

Interferon Inducers and Foot-and-Mouth Disease Vaccines: Influence of Two Synthetic Polynucleotides on Antibody Response and Immunity in Guinea Pigs and Swine

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ABSTRACT

Polyriboadenylic-polybouridylic acid enhanced the immunological response of guinea pigs to aqueous foot-and-mouth disease virus vaccine. Polyriboninosinic-polyribocytidylic acid enhanced the early antibody production of swine to oil emulsified foot-and-mouth disease virus vaccine. Polyriboninosinic-polyribocytidylic acid alone did not stimulate resistance to foot-and-mouth disease in swine.

RÉSUMÉ

Cette expérience a démontré que l'acide polyriboadénylique-polyribouridylique intensifie la réaction immunitaire, chez des cobayes ayant reçu un vaccin aqueux contre la fièvre aphteuse. Par ailleurs, l'acide polyriboninosinique-polyribocytidylique intensifie la production initiale d'anticorps, chez des porcs ayant reçu un vaccin contre la fièvre aphteuse, préalablement émulsifié dans de l'huile. L'acide polyriboninosinique-polyribocytidylique n'augmente pas par lui-même la résistance des porcs à l'endroit de la fièvre aphteuse.

INTRODUCTION

Synthetic polyanionic compounds, oligonucleotides and polynucleotides stimulate interferon induction, enhance the early production of antibody forming cells (10) and are immunological adjuvants for selected antigens (1). Interest at this Center in the development of vaccines effective against foot-and-mouth disease (FMD) virus (5-9, 12, 13, 20) led us to develop a mouse model system for assessing the role of polyanions alone (15, 17) or in the presence of FMD virus (FMDV) vaccines (16) to stimulate resistance to FMD. Polyriboninosinic-polyribocytidylic (poly I·C) (17), divinyl ether-maleic anhydride (DVE/MA) and itaconic-acrylic acid (IAA) (3, 15) alone stimulated resistance to the lethal effects of this virus in infant mice. The carboxylic acid polyanions DVE/MA and IAA also enhanced the FMDV vaccine induced resistance to infection in mice (3) and increased early antibody production (16).

However, when these compounds were tested in animals considered to be natural hosts for this virus (bovine, porcine and ovine species), little (18) or no (4, 13) resistance to FMD was observed. Furthermore, when DVE/MA was administered with FMDV vaccines in swine, resistance was not enhanced even though slight increases in early antibody were detected (4).

This study was initiated to evaluate a guinea pig model system for assessing induced resistance and antibody formation by FMDV vaccines containing polyribonucleotides. Similar studies were done in swine because of the reported difficulties in immunizing this species with FMDV vaccines (7, 12).

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Submitted May 25, 1976.

MATERIALS AND METHODS

POLYNUCLEOTIDES

Polyriboadenylic-polyribouridylic acid copolymer (poly A•U)¹ was supplied as a 1x solution (1.2 mg/ml of pyrogen-free saline) or a 2x solution (2.4 mg/ml), the latter was used in vaccine preparation. Poly I•C² was obtained as a 1-mg/ml sterile saline solution. Both polynucleotides were stored at 4°C.

EXPERIMENTAL ANIMALS

The animals used were Hartley strain guinea pigs, weighing approximately 500 g and young linebred Tamworth swine, weighing approximately 20 kg. Blood for serum antibody assays was obtained from guinea pigs by heart puncture and from swine by precaval venipuncture.

VIRUS PRODUCTION

FMDV, type O, subtype 1, strain Brugge (FMDV, O₁-Br) was grown in baby hamster kidney (BHK-21) cell cultures in 2 liter roller culture vessels (14). Virus was concentrated and purified by the procedures of Wagner *et al* (20) for vaccine production. The antigen mass concentration of purified virus was determined by complement-fixation and spectrophotometric procedures (5). Purified virus was inactivated with 0.05% acetyleneimine at 37°C for 24 hours and tested for innocuity as described by Graves *et al* (8). Some of the clarified virus harvest was stored at -84°C in 1 ml aliquots and used in mouse assays for virus neutralizing (VN) antibody determinations.

VACCINE FORMULATION

Vaccines contained various combinations of purified virus antigen, poly A•U and Freund's incomplete adjuvant (nine parts light mineral oil, one part mannide monooleate). The mass concentration of FMDV, O₁-Br antigen was adjusted to 5 µg per 2 ml dose of vaccine. Various concentrations of poly A•U were incorporated (as indicated) in the aqueous phases of some vac-

cines. Vaccines were emulsified in incomplete Freund's adjuvant by repeatedly passing equal volumes of the aqueous and oil phases between two syringes joined by a double hubbed coupler.

ANTIBODY ASSAYS

The VN antibodies in guinea pig and swine serums were titered by a constant virus:variable serum dilution test in suckling mice (6). Serum virus mixtures (0.03 ml) were inoculated intraperitoneally (IP) into each of eight suckling mice. Serum titers (PD₅₀) of VN antibody are expressed as the common logarithm of the reciprocal of the serum dilution that protected 50% of the mice against 100 LD₅₀ of FMDV, O₁-Br.

EXPERIMENTS AND RESULTS

EXPERIMENT 1A

Effect of poly A•U on the immunological response of guinea pigs to aqueous FMDV, O₁-Br — Aqueous FMDV, O₁-Br vaccine alone or in combination with one of the various milligram quantities of poly A•U indicated in Table I, were each inoculated subcutaneously (SC) into 20 guinea pigs. Serum from five guinea pigs of each vaccine group was obtained at seven, 14 and 35 days postvaccination (DPV). Mean VN antibody titers shown in Table I indicate that aqueous FMDV vaccine alone stimulated substantial amounts of serum antibody. The same vaccine containing 1500 µg of poly A•U induced lower levels of serum antibody.

At 35 DPV, the guinea pigs remaining in each group were challenged by intradermal lingual inoculation of virulent FMD, O₁-Br virus (9) and observed seven days for development of local and generalized FMD lesions. The results are summarized in Table I. Three of five guinea pigs receiving vaccine alone developed localized tongue lesions but none developed generalized lesions of the foot pads. When 1500 or 7500 µg of poly A•U were included with the vaccine no tongue or foot pad lesions developed. However, two of six guinea pigs receiving vaccines containing 15000 µg of poly A•U developed generalized lesions. Results in the control group indicate that the challenge virus dose contained approximately one guinea pig ID₅₀.

¹Merck, Sharp & Dohme, Division of Merck & Co., Inc., Rahway, New Jersey.

²Miles Laboratories, Inc., Elkhart, Indiana.

TABLE I. Effect of Poly A·U on the Immunological Response of Guinea Pigs to Aqueous FMDV Vaccine

µg Poly A·U	Days postvaccination ^a			Challenge results ^b	
	7	14	35	Tongue	Footpads
15000	0.9	0.9	1.4	3/6	2/6
7500	1.5	1.3	1.4	0/5	0/5
1500	1.6	2.0	1.6	0/5	0/5
0	1.8	1.3	1.5	3/5	0/5
				Controls	11/17

^aMean (N = 5) virus neutralizing antibody (PD₅₀) titers

^bNumber of positive reactions/number of animals inoculated

EXPERIMENT 1B

Effect of poly A·U on the immunological response of guinea pigs to emulsified FMDV, O₁-Br antigen — Guinea pigs were inoculated SC with emulsified vaccines containing graded doses of poly A·U and six from each group were bled at eight and 35 DPV. Results (Table II) of the VN antibody assays indicate that low levels of poly A·U did not increase the antibody response over that of the control vaccine not containing poly A·U. Although two to fourfold differences in serum antibody levels were observed among the groups, no correlation was found between dose and response.

EXPERIMENT 2

Effect of 600 µg of poly A·U on immunological response of guinea pigs and swine to emulsified FMDV vaccines — Three vaccines, each containing 5 µg of FMDV, O₁-Br per dose, were prepared to assay possible adjuvant potentiation by poly A·U in swine and guinea pigs. Two were prepared as emulsified vaccines, one contained 600 µg poly A·U per 2 ml and the other contained no poly A·U. The third vaccine contained 600 µg of poly A·U per 2 ml and was used as an aqueous control vaccine.

Each vaccine was inoculated SC into four swine and 24 guinea pigs. Animals were bled at the intervals indicated in Table III and VN antibody titers were determined. Results show that poly A·U in emulsified or aqueous vaccines had no discernible potentiation of adjuvant activity in swine or guinea pigs. All swine were challenged by inoculation with virulent FMDV, O₁-Br at 90 DPV and observed for 14 days. Three of the four swine vaccinated with aqueous vaccine containing poly A·U, one of four vaccinated with emulsified vaccine containing poly A·U and two of four vaccinated with emulsified vaccine containing

no poly A·U developed FMD.

EXPERIMENT 3A

Effect of poly I·C on the immunological response of swine to emulsified FMDV, O₁-Br vaccine — Five swine were inoculated SC with emulsified vaccine, five additional swine were inoculated SC with emulsified vaccine and intraperitoneally (IP) with poly I·C (1 mg/kg). One of the pigs in the latter group died two days later of conditions unrelated to experimental treatments. The swine were bled at various times and the mean VN antibody titers are reported in Table IV. The data indicate that twofold to threefold higher VN antibody titers occurred during the first seven days in the group receiving vaccine plus poly I·C than in the group receiving vaccine alone. Antibody titers were similar in both groups thereafter.

EXPERIMENT 3B

Effect of poly I·C in emulsified FMDV vaccines on resistance of swine to FMD — Twenty-eight days after the above two groups were vaccinated, two additional pigs housed in a separate room were infected by

TABLE II. Effect of Poly A·U on the Immunological Response of Guinea Pigs to Emulsified FMDV Vaccine

µg Poly A·U ^a	Days Postvaccination ^b	
	8	35
0	1.5	3.3
0.2	1.1	3.6
2.0	1.3	3.8
20.0	1.4	3.6
200.0	1.0	3.4
2000.0	1.1	3.9

^aPoly A·U = poly A·U in aqueous phase of vaccine emulsion

^bMean (N = 6) virus neutralizing antibody (PD₅₀) titers

TABLE III. Antibody Response of Guinea Pigs and Swine to Emulsified Foot-and-Mouth Disease Vaccine Containing Poly A·U

DPV ^a	Emulsified vaccines ^b		Aqueous Control Vaccine + poly A·U ^c
	Oil alone	Oil + poly A·U ^c	
Swine			
7.....	≤0.3	<0.3	≤0.3
14.....	0.8	≤0.6	<0.3
28.....	1.4	0.8	<0.3
56.....	1.6	1.4	<0.3
90.....	1.9	1.8	<0.3
Guinea pigs			
7.....	<0.3	<0.3	<0.3
14.....	<0.3	≤0.3	≤0.7
35.....	1.5	1.4	0.4
70.....	1.9	2.0	0.7

^aDPV = days postvaccination

^bMean (swine N = 4; guinea pigs N = 6) virus neutralizing antibody (PD₅₀) titer

^c600 µg per 2 ml dose

injecting each in a foot pad (4) with 10⁴ PFU of virulent FMDV, O₁-Br. The next day, when both donors were febrile, they were transferred to the room containing the vaccinated pigs. Two other groups of swine were also introduced to the room at the same time. One group contained three untreated control pigs. The second group contained five pigs which had been injected IP with 1 mg/kg poly I·C on the previous day. All pigs were observed for clinical signs of FMD for 14 days after this contact exposure.

The two donor pigs had generalized vesicular lesions and other clinical signs of severe FMD 48 hours after being injected with virus. In the untreated control group, one pig exhibited FMD three days after exposure to the donors, the other two re-

acted the next day. One pig which received only poly I·C had FMD lesions four days after contact exposure and the remaining four pigs in that group had lesions on the seventh day after exposure. FMD lesions were not observed in pigs treated with vaccine alone or with vaccine plus poly I·C throughout the entire 14-day exposure period.

Preliminary experiments with pigs indicated that no toxicity reactions (19) followed IP injection of 1 mg/kg poly I·C. However, rather severe but transient reactions occurred in this group immediately after poly I·C administration. Without exception, these pigs were seized by an intense, generalized pruritus followed by furious scratching against any available object. This reaction quickly progressed to trembling, urination, defecation and ataxia, followed by a brief period of collapse. Soon after the pigs regained their feet, the episode apparently passed and the affected pigs began to eat and behave normally. No further signs of toxicity were observed in these pigs.

TABLE IV. Effect of Poly I·C Treatment on Antibody Response^a and Immunity in Swine to Emulsified FMDV Vaccine

Day(s) postvaccination	Poly I·C ^b + vaccine ^a	Vaccine alone ^a
1	<1.7	<1.65
2	<1.9	<1.65
3	2.7	2.2
4	3.2	2.8
7	3.9	3.6
14	4.4	4.3
21	4.6	4.6
28	4.8	4.8
Immunity	0/5 ^d	0/5

^aMean PD₅₀ values

^bPoly I·C injected intraperitoneally

^cVaccine injected subcutaneously

^dNumber of pigs with FMD lesions/number exposed

DISCUSSION

Simultaneous inoculation of synthetic polynucleotides and antigen stimulate earlier and greater antibody responses in mice (2) than antigen alone. Both poly A·U and poly I·C incorporated in emulsified viral vaccines produced antibody responses that greatly exceeded the expected additive effects of adjuvant and polynucleotides tested singly (21). However, in the present

experiments comparing the immunological response of guinea pigs and swine following the administration of FMDV vaccines combined with poly A·U the results were disappointing.

There was an indication that certain doses of poly A·U combined with aqueous FMDV vaccine altered the guinea pig resistance to FMD (Table I). Poly A·U concentrations of 1500 and 7500 μg incorporated in the vaccine prevented the primary site lesion development observed in guinea pigs which had received vaccine alone. However, when the poly A·U dose was increased, inoculation site and generalized lesions developed, suggesting this polynucleotide dose may have interfered with the immunological resistance of guinea pigs to FMDV, O₁-Br.

In contrast, there was no indication that poly A·U enhanced the immunological response of guinea pigs or swine to emulsified FMDV vaccines (Tables II and III) at the dose and time intervals tested. These observations differ from those reported for adult mice or swine treated with DVE/MA and emulsified FMDV, O₁-Br vaccine (4), indicating that the mechanism of antibody enhancement by poly A·U and DVE/MA may be different.

However, early antibody produced in response to emulsified FMDV vaccines was enhanced by simultaneously injecting swine with poly I·C (Table IV), although this enhanced response was not sustained. As similarly reported for cattle and goats (13), pretreating swine with poly I·C alone failed to induce resistance to FMD.

Pyrogenic response in calves, rabbits, dogs, adult cattle and goats (4) has been reported following inoculation of poly I·C intravenously. Since preliminary trials with varying doses of poly I·C in swine had predicted that a 1-mg/kg level was non-toxic when given intraperitoneally, the observed transient toxicity manifestations were surprising.

Enhanced antibody synthesis, increased resistance to viral infection or both, in an unnatural host does not necessarily predict a similar reaction in the natural host species (1, 3, 4, 11, 13, 15, 17, 18). Although poly A·U appears to have some adjuvant activity in guinea pigs, it cannot be concluded that this species will be a useful model in other tests of the efficacy of different polynucleotides as modifiers of the immune response in domestic animals exposed to foot-and-mouth disease vaccines.

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