Interferon Inducers: Enhancement of Viral Oncogenesis in Mice and Rats[1] (36314)

A. F. GAZDAR, A. D. STEINBERG, G. F. SPAHN, AND S. BARON

National Cancer Institute, National Institute of Arthritis and Metabolic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014; and Microbiological Associates, Walkersville, Maryland 21793

Interferon and interferon inducers can protect experimental animals from several virus-induced tumors (1–5), but prolonged administration is usually required. Treatment with double-stranded RNA inducers does not always prevent viral oncogenesis, and under certain conditions may enhance tumor induction (6–9). Viral oncogenesis is also enhanced by co-infection with several nononcogenic viruses (10–12). However, it has not been determined whether enhancement of virus-induced tumors is a general property of interferon and interferon inducers. We herein report that pretreatment with a single dose of several different interferon inducers enhances the effects of murine sarcoma and leukemia viruses in mice and rats.

Materials and Methods. Weanling mice (16–20 g) were obtained from the National Cancer Chemotherapy Service (Cr), National Cancer Institute, Bethesda, MD; Division of Research Services (N), National Institutes of Health, Bethesda, MD, and Microbiological Associates (Mai), Walkersville, MD. Suckling Osborne-Mendel rats (16–18 g) were obtained from the National Institutes of Health.

Interferon was produced by stimulation of a transformed mouse cell line by Newcastle disease virus (NDV). The supernatant fluids were harvested 48 hr after infection and concentrated by dialysis against Aquacide (Calbiochem, Los Angeles, CA). The final concentration of fetal bovine serum in the preparation, prior to dilution, was 20%. Concentrations of interferon were expressed as the reciprocal of the highest dilution which inhibited the hemagglutinin yield of GD VII virus by $10^{0.5}$ in mouse L cells (13).

The synthetic polyribonucleotides polyinosinic·polycytidylic acid (poly I·poly C) and polyriboadenylic·polyribouridylic acid (poly A·poly U) were purchased from P-L Biochemicals, Milwaukee, WI. Tilorone hydrochloride was provided by Dr. R. F. Krueger, William Merrell Co., Cincinnati, OH. Dr. John Niblack, Charles Pfizer Co., Groton, CT, donated pyran copolymer. Statolon was a gift from Dr. W. Kleinschmidt, Lilly Research Laboratory, Indianapolis, IN.

A concentrate of the Moloney strain of murine sarcoma virus (MSV) was prepared from virus-induced tumors in BALB/c mice by differential centrifugation (14) and was a 1 g of tumor/ml equivalent. The MSV pool contained lactic dehydrogenase virus (LDH virus). MSV free of all known extraneous murine viruses was prepared from virus-inoculated secondary mouse embryo cell cultures. MSV was inoculated (vol = 0.1 ml) sc into the left thigh. Mice were examined 4, 5, 7, 12, 15, 20, 25, and 30 days after virus inoculation and tumor size was carefully graded 0–4 arbitrary units, using the criteria of Blumenschein and Moloney (15). Rauscher leukemia virus (RLV) (0.1 ml) was injected ip; the animals were sacrificed 21 days later, and their spleens were weighed (16). NDV was obtained by harvesting the allantoic fluid of embryonated eggs 72 hr after virus inoculation. Semliki forest virus (SFV) was grown in primary chick embryo cell culture.

The adjuvant properties of poly I·poly C were studied by injecting 2.5×10^8 sheep erythrocytes (SRBC) and 250 μg of poly I·poly C iv using the same syringe (but with-

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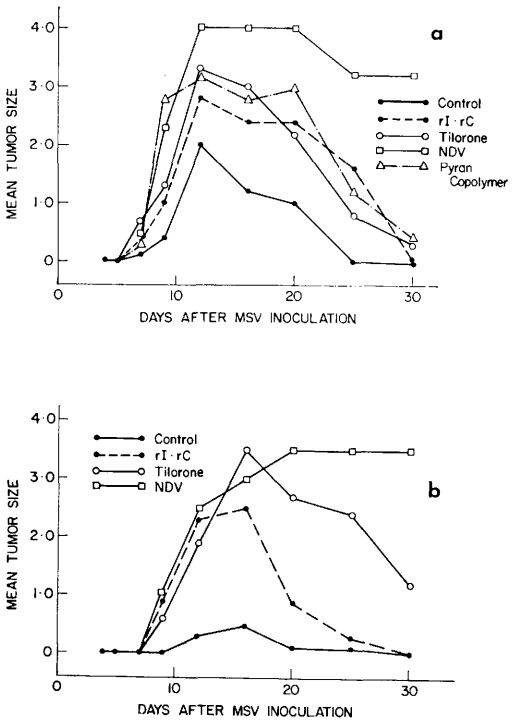

FIG. 1. Enhancement of MSV tumor induction by interferon inducers in weanling AL/N mice (a); and suckling Osborne-Mendel rats (b). Each experimental and control group consisted of 10 animals. The animals were pretreated with poly I·poly C (200 μg subcutaneously), tilorone hydrochloride (2.5 mg orally), or NDV ($10^{8.5}$ plaque forming units iv). Mice were also pretreated with pyran copolymer (10 mg ip). MSV ($10^{4.5}$ tumor inducing doses, as titered in newborn BALB/c mice), was injected into the left thigh 24 hr later. Tumor size was graded in arbitrary units using the criteria of Blumenschein and Moloney (15).

out mixing). The spleens were harvested 72 hr later and the antibody forming cells to SRBC were enumerated by the Jerne plaque assay (17).

Results. The effects of interferon inducers on MSV tumor induction in weanling AL/N mice and suckling Osborne-Mendel rats are shown in Fig. 1. MSV rapidly induces tumors which, in the majority of immunologically competent animals, spontaneously regress (18). Pretreatment with a single dose of an interferon inducer 24 hr prior to virus inoculation significantly enhanced MSV tumor induction, as manifested by shorter latent time to tumor appearance, higher incidence to tumors, larger mean tumor size, and longer regression time. Enhancement of tumor induction by inducers was greater in rats than in mice. Rats are relatively resistant to MSV, and only 2/10 controls developed moderate sized tumors which regressed within a few days. All rats pretreated with inducers developed large tumors which regressed slowly. Of the rats pretreated with NDV, 3/10 died with progressively growing tumors. Pretreatment of AL/N mice with NDV increased the minimal tumor inducing dose of the MSV preparation, as calculated by the Reed–Muench formula (19), by 11-fold, and poly I·poly C pretreatment increased it 5-fold. In addition to the inducers illustrated in Fig. 1, pretreatment of AL/N mice with poly A·poly U (200 μg injected subcutaneously) shortened the latent time to tumor appearance and increased the mean tumor size, but did not lengthen the regression time.

These results were confirmed by pretreating AL/N mice with interferon inducers 24 hr prior to MSV inoculation and harvesting the tumors 14 days later. The MSV was inoculated sc in the back to aid separation of the tumors from surrounding tissues. The tumors so induced grew at a considerably slower rate than those resulting from MSV inoculation into the thigh. The results of the mean tumor weights were as follows: control mice, 380 mg; poly I·poly C-pretreated mice, 1670 mg; tilorone-pretreated mice, 1720 mg; NDV-pretreated mice, 2260 mg.

Enhancement of MSV tumors occurred if poly I·poly C was injected into weanling mice 24 hr prior to or simultaneously with the virus. Injection of poly I·poly C 24 hr after or 48 hr prior to MCV had no effect on the tumors. However, pretreatment of 1–3-day-old AL/N and C3H mice with poly I·poly C (10 μg subcutaneously) and tilorone (125 μg orally), respectively, had no effect on MSV tumor induction.

As demonstrated in Table I, the enhancing effect of poly I·poly C varied in a number of different mouse strains. Pretreatment with poly I·poly C had no effect on MSV tumor induction in BALB/c mice at any virus dilu-

TABLE I. Strain-Dependent Enhancement in Mice of MSV Tumor Induction by Poly I·Poly C.[a]

Mouse strain	Sex	Source[b]	Interferon[c] titer	Enhancement of tumor induction[d]; MSV dose[e]: 1.5	2.5	3.5
BALB/c	M, F	Cr, N	3.2–3.7	—	—	—
AL/N	M, F	Cr, N	3.8–4.1	+	+	+
BALB/c × AL/N F_1	Mixed	N	3.5	+	+	
NZB	M	Cr	4.2	+		
NZW	M	N	3.4	+		
NZW × NZB F_1	Mixed	Cr	3.6	+		
A/J	M	Cr	3.2	+		
A/He	M	N	3.6	—	—	
C57BL/6	M	N	3.9	+	—	
DBA/2	M	N	3.9	—		
N:NIH(SW)	M	N	4.4	—	—	
AKR	M	N	3.9	—		
C3H	M	N	4.1	+		
SM/J	M	Cr	3.4	+		
SJL/J	F	Cr	3.1	+		
CBA	F	Cr	4.3	+		

[a] Each experimental and control group consisted of 10 mice. Mice were injected in the right leg with 200 μg of poly I • poly C 24 hr prior to virus injection in the left leg.

[b] Mice were obtained from the National Cancer Institute (Cr) or the National Institutes of Health (N).

[c] Mean serum interferon titer ($\log_{10}$) 6 hr after a subcutaneous injection of 200 μg of poly I • poly C.

[d] Enhancement of MSV tumor induction at different virus doses. Enhancement defined as increase in mean tumor size by more than 0.8 arbitrary units for at least 3 consecutive readings.

[e] MSV dose ($\log_{10}$) as titered in newborn BALB/c mice.

tion, while poly I·poly C pretreatment enhanced tumor induction in AL/N mice at all virus dilutions. MSV tumor induction in all the BALB/c × AL/N F_1 hybrid mice was enhanced by poly I·poly C. The greatest degree of MSV tumor enhancement by poly I·poly C occurred in NZW mice. While only 4 control NZW mice developed small tumors which regressed after a few days, all of the poly I·poly C-injected mice developed progressively growing tumors and died within 25 days of virus injection. A surprising finding was the different effects of poly I·poly C in the A mice substrains. While poly I·poly C pretreatment enhanced MSV tumor induction in A/J mice, it had no effect in A/He mice. Sex differences were not noted in the poly I·poly C-induced enhancement of MSV tumors. The mouse strain-dependent action of poly I·poly C on MSV tumors did not correlate with the H-2 type or the allotypes of the Fv-1 locus, which controls the sensitivity to murine leukemia viruses (20).

The data in Table I also indicates a lack of correlation between poly I·poly C-mediated tumor enhancement and the induced circulating interferon responses. Because poly I·poly C enhanced MSV tumors in AL/N mice but not in BALB/c mice, the interferon responses of these 2 strains were studied in greater detail. The circulating interferon levels 3, 6, 12, 18, 24, and 30 hr after a single subcutaneous dose of 200 μg of poly I·poly C were measured. The magnitude and duration of the induced serum interferon levels were similar in both mouse strains.

Strain differences in the hyporeactive period to interferon induction following poly I·poly C injection (21) were studied in AL/N and BALB/c mice by restimulating

TABLE II. Effect of Interferon and Interferon Inducers on RLV-Induced Splenomegaly.[a]

Mouse strain	Experimental group	Dose of RLV[b] (log_{10})	Mean spleen wt (mg)	Coefficient of variation (%)	Change (%) in spleen wt	p
BALB/c	Control	2.2	492	58	—	—
BALB/c	Tilorone	2.2	388	55	−21	>.05
BALB/c	NDV	2.2	701	50	+42	>.05
BALB/c	Poly I • poly C	2.2	916	45	+86	<.01
BALB/c	Interferon, 10^5 units	2.2	908	74	+85	<.05
BALB/c	Interferon, 10^4 units	2.2	646	52	+31	>.05
BALB/c	Interferon, 10^3 units	2.2	671	44	+36	>.05
C3H	Control	2.7	408	52	—	—
C3H	Poly I • poly C	2.7	934	64	+129	<.05
C3H	Control	1.7	222	29	—	—
C3H	Poly I • poly C	1.7	463	39	+109	<.01

[a] Each experimental and control group consisted of 10 male mice. BALB/c mice were obtained from the National Cancer Institute (Cr) and C3H mice from Microbiological Associates (Mai). Weanling mice were injected intraperitoneally with interferon, injected subcutaneously with poly I • poly C (200 μg), given 2.5 mg of tilorone hydrochloride orally, or injected intravenously with $10^{8.6}$ plaque forming units of NDV. RLV was injected intraperitoneally 24 hr later. Mice were sacrificed after 21 days; and their spleens were weighed.

[b] RLV expressed as the 50% spleen enlarging dose, as titered in weanling BALB/c mice.

them with 10 or 200 μg of poly I·poly C, 24 and 48 hr after an injection of 200 μg of poly I·poly C. Similar levels of interferon were induced in both strains. The minimal protective dose of poly I·poly C against 1 LD_{100} challenge dose of SFV was 6 μg in both AL/N and BALB/c mice.

The stock MSV preparation induced 80–120 units of circulating interferon 24 hr after injection into BALB/c and AL/N mice. MSV free of LDH virus failed to induce detectable circulating interferon 24 and 48 hr after inoculation.

The effect of pretreatment with potent interferon preparations on MSV tumor induction was investigated in AL/N mice. Mice were injected with 10^5, 10^4, and 10^3 units of interferon (vol = 0.1 ml) 18 hr prior to MSV injection into the same site. Mice pretreated with 10^5 and 10^4 units of interferon developed tumors 1.5 days earlier and the tumors reached a maximum size 2 days earlier than controls. Mean tumor size and tumor regression time were not influenced by interferon pretreatment. Local pretreatment with 10^3 units of interferon and intraperitoneal injection of 10^4 and 10^3 units of interferon 18 hr prior to MSV injection had no effect on MSV-induced tumors.

The effects of interferon and interferon inducers on RLV-induced splenomegaly are presented in Table II. Pretreatment with a high dose of interferon or with poly I·poly C enhanced virus-induced splenomegaly, but lower doses of interferon, tilorone, and NDV had no significant effect. Surprisingly, poly I·poly C pretreatment of BALB/c mice increased RLV-induced splenomegaly but did not enhance MSV tumor induction in the same mouse strain.

In BALB/c mice, pretreatment with NDV, tilorone, and pyran copolymer also enhanced MSV tumor induction; but poly I·poly C had no effect. Pretreatment of BALB/c mice with tilorone resulted in the death of 6/10 mice within 25 days of virus injection with progressively growing tumors and multiple splenic metastases. All the MSV-inoculated controls appeared free of tumors 28 days after virus injection. Pretreatment with statolon (100 μg injected subcutaneously) had no effect on MSV tumor induction in AL/N or BALB/c mice.

The adjuvant properties of poly I·poly C in the 3-day antibody response to SRBC immunization are presented in Table III.

TABLE III. Effect of Poly I·Poly C on Mouse 3-Day Response to SRBC.

Mouse strain	Plaque forming cells/spleen[a]		
	SRBC	SRBC + poly I·poly C	Adjuvant effect
BALB/c	1375	3125	2.3
AL/N	6900	4900	0.8
C57BL/6	2200	9200	4.2
NZB/NZW F_1	55,000	44,000	0.8
DBA/2	1490	1550	1.0

[a] Each group consisted of 6 mice. The data represent the mean values of duplicate experiments.

Considerable strains differences were noted in the adjuvant action of poly I·poly C, and there was no correlation between the enhancement of humoral immunity by poly I·poly C and its enhancement of MSV-induced tumors.

Discussion. Our results demonstrate that pretreatment with 5 of 6 interferon inducers of diverse origin and chemical composition enhanced viral oncogenesis. Statolon did not enhance MSV-induced tumors, but depending on the circumstances, can enhance or suppress splenomegaly induced by Friend leukemia virus (I. Gressor, personal communication).

Enhancement of MSV-induced tumors by poly I·poly C was mouse strain dependent. Even minor substrain differences among A strain mice influenced the poly I·poly C effect. The strain-dependent effect of poly I·poly C could not be explained by differences in circulating interferon induction, the extent or duration of the hyporesponsive period to interferon induction, or in the induced antiviral state. MSV that was free of extraneous viruses did not induce circulating interferon.

Tumor enhancement by interferon inducers could be due to several mechansims which include: (a) alteration of the immune response; (b) increased cell penetration or growth of tumor viruses; (c) increased transformation rate; and (d) increased rate of replication of tumor cells.

Polyribonucleotides can act as nonspecific immunological enhancers (22, 23). Blocking antibodies, capable of preventing regression of MSV-induced tumors, are present in the sera of some mice with progressively growing tumors (24). The failure of two interferon inducers to enhance MSV tumors in newborn mice provides support to the concept that immunological competence may be required for enhancement. However, there was no correlation between the ability of poly I·poly C to enhance the SRBC response and its effect on MSV tumor induction.

While continuous treatment with interferon and interferon inducers can suppress replication of oncornaviruses (25, 26), there is insufficient information to determine whether a single pretreatment with inducer could increase cell penetration or replication by oncornaviruses. Similarly, the possible mechanism of increased transforming activity cannot be fully evaluated at present, although preliminary findings indicate that MSV focus formation in mouse cell cultures is increased by pretreatment with low doses of polynucleotides (A. Gazdar and Y. Ikawa, unpublished data).

The growth of transplanted syngeneic tumors may be both hindered or accelerated by poly I·poly C treatment [Refs. (27, 28); and A. Gazdar, unpublished data]. These effects need further study to evaluate a possible effect on tumor cell growth as a mechanism mediating enhancement of viral oncogenesis.

The role of interferon in tumor enhancement by interferon inducers requires further study. High doses of interferon increased RLV-induced splenomegaly but only had a minimal enhancing effect on MSV tumor induction. There was no correlation between the induced circulating interferon levels and the mouse strain-dependent tumor-enhancing effect of poly I·poly C.

Enhancement of certain oncornaviruses may be a general property of interferon inducers. Tumor enhancement is dependent on multiple factors including the age and strain of the test animals, the inducer and the time of its administration, and the oncornavirus. Although the effect of the inducer on the immune response may not be the mediating factor, its effects on virus penetration, multi-

plication, and transforming ability, and on the rate of growth of tumor cells require further study. Inteferon inducers may represent a double-edged sword, providing protection against many viruses, but, depending on the conditions, capable of either enhancing or inhibiting viral oncogenesis.

Summary. Pretreatment with several interferon inducers enhanced virus-induced sarcomas and leukemias in mice and rats. Enhancement was dependent on multiple factors including the animal strain, age of mice, and time of treatment. The strain-dependent effects of poly I·poly C could not be explained by differences in circulating interferon induction, the extent and duration of the hyporesponsive period to interferon induction, or in the induced antiviral state. Also, there was no correlation between the strain-dependent adjuvant properties of poly I·poly C on humoral immunity and its effect on MSV tumor induction. Depending on the conditions, interferon inducers are capable of either enhancing or inhibiting viral oncogenesis.

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