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Candidate peptide-vaccine induced potent protection against CSFV and identified a principal sequential neutralizing determinant on E2

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Abstract

Previously, two candidate multi-peptide-vaccines (MPVs) consisted of five overlapping synthetic peptides covering the antigenic domain B/C (aa693–777) on envelope protein E2 were prepared in our lab. And they successfully induced peptide-specific neutralizing antibodies and provided pigs with complete protection from the lethal challenge of virulent classical swine fever virus (CSFV) strain Shimen. In this study, these five peptides were conjugated to bovine serum albumin (BSA), with which five groups of pigs ($n = 10$) were inoculated, respectively. Among these candidate peptide-vaccines (PVs), PV-BC1 (BC1: aa693–716) exhibited the most potent protective activity, PV-BC3, PV-BC4 and PV-BC5 (BC3: aa723–745; BC4: aa741–760; BC5: aa757–777) had weaker effects, while no effect of PV-BC2 (BC2: aa712–727) had been detected. Moreover, the polyclonal antibodies induced by PV-BC1 and PV-BC4 were capable of neutralizing C-strain virus in vitro. Thus, a principal sequential neutralizing determinant (aa693–716) and a minor sequential neutralizing determinant (aa741–760) were proved to lie in the antigenic domain B/C, which can be recruited into developing more effective "marker vaccine" by epitope-vaccine strategy. Our study also indicates that scanning with a panel of sequential peptide-immunogens is an effective method to locate sequential neutralizing epitopes.

Keywords: Classical swine fever virus (CSFV); Marker vaccine; Peptide vaccine; Sequential neutralizing epitope; Immunodominant

1. Introduction

Classical swine fever virus (CSFV), the pathogen of classical swine fever (CSF), is a small (40–60 nm in diameter) enveloped positive-stranded RNA virus belonging to the genus *Pestivirus* of the *Flaviviridae* family [1]. CSFV contains a genome about 12.3 kb, coding for four structural proteins (nucleocapsid protein C and envelope glycoproteins E0, E1 and E2) and seven nonstructural proteins [2]. Three envelope-associated glycoproteins are derived from glycoprotein precursor E012 via post-translational processing [3,4]. Envelope glycoprotein E2 (formerly termed E1 or gp51–54) and E0 (also termed E^{rns}) reside on the outer surface of the virion [5] and are believed to be involved in the attachment of CSFV to susceptible cells and the viral entry [6]. Although immunity against other envelope glycoproteins contributes to neutralization of the virus [7], immunogenic response against E2 alone was proved sufficient for complete protection. The pigs were protected from the lethal challenge of virulent CSFV strains after vaccination with immuno-affinity-purified E2 expressed in insect cells [8] or recombinant E2-expressing viruses, such as recombinant pseusdorabies virus [9] and recombinant vaccinia virus [10,11]. In 1993, Kimman et al. demonstrated that affinity-purified or PRV-expressed E2 was not a major

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T-cell antigen, while it was capable of inducing neutralizing antibodies and ensuring complete protection from CSFV [12]. All these findings indicated that E2-induced neutralizing antibodies, rather than the cellular immune response, play a key role in the CSFV-directed protective immunity.

Four distinct antigenic domains (A, B, C and D) were successfully mapped to the N-terminal of E2 in the 1980s [13], within which domain B and C were found adjacent while domain A stayed some distance from them [14]. In 1993, van Rijn et al. found that the binding of the MAbs to domain B and C was not affected when domain A was mutated at some amino acid positions or completely deleted and vice versa [15,16]. In 1996, they further confirmed that modified E2 containing either of the two structural antigenic units, domain B/C or domain A, was able to separately protect pigs from lethal challenge of CSFV [17]. All these discoveries confirmed the structural and functional independence of antigenic domain B/C and A [15].

There has been an on-going argument over whether conformation-independent epitopes exist in E2, and the neutralizing epitopes are not yet to be carefully investigated, which greatly limited the development of effective peptide-vaccine (PV). In fact, many earlier findings did not indicate the existence of sequential epitope in E2 N-terminal [15,16,18]. In our previous study, five overlapping peptides (BC1–BC5) covering the N-terminal part (aa693–777) of E2 were synthesized and coupled to carrier protein together. Interestingly, this conjugant successfully induced high titer of peptide-specific neutralizing antibodies against CSFV virulent strain Shimen and resulted in complete survival of immunized pigs [19,20]. So some sequential neutralizing epitopes probably lie on antigenic domain B/C. Here, these five peptides were separately conjugated to BSA, then injected to pigs to map the linear neutralizing epitopes in more detail.

2. Materials and methods

2.1. Peptides

Five overlapping peptides covering amino acids 693–777 (unit B/C) on glycoprotein E2 of CSFV strain Shimen (Sequence number in GenBank: AF092448) were commercially synthesised by Genemed Synthesis Inc. (USA):

BC1 (aa693–716, CKEDYRYAISSTNEIGLLGAGGLT),
BC2 (aa712–727, CAGGLTTTWKEYSHDLQ),
BC3 (aa723–745, CSHDLQLNDGTVKAICVAGSFKVT),
BC4 (aa741–760, CSFKVTALNVVSRRYLASLHK),
BC5 (aa757–777, CSLHKGALLTSVTFELLFDGTN),

The Cys (underlined) on the N-terminal of BC2, BC3, BC4 and BC5 was added for conjugation. Each peptide was chemically linked to carrier protein bovine serum albumin (BSA; obtained from Sigma, USA) by MBS-method.

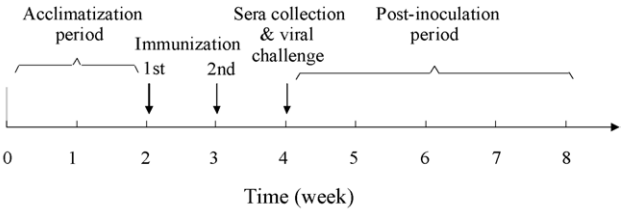

Fig. 1. Schedule of the whole experiment: the acclimatization period (2 weeks) and the experiment period (6 weeks).

2.2. Construction of immunogen and Immunization

Five candidate peptide-vaccines (PVs), designated as PV-BC1, PV-BC2, PV-BC3, PV-BC4 and PV-BC5, respectively, were mixed with complete Freund's adjuvant (CFA) for a first immunization and with incomplete Freund's adjuvant (IFA) for a second time. The commercial vaccine against CSFV (C-strain) was obtained from Nanjing Medical Machinery (PR China).

A total number of 70 healthy pigs aged 8–10 weeks were randomly divided into seven groups (10 pigs per group). Each group was raised separately and fed ab libitum. During the 2-week acclimatization period (Fig. 1), environmental temperatures and individual body temperatures were monitored once a day. The normal rectal temperature was defined at about $38.5 \pm 0.5\,^{\circ}C$, and a temperature exceeding $40\,^{\circ}C$ was considered as fever. Pigs were injected in buttock with the prepared vaccines twice in an intra-muscular way. The dose for each pig was 50 μg peptide (in conjugant) per injection. A sixth group was immunized with the commercial vaccine against CSFV (C-strain) under instruction to serve as a positive control. A seventh group not immunized was prepared as a negative control. One pig in group 4 died for non-experimental error reason during the immunized period.

2.3. Detection of peptide-specific antibodies by ELISA

The anti-sera (AS) from pigs were all tested with the enzyme-linked immunosorbent assay (ELISA). The peptides (5 μg/ml) were coated overnight in a microtiter plate at $4\,^{\circ}C$. Nonspecific binding was blocked by incubated with 0.25% gelatin-PBS for more than 2 h. After washing one time with PBS-Tween 20 (0.05% Tween 20), normal sera and anti-sera of different dilution levels were added, respectively, to the wells according to experiment design. After incubation for an hour and a second wash, peroxidase-conjugated rabbit anti-swine antibody (P0164, DAKO) was added. When incubation for another 45 min and a further wash were finished, freshly prepared OPD-peroxide solution was added and the optical density was measured (o.d. 450 nm).

2.4. Cells and flow cytometry (FCM)

PK15, the pig endothelial cell line, was cultured in complete medium of DMEM with 10% fetal bovine serum, and