Comparative Study of the Efficacy of Different Forms of Interferon Therapy in the Treatment of Mice Challenged Intranasally with Vesicular Stomatitis Virus (VSV) (35884)

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Different approaches of interferon therapy have been proposed for the treatment of virus infections: (a) exogenous interferon administration; (b) endogenous induction of interferon with interferon inducers; and (c) more recently (1), transfer of interferon-producing macrophages. Whether exogenous or endogenous, interferon has generally been found more effective when used prophylactically instead of therapeutically. In a variety of experimental virus infections, however, protective effects were also obtained with either exogenous (2–5) or endogenous (6–13) interferon, or interferon-producing macrophages (1), administered at 1, 2, 3, or as late as 5 days after virus challenge. Exogenous interferon proved superior in some studies (3, 5), endogenous interferon in others (13). The recent description of another approach of interferon therapy (interferon-producing macrophages) (1) raises the question which form of interferon treatment might offer the greatest protection in established virus infections. Therefore, different forms of interferon therapy [exogenous interferon; interferon induced endogenously with poly I:C (polyriboinosinic acid:polyribocytidylic acid complex), and chlorite-oxidized oxyamylose (COAM); peritoneal macrophages treated with interferon or interferon inducers (poly I:C and COAM)] were compared in their ability to protect mice against meningoencephalitis, induced by intranasal challenge with VSV (vesicular stomatitis virus). All treatment regimens were given at the same time (5 days after virus challenge when the first symptoms of disease appear) and by the same route (intraperitoneally).

Materials and Methods. Animals. All experiments were carried out with young (20 day old) male NMRI mice weighing 11–13 g. They were housed in an air-conditioned room at 25°.

Compounds. Chlorite-oxidized oxyamylose (COAM) was synthesized by Dr. P. Claes of our Institute (14). The polymer was dissolved at 10 mg/ml in Eagle's minimal medium (MEM) and stored at 4°.

Polyriboinosinic acid:polyribocytidylic acid complex (poly I:C) was prepared by annealing the individual homopolynucleotides (both purchased from P-L Biochemicals, Inc., Milwaukee, Wisconsin) in equal amounts (1 mg/ml) in saline (0.15 *M* NaCl) at 45° for 1 hr. Duplex formation was demonstrated by procedures described previously (8, 9). Poly I:C was stored frozen in aliquots of 1 mg/ml in saline at −20°.

Interferon. Mouse interferon was produced in L 929 cells with NDV (Newcastle disease virus, Kumarov strain), as described elsewhere (15). The interferon preparations used titered 1000 to 2000 units/10 μg of protein. The interferon titers were measured by plaque reduction of VSV (Indiana strain) in L cell monolayers and protein determinations were performed according to Lowry *et al.* (16).

Peritoneal macrophages treated with interferon or interferon inducers. Peritoneal cells were harvested from normal donor mice or mice which had received an intraperitoneal injection of COAM (3 mg/mouse) 18 hr before. The cells were collected by rinsing the peritoneal cavity with MEM, supplemented with 10% calf serum and heparin (100 units/ml). The macrophages from COAM-treated animals and a first set of control macrophages (from normal donors) were washed, resuspended in MEM (+10% calf serum) at approximately 1.5 to 2.0 × 10^7

[1] "Aangesteld Navorser" of the Belgian N.F.W.O.

cells/ml); 0.3 ml of this cell suspension was injected without further processing. Another set of macrophages from normal mice was washed and resuspended at approximately 10^7 cells/ml in either MEM (+10% calf serum) (second set of control macrophages) or MEM (+10% calf serum + 1000 units/ml of interferon) (interferon-treated macrophages), or MEM (+100 μg/ml of poly I:C) (poly I:C-treated macrophages). The interferon-treated macrophages and the second set of control macrophages were incubated at 37° for 18 hr; the poly I:C-treated macrophages were incubated at 37° for 2.5 hr. All cells were then washed thoroughly, resuspended in MEM (+10% calf serum) at approximately 1.5 to 2.0 × 10^7 cells/ml; 0.3 ml of this cell suspension was injected.

Intranasal VSV assay. Mice were inoculated intranasally with 10^5 PFU (plaque-forming units, measured in L 929 cell monolayers) of VSV according to previously described procedures (7, 8). Mortality was recorded daily for 15 days. Animals surviving 15 days after infection without visible signs of illness were counted as survivors. Clinical symptoms of VSV meningoencephalitis appeared from day 5 after infection: circling (predominantly to the left), paralysis (especially flaccid paralysis of the hind legs) convulsions, prostration. Most animals died between days 5 and 9 after infection.

Statistical significance was assessed by the χ^2 test (with Yates' correction). The χ_Y^2 was computed for the final numbers of mice that died or survived in treated and control groups.

Results. Exogenous interferon treatment. A single intraperitoneal injection of a relatively low dose of interferon (300 units/mouse) afforded a slightly significant protection ($p<.20$) against intranasal VSV challenge, whether interferon was administered at 1 day before, or, 1, 3, or 5 days after infection (Table I). With a single dose of 300 units, the most marked protection ($p<.02$) was obtained when the injection was given 1 day after virus challenge, that is 1 day before virus multiplication in the brain reaches its peak value (7). A single dose of 3000 units injected 5 days after infection was slightly more effective than a single dose of 300 units, as were four daily doses of 300 units started 5 days after infection (Table I). As might be expected, repeated administration of 300 units of interferon on alternating days from 1 day before until 5 days after infection proved superior to any other regimen of ex-

TABLE I. Antiviral Protection with Single or Repeated Intraperitoneal Injections of Exogenous Interferon Administered at Different Times Before (—) or After (+) Intranasal VSV Challenge.

Treatment (days)	Survivors/ no. challenged	Survival (%)	Significance p
Single injection of 300 units of interferon/mouse			
—1	5/20	25	<.20
+1	10/30	33	<.02
+3	8/30	27	<.10
+5	11/50	22	<.20
of 3000 units of interferon/mouse			
+5	6/22	27	<.10
Repeated injections of 300 units of interferon/mouse			
—1, +1, +3, +5	12/30	40	<.001
+5, +6, +7, +8	7/22	32	<.05
Control	9/85	10.5	—

TABLE II. Antiviral Protection with Single or Repeated Intraperitoneal Injections of COAM Administered at Different Times Before (—) or After (+) Intranasal VSV Challenge.

Treatment (days)	Survivors/ no. challenged	Survival (%)	Significance p
Single injection of 3 mg of COAM/mouse			
—1	10/20	50	$<.001$
+1	12/20	60	$<.001$
+3	13/21	62	$<.001$
+5	12/41	29	$<.01$
of 1 mg of COAM/mouse			
—1	4/20	20	NS[a]
Repeated injections of 1 mg of COAM/mouse			
+1, +3, +5	2/20	10	NS
Control	9/85	10.5	—

[a] Nonsignificant.

ogenous interferon treatment.

Endogenous interferon treatment. The protective effects of intraperitoneal and intravenous injection of poly I:C against intranasal VSV challenge have been described previously (7, 8): maximum protection was obtained if treatment was initiated shortly before virus challenge; treatment begun at 3 days after infection was still effective, but treatment delayed until day 5 did not cause a significant increase of the number of surviving mice [Ref (8), Fig. 3]. As shown in Table II, intraperitoneal administration of COAM resulted also in a significant protection against intranasal VSV inoculation. In contrast with poly I:C (8), a single dose of COAM, administered 1 or 3 days after virus challenge, offered a greater protection than a single dose 1 day before virus challenge (Table II). A slight but significant ($p<.01$) protection was observed with 3 mg of COAM injected at 5 days after infection. Although a single dose of 3 mg of COAM given at either 1, 3, or 5 days after virus inoculation afforded a marked protection, there was no protection at all if the same amount of COAM was administered in three repeated doses of 1 mg at 1, 3, and 5 days after infection. A single dose of 1 mg of COAM injected 1 day before virus challenge was also insufficient to confer protection.

Treatment with macrophages pretreated with interferon. A rather small delay in mortality and increase of the number of surviving mice was noted with peritoneal cells, which had been exposed to interferon *in vitro* for 18 hr, and which were injected intraperitoneally at 5 days after intranasal VSV challenge (Fig. 1). Peritoneal cells which were incubated with control medium for 18 hr did not show such an effect. Interferon-treated macrophages were not more effective than corresponding doses of exogenous interferon, administered directly (Fig. 1).

Treatment with macrophages pretreated with interferon inducers. Intraperitoneal injection of peritoneal cells from COAM-treated mice at 5 days after intranasal VSV challenge, caused a 20% increase in survival rate (Fig. 2); an injection of poly I:C-treated macrophages reduced the final deaths by more than 30%.

Control peritoneal cells (which were not preincubated *in vitro*) failed to induce any protective activity. COAM and COAM-treated macrophages did not differ in activity, whereas poly I:C-treated macrophages were remarkably more effective than poly I:C itself (Fig. 2).

Discussion. The intranasal VSV assay system in mice is an experimental virus infection which closely resembles certain natural virus infections in animals and man (*e.g.*, herpes simplex encephalitis, rabies, enterovirus in-

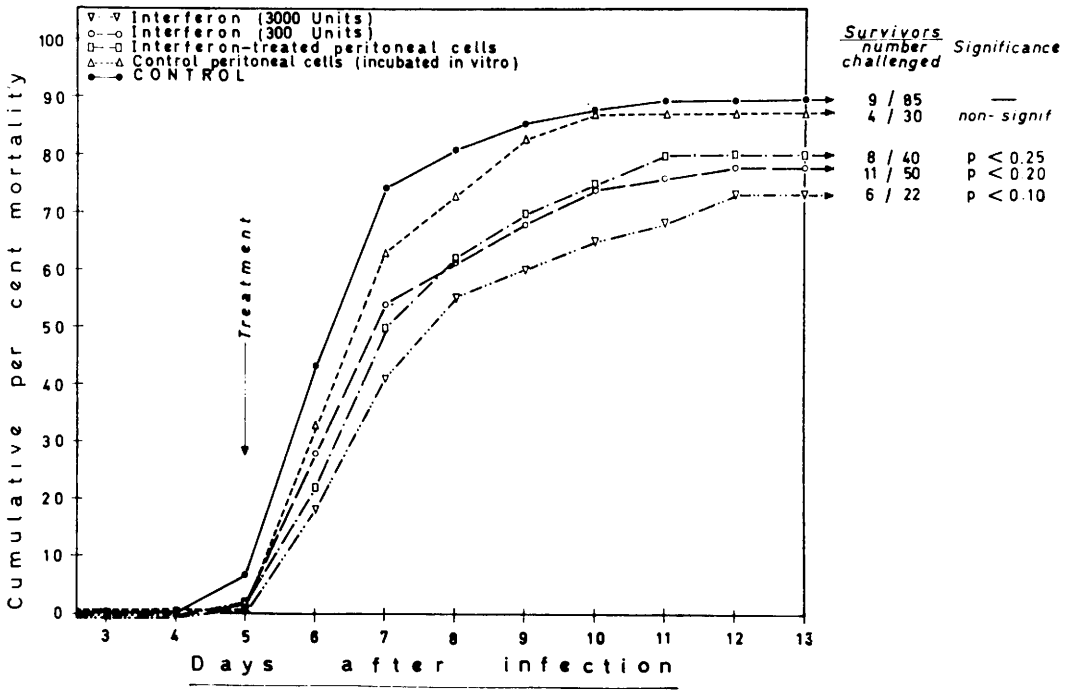

FIG. 1. Antiviral protection with a single intraperitoneal injection of interferon or interferon-treated peritoneal macrophages at 5 days after intranasal VSV challenge. Interferon injected at 300 or 3000 units/mouse. Interferon-treated and control peritoneal macrophages were injected at 5 to 7 × 10^6 cells/mouse; the cells were preincubated *in vitro* for 18 hr at 37° with either 1000 units of interferon/10^7 cells/ml or with interferon-free medium (see Materials and Methods).

fections) in that the virus spreads from a respiratory tract site (olfactory mucosa) along the neural route (olfactory nerves) to the brain (17). The prophylactic and therapeutic efficacy of several synthetic interferon inducers has been assessed in this system: pyran (maleic anhydride/divinyl ether) copolymer (7), poly I:C (7, 8) and tilorone (bis-DEAE-fluorenone) hydrochloride (9). Either compound proved most effective in reducing mortality when given shortly (within 1 day) before VSV challenge; the protective activity decreased the later treatment was started after virus inoculations. However, with COAM, a polyacetal carboxylic acid which stimulates interferon production and resistance to several virus infections *in vivo* (14, 18), protection was not diminished if the polymer was given 1 or 3 days after, instead of 1 day before intranasal VSV challenge (Table II). These findings contrast with the lack of activity noted with COAM treatment started after infection in other experimental virus models, *e.g.*, intraperitoneal Mengo virus infection. In this system, where both COAM and virus were administered by the same route (intraperitoneally), there was no protection if COAM was injected later than 16 hr prior to infection (19).

If three separate doses of 1 mg of COAM were administered at 1, 3, and 5 days after infection instead of a single dose of 3 mg at either 1, 3 or 5 days after infection, COAM failed to reduce the mortality rate. Similar failures have been reported with repeated administration of pyran copolymer (8) and tilorone hydrochloride (9) in the intranasal VSV system, and with repeated injection of polyacrylic acid in the vaccinia tail lesion system (20). It is not immediately clear why polycarboxylates and tilorone hydrochloride loose their protection activity upon repeated ad-

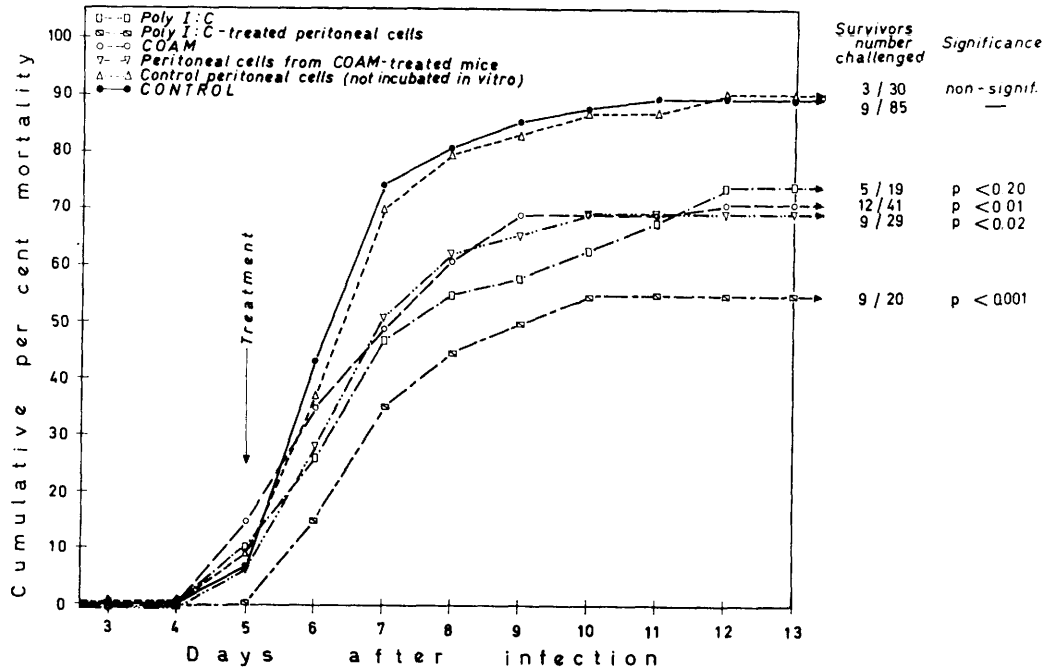

FIG. 2. Antiviral protection with a single intraperitoneal injection of poly I:C, COAM, or poly I:C- or COAM-treated peritoneal macrophages at 5 days after intranasal VSV challenge. Poly I:C and COAM injected at 100 μg and 3 mg/mouse, respectively. Control and poly I:C- or COAM-treated peritoneal macrophages were injected at 5 to 7 $\times$ 10^6 cells/mouse; control cells and COAM-treated peritoneal cells were directly harvested from either normal donor mice or mice which received an intraperitoneal injection of 3 mg of COAM 18 hr previously; poly I:C: treated peritoneal cells were obtained from normal donors and exposed to 100 μg/ml of poly I:C for 2.5 hr at 37° *in vitro* (see Materials and Methods).

ministration. The loss of protection may be related to hyperreactivity to the toxic effects or hyporeactivity to the interferon inducing ability of the compound. Repeated injections of poly I:C did not result in a decreased protective activity (7, 8), nor did exogenous interferon administrations (Table I). In fact, both poly I:C and interferon were more effective when injected in repeated doses instead of a single dose.

The data presented in Tables I and II and Figs. 1 and 2, and summarized in Table III show (a) that different forms of interferon therapy (endogenous or exogenous interferon, peritoneal macrophages treated with interferon or interferon inducers), administered intraperitoneally at 5 days after infection (at the time that the clinical symptoms appear) are all capable of delaying mortality and increasing the survival rate of mice infected intranasally with VSV; (b) that at the concentrations used, exogenous interferon and interferon inducers proved equally active; (c) that in the given conditions, no significant differences were noted in the protection afforded by interferon and interferon-treated macrophages, no differences in the protection afforded by COAM and COAM-treated macrophages, but a significant difference in the activities exhibited by poly I:C and poly I:C-treated macrophages. Poly I:C-treated macrophages proved markedly more protective than poly I:C itself. It should also be pointed out that COAM and exogenous interferon were used at concentrations which gave similar interferon titers in the circulation of mice, whereas poly I:C was injected at a dose which induced considerably higher interferon titers in the blood stream (data not reported in detail). At this dose, however,

TABLE III. Comparison of the Efficiency of Different Forms of Interferon Therapy at 5 Days After Intranasal VSV Challenge.

Treatment (intraperitoneally)	Increase in survival compared to control[a] (%)	Increase of mean survival time, compared to control[a] (days)
Exogenous interferon		
300 units/mouse	11.5	1
3000 units/mouse	17	1.5
Endogenous interferon		
poly I:C (100 μg/mouse)	16	1.3
COAM (3 mg/mouse)	19	1
Peritoneal macrophages treated with interferon		
5 to 7 $\times$ 10^6 cells (equiv to 500 to 700 units of interferon/mouse	9	1
Peritoneal macrophages treated with interferon inducers		
with poly I:C		
5 to 7 $\times$ 10^6 cells (equiv to 50 to 70 μg of poly I:C)/mouse	34	3
with COAM		
5 to 7 $\times$ 10^6 cells (equiv to 3 mg of COAM)/mouse	20	1

[a] Data calculated from Figs. 1 and 2.

poly I:C was not more protective than COAM or exogenous interferon. The findings that (a) poly I:C is less active therapeutically than might be expected from the titers of interferon induced endogenously; and (b) poly I:C-treated macrophages are more effective than poly I:C itself, suggest that poly I:C, in addition to its beneficial effects mediated by interferon and probably transferred through (peritoneal) macrophages, may impart other, rather adverse effects on the recovery from virus infections.

Summary. Different forms of interferon therapy, (i) exogenous interferon; (ii) interferon inducers: poly I:C (homopolymer pair of polyriboinosinic acid and polyribocytidylic acid) and COAM (chlorite-oxidized oxyamylose); (iii) peritoneal macrophages treated with interferon; and (iv) peritoneal macrophages treated with interferon inducers (poly I:C and COAM), have been compared in their capacity to protect mice from a lethal intranasal challenge with vesicular stomatitis virus (VSV). All treatment regimens were given by the same route (intraperitoneally) at the same day (5 days after infection, at the time that the clinical symptoms appear) and at comparable doses. All treatment regimens afforded a partial protection. This protection was greatest with poly I:C-treated macrophages. There was no essential difference in the protection conferred by exogenous interferon, COAM, interferon-treated and COAM-treated macrophages. However, poly I:C was therapeutically less effective than poly I:C-treated macrophages and also less active than might have been expected from the titers of interferon that it induced endogenously.

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