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Recombinant adenovirus expressing type Asia1 foot-and-mouth disease virus capsid proteins induces protective immunity against homologous virus challenge in mice

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ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious disease worldwide affecting cloven-hoofed animals that is caused by foot-and-mouth disease virus (FMDV). The FMDV capsid polyprotein and 3C proteinase are required for capsid precursor processing and assembly. The FMDV capsid protein, which contains the entire repertoire of immunogenic sites, can stimulate both humoral immunity and T-cell-mediated immune responses. In this study, we constructed a recombinant adenovirus, rAdV-Asi-05, that expresses the P1–2A and 3C genes of the type Asia1 FMDV strain Asia1/YS/CHA/05. The humoral immune responses elicited by the Ad5-vectored capsid protein of type Asia1 FMDV in BALB/c mice and the ability of rAdV-Asi-05 to rapidly induce protection against challenge with FMDV Asia1/YS/CHA/05 in C57BL/6 mice were evaluated. The processing of polyprotein P1 into the structural proteins VP0, VP3, and VP1 in rAdV-Asi-05-infected HEK 293 cells was detected by Western blotting. BALB/c mice immunised with rAdV-Asi-05 produced type Asia1 FMDV-specific neutralising antibodies, and the neutralisation titres increased significantly after the boost. Importantly, C57BL/6 mice immunised with a single 10^7 PFU dose of rAdV-Asi-05 exhibited protective immunity against challenge with 100 times the lethal dose of FMDV Asia1/YS/CHA/05. In summary, rAdV-Asi-05 elicited a high titre of neutralising antibodies against type Asia1 FMDV in BALB/c mice. Moreover, rAdV-Asi-05 provided complete protection against FMDV Asia1/YS/CHA/05 challenge in C57BL/6 mice. This study highlights the potential of rAdV-Asi-05 to serve as a type Asia1 FMDV vaccine.

1. Introduction

Foot-and-mouth disease virus (FMDV) is a positive-sense single-stranded RNA virus belonging to the genus *Aphthovirus* within the *Picornaviridae* family (Grubman and Baxt, 2004). It is highly contagious and spreads extremely rapidly. The P1, 2A and 3C regions of the viral genome are required for the processing and assembly of the viral polyproteins into the capsid, which is necessary for the induction of neutralising and protective immunity (Grubman and Mason, 2002). Thus, this immunogen contains the entire repertoire of immunogenic sites present on the intact FMDV particle.

Chemically inactivated FMD vaccines emulsified with adjuvant have been effectively used to control the disease in enzootic countries, and regular vaccination has contributed to the eradication of FMD in some areas of the world (Brownlie, 2001; Doel, 2003). However, the production of such vaccines requires containment facilities to grow and inactivate live virus, and there is a potential risk of escape of the live virus from those facilities or from improper vaccine preparation (Barteling and Vreeswijk, 1991; King et al., 1981). In addition, current inactivated vaccines contain nonstructural viral proteins, making it difficult to differentiate infected from vaccinated animals. Thus, there is an urgent need to develop vaccines that overcome these disadvantages related to the conventional inactivated vaccine. Ideal FMD vaccines target immunogens that contain the entire repertoire of immunogenic sites present on intact virus but lack infectious nucleic acid. Various approaches have been investigated to develop alternative FMD vaccines, including the construction of modified live viruses (Almeida et al., 1998), biosynthetic proteins (Kleid et al., 1981), synthetic peptides (Bittle et al., 1982; DiMarchi et al., 1986; Taboga et al., 1997), naked DNA vectors (Chinsangaram et al., 1998), and recombinant viruses (Sanz-Parra et al., 1999). Currently, the most efficient delivery system is a replication-defective human adenovirus serotype 5 (Ad5) vector (Grubman et al., 2010; Mayr et al.,

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1999; Moraes et al., 2011). Ad5 was chosen as an ideal vector for the expression of the FMDV capsid protein because of the absence of pre-existing anti-Ad5 immunity in swine (Wesley et al., 2004).

The viral capsid mimics the structure of authentic virus particles, which are readily recognised by the immune system and present viral antigens in a more authentic conformation than other subunit vaccines (Rweyemamu et al., 1979). Moreover, empty capsids have been shown to be highly effective at stimulating CD4 proliferative responses and cytotoxic T lymphocyte (CTL) responses (Beyer et al., 2001; Murata et al., 2003; Paliard et al., 2000). Human papillomavirus VLP-based bivalent HPV16/18 or quadrivalent HPV6/11/16/18 vaccines have been successfully introduced into the market (Garland et al., 2007; Harper et al., 2006; Schiller et al., 2008). For FMDV, the capsid protein represents a novel class of subunit vaccines that are able to stimulate efficient cellular and humoral immune responses, and these vaccines have shown dramatic effectiveness as candidate vaccines for FMD (Mayr et al., 1999, 2001; Moraes et al., 2002; Pacheco et al., 2005; Pena et al., 2008). Animals immunised with this marker vaccine can readily be differentiated from infected animals. One vaccination with this subunit vaccine delivered by a replication-defective human adenovirus vector can induce rapid (within 7 days) and relatively long-lasting protection in swine (Pacheco et al., 2005). Similarly, cattle immunised with this recombinant vectored capsid protein developed high levels of FMDV-specific neutralising antibodies and were rapidly protected against infection when exposed to virulent virus either directly or through contact (Pacheco et al., 2005; Wu et al., 2003).

Serotype Asia1 FMDV is normally found only in Asia. However, this type of FMDV has previously spread into Europe, causing outbreaks in Turkey in 1999 and in Greece in 2000. Recently, outbreaks of type Asia1 FMD occurred in 2005, with cases detected in Pakistan, Iran, Tajikistan, China and Russia (Valarcher et al., 2005a). The epidemics of this type Asia1 FMDV demonstrate the need to develop a safe and effective FMDV vaccine. Our approach uses Ad5 as a vector to express the P1–2A capsid region and the complete 3C protease of type Asia1 FMDV. We assessed the humoral immune responses elicited by the Ad5-vectored capsid protein of type Asia1 FMDV in BALB/c mice and the ability of the candidate vaccine to rapidly achieve protection against FMDV Asia1/YS/CHA/05 challenge in C57BL/6 mice. The results showed that the immunisation of BALB/c mice with the recombinant adenovirus induced a high titre of neutralising antibodies against type Asia1 FMDV, and complete protective immunity in C57BL/6 mice developed as early as 5 days after vaccination with a single 10^7 PFU dose of rAdV-Asi-05.

2. Materials and methods

2.1. Plasmids, cell lines, virus and MAb

The plasmids pAdEasy-1 and pShuttle-CMV, Ad-BJ5183 *Escherichia coli* competent cells and HEK 293 cells were all purchased from Stratagene (Stratagene, USA). Replication-defective human adenovirus type 5 (Ad5) was kept in our laboratory. FMDV strain Asia1/YS/CHA/05 (Wang et al., 2011) (GenBank accession number, GU931682) was propagated and titred in BHK-21 cells. FMDV serotype-independent MAb 4B2 was described previously (Yu et al., 2011).

2.2. Construction of recombinant adenovirus

The complete P1 and 2A coding regions and the partial 2B coding region of FMDV Asia1/YS/CHA/05 were amplified by PCR from the plasmid pAsi, which contains an infectious cDNA clone (Wang et al., 2011), using the primers P12A–F1 and P12A–R1 (Table 1). A portion of the 3B and 3C complete coding sequence was amplified by PCR using primers 3C–F1 and 3C–R1 (Table 1). The PCR products of P1–2A were digested with KpnI and XhoI and cloned into pBluescript II SK(+) (Stratagene, USA). The resulting vector was named pBlue-P12A. The PCR products of 3BC were digested with XhoI and SphI, and the resulting fragment was cloned into pBlue-P12A. The resulting plasmid was named pBlue-P12A3C. Thus, the recombinant plasmid pBlue-P12A3C contained the complete P1, 2A and 3C coding regions of FMDV Asia1/YS/CHA/05. pBlue-P12A3C was PCR amplified using primers P1-F1 and P2-R1 (Table 1). The primer P1–F1 contains a Kozak sequence (CCACCATGG) and an ATG codon immediately upstream of the P1 coding region, and primer P2–R1 contains a TAG termination codon immediately downstream of the 3C coding region. Subsequently, the type Asia1 FMDV P12A3C cassette was cloned into the pShuttle-CMV vector between the cytomegalovirus (CMV) promoter and the simian virus 40 (SV40) polyadenylation signal using KpnI and NotI digestion. The resulting plasmid, pShuttle-Asia1 P12A3C, was linearised with PmeI and was transformed into *E. coli* BJ5183 cells to generate the final plasmid, pAdV-Asia1 P12A3C. Finally, recombinant adenovirus was rescued by the transfection of PacI-linearised pAdV-Asia1 P12A3C into HEK 293 cells using Effectene® Transfection Reagent (Qiagen, USA).

Table 1
Primers used to construct recombinant adenovirus expressing P12A3C of FMDV Asia1/YS/CHA/05.

Primer[a]	Sequence (5' to 3')
P12A–F1	5' TAT GGA TCC GGT ACC ATG GGA GCC GGG CAA TCC AGT 3'
P12A–R1	5' GAA ACT CGA GGC GAC TTT GAC CAA C3'
3C–F1	5' CCA CTC GAG TCG TCA GAA ACC TCT GGA AGT 3'
3C–R1	5' TAT GCA TGC TCT AGA CTA CTC GTG GTG TGG TTC GGG ATC 3'
P1–F1	5' TCT GGT ACC ACC ATG GGA GCC GGG CAA TCC AGT CC 3'
P2–R1	5' TAT GCG GCC GCC TAC TCG TGG TGT GGT TCG G 3'

[a] Primer pairs P12A–F1/P12A–R1 and 3C–F1/3C–R1 were used for amplifying the P12A coding region and 3C protease gene, respectively.

2.3. Indirect immunofluorescence assay

HEK 293 cells in 96-well plates were infected with rAdV-Asi-05 or Ad5, fixed with ice-cold anhydrous ethanol for 15 min at 4 °C and air dried. Fixed cells were treated with 50 μL/well of MAb 4B2 at a 1:200 dilution in PBS and incubated for 1 h at 37 °C. After washing the plates with PBS, 50 μL/well of FITC-conjugated goat anti-mouse IgG (Sigma, USA) at a 1:200 dilution was added and incubated for 1 h at 37 °C. The plates were washed three times with PBS and examined under an Olympus microscope connected to a Leica DFC 490 digital colour camera.

2.4. SDS–PAGE and Western blotting

HEK 293 cells infected with rAdV-Asi-05 were subjected to 12% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (12% SDS–PAGE). FMDV Asia1/YS/CHA/05 and HEK 293 cells infected with Ad5 were used as positive and negative controls, respectively. The separated proteins were electrophoretically transferred to a nitrocellulose membrane. After blocking the membrane with 5% nonfat milk in PBS overnight at 4 °C, we incubated the membrane with bovine FMDV convalescent serum (diluted 1:2000 in PBS) at 37 °C for 1 h, washed it three times with PBST, and probed it with a 1:5000 dilution of HRP-conjugated goat anti-bovine IgG (Sigma, USA) at 37 °C for 1 h. The reactivity was visualised with the substrate 3,3'-diaminobenzidine (Sigma, USA).