RESEARCHREPORT

SWINE HEALTH

Title: Characterization of the Taiwanese strain of foot-and-mouth disease virus (FMDV) - **NPB #98-048**

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I. Abstract:

Evaluation of the genetic and antigenic properties of the highly swine-virulent strain of FMDV that devastated the Taiwanese pork industry (OTai) has helped us to understand this new virus. OTai is characterized by a porcinophilic nature (inability to cause disease in bovines), which may be related to its hyper-virulence in swine. Immunological tests on virus preparations and genetic analyses we performed on the virus capsid-encoding regions of OTai have revealed substantial differences from South American and European isolates of FMDV. These changes suggest that vaccine seeds specific for the Asian subtype will be needed for effective control of outbreaks it causes. In addition, we have determined that OTai contains a significant mutation in a non-structural protein 3A, which has not been observed in natural isolates of FMDV from Asia, Africa, or South America.

Using a combination of tissue culture and live bovine challenge, we were able to demonstrate that the altered 3A protein **is** responsible for the altered tropism of OTai. Specifically, we have tested the role of this mutated 3A protein in host-range in cells and in animals using genetically engineered forms of FMDV, which have exchanged 3A-encoding regions of their genomes. These experiments showed that the addition of a European 3A protein-encoding region to a genetically engineered OTai virus produced a virus that is able to replicate in bovine cells and bovines. Current studies are aimed at determining if the altered 3A gene is responsible for the hypervirulence of OTai in swine.

The research supported by this project has extended the USDA's research programs on FMD into studies of this particularly dangerous Asian subtype of FMDV. Furthermore, the information learned from these studies will contribute to our knowledge of host/virus interactions that cause disease and outbreaks, and could contribute to the development of better vaccines for FMD.

II. Introduction:

Foot-and-mouth disease (FMD), a debilitating and highly contagious illness of cattle, swine, and other cloven-hoofed animals, poses a catastrophic threat to US livestock. The disease is caused by a virus that exists in seven recognized serotypes that are found throughout much of the world, although the US has been FMD-free since 1929. FMD has been controlled in many countries using vaccines prepared from inactivated virus, however, widespread vaccination is expensive, and countries that have eradicated the disease stop vaccination. Banked vaccines can be used to assist in the control of outbreaks, but the highly variable nature of the virus means that vaccines banked for this purpose could become outdated. Moreover, there is no US facility approved for manufacture of these vaccines, which could place our livestock at risk when new types of virus emerge.

In the Spring of 1997 a particularly virulent strain of FMDV serotype O (OTai) emerged and caused a rapidly spreading epizootic in Taiwan. Failure to quickly identify the outbreak, and inability to obtain vaccine during the early stages of the epizootic, contributed to the extent of the destruction. The epizootic devastated the country's pork industry, resulting in destruction of over 4 million swine and costing billions of dollars for decontamination and carcass disposal. Some costs, including the reluctance of consumers to eat pork (fearing for their own health), cannot be accurately measured. Others, such as the loss of international trade, will exceed $1.5 billion per year, with an estimated final price tag of over $6 billion.

Molecular epidemiology analyses have related OTai to viruses from several areas in Southeast Asia, including Hong Kong (N. Knowles, Pirbright, UK, personal communication). Interestingly, OTai, although highly virulent (and in many cases deadly) for swine, did not cause disease in cattle during the Taiwanese epizootic. Moreover, work at the UK's Pirbright Laboratory has confirmed the difficulty of transmitting OTai to cattle and showed that bovine cells will not replicate the virus (Dunn and Donaldson, 1997, Vet. Record 141:174-5). The difficulty of growing OTai in cells derived from ungulates, which are usually highly sensitive to FMDV (House and House, 1989, Vet. Microbiol. 20:99-109), and differences in its genome, interfered with attempts to identify virus in samples sent to PIADC from Taiwan.

III. Objectives:

1. To evaluate growth and monitor cell-culture selection of the Genetically Engineered OTai (OTaiGE) and Animal-Derived virus (OTaiAD), during preparation of large seeds of virus, similar to those needed for vaccine preparation.
2. To determine the antigenic properties of OTaiGE/OTaiAD, and Cell Culture-adapted OTai (OTaiCC).
3. To determine the complete nucleotide sequence of the capsid region of the OTaiGE/OTaiAD, and OTaiCC, and design oligonucleotide primers for the efficient identification and characterization of OTai isolates using the polymerase chain reaction (PCR).
4. To determine the immunogenicity of vaccines prepared from OTaiCC and OTaiGE in a swine vaccination/challenge model, and compare these to immune responses produced by O1 strains from South America and Europe.
5. To determine if isolates and sera obtained from these animals are identified in diagnostic tests currently in use at PIADC, and if not, to improve these tests.

IV. Procedures:

The studies performed utilized the following four procedures: 1) Propagation and evaluation of viruses in cell cultures, 2) Analyses of nucleotide sequences, 3) Genetic engineering of viruses from cloned cDNA molecules, and 4) Swine and bovine inoculations.

1) Propagation and evaluation of viruses in cell cultures:

Viruses were propagated and infectious virus titers were evaluated in BHK 21 cells using standard techniques (Rieder et al., 1993, J. Virol. 67:5139-45). Enzyme-linked immunosorbent assay (ELISA) evaluation of individual viruses were performed using the method of Ferris and Donaldson, 1992, Rev. Sci. Tech.11:657-84.

2) Analysis of nucleotide sequences:

Nucleotide sequence data were generated from polymerase chain reaction (PCR) -amplified reverse transcriptase products (Rieder et al., 1993, J. Virol. 67:5139-45, Tellier et al., 1996, PNAS 93:4370-4371) prepared from RNA extracted from swine lesions using standard techniques (Rieder et al., 1993, J. Virol. 67:5139-45).

3) Genetic engineering of viruses from cloned cDNA molecules:

Genetically engineered chimeric viruses were produced from *in vitro*-generated RNA transcripts of genome-length cDNAs using standard techniques (Rieder et al., 1993, J. Virol. 67:5139-45 and Mason et al., 1994, PNAS 91:1932-1936). Genome-length cDNA containing plasmids were derived from serotype A12 cDNAs (Rieder et al., 1993, J. Virol. 67:5139-45) containing fragments of OTai cDNA prepared as described above. Chimeric viruses uses in these studies were generated by high-efficiency transfection of BHK cells (Mason et al., 1994, PNAS 91:1932-1936), and experiments were performed with low-passage stocks of virus (unless otherwise indicated).

4) Swine and bovine inoculations:

Swine were inoculated with viruses using the method of Almeida et al., 1999, Virus Res. 55:49-60, and bovine were inoculated using the method of Sá-Carvalho et al., 1997, J. Virol. 71:5115-23.

V. Results:

Overall accomplishments:

Following discovery of a novel 3A protein-encoding region in the OTai genome, resources were re-directed from objectives 4 and 5 to a new objective (please see interim progress report): determining the genetic basis for the altered virulence (the porcinophilic nature) of OTai. Accomplishments in this final area are listed after the accomplishments of objectives 1 – 5. Portions of our accomplishments will be published in the *Journal of Virology* in a manuscript entitled “Genetic determinants of altered virulence of the Taiwanese foot-and-mouth disease virus” (by C.W. Beard and P.W. Mason, accepted for publication on October 6, 1999).

Objective #1:

Serial passages in BHK cells have been accomplished for both animal-derived (OTaiAD) and genetically engineered OTai (OTaiGE). Endpoint titers for viruses obtained from these studies show similar titers for the genetically

engineered and propagated forms of OTai (see Table 1). Furthermore, end-point dilutions in antibody-capture ELISA tests (see also objective 2) demonstrated that both the passaged OTaiAD (OTaiCC) and the OTaiGE grow to similar amounts (in terms of antigenic mass) in BHK cells. Thus both the genetically engineered and tissue culture-grown animal virus could be used for inactivated antigen preparation for use as vaccines.

Objective #2:

Using an ELISA test, the antigenic properties of several OTai strains were compared to a genetically engineered, animal (bovine and swine) -virulent O1 Campos virus (vCRM8; Sá-Carvalho et al., 1997; J. Virol. 71:5115-5123) and its tissue culture-passaged derivative (Table 2). These studies demonstrated that with the limited selection of monoclonal antibodies (MAbs) at our disposal, that the genetically engineered OTai only differed at only a single antigenic site from tissue culture-passaged animal-derived OTai virus (Table 2). Interestingly, these studies were not able to detect the accumulation of any changes in antigenic structure during propagation of the viruses in BHK cells (Table 2). However, significant differences in MAb reactivities were detected between the Asian and South American viruses.

Objective #3:

The complete nucleotide sequence of the capsid-encoding region of OTai has been determined. Numerous nucleotide and predicted amino acid differences were detected between OTai and O1 Campos (Table 3). Application of the OTai data to the known three-dimensional structure (Logan et al., 1993; Nature 362:566-568) of a European O1 virus (essentially identical to O1 Campos) demonstrated that many of these changes are in surface-exposed residues on the viral capsid (Table 3). These surface differences are found in all four of the known antigenic sites on the viral capsid, consistent with the antigenic differences detected between the Tai and Campos viruses using ELISA (see Objective #2, Table 2).

Objective 4:

Several swine have been infected with Asian and South America type O viruses, or given vaccines prepared from these viruses. The ELISA reactivities of two selected animals are shown in Table 2. These data show that despite the large differences in individual antigenic sites, polyclonal sera from each of these viruses react well with inactivated antigens prepared from the other.

Objective 5:

As shown in Table 2, sera from infected animals reacted well with antigen prepared from either serotype O virus. Similar data was obtained from sera produced from animals vaccinated with chemically inactivated derivatives of these viruses (results not shown). Thus, the ELISA test is suitable for detecting sera to these apparently quite dissimilar subtypes of serotype O virus.

New Objective:

New information on the genetic structure of the OTai genome, has demonstrated that this virus has a unique 3A protein that differs from other well-characterized FMDV types (Table 4). Fig.1 shows that this large difference in encoded amino acids is due, in part, to a dramatic deletion and mutation found in

the protein encoded by OTai. Interestingly, a similar deletion (but not a mutation), have been previously identified in the 3A protein of egg-adapted FMDV used as vaccines in South America (Giraudo et al., 1990; Virol. 177:780-783). This exciting new data is being further evaluated, by examination of the 3A-coding region of several related Asian isolates of serotype O. These data will be critical for defining the origins of the OTai virus, and in determining if this altered 3A protein has been associated with particularly virulent outbreaks of disease in the past.

Using our ability to genetically engineer FMDV from cDNA molecules, we have successfully demonstrated that the deleted 3A protein of OTai is responsible for its bovine attenuation. Specifically, we have shown that a chimeric virus generated by substituting the serotype A12 3A coding region (see Fig.1) into the OTai genome produces a chimeric virus able to cause disease in bovines (Beard and Mason, J. Virol. 1999, in press). Thus, the genetic basis of bovine attenuation of OTai has been identified. Future studies will relate this genetic alteration to the observed hypervirulence of the OTai in swine and determine the mechanism by which the altered 3A protein influences the host-range of FMDV.

Table 1: Infectivity titers of vCRM8, vOTaiGE, and swine-derived virus on BHK cells.

virus	titer on BHK cells[1]
p1 vCRM81[2]	1.3×10^7
p10 vCRM8[2]	1.6×10^7
p1vOTai.GE[3]	0.55×10^7
p10vOTaiGE[3]	1.75×10^7
p1vOTaiCC[4]	1.05×10^7
p10vOTaiCC[4]	1.15×10^7

1 Titer in plaque forming units (pfu).

2 Genetically engineered O1 Campos virus (Sa-Carvalho et al., 1997; J Virol. 71:5115-5123), BHK passage 1 or 10 (p1 or p10).

3 Genetically engineered OTai virus, BHK passage 1 or 10 (p1 or p10).

4 BHK passage 1 and 10 of virus (p1 or p10) harvested directly from an infected swine #196.

Table 2: Summary of ELISA data comparing MAb reactivities of genetically engineered O1 Campos, OTaiGE, and swine-derived virus.

	αO1 Brugge MAb[1]		α O1 Campos MAb[2]							convalescent sera[3]	
virus	12 FE	10 GA	11 HC	34 HC	12 BB	12 DB	12 FA	11 AD	13 G1	α-O TaiGE	α-O Camp
p1vCRM8[4]	2.6	3.2	3.2	2.6	2.9	3.8	2.9	2.6	2.3	3.2	3.8
P10vCRM8[4]	2.9	3.5	3.2	2.6	2.9	3.5	2.9	2.6	2.3	3.2	3.8
p1vOTai GE[5]	2.3	3.2	2.9	-[7]	-	-	-	2.3	2.3	3.2	3.5
p10vOTaiGE[5]	2.3	3.2	2.9	-	-	-	-	2.3	2.3	3.5	3.5
p1vOTaiCC[6]	2.3	3.2	2.9	-	-	-	-	-	2.6	3.5	3.5
p10 vOTaiCC[6]	2.6	3.2	2.9	-	-	-	-	-	2.9	3.5	3.5

1 Log of reciprocal titer in reaction with the MAbs of Stave et al., 1988; Virol. 162:21-29.

2 Log of reciprocal titer in reaction with the MAbs of Alonso et al., 1994; Vaccine 12:682-696.

3 Log of reciprocal titer in reaction with sera from swine infected with viruses carrying O1 Campos (swine #160) or OTai (swine #204) capsids.

4 Genetically engineered O1 Campos virus (Sa-Carvalho et al., 1997; J. Virol. 71:5115-5123), BHK passage 1 or 10 (p1 or p10).

5 Genetically engineered OTai virus, BHK passage 1 or 10 (p1 or p10).

6 BHK passage 1 and 10 (p1 or p10) of virus harvested directly from an infected swine #196.

7 No specific reaction detected.

Table 3: Summary of differences in nucleotide/predicted amino acid sequences between the capsid-encoding regions of O1 Campos (Sa-Carvalho et al., 1997; J. Virol. 71:5115-5123) and O1 Tai.

	VP4	VP2	VP3	VP1
Nucleotide changes (% diff)	40/255 (15.7)	113/654 (17.3)	103/660 (15.6)	143/633 (22.6)
Amino acid changes (% diff)	4/85 (4.7)	7/218 (3.2)	7/220 (3.2)	32/211 (15.2)
Percent surface-exposed[1]	N.A.[2]	42.9%	57.1%	75.0%

[1] Determined by positioning OTai sequence data on the 3-dimensional structure data of Logan et al., 1993 (Nature 362:566-568).

[2] Not applicable; VP4 is not surface-exposed on the FMDV capsid.

Table 4: Comparison of differences between nonstructural protein encoding segments of the FMDV genomes of OTai, O1K and A12

	% Differences at the amino acid level relative to O1K[1]					
Virus	2B	2C	3A	3B3	3C	3D
OTai[2]	4.5	3.1	28.6	2.8	2.8	1.4
A12[3]	3.9	1.9	4.6	4.2	3.8	2.6

[1] Based on amino acids encoded in the nucleotide sequences of O1K (Forss et al., 1984, Nucleic Acids Res. 12:6587-6601).

[2] Amino acids encoded by OTai.

[3] Amino acids encoded in the nucleotide sequences of A12 (Robertson et al., 1985, J. Virol. 54:651-660).

Fig. 1. Alignment of the deduced 3A amino acid sequences (shown in single-letter code) of OTai, O1 Campos (Giraudo et al., 1990; Virol. 177:780-783), A12 (A12 (Robertson et al., 1985, J. Virol. 54:651-660), and an egg-adapted O1 Campos (Giraudo et al., 1990; Virol 177:780-783), reveal significant differences among their C-terminal halves ("." = identity to O1 Campos, "-" = deletion). The hydrophobic domain located at residues #61-76 (underlined) is thought to function in membrane binding

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O1Campos  ISIPSQKSVLYFLIEKGQHEAAIEFFEGMVHDSIKEELRP  40
A12       ........................................  40
O1C-O/E   ........................................  40
OTai      ........................................  40

O1Campos  LIQQTSFVKRAFKRLKENFEIVALCLTLLANIVIMIRETR  80
A12       ........................................  80
O1C-O/E   ........................................  80
OTai      ..................V.............L.QA.  80


O1Campos  KRQKMVDDAVNEYIEKANITTDDKTLDEAEKSPLETSGAS 120
A12       ......................T.......N........ 120
O1C-O/E   ...N-------------------................. 101
O1 Tai:   ..YQS...PLDG----------.V..GD...N........ 110

O1Campos  TVGFRERTPPGQKACDDVNSEPAQPVEEQPQAE 153
A12       ........LT..R..N.......R.A....... 153
O1C-O/E   ..............................  134
OTai      A......S.TE.GTRE.A.A..VVFGR...R.. 143
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