ACTIVATION OF MEMBRANE-TOXICITY AND CYTOTOXICITY OF TUMOR CELLS BY LEUKOCYTIC AND FIBROBLASTIC INTERFERON

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The writers showed previously [4] that tumor cells of transplantable sarcomas and leukemias of mice can injure the membrane of lymphocytes and also of certain other cells cultured *in vitro*. The degree of injury to the membrane of target cells depends on the level of RNA and protein synthesis in these cells [2]. It has been shown, in particular, that a significant lowering of RNA and protein synthesis in K-562 and IAC-1 target cells enables the cytotoxic action (cytotoxicity index up to 45%) of SA-1 ($H\text{-}2^a$) and IAC ($H\text{-}2^a$) cells to be observed in the test with ^{51}Cr [9]. In the investigations cited above, cytostatic, membrane-toxic, and cytotoxic interaction was thus observed between natural killers and tumor cells.

There is evidence in the literature [5, 12] of an increase in resistance of the tumor cell to the action of normal killers (NK) under the influence of exogenous interferon. The present writers have suggested that this is the result of potentiation of the harmful action of the tumor cell by interferon.

In the investigation described below the effect of interferon (leukocytic and fibroblastic) on the membrane-toxicity and cytotoxicity of tumor cells was studied.

EXPERIMENTAL METHOD

The following tumor effector cells were used: sarcoma MCh-11 ($H\text{-}2^b$), SA-1 ($H\text{-}2^a$), thymoma ($H\text{-}2^k$), and lymphoma ICA-1 and IAC ($H\text{-}2^a$). In control experiments, splenocytes of C57BL/6 mice were used as effectors. IAC-1 cells (IAC cells maintained *in vitro* for 24 h) were used as target cells. The technique for testing cell-mediated cytotoxicity was that described by the writers previously [1]. To prevent reutilization of degradation products of [^{3}H]-RNA the effector cells were treated with actinomycin D (1 μg/ml at 37°C for 1 h). The present writers and others [8] have shown that treatment in this way does not affect the cytotoxicity of NK. We know [9] that the cytotoxicity of peritoneal macrophages can be activated by interferon, and for that reason tumor cells for intraperitoneal transplantation were purified by adsorption twice on plastic Petri dishes (Flow Laboratories, England, No. 120307) for 35 min in medium RPMI-1640 with 10% fetal serum, in air with 5% CO_2. Fractionation was then carried out on a Ficoll–Hypaque gradient (density 1.077 g/cm^3, Pharmacia, Sweden). The writers showed previously that fewer than 1% of other cells still remain in a suspension of peritoneal tumor cells after treatment in this way [3].

The effector cells were treated with leukocytic or fibroblastic interferon. They were obtained as follows. Mouse fibroblasts were explanted after trypsinization of skin and muscle tissue from adult mice, and then grown by roller culture in bottles. Interferon synthesis was induced 5-6 days after monolayer formation by means of Newcastle disease virus (strain H) in a dose of 100 CPD_{50} per cell. The virus-containing liquid was poured off after 1 h and the cells were thoroughly washed and then incubated at 37°C in medium 199 with 10% adult mouse serum. The culture fluid was collected 18 h later and its interferon content determined. The interferon was purified by adsorption chromatography on highly porous glass (100-120 mesh). After purification the preparation was concentrated by reversed dialysis against polyethyleneglycol (mol. wt. 40,000 daltons, from Serva, West Germany) until the protein concentration in

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TABLE 1. Activation (in %) of Membrane-Toxicity and Cytotoxicity of Tumor Cells by Leukocytic and Fibroblastic Interferon

Effector cells	IM, %					IC, %		
	without treatment with IF	treatment with LIF	Activation of IM after treatment with LIF	IM after treatment with FIF, %	Activation of IM after treatment with FIF	without treatment with IF	treatment with LIF	Activation of IC after treatment with LIF
MCh-11	48±1	68±2	42	64±2	33			
SA-1	47±2	64±2	36	60±1	29	11±1	17±1	47
IAC	36±1	47±2	30	44±3	27	19±2	24±1	26
Thymoma	34±2	49±1	45	46±2	36			
NK	44±2	65±1	42	62±1	41			

Legend. Here and in Tables 2 and 3: IF) interferon, LIF) leukocytic interferon, FIF) fibroblastic interferon, IM) index of membrane-toxicity, IC) index of cytotoxicity.

TABLE 2. Abolition of Activation of Membrane-Toxicity of Tumor Cells by Anti-interferon Serum

Experimental conditions	IM of tumor effector cells and NK, %		
	SA-1	IAC	NK
Without treatment with IF	45±1	34±2	43±2
After treatment with FIF	61±2	52±2	62±3
After treatment with FIF and anti-interferon serum:			
titer 1:20	47±1	33±2	45±2
titer 1:400	49±1	34±2	46±2
titer 1:8000	50±1	36±2	49±1

TABLE 3. Effect of Interferon on Membrane-Toxicity and Resistance of Tumor Cells

Effector cells	Target cells	Effector/target ratio	IM, %	Increase in resistance of target cells treated with LIF
IAC	IAC-1	50:1	37±2	45
IAC	IAC-1 (LIF)	50:1	21±2	—
IAC-1 (LIF)	IAC-1	50:1	14±2	—
IAC-1 (LIF)	IAC-1 (LIF)	50:1	1,5±1	—

the preparations was 1.5 mg/ml. By this method the interferon was purified a hundredfold in terms of protein. Leukocytic interferon was obtained by injecting 1 ml of Newcastle disease virus (strain H) in a titer of 10^8 CPD_{50} into sexually mature mice. Serum was obtained from these mice 4-6 h later and the virus was inactivated for 3 days by lowering the pH to 2.0. The pH was then adjusted to the physiological value, the interferon titer determined, the product was divided into 1-ml batches, and lyophilized.

The interferon titer was determined by a micromethod on L cells against 100 CPD_{50} of vesicular stomatitis virus (Indiana strain), using plastic plates. Reference reagent (002: 904511, USA) was used in each measuring system and all the results were expressed in International Units, using this reagent.

The titer of the purifed preparations of fibroblastic interferon was 7000 IU, and the titer of leukocytic interferon 30,000 IU. The effector cells were incubated with different

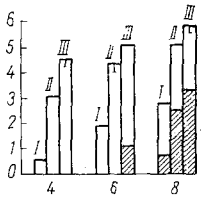

Fig. 1. Growth of subcutaneous tumor (thymoma H-2^k) in AKR mice after injection of tumor cells treated (unshaded column) and not treated (shaded columns) with leukocytic interferon (300 IU). Number of tumor cells injected into mice: I) 10^4, II) 10^5, III) 10^6. Abscissa, time (in days); ordinate, diameter of tumor (in mm).

doses of interferon (250-2000 IU) for 1 h after 37°C, then washed 3 times with large volumes of medium 199 with 10% fetal serum. Antiserum against fibroblastic interferon was obtained from Professor F. I. Ershov, to whom the authors are grateful.

EXPERIMENTAL RESULTS

Table 1 gives results showing activation of membrane-toxicity and cytotoxicity of tumor cells by leukocytic and fibroblastic interferon in a final concentration of 250 IU/ml of cell suspension (3×10^6 cells/ml). As Table 1 shows, under the influence of leukocytic interferon the membrane-toxicity of the tumor cells was increased by 30-44%, and the cytotoxicity was increased by 26-47%. Under the influence of fibroblastic interferon, membrane toxicity was increased by 27-40%. In the next experiments three different doses of leukocytic interferon were used. The results showed that if interferon was used in doses of 50 and 300 IU an effect of activation of membrane-toxicity of the tumor cells and splenic effectors was obtained, similar in magnitude to that shown in Table 1. On treatment with leukocytic interferon in a dose of 2000 IU the opposite effect was observed: inhibition of membrane-toxicity of the effector cells. Large doses of interferon thus suppress the effector properties both of tumor cells and of natural killers. This result agrees with data in the literature [7] on inhibition of the functions of T and B lymphocytes by large doses of interferon and the direct destructive effects of large doses of interferon on tumor cells during prolonged exposure [8].

The results of an experiment to abolish the activating action of fibroblastic interferon by treatment with anti-interferon serum are given in Table 2. With the serum in a dilution of 1:20 the activating action of fibroblastic interferon was completely abolished. With serum in a dilution of 1:8000 this effect was substantially less.

Table 3 gives the results of experiments carried out on cells of the same tumor (IAC-1 and IAC) in order to remove some of the variables. It was found that the membrane-toxicity of IAC-1 cells, treated with leukocytic interferon, in relation to intact IAC-1 cells increased simultaneously with an increase in their resistance relative to IAC cells.

The results of experiments *in vivo* deserves special attention. Injection of thymoma cells and sarcoma SA-1 cells, treated with leukocytic interferon, into mice (Fig. 1) caused the formation of large subcutaneous tumors earlier than when intact cells of the same tumor were injected.

Previously [2, 3] the writers demonstrated on different models the cytostatic, membrane-toxic, and cytotoxic interaction of NK and tumor cells. A series of investigations indicating increased resistance of the tumor cell to the action of NK under the influence of exogenous interferon also has been published recently [4, 11]. In particular, it was shown that if NK are treated with interferon before the formation of pairs with the target cell the cytotoxicity of the NK is sharply increased, but if previously formed NK cell—target cell pairs are treated with interferon the level of lysis of the tumor cells in the pairs does not rise. Treatment with interferon has been shown not to change the number of effector—target conjugates and not to raise the cytotoxicity level in the case of separate treatment of effectors and targets with interferon before pair formation, but it does raise the level of lysis in pairs if only the effector cells are pretreated with interferon.

It is suggested that the facts now described and data in the literature are interconnected. For instance, it may be postulated that the tumor cell, when stimulated by interferon, attacks NK more actively, and that this may account for the phenomenon, which some workers have described, of increased resistance of the tumor cell to NK under the influence of exogenous interferon. In our view, the increase in membrane-toxicity and cytotoxicity of the tumor cell observed under the influence of doses of interferon usually used to activate NK (up to 300 IU) explains the increased resistance of the tumor cell to natural killers and may serve as the starting point for a search for more effective and safer schemes of interferon treatment in medical practice.

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