Inhibitory Effect of Polyinosinic:Polycytidylic Acid on the Growth of Transplantable Mouse Mammary Carcinoma[1] (36370)

MILAN POTMESIL AND ANNA GOLDFEDER

Cancer and Radiobiological Research Laboratory, Departments of Health and Hospitals, Frances Delafield Hospital and New York University, New York, New York 10032

The double-stranded synthetic RNA, polyinosinic:polycytidylic acid (poly I:poly C), inhibits the growth of a variety of mouse tumors and leukemias, both of unknown and viral etiology, spontaneous and transplantable (1–7). Poly I : poly C also blocks carcinogenesis by dimethylbenzanthracene (8). The mechanism of the antineoplastic activity is not clear and appears to be rather complex (2, 5, 9). The interferon induced by poly I : poly C may contribute to the inhibition of the growth of certain tumors (5, 10, 11). An apparent lack of correlation between serum interferon levels and antitumor action, however, has also been observed (12). Poly I : poly C is capable of a strong enhancement of both humoral antibody formation (13, 14) and cell-mediated rejection of grafts (14, 15). It was suggested that the compound could possibly exert an antitumor action by enhancing the cell-mediated immune response (2, 7, 14, 16). A direct chemotherapeutic effect of the inducer through the inhibition of RNA and protein synthesis in mouse tumors was also reported (17).

In the present study poly I : poly C decreased the growth rate of tumor implants and induced a temporary regression of established tumors. The morphologic examination of treated tumors did not reveal the presence of lymphoid cells as an indication of cell-mediated immune response. Increased serum interferon levels were observed in the early phase of the treatment, when the regression of tumors was most significant.

Materials and Methods. Tumor. A mouse mammary adenocarcinoma DBA (200th and 205th pass.) propagated in isologous hosts of the DBA/2J strain was used in these experiments. Females, 2–3 months of age, were recipients of tumor implants.

Poly I : poly C. Poly I : poly C (double-stranded sodium salt, lyophilized), purchased from P-L Biochemicals, Milwaukee, WI, was dissolved at 1 mg/ml in pyrogen-free 0.85% NaCl containing 0.01 *M* phosphate buffer (pH 7.4) at 45° and kept at 4° until used.

Interferon assays. The plaque-inhibition test (18) was employed, using L 929 cells and vesicular stomatitis virus. Interferon titer was expressed as the reciprocal of the highest dilution of the tested sera causing a 50% inhibition of the control plaque count.

Light microscopy techniques. Pieces of the tumor tissue were fixed in Bouin's solution, routinely processed, and stained with hematoxylin and eosin. Other parts of the tumor were used for contact and smear preparations; these were fixed in methyl alcohol and stained with May-Grünwald–Giemsa.

Experimental design. Two schedules of treatment were used: (a) 150 μg of poly I : poly C in 0.15 ml phosphate-buffered saline (PBS) was injected intraperitoneally at 48-hr intervals starting 24 hr after implantation of the tumor; (b) the same dose and time schedule was used for the treatment of developed tumors, 0.6–1.0 cm^3 or 1.8–2.0 cm^3 in size. Control tumor-bearing mice were injected with PBS (placebo). Each series consisted of 10 treated and an equal number of untreated control mice. In addition, groups of five mice with developed DBA tumors, injected once or repeatedly with poly I : poly C, and a corresponding number of control

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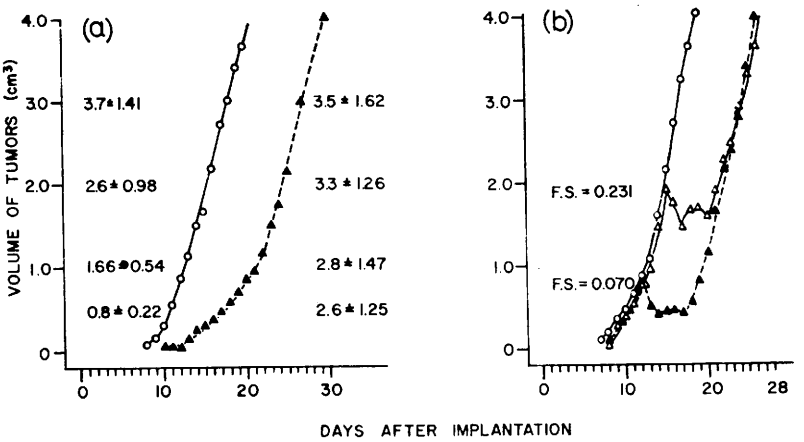


FIG. 1. Effect of poly I:poly C treatment on the growth rate of DBA tumors. O—O placebo (PBS); ▲- - -▲ treatment with poly I:poly C of tumor implants (a) or of developed tumors 0.6–1.0 cm³ in size (b); △—△ treatment with poly I:poly C of developed tumors 1.8–2.0 cm³ in size. Volume-doubling time ± SD of controls (left-hand side) and of treated tumors (right-hand side). Each point represents the mean value of 10 measurements. F.S. = fractional survival of the tumor tissue.

mice, were killed. Blood serums of mice in each group were pooled and used for interferon assays. Histologic sections were prepared (a) from DBA tumors of mice treated with poly I : poly C after the implantation of the tumor tissue; (b) from developed DBA tumors, poly I : poly C treated, at the time of their regression; (c) from DBA tumors of untreated control mice.

Evaluation of the treatment. The latent period of the tumor growth (time between implantation and appearance of a palpable nodule) in treated and untreated mice was recorded and the size of tumors was measured in three dimensions with calipers. The volume was calculated in cubic centimeters:

$$V = (\pi/6) \times D_1 \times D_2 \times D_3$$

($D_{1\text{-}3}$: three different diameters in millimeters). The average growth in volume was expressed by the slope of the line obtained by plotting tumor volumes, calculated for different days after implantation. The volume-doubling time was calculated by regression analysis (19). The treatment of developed tumors was evaluated using the extrapolation of regrowth curves to the first day of treatment and estimating the fractional survival of the tumor tissue (20). The survival of treated and untreated mice was expressed in terms of cumulative days of survival and the number of dead animals as a function of time (2).

TABLE I. Effect of Poly I: Poly C Treatment on the Survival of Mice with DBA Tumors.

Size of tumors in time of the 1st injection	Treatment (no. of inject.)	No. of animals	Cumulative survival days[a]	Mean day of death ± SD
Implant	Poly I: poly C (11)	10	6,203	35.7 ± 12.2
	PBS (11)	10	3,894	27.9 ± 5.8
0.6–1.0 cm³	Poly I: poly C (10)	10	8,882	42.4 ± 5.2
1.8–2.0 cm³	Poly I: poly C (10)	10	9,080	40.0 ± 17.9
0.6–1.0 cm³	PBS (10)	5	2,431	23.9 ± 6.1
1.8–2.0 cm³	PBS (10)	5		

[a] Cumulative survival days$_n = \sum_{i}^{n} (i) \times$ no. of animals alive on each day i; $i =$ the number of each experimental day up to day$_n$.

TABLE II. Effect of Poly I: Poly C Treatment on Serum Interferon Level of Mice with DBA Tumors.

Size of tumors in time of the 1st injection	Treatment (no. of inject.)[a]	No. of animals	Interferon titer in pooled sera
—	Poly I: poly C (1)	5	250
0.6–1.0 cm^3	Poly I: poly C (1)	5	240
	PBS (1)	5	<16
	Poly I: poly C (10)	5	<16
	PBS (10)	5	<16
1.8–2.0 cm^3	Poly I: poly C (1)	5	180
	PBS (1)	5	<16
	Poly I: poly C (10)	5	<16
	PBS (10)	5	<16

[a] The sera were collected 5 hr after the last injection of poly I : poly C or PBS.

Results. The effect of poly I : poly C treatment on the growth of mouse mammary carcinomas is presented in Fig. 1. The latent period was longer in treated tumors (11.1 ± 2.64 days) as compared with controls (6.9 ± 0.31 days). Treatments of tumors starting 24 hr after implantation resulted in a significantly decreased volume-doubling time (*t* test: $p < .001$). The effect was most apparent during the early stages of the treatment (Fig. 1a). The treatment of established tumors was more effective in small tumors: the fractional survival of the tumor tissue after therapy was lower in tumors 0.6–1.0 cm^3 (0.070) than in tumors 1.8–2.0 cm^3 (0.231) (Fig. 1b). All treated groups of mice showed a significantly longer survival time (*t* test: $p < .01$); however, differences between treated groups were not significant (*t* test: $p \geqq .05$) (Table I).

Interferon levels were measured in serum of control mice without tumors and in serum of tumor-bearing mice that had received either poly I : poly C or PBS (Table II). Mice of all groups responded to the first injection of the inducer but did not respond after a series of injections. Interferon levels after the first injection of poly I : poly C did not show significant differences in any of the groups studied.

In histologic sections, the DBA tumor consists of epithelial cells arranged in solid nests with no apparent formation of acini. Nests of variable size are separated by narrow septa of connective tissue (Fig. 2a). The vascular bed of the tumor is located in the major septa. Necrobiotic cells are rarely present. However, certain areas of poly I : poly C-treated tumors showed lesions of minor extent located in septa and spreading from these areas centrifugally (Fig. 2b). In other parts of treated tumors extensive lesions with occasional hemorrhages were present. Lesions of the tumor tissue exhibited pyknosis, karyolysis and fragmentation of nuclei, shrunken cords of featureless cells (Fig. 2c, e), and in some areas extensive empty spaces indicating loss of cells with preserved nets of tumor stroma (Fig. 2d). The extent of lesions was significantly lower in tumors treated immediately after implantation than in treated developed tumors (Table III). Only a few lymphoid cells were observed in the lumens of large thin-walled vessels (Fig. 2f) and their incidence did not exceed the incidence of these cells in vessels of untreated control tumors. Thrombi were not present. Lymphoid cells were not observed in connective tissue septa carrying the large vessels and their branches or in nests of tumor cells and did not accompany the destruction of the tumor tissue. Lymphocytes were rarely present in smear and contact preparations of both treated and untreated tumors.

Discussion. Poly I : poly C treatments of tumor implants extended the latent period and decreased the growth rate, induced the regression of small and large DBA tumors, and thereby extended the life span of animals. Regression of tumors and their de-

TABLE III. Histology of DBA Tumors Treated with Poly I: Poly C.

Size of tumors in time of the 1st injection[a]	Treatment (no. of injections)	No. of animals	Histologic evaluation[b]		
			No. pos.	No. equiv.	No. neg.
Implant	Poly I: poly C (10)	5	—	3	2
	PBS (6)	5	—	—	5
0.6–1.0 cm^3	Poly I: poly C (2)	3	3	—	—
	PBS (2)	3	—	—	3
1.8–2.0 cm^3	Poly I: poly C (2)	3	3	—	—
	PBS (2)	3	—	—	3

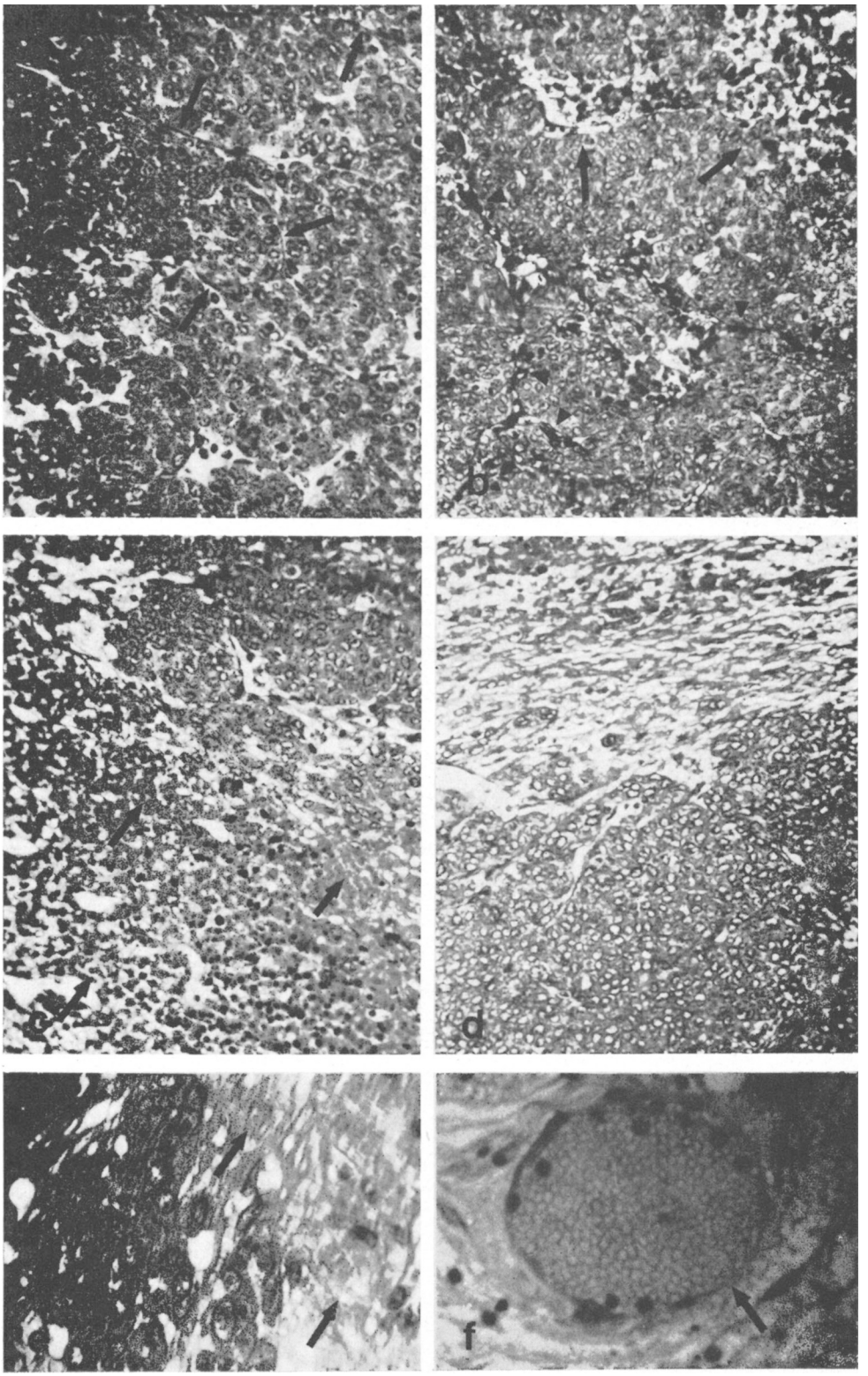
[a] The size of tumors treated with poly I: poly C in time of killing reached 0.57 ± 0.04 cm^3 in the first, 0.44 ± 0.07 cm^3 in the second, and 1.63 ± 0.20 cm^3 in the third group.

[b] Positive = distinct signs of necrosis and tumor-cell degeneration; equivocal = sparse necrosis and cell degeneration; negative = no distinct signs of necrosis or cell degeneration.

creased rate of growth was more apparent at the beginning of treatments. Tumor-bearing mice responded to the first poly I : poly C injections with significantly elevated interferon levels. No detectable interferon was observed after a series of 10 poly I : poly C injections; the decline of interferon levels was apparently due to the inhibition of interferon production after repeated injections of the inducer (hyporeactive period) as was demonstrated in previous studies (21, 22). It is likely that the strain of L cells used for the interferon assay was of low sensitivity to interferon and that a sensitive assay would have revealed continued but diminished production of interferon after repeated injections of poly I : poly C (22).

The results reported in the literature demonstrated that poly I : poly C is capable of stimulating both humoral and cellular immune responses (13–15) and that a strong cellular immunity was established in grafted mice treated even with a single dose of the inducer (14). Based on experimental observations made *in vitro* (23) it was speculated that interferon may modify the surface of cells and alter tumor–host cell interaction (24). The ability of poly I : poly C to stimulate the cell-mediated immune response has been repeatedly considered as an important factor in the mechanism by which this compound exerts its antitumor effect (2, 7, 14, 16, 25). Infiltration of lymphoid cells is a fundamental characteristic of immune reactions of the cellular type (26, 27). In the present study there was no evidence that isografts of treated tumor tissue were destroyed by induced cellular immune response and that lymphocytes play some crucial role in the genesis of the inhibitory effect of poly I : poly C on the growth of DBA tumors.

Summary. This study was designed to test the effect of the sequential administration of poly I : poly C on the growth rate and morphology of DBA mouse mammary adenocarcinoma. Treatments with poly I : poly C (150 μg ip per dose) started (a) 24 hr after implantation; (b) when the size of tumors reached 0.6–1.0 cm^3, or (c) 1.8–2.0 cm^3. Controls were injected with 0.15 ml phosphate-buffered saline (placebo). In all groups the treatment continued at 48-hr intervals for 18–22 days. Treatments starting 24 hr after implantation resulted in a temporary arrest of the growth of tumor implants followed by a significantly decreased volume-doubling time (for tumors 0.5 and 1.0 cm^3 in size $p < 0.001$). The treatment of established tumors was more effective in small tumors. The fractional survival of tumor tissue was significantly lower in tumors measuring 0.6–1.0 cm^3 (0.070) than in tumors 1.8–2.0 cm^3 in size (0.231). All groups of mice treated with poly I : poly C showed a significantly longer survival time ($p < .01$). Increased interferon levels were observed after the first injection of poly I : poly C but not after a series of injections. The regression of tumors was most significant at the beginning of treatment. Extensive necrotic lesions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were observed in histologic sections of tumors treated with poly I : poly C. There was no morphologic evidence suggesting the damage of the tumor tissue by induced cell-mediated immune response, and there was no indication that lymphocytes play some crucial role in the genesis of the inhibitory effect of poly I : poly C on isologous DBA tumors.

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FIG. 2. Histological sections of the DBA tumor: a, nests of epithelial cells separated by septa of connective tissue (arrows), placebo treatment; b, a lesion of minor extent exhibiting pyknosis of cells (arrows) and pyknotic cells in the region of septa (pointers), poly I:poly C treatment after implantation; c, pyknosis and karyolysis, cords of featureless cells (arrows), poly I:poly C treatment of developed tumors; d, nets of tumor stroma, poly I:poly C treatment of developed tumors; e, cords of featureless cells (arrows), and f, large thin-walled vessel (arrow) with nucleated blood cells in a tumor treated with poly I:poly C. H. and E., a–d, ×280, e, ×625, f, ×365.