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Vaccine 23 (2005) 3318–3328
Vaccine
www.elsevier.com/locate/vaccine

Oronasal vaccination with classical swine fever virus (CSFV) replicon particles with either partial or complete deletion of the E2 gene induces partial protection against lethal challenge with highly virulent CSFV

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Received 9 July 2004; received in revised form 7 December 2004; accepted 5 January 2005
Available online 26 January 2005

Abstract

A cDNA clone of the classical swine fever virus (CSFV) strain Alfort/187 [Ruggli N, Tratschin JD, Mittelholzer C, Hofmann MA. Nucleotide sequence of classical swine fever virus strain Alfort/187 and transcription of infectious RNA from stably cloned full-length cDNA. J Virol 1996;70(6):3478–87] was used to construct two E2 deletion mutants lacking either the complete E2 gene or, alternatively, a stretch of 204 nucleotides encoding 68 amino acids located in the C-terminal region of the E2 glycoprotein. The respective in vitro synthesized mutant RNAs replicated in SK-6 cells but no infectious virus was generated. Both replicons could be packaged into virus particles in SK-6 cells constitutively expressing E2 of CSFV. For the resulting CSF virus replicon particles (CSF-VRP) A187-E2del373 and A187-E2del68 titers of 10^6 and 10^7 $TCID_{50}$/ml, respectively, were obtained. Oronasal vaccination with 10^7 $TCID_{50}$ of either of the two CSF-VRP protected pigs against a challenge with a lethal dose of CSFV strain Eystrup. In contrast, after intradermal vaccination VRP A187-E2del68 but not VRP A187-E2del373 lacking the complete E2 gene induced a protective immune response. We conclude that E2-complemented CSF-VRP have the potential to be used as live-attenuated non-transmissible oral vaccines for pigs. In addition, our data suggest that E2 of CSFV is dispensable for the induction of mucosal but not of parenteral immunity.

Keywords: Pestivirus; Classical swine fever virus; Virus replicon particles; Oral vaccination

1. Introduction

Classical swine fever (CSF) is a highly contagious and often fatal viral disease of domestic pigs and wild boar. The causative agent, the CSF virus (CSFV) belongs to the genus *Pestivirus* within the family *Flaviviridae*. The other three pestivirus species are the ruminant viruses bovine viral diarrhea virus type I (BVDV-1) and type II (BVDV-2), and border disease virus (BDV) [2]. Pestiviruses are small enveloped viruses with a single-stranded 12.3 kb RNA genome of positive polarity containing one large open reading frame (ORF) coding for a polyprotein of approximately 4000 amino acids. The polyprotein composed of four structural and eight nonstructural proteins in the order NH_2-N^{pro}-C-E^{rns}-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH is processed co- and posttranslationally by viral and host cell proteases. The structural components of the virion include the capsid protein C and the three envelope glycoproteins E^{rns}, E1, and E2 (for a review see [3]). Animals infected with CSFV develop antibodies against the structural proteins E^{rns}, E2, and the nonstructural protein NS3. Subunit vaccines based on either E2 or E^{rns} have been shown to

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doi:10.1016/j.vaccine.2005.01.076

induce protective immunity in pigs [4,5]. These two envelope proteins also represent targets for neutralizing antibodies [6,7]. Monoclonal antibodies (mAbs) directed against either E2 or E^{rns} are used to differentiate CSFV from other pestiviruses [8].

In countries of the European Union (EU) and in Switzerland the domestic pig population is generally free of CSFV but the virus is prevalent among swine in other parts of the world and in some wild boar populations in Europe [9]. This situation, together with the strict non-vaccination policy pursued in the EU, poses a constant threat to the domestic pig population. Indeed, in Europe the vast majority of the first outbreaks of CSF occur in regions where the virus is endemic in wild boar [9]. Thus, sporadic local outbreaks, i.e. in 1993 in Switzerland in domestic pigs [10] and in wild boars in 1998 [11] but also epidemics like in the Netherlands in 1997/1998 [12] have repeatedly occurred.

The non-vaccination policy was implemented for economical reasons, namely to allow free trade of pigs within Europe. This was required as the available live attenuated vaccine strains of CSFV, although efficient and safe [13], do not allow serological differentiation between immunized pigs and pigs infected with a CSFV field strain. However, the huge economical losses caused by the stamping out policy as well as ethical objections to the mass killing of healthy animals claim for alternatives to the current policy. Thus, the development of marker vaccines allowing differentiation between vaccinated and infected animals is of high priority. Such "Differentiating Infected from Vaccinated" (DIVA) vaccines [14] could be used in the case of an epidemic to support the control of CSF but are also considered for CSF eradication in wild boar.

Several laboratories have recently been involved in the development of CSF marker vaccines [15]. E2 subunit vaccines produced using the baculovirus protein expression system were shown to induce serum antibodies with high virus neutralizing titers and to provide good protection against CSF [4,16,17]. Live attenuated marker vaccines, which are considered to be more effective and also less expensive than vaccines based on the production of recombinant antigen, have been described. One approach is the generation of chimeric CSFV in which either the E2 or the E^{rns} gene is replaced by the corresponding gene of the bovine viral diarrhea virus. These viruses were shown to be protective and to induce a distinguishable antibody response in pigs [18,19]. Also recombinant live attenuated pseudorabies virus [20], vaccinia virus [21], and porcine adenovirus [22] expressing CSFV envelope proteins have been described as potential CSF vaccines. We have recently shown that CSFV lacking the N^{pro} gene was attenuated and protected pigs against challenge with highly virulent CSFV [23,24]. This virus does not represent a marker vaccine as no antibodies against N^{pro} could be detected in pigs infected with wildtype CSFV (Mayer, personal communication) but might be the basis for the production of a potent live attenuated marker vaccine.

The most recent CSF vaccines described are the so-called virus replicon particles (VRP). These particles contain a mutant genomic RNA which is able to replicate and to express the encoded viral proteins but which does not contain the complete information required for particle formation due to deletions in at least one of the genes encoding the viral structural proteins. These virus particles are non-transmissible and, therefore, fulfill one of the requirements for a safe vaccine. Corresponding CSF-VRP with deletions in either the E^{rns} or the E2 gene have been generated in complementing cells constitutively expressing the respective structural protein [25,26]. In the VRP vaccines described by these authors the antigenic domains of the respective structural protein E^{rns} or E2 were deleted to allow differentiation between immunized and infected animals. Both types of marker vaccines were shown to be immunogenic. Protection of pigs against CSF was obtained with the E2-deleted VRP after simultaneous intradermal, intramuscular, and intranasal injection [25]. For the E^{rns}-deleted VRP, protection was obtained after parenteral immunization but not when the intranasal route was used [26].

We describe here the construction of two experimental E2-complemented CSF-VRP vaccines, which lack either the complete or a small part of the E2 gene. The truncated E2 protein expressed from the latter construct presumably contains the complete antigenic repertoire of E2 required for inducing an effective immune response against CSFV. Oronasal immunization with either of the two CSF-VRP protected pigs from severe CSF after challenge with highly virulent CSFV. Protection after intradermal injection was observed only for the CSF-VRP carrying the small E2 deletion.

2. Materials and methods

2.1. Cells and viruses

SK-6 swine kidney cells provided by M. Pensaert (Faculty of Veterinary Medicine, Ghent, Belgium) were grown in Earle's minimal essential medium (EMEM) supplemented with 7% horse serum (EMEM-HS). CSFV vA187-1 was derived from plasmid pA187-1 [1] containing the cDNA consensus sequence of CSFV strain Alfort/187. CSFV strain Eystrup was obtained from H.-J. Thiel (Justus-Liebig-Universität, Giessen, Germany).

2.2. Establishment of SK-6 cells expressing CSFV envelope protein E2

The sequences coding for either E2 or E2-p7 of CSFV including the 48 3′-terminal nucleotides of the E1 gene encoding the E2 signal sequence were amplified from pA187-1 by PCR using *Pfu* Turbo polymerase (Stratagene). The PCR primers were designed to allow subsequent insertion of the respective DNA fragments into expression plasmid pEGFP-N1 (Clontech) from which the sequence encoding