The Role of Interferon in the Protective Effect of a Synthetic Double-Stranded Polyribonucleotide against Intranasal Vesicular Stomatitis Virus Challenge in Mice

ERIK DE CLERCQ, MARC R. NUWER, and THOMAS C. MERIGAN

From the Division of Infectious Diseases, Department of Medicine, Stanford University School of Medicine, Stanford, California 94305

ABSTRACT Intravenous injection of polyinosinic acid/polycytidylic acid [(poly rI)·(poly rC)] offered significant protection against intranasal challenge of young mice with vesicular stomatitis virus (VSV). Optimal protection was obtained when a single dose was administered 2 hr before virus challenge, but repeated doses were effective when started as late as 3 days after virus challenge. The therapeutic ratio or ratio of maximum tolerated dose to minimum effective dose for a single intravenous injection of (poly rI)·(poly rC) 2 hr before virus inoculation was ≥ 8 mg/kg : 0.004 mg/kg or ≥ 200.

Dose-response curves for interferon production and antiviral protection by (poly rI)·(poly rC) were closely parallel. Equivalent doses of poly rI or poly rC alone did not exert any interferon-inducing capacity or protective effect on intranasal VSV challenge. Several factors, which are known to potentiate or antagonize interferon production, increased or decreased the interferon-inducing capacity and antiviral protection of either (poly rI)·(poly rC) or maleic acid/divinyl ether copolymer (MA/DVE) in parallel. Interferon production and antiviral protection by MA/DVE were enhanced by arginine but abolished by prior treatment with MA/DVE; DEAE-dextran (intraperitoneally), kinetin riboside and isopentenyladenosine, and prior injection of endotoxin reduced both interferon production and antiviral protection by (poly rI)·(poly rC).

Treatment with exogenous interferon in amounts which closely mimicked the levels of circulating interferon produced endogenously by an effective dose of (poly rI)·(poly rC) gave protection against intranasal VSV which was identical with that dose of (poly rI)·(poly rC). This strongly suggests that interferon production accounts for the whole protective effect of (poly rI)·(poly rC) in the intranasal VSV assay.

Dr. De Clercq is a Fellow of the Damon Runyon Memorial Fund for Cancer Research and "Aangesteld Navorser" of the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek.

Received for publication 20 February 1970.

INTRODUCTION

The synthetic polyanionic interferon inducers maleic acid/divinyl ether copolymer (MA/DVE) (1) and polyinosinic/polycytidylic acid [(poly rI)·(poly rC)] (2) have been shown effective in protecting young mice from intranasal challenge with vesicular stomatitis virus (VSV) (3). This experimental virus infection, described by Sabin and Olitsky in 1938 (4), resembles certain natural virus infections in animals and man (e.g. poliomyelitis, rabies, and herpes simplex encephalitis) (5), in that the virus spreads from a respiratory tract site (olfactory mucosa) through the olfactory nerves to the brain.

As both MA/DVE and (poly rI)·(poly rC) offered antiviral protection at a distant site from where they were injected, their antiviral action was assumed to be mediated by interferon production (3). Protection at a distant site has also been demonstrated for MA/DVE, (poly rI)·(poly rC), and related synthetic polyanions in other acute viral infections such as vaccinia tail lesions in the mouse (6–9,[1,2]), herpes simplex infection of the rabbit eye (10), and lethal infections with Columbia SK virus (9, 11), encephalomyocarditis virus (12), influenza virus,[2] and foot and mouth disease in mice (13). Additional support for the role of interferon in the protection observed in these model infections came from the finding that antiviral protection was

[1] Claes, P., A. Billiau, E. De Clercq, J. Desmijter, E. Schonne, H. Vanderhaeghe, and P. De Somer. 1970. *J. Virol.* **5**: 313.

[2] Billiau, A., J. Desmijter, and P. De Somer. 1970. *J. Virol.* **5**: 321.

maximal when virus challenge was given shortly (2 hr to 1 day) after administration of the polymer, that is, at the time interferon production reached its peak. Polyanionic macromolecules have a wide variety of effects in the whole animal (see Discussion), only one of which is interferon production; hence a temporal relationship between the presence of interferon and antiviral protection is not adequate to prove a causal relationship. More substantial support for the role of interferon in the antiviral activity of synthetic polyanions, came from studies comparing the activity of polycarboxylates (maleic anhydride copolymers, polyacrylic acid, polyacetal carboxylic acids) with different densities of charge. Both interferon production and antiviral protection required a high density of free negative groups and decreased in parallel upon esterification or amidation of the carboxyl groups (maleic anhydride copolymers, polyacrylic acid) or insufficient oxidation of the parent compound (polyacetal carboxylic acids) (6, 14).[1]

The role of interferon in the antiviral protection conferred by MA/DVE and (poly rI)·(poly rC) is further assessed in the present report. First, optimal dose and time were determined for intravenously injected (poly rI)·(poly rC) to protect mice against intranasal VSV challenge. Interferon production and antiviral protection were then compared following injection of graded doses of (poly rI)·(poly rC). Secondly, several factors which specifically antagonize or potentiate interferon production, were examined to see whether they influenced resistance of mice to VSV challenge in parallel. Finally, conclusive evidence for the role of interferon in this protective effect was afforded by simulating serum levels of (poly rI)·(poly rC)–induced interferon with intravenously administered exogenous interferon.

METHODS

Animals. Randomly bred Swiss-Webster albino mice were used throughout this study. They were kept in an air-conditioned room at 20°C. Young (14 day to 24 day) mice were used for intranasal inoculation with VSV as older mice developed resistance to intranasal inoculation of the virus (3, 4).

Compounds. Maleic acid/divinyl ether copolymer NCS-46015-C (MA/DVE) (mol wt 17,000) was synthesized and characterized at Hercules Incorporated, Wilmington, Del., and provided by Dr. W. Regelson, Division of Oncology, Medical College of Virginia, Richmond, Va. The polymer was made up in minimal Eagle's medium (MEM) immediately before use.

Polyriboinosinic acid (poly rI)[3] and polycytidylic acid (poly rC) were purchased from P-L Biochemicals, Inc., Milwaukee, Wis. The homopolymers were dissolved in saline at 1 mg/ml; equal amounts of both polynucleotides were mixed and allowed to react for 1 hr at 25°C. Formation of double-stranded (poly rI)·(poly rC) was demonstrated by a marked hypochromic effect at 260 nm and a sharp transition (Tm) at 62°C. Small volumes of the stock solution (1 mg/ml) were stored at −20°C. Immediately before use a sample was thawed and diluted in PBS or saline to the appropriate concentration.

Arginine (L-arginine, free base A-grade) and diethylaminoethyl-dextran (DEAE-dextran) were purchased from Calbiochem, Los Angeles, Calif., and Pharmacia, Uppsala, Sweden respectively. Arginine was dissolved in MEM and DEAE-dextran in distilled water or saline just before use.

Salmonella enteritidis endotoxin was obtained from Difco Labs, Detroit, Mich. It was stored at 1 mg/ml or 0.1 mg/ml in saline at 4°C.

Kinetin riboside (6-furfurylaminopurine riboside) (Cyclo Chemical Corp., Los Angeles) was dissolved in warm 95% ethanol (15) and diluted to the appropriate concentration in saline. (Maximum ethanol concentration in the injected kinetin riboside solutions: 1%; 0.2 ml injected intravenously per mouse.) N^6-(Δ^2-isopentenyl) adenosine [N^6-(γ,γ-dimethylallyl) adenosine] was kindly supplied by Dr. M. H. Fleysher, Roswell Park Memorial Institute, Buffalo, N. Y. It was diluted to the appropriate concentration in saline before use.

Interferon. Mouse interferon was produced in L 929 cells as described elsewhere (16) with the E4 Herts strain of Newcastle disease virus. After acid inactivation of residual-inducing virus the tissue culture fluid was 10-fold concentrated by pressure dialysis in a pressure cell (Amicon Corp., Cambridge, Mass.) employing a UM-10 filter.

Interferon assay. Interferon titers in the serum were measured with a plaque reduction technique on mouse L cell monolayers using bovine vesicular stomatitis virus (VSV) (Indiana strain) as the challenge virus (6). The interferon titer corresponded to the reciprocal of the highest dilution of the sample which reduced virus plaque formation by 50%. Interferon was characterized by its sensitivity to trypsin (1.0 mg/ml, 37°C, 1 hr), thermolability (56°C, 1 hr), non-sedimentability (100,000 g, 2 hr), and species specificity (lack of activity in human skin fibroblasts).

Intranasal VSV assay. The Indiana strain of bovine vesicular stomatitis virus (VSV) was propagated and titrated in primary chick embryo fibroblasts. Titer of stock virus was 10^9 p.f.u. (plaque-forming units) per ml. Small volumes (0.5 ml) of stock virus were sealed in 2-ml glass vials and stored at −70°C. Before inoculation a sample was thawed and diluted in MEM. Unanesthetized mice were inoculated with the virus by applying 0.01 ml (14 day old mice) or 0.02 ml (older mice) of the virus suspension in four to six drops to the external nares from a number $27 \times \frac{1}{2}$ inch needle mounted on a 0.25 ml syringe. Mortality was recorded daily for 15 days. Animals surviving 15 days after injection without visible signs of illness were counted as survivors. Most animals died between the 4th and 7th days after virus challenge (14 day old mice), or between the 5th and 8th to 9th days (18 to 20 day old mice), or between the 6th and 10th days (24 day old mice). Illness became clinically evident on the 3rd to 4th day (14 day old mice) or the 4th to 5th day (18 to 20 day old mice) or the 5th to 6th day (24 day old mice). Characteristic signs of disease were fever, circling (predominantly to the left), flaccid paralysis of the hind legs, and convulsions.

Statistical significance was assessed by the χ^2 test (with Yates correction). χ^2_Y was computed for the *final* numbers

[3] Designation of polynucleotides according to the Rules of the Office of Biochemical Nomenclature, National Research Council (20 January 1969).

of mice which died or survived in treated and control groups, unless stated otherwise.

RESULTS

Dose response effect. Doses of (poly rI)·(poly rC) ranging from 0.016 to 8 mg/kg were injected intravenously 2 hr before intranasal challenge with VSV (Fig. 1). The protection observed was directly proportional to the dose of (poly rI)·(poly rC) employed: per cent survival rose from 10 [control and 0.016 mg/kg of (poly rI)·(poly rC)] to 36 for 0.08 mg/kg of (poly rI)·(poly rC), 63 (for 0.4 mg/kg), and to 80 (for 1.6 mg/kg). Raising the dose of (poly rI)·(poly rC) to 8 mg/kg did not further increase the number of surviving mice. From these data a minimum effective dose of 0.04 mg/kg may be calculated and, as (poly rI)·(poly rC) did not show signs of toxicity at the highest dose tested (8 mg/kg), its therapeutic index (with intravenous administration) is greater than 200. The dose of (poly rI)·(poly rC) found to protect mice from a lethal infection with VSV, 2 hr after the intravenous injection of the polymer, was much lower than that reported to confer protection 1 day after intraperitoneal injection (3).

When a range of (poly rI)·(poly rC) dosages were compared for interferon production and antiviral protection, parallel curves were obtained for the dose–interferon production and the dose–antiviral protection effects (Fig. 2). Both responses as plotted in Fig. 2 were nearly linear from 0.016 mg/kg to 1.6 mg/kg but leveled off at 8 mg/kg, the highest dose tested. No antiviral protection and only minimal interferon production (50 U/4 ml serum) were noted for 0.016 mg/kg of (poly rI)·(poly rC). The closely parallel dose-response curves suggest that the protective activity of (poly rI)·(poly rC) in this assay is closely related to interferon production. A direct relationship between the dose of (poly rI)·(poly rC), serum interferon titers, and survival was also established in mice inoculated with foot and mouth disease virus (13).

Requirement of complementary base-paired complex formation for interferon production and antiviral protection by (poly rI)·(poly rC). The homopolymer pair (poly rI)·(poly rC) has been reported to induce interferon production (2, 17), pyrogenicity (17), and antiviral protection (13) in animals at doses the homopolymers poly rI and poly rC alone did not (though Baron et al. [18] found some preparations of poly rI and poly rC active in producing interferon in rabbits). Although poly rI and poly rC alone have little, if any, interferon-inducing capacity, they reduced the occurrence of internal adenovirus type 12 tumors in hamsters (19). Stimulation of immune mechanisms by the homopolymers was suggested as an explanation of the latter effect (19).

To establish the role of interferon in the resistance induced by (poly rI)·(poly rC) against intranasal

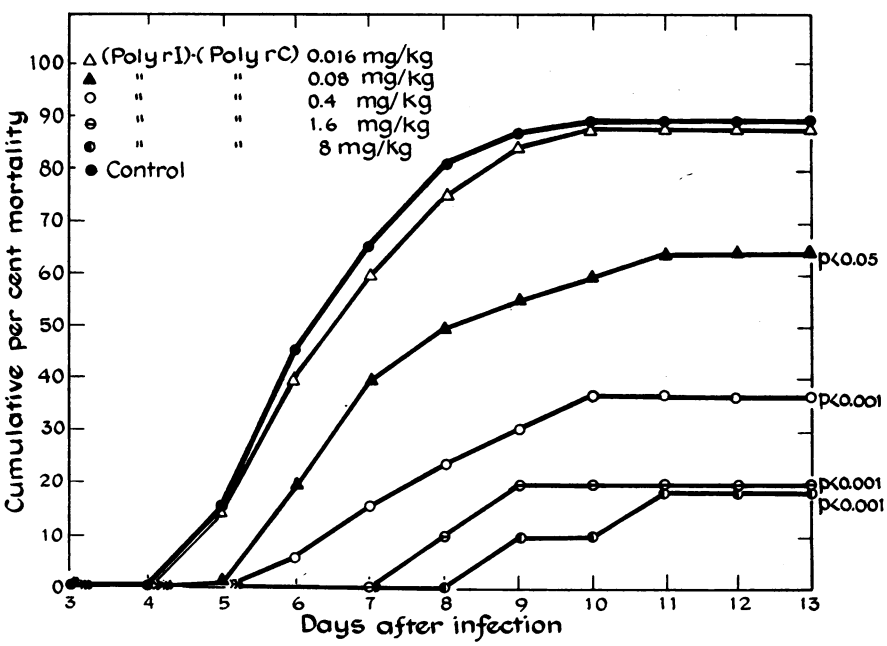

FIGURE 1 Effect of various doses of (poly rI)·(poly rC) on mortality produced by 5.10^6 p.f.u. of VSV in 20 day old mice (10–12 g). (Poly rI)·(poly rC) injected intravenously 2 hr before virus challenge. 30 mice per group.

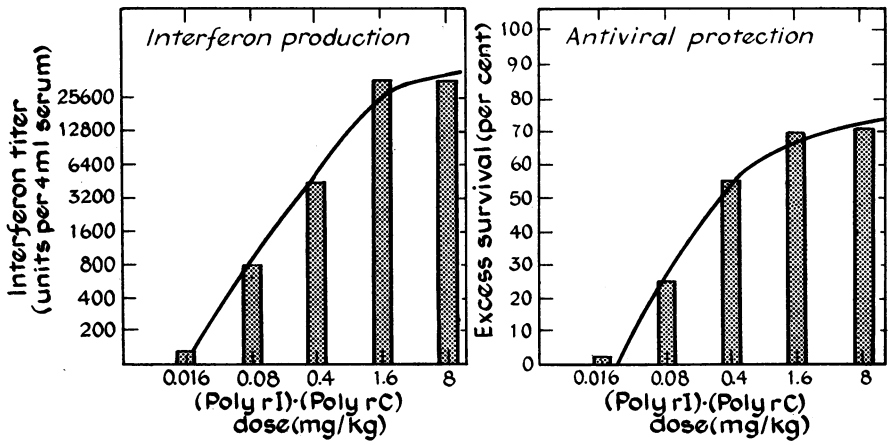

FIGURE 2. Interferon production and antiviral protection for graded doses of (poly rI)·(poly rC) injected intravenously into 20 day old mice (10–12 g). Antiviral protection presented as excess survival (%): data taken from Fig. 1. Interferon production 2 hr after injection of (poly rI)·(poly rC): mean values for three experiments with two mice per group.

VSV challenge in mice, poly rI and poly rC were tested for both interferon production and antiviral protection under conditions were (poly rI)·(poly rC) induced high serum interferon titers and conferred significant resistance to virus infection (Fig. 2). Table I shows that the single homopolymers poly rI and poly rC did not stimulate either interferon production or protection against intranasal VSV inoculation, whereas (poly rI)·(poly rC) did so. These findings indicate the importance of complementary base-pairing for the protective and interferon-inducing activity of (poly rI)·(poly rC) and relate the protection against VSV infection to the interferon produced.

Time response effect. Single or repeated doses of 0.4 mg/kg of (poly rI)·(poly rC) were administered intravenously several times either before or after intranasal challenge with VSV (Fig. 3). A single injection 2 hr before virus challenge was as effective as daily treatment starting 1 day after infection and continued until the 7th day after infection: with either regimen survival increased by 60%.

Treatment begun 3 days after infection and continued daily until the 7th day, increased the number of survivors by 30%. When treatment was delayed until first symptoms of disease (fever, circling, paralysis, convulsions) occurred (5th to 6th day) (3), there was

TABLE I

*Interferon Production and Antiviral Protection by Poly rI, Poly rC, and (Poly rI)·(Poly rC)**

Treatment‡	Interferon production§	Protection against intranasal VSV challenge‖		
		Survivors/ number challenged	Per cent survival	Significance
	U/4 ml serum			
(Poly rI)·(poly rC)	3200	13/21	62	$P < 0.02$
Poly rI	<30	4/21	19	NS
Poly rC	<30	3/21	14	NS
Control	<30	5/23	22	—

* Poly rI, poly rC, and (poly rI)·(poly rC) injected intravenously: 0.2 mg/kg for interferon determination, 0.4 mg/kg for antiviral protection.

‡ 2 hr before virus challenge or bleeding of animals for interferon determination.

§ In 20-g mice; mean values for two experiments (two mice per group).

‖ 2×10^6 p.f.u. in 20 day old mice (10–12 g).

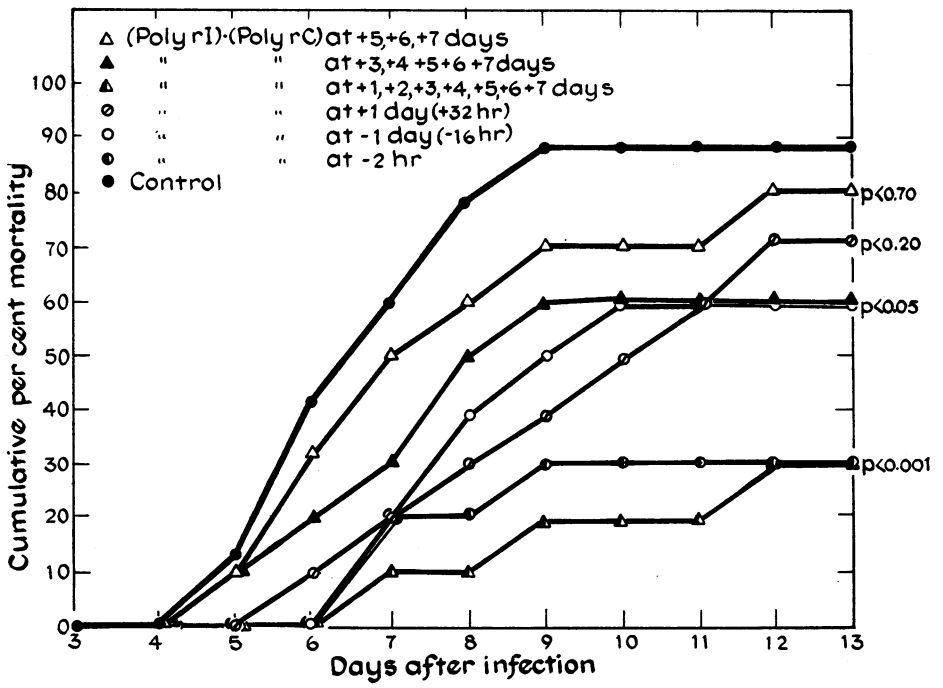

FIGURE 3 Effect of a single or repeated intravenous injections of (poly rI)·(poly rC) on mortality produced by 5.10^6 p.f.u. of VSV in 20 day old mice (10–12 g). A single dose of 0.4 mg/kg of (poly rI)·(poly rC) was administered either 2 hr or 16 hr before, or 32 hr after virus challenge. Repeated doses of 0.4 mg/kg of (poly rI)·(poly rC) injected daily, starting either 1 day, 3 days, or 5 days after infection, and continued until 7th day. 20 mice per group (40 mice for control group).

only a minor increase of the mean survival time and percentage of surviving mice. Thus, the protection obtained was closely related to the time treatment was initiated, as was noted previously (3) with intraperitoneal injection of (poly rI)·(poly rC). However, intravenous administration proved more effective than intraperitoneal administration when treatment was started after virus challenge, e.g., 5–10 mg/kg of (poly rI)·(poly rC), injected intraperitoneally at the 3rd and 5th days delayed mortality but did not prevent the eventual death of all mice (3), whereas 0.4 mg/kg of (poly rI)·(poly rC), injected intravenously daily from the 3rd until the 7th day, reduced the final deaths by 30%.

The optimal time for a single intravenous administration of (poly rI)·(poly rC) appeared to be 2 hr before virus inoculation; the antiviral effect diminished when the time of injection was shifted backwards (16 hr before virus infection) or forwards (32 hr after virus infection) (Fig. 3).

Effect of cations on interferon production and antiviral protection. Several factors, which are known to either antagonize or potentiate interferon production, were studied for their effect on interferon production and antiviral protection by MA/DVE and (poly rI)·(poly rC).

Basic substances such as DEAE-dextran (20–25), neomycin (25, 26) and other polyamines (26) have been shown active in potentiating induction of interferon or cellular resistance to virus infection by (poly rI)·

TABLE II

*Effect of Arginine on Interferon Production and Antiviral Protection by MA/DVE**

Treatment‡	Interferon production§	Protection against intranasal VSV challenge‖		
		Survivors/ number challenged	Per cent survival	Significance
	U/4 ml serum			
MA/DVE +arginine	245	9/22	41	$P < 0.005$
MA/DVE	95	5/20	25	$P < 0.05$
Control	<30	0/24	0	—

* MA/DVE: 100 mg/kg intraperitoneally; arginine: 300 mg/kg intraperitoneally; arginine injected simultaneously or 2–4 hr before MA/DVE

‡ 18–24 hr before virus challenge or bleeding of animals for interferon determination.

§ In 20-g mice; mean values for three experiments (two mice per group).

‖ 2×10^6 p.f.u. in 18 day old mice (9–11 g).

(poly rC) in vitro, though not in all cell cultures (26). DEAE-dextran has also been reported to increase interferon induction by (poly rI)·(poly rC) in the mouse (27). However, neomycin failed to increase interferon induction by (poly rI)·(poly rC) in the rabbit (26). So far, there is no evidence that basic substances (DEAE-dextran or neomycin) potentiate the activity of (poly rI)·(poly rC) against experimental viral infections in the whole animal.

Arginine has been observed to increase interferon production in mouse peritoneal macrophages in vitro by MA/DVE, when complexed to MA/DVE at a 3:1 weight ratio (charge equivalence) (6). Arginine also increased interferon production by MA/DVE in vivo and increased the antiviral effect of MA/DVE against intranasal VSV challenge in parallel (Table II). For this effect, both arginine and MA/DVE were injected intraperitoneally. The route of administration may be critical for the enhancing effect of arginine on the antiviral activity of MA/DVE, as arginine failed to increase the protective activity of MA/DVE when both were given intranasally (3).

Similar experiments were carried out to evaluate the effect of DEAE-dextran on interferon production and antiviral protection by (poly rI)·(poly rC) (Table III). Intravenous injection of DEAE-dextran had no effect on either induction of interferon or antiviral protection by (poly rI)·(poly rC) when they were given simultaneously by the same route. However, intraperitoneal injection of DEAE-dextran markedly reduced the capacity of (poly rI)·(poly rC) to stimulate both interferon production and protection against VSV infection (Table III). Intraperitoneally injected DEAE-dextran also diminished interferon response to MA/DVE (two-

TABLE III

*Effect of DEAE-Dextran on Interferon Production and Antiviral Protection by (Poly rI)·(Poly rC)**

Treatment	Interferon production‡	Protection against intranasal VSV challenge§		
		Survivors/ number challenged	Per cent survival	Significance
	U/4 ml serum			
Both DEAE-dextran and (poly rI)·(poly rC) injected intraperitoneally‖				
(Poly rI)·(poly rC) + DEAE-dextran	120	1/20	5	NS
(Poly rI)·(poly rC)	1800	11/21	52	$P < 0.005$
DEAE-dextran	<30	0/10	0	NS
Control	<30	1/23	4	—
Both DEAE-dextran and (poly rI)·(poly rC) injected intravenously¶				
(Poly rI)·(poly rC) + DEAE-dextran	2700	7/10	70	$P < 0.001$
(Poly rI)·(poly rC)	2400	7/10	70	$P < 0.001$
DEAE-dextran	<30	—	—	—
Control	<30	5/50	10	—

NS = not significant.

* (Poly rI)·(poly rC): 5 mg/kg intraperitoneally for interferon production, 15 mg/kg intraperitoneally for antiviral protection, 0.4 mg/kg intravenously for both interferon production and antiviral protection. DEAE-dextran: 50 mg/kg intraperitoneally for interferon production, 75 mg/kg intraperitoneally for antiviral protection, 15 mg/kg intravenously for both interferon production and antiviral protection.

‡ In 20-g mice; mean values for three experiments (two mice per group).

§ 5.10^4 p.f.u. in 14 day old mice (6–7 g) for DEAE-dextran and (poly rI)·(poly rC) injected intraperitoneally; 5.10^6 p.f.u. in 20 day old mice (10–12 g) for DEAE-dextran and (poly rI)·(poly rC) injected intravenously.

‖ DEAE-dextran 4 hr before (poly rI)·(poly rC); (poly rI)·(poly rC) 16 hr before bleeding of animals for interferon determination and 24 hr before virus challenge.

¶ DEAE-dextran and (poly rI)·(poly rC) injected simultaneously 2 hr before virus challenge or bleeding of animals for interferon determination.

to threefold) and concomitantly diminished the antiviral protection by MA/DVE (reduction of mean survival time by 24 hr) (data not shown on Table III). DEAE-dextran alone did not change the mortality rate of VSV-infected mice, suggesting that its effect on the antiviral activity of (poly rI)·(poly rC) and MA/DVE was due to an interaction with the polyanion itself.

Hyporeactivity to both interferon production and antiviral protection. Prior injection of animals with endotoxin, various viruses, statolon, MA/DVE, polyacrylic acid, or (poly rI)·(poly rC) results in reduction of the interferon response to a second injection of the homologous stimulus several days later (homologous hyporeactivity or tolerance) (6, 10, 14, 28–37). Heterologous hyporeactivity has been observed between endotoxin and virus (28, 29, 31), virus and statolon (33), endotoxin and (poly rI)·(poly rC) (33, 35), but not between statolon and endotoxin (32) or (poly rI)·(poly rC) (34). A gradient in the ability to produce tolerance has recently been proposed by Ho, Breinig, Postic, and Armstrong (36), endotoxin being the most effective agent, virus the least effective and (poly rI)·(poly rC) of intermediate activity.

We were interested in whether hyporeactivity to interferon induction was associated with hyporeactivity to antiviral protection. The effects of repeated injections of interferon inducers were explored in mice given two doses of MA/DVE 5 days apart, and mice given (poly rI)·(poly rC) 2 days after endotoxin. Both treatment schedules are known to significantly diminish the interferon response to the second stimulus (6, 34, 35). Pretreatment of mice with MA/DVE not only reduced the interferon-inducing capacity but also abolished the protective activity of a second dose 5 days later (Table IV). A single injection of MA/DVE conferred maximal protection when given 1 day before VSV challenge (Table IV) and was slightly active when given 10 days before virus inoculation (3). The injection of MA/DVE showed very little, if any, activity when given 6 days before virus inoculation (3), (Table IV). Similarly, prior injection of endotoxin produced a marked decrease in the interferon response to (poly rI)·(poly rC) 2 days later, as well as a decrease in its antiviral effect in the intranasal VSV assay (Table V). Injection of endotoxin alone, 3 days before virus challenge, did not influence the mortality rate.

The parallelism between hyporeactivity to interferon production and hyporeactivity to antiviral protection, as examined in the two different systems, suggests that both mechanisms are interrelated, the simplest relationship obviously being that interferon production underlies the protective activity of MA/DVE and (poly rI)·(poly rC) against intranasal VSV infection in the mouse.

Effect of the nucleoside analogs kinetin riboside and isopentenyladenosine on interferon production and antiviral protection. The plant cytokinines, kinetin riboside and isopentenyladenosine, reverse the stimulatory effects of oligodeoxyribonucleotides and double-stranded RNA's such as (poly rA)·(poly rU) on antibody production (38–40) and phagocytosis (41); kinetin riboside also antagonizes the effect of endotoxin on host resistance to infection with *Pseudomonas aeruginosa* (15). Kinetin riboside and isopentenyladenosine do not inhibit the normal immune response, but only the adjuvant-like effects on antibody production.

As double-stranded RNA has an adjuvant-like effect on normal antibody production (42), as well as initiates

TABLE IV

*Tolerance to Interferon Production and Antiviral Protection by MA/DVE**

Time between injections of polymer and virus challenge or bleeding of animals for interferon determination			Protection against intranasal VSV challenge§		
First injection	Second injection	Interferon production‡	Survivors/ number challenged	Per cent survival	Significance
days		*U/4 ml serum*			
—	−1	150	36/51	70	$P < 0.001$
−6	—	<30	20/52	38	NS
−6	−1	<30	9/22	41	NS
—	—	<30	36/106	34	—

NS = not significant.

* 125 mg/kg intraperitoneally.

‡ In 25-g mice, as reported earlier (6).

§ 2×10^6 p.f.u. in 24 day old mice (12–14 g).

interferon production and confers antiviral protection, we examined the effects of kinetin riboside and isopentenyladenosine on interferon production and antiviral protection by (poly rI)·(poly rC). Both nucleoside analogs caused a 4- to 5-fold decrease of the interferon response to (poly rI)·(poly rC) (Fig. 4 a) and decreased the activity of (poly rI).(poly rC) against intranasal VSV challenge (Fig. 4 b). Kinetin riboside and isopentenyladenosine did not increase the mortality rate of (poly rI)·(poly rC)–treated mice during the early mortality period (4th to 7th day) but did so during the later period (8th to 14th day). Neither material blocked interferon production or antiviral protection completely.

The parallel effects of kinetin riboside and isopentenyladenosine on both interferon production, antiviral protection (Fig. 4), phagocytosis (41), and production of specific antibody (38–40) support the hypothesis that a common mechanism (release of oligonucleotides ? [43]) might underlie interferon production, antiviral protection, and the other host resistance mechanisms.

Simulation of the antiviral effect of endogenous interferon with exogenous interferon. To directly evaluate the role of interferon in the antiviral protection conferred by (poly rI)·(poly rC), attempts were made to simulate the endogenously produced interferon with exogenous interferon. When the integrated areas under the time response curves were considered, four repeated doses of 400 U of interferon per mouse, injected intravenously 2 hr apart, produced an interferon response in the circulation, which was intermediate between the endogenous interferon levels induced by 0.08 mg/kg and 0.016 mg/kg of (poly rI)·(poly rC) (Fig. 5, a, b, and c: left side). It is of interest that the intravenously administered interferon was cleared rapidly from the circulation but its rate of removal was slower than observed by others (44, 45). Disappearance was far from complete 2 hr after the injections and about 10% of the original serum titer was detected 2 hr after the 4th interferon injection. Brain interferon titers were measured after injection of interferon and (poly rI)·(poly rC) (0.08 mg/kg) and, though smaller amounts of interferon were found in the brain tissue than in the serum, these levels increased and decreased in parallel (Fig. 5, a and b: left side).

The protection against intranasal VSV challenge was closely related to the levels of circulating interferon, no matter where the interferon originated from (exogenous or endogenous): 0.08 mg/kg of (poly rI)·(poly rC) increased the number of surviving mice by 26% ($P < 0.05$), 0.016 mg/kg of (poly rI)·(poly rC) did not affect survival, but passive interferon treatment increased the survival by 12% ($P < 0.30$) (Fig. 5, a, b, and c: right side). When the daily death and survival counts (as determined from the day the first animal died to the day to the last animal died in either group) were compared, protection conferred by 0.08 mg/kg of (poly rI)·(poly rC) was significant at the $P < 0.001$ level and for interferon at the $P < 0.005$ level, whereas protection by 0.016 mg/kg of (poly rI)·(poly rC) was not significant.

The circulating interferon titers and the antiviral protection obtained with exogenous interferon were both intermediate between the circulating interferon

TABLE V

*Effect of Prior Injection of Endotoxin on Interferon Production and Antiviral Protection by (Poly rI)·(Poly rC)**

Treatment‡		Interferon production§	Protection against intranasal VSV challenge‖		
First injection	Second injection		Survivors/number challenged	Per cent survival	Significance
		U/4 ml serum			
—	(Poly rI)·(poly rC)	480	5/10	50	$P < 0.025$
Endotoxin	(Poly rI)·(poly rC)	<30	1/9	10	NS
Endotoxin	—	<30	0/10	0	NS
—	—	<30	0/12	0	—

NS = not significant.

* Both endotoxin and (poly rI)·(poly rC) intraperitonelly. Endotoxin: 5 mg/kg for interferon determinations, 3 mg/kg for antiviral protection. (Poly rI)·(poly rC): 2.5 mg/kg for interferon production, 15 mg/kg for antiviral protection.

‡ First injection 2 days before second injection; second injection 6 hr before bleeding of animals for interferon determination or 1 day before virus challenge.

‖ 5.10^4 p.f.u. in 14 day old mice (6–7 g).

§ In 20-g mice; mean values for two experiments (two mice per group).

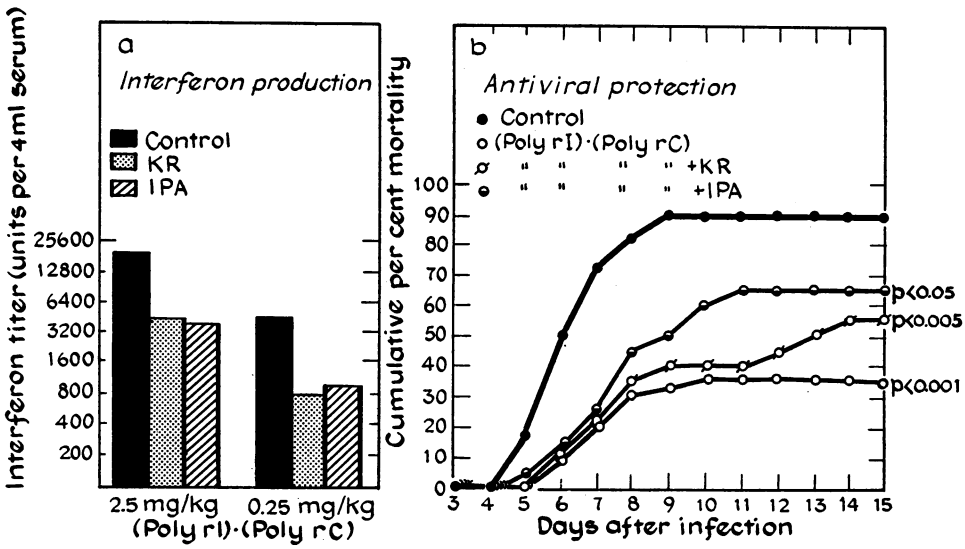

FIGURE 4 Effect of kinetin riboside and isopentenyladenosine on interferon production and antiviral protection by (poly rI)·(poly rC). Mortality was produced by 5.10^6 p.f.u. of VSV in 20 day old mice (10–12 g) (30 mice per group). Interferon titers were determined in 20-g mice (mean values for three experiments with two mice per group). Both kinetin riboside, isopentenyladenosine, and (poly rI)·(poly rC) were injected intravenously 2 hr before virus challenge or bleeding of animals for interferon determination. Doses: (Poly rI)·(poly rC): as indicated for interferon production, 0.4 mg/kg for antiviral protection; kinetin riboside (KR) and isopentenyladenosine (IPA): 50 mg/kg for interferon production, 30 mg/kg for antiviral protection.

production and protective effect of 0.08 and 0.016 mg/kg of (poly rI)·(poly rC), and appeared to be equivalent to the interferon and resistance-inducing capacity of approximately 0.04 mg/kg of (poly rI)·(poly rC). Thus, under our experimental conditions, (poly rI)·(poly rC) did not offer a better protective effect against intranasal VSV than could be expected from the interferon it produced, indicating that its whole activity was due to interferon production.

DISCUSSION

The activity of (poly rI)·(poly rC) and other synthetic polyanions has been evaluated against a variety of acute viral infections in experimental models (2, 3, 6–13, 46, 47),[1, 2] some of which resemble natural virus infections in animals and man (e.g., herpetic keratoconjunctivitis [10], viral meningo-encephalitis [3]). Whenever a dose-response relationship for (poly rI)·(poly rC) was established, its minimum effective dose proved far below the maximum tolerated dose: graded doses of (poly rI)·(poly rC) were given prophylactically to mice, infected subcutaneously with Columbia SK virus (11) or intranasally with VSV (Fig. 1). Intraperitoneal (poly rI)·(poly rC) was effective at 0.2 mg/kg in the first model and intravenous (poly rI)·(poly rC) at 0.04 mg/kg in the second. The maximum tolerated doses of (poly rI)·(poly rC) were 12.5 mg/kg and $\geq$ 8 mg/kg respectively, indicating a margin of safety (or therapeutic index: ratio of maximum tolerated dose to minimum effective dose) of 60 (first model) and $\geq$ 200 (second). Intranasal (poly rI)·(poly rC) prevented death in mice infected with mouse pneumonia virus or Columbia SK virus given by the same route, at a dosage level as low as 0.006 mg/kg (9, 46). As mice did not show a significant behavioral change when given 1 mg/kg of (poly rI)·(poly rC) by the respiratory route a favorable activity to toxicity ratio was also achieved for intranasal administration (46).

The route of administration of (poly rI)·(poly rC) has been found to be critical as was the time of initiation of treatment and the type of infection in which it was used. Intranasal (poly rI)·(poly rC) increased survival ratio of mice infected with intranasal VSV when given shortly before virus, but failed to protect when given after virus challenge (3). In contrast, intraperitoneal (poly rI)·poly rC) (3) and especially intravenous (poly rI)·(poly rC) (Fig. 3) conferred significant protection when applied several days after intranasal VSV challenge. However, intraperitoneal (poly rI)·(poly rC), 1 day after virus inoculation, did not decrease the lethal effect of a subcutaneous Columbia SK virus infection (11). Against intranasally inoculated influenza virus, intranasal (poly rI)·(poly rC) was active, whereas intraperitoneal or intravenous (poly rI)·(poly rC) was

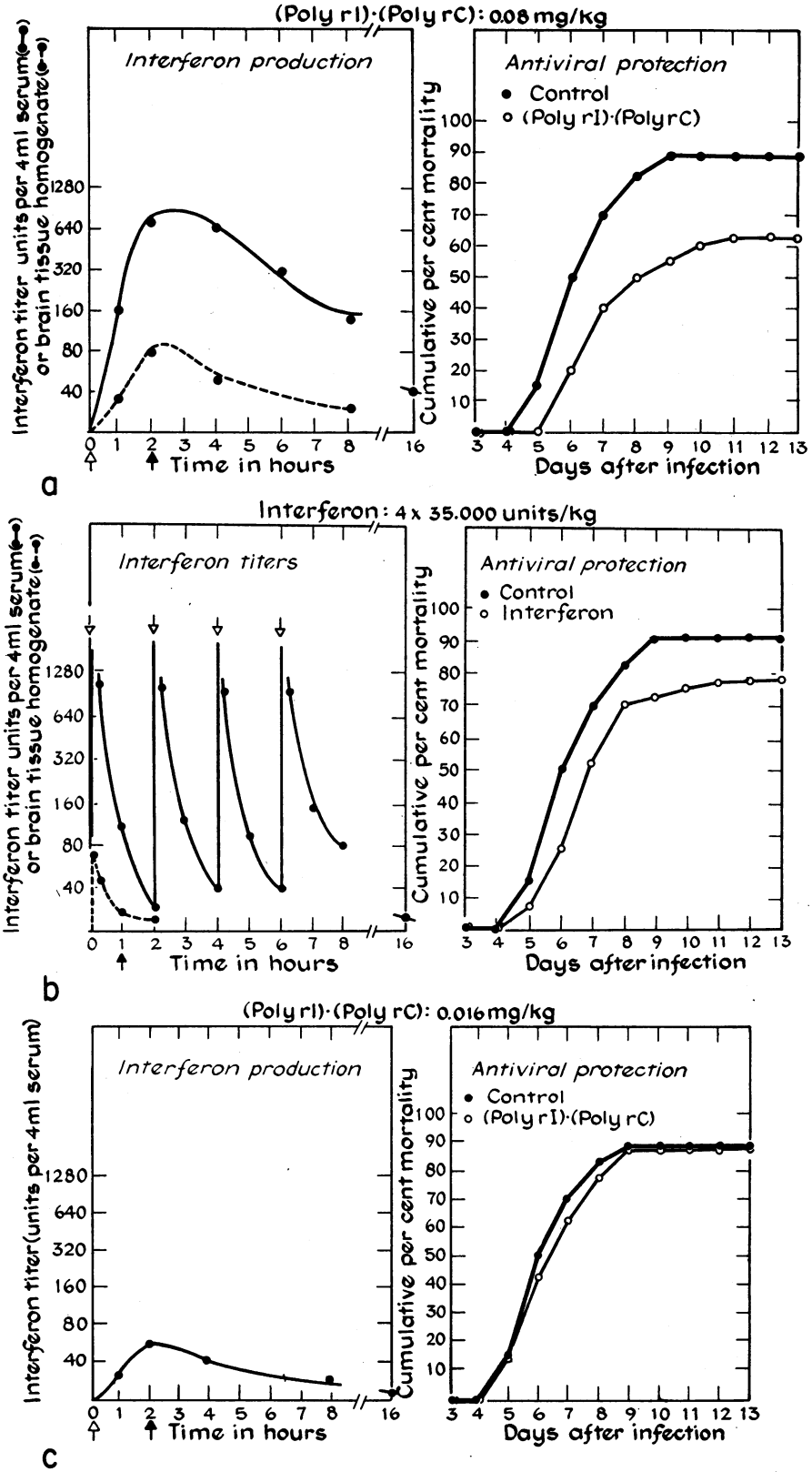
(Poly rI)·(Poly rC): 0.08 mg/kg
Interferon production
Interferon titer units per 4 ml serum(●–●) or brain tissue homogenate(○–○)
1280
640
320
160
80
40
0 1 2 3 4 5 6 7 8 16
Time in hours
Antiviral protection
● Control
○ (Poly rI)·(Poly rC)
Cumulative per cent mortality
100 90 80 70 60 50 40 30 20 10
3 4 5 6 7 8 9 10 11 12 13
Days after infection
a
Interferon: 4 x 35.000 units/kg
Interferon titers
Interferon titer units per 4 ml serum(●–●) or brain tissue homogenate(○–○)
1280
640
320
160
80
40
0 1 2 3 4 5 6 7 8 16
Time in hours
Antiviral protection
● Control
○ Interferon
Cumulative per cent mortality
100 90 80 70 60 50 40 30 20 10
3 4 5 6 7 8 9 10 11 12 13
Days after infection
b
(Poly rI)·(Poly rC): 0.016 mg/kg
Interferon production
Interferon titer (units per 4 ml serum)
1280
640
320
160
80
40
0 1 2 3 4 5 6 7 8 16
Time in hours
Antiviral protection
● Control
○ (Poly rI)·(Poly rC)
Cumulative per cent mortality
100 90 80 70 60 50 40 30 20 10
3 4 5 6 7 8 9 10 11 12 13
Days after infection
c

FIGURE 5

not (11). In some virus infections, such as herpetic keratoconjunctivitis (10), vaccinia tail lesions (9), and Columbia SK virus infection, (9), (poly rI)·(poly rC) afforded essentially the same level of protection, regardless of its route of administration.

Interferon is thought to mediate the protective effect of (poly rI)·(poly rC) and other synthetic polyanions in acute viral infections, but there is no direct proof of its role. (Poly rI)·(poly rC) or MA/DVE were also reported to block in mice or hamsters the growth of a variety of malignant tumors, whether or not they are known to contain or to depend on a virus (Friend leukemia [48–50], Moloney sarcoma [51, 52], L-1210 leukemia [53, 54], adenovirus 12 and SV_{40} virus–induced tumors [19, 55], malignant melanoma [56], RC 19 [Rauscher virus–induced transplantable] tumor [57], and other transplant tumors [54]) and to protect mice from lethal infections with bacteria (*Listeria monocytogenes* [58], *Escherichia coli*,[4] pneumococcus [59]), fungi (*Cryptococcus neoformans* [59]) or protozoa (*Plasmodium berghei* [60], *Leishmania donovani*[5, 6]). The possible role of interferon in the anti-tumor, antibacterial, and anti-fungal activity of (poly rI)·(poly rC) is even more questionable than its role in the antiviral activity of (poly rI)·(poly rC), as interferon has not yet been shown effective against some tumors, fungal and bacterial infections which are inhibited by (poly rI)·(poly rC) (e.g., malignant melanoma [56], *Listeria monocytogenes* [58], *Escherichia coli*[4]). Other mechanisms such as stimulation of the reticuloendothelial activity (58, 59, 61), humoral (42), and cellular (62) immunity may explain the anti-tumor, -bacterial, and -fungal action of (poly rI)·(poly rC).

Although mechanisms not involving interferon might be quantitatively more important than interferon in the regression of tumors and the outcome of nonviral infections treated with (poly rI)·(poly rC), their part in the protective effect of (poly rI)·(poly rC) against acute viral infections such as intranasal VSV challenge does not seem to be significant. This study presents several points suggesting that the whole protective effect of (poly rI)·(poly rC), injected intravenously 2 hr before intranasal VSV inoculation, is attributable to interferon production: (*a*) a single intravenous injection of (poly rI)·(poly rC) conferred maximal protection when given 2 hr before virus challenge, that is at the time of maximal interferon production (Fig. 3); (*b*) interferon production and antiviral protection increased almost linearly with increasing doses of (poly rI)·(poly rC), went up at the same dose and leveled off at the same dose (Fig. 2); (*c*) at a dosage level where (poly rI)·(poly rC) was highly active in inducing interferon and protection against intranasal VSV challenge, the single homopolymers poly rI and poly rC failed to stimulate interferon production or confer antiviral protection (Table I); (*d*) several factors which specifically increased or decreased the interferon response (arginine, DEAE-dextran, kinetin riboside, isopentenyladenosine, and repeated administration of the interferon inducer) enhanced or reduced the antiviral protection afforded by either (poly rI)·(poly rC) or MA/DVE in parallel (Tables II, III, IV, and V, Fig. 4); (*e*) administration of exogenous interferon in amounts which closely mimicked the levels of endogenous interferon production by (poly rI)·(poly rC) gave essentially the same antiviral protection as the corresponding dose of (poly rI)·(poly rC) (Fig. 5).

In the latter experiments, exogenous interferon proved as effective as endogenous interferon at similar serum levels. In studies performed by Gresser, Fontaine-Brouty-Boyé, Bourali, and Thomas (12) and Gresser and Bourali (57) exogenous interferon therapy was even more efficacious than treatment with inducers of endogenous interferon (in mice inoculated with encephalomyocarditis virus or RC 19 tumor cells). However,

[4] Weinstein, M. J., J. A. Waitz, and P. E. Came. 1970. *Nature (London)*. **226**: 170.

[5] Actor, P., D. Kunkel, M. Goore, S. Garg, and J. Pagano. 1969. Personal communication.

[6] Herman, R., and S. Baron. 1970. *Nature (London)*. **226**: 168.

FIGURE 5 Comparison of interferon titers in serum and brain tissue and degree of protection against intranasal VSV challenge, following intravenous injection of either (poly rI)·(poly rC) or interferon. *Doses:* (poly rI)·(poly rC): single injection of either 0.08 mg/kg (Fig. 5 *a*) or 0.016 mg/kg (Fig. 5 *c*) 2 hr before virus challenge; interferon: repeated injections of 400 U per mouse (35.000 U/kg) (Fig. 5 *b*) at 1 hr before and 1 hr, 3 hr, and 5 hr after virus challenge. Time of injections of (poly rI)·(poly rC) or interferon indicated by white arrows; time of VSV challenge indicated by black arrows. All experiments performed with 20 day old mice (10–12 g): interferon titers are mean values for three experiments with two mice per group, antiviral protection data are obtained with 30 mice per group. Mortality was induced with 5.10^6 p.f.u. of VSV per mouse. Black arrows indicate time(s) of VSV infection in relationship to determination of interferon titer. Interferon titers were determined at 15 min, 1 hr, and 2 hr after the injection of interferon, and 1, 2, 4, 6, and 8 hr after the injection of (poly rI)·(poly rC). Interferon was also measured 16 hr after the injection of (poly rI)·(poly rC) or first injection of interferon. Brain tissue homogenate was prepared by Dounce homogenizing brain tissue in 5 volumes MEM.

exogenous interferon and interferon inducers were administered in arbitrarily chosen dosages and the treatment regimen used for (poly rI)·(poly rC) (and Newcastle disease virus) was likely to lead to hyporeactivity (10, 28, 36, 37). Moreover, circulating interferon titers were not determined, so that the efficacy of exogenous versus endogenous interferon treatment could not be compared on the basis of interferon titers obtained in the animal.

ACKNOWLEDGMENT

This investigation was supported by U. S. Public Health Service Grant AI 05629.

REFERENCES

1. Merigan, T. C. 1967. Induction of circulating interferon by synthetic anionic polymers of known composition. *Nature (London)*. **214**: 416.
2. Field, A. K., A. A. Tytell, G. P. Lampson, and M. R. Hilleman. 1967. Inducers of interferon and host resistance. II. Multistranded synthetic polynucleotide complexes. *Proc. Nat. Acad. Sci. U.S.A.* **58**: 1004.
3. De Clercq, E., and T. C. Merigan. 1969. Local and systemic protection by synthetic polyanionic interferon inducers in mice against intranasal vesicular stomatitis virus. *J. Gen. Virol.* **5**: 359.
4. Sabin, A. B., and P. K. Olitsky. 1938. Influence of host factors on neuro-invasiveness of vesicular stomatitis virus. III. Effect of age and pathway of infection on the character and localization in the central nervous system. *J. Exp. Med.* **67**: 201.
5. Johnson, R. T., and C. A. Mims. 1968. Pathogenesis of viral infections of the nervous system. *N. Engl. J. Med.* **278**: 23.
6. Merigan, T. C., and M. S. Finkelstein. 1968. Interferon-stimulating and *in vivo* antiviral effects of various synthetic anionic polymers. *Virology*. **35**: 363.
7 De Clercq, E., and P. De Somer. 1968. Effect of interferon, polyacrylic and polymethacrylic acids on tail lesions in vaccinia-infected mice. *Appl. Microbiol.* **16**: 1314.
8. De Clercq, E., and P. De Somer. 1969. Prolonged antiviral protection by interferon inducers. *Proc. Soc. Exp. Biol. Med.* **132**: 699.
9. Nemes, M. M., A. A. Tytell, G. P. Lampson, A. K. Field, and M. R. Hilleman. 1969. Inducers of interferon and host resistance. VI. Antiviral efficacy of poly I:C in animal models. *Proc. Soc. Exp. Biol. Med.* **132**: 776.
10. Park, J. H., and S. Baron. 1968. Herpetic keratoconjunctivitis: therapy with synthetic double-stranded RNA. *Science (Washington)*. **162**: 811.
11. Lindh, H. F., H. L. Lindsay, B. R. Mayberry, and M. Forbes. 1969. Polyinosinic-cytidylic acid complex (poly I:C) and viral infections in mice. *Proc. Soc. Exp. Biol. Med.* **132**: 83.
12. Gresser, I., D. Fontaine-Brouty-Boyé, C. Bourali, and M. T. Thomas. 1969. A comparison of the efficacy of endogenous, exogenous, and combined endogenous-exogenous interferon in the treatment of mice infected with encephalomyocarditis virus. *Proc. Soc. Exp. Biol. Med.* **130**: 236.
13. Richmond, J. Y., and L. D. Hamilton. 1969. Foot-and-mouth disease virus inhibition induced in mice by synthetic double-stranded RNA (polyriboinosinic and polyribocytidylic acids). *Proc. Nat. Acad. Sci. U.S.A.* **64**: 81.
14. De Somer, P., E. De Clercq, A Billiau, E. Schonne, and M. Claesen. 1968. The antiviral activity of polyacrylic and polymethacrylic acids. II. Mode of action in vivo. *J. Virol.* **2**: 886.
15. Kessel, R. W. I., and W. Braun. 1966. Endotoxin-enhanced host resistance: reversal by kinetin riboside. *Nature (London)*. **211**: 1001.
16. Hanna, L., T. C. Merigan, and E. Jawetz. 1966. Inhibition of TRIC agents by virus-induced interferon. *Proc. Soc. Exp. Biol. Med.* **122**: 417.
17. Lindsay, H. L., P. W. Trown, J. Brandt, and M. Forbes. 1969. Pyrogenicity of poly I: poly C in rabbits. *Nature (London)*. **223**: 717.
18. Baron, S., N. N. Bogomolova, A. Billiau, H. B. Levy, C. E. Buckler, R. Stern, and R. Naylor. 1969. Induction of interferon by preparations of synthetic single-stranded RNA. *Proc. Nat. Acad. Sci. U.S.A.* **64**: 67.
19. Larson, V. M., W. R. Clark, and M. R. Hilleman. 1969. Influence of synthetic (poly I:C) and viral double-stranded ribonucleic acids on adenovirus 12 oncogenesis in hamsters. *Proc. Soc. Exp. Biol. Med.* **131**: 1002.
20. Dianzani, F., P. Cantagalli, S. Gagnoni, and G. Rita. 1968. Effect of DEAE-dextran on production of interferon induced by synthetic double-stranded RNA in L cell cultures. *Proc. Soc. Exp. Biol. Med.* **128**: 708.
21. Vilcek, J., M. H. Ng, A. E. Friedman-Kien, and T. Krawciw. 1968. Induction of interferon synthesis by synthetic double-stranded polynucleotides. *J. Virol.* **2**: 648.
22. Falcoff, E., and R. Perez-Bercoff. 1969. Interférons humains induits in vitro par des polynucléotides synthétiques. *Biochim. Biophys. Acta.* **174**: 108.
23. Colby, C., and M. J. Chamberlin. 1969. The specificity of interferon induction in chick embryo cells by helical RNA. *Proc. Nat. Acad. Sci. U.S.A.* **63**: 160.
24. Bausek, G. H., and T. C. Merigan. 1969. Cell interaction with a synthetic polynucleotide and interferon production in vitro. *Virology*. **39**: 491.
25. Billiau, A., C. E. Buckler, F. Dianzani, C. Uhlendorf, and S. Baron. 1969. Induction of the interferon mechanism by single-stranded RNA: potentiation by polybasic substances. *Proc. Soc. Exp. Biol. Med.* **132**: 790.
26. Lampson, G. P., A. A. Tytell, A. K. Field, M. M. Nemes, and M. R. Hilleman. 1969. Influence of polyamines on induction of interferon and resistance to viruses by synthetic polynucleotides. *Proc. Soc. Exp. Biol. Med.* **132**: 212.
27. Dianzani, F., G. Rita, P. Cantagalli, and S. Gagnoni. 1969. Effect of DEAE-dextran on interferon production and protective effect in mice treated with the double-stranded polynucleotide complex polyinosinic-polycytidylic acid. *J. Immunol.* **102**: 24.
28. Youngner, J. S., and W. S. Stinebring. 1965. Interferon appearance stimulated by endotoxin, bacteria, or viruses in mice pre-treated with *Escherichia coli* endotoxin or infected with *Mycobacterium tuberculosis*. *Nature (London)*. **208**: 456.
29. Ho, M., and Y. Kono. 1965. Tolerance to the induction of interferons by endotoxin and virus. *J. Clin. Invest.* **44**: 1059.
30. Ho, M., Y. Kono, and M. K. Breinig. 1965. Tolerance to the induction of interferons by endotoxin and virus: role of a humoral factor. *Proc. Soc. Exp. Biol. Med.* **119**: 1227.

31. De Somer, P., and A. Billiau. 1966. Interferon production by the spleen of rats after intravenous injection of Sindbis virus or heat-killed Escherichia coli. *Arch. Gesamte Virusforsch.* **19**: 143.
32. Youngner, J. S., and W. R. Stinebring. 1966. Comparison of interferon production in mice by bacterial endotoxin and statolon. *Virology.* **29**: 310.
33. Youngner, J. S. 1968. Interferon production in mice injected with viral and nonviral stimuli. *In* Medical and Applied Virology. M. Sanders and E. H. Lennette, editors. Warren H. Green, Inc., St. Louis. 210.
34. Absher, M., and W. R. Stinebring. 1969. Endotoxin-like properties of poly I. poly C, an interferon stimulator. *Nature (London).* **223**: 715.
35. De Clercq, E., and T. C. Merigan. 1969. An active interferon inducer obtained from *Hemophilus influenzae* type b. *J. Immunol.* **103**: 899.
36. Ho, M., M. K. Breinig, B. Postic, and J. A. Armstrong. 1970. Tolerance to the interferon-stimulating effect of poly I:C, virus and endotoxin. *Ann. N. Y. Acad. Sci.* In press.
37. Baron, S., H. duBuy, C. E. Buckler, M. L. Johnson, J. Park, A. Billiau, P. Sarma, and R. J. Huebner. 1970. Induction of interferon and viral resistance in animals by polynucleotides. *Ann. N. Y. Acad. Sci.* In press.
38. Braun, W., and M. Nakano. 1965. Influence of oligodeoxyribonucleotides on early events in antibody formation. *Proc. Soc. Exp. Biol. Med.* **119**: 701.
39. Braun, W., and M. Nakano. 1967. Antibody formation: stimulation by polyadenylic and polycytidylic acids. *Science (Washington).* **157**: 819.
40. Braun, W., M. Nakano, L. Jaraskova, Y. Yajima, and L. Jimenez. 1968. Stimulation of antibody-forming cells by oligonucleotides of known composition. *In* Nucleic Acids in Immunology. O. J. Plescia and W. Braun, editors. Springer-Verlag New York Inc., New York. 347.
41. Friedman, H. H., and W. Braun. 1965. Influence of oligodeoxyribonucleotides on phagocytic activity of the reticuloendothelial system. *Proc. Soc. Exp. Biol. Med.* **120**: 222.
42. Woodhour, A. F., A. Friedman, A. A. Tytell, and M. R. Hilleman. 1969. Hyperpotentiation by synthetic double-stranded RNA of antibody responses to influenza virus vaccine in adjuvant 65. *Proc. Soc. Exp. Biol. Med.* **131**: 809.
43. Braun, W., and W. Firshein. 1967. Biodynamic effects of oligonucleotides. *Bacteriol. Rev.* **31**: 83.
44. Baron, S., C. E. Buckler, R. V. McCloskey, and R. L. Kirschstein. 1966. Role of interferon during viremia. I. Production of circulating interferon. *J. Immunol.* **96**: 12.
45. Ho, M., and B. Postic. 1967. Prospects for applying interferon to man. First International Conference on Vaccines against Viral and Rickettsial Diseases of man. Pan American Health Organization, Washington, D. C. 632.
46. Hilleman, M. R. 1970. Prospects for the use of double-stranded ribonucleic acid (poly I:C) inducers in man. *J. Infec. Dis.* **121**: 196.
47. De Clercq, E., and P. De Somer. 1968. Protective effect of interferon and polyacrylic acid in newborn mice infected with a lethal dose of vesicular stomatitis virus. *Life Sci.* **7**: 925.
48. Regelson, W. 1967. Prevention and treatment of Friend leukemia virus (FLV) infection by interferon-inducing synthetic polyanions. *Adv. Exp. Med. and Biol.* **1**: 315.
49. Chirigos, M. A., W. Turner, J. Pearson, and W. Griffin. 1969. Effective antiviral therapy of two murine leukemias with an interferon-inducing synthetic carboxylate copolymer. *Int. J. Cancer.* **4**: 267.
50. Larson, V. M., W. R. Clark, G. E. Dagle, and M. R. Hilleman. 1969. Influence of synthetic double-stranded ribonucleic acid, poly I: C, on Friend leukemia in mice. *Proc. Soc. Exp. Biol. Med.* **132**: 602.
51. Sarma, P. S., G. Shiu, R. H. Neubauer, S. Baron, and R. J. Huebner. 1969. Virus-induced sarcoma of mice: inhibition by a synthetic polyribonucleotide complex. *Proc. Nat. Acad. Sci. U.S.A.* **62**: 1046.
52. Pearson, J. W., W. Turner, P. S Ebert, and M A. Chirigos. 1969. Effectiveness of therapy with a multistranded synthetic polynucleotide complex against a murine sarcoma virus. *Appl. Microbiol.* **18**: 474.
53. Zeleznick, L. D., and B. K. Bhuyan. 1969. Treatment of Leukemic (L-1210) mice with double-stranded polyribonucleotides. *Proc. Soc. Exp. Biol. Med.* **130**: 126.
54. Levy, H. B., L. W. Law, and A. S. Rabson. 1969. Inhibition of tumor growth by polyinosinic-polycytidylic acid. *Proc. Nat. Acad. Sci. U.S.A.* **62**: 357.
55. Larson, V. M., P. N. Panteleakis, and M. R. Hilleman. 1970. Influence of synthetic double-stranded ribonucleic acid (poly I:C) on SV_{40} viral oncogenesis and transplant tumor in hamsters. *Proc. Soc. Exp. Biol. Med.* **133**: 14.
56. Bart, R. S., and A. W. Kopf. 1969. Inhibition of the growth of murine malignant melanoma with synthetic double-stranded ribonucleic acid. *Nature (London).* **224**: 372.
57. Gresser, I., and C. Bourali. 1969. Exogenous interferon and inducers of interferon in the treatment of Balb/C mice inoculated with RC_{19} tumour cells. *Nature (London).* **223**: 844.
58. Remington, J. S., and T. C. Merigan. 1970. Synthetic polyanions protect mice against intracellular bacterial infection. *Nature (London).* **226**: 361.
59. Regelson, W., and A. E. Munson. 1970. The reticuloendothelial effects of interferon inducers: polyanionic and non-polyanionic phylaxis against microorganisms. *Ann. N. Y. Acad. Sci.* In press.
60. Jahiel, R. I., J. Vilcek, R. Nussenzweig, J. Vanderberg. 1968. Interferon inducers protect mice against Plasmodium berghei malaria. *Science (Washington).* **161**: 802.
61. Regelson, W., A. E. Munson, W. R. Wooles, W. Lawrence, Jr., and H. Levy. 1970. Reticuloendothelial response to interferon inducers: the biphasic action of pyran copolymer and polynucleotides on phagocytic and immunologic response. International symposium on Interferon, Lyon, France. In press.
62. Levy, H. B., L. W. Law, A. Rabson, F. Riley, A. Garapin, and R. Asofsky. 1970. The antitumor action of polyinosinic·polycytidylic acid. *Ann. N. Y. Acad. Sci.* In press.