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Variable interference with interferon signal transduction by different strains of porcine reproductive and respiratory syndrome virus

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is a positive-sense single-strand RNA virus that has contributed to substantial losses to the swine industry. The objective of this study was to examine the interference of interferon (IFN)-activated signaling by PRRSV viral proteins and compare the effects of several PRRSV strains on the IFN signaling. Three non-structural proteins (nsp 1β, 7 and 12) and two structural proteins (GP3 and N) of VR-2385 were found to significantly inhibit the expression of IFN-stimulated response element (ISRE) luciferase reporter. Nsp1β and N showed robust inhibition and their-encoding sequences from the six PRRSV strains (VR-2385, Ingelvac PRRS MLV, VR-2332, NVSL97-7895, MN184, and Lelystad) were cloned for further characterization. The nsp1βs of all the strains except MLV inhibited expression of IFN-induced ISRE reporter, interferon-stimulated gene 56 (ISG56) and signal transducer and activator of transcription 2 (STAT2). The N proteins inhibited the IFN-induced ISRE reporter expression and STAT2 elevation and blocked nuclear translocation of STAT1. In MARC-145 cells, all the six PRRSV strains with the exception of MN184 blocked the activity of exogenous IFN-α. In primary porcine pulmonary alveolar macrophages (PAMs), only MLV and NVSL failed to inhibit the activity of IFN-α. These results indicate that some PRRSV strains, such as NVSL and MN184, have variable effects on IFN-activated signaling in the two types of cells.

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1. Introduction

PRRSV is an enveloped, single-stranded positive-sense RNA virus and has contributed to substantial loss to the swine industry since it was reported in 1987 (Conzelmann et al., 1993; Meulenberg et al., 1993; Neumann et al., 2005). PRRSV is classified into the family *Arteriviridae*. The genome of PRRSV is approximately 15 kb in length with ten open reading frames (ORFs) (Faaberg et al., 2011). ORFs 1a and 1b comprise 80% of the viral genome and encode viral enzymes for RNA synthesis, including polymerase, protease, and helicase. The polypeptides from the two ORFs are processed into 14 non-structural proteins (nsps) in infected cells. ORFs 2, 2a, 3, and 4 of PRRSV encode minor membrane-associated proteins GP2, E, GP3, and GP4, respectively. ORFs 5, 6, and 7 encode a major envelope glycoprotein (GP5), a membrane protein (M) and a

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nucleocapsid protein (N), respectively (Faaberg et al., 2011). Recently, a new ORF5a was found to encode a small protein (Firth et al., 2011; Johnson et al., 2011) and additional ribosomal frame shifting was found to yield a new transframe fusion protein (nsp2TF) (Fang et al., 2012). The main target cells for PRRSV infection *in vivo* are porcine pulmonary alveolar macrophages (PAMs) (Wensvoort et al., 1991). PRRSV can be propagated *in vitro* in the epithelial-derived monkey kidney cells MARC-145 (Kim et al., 1993) and in PAMs.

PRRSV infection in pigs causes the delayed appearance and low titer of neutralizing antibodies (Labarque et al., 2000), as well as weak cell-mediated immune response (Xiao et al., 2004). PRRSV appears to inhibit synthesis of type I interferons (IFNs) in infected pigs (Albina et al., 1998; Buddaert et al., 1998). IFNs could not be detected in the lungs of pigs in which PRRSV actively replicated. In addition, PRRSV infection of PAMs and MARC-145 cells *in vitro* leads to very low IFN-α expression (Albina et al., 1998; Miller et al., 2004). The suppression of innate immunity is an important contributing factor to the modulation of host adaptive immune responses.

Type I IFNs, including IFN-α and β, are critical components of innate immunity against viral infections and play an important role in the stimulation of adaptive immune response (Gonzalez-Navajas et al., 2012; Takaoka and Yanai, 2006). The signaling of type I IFNs is initiated after they bind to their receptors on the cell surface (Darnell et al., 1994; Schindler and Darnell, 1995; Stark et al., 1998). The receptor binding activates Janus kinases (JAK), which then phosphorylate both the signal transducer and activator of transcription 1 (STAT1) and STAT2. The phosphorylation of STAT1 and STAT2 results in formation of STAT1/STAT2 heterodimers, which interact with interferon regulatory factor 9 (IRF9) and subsequently form STAT1/STAT2/IRF9 heterotrimers, also known as interferon-stimulated gene factor 3 (ISGF3). The ISGF3 is consequently translocated into the nucleus and binds to consensus interferon-stimulated response element (ISRE) in chromosome DNA to activate expression of IFN-stimulated genes (ISGs), many of which encode antiviral proteins. PRRSV inhibits the IFN-activated JAK/STAT signal transduction and ISG expression in both MARC-145 and PAM cells (Patel et al., 2010). The nuclear translocation of ISGF3 is blocked in the PRRSV-infected cells and nsp1β is responsible for the inhibition (Chen et al., 2010; Patel et al., 2010). It is not known whether other PRRSV proteins are also involved in this interference and whether different PRRSV strains have similar effects on the IFN-activated signaling in MARC-145 and PAM cells.

In this study, PRRSV viral proteins were screened for potential antagonists of IFN-activated signaling and the effect of different PRRSV strains on type I IFN activity was examined. Nsp1β, nsp7, nsp12, GP3 and N were found to be involved in inhibition of the IFN signaling. The nsp1βs of the seven strains except for MLV were able to block the IFN-activated gene expression. The N protein was also found to be able to inhibit the IFN-activated signaling. Several PRRSV strains were found to have variable inhibitory effects on the activity of IFNs in MARC-145 and PAM cells. Our results suggested that some PRRSV strains have variable effects on the IFN signaling in PAM and MARC-145 cells and that several viral proteins are involved in the inhibition.

2. Materials and methods

2.1. Cells and viruses

MARC-145 (Kim et al., 1993), HEK293 (ATCC CRL-1573), and HeLa (ATCC CCL-2) cells were maintained in DMEM supplemented with 10% fetal bovine serum. Porcine alveolar macrophages were collected and maintained in RPMI1640 medium supplemented with 10% fetal bovine serum as described (Patel et al., 2010). The Institutional Animal Care and Use Committee (IACUC) of the University of Maryland approved collecting porcine alveolar macrophages from 4-week-old healthy piglets in this study according to relevant guidelines and policies for the care and use of laboratory animals of USDA and NIH. PRRSV strain VR-2385 (Meng et al., 1994), VR-2332, Ingelvac PRRS MLV, NVSL97-7895 (GenBank accession no. AY545985), Lelystad, A2MC2 (Nan et al., 2012), and MN184 (Han et al., 2006) were used in this study.

2.2. Virus infection and IFN treatment

MARC-145 cells were plated in 12-well cell culture plates with a density of 200,000 cells per well and were grown overnight. The cells were inoculated with PRRSV at 1 multiplicity of infection (MOI). At 48 h post infection, the cells were treated with Universal Type I IFN (R&D Systems, Minneapolis, MN) at 1000 U/mL. At 12 h post IFN treatment, the cells were harvested by lysis in TRIzol for RNA isolation or Laemmli sample buffer for Western blot analysis. For PAM cells, plating was done in 12-well cell culture plates with a density of one million cells per well, followed by overnight pre-incubation. The cells were inoculated with PRRSV at 0.05 MOI and, at 15 h post infection, treated with recombinant porcine IFN-α (R&D Systems) for 8 h before being harvested for further analysis.

2.3. Plasmids

The pEGFP-C1-STAT1, for STAT1-GFP expression, was obtained from Addgene. ISRE reporter plasmid was obtained from Stratagene (Agilent Technologies, Inc., Clara, CA). The cDNAs of encoding sequences of nsp1α, nsp1β, nsp2, nsp3, nsp4, nsp5, nsp7, nsp9, nsp10, nsp11, nsp12, ORF2a, ORF2, ORF3, ORF4, ORF5a, ORF5, ORF6 and ORF7 of PRRSV VR-2385 were cloned into pCAGEN vector, individually, as described (Patel et al., 2010). The cDNAs of nsp1βs of VR-2385, NVSL, VR-2332, MLV, MN184, Shaanxi-2 and Lelystad strains were cloned into both VenusC1 and pCAGEN vector, respectively. The ORF7 cDNAs of VR-2385, NVSL, MN184 and Lelystad strains were cloned into both VenusC1 and pCAGEN vectors. The resulting recombinant plasmids were confirmed *via* restriction enzyme digestion and DNA sequencing.

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