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Short communication

Candidate multi-peptide-vaccine against classical swine fever virus induced potent immunity with serological marker

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Abstract

Our previous study proposed a protective multi-peptide-vaccine (MPV) with Freund's adjuvant against classical swine fever virus (CSFV). In this study, another candidate MPV, using aluminum adjuvant, was further examined. All immunized pigs kept healthy during the experimental period, while the control group rapidly showed clinical symptoms and died. Moreover, anti-sera from MPV-immunized pigs could interact with peptides involved in the MPV, in contrast to anti-sera from non-immunized or infected ones. This property permits MPV-immunized pigs to be easily differentiated from infected ones with simple serological method. Therefore, this new MPV is suitable to act as a candidate marker vaccine against CSFV.

Keywords: Classical swine fever virus (CSFV); Multi-peptide-vaccine (MPV); Marker vaccine; Aluminum adjuvant

1. Introduction

Spread of classical swine fever (CSF) has caused huge economic loss to animal husbandry industry, exemplified by the 1997–1998 CSF outbreaks in Netherlands [1,2]. Classical swine fever virus (CSFV), member of the genus *Pestivirus* of the *Flaviviridae* family [3], has been identified as the pathogen of CSF. Two different strategies, systematic vaccination campaigns (e.g. with traditional live attenuated virus) in Asia and a non-immunization "stamping out" policy adopted by some European countries, both played crucial roles in controlling the epidemic, but the result was less satisfying than might be expected. Moreover, though the emergency vaccination policy, as well as measures for early diagnosis, basically has not been prohibited by EU Council Directive 80/217/EEC [4], in many EU countries, it has not been fully applied in the last 15 years even during the spread of epidemic. Once the emergency vaccination is carried out, such regions would be banned from international trade for 1–2 years, for immunized pigs could not be easily differentiated from infected ones, and the following economic loss could be more severe than CSF epidemic itself [5]. Due to such considerations, a safe "marker vaccine" was proposed as a way out of this dilemma [6]. It offers an advantage over other vaccines that cannot differentiate immunized pigs from infected ones.

In our previous study, five overlapping peptides, P1–P5 (Fig. 1), from antigenic domain B/C on envelope protein E2 of CSFV virulent strain Shimen, were conjugated to bovine serum albumin (BSA), respectively. These conjugants together as a candidate multi-peptide-vaccine (MPV), were demonstrated to have high immunogenicity and successfully induced potent protective immunity [7]. However, the DIVA (differentiating infected from vaccinated animals) ability of this MPV is yet to be determined, and it is of importance to reduce the high cost and potential toxicity of Freund's adjuvant before this candidate MPV can be widely applied.

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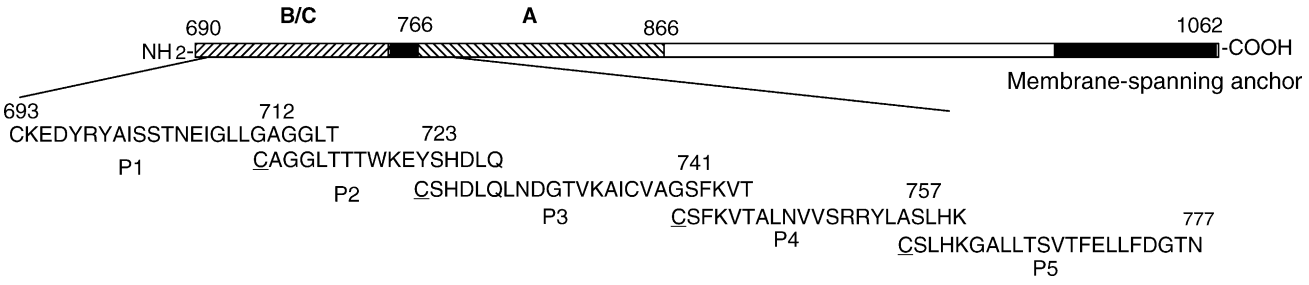

Fig. 1. Diagram of five overlapping peptides (P1–P5) from the antigenic domain B/C on E2 of CSFV virulent strain Shimen.

2. Materials and methods

2.1. Construction of immunogen

Five overlapped peptides (Fig. 1) from unit B/C (aa693–777) on glycoprotein E2 of CSFV strain Shimen (Sequence number in GenBank: AF092448) were commercially synthesised in Genemed Synthesis Inc. (USA). The Cys (underlined) on the N-terminal of P2, P3, P4 and P5 is added for conjugation by MBS-method. Each peptide was chemically linked to carrier protein BSA (bovine serum albumin; obtained from Sigma, USA) by GA-method. The commercial vaccine against CSFV (C-strain) was gained from Nanjing Medical Machinery (PR China).

2.2. Immunization of pigs

Thirty healthy SPF (special pathogen free) pigs aged 8–10 weeks were randomly allotted into three groups (10 pigs per group). Each group was housed individually in a pen and fed ab libitum. During a 2-week acclimatization period, environmental temperature and body temperature were measured once a day. Pigs were immunized intra-muscularly on the tip of buttocks. Group 1 was immunized with candidate MPV at day 0 and day 14. The dose for each pig in this group is 50 μg per peptide (in conjugant) every injection. Group 2 was immunized with the recommended dose of the commercial Chinese vaccine strain (C-strain) one time at day 0 according to the specification. Group 3 (negative control group) was non-immunized.

2.3. Detection of peptide-specific antibodies in ELISA and FACS

The peptide-specific antibodies in pig sera were first tested in the enzyme-linked immunosorbent assay (ELISA). The peptides (5 μg/ml) were coated overnight on a microtiter plate at 4 °C. Nonspecific binding was blocked for more than 2 h by incubation with 0.25% gelatin in PBS. After washing one time with PBS-Tween 20 (0.05% Tween 20), pig anti-sera or normal serum with different dilution were added and incubated for 1 h at room temperature. After washing, peroxidase-conjugated rabbit anti-swine antibody (P0164, DAKO) was added. After further washing, freshly prepared 2,2′-azino-di-(3-ethylbenzthiazoline sulfonate)-peroxide solution was added The optical density was measured and the cutoff value was 0.2.

The pig endothelial cell line PK15 was cultured in complete medium of DMEM with 10% fetal bovine serum, and infected with live vaccine strain Thiversval. After the establishment of an effective infection, the infected PK15 cells (2×10^6) were incubated on ice with PBS, normal serum (NS) or different anti-sera (AS) for 45 min separately. NS and AS were diluted at 1:50 with PBS. Each serum was a mixture of corresponding group. Subsequently they were carefully washed with PBS and stained with a FITC-labeled secondary antibody (DAKO, F0235) for half an hour. Flow cytometry (FCM) analysis was carried out with BD FACSCalibur.

2.4. Virus and experimental inoculation

Pigs immunized with the candidate peptide-vaccine or the Chinese vaccine strain (C-strain) and non-immunized pigs (control group) were inoculated with lethal dose (100 LD50) of CSFV strain Shimen (gained from National Institute of Veterinary Drug Control, PR China; Sequence number in Genbank: AF092448) intranasally as natural infection at day 7 after the second immunization. The ambient temperature, body temperature (rectal temperature), clinical signs and food intake were observed every day during the experiment. Fever was defined as a rectal temperature exceeding 40 °C.

3. Results and discussion

Being the only adjuvant licensed by FDA so far, aluminum salts has established a safety record with rare adverse events reported during the past 70 years [8], and aluminum compounds have been widely used in FDA-licensed vaccines [9]. So we chose aluminum adjuvant as a safe and inexpensive alternative to Freund's adjuvant.

After separately conjugated to BSA, the five overlapping peptides (Fig. 1) were mixed and absorbed into aluminium hydroxide adjuvant, together as a MPV, and then were injected to pigs ($n = 10$) intra-muscularly on the tip of buttocks. After two times immunization, anti-sera were collected and further studied with fluorescent activated cell sorter (FACS). An obvious increase in fluorescent intensity of Thiveral-