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Identification of a conserved linear B-cell epitope at the N-terminus of the E2 glycoprotein of Classical swine fever virus by phage-displayed random peptide library

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ABSTRACT

The E2 protein of Classical swine fever virus (CSFV) is an important envelope glycoprotein, which is responsible for inducing protective immune response in swine. Four antigenic domains, A–D, have been mapped on the E2 protein. The present study describes the identification of a linear B-cell epitope at the N-terminus of the E2 protein by screening a phage-displayed random 12-peptide library with the neutralizing monoclonal antibody (mAb) HQ06 directed against the E2 protein. HQ06 was found to bind to the phages displaying a consensus motif LFDGSNP, which is highly homologous to ^{772}LFDGTNP^{778} of the CSFV polyprotein, locating on the border between antigenic domains B/C and A of the E2 protein. Considering that HQ06 showed reactivity with the motif ^{772}LFDGTNP^{778} expressed as GST fusion in Western blot and indirect ELISA, we propose that the motif ^{772}LFDGTNP^{778} represents a linear B-cell epitope of the E2 protein. The motif ^{773}FDGTNP^{778} is the minimal requirement for the reactivity as demonstrated by analysis of the reactivity of HQ06 with several truncated peptides derived from the motif. Alignment of amino acid sequences from a number of CSFV isolates indicated that the epitope is well conserved among different subgroups of CSFV. Substitutions of the individual residues within the epitope ^{773}FDGTNP^{778} demonstrated that residues ^{773}F, ^{775}G, and ^{778}P constitute the core of the epitope. The identified epitope will be useful for development of diagnostic assays and epitope-based marker vaccines against CSFV.

1. Introduction

Classical swine fever virus (CSFV) is the etiological agent of Classical swine fever (CSF), one of OIE Listed diseases. CSFV is a small, enveloped, positive-stranded RNA virus that belongs to the genus *Pestivirus* within the family *Flaviviridae*. The genus *Pestivirus* currently comprises six species, CSFV, *Bovine viral diarrhea virus 1* (BVDV-1), BVDV-2, *Border disease virus* (BDV), Pestivirus of Giraffe (a tentative pestivirus species), and D32/00_'HoBi' (an atypical pestivirus) (Heinz et al., 2004; Schirrmeier et al., 2004; Becher et al., 2003). The genome of CSFV contains a single, large open reading frame (ORF) encoding a polyprotein that, upon proteolytic processing, gives rise to four structural proteins: C, E^{rns}, E1, and E2, and seven non-structural proteins: N^{pro}, p7, NS2-3, NS4A, NS4B, NS5A, and NS5B (Moser et al., 1999). E2 is an envelope glycoprotein, which is responsible for eliciting neutralizing antibodies in infected animals and thus protecting pigs against virulent challenge (van Zijl et al., 1991; Konig et al., 1995). The N-terminus of E2 protein was shown to be immunodominant, and several antigenic domains full of epitopes have been identified (van Rijn et al., 1993, 1994).

Though a non-vaccination stamping-out policy is pursued in many countries including European Union member states, vaccination with modified live vaccines (MLV), such as the lapinized C-strain vaccine, has being carried out in endemic or epidemic areas including China. Failed vaccination with MLV, though effective in most cases, occurs occasionally due to the interference of maternally derived antibodies and improper vaccination schedules. Moreover, animals vaccinated with MLV are difficult to be discriminated from those infected with wild-type viruses by conventional diagnostic assays. Thus, development of marker vaccines which enable differentiation of infected from vaccinated animals (DIVA) is currently an urgent task for the control of CSF.

Epitopes or antigenic determinants are the part (contact points) of an antigen involved in specific interaction with the antigen-binding site (the paratope) of an antibody or a T-cell receptor. Detailed analysis of epitopes is important both for the understanding of immunological events and for the development of epitope-based marker vaccines and diagnostic tools for various

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Table 1
The oligonucleotides coding for the wild-type and mutated versions of the motif LFDGTNP

Designations of oligonucleotides	The sequences of oligonucleotides	Coding motifs (designations)
Ep7wt-S	*GATCC*CTGTTTGATGGTACCAATCCG[TAA]*C*	LFDGTNP (Ep7wt)
Ep7wt-R	*TCGAG*[TTA]CGGATTGGTACCATCAAACAG*G*	
Ep7ΔP-S	*GATCC*CTGTTTGATGGTACCAAT[TAA]*C*	LFDGTN– (Ep7ΔP)
Ep7ΔP-R	*TCGAG*[TTA]ATTGGTACCATCAAACAG*G*	
Ep7ΔL-S	*GATCC*TTTGATGGTACCAATCCG[TAA]*C*	–FDGTNP (Ep7ΔL)
Ep7ΔL-R	*TCGAG*[TTA]CGGATTGGTACCATCAAA*G*	
Ep7ΔLF-S	*GATCC*GATGGTACCAATccg[TAA]*C*	––DGTNP (Ep7ΔLF)
Ep7ΔLF-R	*TCGAG*[TTA]cggATTGGTACCATC*G*	
Ep7F773S-S	*GATCC*CTGagcGATGGTACCAATCCG[TAA]*C*	LSDGTNP (Ep7F773S)
Ep7F773S-R	*TCGAG*[TTA]CGGATTGGTACCATCgctCAG*G*	
Ep7D774G-S	*GATCC*CTGTTTggcGGTACCAATCCG[TAA]*C*	LFGGTNP (Ep7D774G)
Ep7D774G-R	*TCGAG*[TTA]CGGATTGGTACCgccAAACAG*G*	
Ep7G775S-S	*GATCC*CTGTTTGATagcACCAATCCG[TAA]*C*	LFDSTNP (Ep7G775S)
Ep7G775S-R	*TCGAG*[TTA]CGGATTGGTgctATCAAACAG*G*	
Ep7T776S-S	*GATCC*CTGTTTGATGGTagcAATCCG[TAA]*C*	LFDGSNP (Ep7T776S)
Ep7T776S-R	*TCGAG*[TTA]CGGATTgctACCATCAAACAG*G*	
Ep7N777S-S	*GATCC*CTGTTTGATGGTACCagcCCG[TAA]*C*	LFDGTSP (Ep7N777S)
Ep7N777S-R	*TCGAG*[TTA]CGGgctGGTACCATCAAACAG*G*	
Ep7P778F-S	*GATCC*CTGTTTGATGGTACCAATttt[TAA]*C*	LFDGTNF (Ep7P778F)
Ep7P778F-R	*TCGAG*[TTA]aaaATTGGTACCATCAAACAG*G*	

Notes: Introduced bases for cloning (to form the overhanging ends of BamHI and XhoI after annealing the two complementary oligonucleotides) are shown in italics; stop codons are boxed; mutated bases are shown in lowercases; deleted residues are shown as dashes; mutated residues are underlined; the designations of the motifs are shown in parentheses.

diseases. Phage display, using either random peptide libraries or gene-targeted libraries, provides a powerful technique for epitope identification. This technology can identify amino acids on protein antigens that are critical for antibody binding and, further, the isolation of peptide motifs that are both structural and functional mimotopes of both protein and non-protein antigens (Rowley et al., 2004; Wang and Yu, 2004). Several studies have reported the applications of the technology in epitope mapping in flaviviruses (Bugli et al., 2001; Zhang et al., 2006; Herrmann et al., 2007).

In a previous study, we developed a neutralizing monoclonal antibody (mAb) against the E2 protein of CSFV. The mAb was shown to recognize CSFV in immunofluorescence assay and Western blot (Hou et al., 2008). This paper describes the identification of a novel conserved linear B-cell epitope on the E2 protein of CSFV by screening a phage-displayed random library with the anti-E2 mAb.

2. Materials and methods

2.1. Phage-displayed library

The Ph.D.-12™ Phage Display Peptide Library Kit was purchased from New England BioLabs Inc. The dodecapeptide library consists of 2.7×10^9 electroporated sequences (1.5×10^{13} pfu/ml).

2.2. Monoclonal antibody

The mAb HQ06 directed against the E2 protein of CSFV was generated by immunization of BALB/c mice with the recombinant truncated E2 protein (aa 754–881) of CSFV Shimen/HVRI strain (GenBank accession no. AY775178) expressed in *Escherichia coli*. The mAb showed reactivity and neutralizing activity with Shimen/HVRI strain in infected PK15 cells in virus neutralization test (Hou et al., 2008). The mAb was purified from the ascites fluid of mice inoculated with the hybridma cells secreting HQ06 by affinity chromatography using rProtein G (Sigma, USA) according to the manufacturer's instructions, and the concentration was determined. The mAb HQ06 with high purity (>90%, determined by SDS-PAGE) was obtained and used for biopanning.

2.3. Biopanning

Three successive rounds of biopanning were carried out according to the manufacturer's instruction manual. Briefly, one well of a 96-well microtiter plate was coated with 15 μg of HQ06 in coating buffer (0.1 M $NaHCO_3$, pH 8.6), followed by blocking with blocking buffer (0.1 M $NaHCO_3$, pH 8.6, 0.02% NaN_3, and 5 mg/ml BSA) for 2 h at 4 °C. About 1.5×10^{11} pfu (4×10^{10} phages, 10 μl of the original library) were added to the well and incubated for 1 h at room temperature. The unbound phages were removed by successive washings with TBS buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl) containing gradually increased concentrations (0.1%, 0.3%, and 0.5%) of Tween-20, and the bound phages were eluted with elution buffer (0.2 M glycine–HCl, pH 2.2) containing 1 mg/ml BSA. The eluted phages were amplified in early-log *E. coli* ER2738 strain cells.

2.4. Phage ELISA

After three rounds of biopanning, eight individual phage clones were selected and assayed for target binding by sandwich ELISA as described by the manufacturer's instructions. Briefly, 96-well microtiter plates were coated overnight with 2 μg of HQ06 or anti-porcine IFN-γ mAb (Sigma, USA) served as negative controls. After 2 h of blocking with blocking buffer at 4 °C, phage clones were added to the wells (2×10^{11} pfu in 100 μl per well) and incubated with agitation for 2 h at room temperature. Bound phages were subjected to reaction with horseradish peroxidase (HRP)-conjugated anti-M13 antibody (Pharmacia, USA), followed by color devel-