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Research article

Identification of a conformational epitope on the VP1 G-H Loop of type Asia1 foot-and-mouth disease virus defined by a protective monoclonal antibody

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ABSTRACT

Although neutralizing antigenic sites of foot-and-mouth disease virus (FMDV) can be defined by selection of monoclonal antibody (MAb) escape mutants, no conformational neutralizing epitope on the major antigenic site located on the G-H loop of type Asia1 FMDV has been precisely mapped. In this study, we generated a potent neutralizing MAb 3E11, which recognized a conformation-dependent epitope and neutralized FMDV Asia1/YS/CHA/05 *in vitro*. Importantly, a dose of 5.5 NT_{50} of the MAb 3E11 completely protected suckling mice from a dose of 10 LD_{50} of homologous virus challenge *in vivo*. Through a 12-mer random peptide phage display, synthetic peptide analysis and constructing a series of FMDV Asia1/YS/CHA/05 mutants using reverse genetic system, we finely mapped the neutralizing epitope as the 12-amino acid peptide ^{141}SXRGXLXXLXRR^{152}. These results provide additional insights into the virus–MAb interaction at the amino acid level and may help in the development of an epitope-based Asia1 FMDV vaccine.

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1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious and economically important disease that affects cloven-hoofed animals worldwide. The disease is caused by the foot-and-mouth disease virus (FMDV), which belongs to the genus *Aphthovirus* within the *Picornaviridae* family (Grubman and Baxt, 2004). There are seven distinct serotypes of FMDV; namely, O, A, C, Southern African Territories 1 (SAT1), SAT2, SAT3 and Asia1. The virus consists of a non-enveloped icosahedral capsid and a single-stranded positive sense RNA genome of about 8.5 kb. The capsid is composed of 60 copies of four virus-encoded structural proteins, VP4, VP2, VP3 and VP1, of which VP1 is the major viral protein on the surface of the intact virus. Due to the error-prone nature of the RNA-dependent RNA polymerase, FMDV exhibits a high potential for genetic and antigenic variation, and multiple genotypes exist within each serotype (Bachrach, 1968; Domingo et al., 2003). Antigenic diversity is one of the major obstacles to vaccinating against FMD.

Some antigenic sites have been defined by the selection and sequence analysis of monoclonal antibodies (MAbs) escape mutants FMDV type O (Crowther et al., 1993; Parry et al., 1989; Pfaff et al., 1988), type A (Moore et al., 1989; Saiz et al., 1991) and type C (Lea et al., 1994). For type Asia1 FMDV, four functionally independent neutralizing antigenic sites have been detected (Butchaiah and Morgan, 1997; Marquardt et al., 2000; Sanyal et al., 1997). Among all the defined antigenic sites, the G-H loop (encompassing residues 140–160) (Lea et al., 1994; Logan et al., 1993), which is located on the surface of the capsid protein VP1, the major neutralizing antigenic site (Ochoa et al., 2000; Thomas et al., 1988). However, multiple overlapping

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epitopes exist in the G-H loop antigenic site (VP1 residues 138–150) of FMDV C-S8c1(Mateu et al., 1990). Thus, fine mapping of the FMDV epitopes instead of determining the antigenic site remains a challenging task. Although the flexible G-H loop in VP1 contains a highly conserved Arg-Gly-Asp (RGD) triplet which interacts with cell surface integrin receptors (Jackson et al., 1997; Monaghan et al., 2005; Neff et al., 1998), single amino acid substitution affects multiple overlapping epitopes in the major antigenic site of type C FMDV (Mateu et al., 1990). The antigenic sites of FMDV can vary among the different serotypes, the antigenic sites can even vary within the same genotype. Therefore, fine mapping of the neutralizing epitopes is needed to select the possible targets in the rational design of epitope-based vaccine (Gershoni et al., 2007).

All four of the antigenic sites of type Asia1 FMDV are dependent on the native conformation of the virus (Sanyal et al., 1997). In contrast to linear epitopes, conformational or discontinuous epitopes are formed when remote amino acids in the primary sequence of a protein are brought into close proximity in the folded protein (Gustafsson et al., 2009). Thus, discontinuous epitopes are relatively difficult to map using synthetic peptides or selection of escape mutants. Functional mimicry of FMDV discontinuous antigenic sites could be achieved by means of synthetic peptide constructions (Parry et al., 1989; Villen et al., 2001, 2002) and selection of neutralization escape variants (Barnett et al., 1989; Saiz et al., 1991). However, random peptide phage display and reverse genetic system as powerful means for FMDV epitope mapping have rarely been used.

Here we describe the generation and characterization of a strong neutralizing monoclonal antibody, entitled 3E11, against type Asia1 FMDV Asia1/YS/CHA/05. Importantly, by using a combined approach of phage display, site-directed mutagenesis based on synthetic peptides and a reverse genetic system, we finely mapped the conformational epitope recognized by the MAb 3E11. The relationship between the identified conformational neutralizing epitope in this study and the antigenic sites described previously in other serotypes is also discussed.

2. Materials and methods

2.1. Viruses and cells

Serotype Asia1 FMDV Asia1/YS/CHA/05(GenBank accession number, GU931682), isolated during the pandemic of China in 2005 (Valarcher et al., 2005; Zhang et al., 2008), was used in this study. The baby hamster kidney cell line BHK-21 was maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Gibco-BRL, Grand Island, NY, USA).

2.2. Monoclonal antibodies

Six-week-old female BALB/c mice were subcutaneously immunized with 100 μg of inactivated purified FMDV Asia1/YS/CHA/05 antigen emulsified with an equal volume of adjuvant VG206 (SEPTIC, France). Two boosters of the adjuvant emulsified antigen were given at 2-week intervals. Two weeks after the third immunization, the mice were intraperitoneally boosted with 100 μg of antigen alone. Three days later, immunized spleen cells were fused with SP2/0 myeloma cells using 50% (w/v) polyethylene glycol and 10% dimethyl sulfoxide (DMSO) (v/v) (Sigma, St. Louis, MO, USA). Hybridomas were screened by an enzyme-linked immunosorbent assay (ELISA) and verified by an indirect immunofluorescence assay (IFA) and Western blotting. The hybridoma producing MAb was cloned three times by limiting dilution of the cells. Antibody subtype identification was performed using the SBA Clonotyping™ System/HRP Kit (Southern Biotech, Birmingham, AL, USA). FMDV serotype-independent MAb 4B2 was described previously (Yu et al., 2010).

2.3. Indirect immunofluorescence assay

BHK-21 cells in 96-well plates were infected with FMDV, fixed with ice-cold anhydrous ethanol for 15 min at 4 °C and air dried. Then 50 μL/well MAb at 1:200 dilutions in PBS was added for 1 h at 37 °C. After washing with PBS, 50 μL/well of FITC-conjugated goat anti-mouse IgG (Sigma, St. Louis, MO, USA) at a 1:200 dilution was added and incubated for 1 h at 37 °C. Plates were washed three times with PBS and observed under an OLYMPUS microscope connected to a Leica DFC 490 digital color camera.

2.4. Biopanning

The MAb 3E11 was preliminarily purified by the caprylic acid–ammonium sulfate method, followed by purification with the NAb™ Protein G Spin Purification Kit (Pierce, Rockford, IL, USA). The M13 phage display library displaying 12-mer random peptides (Ph.D-12 Phage Display Peptide Library Kit, New England Biolabs) was used. The affinity selection of the phage clones from the random peptide library was conducted following the manufacturer's recommendations with minor modifications. MAb 4C6 was used to remove any phages that specifically bound to the Fc fragment of the antibody. In the first round of the biopanning, a 96-well plate was coated with 10 μg of MAb 3E11 in 0.1 M $NaHCO_3$ buffer (pH 8.6) at 4 °C for 12 h. The coated wells were washed with Tris-buffered saline (50 mM Tris–Cl [pH 7.5], 150 mM NaCl) containing 0.1% (v/v) Tween 20 (TBST) followed by blocking with 1 mg/mL bovine serum albumin (BSA) in 0.1 M $NaHCO_3$ buffer. The phage library (1.5×10^{11} phages/100 μL) was incubated with the blocked wells for 1 h at room temperature. Unbound phages were washed away with 10 washes of TBST. The bound phages were eluted by 0.2 M glycine–HCl containing 1 mg/mL BSA (pH 2.2) and immediately neutralized with 1 M Tris–HCl (pH 9.1). The eluted phages were amplified in *E. coli* (ER2738) and titered on LB/IPTG/Xgal plates. Second and third rounds of selection were performed similarly to the first round except the concentration of Tween 20 was raised to 0.5% while the concentrations of MAb 3E11 were reduced to 5 μg/mL and 1 μg/mL, respectively.

For the fourth round of biopanning, 300 ng of purified MAb 3E11 was mixed with the amplified third-round

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