REGULATION OF HUMAN NATURAL KILLER CELL ACTIVITY BY AN INTERFERON INHIBITOR

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The results of a study of the sensitivity of various lines of target cells (TC) to the antiviral action of interferon (IFN) point to the existence of negative regulation, exerted through the action of interferon inhibitors (IFNI), which are produced simultaneously with IFN when the latter is induced [4, 10]. IFNI, which inhibit the antiviral activity of human IFN relative to TC of the M-19 line and the corresponding activity of murine serum IFN on L 929 cells, have a marked antiprolierative action, which is manifested by a greater degree against TC of malignant lines and which is independent of species and also of the sensitivity of the TC to homologous IFN [2].

Previous findings suggest that IFNI may possess definite regulatory properties and may be involved in the mechanism of regulation of the sensitivity of TC to the cytotoxic action of natural killer cells, for which IFN is the key factor of differentiation and activation [12].

The aim of this investigation was to study the action of murine IFNI on resistance of murine TC of transplantable lines in the natural cytotoxicity test with human natural killer cells.

EXPERIMENTAL METHOD

Mononuclear cells (MNC) were isolated from peripheral venous blood of four healthy individuals in a one-step Ficoll-Paque density gradient ("Pharmacia Fine Chemicals." Sweden), d = 1.077 g/cm^3, by the method in [7].

The cytotoxic activity of natural killer cells in a suspension of MNC of lines L 929 (a transplantable line of mouse fibroblasts, highly sensitive to murine IFN [8]) and MCB (an embryonic cell line obtained from C57B mice, insensitive to the action of IFN [1]), labeled with ^{3}H-uridine in a dose of 3 μCi/ml, by means of a radiometric technique [9] in the modification in [3]. Combined incubation of MNC and TC was carried out for 14 h at 37°C in a humid atmosphere containing 5% CO_2. The composition of the complete nutrient medium prepared on the basis of RPMI-1640 was: RPMI-1640 ("Amimed," Switzerland) – 88 ml, fetal calf serum (N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, – 12 ml) HEPES ("Serva," West Germany) – 10 mM, glutamine – 2 mM, gentamicin ("Pharmachim," Bulgaria) – 40 μg/ml. The initial suspensions contained 10^7 MNC in 1 ml and 10^5 TC in 1 ml medium. Activity of natural killer cells was tested with ratios of effector and target cells of 100:1, 50:1, 25:1, 12:1, and 6:1.

The cytotoxic action (CTI) was calculated by the equation:

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

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

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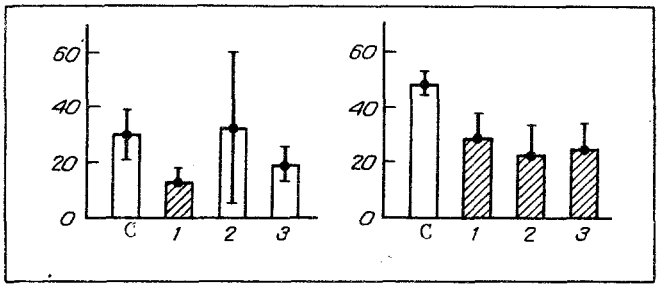

Fig. 1 Fig. 2

Fig. 1. Action of IFN (1), IFNI (2), and a combination of both (3) on sensitivity of L 929 TC to indirect cytolysis by natural killer cells. Here and in Fig. 2: ordinate, cytolysis (in per cent). C) control, shaded columns – $p < 0.05$ compared with control. Effector:target ratio 100:1.

Fig. 2. Action of IFN (1), IFNI (2), and a combination of both (3) on sensitivity of MCB TC to indirect cytolysis by natural killer cells.

IFNI, generously provided by V. V. Parfenov and T. A. Tikhonova, was contained in the ultrafiltrate of the culture fluid obtained by induction of IFN on MCB cells with Newcastle disease virus (100 CPD_{50} per cell) and concentrated after neutralization of the inducing virus in thin-channel wells of the TCF-10 apparatus ("Amicon," The Netherlands) by the method in [5].

To study the action of IFNI on sensitivity of TC to indirect cytolysis by natural killer cells, murine serum IFN-α/β in a dose of 100 U/ml, IFNI in a dose of 8 U/ml, and also IFN with IFNI in combination (in the above-mentioned doses) were added to a culture of TC for 24 h at 37°C followed by rinsing the preparations, labeling of the TC, and performance of the cytotoxic test.

The significance of the difference in the mean values was determined by Student's t test.

EXPERIMENTAL RESULTS

In agreement with the well documented protective action of IFN on TC, sensitive to the action of MNC in the natural cytotoxicity test [11], IFN reduced lysis of the L 929 TC during incubation of the latter with human MNC, possessing natural killer cell activity, by 2.2 times ($p < 0.05$; Fig. 1). The results correlate with other observations made with different ratios of effector and target cells, and which showed that under these circumstances natural killer cell activity was reduced in 6 of the 7 (i.e., in 86%) cases. Treatment of TC with IFNI did not change the cytotoxicity of the natural killer cells, which was observed also during the action of the combination of IFN with IFNI on TC. The effects observed are unconnected with variation of the intensity of incorporation of ^{3}H-uridine into L 929 TC: under the influence of IFN it was unchanged, whereas treatment of the TC with IFNI and also with a combination of IFN and IFNI led to an increase in the intensity of incorporation of the isotope into TC (Table 1).

Consequently IFNI, which is active against the antiviral effect of IFN, abolishes the protective action of the latter on TC of the L 929 line, which has marked sensitivity to IFN and to indirect cytolysis by natural killer cells [6], evidence that IFNI has immunoregulating properties, and suggesting that it may be regarded as an inhibitor of the regulatory action of IFN (IRAI) relative to the cytotoxicity of natural killer cells. When cells of the MCB line, resistant to the antiviral action of IFN, were used as TC, abolition of the protective effect of IFN in the natural cytotoxicity test on treatment of TC with IFNI could not be recorded. On the contrary, as is shown in Fig. 2, IFN, IFNI, and also a combination of both acted in the same way on sensitivity of the MCB TC to indirect cytolysis by natural killer cells. In all three cases activity of natural killer cells was reduced by 1.7 ($p < 0.05$), 2.1 ($p < 0.05$), and 1.9 times ($p < 0.05$) respectively. The effects discovered, just as in the case with L 929 TC, were unconnected with any variation in the intensity of incorporation of ^{3}H-uridine into TC. It was reduced by the action of IFNI and was unchanged on treatment of the TC with IFN and a combination of IFN with IFNI (Table 1).

TABLE 1. Effect of IFN, IFNI, and a Combination of Both on Incorporation of ^{3}H-Uridine into TC (M ± m; n = 7-8)

TC	Experimental conditions	Incorporation of ^{3}H-uridine, cpm
L929	Control	1903±339
	IFN	2910±382
	IFNI	4105±360*
	IFN + IFNI	5962±535*
MCB	Control	567±52
	IFN	773±205
	IFNI	266±21*
	IFN + IFNI	785±243

Legend. Asterisks indicate values for which $p < 0.01$ compared with control; $P_{IFN+IFNI/IFNI} < 0.01$ for L 929 TC; $P_{IFNI/IFN} < 0.05$ for MCB TC.

Consequently IFNI produced in response to induction of IFN possess marked regulatory properties relative to natural killer cell activity after preliminary treatment of TC with them. The regulatory action of IFNI on TC in the natural cytotoxicity test depends on their sensitivity to the antiviral effect of IFN. IFNI function as an IRAI, abolishing the protective action of IFN on cells of the L 929 line, highly sensitive to it, and as IFN, protecting to a certain degree TC of the MCB line, resistant to IFN, against indirect cytolysis by natural killer cells. Under these circumstances both lines of TC tested are highly sensitive to lysis in the natural cytotoxicity test [6], evidence that the mechanism of action of IFNI is linked with a change in expression of the surface structures of TC, facilitating or, correspondingly, impeding recognition of TC by natural killer cells and their subsequent lysis.

On the other hand, it was stated in [5] that a high rate of production of IFNI is observed during induction of IFN in MCB cells, resistant to IFN. A special form of substitution of function arises, in which a cell resistant to the antiviral action of IFN and producing high titers of IFNI proves to be sensitive to IFNI and, on treatment both with IFN and (which is important) with IFNI also, becomes resistant to indirect cytolysis by natural killer cells.

Thus information was obtained for the first time about the immunoregulatory properties of IFNI, which can function as IFN or as IRAI, and which evidently modify IFN receptors, as is confirmed by the absence of cumulation of protective action relative to MCB TC in the case of the combined use of IFN and IFNI (Fig. 2). In the case of high specificity of IFN receptors, IFNI block its reception, but if low-affinity receptors are involved in the mechanism of regulation of sensitivity of the cells in the natural cytotoxicity test, they lead to increased resistance of TC to indirect cytolysis by natural killer cells.

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