POTENTIATION OF EFFECTIVE IMMUNOCYTOTHERAPY OF LEUKEMIAS IN MICE BY A NEW INTERFERONOGEN AND LEUKOCYTIC INTERFERON

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Soon after the discovery of natural (normal) killers (NK) their role in resistance to neoplasms was demonstrated [11, 2]. NK are now considered to be the first line of defense of the body against mutating and transformed cells [7]. Investigation of the heterogeneity of cells responsible for natural resistance has shown [4] apparent dissociation between the functional activity of cells mediating antitumor activity *in vivo*, *in vitro*, and in the tumor neutralization test [5]. The problem of the nature and origin of cells responsible for antitumor activity *in vivo* awaits solution [6].

Since a detailed study of the mechanisms of this phenomenon is of evident practical importance in the development of the principles of oriented immunocytotherapy of tumors, it was decided to investigate the possibility of potentiating the immunocytoeffector splenocytes *in vitro* with leukocytic interferon or by administration of interferonogen into the splenocyte donors.

EXPERIMENTAL METHOD

Inbred male C57Bl/6 (H-2^b), BALB/c (H-2^d), and A/Sn (H-2^a) mice weighing 16-18 g, obtained from the "Svetlye Gory" Laboratory Animals Nursery, Academy of Medical Sciences of the USSR, were used. Tumor cell lines EL-4 (H-2^b) (leukemia in C57Bl/6 mice), L-1210 (H-2^d) (leukemia in DBA/2 mice), and YAC (H-2^a) (leukemia in A/Sn mice) were maintained by subculture in mice of the corresponding haplotypes. The YAC-1 tumor cell line was maintained in culture *in vitro* [12].

Model of Adaptive Immunocytotherapy. Tumor cells of strains EL-4 (2×10^4) and L-1210 (5×10^4) were injected intraperitoneally into C57Bl/6 and BALB/c mice of the corresponding haplotype. On the 5th day the animals were given cyclophosphamide (180 mg/kg) by intraperitoneal injection and 6 h later they were given 2×10^7 syngeneic intact splenocytes or splenocytes treated with the various preparations, by the same route.

Cytotoxic Test *In Vitro*. The [^{3}H]-uridine method, developed and suggested previously [6], was used. YAC-1 (H-2^a) lymphoma cells were used as target cells. The tumor neutralization test was carried out both in the classical version and in the modification in [4].

The interferonogen, double-helical synthetic RNA — poly(I:C) in liposomes [2, 3] — was injected into the donors (0.25 ml per mouse, intraperitoneally) of the spleen cells 10-12 h before sacrifice or directly into mice with tumors, immediately after transplantation of splenocytes. This interferonogen was used previously with success by other workers for inducing interferon *in vivo* [2, 3].

Exposure to interferon was carried out *in vitro* (300 units/ml) at 37°C for 1 h, and this was followed by repeated washing with medium No. 199 [11]. The leukocytic interferon was generously provided for study by V. V. Malinovskaya (N. F. Gamaleya Research Institute of Epidemiology and Microbiology).

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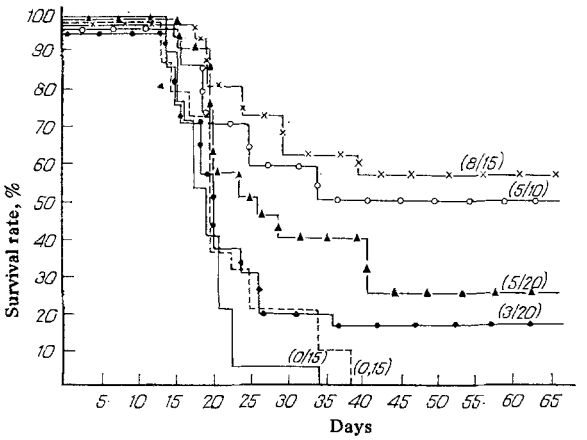

Fig. 1. Immunocytotherapy of EL-4 leukemia in C57Bl/6 mice. Broken line — mice injected with 2×10^4 + cyclophosphamide (CP), 180 mg/kg; triangles — 2×10^4 EL-4 + CP + 2×10^7 splenocytes; empty circles — 2×10^4 EL-4 + CP + 2×10^7 splenocytes treated *in vitro* with interferon; crosses — 2×10^4 EL-4 + CP + 2×10^7 splenocytes from mice treated previously with interferonogen; continuous line — 2×10^4 EL-4; filled circles — interferonogen injected into mice with tumors after transplantation into them of splenocytes from intact C57Bl/6 mice. In parentheses: numerator — number of animals surviving until 60th day, denominator — total number of animals used in experiment. Here and in Figs. 3 and 4: abscissa, duration of experiment (in days); ordinate, survival rate (in percent).

The preparation of double helical RNA was dissolved in physiological saline containing 10 mM phosphate buffer, pH 7.3. To prepare liposomes (ovolecithin from Sigma, USA) with RNA the method of mechanical dispersion of a mixture of lipids and RNA followed by ultrasonic treatment was used [8]. The milky emulsion thus formed contained 58 mg lipid and 3 mg RNA in a volume of 3 ml. After ultrasonic treatment the suspension was centrifuged at 30,000g for 30 min to remove all unincorporated polynucleotide from the liposomes and resuspended in the original buffer to the required concentration. "Empty" liposomes were prepared in the same way but without addition of RNA.

EXPERIMENTAL RESULTS

Injection of 2×10^4 EL-4 tumor cells into C57Bl/6 mice was followed by death of all the animals by the 35th day (Fig. 1). A single injection of cyclophosphamide had virtually no effect on the survival rate although there was some increase in the length of survival of the experimental animals. Injection of intact syngeneic spleen cells into the mice with tumors prevented death of 25% of the animals (5 of 20). It was shown previously that enrichment of the splenocyte suspension with NK by fractionation on a column packed with nylon wadding potentiates the effect of immunocytotherapy. This eliminates any role of macrophages and B cells in this phenomenon. The possibility of stimulating cells mediating antitumor activity was studied. Treatment of the cells to be transplanted with interferon *in vitro* led to a marked increase in the number of surviving animals (Fig. 1). The mean length of survival of the mice which died in this group was 22.5 days, compared with 17.4 days for mice receiving intact splenocytes.

Experiments conducted in a model system [17] (Fig. 2) and its modification [6] (Fig. 3) yielded evidence that splenocytes are ineffective in the tumor neutralization test. A study of the effect of interferonogen on the development and course of leukemia L-1210 in

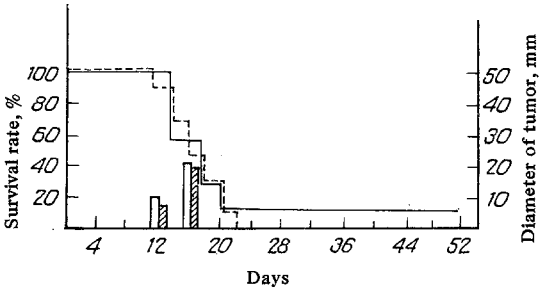

Fig. 2

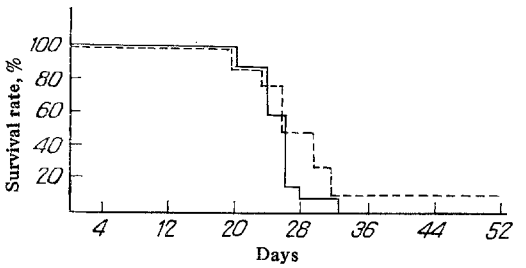

Fig. 3

Fig. 2. Neutralization of EL-4 tumor by splenocytes in C57Bl/6 mice. Broken line — mice given subcutaneous injection of 2×10^4 EL-4; continuous line — 2×10^4 EL-4 + 2×10^6 splenocytes from intact C57Bl/6 mice. Unshaded columns — diameter of tumor after injection of 2×10^4 EL-4; shaded columns — diameter after injection of mixture of 2×10^4 EL-4 and 2×10^6 splenocytes. Abscissa, duration of experiment (in days); ordinate, left — survival rate (in percent), right — diameter of tumor (in mm).

Fig. 3. Neutralization of tumor in C57Bl/6 mice. Broken line — mice injected intraperitoneally with 2×10^4 EL-4; continuous line — 2×10^4 EL-4 + 2×10^6 splenocytes.

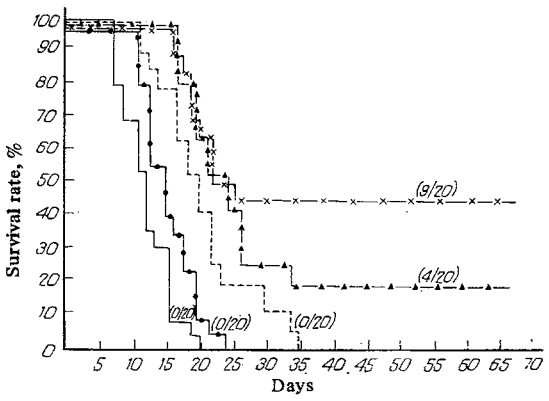

Fig. 4. Immunotherapy of leukemia in BALB/c mice (mice injected with 5×10^4 L-1210 cells). Legend as to Fig. 1.

BALB/c mice yielded similar results (Fig. 4). This series of experiments is more important, if the greater invasiveness of leukemia L-1210 is taken into account.

The next step was to analyze the effect of interferonogen on the cytotoxic activity of NK *in vitro*. As a result of two independent experiments, significant potentiation of the toxic activity of NK was revealed in A/Sn mice receiving interferonogen (Table 1).

The existence of interlinear differences in the manifestation of the immunocytotherapeutic activity of lymphocytes "working" *in vivo*, indirect evidence that they were identical with NK, was demonstrated by the writers previously. Significant potentiation of their activity by the action of interferon and interferonogen likewise is an argument in support of this view. Considering the presence of natural cytophilic antibodies and, more especially, the possibility of synthesis of antibodies against antigens of leukemic cells [15], during the period of immunocytotherapy, the role of K-lymphocytes in this phenomenon must be borne in mind. However, if the data on the resistance of K-cells to the action of interferon are taken into consideration [9], this view becomes improbable.

In a series of recently published investigations [5, 11, 16] the protective action of interferon on tumor cells has been demonstrated. According to Trinchieri et al. [16], not all tumor cells are protected by interferon. In the investigation cited, the tumors used

TABLE 1. Effect of Double Helical RNA on Functional Activity of NK from A/Sn Mice

Experimental conditions			Index of membrane toxicity, percent			
			ratio effectors/targets			
			100:1	50:1	25:1	12,5:1
Expt. 1	A/Sn	—	18,0	12,6	4,9	0
	A/Sn + DH RNA	+	48,7	24,5	7,7	0
Expt. 2	A/Sn	—	6,2	—	—	—
	A/Sn	+	20,0	—	—	—

Legend. Pools of splenocytes from five mice were used as effectors. Leukemia YAC-1 ($H\text{-}2^a$) cells were used as targets.

were PDMC, SW690, and SW691. The conclusion drawn by these workers is to some extent contradictory to the results of the investigation cited above. In the light of these findings, the results obtained by injection of interferonogen directly into the mice with tumors are somewhat unexpected. However, other possibilities cannot be ruled out, for example, activation of prostaglandin synthesis by interferon with, as a result, inhibition of NK [5].

The results obtained in the membrane toxic test with splenocytes of A/Sn mice injected with interferonogen demonstrate its marked stimulating action on NK in A/Sn mice. However, these data are in some disagreement with the results obtained by Orn et al. [13], who found no significant changes in the functional activity of NK in AKR mice under the influence of fairly active inducers of interferon production (tilorone, statolone). The possibility cannot be ruled out that this is connected with the use of test systems which differ in sensitivity (the test with ^{51}Cr and the test with [^{3}H]uridine).

The results are thus indirect evidence of the leading, if not exclusive, role of NK in the phenomenon of cytotherapeutic activity of nonimmune splenocytes *in vivo*. So far as the apparent dissociation between the antitumor activity of these cells *in vivo* and *in vitro* is concerned, it is tempting to suggest that the explanation of this phenomenon may be founds in communications on the cytostatic [8] and membrane-toxic [1] action of NK on the tumor cells.

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