Neutralizing antibody to Porcine epidemic diarrhea virus

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Abstract

Porcine epidemic diarrhea (PED) is a highly contagious devastating enteric disease of pigs caused by porcine epidemic diarrhea virus (PEDV). Pigs of all ages can be infected, but newborn piglets during the first week of life are highly susceptible to the virus and the fatality rate among them can reach 80-100%. PED inflicts severe economic damage to the pig industries all over the world including Thailand. PEDV vaccines are limitedly available with unreliable efficacy. There is, currently, no specific treatment for PEDV infection. This study aimed to produce ready-to-use protective monoclonal antibodies (MAbs) for passive immunization of the newborn piglets against the PEDV during their susceptible age. A hybridoma clone (A3) that secretes IgM MAb specific to domain 1 of PEDV spike protein was generated. The antibody significantly neutralizes the PEDV infection in vitro. The epitope is shared by the PEDV G1 and G2 strains. Further testing of the MAb in the piglet challenge study, towards the real application is in progress.

Keywords: Porcine epidemic diarrhea (PED) virus (PEDV), Monoclonal antibodies (MAbs), Virus neutralization, Passive immunization, Spike protein

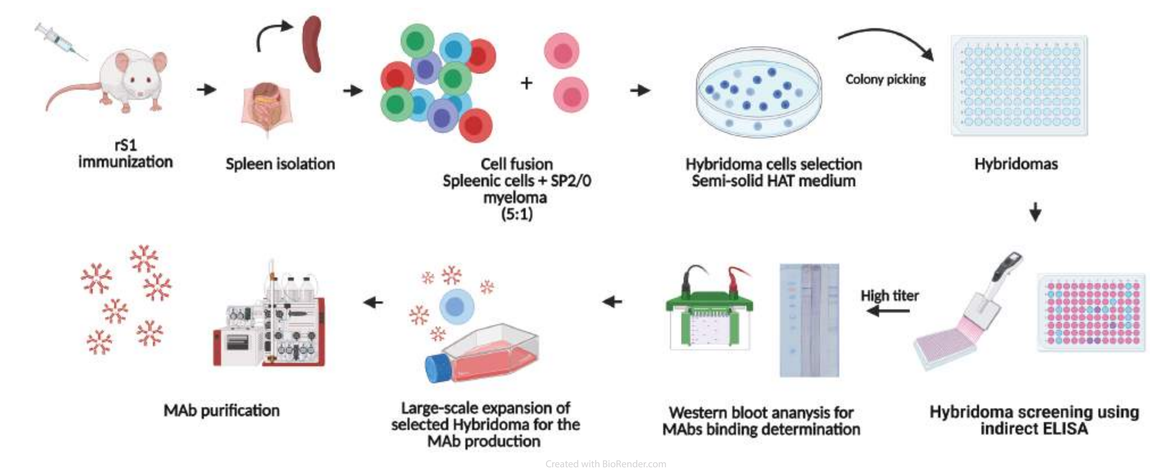
Introduction

Porcine epidemic diarrhea (PED) caused by PED virus (PEDV) is a highly contagious disease of pigs. The PEDV infects pigs of all ages, but newborn piglets (during the first week of their lives) are highly susceptible which the PED mortality rate among them can reach 80-100% (Antas et al., 2019; Stadler et al., 2018). PED is characterized by severe diarrhea, vomiting and anorexia that lead to severe dehydration and death (Lee, 2015). Strict biosecurity in farms is the key to prevent this virus infection and spreading. PEDV vaccines are available in some countries for sow immunization to induce maternal antibodies that passively transferred to the newborn piglets, but the efficacy of these vaccines are unreliable while the vaccine administration protocol is complicated and unstandardized. The PEDV outbreaks occur in all parts of the world (Volker, 2011; Song et al., 2015) causing negative economic impact to the pig industries. The situation indicates the needs of effective vaccines and therapeutic regimen against the PEDV infection. This study aims to produce ready-to-use neutralizing monoclonal antibodies (MAbs) for passive immunization of the newborn piglets against the PEDV infection during their susceptible age (Thavorasak et al., 2022).

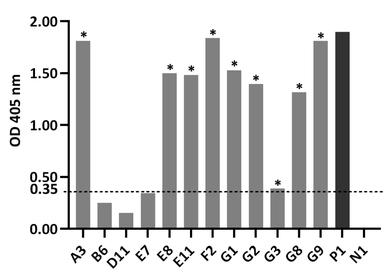
Methods and Results

1. Preparation of MAbs to PEDV spike protein using Hybridoma technology

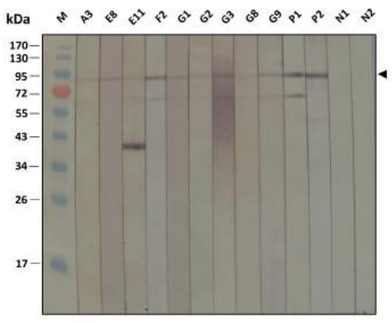
1A



1B



1C



1D

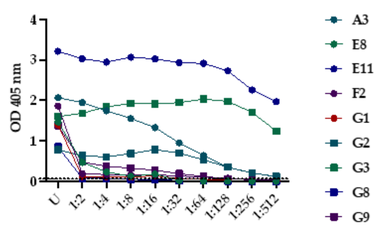


Fig. 1A: Schematic representation of hybridoma generation and monoclonal antibody production.

Fig. 1B: Indirect ELISA results of culture supernatants of hybrodomas against rS1 using mouse immune serum (1:50,000) as positive control (P1) and SP2/0-Ag14 culture supernatant as negative control (N1). Asterisks indicate the clone that gave OD 405 nm ≥ 0.35; they were selected for further experiments.

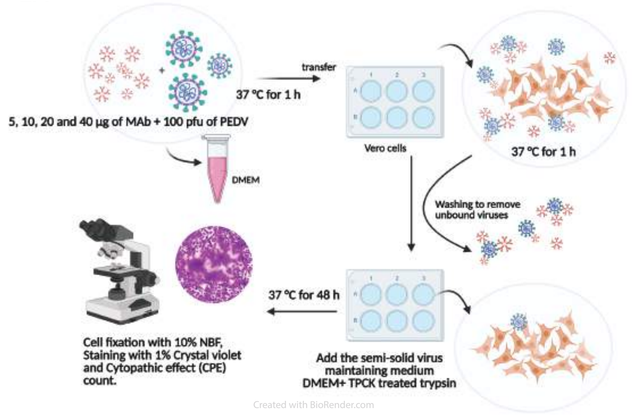
Fig. 1C: Results of Western blot analysis to demonstrate binding of the MAbs from the selected hybridomas to SDS-PAGE-separated rS1. The antigen-antibody reactive bands were revealed by using BCIP-NBT reaction. P1 is mouse immune serum (1:10,000); P2, mouse anti-His antibody; N1, SP2/0-Ag14 spent medium; N2, PBS.

Fig. 1D: Indirect ELISA titers of the rS1-specific mAbs secreted from hybridoma clones.

Methods and Results

2. Neutralization of PEDV infectivity

2A



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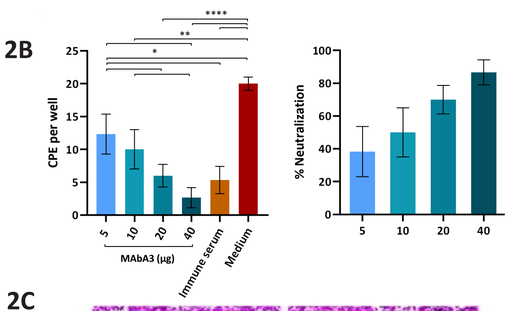

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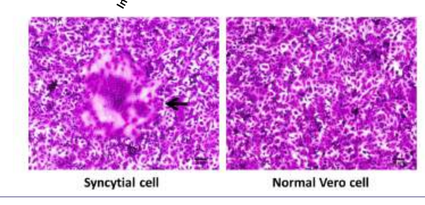


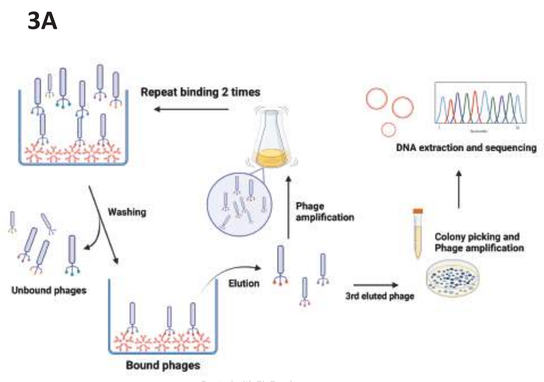
Fig. 2A: Schematic representation of neutralization assay

Fig. 2B: Neutralization assay to demonstrate PEDV neutralizing activity of MAbA3 (at 5, 10, 20 and 40 µg) using mouse immune serum (PAbs) and medium alone (No treatment) as positive and negative neutralization controls, respectively (left panel). Percent neutralization of the MAb (right panel).

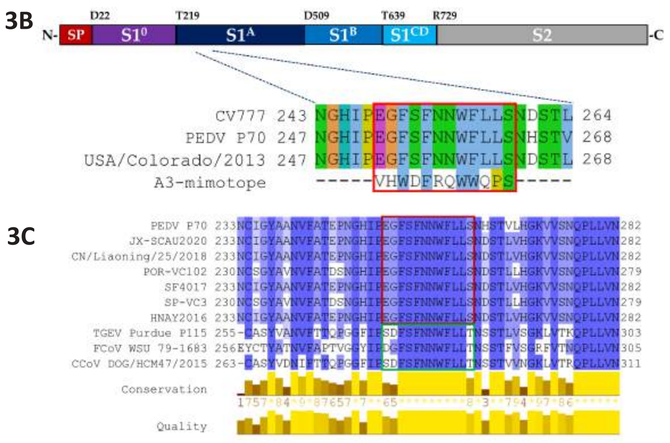
Fig. 2C: Cytopathic effect (CPE) of Vero cells (syncytial formation) caused by PEDV infection (arrow) (left panel). Normal non-infected Vero cells (right panel).

3. Epitope mapping of MAbA3

3A



3B



3C

Fig. 3A: Schematic representation of epitope identification using Ph.D.-12™ phage display peptide library

Fig. 3B: Domain organization of spike protein of PEDV P70 strain (Upper panel) and multiple sequence aligment of A3 mimotope with PEDV CV777 (classical strain, G1), PEDV P70 (G2 strain that used in this study) and USA/Colorado/2013 strain (lower panel).

Fig. 3C: Multiple alignments of the amino acid sequences of spike proteins of various strains of PEDV that were published in PubMed during the past 5 years and other alphacoronaviruses. The amino acid sequences of PEDV that caused PED outbreaks included JX-SCAU2020 (GenBank: QUE39276.1); CN/Liaoning/25/2018 (GenBank: QCW07226.1); POR-VC102 (GenBank: QQO86882.1); SF4017 (GenBank: QEQ55819.1); SP-VC3 (GenBank: QQO86847.1); and HNAY2016 (GenBank: QQS74747.1). The sequences of other alphacoronaviruses include TGEV Purdue P115 (GenBank: ABG89325.1); FCoV WSU 79-1683 (GenBank: JN634064.1); and CCoV DOG/HCM47/2015 (GenBank: LC190907.1).

Conclusion

PEDV S1 specific MAb of hybridoma clone A3 could significantly neutralize infectivity of the PEDV genotype G2 field isolate *in vitro*. The A3-epitope is located on S1A subdomain of spike protein and shared by the PEDV G1 and G2 strains, as well as other alphacoronaviruses including canine and feline-coronaviruses and porcine transmissible gastroenteritis virus. The MAbA3 awaits evaluation *in vivo* before developing further to the real application against PEDV in piglets.

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