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Short communication

Porcine reproductive and respiratory syndrome virus activates the transcription of interferon alpha/beta (IFN-α/β) in monocyte-derived dendritic cells (Mo-DC)

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is known to be a poor inducer of interferon alpha/beta (IFN-α/β), which may contribute to the delayed development of adaptive immunity and the resultant viral persistence. However, the exact mechanism by which PRRSV inhibits the induction of IFN-α/β during infection of its natural host cells remains less well defined. Here, we show that PRRSV efficiently activates the transcription of IFN-α/β in porcine monocyte-derived dendritic cells (Mo-DC) in a time-dependent and transient manner; and this effect is dependent on the activation of phosphatidylinositol 3-kinase (PI3K). Despite the abundant IFN-α transcripts detected in PRRSV-infected Mo-DC, little or no detectable IFN-α is found in the supernatants and cell lysates of PRRSV-infected Mo-DC, suggesting that PRRSV either blocks the translation of IFN-α or inhibits the RNA processing and transport. Furthermore, we observed that PRRSV infection significantly reduced the induction of IFN-α by Poly I:C treatment; and virus replication is essential to the effect since heat-inactivated PRRSV has no effect on IFN-α induction by Poly I:C. Overall, our data provide evidence for the possible role of PI3K in the activation of the transcription of IFN-α/β by PRRSV. We conclude that PRRSV inhibits the induction of IFN-α in Mo-DC by as yet undefined post-transcriptional mechanisms.

1. Introduction

Interferon alpha/beta (IFN-α/β) production by virus-infected cells represents a critical part of host's innate immunity. IFN-α/β not only induces an antiviral state in cells by activating the expression of IFN stimulated genes (ISGs) but also modulates the subsequent development of adaptive immunity (Iwasaki and Medzhitov, 2004; Welsh et al., 2012). However, a number of viruses have evolved strategies to block the induction of IFN-α/β by interfering with the transcriptional and/or translational regulatory mechanisms (Lyles, 2000; Mibayashi et al., 2007; Ruggli et al., 2005). In most of the cases, the interference with the IFN-α/β induction pathway contributes to the establishment of persistent virus infections in hosts (Haller et al., 2006).

Porcine reproductive and respiratory syndrome virus (PRRSV) is a plus-sense, single-stranded RNA virus with a genome length of approximately 15 kb. PRRSV infection is characterized by reproductive failure including abortion and stillborn piglets in infected pregnant sows and by acute respiratory disease in neonatal and young pigs. Approximately 10 million animals are infected with PRRSV each year in the US with the economic impact reaching 560 million dollars (Neumann et al., 2005). PRRSV primarily

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infects immune cells including alveolar macrophages and dendritic cells, which is believed to contribute to the immune suppression and viral persistence (Halbur et al., 1996; Suarez, 2000; Wang et al., 2007). PRRSV infections induce little or no IFN-α/β *in vivo* and *in vitro* (Albina et al., 1998; Buddaert et al., 1998). Studies have further suggested that PRRSV not only failed to induce IFN-α, but also was capable of blocking the IFN-α induction ability of other viruses such as porcine transmissible gastroenteritis coronavirus (TGEV) or dsRNA (Poly I:C) *in vitro* (Albina et al., 1998; Calzada-Nova et al., 2011; Miller et al., 2004). Overall, the existing evidence clearly suggests that PRRSV may have intrinsic properties to inhibit or reduce the induction of IFN-α/β. Several recent studies have shown the role of nonstructural proteins of PRRSV, especially Nsp1α and Nsp1β, in antagonizing the transcriptional activation of IFN-β *in vitro* (Beura et al., 2010; Chen et al., 2010; Kim et al., 2010; Song et al., 2010), but their relevance to the natural host cells such as macrophages and Mo-DC remains unknown. Here, we intended to explore the mechanisms by which PRRSV inhibits the induction of IFN-α/β in its natural host cells, porcine Mo-DC.

2. Materials and methods

2.1. Cells and viruses

Porcine monocyte-derived dendritic cells (Mo-DC) were prepared as described previously (Wang et al., 2007). Briefly, pig peripheral blood was obtained from the state-inspected South Dakota State University Meat Laboratory. Serum samples were examined for the presence of PRRSV specific antibodies using the IDEXX HerdChek® PRRS X3 Ab ELISA (Westbrook, MA) at the South Dakota State University's Animal Disease Research and Diagnostic Laboratory. Peripheral blood mononuclear cells (PBMC) were isolated from pig peripheral blood by density centrifugation using Histopaque-1077 (Sigma Inc., St. Louis, MO). CD14 positive monocytes were purified from PBMC by staining with mouse-anti-swine CD14 (AbD Serotec, UK) and goat-anti-mouse IgG microbeads (Miltenyi Biotec, Auburn, CA) followed by an immunomagnetic separation method. Purified CD14 positive monocytes were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 1% Penicillin-Streptomycin solution, 25 ng/ml of IL-4 (R&D systems Inc., Minneapolis, MN) and 10 ng/ml of GM-CSF (R&D systems Inc.) for 7 days at 37 °C in a 5% CO_2 atmosphere.

PRRSV-23983 was used in this study. It was propagated in MARC-145 cells and the supernatant of infected cells was collected and stored at −80 °C. Virus titers were determined by transferring a 10-fold serially diluted supernatant to MARC-145 cells and incubating for 5–7 days. 50% tissue culture infective doses were then calculated on the basis of the cytopathic effects caused by the virus-containing supernatants at different dilutions. Heat inactivated virus was obtained by incubating virus at 56 °C for 1 h. The loss of infectivity was confirmed by its inability to cause a cytopathic effect on MARC-145 cells.

2.2. Real-time RT-PCR

1×10^6 Mo-DC were either mock infected or infected with 0.05 MOI of PRRSV-23983. In some of the groups, cells were infected with heat-inactivated PRRSV-23983 to investigate the effect of virus replication on cellular gene transcription. In other groups, cells were treated with 25 μg/ml poly I:C alone or together with either PRRSV-23983 or heat-inactivated PRRSV-23983. Cells treated with 1 μg/ml Pam3Csk4, a TLR1/2 agonist, (Invivogen, San Diego, CA) were also included as a negative control. At 4, 12, 24, and 48 h after infection, cells were harvested and total RNAs were extracted using RNeasy protect mini kit (Qiagen, Valencia, CA) by following the manufacturer's instructions. The concentrations of RNA were determined using a NanoDrop ND-1000 spectrometer (Thermo Scientific). The same amount of RNA in each treatment group was reverse transcribed into complementary DNA (cDNA) using high-capacity cDNA reverse transcription kits (Applied Biosystem, Foster City, CA) by following the manufacturer's instructions. Real-time PCR was then performed on a Mx3000P Real-time thermocycler (Agilent Technologies). Primer sequences used in this study are shown in Table 1. For PCR amplifications, 4 μl of cDNA was added to a mixture containing 10 μl of SYBR green master mix (Brilliant II SYBR green QPCR master mix, Stratagene, Lajolla, CA), 0.3 μl of ROX reference dye (Stratagene), 0.2 μl of forward primer (50 pmol/μl) and 0.2 μl of reverse primer (50 pmol/μl). All samples were tested in duplicate and the cycling conditions were 90 °C for 10 min followed by 40 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. At the end of amplifications, cycle threshold (Ct) values were obtained. Transcripts of porcine GAPDH were also examined to normalize the amount of input RNA. Relative transcript levels were quantified by the ΔΔCT method as described previously (Guo et al., 2008).

2.3. ELISA for IFN-α

Mo-DC were treated as described above in 2.2. At 4, 12, 24, and 48 h after infection, supernatants and cells were both collected. Cytoplasmic extracts were prepared with Nucbuster protein extraction kit (EMD Bioscience, San Diego, CA) by following the manufacturer's instruction. The concentration of IFN-α in both of the supernatants and cytoplasmic extracts were measured with a porcine IFN-α specific ELISA as previously described (Diaz de Arce et al., 1992). Briefly, HRP was conjugated to the K9 MAb (PBL InterferonSource Inc., Piscataway, NJ), a porcine IFN-α specific monoclonal antibody, with a labeling kit (Lightning

Table 1
Primers used for real time RT-PCR analysis.

Primers	5′-3′ sequence	Gene accession #
GAPDH FP	AGG TCA TCC ATG ACA ACT TCG GCA	AF017079
GAPDH RP	AGC ACC AGT AGA AGC AGG GAT GAT	AF017079
IFN-α FP	ACT CCA TCC TGG CTG TGA GGA AAT	NM214393
IFN-α RP	ATC TCA TGA CTT CTG CCC TGA CGA	NM214393
IFN-β FP	TGC AAC CAC CAC AAT TCC AGA AGG	NM001003923
IFN-β RP	TCT GCC CAT CAA GTT CCA CAA GGA	NM001003923