OIL ADJUVANTED FOOT-AND-MOUTH DISEASE VACCINE: A COOPERATIVE STUDY

Argentina (Instituto Nacional de Tecnología Agropecuaria [INTA])[1]
and the
United States (Plum Island Animal Disease Center [PIADC])[2]

ABSTRACT

A summation of cooperative studies on foot-and-mouth disease virus vaccines between the Argentine Instituto Nacional de Tecnología Agropecuaria (INTA), the Pan American Foot-and-Mouth Disease Center, Brazil, and the United States Plum Island Animal Disease Center, is presented. These studies involve the immune response obtained in steers from (1) Frenkel virus vaccines prepared in oil and aluminum hydroxide adjuvants. When the immunity of the steers was challenged at 30 days, the Frenkel vaccine adjuvanted with aluminum hydroxide and saponin gave the best protection. In the major study (11), the response of steers from 3 BHK produced virus vaccines emulsified with oil adjuvant was studied. The three vaccines were prepared: one in which the virus was used in the concentration in which it was harvested; the other two from virus which was concentrated 100-fold by the polyethylene-glycol technique and then diluted to a 20X and 1X concentration. The antibody response and protection afforded by the 3 vaccines were similar. It was concluded that animals in the three groups were afforded similar protection when their immunity was challenged, indicating that these differing concentrations of antigen provided no significant difference in protection.

INTRODUCTION

A summation of results obtained in cooperative studies on foot-and-mouth disease virus (FMDV) vaccines conducted in Argentina by the Instituto Nacional de Tecnología Agropecuaria (INTA), the Pan American Foot-and-Mouth Disease Center (PAFMDC), Brazil, and the United States Plum Island Animal Disease Center (PIADC) over a period of several years (1968-1975) is reported (9, 11, 12). In this report, the immune response of oil adjuvant and aluminum hydroxide vaccines in cattle (part I), and the immune response after vaccination and revaccination with an FMD vaccine emulsified with oil adjuvant (part II) are evaluated. In the preliminary studies workers compared results of vaccines in which the viruses used for antigen were grown in baby hamster kidney (BHK) cells, inactivated with acetylethyleneimine (AEI) and emulsified with oil adjuvant (PIADC and INTA) and vaccines in which the virus used as antigen was grown in Frenkel cultures, inactivated with formaldehyde and combined with aluminum hydroxide and saponin adjuvant. The vaccinated cattle were kept at a holding unit in the Valdés Peninsula, Chubut Province, Argentina.

MATERIALS AND METHODS

Selection of strains of FMDV

On the advice of representatives of the Pan American Foot-and-Mouth Disease Center, the strains of FMDV subtype O_1 Caseros, subtype A_{24} strain Cruzeiro, and subtype C_3 Resende selected for the vaccine preparation were the same as those previously reported (9, 11).

[1]S. Rivenson; O. Ibarra; O.P. Gaggino; O. Laporte; H. García Olano; J.C. Pizzi; L. Marangunich. Instituto Nacional de Tecnología Agropecuaria, Servicio Nacional de Programación y Evaluación Técnica, San José 151, 2° Piso, Buenos Aires, Argentina.

[2]J.J. Callis; P.D. McKercher; J.H. Graves; H.L. Bachrach; K.M. Cowan; H.R. Cunliffe. USDA, Science and Education Administration, Federal Research, Plum Island Animal Disease Center, P.O. Box 848, Greenport, New York 11944, U.S.A.

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Vaccines

Four trivalent vaccines were prepared for the initial part (I) of these studies: 1) FMDV grown in Frenkel cultures, inactivated with formaldehyde and combined with aluminum hydroxide saponin adjuvant; 2) FMDV grown as in 1) but combined with INTA oil formulation; 3) FMDV grown in BHK cell cultures, inactivated with AEI and emulsified with PIADC oil adjuvant; and 4) similar to 3) but emulsified with the INTA oil adjuvant (1, 2, 10).

Three trivalent vaccines were used in II, the second major part of the study as reported (9) and consisted of: 1) Vaccine 1 - virus grown in BHK cells, inactivated with AEI and emulsified with oil adjuvant (INTA formulation) (2); 2) Vaccine 2 - virus grown as above, concentrated 100-fold by the polyethylene glycol technique (13) and then reconstituted to contain approximately the same antigen mass as Vaccine 1; and 3) Vaccine 3 - virus processed as in Vaccine 2, but adjusted to contain 20-fold the antigenic mass as Vaccines 1 and 2.

Safety testing

The infectivity of each antigen prepared at PIADC was tested by inoculating the tongues of each of 6 steers at 20 sites with 0.1 ml of the inactivated virus (8). All antigens and final products were further tested in a similar manner in Argentina before use in part I and at PIADC before use in part II.

Potency tests

The BHK antigens prepared at PIADC for part I were tested as monovalent and trivalent vaccines before their transportation to Argentina. The monovalent vaccines were each inoculated into 6 steers whose immunity was challenged at 30 days postvaccination (DPV) with their homologous viruses. The same antigens were formulated into a trivalent vaccine, and a 5-ml dose of this vaccine was inoculated into each of 6 steers whose immunity was also challenged at 30 DPV.

In Argentina, a 30-day potency test was conducted with each vaccine--1, 2, 3 and 4. Twenty-seven steers were inoculated with each vaccine, and these animals, totalling 108, with controls were kept in the isolation station at Puesto Larralde in Peninsula Valdés.

In the potency test for II, the second part of the study, 12 steers were inoculated subcutaneously, each with a 5-ml dose of vaccine, 4 steers were used with each vaccine--1, 2 and 3. A 50% bovine protective dose for O virus was determined for Vaccines 1 and 2 (9).

Vaccination

A total of 371 steers between 1 and 2 years old were selected for the study (II). They were from Chubut, the southern province of Argentina, situated in the FMD-free area of Patagonia, and were transferred to the cattle holding station in the Valdés Peninsula. Serums from the selected steers were examined for FMD antibodies at INTA by the mouse protection test (5). Only those with a serum-neutralizing index of less than 1 were selected for use in the study. They were divided into groups of 52--a group of 52 animals for each of the 3 vaccines and a group of 52 as nonvaccinated controls. Each group of 52 animals was selected from the 371 animals by pseudo random number selection on a computer (9).

Serology

Blood samples were collected from these vaccinated animals and appropriate control animals at 0, 30, 60, 120, 180, 240, 300, 365, 455 and 545 DPV. Serums were assayed for FMDV O, A and C antibodies at PIADC by a variety of serological techniques, including radial immunodiffusion (4), microneutralization (14), plaque reduction (3), mouse PD_{50} (6) and serum protection test in suckling mice (5).

The bioassay values against FMDV A for the 5 assay techniques (9), in conjunction with the results of immunity, were analyzed by stepwise linear discriminant analysis. With analysis, the assay techniques that most effectively discriminated between protected and unprotected animals were the mouse protection and the PD_{50} test in suckling mice.

Challenge of immunity

The steers in the potency study conducted in Argentina (initial, part I) were transported by truck from the Valdés Peninsula to Buenos Aires, divided into 3 groups and kept in isolation barns designated for the appropriate virus type (O, A or C) to be used in the challenge of immunity. Each group, including the control group, consisted of 9 randomly selected animals from the different vaccine groups. The challenge virus consisted of bovine tongue epithelium passaged in the bovine within 60 days, and recently titrated in mice. The dose of challenge virus consisted of 10,000 mouse LD_{50} contained in a 1-ml volume and inoculated at 4 sites in the tongue intradermalingually, 0.25 ml/site. At 48 hours postchallenge, the control animals from each group were examined to assure that the challenge dose of virus was effective, and tissue was collected for complement-fixation (CF) typing to ascertain that the infection was caused by the correct challenge virus. All animals were examined at 5 and 10 days postchallenge, and final results were summarized.

In the major study (II), each vaccinated group of steers was divided into 3 groups of 16 animals each by random number selection on a computer. Four spare animals in each group of 52 constituted replacements for animals which died or were injured. The immunity of all groups was challenged by exposure to FMDV A as follows: one group at 6 months, one group at 12 months, and one group revaccinated at 6 months and challenged 12 months later.

RESULTS

Safety test

All antigens and vaccine samples from the initial study (I) and the major study (II) proved innocuous to the inoculated steers.

Potency tests

The results of the challenge of immunity obtained in study I are presented in Table 1.

In study II, 1 of 4 steers inoculated with Vaccine 1 developed one secondary lesion on the gum; the steers inoculated with Vaccines 2 and 3 were resistant to infection.

Serology

The antibody assays of the steer serums (part I) were measured by a seroneutralization test in tissue culture and recorded as neutralization indices (Table 2).

TABLE 1. *Initial study results, portion of animals protected from challenge, infected with O, A and C FMDV at 30 days postvaccination*

Challenge FMDV type	Vaccines				
	Frenkel, $Al(OH)_3$ & saponin	Frenkel, INTA oil	BHK, PIADC oil	BHK, INTA oil	Controls
O	9/9[a]	1/9	6/9	3/8	0/9
A	9/9	3/9	5/9	5/9	0/9
C	9/9	7/9	8/9	9/9	1/9

[a]Code: Numerator, number of animals protected; denominator, number of animals challenged.

TABLE 2. *Neutralizing indices of bovine serums from study I against A_{24}, A_{24-25} and A_{25} FMDV*

Vaccines	Index with FMDV[a]		
	A_{24}	A_{24-25}	A_{25}
Frenkel, Al $(OH)_3$ and saponin	3.32	2.02	1.73
Frenkel, INTA oil	1.01	1.78	1.27
BHK, PIADC oil	3.86	0.62	0.73
BHK, INTA oil	4.04	1.42	0.45

[a] A_{24} = virus grown in BHK cells; A_{24-25} = viruses grown in Frenkel cultures.

The titers of the serums against A by the 5 techniques (part II), in conjunction with the results of the challenge of immunity, were analyzed by discriminant analysis.

The results of the analysis of the data for the 3 challenge-of-immunity groups at 180, 365 and 545 DPV indicate that the mouse protection test was the most effective discriminator between the protected and unprotected animals.

With the same data, which were tested by the 5 serological techniques but including an additional 34 animals that were not assayed by radioimmunodiffusion, the PD_{50} test in suckling mice appeared to be the most effective discriminator.

Immunity

The results of the challenge of immunity of the animals (part II) are shown in Table 3.

In general, although all groups had considerable protection when their immunity was challenged with FMDV A, there was little difference between vaccines, time of exposure and results of revaccination.

The results of the challenge data were examined by Chi-square techniques in comparing vaccines, duration of immunity and possible differential behavior of the vaccines over time or the holding periods under different vaccines. No Chi-square value approached significance. Immunity was the same at 12 months as at 6 months and was the same after revaccination at 6 months and holding for an additional 12 months as at 12 months. The response to the vaccines over time did not change nor did the immunity at different times differ among vaccines. The vaccines protected against FMDV in about three-fourths of vaccinated steers for at least 1 year.

DISCUSSION

In study I, the best results were obtained with vaccine produced from Frenkel cultures and combined with aluminum hydroxide and saponin adjuvant (Tables 1 and 2). All the steers vaccinated with this vaccine were protected when their immunity was challenged at 30 DPV. Under the conditions of the study, these animals were completely protected against FMDV, types O, A and C.

The vaccines prepared from antigens grown in BHK cultures, inactivated with AEI and emulsified with oil adjuvants (PIADC and INTA formulations) protected 67% and 63% respectively, of the test animals.

The vaccine prepared from the antigens grown in Frenkel cultures, inactivated with formaldehyde and emulsified with oil (INTA) protected only 42% of the steers and produced the poorest response of the 4 vaccines.

TABLE 3. *Results of challenge of immunity with FMDV A*

Vaccine No.[a]	Days postvaccination			
	180	365	545[b]	Total
1	13/16[c]	11/16	9/16	33/48
2 (1X)	12/16	10/16	13/16	35/48
3 (20X)	15/16	13/16	11/16	39/48
Total	40/48	34/48	33/48	107/144

[a] Vaccine No. 1, virus grown in BHK cells (not concentrated); vaccine No. 2, virus grown in BHK cells, concentrated 100-fold and reconstituted to contain the same antigen mass as No. 1; vaccine No. 3, virus as in No. 2, but adjusted to contain 20-fold the antigenic mass of No. 1 and No. 2.

[b] Revaccinated at 180 days after initial vaccination.

[c] Code: Numerator, number of animals protected; denominator, number of animals challenged.

Before the challenge of immunity of study I, CF tests at PAFMDC and at INTA indicated that the FMDV used for the A antigen in the Frenkel vaccines included 2 strains of A virus--A_{24} and A_{25}. The information from PAFMDC indicated that the A antigen in both BHK vaccines was A_{24} and did not include A_{25} as in the vaccines made from the Frenkel antigens. The average neutralization indices for the 4 vaccines obtained with each of the 3 virus types are given in Table 2. With type A_{24} virus, the average protection index of the 2 BHK vaccines (which were uniformly high and very similar) was significantly higher than that of either of the 2 Frenkel vaccines.

The actual effect of the challenge with FMDV A_{24} that contained A_{25} is rather difficult to determine. However, Graves *et al.* (7) reported that immunity is highly dependent on the relatedness of the subtype of the virus used for the exposure to that used to prepare the vaccine. Possibly, therefore, the poorer protection afforded by the BHK antigens, which contained only FMDV A_{24}, was related to the challenge virus which consisted of FMDV A_{24-25}.

In study II, reactions obtained at the sites of vaccination, particularly when the animals were revaccinated, were severe although they disappeared within 30 days. The immunity of the steers was challenged with A virus; however, from the antibody measurements as assayed by a number of different methods, one could assume that the test was reasonably successful for 2 of the 3 virus types tested--A and C. The antibody response obtained with the O virus with the exception of the potency test was disappointing (9). One could speculate that this virus is more sensitive and fragile than types A and C and thus did not remain as stable in the emulsified vaccine.

The results of the challenge of immunity at 6 months after vaccination, 12 months after vaccination, and 12 months after revaccination (at 6 months) were only slightly different, indicating that further studies to determine the optimal time of revaccination with such products are needed.

The response obtained with the 3 vaccines (part II) was very similar. The 20-fold concentrated vaccine did not produce a greater antibody response nor any greater degree of protection than either the crude or the 1X vaccine.

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