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Original Article

Function of CD163 fragments in porcine reproductive and respiratory syndrome virus infection

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Abstract: Monocyte/macrophage scavenger receptor CD163 plays an important role in porcine reproductive and respiratory syndrome virus (PRRSV) infection. To identify the domains of CD163 involved in PRRSV infection, CD163 fragments P1 (1-798 bp), P2 (790-2046 bp), P3 (2023-3345 bp), Y-P1 (160-798 bp), Y-P2 (790-2046 bp) and Y-P3 (2143-3084 bp) were expressed by eukaryotic and prokaryotic expression systems, respectively. Infection experiments revealed that non-permissive BHK-21 cells transfected with pCD163 could be infected by PRRSV. However, cells with truncated CD163 (P1, P2, or P3) were not susceptible to PRRSV. Meanwhile, Y-P1, Y-P2, and Y-P3 were expressed in *E. coli* and antisera to these peptides were prepared in mice. A virus blocking test showed that Y-P2 protein and anti-Y-P2 mouse serum could block PRRSV infection in a dose-dependent manner, while Y-P3 protein could improve virus infection.

Keywords: PRRSV, CD163 fragments, infection, virus blocking

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important swine infectious diseases worldwide [1, 2]. It is characterized by reproductive failure in sows and gilts, and respiratory disorders in pigs of all ages [3, 4]. PRRS virus (PRRSV), the causative agent of PRRS, belongs to the family *Arteriviridae*, within the order *Nidovirales* [5]. The genome of PRRSV is 15 kb in length and includes at least nine open reading frames (ORFs) [6]. The major structural proteins are the envelope glycoprotein (GP5), matrix (M) protein and nucleocapsid (N) protein encoded by ORF 5, 6 and 7, respectively. The minor structural proteins associated with the viral envelope are GP2a, GP2b, GP3 and GP4 encoded by ORF 2a, ORF 2b, ORF 3 and ORF 4, respectively.

PRRSV has a highly restricted tropism for cells of the monocyte-macrophage lineage and porcine alveolar macrophages (PAMs) in the natural host [7-10]. Entry of PRRSV is mediated by attachment to one or more cellular receptors and/or co-receptor, which are important determinants of the highly restricted cell tropism [11, 12]. Several cellular factors have been studied for their involvement in PRRSV binding and internalization. Heparin-like molecules can bind to the PRRSV M protein and may serve as attachment factors. This interaction is followed by the internalization of virions by clathrin-coated vesicles and release of viral RNA from acidified vesicles via the attachment of sialoadhesin (CD169) to the GP5/M dimer on the surface of the virion [13]. Several studies have confirmed that monocyte/macrophage scavenger receptor CD163 plays an important role in PRRSV infection.

CD163, a member of the cysteine-rich scavenger receptor (SRCR) superfamily, is expressed selectively on the cell surface of monocytes/macrophages [14]. It comprises a large extracellular region containing a signal peptide followed by nine SRCR domains with a 35 amino acid proline-serine-threonine (PST) rich region separating SRCR 6 and 7. The second PST rich region connects SRCR 9 with the transmembrane domain and a short cytoplasmic tail, which contains a functional internalization motif [15-19]. The extracellular region of CD163 includes the essential component for ligand-binding processes [20-22].

Table 1. Primers for amplification of CD163 full length and fragment genes

Fragment Name	Primer Nucleotide Sequences[a)]	Size and Characterization of Fragments
P1	Z-1F: CCGAAGCTTATGGACAAACTCAGAATGGTGC	798 bp (mainly containing SRCR1-2)
	Z-1R: CGCGTCGACTGCTCTCAGTTTCAGGTCTGCTCCA	
P2	Z-2F: CCGAAGCTTATGAAACTGAGAGTGGTAGATGGAGT	1254 bp (mainly containing SRCR3-6)
	Z-2R: CGCGTCGACTGGCAGATTACAGAGGCCACTTGC	
P3	Z-3F: CCGAAGCTTATGCAAGTGGCCTCTGTAATCTGCT	1323 bp (mainly containing SRCR7-9, transmembrane domain and the cytoplasmic tail)
	Z-3R: CGCGTCGACTGTTGTACTTCAGAGTGGTCTCCTG	
Y-P1	Y-1F: GAGCTCACGGGTGGTGAAAACAAGTGCT	639 bp (containing SRCR1-2 but no signal peptide)
	Y-1R: CTCGAGTCTCAGTTTCAGGTCTGCTCCA	
Y-P2	Y-2F: CGGGATCCAAACTGAGAGTGGTAGATGGAGT	1254 bp (mainly containing SRCR3-6)
	Y-2R: CCGCTCGAGTCAGCAGATTACAGAGGCCACTTGC	
Y-P3	Y-3F: GAGCTCAGTGGTCAACTTCGCCTGGTC	942 bp (mainly containing SRCR7-9 and transmembrane domain)
	Z-3R: CGCGTCGACTGTTGTACTTCAGAGTGGTCTCCTG	
CD163	Forward: 5'-CGGGATCCATGGACAAACTCAGAATGGTGCTACATGAAAACTCT-3'	3345 bp
	Reverse: 5'-GCTCTAGATTGTACTTCAGAGTGGTCTCCTGAGGGATT-3'	

a): Primer sequences are in 5' to 3' direction. Restriction enzyme sites in the primers are underlined.

To identify the specific domains of CD163 required for PRRSV infection of cells, a eukaryotic expression plasmid pcDNA3.1/V5-His A-CD163 with full length CD163 gene and its fragments were constructed and transfected into non-susceptible BHK-21 cells. Meanwhile fragments of CD163 were expressed in *E.coli* and antisera to these peptides were prepared in mice. The relationship of these proteins and their antisera with PRRSV were analyzed using a virus blocking method.

Materials and methods

Animals

Adult female BALB/c mice were purchased from Laboratory Animal Center of Shandong University (Shandong, China) and used to generate polyclonal antibodies against fragmented CD163 proteins. Experiments were carried out in accordance with guidelines issued by the Ethical Committee of Institute of Animal Husbandry and Veterinary Science, Shanghai Academy of Agricultural Sciences.

Cells and reagents

Primary PAMs were harvested from 4- to 6-week-old PRRSV-negative pigs by alveolar lavage with PBS. The collected fluid was centrifuged at 500× *g* for 15 min at 4°C. MA104 derived monkey kidney (MARC-145) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, USA) containing 10% fetal bovine serum (FBS). Baby hamster kidney-21 (BHK-21) cells were maintained in minimal essential media (MEM) (Invitrogen) containing 10% fetal bovine serum (FBS). Cells were grown in an incubator with 5% CO_2 at 37°C. Porcine anti-CD163 monoclonal antibody was purchased from AbD Serotec (USA) [23, 24]. Monoclonal antibody 6D10 against PRRSV nucleocapsid was prepared in our laboratory.

Preparation of PRRSV

North American type PRRSV strain TA-12 was isolated and identified by our laboratory (GenBank accession no. HQ416720). MARC-145 cells were inoculated with PRRSV TA-12 at 10^3 $TCID_{50}$/ml, incubated for 1 hr at 37°C and propagated in DMEM supplemented with 3% FBS until 80-85% cytopathic effect appeared. Then the virus was harvested by freezing and thawing infected cells three times and centrifuging at 10,000× *g* for 10 min. The supernatant was filtered and virus titer was determined as $TCID_{50}$ mL^{-1} according to the Reed and Muench method.

Cloning and sequencing of the CD163 gene

Total cellular RNA was isolated from PAM using Trizol reagent (Invitrogen) according to the manufacturer's instructions, and full length CD163 mRNA was amplified by reverse transcription polymerase chain reaction RT-PCR. Seven primer pairs (**Table 1**) were designed using DNASTAR according to a CD163 sequence published in GenBank (accession no. EU016226). The amplification was performed with a 50 μL reaction mixture containing 1.5 mM $MgCl_2$, 1×PCR buffer, 0.2 mM of each dNTP, 0.2 μM of each primer, 2.5 units TransTaq HiFi DNA Polymerase (TransGen Biotech, China) and 1 ng

of cDNA template. For full length CD163 amplification, the PCR parameters were as follows: denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 3.5 min, with a final extension step at 72°C for 10 min. PCR products were purified using a PCR extraction kit (TransGen Biotech, China) and ligated into pMD18-T for sequencing. For CD163 fragment amplification, the full length CD163 gene was used as a template. PCR parameters were as follows: denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min (Y-P1 and P1) or 2 min (Y-P2, Y-P3, P2 and P3). The final extension step was at 72°C for 10 min.

Construction of the expression vectors

Fragments of CD163 were amplified using the templates of pMD18-T-CD163 with the primers listed in **Table 1**. PCR products were digested with matching restriction enzymes and four fragmented genes were inserted into vector pcDNA3.1/V5-HIS A (Invitrogen) which were designated pCD163, P1, P2, P3. Three fragmented genes were inserted into pET-28a-(+) (Novagen, USA), and were designated Y-P1, Y-P2 and Y-P3 with the predicted molecular weights of 27 KD, 46 KD, and 39 KD, respectively. The recombinant plasmids were confirmed by PCR, enzyme digestion and sequence analysis. The concentration of plasmid was measured using spectrophotometer ND-1000 V3.2 (NanoDrop, USA).

Transfection of non-permissive cells

The plasmids of pCD163, P1, P2, and P3 were transfected into BHK-21 cells with lipofectamine 2000 (Invitrogen, USA) according to the manufacturer' instructions. Briefly, cells were plated in a 24-well cell culture plate (Haimen, China). When the cells were 80-90% confluent, the mixtures of plasmid and transfection reagent were added into the cell culture. Cells were examined 24 h post-transfection by fluorescence microscopy (LEICA, HC, Germany).

Indirect immunoflurescence assay (IFA)

Twenty-four hours after transfection with plasmids pCD163, P1, P2, and P3, BHK-21 cells were washed three times with DMEM and incubated with PRRSV at 100 $TCID_{50}$/mL for 1 h at 37°C. Then the cells were washed three times with PBS and cultured for another 24 h. Transfected cells were fixed with 75% ice-cold methanol for 30 min at 4°C and incubated with 6D10 for 1 h at 37°C, then washed three times with PBS and incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (H+L) antibody (JACKSON, USA) for 1 h at 37°C. After washing three times with PBS and one time with deionized water, the cells were examined under fluorescence microscopy (LEICA, HC, Germany). Normal cells infected with virus, cells transfected with the pcDNA3.1/V5-HIS A vector and non-infected cells were used as controls.

Expression and identification of CD163 recombinant proteins

The recombinant expression vectors were transformed into Rosetta expression bacteria and propagated in Luria-Bertani (LB) medium containing 100 μg/mL kanamycin at 37°C for 2.5 h until the OD_{600} reached 0.6-0.8. Isopropyl β-D-thiogalactopyranoside was then added in a final concentration of 1 mM. The culture was incubated for an additional 6 h at 37°C. Bacterial culture was collected and centrifuged at 12,000× *g* for 10 min at 4°C. The induced proteins (Y-P1, Y-P2, and Y-P3) were denatured and separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (12%). The proteins in the gel were transferred electrophoretically onto polyvinylidene difluoride (PVDF) membrane (Millipore, USA). Western blot analysis was performed according to the method described by Zhou *et al*. [25]. Anti His-Tag mouse monoclonal antibody (CWBIO, China) bound to the proteins was detected by horse radish peroxidase (HRP)-goat anti-mouse IgG (JACKSON, USA) and visualized with the substrate DAB (TIANGEN, China).

Generation of antibodies against CD163 recombinant proteins

Adult BALB/c mice (3 mice/group) were immunized with CD163 recombinant proteins (Y-P1, Y-P2, Y-P3) (100 μg/50 μL of PBS) mixed with equal amount Freund's adjuvant (SIGMA, USA). A total of 100 μL of the mixture was injected into each mouse via intraperitoneal injection. Freund's complete adjuvant was used for the first immunization and Freund's incomplete adjuvant was used for the rest of three injections at biweekly intervals. Mouse sera were

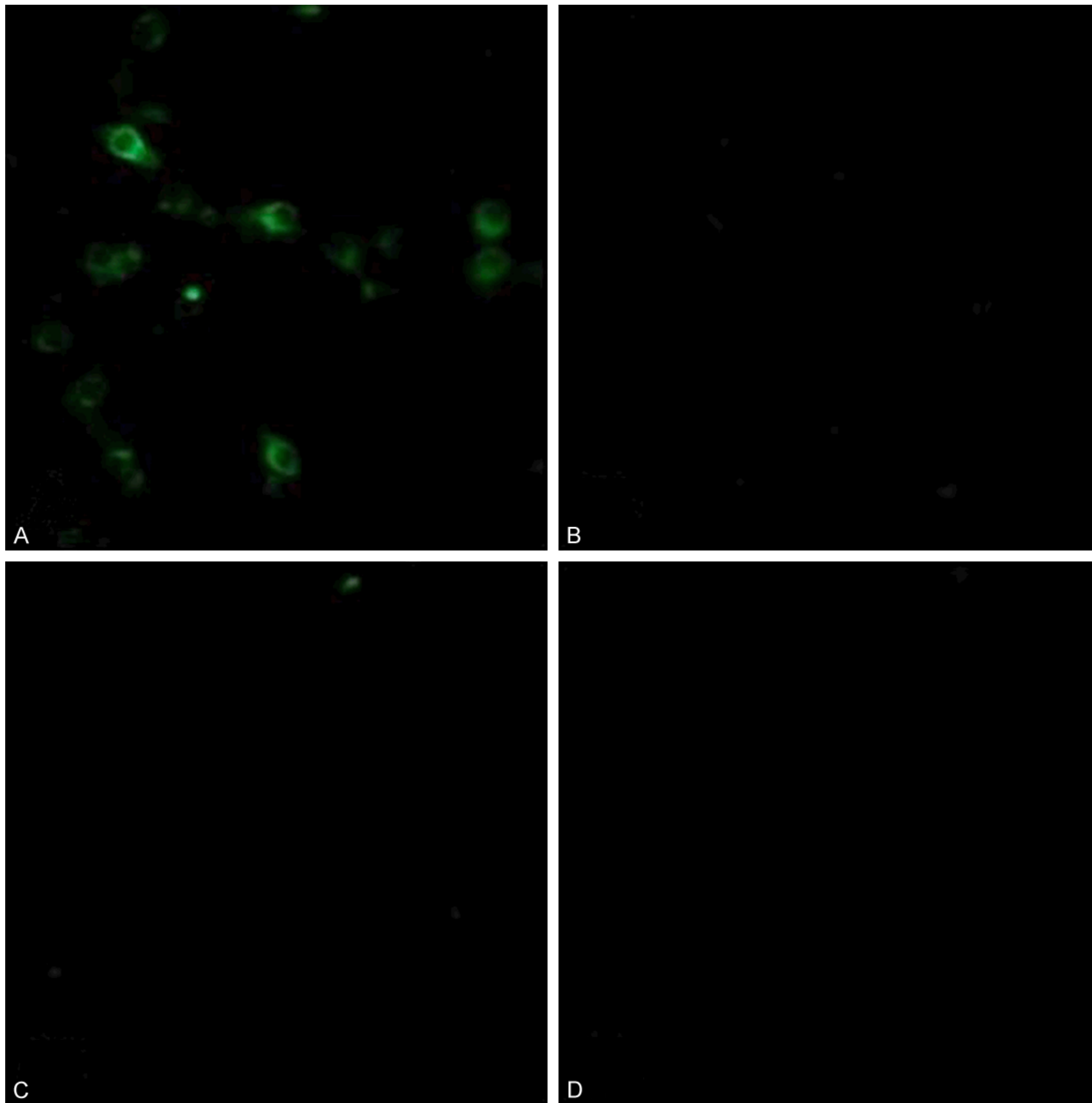

Figure 1. IFA results of PRRSV infection of pCD163 transfected BHK-21 cells. Cells were infected with PRRSV TA-12 strain at 24 h post transfection and detected with monoclonal antibody 6D10. A: BHK-21 cells transfected with plasmid pcDNA3.1/V5-His A-CD163; B: pcDNA3.1/V5-His A vector control; C: BHK-21 cells were infected with PRRSV; D: BHK-21cells were not infected with PRRSV.

collected before each immunization and serum anti- Y-P1, Y-P2, and Y-P3 were detected using an indirect ELISA. Y-P1, Y-P2, and Y-P3 were coated onto ELISA plates (NUNC, Denmark) at 2 μg/mL (100 μL/well) in PBS (0.01 M, pH 7.2) at 4°C, overnight. After washing three times with 0.01 M PBS containing 0.05% (v/v) of Tween-20 (PBS'T), the plates were blocked with 2.5% dry milk in PBS'T (200 μL/well) for 1 h at room temperature (RT). Mouse antisera diluted 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} in 2.5% dry milk in PBS'T were added at 100 μL/well and incubated for 1 h at RT. HRP-goat anti-mouse IgG (JACKSON, USA) was used to detect bound antibodies. After the final washing step, TMB (0.01% 3', 3', 5', 5' tetramethybenzidine in 0.1 M citric acid, 0.2 M Na_2HPO_4 and 0.1% H_2O_2) substrate was used to develop the colorimetric reaction for 15 min at RT. The reaction was stopped by adding 3 M H_2SO_4 in each well (50 μL/well) and read at $OD_{450\ nm}$ on an automatic ELISA plate reader.

Protein refolding by urea concentration and pH gradient

CD163 recombinant proteins were purified using the Ni-NTA column (GenScript, China) according to the manufacturer's instructions.

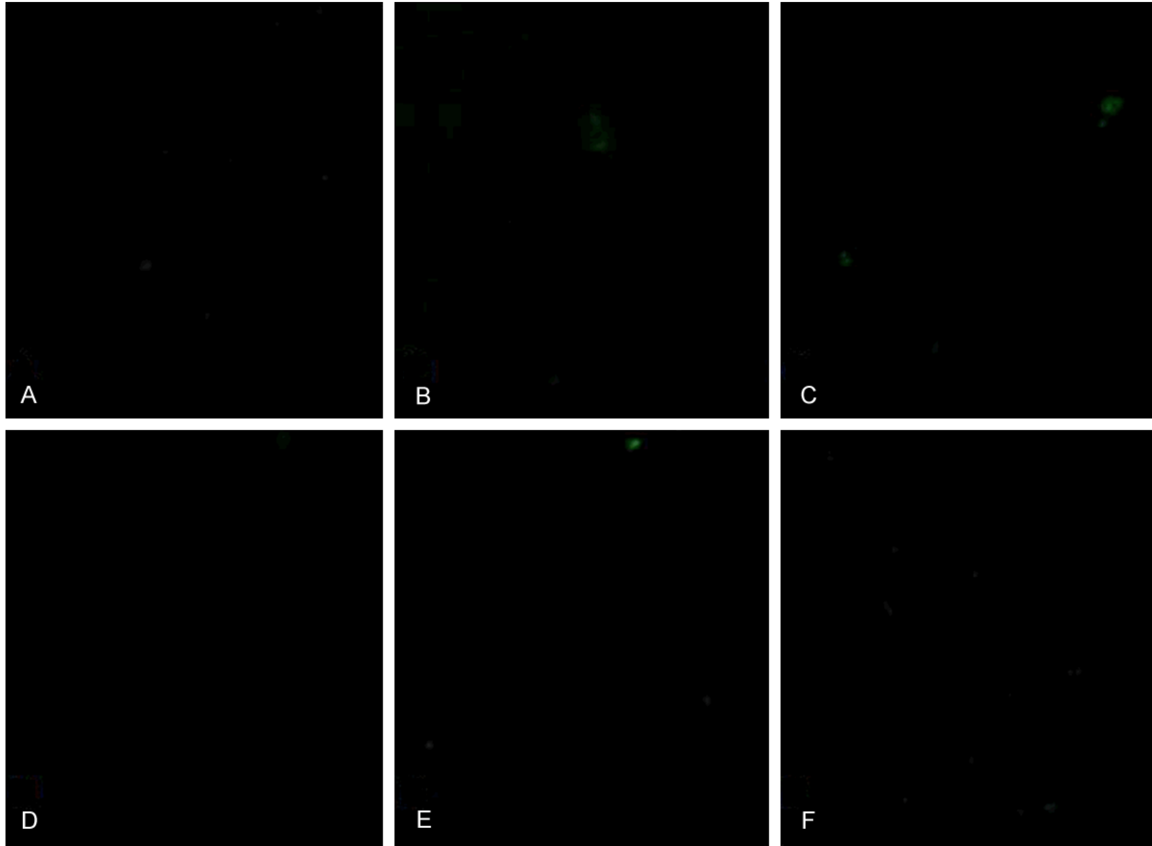

Figure 2. IFA results of PRRSV infection of P1, P2 and P3 transfected BHK-21 cells. BHK-21 cells transfected with P1 (A), P2 (B), P3 (C) were infected with PRRSV 24 hr post transfection and detected using monoclonal antibody 6D10. (D) pcDNA3.1/V5-His (A) vector control; (E) BHK-21 cells were infected with PRRSV; (F) BHK-21 cells were not infected with PRRSV.

Proteins in the elution buffer containing 3 mM reduced glutathione (GSH), 0.3 mM oxidized glutathione (GSSG) and 0.5 mM L-arginine were dialyzed in buffer A (0.01 M PBS, pH 6.0, containing 4 M urea) for 12 hr at 4°C, buffer B (0.01 M PBS, pH 7.0, containing 3 M urea), buffer C (0.01 M PBS, pH 8.0, containing 2 M urea), and buffer D (0.01 M PBS, pH 8.5, containing 1 M urea). Dialysis was carried out for 12 h at 4°C. The concentration of each recombinant protein was determined using the Bradford buffer (TIANGEN, China).

Antibody blocking assay

MARC-145 cells at a density of 1×10^5/mL were seeded into 96-well cell culture plates at 100 μL per well and incubated for 24 hr at 37°C in 5% CO_2 to reach a confluent monolayer. Mouse antisera against Y-P1, Y-P2, and Y-P3 at dilutions of 1:20, 1:40, 1:80, 1:160, and 1:320 were added into wells, in duplicate, at 100 μL per well. Equivalent amounts of pre-immune mouse sera were used as controls. Following 1 hr incubation at 37°C, PRRSV TA-12 at 100 $TCID_{50}$/well was added. After additional incubation for 1 h at 37°C, cells were washed with DMEM three times and cultured in DMEM supplemented with 3% FBS at 37°C in an incubator with 5% CO_2. Cytopathic effect (CPE) was observed and recorded every day.

Fluorescence focus assay

Monolayered MARC-145 cells were incubated with CD163 recombinant proteins and a control avian HEV ORF3 recombinant protein at concentrations of 10 μg/mL, 20 μg/mL, and 40 μg/mL for 1 h at 37°C. After washing with DMEM, TA-12 PRRSV at 100 $TCID_{50}$ in DMEM was added and incubated for 1 h at 37°C. After removing DMEM, the cells were cultured in DMEM containing 3% FBS for 24 h at 37°C and were fixed for 30 min in 75% ice-cold methanol.

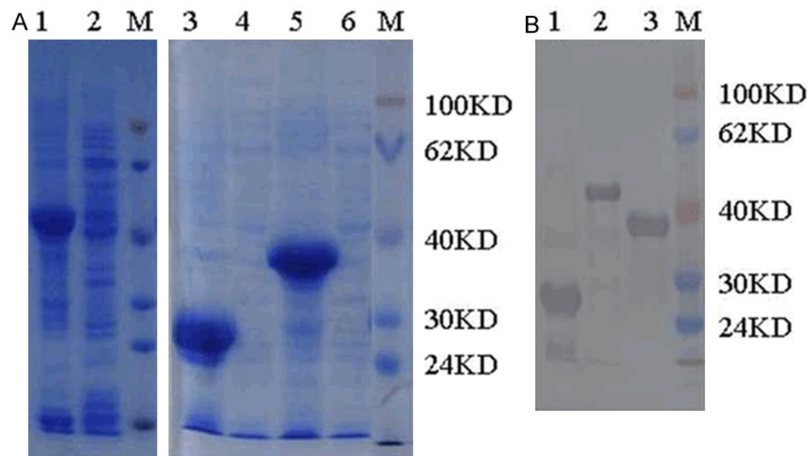

Figure 3. Identification of the fragmented recombinant CD163 proteins. SDS-PAGE results revealed the expression of Y-P1, Y-P2 and Y-P3 with MW of 27 kD, 46 kD and 39 kD (Lane 3, 1, and 5) respectively, and western blot analysis of the expression of the recombinant protein of CD163 fragments in *E.coli* (Rosetta). For (A): Lane 1 was induced Rosetta cells containing pET-28a-(Y-P2); Lane 3 was induced Rosetta cells containing pET-28a-(Y-P1); Lane 5 was induced Rosetta cells containing pET-28a-(Y-P3); Lane 2, 4 and 6 was induced Rosetta cells containing pET-28a expression vector; For (B): Y-P1, Y-P2, and Y-P3 protein (Lane 1, 2 and 3) was detected by anti-His tag monoclonal antibody, respectively; M was molecular mass standard in kilodaltons.

The remaining procedure was the same as that described for IFA. Fluorescent focus units (FFU) were counted.

Results

Construction of pCD163, P1, P2, P3, Y-P1, Y-P2 and Y-P3

Sequence analysis revealed that the full length of CD163 transcripts from PAM was amplified by RT-PCR. The amplified CD163 encoded 1115 amino acids and was 99.37% identical at the amino acid level to the sequence in GenBank (accession no. EU016226). PCR, enzyme digestion and sequence analysis showed that pCD163, P1, P2 and P3 were cloned successfully into pcDNA3.1/V5-His A and Y-P1, Y-P2, Y-P3 were cloned into pET28a-(+).

BHK-21 cells transfected with CD163 gene conferred susceptibility to PRRSV

BHK-21 cells transfected with pCD163 containing the full-length CD163 gene could be infected with PRRSV (**Figure 1A**), while cells transfected with the control vector (pcDNA3.1-HIS-A) and non-transfected cells were not infected with PRRSV (**Figure 1B-D**).

To identify the functional domains of CD163 that were required for PRRSV infection, fragmented CD163 genes (P1, P2 and P3) were cloned into the pcDNA3.1-His-A vector used to transfect BHK-21 cells. IFA results showed that BHK-21 cells transfected with P1, P2 and P3 could not be infected by PRRSV (**Figure 2A-C**). BHK-21 cells that were either transfected with the control vector or that were non-transfected could not be infected with PRRSV (**Figure 2D**, **2E**).

Expression of CD163 fragmented proteins and generation of polyclonal antibodies

To investigate the role of CD163 fragmented proteins, Y-P1, Y-P2 and Y-P3 were amplified and cloned into a prokaryotic expression system, pET-28a (+), and transformed into *Escherichia coli* with 1 mM isopropyl β-D-thiogalactoside at 37°C for the expression of recombinant proteins. The expressed CD163 proteins Y-P1, Y-P2, and Y-P3 were examined by SDS-PAGE for molecular weights of 27 KD, 46 KD, and 39 KD, respectively, (**Figure 3A**) and were confirmed by western blot assay with the use of anti-His Tag antibody (**Figure 3B**). The purified recombinant proteins were used to immunize mice for the generation of polyclonal antibodies. We calculated the ratio of absorbance of the positive serum to that of negative serum (P/N). If the ratio of P/N was less than 1.5, the result was taken as negative. If $1.5 \leq P/N < 2.1$, of the dilution of the serum will increase, the dilution point when the ratio of P/N decreases to below 2.1, is just the titer of antibody for the antiserum. The antisera against Y-P1, Y-P2, and Y-P3 were tested using an indirect ELISA with a P/N value at an $OD_{450\ nm}$ of 2.1 at a dilution of 10^{-5} (data not shown).

Blocking of PRRSV infection with antibodies against CD163 fragmented proteins

To examine the effect of polyclonal anti-CD163 fragment antibody on PRRSV infection, the monolayer MARC-145 cells were incubated with different concentrations (10, 5, 2.5, 1.25,

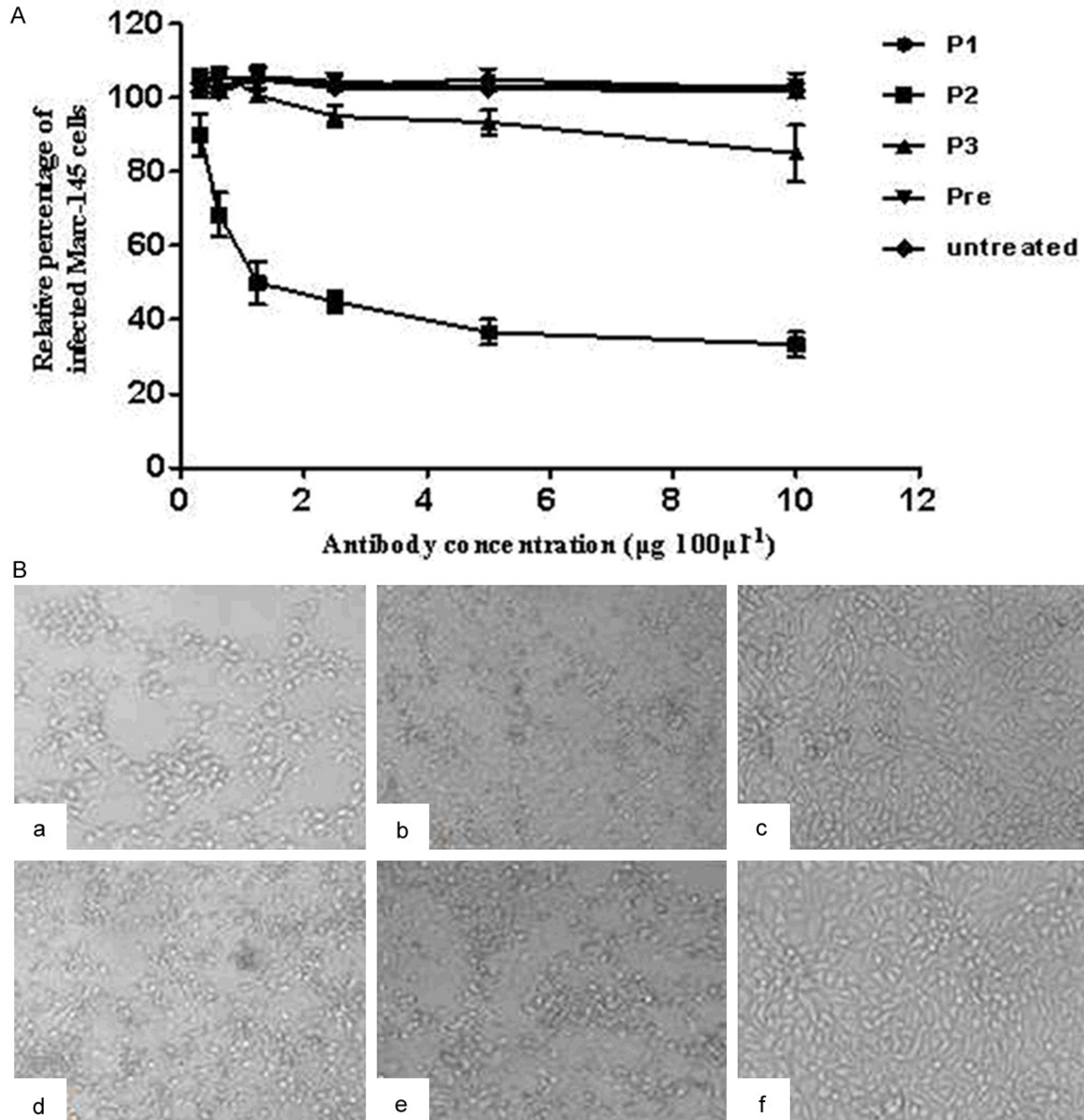

Figure 4. Effect of CD163 fragment-specific antibodies on PRRSV infection of MARC-145 cells. A: MARC-145 cells were treated with different concentrations (10, 5, 2.5, 1.25 and 0.625 μg/100 μl) of pre-immunized (▼), Y-P1 (●), Y-P2 (■), and Y-P3 (▲) specific antibodies. After inoculation with PRRSV, the relative percentage of infected MARC-145 cells was calculated, with untreated cells as reference (♦). Each value represents the mean ± SD of three experiments. B: Representative light microscopic images of MARC-145 cells infected with PRRSV. a-c: stands for Y-P1, Y-P2 and Y-P3, it was incubated with mouse serum and infected by PRRSV; d: was incubated with pre-immunized mouse serum and infected by PRRSV; e: was infected by PRRSV and no mouse serum was incubated; f: was no mouse serum and no virus.

and 0.625 μg/100 μL) of anti-CD163 fragment mouse serum at 37°C to evaluate effects on PRRSV infection. Relative percentages of infected MARC-145 cells are given in **Figure 4A**. The anti-Y-P3-specific serum reduced PRRSV infection in a dose-dependent manner (10, 5, 2.5, and 1.25 μg/100 μL). In contrast, no inhibition was observed with the anti-Y-P1 and anti-Y-P2 and pre-immunized mouse sera. Anti-Y-P3 antibody at a dilution of 1/20 could completely block PRRSV infection (**Figure 4B**).

Effect of CD163 protein fragments on PRRSV infection

To confirm whether CD163 protein fragments could block PRRSV infection, a MARC-145 cell monolayer was incubated with refolded protein

Table 2. The inhibition of proteins for PRRSV infected Marc-145 cells

Designation	Titer Determined by $\log_{10}^{FFU/mL}$						PRRSV Infection[b)]
	1st	2nd	3rd	4th	5th	Average	
Y-P1	6.2	6.0	6.5	6.2	6.1	6.20	++
Y-P2	5.1	5.0	4.5	4.3	4.1	4.60	+
Y-P3	7.5	7.3	7.0	6.5	6.8	7.02	+++
Unrelated Protein	6.2	6.1	5.9	6.3	6.3	6.16	++
No Protein	6.3	6.0	5.9	6.5	6.2	6.18	++

b): +++ = Highly positive; ++ = Moderately positive; + = Slightly positive.

at 37°C for 1 h. FFU results showed that Y-P3 protein inhibited PRRSV infection at concentrations of 200 μg/mL and 400 μg/mL, but that Y-P2 enhanced PRRSV infection at concentrations of 200 μg/mL and 400 μg/mLs when compared with ORF_3 protein from HEV, an unrelated protein. For Y-P1 protein, there was no difference in infection from the control (**Table 2** shows a concentration of 200 μg/mL).

Discussion

Enveloped viruses enter into cells via a series of steps involving interaction between viral surface proteins and cell surface molecules, followed by activation of cellular pathways that lead either to fusion at the plasma membrane or internalization of the virus particle and release of the virus genome into the cytoplasm. With an increased understanding of PRRSV entry into porcine macrophages, it is clear that multiple attachment factors, receptors and entry mediators are used in parallel or in succession [26, 27]. CD163 was shown previously to be a cellular receptor that mediated infection of MARC-145 cells and allowed PRRSV infection of some continuous cell lines upon expression of a recombinant form [28]. Furthermore, susceptibility of macrophages to PRRSV infection has been shown to be associated with high levels of CD163 expression [29].

Although CD163 is clearly essential during PRRSV entry, the exact entry mechanism is not known. Incubation of macrophages with CD163 specific antibodies at 37°C depressed PRRSV infection. However, when the cells were incubated with these antibodies at 4°C, there was no obvious inhibitory effect. This suggested that CD163 was involved in PRRSV infection of marcophages; however, probably not as attachment receptor. This is supported by analysis of PRRSV attachment and internalization in cells expressing recombinant CD163, revealing a low efficiency for PRRSV binding and internalization in these cells [30].

In this study, non-permissive cells expressing the truncated CD163 (P1, P2, or P3) did not become susceptible to PRRSV. Deletion of all extracellular SRCR domains resulted in complete loss of infection, which was in agreement with results reported previously by Van Gorp *et al.* [31]. However, this virus blocking experiment showed that anti-Y-P2 antibody could block PRRSV infection in a dose-dependent manner. These results suggested that the Y-P2 fragment (790-2,046 bp) was also involved in PRRSV infection of MARC-145 cells. Conversely, Y-P3 (2,143-3,084 bp) protein could improve PRRSV infection. It is possible that the Y-P3 protein was improperly folded, leading to a modification in its function as the PRRSV receptor. Alternatively, the CD163 cytoplasmic tail might have interacted with the host signaling mechanism to modulate PRRSV infection. Although there is no evidence to support the hypothesis that a cytoplasmic-tail-mediated signaling pathway exists during PRRSV infection and that it regulates viral replication, it is remarkable that CD163 is known to be involved in intracellular signaling, triggered by ligand binding to CD163 at the cell surface, and the induction of the protein tyrosine kinase-dependent signal pathway [21, 32, 33]. Therefore, new perspectives are needed to address this potential correlation between PRRSV infection and a host signaling pathway. Although some cellular and viral factors have been implicated in the process of PRRSV infection [34-36], their precise functions and possible interaction with the PRRSV virion have not yet been elucidated. Further studies of the interactions between different cellular and viral factors are necessary to obtain a clearer picture of the PRRSV infection process.

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Disclosure of conflict of interest

None.

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