INHIBITION OF TUMOR GROWTH BY POLYINOSINIC-POLYCYTIDYLIC ACID

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Abstract.—The synthetic double-stranded RNA, polyinosinic-cytidylic acid, inhibits the growth of some tumors in mice. Two days after implantation of a reticulum cell sarcoma, a lymphatic lymphoma, a fibrosarcoma, two leukemias, and a human adenovirus 12-induced tumor, treatment of groups of mice resulted in decreased growth rates of the tumors and increased survival times of the animals. In the two tumors tested (the reticulum cell sarcoma and the adenovirus 12-induced tumor) initiation of treatment after the tumor was grown to moderate size caused a regression of the tumor. In the case of the reticulum cell sarcoma, the tumor had not reappeared in some of the animals two months after cessation of treatment.

The synthetic double-stranded RNA, polyinosinic-polycytidylic acid (polyrI:rC), can induce cells to synthesize interferon and to develop a high degree of resistance to virus replication,[1] presumably by mechanisms analogous to, but not necessarily identical with, those involved in the interferon system.[2–5]

The antiviral action of interferon appears to be attributable to derepressions of cell genome function, leading to the synthesis of modified ribosomal subunits and ribosomes in interferon-treated cells.[6–9] Interferon-type ribosomes and subunits can distinguish between cell messenger RNA and viral RNA, and can bind and translate the former as well as do normal ribosomes, but they bind and translate viral RNA poorly. Interferon-type ribosomes support normal cell growth as well as do control ribosomes.[10–13] PolyrI:rC does not produce visual cell toxicity at levels that induce strong resistance to virus,[14] but it has been found to inhibit the growth of a variety of tumors in mice. These experiments are reported here.

Materials and Methods.—*PolyrI:rC:* Polyriboinosinic acid and polyribocytidylic acid were purchased from P-L Biochemicals. They were heterogeneous in size, with molecular weights in excess of 10^5. They were dissolved at a concentration of 1 mg/ml in 0.85% NaCl containing 0.01 M phosphate buffer at pH 7.2. A Dounce homogenizer was used to facilitate solution. The solutions were warmed to 30°C and the polyrC was poured into the polyrI while constantly being mixed. In some experiments, equal volumes of solution were mixed. This mixture contained a slight molar excess of polyrC. In some later experiments, equimolar mixtures were used. One M $MgCl_2$ solution was added to a final concentration of 5×10^{-3} M. There was a hypochromic shift of about 35%, indicating that the base-paired double-stranded structure had formed.

Only one schedule of treatment was used in these studies: 100 μg were given each mouse on Mondays, Wednesdays, and Fridays 24–48 hr after implantation of the tumor, except for the L1210 leukemia, where injections were given daily. The route of administration was intravenous or intraperitoneal, as indicated in the figures.

Tumors: Neoplasm J96132 arose spontaneously in a female 105 C57 BL/KaLw mouse, $11^1/_2$ months of age. Spleen and liver involvement were noted at necropsy. The neo-

plasm is classified as a reticulum cell sarcoma type A.[14b] It has been transplanted through 18 generations in syngeneic mice. At the 12th generation the neoplasm was frozen in liquid nitrogen and recovered 21 months later. It is now carried in the solid form (subcutaneous transfer) or in the ascites form (intraperitoneal transfer).

Neoplasm B1237 arose in an SJL strain mouse ten months of age. It has been transplanted subcutaneously through five generations and is classified as a generalized lymphocytic lymphoma.

The MT-1 tumor was induced by human adenovirus 12 in neonatally thymectomized Balb/C mice.[15] It was passaged as a 50% suspension of tissue in 199 medium in weanling male Balb/C mice; 0.2 ml of the material was injected subcutaneously. The tumor was in the 50th-generation passage.

The fibrosarcoma was a spontaneous tumor that originated in Balb/C mice. When used, it was in the 30th-generation passage.

The L1210 leukemia was carried in strain DBA/2 and tested in (C57B1/6 × DBA/2) F_1 hybrid male mice. Implantation of the tumor was by intraperitoneal injection of 10^5 cells per mouse.

Each experiment consisted of eight to ten treated and an equal number of untreated mice. Two or more independent experiments were performed with each tumor, except for the fibrosarcoma on which only one was done. In general, the mice weighed about 20 gm.

Expression of results: One expression of the data is as cumulative days of survival; the number of cumulative survival days on day n for a group of mice on day n is obtained from the expression:

$$\text{Cum. surv. days}_n = \sum_{0}^{n} (i) \times \text{no. of animals alive on each day}_i,$$

where i is the number of each experimental day up to n. Thus, 9 animals surviving for 3 days contributed $(9 \times 1) + (9 \times 2) + (9 \times 3) = 54$ cumulative survival days.

Other methods of data presentation are the percentage of the animals dead as a function of time and mean day of death ± standard error of the mean.

Results.—Three effects of polyrI:rC were noted: (1) a decrease in growth rate of the tumor, (2) an increase in survival time of animals, and (3) regression of an already grown tumor. All tumors tested showed one or more of these effects.

It is difficult to quantify tumor volume accurately, particularly when the tumor has become large and irregular in shape. The MT-1 tumor could be measured for about the first two weeks of its development. An estimate was made of its length, width, and height, and the volume was calculated as the product of these three dimensions. Figure 1 shows that treatment with polyrI:rC, beginning two days after transplant of the tumor, inhibits its growth. Initiation of treatment after the tumor had obtained modest size led to massive necrosis and sloughing in four of the ten animals treated and a marked reduction in tumor size in the six others. After a few weeks, however, these tumors increased in volume again, despite continued treatment. No studies were made to determine if larger doses of polyrI:rC could prevent regrowth or cause another rejection of the tumor once it had regrown. In one experiment with three-week-old mice, the hemorrhagic regression was accompanied by sudden death of all the treated mice, possibly because of the resorption of the necrotic tissue.

The most marked effect of the drug on survival time was in tumor J96132 (a reticulum cell sarcoma in C57 Kaplan black mice), either in ascites form or as a

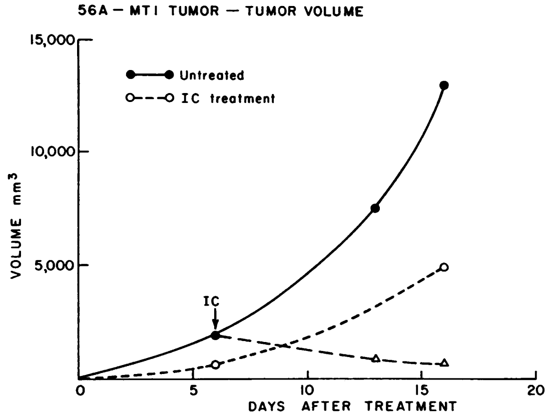

FIG. 1.—Effect of polyrI:rC on growth rate of MT-1 tumor. Forty animals were inoculated with MT-1 tumor; 2 days later I.V. treatment was initiated in half the mice (day 0), as outlined in *Materials and Methods;* 6 days later, ten of the untreated animals began receiving treatment.

solid subcutaneous tumor. Figure 2 plots cumulative survival days as well as percentage dead for the subcutaneous form of the tumor. It can be seen that all the untreated animals were dead by day 41, and all the treated were alive. When the tumor was in the ascites form, the untreated animals were all dead at 42 days and all the treated animals were still alive 6 weeks later. Two weeks after the cessation of treatment, microscopic examination of the site of the primary transplant revealed a few viable tumor cells surrounded by a mass of necrotic cells. Metastatic cells could not be found in the liver or spleen. About 30 per cent of all the animals originally inoculated with this tumor were still alive two months after treatment was stopped and showed no gross evidence of tumor.

In one experiment, the J96132 subcutaneous tumor was allowed to grow to

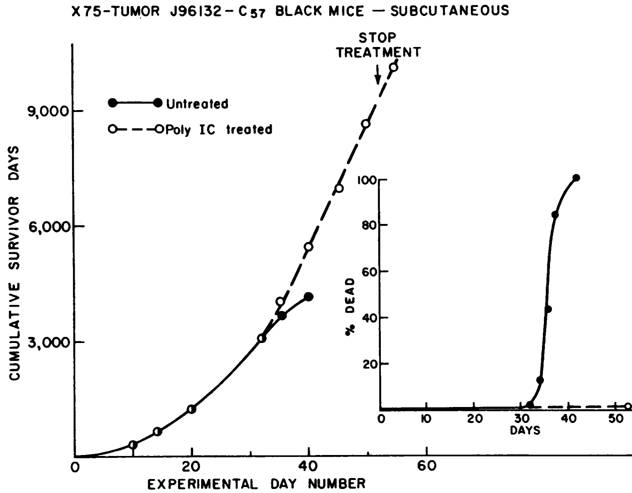

FIG. 2.—Effect of polyrI:rC on survival of animals bearing tumor J96132. Ten tumor-bearing mice were treated with 100 μg of polyrI:rC, intraperitoneally, three times weekly, starting 2 days after tumor implantation.

about 2–4 mm in diameter before initiation of treatment with 200 μg of polyrI:rC intraperitoneally every other day. Within eight days, the tumors were no longer palpable in any of the ten animals, while the tumors in ten untreated animals were slowly increasing in volume. After 34 days, nine of the ten untreated animals were dead, but all the treated animals were alive.

Table 1 shows data for other tumors. Although they were less responsive, the drug exerted protective action in all cases. Only one treatment schedule was used—100 μg per mouse three times weekly. It is not known whether other schedules would be more effective. There was no gross evidence of toxicity of the polyrI:rC at these levels.

TABLE 1. *Effect of poly I:C on mouse tumors.*

Tumor	Treatment route	Cumulative Survival Days (no. of animals)		Mean Day of Death ± S.E. of Mean	
		Treated	Untreated	Treated	Untreated
J96132-reticulum cell sarcoma (ascites)	I.P.	(10)	(10)	77.0* ± 4.8	39.2 ± 1.2
J96132-reticulum cell sarcoma (subcutaneous)	I.P.	(40)	(40)	87.0[1] ± 6.3	38.0 ± 1.7
Fibrosarcoma	I.V.	7516 (10)	6266 (10)	38.0 ± 1.3	25.3 ± 2.5
B1237-lymphoma (ascites)	I.V.	900 (18)	420 (18)	15.2 ± 0.54	10.5 ± 0.51
MT-1 tumor (subcutaneous)	I.V. or I.P.	4854 (20)	3079 (20)	32.9 ± 0.82	26.2 ± 0.28
B1237-lymphoma (subcutaneous)	I.V.	1380 (20)	1180 (20)	16.9 ± 0.41	13.2 ± 0.54
L1210 leukemia†	I.P.	(30)	(30)	12.9	9.1

With the exception of the J96132 reticulum cell sarcoma, all animals ultimately died. (See also Fig. 2.) I.V., intravenous; I.P., intraperitoneal.

* Mean day of death of the animals that died. About 30% of all the animals treated have survived, although treatment had been stopped at about day 50.

† Tested by the Cancer Chemotherapy National Service Center, National Cancer Institute, in their standard screening procedure. In this test system, differences of this magnitude are highly significant.

Discussion.—The mechanism of this antitumor action is under investigation. For several reasons it is not thought likely that the effect is attributable solely, if at all, to the antiviral action of the interferon induced. These tumors are not known to contain infectious oncogenic viruses. The MT-1 tumor was originally induced by adenovirus 12. This virus cannot be recovered from the tumor, but adenovirus-12 specific T antigen is present. T antigens of transformed cells, specifically SV40 T antigen, are not affected by interferon,[16] even though infection by SV40 viruses is quite sensitive to inhibition by interferon. In addition, adenovirus 12 is relatively resistant to the action of interferon. There is no evidence that the other tumors we have used were induced by virus. Interferon can retard progression of the leukemia induced by Friend virus where tumor growth is dependent on continued replication of the virus,[17] but such dependence does not appear to pertain to the tumors studied here. One explanation for the antitumor action might relate to possible enhanced immunological rejection of foreign antigens. Another possible mechanism might be a direct action of the

polyrI:rC on the tumor.[18] Such a mechanism might involve, for example, the induction of modified ribosomes able to make even finer distinctions than those made by interferon-type ribosomes. Preliminary evidence indicates that treatment of primary mouse cells or mouse L cells with polyrI:rC modifies RNA and protein synthesis (unpublished observations). A third possibility is that polyrI:rC produces changes in the blood supply to the tumor with subsequent isohemic necrosis. Examination of these three possibilities is in progress.

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