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Short communication

Porcine reproductive and respiratory syndrome virus attachment is mediated by the N-terminal domain of the sialoadhesin receptor

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ABSTRACT

Sialoadhesin (Sn) is an important receptor for viral attachment and internalization of porcine reproductive and respiratory syndrome virus (PRRSV) to porcine alveolar macrophages (PAM). To investigate whether the N-terminal domain of Sn is sufficient and/or necessary for PRRSV attachment, we constructed a series of truncated fragments of porcine Sn and expressed these in the non-permissive PK15 cell line. The first 150 amino acids comprising the entire first domain of the Sn N-terminal region was necessary for PRRSV binding to cells, and the N-terminal domain alone was sufficient for virus attachment. The attachment of PRRSV to PAM cells was inhibited by polyclonal anti-serum against the N-terminal region of porcine Sn in a dose-dependent manner. The present study demonstrates that the first domain at the N-terminus of Sn mediates PRRSV attachment to PAM cells and contributes to better understanding the interaction between PRRSV and its host cells.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important diseases affecting the swine industry worldwide and is characterized by the reproductive failure in late-term gestation in sows and respiratory disease in pigs of all ages. PRRS virus (PRRSV), the causative agent of the disease, is an enveloped virus containing a 15 kb positive-strand RNA genome (Meulenberg et al., 1997). PRRSV belongs to the family *Arteriviridae* and grouped along with the *Coronaviridae* and the *Roniviridae* in the order of the *Nidovirales* (Cavanagh, 1997; Mayo, 2002). Classically, the *Arteriviridae* share a marked *in vivo* tropism for cells of the monocyte/macrophage lineage (Plagemann and Moennig, 1992; Snijder and Meulenberg, 1998) and, in the case of PRRSV infection, porcine alveolar macrophages (PAM) are the primary target cells (Mardassi et al., 1994).

Presently, three PRRSV receptors have been identified on PAM: heparan sulphate (HS), sialoadhesin (Sn; CD169) and CD163 (Delputte et al., 2002; Vanderheijden et al., 2003; Calvert et al., 2007). Early attachment of PRRSV is mediated mainly via interaction with HS, while Sn is sufficient for both PRRSV attachment and internalization. Although HS is not essential for Sn-mediated internalization, it has been shown to enhance internalization and, thus, infection (Delputte et al., 2005). It is possible that binding Sn is a necessary first step in a pathway that also includes CD163, leading to uncoating and release of viral RNA into the cytoplasm (Calvert et al., 2007). Both the attachment of PRRSV and the infection of PAM have been shown to be dependent on the presence of sialic acid on the

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virus exterior (Delputte and Nauwynck, 2004), suggesting Sn on target cells is at least involved in virus attachment. The development of a monoclonal antibody (MAb41D3) directed against Sn that is able to completely block infection is further evidence of the essential role of Sn in PRRSV infection. Furthermore, expression of recombinant Sn in cells typically non-permissive to PRRSV infection are also able to internalize virus (Duan et al., 1998; Vanderheijden et al., 2003).

Sn is a type 1 membrane glycoprotein of the Ig superfamily (Williams and Barclay, 1988) and has a complex structure with 17 domains (Crocker et al., 1994). The porcine Sn gene is 5193 bp in length and encodes a large protein of 210 kDa that is markedly diverse from both mouse and human homologs. Recently, the N-terminal domain of Sn was considered to be involved in PRRSV attachment to macrophage cells (Delputte et al., 2007) and this led us to investigate the binding domain of porcine Sn using a series of deletion mutants.

2. Materials and methods

2.1. Cells, virus and monoclonal antibodies

PAM cells were harvested from four-week old PRRSV-negative piglets by alveolar lavage with sterile phosphate-buffered saline (PBS) containing 0.25% EDTA (Guo et al., 1996). The PK15 cell line, which is non-permissive to PRRSV infection, was maintained in DMEM (Invitrogen, USA) supplemented with 5% fetal bovine serum (FBS; Invitrogen, USA) in a humidified 5% CO_2 atmosphere at 37 °C. The PRRSV CH-1a strain (GenBank accession no. AY032626) was maintained in our laboratory. Three monoclonal antibodies directly against the nucleocapsid protein (N), matrix protein (M), and GP5 protein were generated by Dr. Zhou at HVRI, CAAS (Zhou, 2005).

2.2. Generation of the pSn recombinant vector

Total RNA was isolated from porcine alveolar macrophages (PAM) using a commercial RNeasy mini kit (QIAGEN, USA) according to the manufacturer's instructions. An oligo$(dT)_9$ primer was used for reverse transcription (RT) and the Sn gene was amplified by PCR based on the porcine Sn sequence in GenBank (accession no. AF509585) and the methods described by Vanderheijden et al. (2003). The amplified PCR product was inserted into the eukaryotic expression vector pcDNA3.1(+) (Invitrogen, USA) to yield recombinant plasmid, pSn. The plasmid pSn was purified using a kit (Promega, USA) and the concentration measured.

2.3. Transfection of non-permissive cells and immunofluorescence assay

PK15 cells were seeded into a 6-well culture plate (Nunc, USA) 1 day before transfection. When the cells were at 70–80% confluence, 2 μg purified pSn plasmid were transfected by Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions. Cells were washed three times with DMEM media (FBS-free) at 48 h post-transfection and exposed to PRRSV at an m.o.i. of 1 $TCID_{50}$. After binding for 1 h at 37 °C, cells were washed three times with PBS.

For the indirect immunofluorescence assay (IFA), the plate was fixed with 70% cold ethanol for 30 min at 4 °C. Following several washes with PBS, the fixed samples were incubated with a monoclonal antibody against the N protein of PRRSV for 1 h at 37 °C. Cells were washed three times and incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Sigma–Aldrich, USA) at a dilution of 1:100, for 1 h at 37 °C. After three washes with PBS and one final wash with deionized H_2O, samples were analyzed by fluorescence microscopy (Leica, HC). Three controls were included in the assay: cells transfected with pcDNA3.1(+) vector or PBS and exposed to PRRSV and normal PK15 cells without transfection and not exposed to virus. The process was repeated three times.

2.4. Construction of the Sn fragments

A pair of complementary oligonucleotides encoding the Sn signal peptide were synthesized, annealed, and then cloned into the Nde I and EcoR I sites of pcDNA3.1(+) to yield the pSS plasmid. Four pairs of primers were synthesized to amplify the N-terminus (49–1755 nt; designated Sia1A), middle-section (1706–3423 nt; designated Sia2B), the C-terminus region upstream of the transmembrane domain (3359–4885 nt; designated Sia3C), and the C-terminus region including the transmembrane domain and the cytoplasmic tail (4924–5190 nt; designated TMTA) (Table 1). TMTA was treated with the restriction enzymes Not I and Xba I and cloned into plasmid pSS to generate the plasmid pSSTM (Fig. 1A),

Table 1
RT-PCR primers for the amplification of the sialoadhesin fragments.

Name	Sequence (5′–3′)	RE site	Amplified fragments
SiaSignal-F	CTAGCATGGACTTCCTGCTCCTGCTCCTCCTCCTGGCTTCATCTGCTCTAGCAG	Nhe I	1–48
SiaSignal-R	AATTCTGCTAGAGCAGATGAAGCCAGGAGGAGGAGCAGGAGCAGGAAGTCCATG	EcoR I	
Sia 1F	CGGGAATTCGGCCTGGCCTCGTGGACGG	EcoR I	49–1755
Sia 1R	CGGGCGGCCGCCCCGCTGGTGCTGTGGCTG	Not I	
Sia 2F	CGGGAATTCGATGCCGGCTCATACCAC	EcoR I	1706–3423
Sia 2R	CGGGCGGCCGCGTAGGAGGCGGCATCTG	Not I	
Sia 3F	CGGGAATTCTGGTACCGAGACCAGCAGCAGC	EcoR I	3359–4885
Sia 3R	CGGGCGGCCGCAGGGCTCTGGTTCCGAAG	Not I	
TMTA-F	AGAGCGGCCGCCCACCTTCTCTGGTTCCTG	Not I	4924–5190
TMTA-R	CGGTCTAGAGACTGTGCTTTTCACAGACTG	Xba I	
Sia 150R	CGGGCGGCCGCGCACACATAGGGAG	Not I	49–498