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Vaccine 23 (2005) 3236–3242
Vaccine
www.elsevier.com/locate/vaccine

Immune response in guinea pigs vaccinated with DNA vaccine of foot-and-mouth disease virus O/China99

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Received 8 December 2003; received in revised form 11 March 2004; accepted 14 March 2004
Available online 12 November 2004

Abstract

In order to obtain the gene P12X3C of foot-and-mouth disease virus (FMDV O/China99) that includes full length P1, 2A, 3C and part of 2B and 3B, the site mutation strategy was used. The recombinant plasmid pcDNA3.1/P12X3C was transfected into BHK-21 cells. The capsid proteins of FMDV expressed in BHK-21 cells were confirmed by sandwich-ELISA and indirect immunofluorescence test. Then the plasmid pcDNA3.1/P12X3C was administered to guinea pigs intramuscularly, and purified FMDV O/China993D protein expressed in yeast cells was injected together with pcDNA3.1/P12X3C. Anti-FMDV antibodies were detected by indirect ELISA, the T-lymphocyte proliferation response was tested by MTT assay, and neutralizating antibodies titers were analyzed by micro-neutralization assay. The result showed that the plasmid pcDNA3.1/P12X3C was able to express immunocompetent proteins of FMDV in BHK-21 cells. Furthermore, anti-FMDV antibodies were elicited and increased by plasmid pcDNA3.1/P12X3C in the second week after vaccination. Neutralizating antibodies were induced and the T-lymphocyte proliferation response was enhanced after vaccination. In the challenge test, all of guinea pigs vaccinated with pcDNA3.1/P12X3C were fully protected from FMDV challenge. However, the result obtained from animals that were injected with protein 3D together with plasmid pcDNA3.1/P12X3C was not satisfied. In conclusion, the results encouraged further work towards the development of a DNA vaccine against FMDV and provided the basis of research for DNA vaccine.

Keywords: Foot-and-mouth disease virus (FMDV); DNA vaccine; Immune response

1. Introduction

Foot-and-mouth disease is a kind of highly contagious vesicular disease of cloven-hoofed animals. Its pathogen is foot-and-mouth disease virus (FMDV), which is a member of the genus *Aphthovirus* of the family *Picornavidae* and includes seven serotypes. FMDV genome is a positive-sense, single-stranded RNA of 8.5 Kb with one large open reading frame. Its translation yields a polyprotein that is subsequently processed by virus-encoded proteases to produce the structural and non-structural proteins necessary for virus assembly and replication. One of the initial polyprotein cleavages, mediated by the 2A protein, is a co-translational cleavage at the N terminus of the 2B protein. The P1–2A precursor is processed by viral protease 3C to produce the structural proteins VP0, VP1 and VP3. These proteins can then self-assemble to form icosahedral empty capsid particles [1–3], which consist of 60 copies of each protein. Encapsidation of viral RNA to produce mature virions is accompanied by the cleavage of VP0 to VP2 and VP4.

In fact, although the conventional vaccines were approved effective, its disadvantages include thermal instability, the short-term nature of protection and extra cost due to the high-security containment necessary for their preparation. Particularly, several outbreaks in Europe could be related with incomplete inactivation of the virus or with the escape of live virus from vaccine production laboratories. Thus, it is urgent

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doi:10.1016/j.vaccine.2004.03.074

to develop a new vaccine that is safe, effective and low cost in manufacture, storage and administration.

DNA vaccine was developed as one of the most promising alternatives to conventional vaccines. DNA-based immunization is a relatively recent approach in which plasmid DNA encoding a target antigen is introduced into the host and immune responses are generated following in vivo expression of the antigenic gene product. Immunization with plasmid DNA is able to elicit both cell-mediated and humoral immune responses [4–6] against antigens derived from numerous viral, bacterial and parasitic pathogens. Recently, although the use of a DNA vaccine for FMDV has been reported, the plasmid encoded the capsid precursor together with protein 3C of FMDV serotype A had yielded variable protection and antibodies [7,8]. In this study, we reported experiments that used the DNA vaccine for FMDV serotype O/China99 and extended this approach with protein 3D of FMDV, which was expressed in yeast cells. Meanwhile, we have taken advantage of the fact that natural empty particles, lacking nucleic acid and containing VP1, VP3 and VP0, are able to induce an antibody response similar to that induced by infectious FMDV particles in guinea pigs [9]. Significantly, these empty capsids are able to induce neutralizing antibodies of the same specificity as the whole virus. In principle, therefore, empty capsids appear to be a good candidate for the development of a recombinant vaccine. Take this into account, we have constructed a plasmid (pcDNA3.1/P12X3C) that contains all of the structural and non-structural protein genes necessary for the formation of FMDV empty capsids, thereinto the P1 sequence plus the 2A and 3C proteases necessary for processing of the P1 polyprotein into VP1, VP3 and VP0, and 2B, which is a potent source of T-cell stimulator [10].

2. Materials and methods

2.1. Cell and virus

BHK-21 cells were cultured in DMEM supplement with 10% foetal calf serum. FMDV O/China99 was preserved by our labs.

2.2. Animals

Twenty healthy guinea pigs weighing 400–500 g were used for the study. All of the animals were fed in isolation hutch.

2.3. Construction of recombinant plasmid

The P12X3C cassette that contains the complete P1–2A, 3C coding sequences and a part of 2B, 3B coding sequences of FMDV serotype O/China99, was obtained by site mutation technology [11]. One part of P1–2A coding sequences with a mutant site XbaI at 3′ terminal was cloned from pGEM/P12X3C (constructed by our labs). The other part of P1–2A2B3C coding sequences with a mutation site of XbaI at 5′ terminal was cloned from pGEM/P12X3C. Then the full-length P12X3C cassette was obtained from the ligation of P1–2A cassette and P1–2A2B3C cassette by PCR and KpnI and XbaI restriction sites was introduced at each end. The P12X3C sequence and plasmid pcDNA3.1(+) (Invitrogen) were digested by KpnI and XbaI endonucleases, respectively. Then the digested products were ligated, and the resultant plasmid was called pcDNA3.1/P12X3C.

2.4. Expression of transfected recombinant plasmid

Expression of the plasmid pcDNA3.1/P12X3C driven by the CMV promoter was verified by transfection of BHK-21 cells (in 35 mm wells) using Lipofectamine Plus™ Reagent (Invitrogen).

Two days after transfection, cells were analyzed for expression of FMDV proteins by the indirect immunofluorescence test (IFAT) and the sandwich-ELISA [12,13]. For IFAT, monolayers of cells were cultured on cover slips and were fixed in 100% cold acetone (−20 °C for 30 min). Samples were incubated with rabbit serum against FMDV (37 °C for 30 min) in humid box and then stained with fluorescein-conjugated goat anti-rabbit serum for 30 min at 37 °C. For sandwich-ELISA, the cells were washed 48 h after transfected and scraped off from bottom of wells and then lysed with buffer (100 mM Tris–HCl, pH 8.3–8.6, 2% Triton X-100, 150 nm NaCl, 0.6 M KCl, 5 mM EDTA, 1% aprotinin, 3 mM PMSF, 1 μg/ml leupeptin, 5 μg/ml trypsin inhibitor) [14]. The lysate was diluted with two-fold serial dilution and added to 96-well flat-bottomed plates (Nunc), which were coated with rabbit serum against FMDV overnight at 4 °C previously. Subsequently, the plates were washed thoroughly with PBST and guinea pig sera against FMDV were added to each well. The plates were incubated for 60 min at 37 °C and rabbit anti-guinea pig IgG peroxidase conjugate (Sigma) at 1:2000 dilution was then added for 1 h at 37 °C, followed by the substrate (0.01% hydrogen peroxide in phosphate/citrate buffer). Absorbance was determined at 492 nm.

2.5. Vaccination

Recombinant plasmids were purified from *E. coli* JM109 using the S.N.A.P. Miniprep Kit (Invitrogen). Plasmid DNA was diluted to the required concentration (1 μg/μl) in endotoxin-free Dulbecco's PBS (DPBS, Sigma). Purified 3D protein expressed in yeast cells was also diluted in DPBS buffer.

The guinea pigs were divided into five groups at random, each containing four guinea pigs: Group A, conventional FMD vaccine; Group B, plasmid pcDNA3.1/P12X3C; Group C, plasmid pcDNA3.1/P12X3C and 3D protein; Group D, pcDNA3.1(+); Group E, DPBS. Guinea pigs were injected with 0.2 ml conventional FMD vaccine or 200 μg DNA or/and 0.5 mg 3D protein at primary administration,