Inducers of Interferon and Host Resistance
VII. Antiviral Efficacy of Double-Stranded RNA of Natural Origin
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MARJORIE M. NEMES, ALFRED A. TYTELL, GEORGE P. LAMPSON, A. KIRK FIELD, AND MAURICE R. HILLEMAN

Division of Virus and Cell Biology Research, Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486

It has been found in these laboratories that certain double-stranded RNA's of diverse natural (*P. funiculosum*, reovirus 3, MS2 coliphage) and synthetic origin (polyriboinosinic:polyribocytidylic acid) are highly active in inducing interferon and resistance to viral infections *in vivo* and *in vitro* (1–6). A recent detailed evaluation of the efficacy of polyriboinosinic–polyribocytidylic complex, poly I:C (rI:rC, $rI_n \cdot rC_n$) against a variety of viral infections in mice has been presented elsewhere (7). The sources of RNA which are active in inducing interferon and resistance to viruses have now been expanded to include replicative-form double-stranded RNA from *E. coli* cells infected with MU9 (a mutant of MS2 coliphage), double-stranded RNA from rice dwarf virus virions (plant virus) and double-stranded RNA from cytoplasmic polyhedrosis virions (insect virus). The activities of these double-stranded RNA's of natural origin are presented below. Finally, evidence is presented to indicate induction of interferon and of resistance to viral infection by a double-stranded DNA-RNA hybrid of coliphage origin.

Materials and Methods. Interferon inducers. The double-stranded RNA's of *P. funiculosum*, reovirus 3 virions and MS2 coliphage (replicative form) were described earlier (1, 3, 4). Replicative form double-stranded RNA (MU9-RF-RNA) derived from the MU9, amber coat protein mutant of MS2 coliphage, was prepared by Drs. B. Lago and J. Birnbaum of Merck Process Research of Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey. Double-stranded RNA's from rice dwarf virus virion and cytoplasmic polyhedrosis virions were kindly furnished by Dr. K. Miura, Nagoya University, Nagoya, Japan. The hybrid DNA-RNA of F1 phage was obtained through the kindness of Dr. Michael Chamberlin of the University of California, Berkeley, Calif. (8). Poly I:C (rI:rC) was prepared by Drs. Peter Pollak and John Zabriskie of the Merck Process Research.

Bioassays. The methods employed for assaying induction of interferon and resistance to viral infection *in vivo* and *in vitro* have been described in earlier reports (1–5, 7).

Results. Activities of double-stranded RNA's of natural origin. Table I shows that all of the 6 double-stranded RNA's of natural origin as well as the DNA-RNA hybrid were highly active in inducing resistance to cytopathic changes in rabbit kidney cell cultures infected with vesicular stomatitis virus when the inducer was added 18–24 hr prior to virus. Similarly, all these substances were excellent inducers of interferon when given intravenously to rabbits. It should be noted that the amounts of inducer which were used do not necessarily represent the minimum amount required for induction. Finally, the double-stranded RNA's given intranasally were all very effective prophylactically in preventing death in mice infected with pneumonia virus of mice (PVM).

Prophylactic and therapeutic efficacy of MU9-RF-RNA against PVM infection of mice. Figure 1 shows that the response of mice infected with PVM virus to the double-stranded replicative form coliphage RNA was dose-related. The response curve was qualitatively similar to that reported earlier for

TABLE I. Induction of Interferon and Host Resistance to Viral Infection by Various Double-Stranded RNA's of Natural Origin.

Source of double-stranded RNA	Least amount required to protect cell cultures against vesicular stomatitis virus[a] (μg)	Induction of serum interferon in rabbits		Prophylactic induction of host resistance in mice against pneumonia virus[d]	
		Amount given iv (μg)[b]	Interferon titer[c]	(μg/mouse)	% Survival[e]
P. funiculosum (viral ?)	0.3	0.5	1:40	20	90
Reovirus 3 virions	0.04	0.5	1:80	16	89
MS2 coliphage (replicative) (MS2-RF-RNA)	0.04	2.0	1:80	9	75
MU9-mutant coliphage (replicative) (MU9-RF-RNA)	0.125	1.8	1:40	15	65
Rice dwarf virus virions	0.015	20	>1:640	3	65
Cytoplasmic polyhedrosis virions (CPV)	0.04	22	1:160	3	90
DNA-RNA F1 phage hybrid	0.6	5	1:40	3	90
Poly I:C control	0.002	2.0	1:640	4	83

[a] Ten $TCID_{50}$ in primary rabbit kidney cultures.

[b] The amounts shown here are not necessarily threshold values.

[c] Titer in serum 2 hr after inducer was given.

[d] Inducer given intranasally 2 hr prior to intranasal challenge with 100 LD_{50} of virus. Mice weighed 9–11 g; 15–20 mice were used for each compound; and 30–40 mice were used as untreated controls.

[e] Corrected for controls.

poly I:C (7) but a lower level of activity was displayed. Table II demonstrated the protective efficacy of coliphage RF-RNA against varying doses of PVM virus in mice. Simultaneous titrations in mice of the PVM virus seed established the LD_{50} titer of the challenge. It is evident that the RNA was effective against at least 1000 LD_{50} of PVM virus. Table III illustrates the influence of time of administration of MU9 coliphage RF-RNA in relation to PVM infection in mice. These data show that the RNA was active

TABLE II. Relationship of PVM Virus Challenge Level to Prophylactic Efficacy of MU9-RF-RNA.[a]

Dose of virus (mouse LD_{50})	MU9-RF-RNA-treated mice (No. surv./no. treated)	% Survival[b]
1000	11/15	73.3
300	12/17	70.6
100	15/16	93.7
30	17/17	100
10	16/16	100
3	20/20	100

[a] The PVM virus was given intranasally to 9 and 11 g mice 3 hr following intranasal instillation of 60 μg of MU9-RF-RNA.

[b] Measured 14 days following virus.

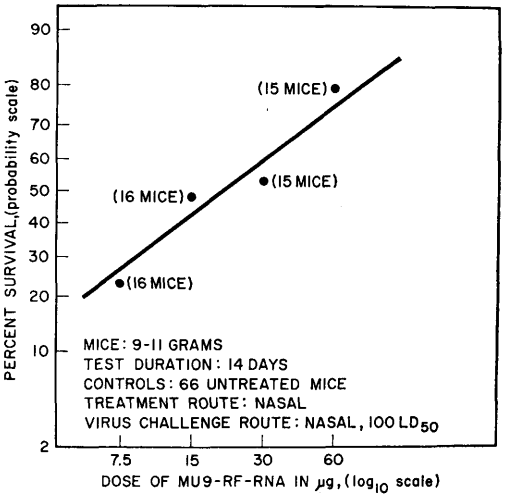

FIG. 1. Relationship of dosage of MU9-RF-RNA to Prophylactic efficacy against PVM virus infection in mice.

TABLE III. Relationship of Time to Prophylactic and Therapeutic Effects of MU9-RF-RNA against PVM Virus Infection in Mice.[a]

Time when RNA was given	Survival: Nos. RNA-treated mice: No. surv./total	RNA-treated mice: % Surv.	Untreated mice[b]: No. surv./total	Untreated mice[b]: % Surv.	Excess survival (%)[c]	Time: Median no. of days: RNA-treated	Median no. of days: Untreated	Excess days of survival
Prior to PVM virus (prophylaxis)								
3 hr	17/17	100	1/33	3.3	96.7	>14	8	>6
1 day	15/16	93.3	3/33	9.9	83.4	>14	7	>7
2 days	16/17	94.1	1/33	3.3	90.8	>14	8	>6
3 days	15/16	93.3	5/36	13.8	79.5	>14	8	>6
4 days	13/16	81.3	1/34	2.9	78.3	>14	8	>6
5 days	8/17	47.1	4/33	12.1	35.0	12	7	5
6 days	8/16	50	4/33	12.1	37.9	11	8	3
7 days	8/17	47.1	3/34	8.8	38.3	12	8	4
8 days	4/16	25.0	1/33	3.3	21.7	10	8	2
Following PVM virus (therapeusis)								
1 day	17/18	94.4	0/33	0	94.4	>14	6	>8
2 days	8/16	50	0/34	0	50.0	10	7	3
3 days	2/17	11.8	0/33	0	11.8	7	7	0

[a] The PVM virus in 100 LD_{50} amount and MU9-RF-RNA in 60 μg amount were given intranasally to 9–11 g mice at the time periods shown.

[b] The control animals received phosphate buffered saline solution.

[c] Treated minus control group as measured 14 days after virus.

both prophylactically and therapeutically. Maximal or near maximal prophylactic effect was observed for 4 days with diminution to an approximate 36% excess survival maintained for 3 additional days. The MU9-RF-RNA was highly active therapeutically when given 1 day following virus but this was reduced to low level when given 3 days after the virus.

Prophylactic efficacy of MU9-RF-RNA against Columbia SK, vaccinia, and Sendai virus infections of mice. Table IV shows that MU9-RF-RNA prevented death from Columbia SK virus infection when the virus was given intranasally 3 hr following intranasal instillation or intraperitoneal injection of the RNA. Intranasal administration of from 2 to 60 μg of MU9-RF-RNA/mouse gave similar protective efficacy. Intraperitoneal administration of from 15 to 60 μg of MU9-RF-RNA/mouse was as active as the similar dose given intranasally. Intraperitoneal administration of MU9-RF-RNA was effective prophylactically in suppressing tail lesions in mice given vaccinia virus intravenously 3 hr after the drug as shown in Table V. Sixty-three μg of MU9-RF-RNA/mouse gave as great an effect as 1000 μg/mouse of Marboran. Eight or 16 μg of MU9-RF-RNA/mouse reduced the efficacy to about 50% suppression of lesions. Table VI shows that MU9-RF-RNA was moderately active when given in 30 or 60 μg dose/mouse prior to parainfluenza 1 virus. Marked reduction of efficacy was noted when the dose of RNA was reduced to 2 or 8 μg.

Discussion. An interpretive summary of the findings in the present study of the efficacy of double-stranded RNA's of natural origin against a variety of viral infections in mice is presented in Table VII. The findings in these studies confirm and extend the observations made in our laboratories (1–7) with synthetic and naturally occurring double-stranded RNA's in relation to induction of interferon and host resistance *in vivo* and

TABLE IV. Prophylactic Protection against Columbia SK Virus Infection in Mice by MU9-RF-RNA Employing Different Routes of Drug Administration and Virus Challenge.[a]

	RNA					
		Dose		Survival data		
Virus route[b]	Route	(μg/mouse)	(mg/kg)	No. surv./total	% Surv.	Excess survival[c] (%)
in	in	60	4	15/17	88.2	74
	in	30	2	17/17	100	85.8
	in	15	1	17/17	100	85.8
	in	8	0.53	16/18	88.9	74.7
	in	4	0.27	14/17	82.3	68.1
	in	2	0.14	13/16	81.3	67.1
	Controls[d]	0	0	5/35	14.2	—
in	ip	60	4	14/14	100	85.8
	ip	30	2	15/15	100	85.8
	ip	15	1	12/15	80	65.8
	Controls[d]	0	0	5/35	14.2	—

[a] The virus dose was 30 mouse LD_{50} of Columbia SK virus given intranasally to 14–16 g mice 3 hr following MU9-RF-RNA.

[b] Abbrev.: in = intranasal; ip = intraperitoneal.

[c] Treated minus control group as measured 14 days after virus.

[d] The controls received phosphate buffered saline solution.

vitro. The present data also show the high el activity of all the double-stranded JA's of natural origin on *in vivo* induction interferon in rabbits and resistance to vesi- ar stomatitis virus infection in rabbit kid- / cell cultures. It is evident from these a, as well as those presented for poly I:C (7), that the double-stranded MU9-RF-RNA was highly efficacious when given therapeutically as late as 2 days after virus. The protective effect of MU9-RF-RNA against PVM infection lasted in mice for at least 7 days although this was reduced after 4 days. Further, protection against vaccinia and

TABLE V. Prophylaxis by MU9-RF-RNA against Vaccinia Virus Given Intravenously in Mice.[a]

	MU9-RF-RNA					
	Dose			Vaccinia tail lesions		% Reduction in median no. of lesions
Route	(μg/mouse)	(mg/kg)	No. of mice	Median	Av $(N)^{1/2}$[b]	
ip	63	5.25	14	1	1.22	93
	32	2.63	13	2	1.52	87
	16	1.81	11	8	2.54	47
	8	0.90	14	5	2.67	67
	0[c]	—	28	15	3.71	—
Marboran[d]	1000	83	14	1	1.56	93

[a] MU9-RF-RNA was administered intraperitoneally to 12 g male mice in variable amount 3 hr prior to intravenous injection of a dose of vaccinia virus calculated from prior titrations to produce 12–30 discernible tail lesions/mouse.

[b] N = number of lesions per mouse, measured 7 days after virus was given.

[c] The controls received phosphate buffered saline solution.

[d] N-methylisatin-β-thiosemicarbazone.

TABLE VI. Prophylactic Protection against Parainfluenza Type 1 (Sendai) Respiratory Infection in Mice by Intranasal Administration of MU9-RF-RNA.[a]

MU9-RF-RNA dose		Survival rate		
(μg/mouse)	(mg/kg)	No. surv./total	% Surv.	Excess survival[b]
60	0.6	11/16	68.7	55.5
30	0.3	13/16	81.3	68.1
8	0.08	7/17	41.3	28.1
2	0.02	7/17	41.3	28.1
0[c]		7/53	13.2	—

[a] MU9-RF-RNA was administered 3 hr prior to intranasal infection of 9–11 g mice of both sexes with 30 mouse LD_{50} of Sendai virus.

[b] Treated minus control groups as measured 14 days after virus.

[c] Controls received phosphate buffered saline solution.

Columbia SK infection was observed even when the MU9-RF-RNA was administered by a route different from that of the virus challenge. This supports the idea that the resistance which is imparted by the RNA is not local but is disseminated to different organs. Finally, the efficacy of drug is dose-related and complete protection may be afforded against very large virus doses. Previous work (9) showed the efficacy of MU9-RF-RNA in preventing internal tumors in hamsters given adenovirus 12 virus by the subcutaneous route.

It is presently conceived that the responsible element for initiating interferon induction in cells infected with RNA viruses is the requirement of a double-stranded RNA complex (1–7). The action of the DNA-RNA hybrid of the F1 DNA-type coliphage in inducing interferon and resistance to viral infection is of extreme interest though the data must be considered preliminary, since insufficient amount of substance was available for extensive confirmatory tests.

The outlook for interferon inducers in relation to acute infectious diseases caused by viruses might be judged to be optimistic in view of the findings to date. This might especially be so, considering that the amount of virus to be combated under natural disease transmission may be far less than the rather severe challenge dosages of virus used in experimental studies.

Summary. Studies in cell cultures, rabbits, and mice demonstrated the prophylactic and therapeutic efficacy against viral infections and of interferon induction by a variety of double-stranded RNA's of natural origin including fungi, animal, plant, insect, and bacterial viruses. All were found active in microgram amounts. RNA and virus were given by a variety of routes in a number of different regimens including variable drug and virus

TABLE VII. Interpretive Summary of Effects of Double-Stranded RNA's of Natural Occurrence on Viral Infections in Mice *in Vivo*.

Virus		Source of double-stranded RNA	Route polynucleotide given	Appraisal of activity
Kind	Route			
PVM	in	*P. funiculosum* (viral ?)	in	Excellent
	in	Reovirus 3 virions	in	Excellent
	in	MS2 coliphage (replicative) (MS2-RF-RNA)	in	Excellent
	in	MU9 mutant coliphage (replicative) (MU9-RF-RNA)	in	Excellent
	in	Rice dwarf virus virions	in	Excellent
	in	Cytoplasmic polyhedrosis virions (CPV)	in	Excellent
Columbia SK	in	MU9 mutant coliphage (replicative) (MU9-RF-RNA)	in	Excellent
	in	MU9 mutant coliphage (replicative) (MU9-RF-RNA)	ip	Excellent
Vaccinia	iv	MU9 mutant coliphage (replicative) (MU9-RF-RNA)	ip	Very good
Parainfluenza 1 (Sendai)	in	MU9 mutant coliphage (replicative) (MU9-RF-RNA)	in	Good

dosage. The most extensive studies were conducted with double-stranded replicative form RNA from MU9 mutant of MS2 coliphage. Remarkably favorable effects were obtained against PVM, Columbia SK, vaccinia, and parainfluenza 1 virus infections. The polynucleotide was therapeutic in mice when given as late as 2 days following PVM virus, and prophylactic when given as long as 7 days before the virus infection. Preliminary test results showed activity of the DNA-RNA hybrid of the F1 DNA coliphage in inducing interferon and resistance to viral infection.

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