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Transmissible gastroenteritis virus and porcine epidemic diarrhoea virus infection induces dramatic changes in the tight junctions and microfilaments of polarized IPEC-J2 cells

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ABSTRACT

Viral infection converts the normal constitution of a cell to optimise viral entry, replication, and virion production. These conversions contain alterations or disruptions of the tight and adherens junctions between cells as part of their pathogenesis, and reorganise cellular microfilaments that initiate, sustain and spread the viral infections and so on. Using porcine epidemic diarrhoea virus (PEDV), transmissible gastroenteritis virus (TGEV) and a model of normal intestinal epithelial cells (IPEC-J2), we researched the interaction between tight and adherens junctions and microfilaments of IPEC-J2 cells with these viruses. In our work, the results showed that IPEC-J2 cells were susceptible to TGEV and PEDV infection. And TGEV could impair the barrier integrity of IPEC-J2 cells at early stages of infection through down-regulating some proteins of tight and adherens junctions, while PEDV cloud cause a slight of damage in the integrity of epithelial barrier. In addition, they also could affect the microfilaments remodelling of IPEC-J2 cells, and the drug-interfered microfilaments could inhibit viral replication and release. Furthermore, PEDV + TGEV co-infection was more aggravating to damage of tight junctions and remodelling of microfilaments than their single infection. Finally, the PEDV and TGEV infection affected the MAPK pathway, and inhibition of MAPK pathway regulated the changes of tight junctions and microfilaments of cells. These studies provide a new insight from the perspective of the epithelial barrier and microfilaments into the pathogenesis of PEDV and TGEV.

1. Introduction

Porcine epidemic diarrhoea virus (PEDV) and transmissible gastroenteritis virus (TGEV) are members of the *Coronaviridae* family (Sestak and Saif, 2002). They both replicate in the differentiated enterocytes covering the villi of the porcine small intestine (Kim and Chae, 2003) and cause acute enteritis in swine of all ages, which is characterised by vomiting, diarrhoea, and dehydration; the mortality rate in seronegative suckling piglets may reach 100% (Chae et al., 2000). Despite the similar clinical diseases and lesions induced, PEDV and TGEV are distinct viral entities. PEDV is unable to grow in porcine cell cultures permissive to the growth of TGEV, such as PK15 cells, and is more closely related to the human respiratory coronavirus HCoV 229E than to TGEV according to the amino acid sequence of the membrane protein (Kim et al., 1999).Enterocytes are connected to each other to form a barrier that separates the inside of the organism from its environment. Essential components of this epithelial fence are the tight and adherens junctions (TJs and AJs) (Cereijido et al., 2008). Some pathogens use tight junction proteins as receptors for their attachment and subsequent internalisation, such as Hepatitis C virus (HCV) and reoviruses (Evans et al., 2007; Guglielmi et al., 2007). Infectious enteric agents that alter tight junctions often elicit inflammatory cascades and cause diarrhoea. Rotaviruses are a major cause of viral gastroenteritis leading to diarrhoea and morbidity in mammals. Its VP8 protein alters the localisation of claudin-3, ZO-1 and occludin, which consequently leads to disruption of the barrier integrity of tight junctions during their infection (Nava et al., 2004). TGEV and PEDV also cause severe diarrhoea in piglets, but it is not known whether this is related to damage to the TJs and AJs of epithelial cells.In addition, TJs and AJs use a variety of transmembrane proteins linked to the microfilaments and to intracellular signalling molecules (Etournay et al., 2007; Gonzalez-Mariscal et al., 2008). Following the changes to TJs and AJs, the microfilaments of the host cell are often co-opted by viruses at many stages of their life cycle,

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such as attachment, internalisation, endocytosis, nuclear targeting, replication, assembly, or cell-to-cell spread. Viruses induce rearrangements of microfilaments so that they can utilise them as tracks or move them aside when they represent barriers. Viral particles recruit molecular motors in order to hitchhike rides to different subcellular sites which provide the proper molecular environment for uncoating, replicating and packaging viral genomes (Burckhardt and Greber, 2009; Vaughan et al., 2009). However, there are few reports about the interactions of PEDV and TGEV with microfilaments of host cells.Mitogen-activated protein (MAP) kinases are Ser/Thr protein kinases that respond to extracellular stimuli such as growth factors and stress, among others. Previous studies have shown that the MAPK signalling pathway cannot only up- or down-regulate the expression of several TJ proteins to alter the molecular composition within TJ complexes, but also plays an important role in microfilament remodelling (Gerits et al., 2007; Melamed et al., 1995).To clearly answer the questions of whether PEDV and TGEV infection could impair the tight and adherens junctions of epithelial cells, how interactions with PEDV, TGEV and microfilaments of epithelial cells occur, and how to change the proteins in the MAPK signalling pathway, we used PEDV and TGEV to infect the porcine intestinal epithelial cell line (IPEC-J2). Results showed that both PEDV and TGEV could infect IPEC-J2 cells, and that they could also down-regulate certain proteins of tight and adherens junctions to alter the epithelial barrier integrity. In addition, they affected the polymerisation and depolymerisation of microfilaments in IPEC-J2 cells, and the disordered microfilaments could also suppress the replication and release of these viruses. Furthermore, PEDV + TGEV co-infection enhanced the damage to tight junctions and the remodelling of microfilaments. Finally, PEDV and TGEV infection impacted on activation of the MAPK signalling pathway in IPEC-J2 cells, and MAPK inhibitor could affect the tight junctions and microfilaments of cells.

2. Materials and methods

2.1. Cell lines and culture

The IPEC-J2 cells (Liu et al., 2010) were donated by Zhanyong Wei from Henan Agricultural University (Henan Province, Zhengzhou, China). ST (pig testis cell line) and Vero (African green monkey kidney cell line) cells were provided by Jiangsu Academy of Agricultural Sciences (JAAS, Jiangsu Province, Nanjing, China). IPEC-J2 cells were cultured and maintained in RPMI 1640 medium (Gibco, USA), supplemented with 7% foetal calf serum (FBS; Gibco, USA), penicillin (100 IU/ml), and streptomycin (100 mg/ml) (Invitrogen, USA). Vero and ST cells were cultured in Dulbecco modified Eagle's medium (Gibco, USA), supplemented with 5% foetal bovine serum, penicillin (100 IU/ml), and streptomycin (100 mg/ml) solutions.

TGEV (STC3 strain) (He et al., 2001), PEDV (CV777 strain) (Brian and Baric, 2005) were also provided by JAAS. TGEV was propagated in ST cells and PEDV was propagated in Vero cells; these were purified as described by Hofmann and Wyler (1988).

Confluent monolayers of IPEC-J2 cells were inoculated with TGEV at a multiplicity of infection (MOI) of 0.1, PEDV at an MOI of 0.1, and PEDV + TGEV both at an MOI of 0.1 for 1 h at 37 °C. Then, the inoculums and unattached viruses were removed and fresh growth medium was added. Infected cells were analysed after the required incubation period.

2.2. Immunofluorescence staining

To examine whether the PEDV/TGEV infected the IPEC-J2 cells, the TGEV-, PEDV-, TGEV + PEDV-infected and mock-infected cells at 0, 6, 12, 24, 48, 72 h post-infection (pi) were determined by indirect immunofluorescence. All group cells grown on glass cover slips in 24-well tissue culture plates were fixed with 3.7% polyoxymethylene, washed 3 times with 0.1 M PBS and permeabilised for 5 min with 0.1% Triton X-100. The cells were incubated with a 1% solution of BSA (30 min, room temperature, RT), then stained with FITC-conjugated TGEV polyclonal antibody (VMRD, USA) and monoclonal mouse anti-PEDV M protein (a gift of Zhang Zhi ban, Harbin Veterinary Research Institute, China) respectively (overnight, 4 °C in dark conditions). Following washing 5 times with 0.1 M PBS, a Cy3-conjugated sheep anti-mouse IgG (A0521, Beyotime, China) was added to the cells (1:400,1 h, RT). The negative control slices were treated in an identical manner except the primary antibodies were omitted. Cells were washed 3 times with 0.01 M PBS and images were acquired using a fluorescence microscope (Zeiss, Germany).

To examine the effects of PEDV/TGEV on the cytoskeletal organisation of IPEC-J2 cells, all groups at 0, 20, 40 minpi, and 1, 2, 12, 24, 48, 96 hpi, were stained with FITC-Phalloidin and determined by immunofluorescence. In short, washed infected cells were fixed with polyoxymethylene, and permeabilised for 5 min with 0.1% Triton X-100. The cells were incubated with a 1% solution of BSA (30 min, RT), and stained with FITC-Phalloidin (30 min, RT, in dark conditions). Images were acquired using a fluorescence microscope (Zeiss, Germany).

2.3. Virus titration assay

TGEV-, PEDV-infected and mock-infected cells were seeded onto 6-well plates (5×10^5) and cultured for 2, 6, 12, 24, 48, 72 h. TGEV and PEDV were collected by freezing and thawing the plates three times, and determined by the tissue culture infectious dose 50 ($TCID_{50}$) in ST and Vero cells, respectively.

2.4. Transepithelial electrical resistance (TEER) assay

TEER, a measure of tight junctions (TJ) integrity, is measured by epithelial tissue volt-ohmmeter (Millicell ERS-2, Millipore Corporation, USA) as described previously (Shatos et al., 1992). In short, IPEC-J2 cells were seeded on 24 collagen-coated Millicell filter inserts with a 0.4 mm pore size (Millipore, USA). When the IPEC-J2 cells reached confluence and were infected TGEV/PEDV at an m.o.i. of 0.1. TEER was calculated from the measured potential difference between the apical and basolateral sides of the cell layer by epithelial tissue volt-ohmmeter. All cells were measured in real-time. Resistance is expressed as both $\Omega\,cm^2$ and percent original TEER value relative to a control:

$$\text{Normal resistance}(\Omega\,cm^2) = (\text{TEER1} - \text{blank}) \times 0.33\,cm^2$$

(for 24 well Millicell Filter)

2.5. Permeability measurements

Permeability was determined by measuring the paracellular passage of 4 kDa fluorescein isothiocyanate dextran (FITC-dextran) (Sigma–Aldrich, USA) dissolved in HBSS (1 mg/ml, Gibco, USA). IPEC-J2 cells were seeded on 24 well collagen-coated Millicell filter inserts with a 0.4 mm pore size (Millipore, USA). After the IPEC-J2 cells reached confluence and infected TGEV/PEDV at an MOI of 0.1. To measure flux in the apical to the basolateral direction, 200 μl of the FITC-dextran tracer solution was loaded onto the apical side of the monolayer and cells were incubated for 20, 40 or 60 min at 37 °C. After this period, 100 μl of the solution was measured for the tracer concentration in the basolateral compartment using a BioTek fluorescence/absorbance microplate reader at an excitation wavelength of 492 nm and emission of 520 nm.