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Cellular entry of the porcine epidemic diarrhea virus

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ABSTRACT

Porcine epidemic diarrhea virus (PEDV), a coronavirus discovered more than 40 years ago, regained notoriety recently by its devastating outbreaks in East Asia and the Americas, causing substantial economic losses to the swine husbandry. The virus replicates extensively and almost exclusively in the epithelial cells of the small intestine resulting in villus atrophy, malabsorption and severe diarrhea. Cellular entry of this enveloped virus is mediated by the large spike (S) glycoprotein, trimers of which mediate virus attachment to the target cell and subsequent membrane fusion. The S protein has a multidomain architecture and has been reported to bind to carbohydrate (sialic acid) and proteinaceous (aminopeptidase N) cell surface molecules. PEDV propagation in vitro requires the presence of trypsin(-like) proteases in the culture medium, which capacitates the fusion function of the S protein. Here we review the current data on PEDV entry into its host cell, including therein our new observations regarding the functional role of the sialic acid binding activity of the S protein in virus infection. Moreover, we summarize the recent progress on the proteolytic activation of PEDV S proteins, and discuss factors that may determine tissue tropism of PEDV in vivo.

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1. Introduction

Porcine epidemic diarrhea virus (PEDV) is the causative agent of porcine epidemic diarrhea (PED), an enteric disease affecting pigs of all ages. The disease is characterized by acute watery diarrhea, dehydration and vomiting, with high mortality in neonatal piglets. Devastating outbreaks of PED in East Asia (since 2010) and in North America (since 2013) have revitalized the research into this porcine coronavirus that was first identified in 1978 (Stevenson et al., 2013). PEDV primarily replicates in the villous enterocytes of the small intestine. Its entry into host cells is mediated by the spike (S) glycoprotein that is exposed on the virion surface. This key entry factor is considered the main determinant of viral host and tissue tropism. Moreover, the S protein is highly immunogenic and the main target for neutralizing antibodies. Understanding this protein's function will thus aid the design of strategies against this enteric swine coronavirus and is fundamental to our understanding of its epidemiology and pathogenesis. In this review, following a brief and general introduction on PEDV, we will describe the structure and function of the spike glycoprotein. In particular, we will report the generation of a recombinant PEDV virus harboring a large deletion in the S protein's N-terminal region for studies to assess the role of sialic acid binding activity of PEDV S in infection. Finally we will discuss the mechanism by which the S protein is proteolytically activated for membrane fusion.

1.1. Coronaviruses infecting swine

PEDV is a member of the *Coronaviridae* family (subfamily *Coronavirinae,* family *Coronaviridae,* order *Nidovirales*). This family of viruses comprises a large group of enveloped viruses with a positive-sense RNA genome of up to 32 kilobases. Coronaviruses infect a broad range of mammalian and avian hosts and can cause respiratory, enteric, hepatic and neurological disease. Pathogenic coronaviruses are found in farm animals as well as in humans and have demonstrated potential to cross the host-species barrier. Two zoonotic coronaviruses – the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) – have emerged over the last two decades, both causing severe and often fatal respiratory disease in humans. Coronaviruses have recently been subdivided into four genera: *Alphacoronavirus*, *Betacoronavirus (lineages A–D)*, *Gammacoronavirus* and *Deltacoronavirus* (de Groot et al., 2013). Pathogenic viruses in each genus include transmissible gastroenteritis virus (TGEV), human coronavirus (HCoV) 229E and HCoV-NL63 (α-CoV), mouse hepatitis virus (MHV, β-CoV, lineage A), SARS-CoV (β-CoV, lineage B), MERS-CoV (β-CoV, lineage C), avian infectious bronchitis virus (IBV, γ-CoV) and porcine deltacoronavirus (PDCoV, δ-CoV). In swine five coronaviruses have been identified, representing three of the four genera. PEDV, TGEV and the natural TGEV deletion mutant porcine respiratory virus (PRCoV) belong to the *Alphacoronavirus* genus. TGEV mainly infects epithelial cells from the small intestine and causes enteritis and fatal diarrhea in piglets; it is clinically indistinguishable from PEDV. Unlike TGEV, PRCoV mostly infects epithelial cells of the respiratory tract and alveolar macrophages causing a mild or often subclinical respiratory disease. The porcine hemagglutinating encephalomyelitis virus (PHEV) belongs to the *Betacoronavirus* genus; it targets respiratory and neuronal tissues and causes vomiting, wasting disease and neurological disorders in seronegative piglets (Straw et al., 2006). The recently identified PDCoV of the *Deltacoronavirus* genus has enteric tropism causing mild to moderate disease in young piglets (Jung et al., 2015).

1.2. PEDV epidemiology

PED was not detected in swine until the 1970s. The first PED outbreak in swine was recognized in England in 1971. Seven years later the etiological agent was identified as a coronavirus and officially named as PEDV (Pensaert and De Bouck, 1978). PED was prevalent throughout Europe causing sporadic, localized outbreaks in the 1980s, 1990s and in subsequent years (Martelli et al., 2008). PED was first reported in Asia in 1982 and since then it has had an increasingly great economic impact on the Asian swine industry. Particularly since 2010, devastating outbreaks have been reported in China and other Asian countries causing up to 100% mortality in suckling piglets (Li et al., 2012; Puranaveja et al., 2009; Sun et al., 2012). PEDV entered the United States (US) for the first time in April 2013 and this virulent strain rapidly spread across the US to 36 states, as well as to other countries in North- and South-America, including Canada (Pasick et al., 2014), Mexico (Vlasova et al., 2014), the Dominican Republic, Colombia and Peru (Ojkic et al., 2015; Oka et al., 2014). A less virulent PEDV strain has been detected in the US characterized by small genomic *in*sertions and *del*etions (S INDEL strain) in the viral spike glycoprotein. Since 2014, PEDV has reemerged in Europe including Germany (Hanke et al., 2015), Italy, Austria (Steinrigl et al., 2015), The Netherlands, Belgium (Theuns et al., 2015), Portugal (Mesquita et al., 2015), France (Grasland et al., 2015) and Ukraine (Dastjerdi et al., 2015).

1.3. PEDV pathogenesis

PEDV mainly infects and replicates in villous enterocytes of the small intestine (duodenum, jejunum and ileum) (Debouck et al., 1981; Ducatelle et al., 1981, 1982). Infection results in destruction of the intestinal epithelium with subsequent villus shortening causing watery diarrhea that lasts for about a week. Other clinical symptoms include vomiting, anorexia and fever. Pigs of all ages are susceptible, but symptoms are most severe in suckling piglets of less than one week old with mortality rates often approaching 100%. Fatality rates in weaned pigs are much lower (1–3%) while mortality has not been observed among fattening pigs (Hou et al., 2007). Many studies indicate that PEDV does not replicate outside the intestinal tract, though PEDV was detected in a recent study by RT-PCR and IHC in other organs of experimentally infected piglets including lung, liver, kidney and spleen (Park and Shin, 2014).

2. Structure, function and antigenicity of the PEDV S protein

The PEDV S protein is the key protein responsible for virus entry into the target cell. It mediates the essential functions of receptor binding and subsequent fusion of the viral and cellular membranes during cell entry, thereby releasing the viral nucleocapsid into the cytoplasm. The PEDV S protein is a ±1383-residues long glycoprotein of 180–200 kilodalton in size. Trimers of these S proteins form the club-shaped, ±20 nm long projections (spikes) on the virion surface that provide the coronavirus its typical crown-like appearance on electron micrographs (Pensaert and De Bouck, 1978). Like other CoV spike proteins, PEDV S is a type I membrane glycoprotein with an N-terminal signal peptide, a large extracellular region, a single transmembrane domain and a short cytoplasmic tail (Fig. 1). The ectodomain of coronavirus spike proteins can be divided into two domains with distinct functions: the N-terminal S1 subunit responsible for receptor binding and the C-terminal membrane anchored S2 domain responsible for membrane fusion. The border between the S1 (residues 1–726, CV777 numbering [GB: AF353511]) and S2 (residues 727–1383) subunit can be deduced from the sequence alignment with *alphacoronavirus* S proteins (e.g.