RESEARCH REPORT
SWINE HEALTH

Title: Development and Testing of a Subunit Vaccine for Foot-and-Mouth Disease. - **NPB #99-019**

Investigator: Marvin J. Grubman

Institution: United States Department of Agriculture, Agricultural Research Service, Plum Island Animal Disease Center

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I. Abstract:

We have tested the potency and efficacy of replication-defective recombinant human adenoviruses containing the capsid and 3C proteinase coding regions of foot-and-mouth disease virus (FMDV) as vaccine candidates in swine. This vaccine, which lacks the coding region of several FMDV nonstructural proteins, has a number of advantages over the current whole virus inactivated vaccine including the ability to readily distinguish vaccinated from infected animals using current technology.

Inoculation of swine with this vaccine resulted in either complete protection from disease after virulent homologous virus challenge or significant reduction in clinical signs as compared to co-housed uninoculated control animals. The optimal vaccine regimen tested was a low dose initial inoculation followed by a high dose boost. All animals inoculated by this regimen were completely free of disease after challenge. Increased efficacy of the two-dose regimen was associated with heightened levels of FMDV-specific IgG1 and IgG2 antibodies. Expression and processing of the FMDV capsid precursor protein, requiring a biologically active 3C proteinase, was essential for induction of FMDV-specific neutralizing antibodies and protection from challenge. This vaccine is safe since there is no evidence of seroconversion of co-housed unvaccinated control animals. These experimental results suggest that the Ad5-FMDV virus vector is a promising vaccine candidate against FMD and additional efforts to improve its potency and efficacy are underway.

II. Introduction:

Foot-and-mouth disease (FMD), a highly infectious and rapidly spreading viral disease of cloven-hoofed animals, is economically the most important disease of domestic animals, and a serious threat to U.S. agriculture. The presence of FMD in a country can have severe economic consequences from the loss of animal productivity and imposition of trade embargoes. Although FMD has not occurred in the U.S. since 1929, it is still a major problem in many parts of the world including South America, Africa, and parts of Asia, and has recently spread to developed countries in Asia which have been free of FMD for many years. For example the outbreak of FMD in the Spring of 1997 in Taiwan, which had been FMD-free for 68 years, resulted in the eventual death or destruction of approximately four million swine, and a multibillion dollar loss due to cleanup costs and lost export revenue. In the Spring of 2000 more limited outbreaks have occurred in South Korea and Japan, countries which had been free of FMD for many decades. Available responses to disease incursion include inhibition of animal and product movement, slaughter, disinfection, and vaccination. The U.S. FMD policy includes vaccination as a control strategy. Whole virus inactivated vaccines are used in FMD control programs, and it has been estimated that worldwide more than a billion doses are used annually. There are seven distinct serotypes of FMDV and numerous subtypes. Current FMD vaccines require production of large quantities of live virus in high containment facilities followed by imine inactivation. These vaccines have been successfully utilized in controlling the disease. However, there are a number of concerns about the safety of the vaccine based, in part, on findings that have linked outbreaks to release of virus from vaccine production plants or presence of residual live virus in chemically inactivated vaccines. Other concerns with the vaccine include selection of antigenic variants upon passage of virus in cell culture for preparation of vaccine stocks and difficulty in differentiating between vaccinated and convalescent animals. In addition, the inability of the U.S. to produce vaccine domestically, since federal law only allows FMDV at the Plum Island Animal Disease Center (PIADC), places us in a vulnerable position to control an outbreak. At the present time the PIADC houses the North American FMD Vaccine Bank that includes small amounts of antigen for only a few of the different serotypes. These reserves are available for an outbreak in the U.S., Canada, or Mexico, but would not be sufficient to control a wide-spread outbreak. To supply the North American FMD Bank, vaccine antigen has been purchased from companies in Western Europe. As a consequence of the above concerns, alternate vaccine approaches are being investigated.

Our approach towards development of safe, effective FMD vaccines is to produce viral subunit vaccines utilizing genetic engineering technology. This approach is based on the demonstration that empty viral capsids, virus particles lacking infectious nucleic acid, are naturally produced in infected cells and are indistinguishable from infectious virus in their ability to stimulate an immune response. The advantages of this approach include, 1) no infectious FMDV required or produced, 2) no tissue culture passage required, 3) no large scale biocontainment facilities needed for production, 4) the possibility of producing this type of vaccine on the U.S. mainland, and 5) the ability to easily distinguish

vaccinated from infected or convalescent animals using current technology, which detects a viral nonstructural protein that is not encoded in our subunit vaccine.

III. Objectives:

The objectives of this proposal are to develop a safe, effective FMD subunit vaccine that can be produced in the U.S. and will allow ready distinction between vaccinated and infected animals with currently available diagnostic procedures. Specifically, a replication-defective recombinant human adenovirus type 5 vector containing the FMDV serotype A12 capsid and 3C proteinase coding regions will be used to vaccinate swine by the intramuscular (IM) and intranasal (IN) routes. Concurrently, second-generation Ad5 vectors designed to induce more efficient FMDV capsid assembly and/or elicit stronger immune responses, will also be constructed.

IV. Procedures:

We previously constructed two adenovirus vectors containing the capsid and 3C proteinase coding regions of FMDV serotype A12, a laboratory strain studied extensively at Plum Island (Mayr et al., Virology, 263:496-506,1999). One vector, Ad5-P12X3CWT, contains the coding region for a biologically active 3C wild-type proteinase (3CWT) which is required for the formation of virus capsid structures, while the second vector contains the coding region for a biologically inactive or mutant 3C proteinase, Ad5-P12X3CMUT. In a previously published study we had demonstrated that the vector containing 3CWT induced a neutralizing antibody response and partial protection in swine (Mayr et al., Virology, 263:496-506,1999).

Three animal studies were performed during this proposal. In all studies six-to eight-week-old Yorkshire/Bluepoint gilts were used in a 3 animals/group format and the PBS inoculated control and vaccinated groups were co-housed.

The objective of our first experiment was to determine if processing of the FMDV capsid protein by the biologically active 3CWT was necessary to induce a neutralizing and protective immune response in swine. In this study 3 groups of swine inoculated IM were used. The first group was inoculated with $1x10^8$ pfu/animal Ad5-P12X3CWT, the second group with PBS (controls), and the third group with $1x10^8$ pfu/animal Ad5-P12X3CMUT. Four weeks later the animals were boosted with $5x10^8$ pfu/animal of the same virus or PBS. The animals were bled prior to and weekly throughout the course of the experiment. Serum samples were assayed for a general FMDV-specific antibody response by radioimmunoprecipitation (RIP), for FMDV- and adenovirus-specific neutralizing antibody response by plaque reduction neutralization (PRN) assays, and for FMDV-specific antibody isotype responses by ELISA. Three weeks following the boost one of the PBS inoculated animals was directly inoculated in the snout and one foot with $5x10^4$ infectious units of animal derived homologous FMDV. The animals were monitored daily for clinical signs and after 2 weeks euthanized.

The objectives of the second swine study were to examine the

effects of the amount and number of Ad5-P12X3CWT vaccine doses on potency and efficacy and involved five groups of animals**.** Two groups of animals were inoculated with a low dose of Ad5-P12X3CWT, $1x10^8$ pfu/animal, one of these two groups boosted 4 weeks post initial inoculation and the second low dose group not boosted. Two groups were inoculated with a high dose of Ad5-P12X3CWT, $5x10^8$ pfu/animal, one group boosted 4 weeks post initial inoculation and the second high dose group not boosted. The fifth group of animals served as PBS inoculated controls. The swine were challenged 4 weeks after the boost using the same protocol as in the first swine study and monitored clinically for two weeks and euthanized. Serum samples were obtained weekly throughout the study and assayed as described above.

The third swine experiment examined two parameters: 1. the effect of a naked DNA vector containing the FMDV empty capsid construct given as an IM boost following an initial IM inoculation with $1x10^8$ pfu/animal Ad5-P12X3CWT vector, 2. the effect of an initial IN inoculation of Ad5-P12X3CWT followed by a IM boost of $1x10^8$ pfu Ad5-P12X3CWT. Animals were challenged, monitored, and serum samples collected throughout the study as already described.

As indicated in the 6-month report, we also began construction of Ad5-P12X3CWT vectors containing the capsid coding region of A24 Cruziero and O1 Campos field strains of FMDV during the later part of the grant period.

V. Results:

Experiment #1: As shown in Fig. 1, at 48 days post inoculation (dpi) all 3 animals inoculated with Ad5-P12X3CWT developed antibodies against the FMDV structural proteins VP0, VP3, and VP1 as detected by RIP, i.e., #95-97. One of the 3 animals inoculated with Ad5-P12X3CMUT developed a significant antibody response against the FMDV structural proteins, i.e., #101, one animal developed a weak response, i.e., #103, and the third animal did not develop an FMDV antibody response, i.e., #102. Significantly none of the control animals developed a FMDV antibody response, i.e., #98, 79, 100. This later result and the absence of an Ad5-specific neutralizing antibody response in the control group (data not shown) supports our previous results demonstrating the inability of the replication-defective Ad5 vector to spread from the inoculated groups to the uninoculated controls (Mayr et al., Virology, 263:496-506,1999) and confirms the safety of this vaccine candidate. The Ad5-P12X3CWT inoculated animals also developed a significant neutralizing antibody response by 2 weeks post inoculation which increased after the boost (Fig. 2), while neither the Ad5-P12X3CMUT nor the control group developed a FMDV-specific neutralizing antibody response. Only the animals inoculated with Ad5-P12X3CWT developed an initial FMDV-specific IgM response as measured by ELISA and after the boost all animals in this group showed a dramatic increase in the levels of IgG1 and IgG2 (data not shown).

Three weeks following the boost one of the PBS inoculated animals, #79, was directly inoculated with virulent homologous FMDV. The animals were monitored daily for clinical signs and after 2 weeks euthanized.

Animal #79 developed a fever by 2 days post challenge (dpc) and lesions on all 4 feet and the snout by 2-3 dpc (Table 1). The remaining 2 control animals and the Ad5-P12X3CMUT inoculated animals developed a fever and lesions on the feet and sometimes the snout by 3-5 dpc. In contrast, all the Ad5-P12X3CWT inoculated animals were completely protected from any clinical signs of disease.

Serum samples were examined 7 and 14 dpc. The directly inoculated control swine as well as the 2 uninoculated control swine and the 3 swine inoculated with Ad5-P12X3CMUT all seroconverted and developed a very high FMDV-specific neutralizing antibody response as well as an antibody response against viral nonstructural proteins (Table 1). The neutralizing antibody titers of the group inoculated with Ad5-P12X3CWT increased after challenge presumably because of the continuous exposure to the control and Ad5-P12X3CMUT inoculated swine which were co-housed in the same room and were actively infected and shedding virus. Significantly the Ad5-P12X3CWT inoculated animals did not develop an antibody response to FMDV nonstructural proteins which is indicative of the absence of virus replication in these animals (Table 1).

Experiment #2: All animals inoculated once or twice with a low or high dose of Ad5-P12X3CWT developed an FMDV-specific neutralizing antibody response and either were completely protected from disease or had significantly reduced signs of disease as compared to uninoculated control animals (Table 2). Animals inoculated twice generally developed higher levels of neutralizing antibodies prior to challenge than once-inoculated animals (Table 2) and developed a secondary immune response as shown by a significant increase in IgG1 and IgG2 levels (data not shown). Animals inoculated with a high dose of Ad5-P12X3CWT generally developed a more rapid FMDV-specific neutralizing antibody response, ie., within one week, than animals inoculated with a low dose of Ad5-P12X3CWT, but the response was essentially equivalent among all inoculated groups by two weeks post initial inoculation.

The results of this experiment clearly demonstrate that inoculation with the Ad5-P12X3CWT vaccine affords protection from challenge with virulent FMDV. Based on experiments #1 and 2 and our previously published data (Mayr, G.A., et al., Virology, 263:496-506, 1999) two inoculations appear to afford a greater degree of protection than either one low or high dose of vaccine.

Experiment #3: One of three swine boosted with a naked DNA containing the FMDV empty capsid construct developed an 8-fold increase in FMDV-specific neutralizing antibodies 1 week postboost, ie., #35 1:40 to 1:360 PRN_{70}, and only this animal was completely protected from virus challenge (Table 3). The other two animals in this group either did not develop an increased neutralizing antibodies response or only a slight increase after DNA inoculation and both developed some signs of disease. Because we only used a small number of animals in this group and since animals inoculated only once with Ad5-P12X3CWT developed similar levels of protection (Experiment 2, Table 2) as those boosted with DNA, it is difficult to draw any firm conclusions from this study about the utility of naked DNA as a viable vaccine candidate. However, we previously reported that naked DNA containing the FMDV empty capsid construct, as the only vaccine tested, was not able to induce FMDV-specific neutralizing antibodies or afford protection in swine (Chinsangaram, J., et al., Journal of Virology, 72, 4454-4457, 1998).

Swine inoculated IN with $1x10^8$ pfu/animal Ad5-P12X3CWT developed only very low levels of FMDV-specific neutralizing antibodies, but one week after an IM boost with the same dose of virus animal #38 developed an 8-fold boost, ie., 1:8 to 1:640 and was the only animal in this group that was completely protected from disease after challenge. Since all animals inoculated initially with $1x10^8$ pfu/animal Ad5-P12X3CWT, ie., 12 animals, develop a neutralizing antibody response only by two weeks postinoculation, this result suggests that swine #38 was pre-exposed to the vaccine by IN inoculation. However, the remaining two animals in this group only developed a neutralizing antibody response two weeks after the IM boost, suggesting that they were not exposed to the vaccine after IN inoculation.

Construction of Ad5-FMDV Field Strains: We have developed new, rapid procedures to produce Ad5 viruses containing capsid coding regions from current FMDV field strains, various swine cytokine genes, or genes from other pathogens of interest. Utilizing these newly developed protocols we have constructed Ad5 virus vectors containing the capsid coding regions from A24 Cruziero and O1 Campos, FMDV strains currently circulating in Brazil. These new viruses have been characterized in cell culture and by radioimmunoprecipitation we detected the expression and processing of the capsid coding regions. We have recently initiated a potency and efficacy study in swine with the Ad5-P12X3CWT virus containing the A24 Cruziero capsid coding region.

In summary, the 3 swine experiments described here as well as our previously published work and subsequent studies clearly demonstrate that vaccination with Ad5-P12X3CWT either completely protects or reduces the clinical signs of disease as compared to uninoculated control animals after contact challenge. The replication-defective Ad5-FMDV virus vector is safe since there is no evidence of seroconversion in the co-housed unvaccinated control animals in any of our studies. There is a direct correlation between processing of the FMDV capsid precursor protein, as occurs in Ad5-P12X3CWT infection, and induction of a FMDV-specific neutralizing antibody response and protection against challenge. The increased efficacy of the two-dose vaccine regimen, specifically a low initial inoculation followed by a high dose boost, is associated with heightened levels of FMDV-specific IgG1 and IgG2 antibodies.

The results described in this report demonstrate the potential of the Ad5-P12X3CWT virus vector as a vaccine candidate. To further improve the potency and efficacy of this vaccine, we plan to utilize our capability to rapidly produce new Ad5 viruses and examine the potential of various immune modulators as vaccine adjuvants.

TABLE 1

Summary of Immune and Clinical Responses to Vaccination and Challenge

	PRN_{70}[a]				
Animal #	**48dpi[b]**	**7dpc[c]**	**14dpc/NS Ab[d]**	**Clinical signs**	**Lesion Score[e]**
95-WT	1:160	1:160	1:8000/-	No fever or lesions.	0
96-WT	1:160	1:320	1:800/-	No fever or lesions	0
97-WT	1:160	1:320	1:8000/-	No fever or lesions	0
98-Control	1:16	1:16,000	1:32,000/+	Fever and lesions on all 4 feet and snout	12
79-Control	1:8	1:32,000	1:32,000/+	Fever and lesions on all 4 feet and snout	16+snout
100-Control	1:8	1:16,000	1:32,000/+	Fever and lesions on all 4 feet and snout	16+snout
101-MUT	1:8	>1:64,000	1:32,000/+	Fever and lesions on all 4 feet and snout	12
102-MUT	1:8	1:32,000	1:64,000/+	Fever and lesions on all 4 feet	13
103-MUT	1:8	1:3200	1:32,000/+	Fever and lesions on all 4 feet	13

[a] PRN_{70}, plaque reduction neutralization assay. The values listed are the serum dilution which resulted in a 70% reduction in the number of plaques.
[b] dpi, days post inoculation
[c] dpc, days post challenge
[d] NS Ab is defined as the presence of antibodies against FMDV nonstructural proteins (2C and/or 3D) as determined by RIP.
[e] The lesion score is the number of digits on which the swine exhibited lesions.

TABLE 2

SUMMARY OF IMMUNE AND CLINICAL RESPONSES TO VACCINATION AND CHALLENGE

	PRN_{70}[a]				
Animal #	**56dpi[b]**	**7dpc[c]**	**14dpc/NS Ab[d]**	**Clinical Signs**	**Lesion Score[e]**
145-l,b[f]	1:400	1:320	1:3200	No fever or lesions	0
146-l,b	1:320	1:160	1:3200	No fever or lesions	0
147-l,b	1:320	1:640	1:3200	No fever or lesions	0
148-l,nb[g]	1:40	1:640	1:16000	No fever or lesions	0
149-l,nb	1:320	1:64000	1:128000	No fever, lesions on one foot	1
154-l,nb	1:160	1:12800	1:128000	Fever, lesions on 3 feet	6
150-h,b[h]	1:320	1:800	1:128000	Fever, lesions on 1 foot	2
151-h,b	1:320	1:640	1:64000	Fever, lesions on 2 feet	2
152-h,b	1:400	1:640	1:12800	No fever or lesions	0
153-h,nb[i]	1:160	1:6400	1:16000	Fever, lesions on 1 foot	1
155-h,nb	1:160	1:320	1:6400	No fever or lesions	0
144-h,nb	1:80	1:1600	1:64000	Fever, lesions on all 4 feet	5
156-control	1:64	1:32000	1:25600	Fever, lesions on all 4 feet	16+snout
157-control	1:8	1:1600	1:16000	No fever, lesion on 1 foot	1
158-control	<1:8	1:32000	1:12800	Fever, lesions on all 4 feet	13

[a]PRN_{70} plaque reduction neutralization assay. The values listed are the serum dilution which resulted in a 70% reduction in the number of plaques.
[b]dpi, days post inoculation
[c]dpc, days post challenge
[d]NS Ab is defined as the presence of antibodies against FMDV nonstructural proteins (2C and/or 3D) as determined by RIP.
[e]The lesion score is the number of digits on which the swine exhibited lesions
[f]l,b, inoculated with low inoculum,$1x10^8$pfu/animal, and boosted with the same inoculum
[g]l,nb, inoculated with low inoculum,$1x10^8$pfu/animal, and not boosted
[h]h,b, inoculated with high inoculum, $5x10^8$pfu/animal, and boosted with the same inoculum
[i]h,nb, inoculated with high inoculum, $5x10^8$pfu/animal, and not boosted

TABLE 3

Summary of Immune and Clinical Responses to Vaccination and Challenge

	PRN_{70}[a]				
Animal #	**49dpi[b]**	**6dpc[c]**	**13dpc/NS Ab[d]**	**Clinical signs**	**Lesion Score[e]**
25-Control	1:64	1:800	1:8000	Fever and lesions on all 4 feet and snout	15+snout
26- Control	1:8	1:25,600	1:16,000	Fever and lesions on all 4 feet and snout	13+snout
27- Control	1:32	1:16,000	1:32,000	Fever and lesions on all 4 feet and snout	16+snout
34-I[f], DNA[g]	1:320	1:200	1:32,000	No fever and 1 lesion	1
35-I, DNA	1:320	1:200	1:25,600	No fever and no lesions	0
36-I, DNA	1:320	1:400	1:64,000	Fever and lesions on 3 feet	5
37-IN[h]/IM[i]	1:160	1:3200	1:12,800	No fever and 1 lesion	1
38-IN/IM	1:320	1:400	>1:25,600	No fever and no lesions	0
39-IN/IM	1:160	>1:25,600	1:32,000	Fever and lesions on all 4 feet	16+snout

[a] PRN_{70}, plaque reduction neutralization assay. The values listed are the serum dilution which resulted in a 70% reduction in the number of plaques.
[b] dpi, days post inoculation
[c] dpc, days post challenge
[d] NS Ab is defined as the presence of antibodies against FMDV nonstructural proteins (2C and/or 3D) as determined by RIP.
[e] The lesion score is the number of digits on which the swine exhibited lesions.
[f] inoculated with low inoculum, $1x10^8$pfu/animal, Ad5-P12X3CWT
[g] boosted at 4 weeks post initial inoculation with 400 ug/animal pCId-P12X3CWT
[h] inoculated intranasally with $1x10^8$pfu/animal Ad5-P12X3CWT
[i] boosted intramuscularly with $1x10^8$pfu/animal Ad5-P12X3CWT

FIGURE 1

RIP OF SERUM FROM SWINE INOCULATED WITH Ad5-P12X3CWT/MUT 48 DPI

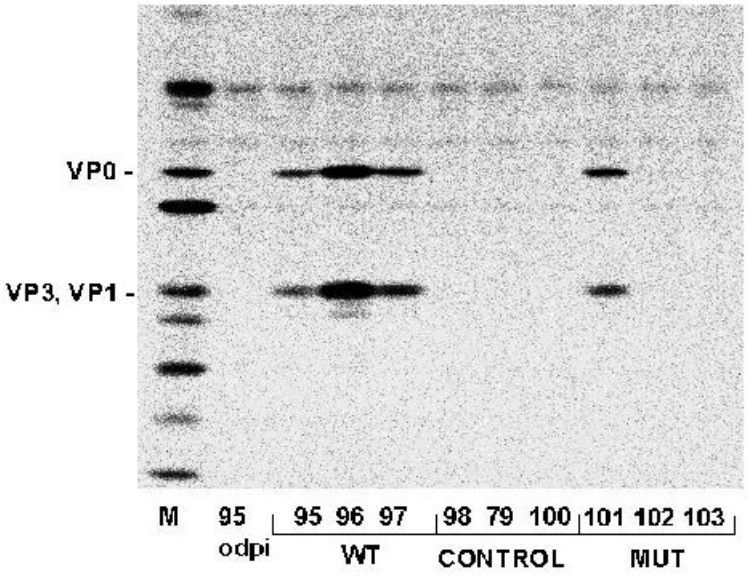

FIGURE 2