for NK cells) are not interconnected on the surface of these TC and cannot form a single micromorphofunctional complex, determining some form of correlation between the sensitivity of these cells to NK cells and IFN.

The similarity of the TC in sensitivity to the action of NK cells also is manifested as a unique change in VI for the parameters of NK-cell activity during dilution of the system from the ratio of E:T of 100:1 to 6:1 (Table 2). By contrast with K-562 TC, lysis of TC of lines I-96 and I-41 is characterized by a successive fall of VI (that for the I-41 line is less marked).

Investigations conducted on a murine model (cells of lines L-929 and MCB, which are highly sensitive and resistant respectively to the action of IFN) have obtained evidence of the reproduction mainly of the situation arising for the human TC lines. As will be clear from Fig. 2, TC of lines L-929 and MCB do not differ from one another in their sensitivity to lysis under the influence of human NK cells (only when the ratio of E:T was 100:1 was a tendency observed for the sensitivity of TC of line MCB to increase, $p < 0.1$), and they correspond to the model human K-562 culture. Consequently, the hematologic characteristics of the TC lines do not determine differences in their interaction with NCT effectors.

The intensity of incorporation of the isotope into MCB TC differs significantly from that of K-562 and L-929 TC (Table 1), further confirmation of the reliability of results obtained on the human model. Finally, values of VI for NK-cell activity in the cytotoxic test with murine TC lines changed in opposite directions (Table 2). This indicates a difference between the fine mechanisms of lysis and interaction of NCT effectors with murine TC, which may perhaps be connected to some degree with the sensitivity of these cells to the action of IFN.

We must dwell on yet another factor of importance from the general biological point of view. The absence of any significant differences in the level of cytotoxic activity of human NK cells against human and murine TC (i.e., in an allogeneic and xenogeneic system) shows that the effectiveness of realization of the cytotoxic potential of NK cells, unlike other cells of the immuni system, reacting specifically to foreign antigens, is not determined by the degree of genetic differences between TC.

Thus murine cell lines, differing in their sensitivity to IFN, do not differ in resistance to lysis on interaction with NCT effectors, evidence of the absence of positive correlation between the molecular structures responsible for reception of IFN, and performing the function of molecular targets recognized by NK cells. These receptor molecules cannot evidently be connected on the surface of TC into a single complex and, consequently, on the evolutionary plane systems of NCT and IFN do not appear simultaneously, but perhaps as mutually compensating systems from the regulatory aspect.

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