*FOOT-AND-MOUTH DISEASE VIRUS INHIBITION INDUCED IN MICE BY SYNTHETIC DOUBLE-STRANDED RNA (POLYRIBOINOSINIC AND POLYRIBOCYTIDYLIC ACIDS)**

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Abstract.—Synthetic 2-stranded RNA—a helical complex formed by duplexing homopolymers of polyriboinosinic and polyribocytidylic acids (poly I:C)—induced host resistance to foot-and-mouth disease virus when microgram quantities were injected into mice. There was a graded response as shown by titrations of polynucleotide complex or virus. Protection was effective for >48 hours after a single injection of polynucleotide complex. Survival and serum interferon titers were directly related.

Interferon induction by foot-and-mouth disease virus (FMDV) in cell cultures has been reported,[1–3] but attenuated strains appeared to induce higher titers of interferon than did virulent strains.[4, 5] Likewise, strains producing large quantities of incomplete virus were also effective interferon inducers.[6] Temporary virostasis, induced *in vivo* by multiple injections of a yeast RNA, was presumably also mediated by induction of an interferon.[7, 8] Other results with another nonviral inducer (phytohemagglutinin) of an interferon effective against FMDV *in vitro* have been reported.[9, 10]

An interest in the structure and function of 2-stranded DNA and RNA[11] and the reports that multistranded RNA[12–14] and multistranded complexes of synthetic polyribonucleotides induced interferon production and host resistance in mice[15] stimulated us to investigate the effects of 2-stranded material in several biological systems, one of which is reported here.

Collaborative work[16–19] involved the preparation and biophysical characterization of double-stranded molecules. X-ray diffraction provided a powerful system for monitoring their double-helicity. The interaction of synthetic polyribonucleotides, e.g., polyriboadenylic acid (poly A) and polyribouridylic acid (poly U), polyriboinosinic acid (poly I) and polyribocytidylic acid (poly C), and polyriboguanylic acid (poly G) and poly C, to form multistranded and especially double-stranded helices has interested us because their structure is very like that of native RNA's and, therefore, they can be used as model compounds to study interaction of nucleic acids with drugs.[20] Moreover, they interact with synthetic polydeoxyribonucleotides. Because of the tendency under certain conditions for these homopolymers to form 3-stranded structures, and also for some homopolymers to aggregate, the fact of being able to monitor the 2-stranded nature of our material in the solid state by X-ray diffraction permitted resolution of the ambiguity attending the 2-strandedness of some of the materials that have been used by others.[15, 20, 21]

We report that a synthetic 2-stranded poly I:C was effective in inducing host resistance to FMDV in mice and that this resistance is at least partly mediated by an interferon.

Materials and Methods.—(1) Synthetic 2-stranded[22] complexes of polyribonucleotide homopolymers were obtained by ethanol precipitation from mixtures of equimolar solutions of homopolymers prepared under optimal conditions for formation of a 1:1 complex and the complexes were stored at −20°C. The complexes were then dissolved in 0.03 *M* NaCl (5 mg/ml) and solutions stored at 4°C until used.

(2) For *in vivo* assays of the effectiveness of these polynucleotides against FMDV serial 2-fold dilutions were prepared in 0.03 *M* NaCl and injected in 0.03 ml amounts intraperitoneally in mice, generally 18–20 hr before virus inoculation. The standard challenge dose of FMDV (strain Asia-1) was 100 LD_{50} per mouse. This dose was determined by preliminary virus titrations in each age group of mice, since increasing age correlates with increasing resistance to this virus.[23]

(3) Interferon titers of pooled mouse sera were assayed by essentially the methods described.[5, 24] Tail vein inoculations of poly I:C preparations were made in 23-day-old mice (0.03 ml). The mice were exsanguinated by cardiac puncture 1.5 hr later. Fifteen to 25 mice were used in each experiment; the pooled sera were frozen (−20°C) until tested. Serial 2-fold dilutions of the sera were made in Hanks' medium containing lactalbumin hydrolysate; 4 confluent primary mouse kidney monolayer cell cultures in roller tubes were overlayered with 1 ml of each dilution. Twenty hours later, the media were decanted and replaced with 1 ml of media containing 100 $TCID_{50}$ FMDV, Asia-1. Interferon titer was the serum dilution giving total suppression of cytotoxicity in half the cultures.

Results.—(1) *Induction of host resistance in mice by synthetic 2-stranded polynucleotides:* The duplex of poly I:C induced host resistance in 9-day-old (suckling) and 23-day-old (weanling) mice when injected intraperitoneally 18 hours before challenge with 100 LD_{50} of FMDV (Table 1). Neither homopolymer protected the mice against similar virus inoculations. At greater dilutions of poly I:C (as little as 0.25 μg per mouse) less than half the mice survived the challenge inoculation.

(2) *Course of host resistance:* To determine the extent of resistance with respect to time of poly I:C injection, virus was inoculated before or after intraperitoneal injection of the polynucleotide complex. Figure 1 shows that 60 per cent or more of the mice survived when poly I:C was injected during the period extending from 48 hours before to 18 hours after virus inoculation, except for intervals immediately preceding virus challenge. This increased mortality associated with near simultaneous injection of poly I:C and virus resembles the

TABLE 1. *Mouse survival after doses of synthetic polynucleotides injected intraperitoneally 18 hr before 100 LD_{50} FMDV.*

Polymer dose (μg/mouse)	Mouse Survival 10 DPVI*					
	9-Day-Old Mice			23-Day-Old-Mice		
	Poly I	Poly C	Poly I:C	Poly I	Poly C	Poly I:C
150	0	0	10	0	0	10
75	3	0	10	0	0	9
32.5	0	0	9	1	3	9
16.3	0	0	8	2	0	3
8.1	0	0	5	2	0	5
0	2	0	2	6	5	1

* Number alive/10 inoculated in experimentals, number alive/50 in controls.

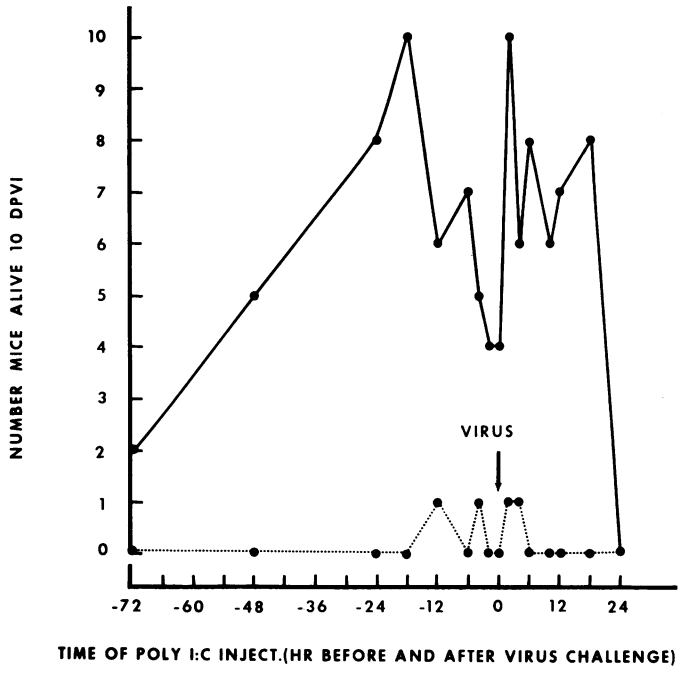

FIG. 1.—Response of suckling mice to intraperitoneal injections of 150 μg/mouse poly I:C in respect to time of virus inoculation 100 LD_{50}, FMDV (Asia-1) shown by the arrow. Solid line indicates number of mice (10 each group) surviving 10 days after virus inoculation (DPVI). Dotted line is control.

results obtained with polysaccharide interferon induction followed by virus infection *in vitro*,[25] interferon induction in mice with successive treatment with cycloheximide and statolon,[26] and the enhancing effect of phytohemagglutinin on subsequent vesicular stomatitis virus multiplication.[27] In the experiments reported here, 100 per cent survival was obtained when the poly I:C was injected 18 hours before the virus. Apparently this interval was necessary for development of maximal resistance when the poly I:C was given intraperitoneally. If the poly I:C injection was delayed until 24 hours after virus, it was too late to arrest the progress of the infection.

(3) *Response of mice to increasing virus concentrations:* Figure 2 shows that 150 μg of poly I:C, given intraperitoneally 18 hours before virus, protected against a wide range of virus concentrations. Even at a challenge dose of 600 LD_{50} more than 50 per cent survived. Repeated experiments have indicated that more than 80 per cent of the mice survived challenge inoculations of up to 500 LD_{50} FMDV, Asia-1.

(4) *Evidence for poly I:C induction of interferon:* Preliminary experiments had indicated that smaller doses of poly I:C as well as much shorter time intervals before virus challenge were required to induce resistance in mice when the polynucleotide complex was injected intravenously rather than intraperitoneally. Table 2 indicates that as little as 1.5 μg/mouse 90 min before virus challenge pro-

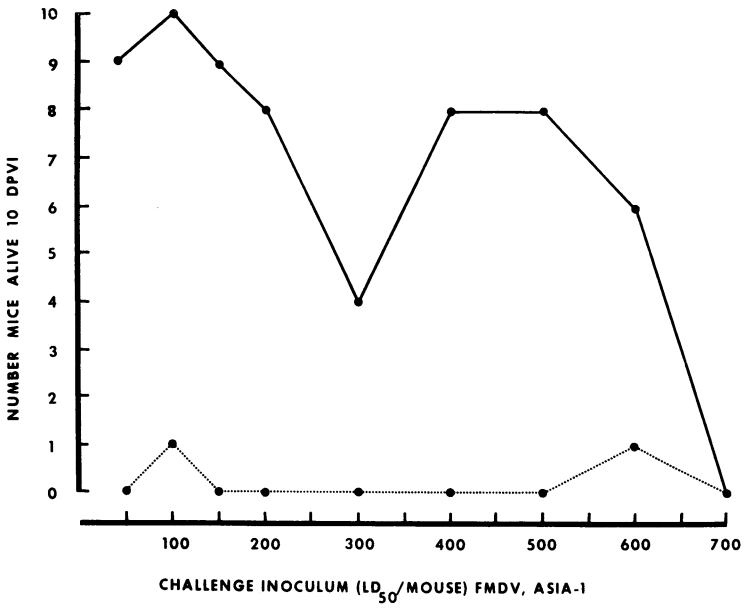

FIG. 2.—Response of suckling mice to intraperitoneal injections of 150 μg/mouse poly I:C given 18 hr before inoculations of various FMDV (Asia-1) concentrations. Solid line indicates number of mice (10 each group) surviving 10 days after virus inoculation (DPVI). Dotted line is control.

tected 50 per cent of the mice. Mice inoculated with varying concentrations of poly I:C were exsanguinated by cardiac puncture 1.5 hours later to test the sera for interferon activity. Poly I:C dose, survival at day 10, and serum interferon titers were directly related (Table 2). When the mouse sera were tested on primary bovine kidney cells or IB-RS-2 cells (a swine kidney stable cell line), no protection against the cytopathic effects of FMDV was seen, thus establishing the species-specificity of this poly I:C-induced interferon.

TABLE 2. *Mouse survival and serum interferon titers after intravenous injections of poly I:C.*

Poly I:C dose (μg/mouse)	10th day survival* (alive/total)	Serum interferon titer† (1.5 hr after poly I:C)
150	10/10	1:2048
15	7/10	1:512
1.5	5/10	1:128
0	0/10	<1:2

* Twenty-three-day-old mice inoculated with 100 LD_{50} 1.5 hr after poly I:C injection.
† Titer is last serum dilution yielding protection against 100 $TCID_{50}$ in half the cultures.

Discussion.—The fact that microgram amounts of poly I:C induce resistance in mice against FMDV while the homopolymers of poly I and poly C did not, indicates that only the double-stranded complex was active in this test system. The correlation of dose of poly I:C, survival at day 10, and interferon titer leads one to assume that the 2-stranded polymer is effective because of its ability to induce interferon. Other mechanisms of protection cannot be excluded.

Foot-and-mouth disease virus is an RNA virus. Protection with 2-stranded polyribonucleotide complexes extends to other kinds of viruses; 2-stranded complexes of poly A:U as well as poly I:C also protected against herpes simplex virus (a DNA virus) in tissue cultures *in vitro*, systemic herpes simplex infection in mice, and locally when applied to rabbits' eyes.[28] This finding confirmed observations on the therapeutic effect of multistranded poly I:C.[21]

It remains to be seen whether even more powerful inducers of interferon exist, particularly whether duplexes of other synthetic homopolymers (including analogues of nucleotides) have equal or greater inducer activity.

This protection after poly I:C may persist for longer than 2 days (Fig. 1) and appears effective against a wide virus dose range (Fig. 2), and very small amounts (as little as 1.5 μg/mouse) given intravenously induced protection as well as appreciable levels of apparent serum interferon (Table 2). Therefore, we carried out protection experiments with 2-stranded complexes in animals normally susceptible to infection with this virus.

Preliminary experiments extending these findings to guinea pigs and cattle indicate that protection against FMDV may be similarly induced with this synthetic 2-stranded polynucleotide complex. Further investigations in these and other animals are under way with this and other double-stranded complexes.

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Abbreviations: Homopolymers, homopolyribonucleotides are long chain polymers composed of identical ribonucleotide units linked by 3′,5′-phosphodiester bonds. The polyribonucleotides are prepared enzymatically using appropriate nucleoside diphosphates as substrates; poly I:C, a synthetic, helical, 2-stranded polynucleotide prepared by complexing single-stranded homopolymers polyinosinic and polycytidylic acids. Each strand of the duplex is thus composed of a single polynucleotide; poly A:U, a similar 2-stranded polynucleotide prepared by complexing for a 1:1 complex single-stranded homopolymers polyadenylic and polyuridylic acids; FMDV, foot-and-mouth disease virus; TCID, tissue culture infective dose; DPVI, days post virus inoculation.

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