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Porcine aminopeptidase N mediated polarized infection by porcine epidemic diarrhea virus in target cells

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ABSTRACT

Infection of polarized intestinal epithelial cells by porcine epidemic diarrhea virus (PEDV) was characterized. Indirect immunofluorescence assay, real-time PCR, and transmission electron microscopy confirmed PEDV can be successfully propagated in immortalized swine small intestine epithelial cells (IECs). Infection involved porcine aminpeptidase N (pAPN), a reported cellular receptor for PEDV, transient expression of pAPN and siRNA targeted pAPN increased and decreased the infectivity of PEDV in IECs, respectively. Subsequently, polarized entry into and release from both Vero E6 and IECs was analyzed. PEDV entry into polarized cells and pAPN grown on membrane inserts occurs via apical membrane. The progeny virus released into the medium was also quantified which demonstrated that PEDV is preferentially released from the apical membrane. Collectively, our data demonstrate that pAPN, the cellular receptor for PEDV, mediates polarized PEDV infection. These results imply the possibility that PEDV infection may proceed by lateral spread of virus in intestinal epithelial cells.

Introduction

Porcine epidemic diarrhea virus (PEDV), a member of the family *Coronaviridae*, is an enveloped virus with a positive-stranded RNA genome that causes diarrhea in pigs and is associated with high mortality in newborn piglets (Song and Park, 2012). Other members of the genus *Alphacoronavirus* include transmissible gastroenteritis virus (TGEV), human coronavirus 229E (HCoV-229E), feline coronavirus (FCoV), canine coronavirus (CCoV) and human coronavirus NL63 (HCoV-NL63) (Adams and Carstens, 2012).

PEDV encodes four structural proteins: a large spike or peplomer glycoprotein (S), a membrane glycoprotein (M), a small envelope protein (E) and a phosphorylated nucleocapsid protein (N) (Cavanagh and Britton, 2008; Egberink et al., 1988). The spike (S) glycoprotein of PEDV is the dominant surface protein and is responsible for initiating infection and for inducing neutralizing antibodies (Duarte and Laude, 1994; Yeo et al., 2003).

APN (CD13) is one of the type II cell surface metalloproteases the large glycosylated ectodomain of which has a zinc metal ion at the active site (Mina-Osorio, 2008). It is known that APN serves as a cellular receptor for several alphacoronaviruses, such as TGEV, HCoV-229E and FCoV (Delmas et al., 1992; Yeager et al., 1992; Tresnan et al., 1996). Only very limited data are available indicating that porcine APN (pAPN) plays a role for PEDV infection. Previously, it has been reported that rabbit anti-pAPN polyclonal antibody inhibited PEDV binding to pAPN protein and pretreatment of Vero E6 cells with a soluble pAPN increased the viral infectivity (Oh et al., 2003). Mature pAPN is a 150-kDa glycosylated protein that is highly expressed in small intestinal mucosa (Oh et al., 2003; Delmas et al., 1992). Li and colleagues demonstrated that MDCK cells, a canine kidney cell line, became susceptible to PEDV infection after transient expression of pAPN; infection was inhibited by anti-pAPN polyclonal antibodies (Li et al., 2007). A swine testicular cell line (ST) that expresses only low levels of the enzyme, is resistant to PEDV infection. However, recombinant ST cells constitutively expressing high levels of pAPN could be infected efficiently (Nam and Lee, 2010). The available data indicate an association between pAPN and PEDV infection,

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although PEDV can be serially propagated in Vero E6 cells, a monkey cell line which does not express pAPN, if a protease is added for release of virions from the cell surface (Hofmann and Wyler, 1988; Shirato et al., 2011).

The primary target of coronaviruses is the respiratory or intestinal epithelium. Epithelial cell layers form a primary barrier to infection by microorganisms entering their host via body cavities such as the respiratory or intestinal tract (Ren et al.,

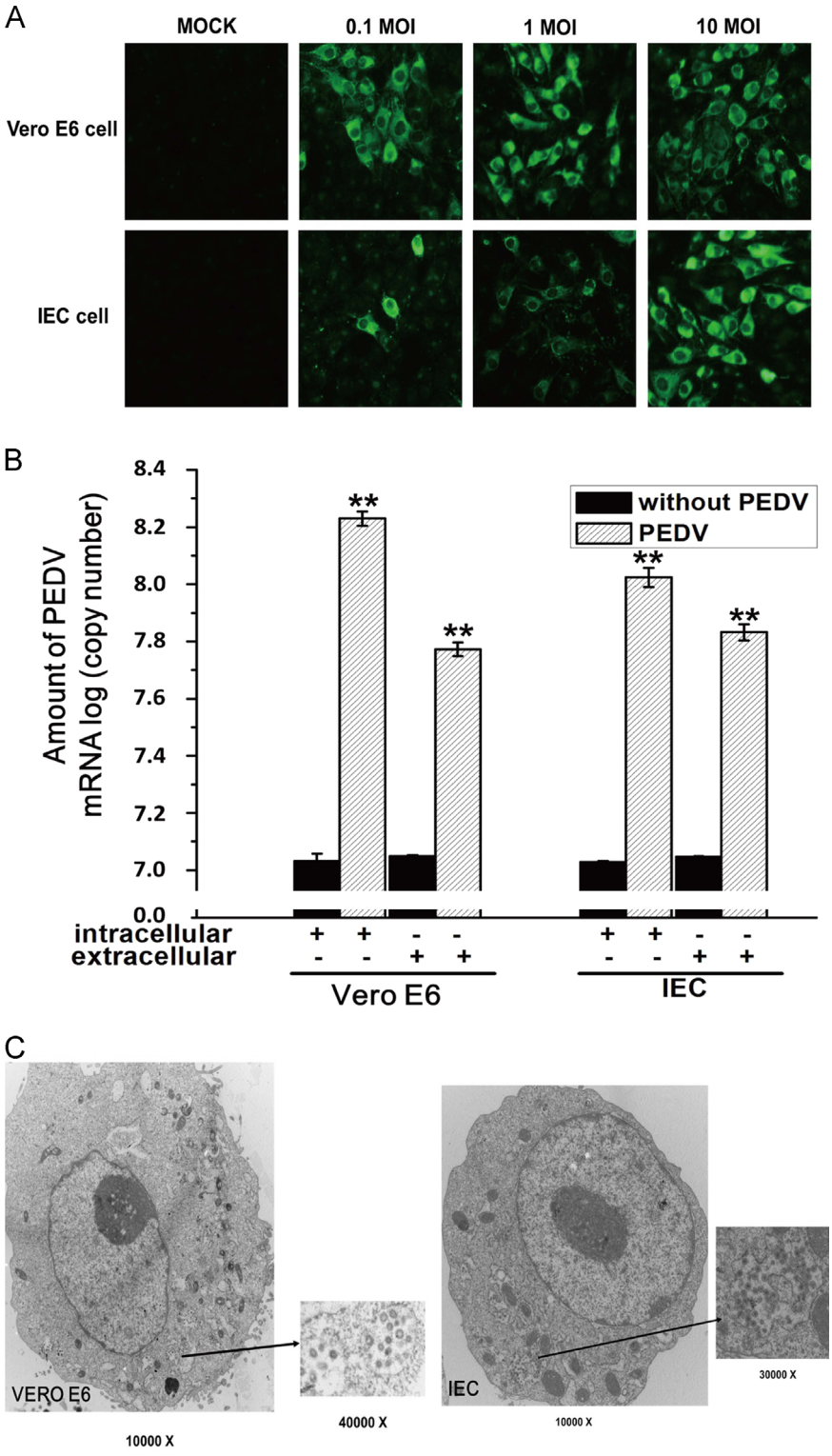

Fig. 1. PEDV can be successfully propagated in IECs. (A) Indirect immunofluorescence analysis of Vero E6 and IECs inoculated with PEDV at different MOI (0.1, 1 and 10) and incubated for 48 h. Cells were fixed in 4% formaldehyde for 15 min at room temperature, permeabilized with 0.1% Triton X-100 for 5 min at room temperature and processed for indirect immunofluorescence using anti-PEDV polyclonal antibody (1:100) and FITC-labeled goat anti-rabbit IgG (1:200). (B) Real-time PCR of the copy numbers of PEDV mRNA in Vero E6 cells infected at an MOI of 1.0 and in IECs infected at an MOI of 10 at 72 h post-infection. All data are expressed as mean $\pm$ SD. $*p < 0.05$; $**p < 0.01$. (C) Vero E6 and IECs were infected by PEDV at an MOI of 1 or 10 and incubated for 48 h. The cells were fixed with glutaraldehyde followed by 4% osmic acid. Sections were visualized by transmission electron microscopy. The PEDV particles are shown (black arrows indicate virus particles).