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Short communication

DNA immunization of pigs with foot-and-mouth disease virus minigenes: From partial protection to disease exacerbation

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ABSTRACT

Despite several attempts to design new vaccines, there are as of yet no available alternatives to the conventional FMDV vaccines. Here, we present the divergent results obtained in pigs after immunization with two experimental DNA vaccines encoding one B and two T cell FMDV epitopes, either expressed alone (pCMV-BTT) or fused to a strong signal peptide (pCMV-spBTT). While all pigs vaccinated with pCMV-spBTT showed both a delay in the disease onset and reduced severity of signs and lesions after FMDV challenge, pigs immunized with pCMV-BTT showed an exacerbation of the disease and most of the pigs remained viremic at 10 days post-infection, the end-point of the experiment, thus opening concerns about FMDV-suboptimal immunization. Interestingly, only one of the four pigs vaccinated with pCMV-spBTT showed neutralizing antibodies before challenge, demonstrating that partial protection against FMDV could be afforded in the absence of preexisting neutralizing antibodies.

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1. Introduction

Foot-and-mouth disease (FMD) is one of the most contagious and economically devastating diseases for animal health that affects several species of cloven-hoofed wild and farm animals. The high genetic variability of Foot-and-mouth disease virus (FMDV) together with its highly contagious nature complicates the successful control of FMD (Domingo et al., 2003). The currently available conventional vaccines, based on chemically inactivated virus, pose several disadvantages including safety issues and the narrow antigenic spectrum they cover (Doel, 2003; Mumford, 2007). Therefore there is a growing demand for the development of novel and safe marker vaccines against FMDV.

DNA immunization is one of the most promising choices accomplishing the above mentioned requisites, thus becoming an attractive vaccination approach for swine and other veterinary species (van Drunen Littel-van den Hurk et al., 2004). Regarding FMDV, several attempts have been made to develop effective DNA vaccines in small animal models and in the natural FMDV hosts (Parida, 2009). In a previous study we showed that DNA vaccines encoding FMDV B and T cell epitopes, either alone (pCMV-BTT) or fused to a signal peptide (pCMV-spBTT), partially protected mice from FMDV challenge (Borrego et al., 2006). Here, we have extended these studies to the pig, an important domestic host for FMDV.

Twelve 8-week-old pigs (Landrace × Large White) were divided into three groups and housed in different isolation boxes. Box 1 contained the control animals for the assay, including 2 pigs (P1 and P2) inoculated with the empty plasmid pCMV and 2 animals (P3 and P4) immunized with 2 intramuscular doses of the conventional vaccine based on the inactivated serotype C FMDV adjuvanted with mineral oil (generously provided by Dr. Paul Barnett, IAH-Pirbright). Animals in boxes 2 and 3 were immunized 3 times with 400 μg of pCMV-BTT (P5–P8) and pCMV-spBTT (P9–P12), respectively (Borrego et al., 2006). One third of each endotoxin-free DNA plasmid dose (Quiagen) was intramuscularly injected in the femoral quadriceps, one third in the tabloid neck and the last third was subcutaneously injected in the ear. In all cases, vaccine doses where administerred three weeks appart and twenty-one days after the last inoculation, animals were challenged by injection of 10^4 pfu of FMDV C-S8c1 into the heel bulbs. Clinical signs of disease (for details see Table 1 legend) were recorded daily after FMDV challenge (Table 1). Appearance and evolution of vesicular lesions in control animals inoculated with the pCMV plasmid (pigs 1 and 2), followed the expected kinetics for this challenge dose (Cubillos

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Table 1
pCMV-spBTT ameliorates while pCMV-BTT exacerbates, FMD clinical signs.

Group (pig number)	Score day 2	Score day 3	Score day 4	Score day 5	Score day 6	Score day 7	Score day 10
(A)							
pCMV (1)	2	4	4	4	4	4	0
pCMV (2)	2	4	4	4	4	4	0
Conventional vaccine (3)	0	0	0	0	0	0	0
Conventional vaccine (4)	0	0	0	0	0	0	0
pCMV-BTT (5)	4	5	6	6	6	6	6[a]
pCMV-BTT (6)	4	5	6	6	6	6	6[a]
pCMV-BTT (7)	4	5	6	7+	–	–	–
pCMV-BTT (8)	4	5	6	6	6	6	6[a]
pCMV-spBTT (9)	1	2	3	3	3	4	0
pCMV-spBTT(10)	1	2	3	3	3	4	0
pCMV-spBTT(11)	0	2	2	3	3	3	0
pCMV-spBTT(12)	0	1	2	3	3	3	0

Group/average	Day 2	Day 3	Day 4	Day5	Day 6	Day 7	Day 10
(B)							
pCMV	2	4	4	4	4	4	0
Conventional vaccine	0	0	0	0	0	0	0
pCMV-BTT	4	0	0	6.25	6	6	6
pCMV-spBTT	0.5	1.75	2.5	3	3	3.5	0

(A) FMD clinical signs were recorded daily and scored individually as follows: (0) no aphthae (vesicles); (1) primary aphthae (at the point of inoculation); (2) secondary aphthae (at least in 2 extremities); (3) aphthae in 3 or 4 extremities; (4) aphthae in 4 extremities, tongue, palate and snout; (5) development of aphthae (>3 cm) in snout, inferior mandible and the four extremities, including the scapular zone of the front legs and the femoral quadriceps of the posterior extremities; (6) large erosions in skin, palate and tongue (due to aphthae breakage), lost of cloven hoofs and presence of bacterial secondary infections; (7) death (+). (B) Average score of FMD clinical signs for each one of the immunization groups.

[a] Clinical signs in pCMV-BTT immunized pigs (pigs 5, 6 and 8), remained until the end of the experiment (day 10 pc), while the rest of the animals were almost fully recovered.

et al., 2008), while conventionally vaccinated pigs (pigs 3 and 4) remained free of disease signs throughout the experiment (Table 1). Remarkably, all pigs inoculated with pCMV-spBTT showed a clear delay of at least 24 h in the development of first signs of disease and both the number and the size of vesicles found between days 4 and 6 post-FMDV challenge (pc) on pig extremities, mouth and snout, showed a clear reduction with regard to control pigs (Table 1). By day 7 pc, pigs within this immunization group exhibited only mild disease signs and were totally recovered by day 10. Conversely, every single animal inoculated with pCMV-BTT developed more severe clinical signs than control pigs in the days after challenge (Table 1), in contrast with results previously obtained in mice, that were protected with this construct (Borrego et al., 2006). These results raised new concerns about the use of mice as an ideal model to test protective vaccines against FMDV, at least when neutralizing antibodies are not involved in the protection afforded. Thus, pigs 5–8 showed a higher number of larger vesicles on the four legs and in the mouth compared to control animals. Also, in contrast with control animals, clinical signs in pCMV-BTT immunized pigs remained until the end of the experiment (day 10 pc) and pig 7 died at day 5 pc due to the severity of the infection. Correlating with the severity of the lesions observed, FMDV-RNA was exclusively detected at the time of sacrifice, in samples obtained from pigs immunized with pCMV-BTT. Thus, FMDV-RNA was detectable by a conventional reverse transcriptase-polymerase chain reaction (RT-PCR) targeted to the 3Dpol FMDV gene (Saiz et al., 2003), in sera and larynx at day 10 pc in 2 of the 3 surviving pigs inoculated with pCMV-BTT (pigs 6 and 8) and in the mesenteric lymph nodes from all pigs immunized with this same plasmid, while viral RNA was undetectable in the rest of the animals at this time-point (Table 2). With the exception of conventionally vaccinated pigs that remained free of virus throughout the experimental challenge, no further differences were found between the rest of the groups regarding the detection of FMDV RNA in sera and nasal swabs (Table 2) or the viral titers in sera, measured by a cytopathic plaque assay using monolayers of IBRS-2 cells (data not shown). Recent publication (Murphy et al., 2010) indicates the development of FMD clinical disease is influenced not only by viral replication but also

Table 2
Virus detection after FMDV challenge in immunized pigs.

Group	Pigs	Day 3 samples			Day 10 samples					
		Serum	Nasal swabs	Pharyngeal swabs	Serum	Nasal swabs	Pharyngeal swabs	Spleen	Larynx	Mesenteric ganglia
pCMV	P1	+	+	+	–	–	–	–	–	–
	P2	+	+	+	–	–	–	–	–	–
Conventional vaccine	P3	–	–	–	–	–	–	–	–	–
	P4	–	–	–	–	–	–	–	–	–
pCMV-BTT	P5	+	+	+	–	–	–	–	–	+
	P6	+	+	+	+	–	–	+	+	+
	P8	+	+	+	+	–	–	+	+	+
pCMV-spBIT	P9	+	+	+	–	–	–	–	–	–
	P10	+	+	+	–	–	–	–	–	–
	P11	+	+	+	–	–	–	–	–	–
	P12	+	+	+	–	–	–	–	–	–

RT-PCR FMDV RNA detection from serum, nasal and pharyngeal swabs either at day 3 or 10 post infection. Virus presence in spleen, tonsil and mesenteric ganglia of immunized pigs was also evaluated by RT-PCR after necropsy at day 10 post-FMDV challenge.

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