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Neutralization of genotype 2 porcine epidemic diarrhea virus strains by a novel monoclonal antibody

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ABSTRACT

Porcine epidemic diarrhea virus (PEDV) has two genotypes, G1 and G2. To research the immunogenicity differences of PEDV G1 and G2 genotype strains and obtain a neutralizing monoclonal antibody (mAb), we inoculated specific-pathogen-free BALB/c mice with a newly emerged strain, PEDV-LNCT2. After immunizations, cells from the spleen of the mice were fused with SP2/0 myeloma cells. Following culturing and subcloning, a strain, 1B9, secreting neutralizing antibody, was obtained. The 1B9 mAb neutralized new variant genotype 2 PEDV strains (LNCT2, LNSY, and Hjms), but it did not neutralize a genotype 1 PEDV strain (CV777), *in vitro*. Results showed that the epitope recognized by the 1B9 mAb lies in the spike protein, and that it is a conformational epitope. These findings confirm that allelic differences in the PEDV S gene between the G1 and G2 genotype strains led to changes in the S protein and, thus, differences in its immunogenicity.

1. Introduction

New variants of porcine epidemic diarrhea virus (PEDV) emerged in China in 2010 (Sun et al., 2012), and they spread to the United States in 2013 (Cima, 2013; Stevenson et al., 2013). These new PEDV variants have caused high morbidity and mortality rates in both newborn and suckling pigs (Wang et al., 2013). These animals present with diarrhea and vomiting, and PEDV infection has resulted in substantial economic losses to the swine industry, as well as increased pork prices in China.

Based on PEDV genome sequences, PEDV strains have been divided into two groups, the G1 genotype (including the prototype European PEDV strain CV777) and the G2 genotype (new variant strains that have emerged since 2011) (Chen et al., 2013; Lee and Lee, 2014). The pathogenesis of G1 and G2 strains has been reported since the 1980s and 2013, respectively (Coussement et al., 1982; Debouck et al., 1981; Stevenson et al., 2013). Both PEDV G1 and G2 strains lead to severe diarrhea and vomiting.

It is well known that the spike (S) protein of coronaviruses plays a crucial role in the induction of neutralizing antibodies, and it has been used to prepare effective vaccines (Gómez et al., 2000; Tuboly and Nagy, 2001). The amino-terminal portion of the S protein of several coronaviruses has been shown to contain key antigenic sites that are responsible for eliciting humoral and cellular immune responses (Delmas et al., 1986; Gebauer et al., 1991; Zhang et al., 2016). The PEDV S gene is often used to evaluate the genetic diversity of coronaviruses (Li et al., 2012; Chen et al., 2013).

Based on research into the PEDV genome (Chen et al., 2013; Lee and Lee, 2014), the main differences between PEDV G1 and G2 strains occur in the S gene. The full length of S gene of G2 strains is 9 nt longer than that of the prototype PEDV strain CV777. In addition to its increased length, the S gene of G2 strains has many insertions and deletions, as well as other mutations, compared with the S gene of G1 strains (Lee and Lee, 2014).

Many pig herds that were vaccinated with inactivated or attenuated CV777 vaccines still experienced high mortality rates among newborn piglets (Li et al., 2012; Sun et al., 2012). A mutation in the S gene may lead to a change in the S protein and, thus, a difference in its immunogenicity, thereby resulting in a different neutralization profile. Therefore, in the present study, PEDV G2 virions were used to immunize BALB/c mice to produce a neutralizing monoclonal antibody (mAb) to test for immunogenicity differences that distinguish G1 and

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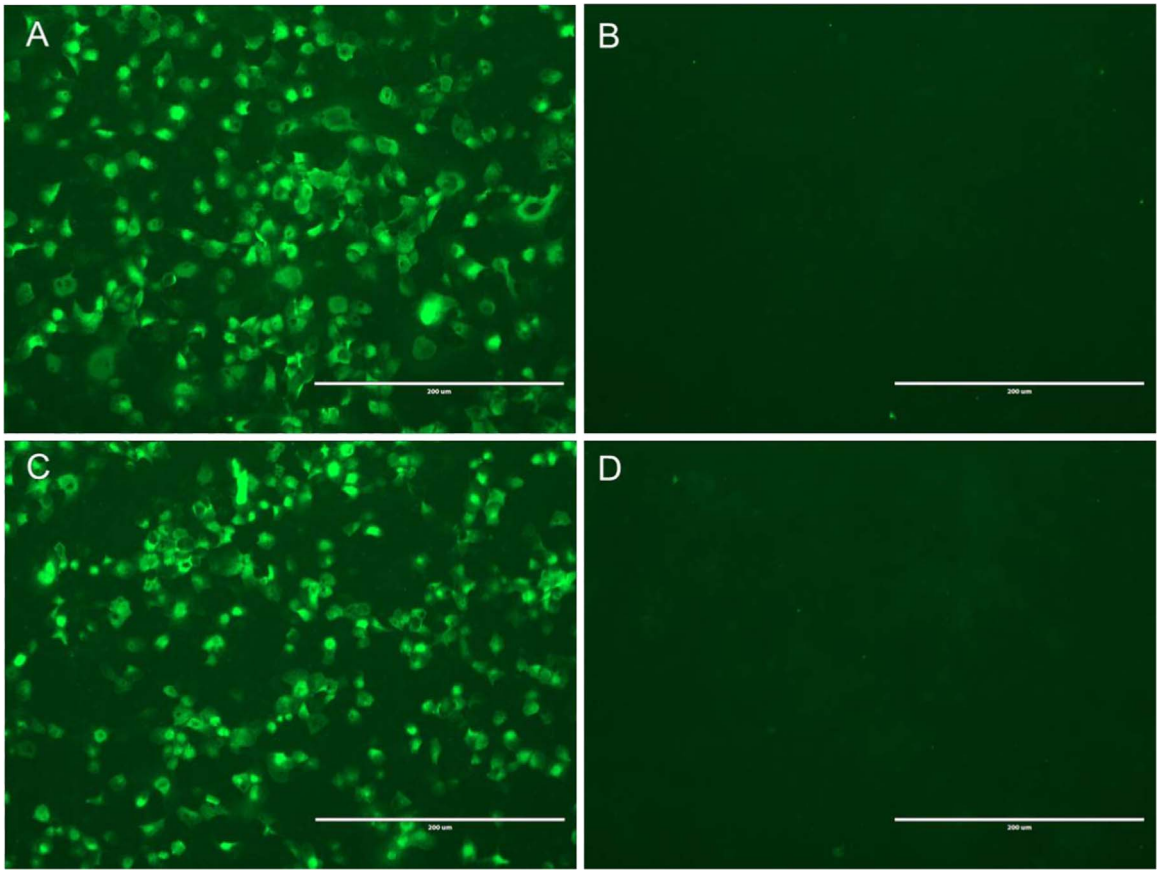

Fig. 1. Reaction of mAb 1B9 with PEDV-positive cells, as detected by an IFA. The cells in A) and C) were Vero E6 cells infected by PEDV strain LNCT2, which yielded positive signals; the cells in B) and D) were healthy Vero E6 cells used as a negative control. In A) and B), mAb 1B9 was used as the primary antibody, while in C) and D), a PEDV polyclonal antibody was used as the primary antibody.

G2 PEDV strains.

2. Results

2.1. Phylogenetic analysis based on PEDV genome and S gene sequences

Two phylogenetic trees of PEDV genomes and S genes were constructed, respectively (Suppl. Fig. S1(a, b)). Both the phylogenetic trees indicated that the LNCT2, LNSY, and Hjms strains belong to the G2 genotype, and the CV777 strain belongs to the G1 genotype. After translating the S genes, the S proteins were analyzed by MegAlign in DNAStar. As shown in Suppl. Fig. S2, we observed that the main differences lie in the S1 portion of the S protein.

2.2. A positive mAb clone against PEDV

After immunizing mice, their spleen cells fused with SP2/0 myeloma cells, and a positive clone of hybridoma cells was selected. A subclone, 1B9, reacted well with PEDV by enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA) (Fig. 1). The heavy chain was IgG2b, and the light chain was kappa, as detected by the SBA Clonotyping System-HRP (Southern Biotech, Birmingham, AL, USA). Western blotting was used to identify the epitope recognized 1B9. However, mAb 1B9 did not react with PEDV strains LNCT2 and CV777, Vero-E6 cells, or the S protein of the LNCT2 strain (Suppl. Fig. S3a). Dot immunoblotting assay shows that mAb 1B9 binds LNCT2 virus stronger than that of CV777 (Suppl. Fig. S3b). These results suggested that the epitope of 1B9 was dependent on the protein conformation. The antibody titer in the culture supernatant was higher than 1:1000, and the titer in the ascites fluid was higher than 1:100,000, as detected by an IFA.

2.3. Neutralization test

Purified mAb 1B9 (diluted to 1 mg per ml) was used in a neutralization test. As shown in Fig. 2, the LNCT2, LNSY, and Hjms strains were neutralized by mAb 1B9, while strain CV777 was not. Additionally, mAb 1B9 completely neutralized the LNCT2, LNSY, and Hjms strains at dilutions of 1:160, 1:80, and 1:160, respectively.

2.4. Identification of the protein recognized by mAb 1B9 using recombinant PEDV S, S1, and S2 proteins

Six plasmids, pcDNA3.1(-)-CV777-S, pcDNA3.1(-)-CV777-S1, pcDNA3.1(-)-CV777-S2, pcDNA3.1(-)-LNCT2-S, pcDNA3.1(-)-LNCT2-S1, and pcDNA3.1(-)-LNCT2-S2, were constructed. The plasmids were transfected into HEK293T cells to identify proteins that reacted with mAb 1B9. As shown in Fig. 3, cells transfected with plasmids pcDNA3.1(-)-CV777-S and pcDNA3.1(-)-LNCT2-S reacted well with mAb 1B9 and PEDV-positive pAb. Cells transfected with the other plasmids did not react with mAb 1B9, but reacted with the positive control antibodies. HEK293T cells transfected with these plasmids did not react with the negative serum (not shown in Fig. 3). These results suggested that mAb 1B9 recognized the S protein of the CV777 and LNCT2 strains. However, neither the S1 nor S2 proteins of CV777 were recognized by 1B9.