Veterinary Microbiology 182 (2016) 156–162

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Replication characteristics of eight virulent and two attenuated genotype 1 and 2 porcine reproductive and respiratory syndrome virus (PRRSV) strains in nasal mucosa explants

Ilias S. Frydas*, Hans J. Nauwynck*

Laboratory of Virology, Department of Virology, Immunology and Parasitology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form 10 November 2015
Accepted 14 November 2015

Keywords:
PRRSV
LV
07V063
08VA
MLV-DV
13V091
VR2332
MLV-VR2332
MN-184
VN
Type 1
Type 2
Nasal mucosa
Sialoadhesin

ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) can spread in between pigs *via* contact and airborne route. It was shown before that the highly pathogenic PRRSV strain Lena was able to replicate 10–100 times more in the nasal mucosa compared to the low pathogenic PRRSV strain LV. In this work, the replication characteristics of four type 1 (LV, 07V063, 08VA, 13V091), three type 2 (VR2332, MN-184, VN) and two attenuated (MLV-DV, MLV-VR2332) PRRSV strains were studied. After 72 hpi, mean virus titers reached $10^{4.5-4.8}$ $TCID_{50}$/ml for LV and 08VA, $10^{5.2-5.4}$ $TCID_{50}$/ml for VR2332 and Lena, and $10^{5.8-6.3}$ $TCID_{50}$/ml for 07V063, 13V091, MN-184 and VN strains, whereas attenuated strains remained below detection limit. The mean number of PRRSV-positive cells/mm^2 at 72 hpi was 1.1 and 1.3 for the attenuated strains and LV, 13.3 for 08VA, 23.5 and 29.3 for VR2332 and 07063, 31.1 and 33.8 for 13V091 and Lena, and, 39.1 and 59.2 for MN-184 and VN respectively. All the LV and MLV-LV infected cells were Sn^+, whereas all other strains also infected Sn^- macrophages. In conclusion, (i) based on the virus shedding in the respiratory explants, PRRSV strains can be categorized as poor (MLV-DV, MLV-VR2332, LV, 08VA), moderate (Lena, VR2332) and strong (07V063, 13V091, MN-184, VN) secretors, and (ii) based on the number of infected cells isolates can be categorized as low (MLV-DV, MLV-VR2332, LV), moderately (08VA, VR2332), highly (07V063, Lena, 13V091) and hyper (MN-184, VN) virulent in the nasal mucosa.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the most devastating disease with massive economic losses in swine producing countries (Nieuwenhuis et al., 2012). The virus is classified into a European genotype (type 1) and a North America genotype (type 2), which show a difference of 40% at the nucleotide level (Wensvoort et al., 1991; Nelsen et al., 1999). Despite the strong progress in pathogenesis and vaccination research, it is still difficult to control PRRS and eliminate the virus. This problem is mainly due to its fast mutation rate, which is one of the highest among all RNA viruses (Hanada et al., 2005).

Previous *in vivo* studies showed that PRRSV primarily infects macrophages in the respiratory tract. Upon a cell-free viremia, the virus replicates in monocytic cells all over the body with a strong tropism for lymphoid and placental residential macrophages (Duan et al., 1997; Karniychuk and Nauwynck, 2013). The host cell receptors for PRRSV Lelystad (LV) were identified in PAM as sialoadhesin (Sn-CD169, binding and internalization receptor) and CD163 (disassembly molecule) (Vanderheijden et al., 2003; Calvert et al., 2007). Both molecules are exclusively expressed by different subsets of monocytic cells.

The virological and clinical outcome of a PRRSV infection is highly variable as it depends on the virulence of the isolate, environmental factors and presence of other pathogens (Brockmeier and Lager, 2002). It has been shown that the virus spreads by direct contact through nasal discharge, saliva, mammary secretions, urine, feces, and by transplacental spread during late gestation (Zimmerman et al., 2006). Airborne transmission is isolate-dependent and has been reported for a distance of 4–9 km, which represents a real danger in areas with a high density of swine farms (Otake et al., 2010). During the last decade, new highly virulent strains emerged in Eastern and Western Europe, and in South-East Asia, characterized by high viral

* Corresponding authors.
E-mail addresses: ilias.frydas@ugent.be (I.S. Frydas), hans.nauwynck@ugent.be (H.J. Nauwynck).

http://dx.doi.org/10.1016/j.vetmic.2015.11.016
0378-1135/© 2015 Elsevier B.V. All rights reserved.

loads in blood and tissues, more severe clinical signs with increased respiratory problems, and increased mortality (Tian et al., 2007; Karniychuk et al., 2010; Frydas et al., 2015).

In a previous study, it was shown that a highly pathogenic type 1 PRRSV strain (Lena) is able to replicate 10–100 times more efficiently than a low pathogenic strain (LV) in nasal mucosa explants (Frydas et al., 2013). Furthermore, Lena displayed a different pattern of infection, as it was able to replicate in intra- and sub-epithelial $CD163^{+}Sn^{-}$ cells, whereas LV could only replicate in lamina propria $CD163^{+}Sn^{+}$ cells (Frydas et al., 2013).

The purpose of this study was to evaluate the replication characteristics in the nasal mucosa of four type 1 (LV, 07V063, 08VA, 13V091) and three type 2 (VR2332, MN-184, VN) virulent PRRSV strains and of two attenuated PRRSV vaccine strains (MLV-DV (type 1) and MLV-VR2332 (type 2). The outcome of the study will provide new information on the replication power of different PRRSV-1 and PRRSV-2 strains in the nasal mucosa and the identification of the target cell (sialoadhesin positive/negative status). This work may help to better understand why some PRRSV strains are more virulent/pathogenic and are spreading easier than others.

2. Materials and methods

2.1. Animals and collection of tissues

Twenty weeks-old conventional Belgian Landrace pigs originating from a PRRSV-negative farm were used. The use of tissues from euthanized animals is in agreement with the laws of the Local Ethical and Animal Welfare Committee of the Faculty of Veterinary Medicine of Ghent University. All pigs were negative for relevant pathogens (SIV, PCV2, PPV). Nasal mucosa was stripped from the septum and conchae, and then immediately placed in transport medium containing phosphate buffer saline (PBS), 0.1 mg/ml gentamicin (Invitrogen), 0.1 mg/ml streptomycin (Certa), and 100 U/ml penicillin (Continental Pharma). Afterwards, the tissues were cut in small square pieces ($1\,cm^2$), placed in six-well plates with the epithelial side facing up on a fine-meshed gauze and cultured for 24 h (37 °C, 5% CO_2) at an air–liquid interface with serum-free medium containing 50% DMEM (Invitrogen), 50% Ham's F-12 GlutaMAX (Invitrogen) and supplemented with 0.1 mg/ml gentamicin (Invitrogen), 0.1 mg/ml streptomycin (Certa), 100 U/ml penicillin (Continental Pharma) and 0.01 mg/ml fungizone (Bristol-Myers Squibb, USA). Six-well plates were overlaid with 3 ml of an agarose solution consisting of a 1:1 mix of 50% of sterile 6% agarose (low gelling agarose, Sigma) and 50% of 2X Ham's F-12 GlutaMAX medium supplemented with 0.2 mg/ml streptomycin (Certa), 200 U/ml penicillin (Continental Pharma) and 0.2 mg/ml gentamicin (Invitrogen). Before inoculation, the tissue explants were transferred from the gauzes on solidified agarose and the exposed lateral edges were sealed with additional 3% agarose. Finally, the explants were incubated in the presence of 1 ml of culture medium at 37 °C, 5% CO_2.

2.2. Viruses

The type 1 07V063 strain was isolated in 2007 from fetal tissues from a Belgian farm experiencing a severe PRRSV-outbreak (Geldhof et al., 2013). PRRSV 08VA was isolated from serum of a 4-week old piglet in a subclinically infected herd. The 13V091 type 1 strain was isolated in 2013 from the serum of a young piglet from a Belgian farm experiencing severe respiratory problems (Frydas et al., 2015). VR2332 is the prototype type 2 PRRSV strain isolated in the US in the late eighties (Benfield et al., 1992). The MN-184 type 2 strain was isolated in 2001 from a commercial swine herd in Minnesota, USA, experiencing a severe outbreak of abortion and pre-weaning, nursery and finisher mortality (Cho et al., 2006). The VN strain was isolated in 2013 from a farm located in an area of South-East Asia experiencing severe problems after the emergence of new highly pathogenic PRRSV (HP-PRRSV) strains with a mortality in piglets reaching up to 91% (Ni et al., 2012). A 4th passage of American type 2 virus VR2332, a 2nd passage of the European type 1 Lelystad virus (LV) and the type 1 subtype 3 Lena, a 3rd passage of the PRRSV vaccine strain MLV-DV, a 1st passage of the vaccine strain MLV-VR2332 a 3rd passage of 07V063, 08VA, 13V091, MN-184 and a 2nd passage of VN was used. All viral stocks were propagated on MARC-145 cells in order to be able to use a high virus titer for virus inoculation and to make a comparison between the different strains. The macrophage grown and the MARC-145 cell grown viruses grew in alveolar macrophages with the same kinetics. Cell-free viral strains were used after centrifugation at 13,000 rpm for 10 min, filtration through a 0.45 μm filter and dilution in serum-free medium to a final titer of $10^{5.8}$ tissue culture infectious dose 50% endpoint ($TCID_{50}$)/ml.

2.3. Virus inoculation and sample collection of nasal explants

Explants were inoculated with 500 μl of PRRSV at a titer of $10^{5.8}$ $TCID_{50}$/ml for 1 h at 37 °C in the presence of 5% CO_2. Afterwards, they were washed two times with warm medium and further incubated with 1 ml of medium. At 0, 24, 48 and 72 hpi, medium was collected for virus titration. Culture medium was titrated on 24 h cultured PAM and on 3 days cultured MARC-145 cells in quadruplicate and a final $TCID_{50}$ was determined after subjecting the cells to a PRRSV-specific immunoperoxidase staining with monoclonal antibody 13E2 against the PRRSV nucleocapsid protein to analyze the presence of PRRSV positive cells (Van Breedam et al., 2011).

2.4. Immunofluorescence microscopy

To quantify, localize and identify PRRSV-positive cells in the nasal mucosa double immunofluorescence (IF) stainings were performed. Several 9 μm cryosections were made at a distance of 50 μm between each other and fixed in 100% methanol at −20 °C for 15 min. The presence of PRRSV-infected cells was determined by indirect immunofluorescence of cryosections from nasal explants collected at 0, 24, 48 and 72 hpi. In order to study the pattern of infection, regions of interest (ROIs) were randomly selected including the epithelium and lamina propria. Samples were incubated with a cocktail of primary antibodies including a PRRSV N-specific monoclonal antibody 13E2 (1:25, IgG_{2a}), and a mouse monoclonal antibody against porcine sialoadhesin (Sn) (41D3, 1:2, IgG_1) (Duan et al., 1998; Van Breedam et al., 2011). Subsequently, a mixture of secondary antibodies was used to reveal the different markers, including a goat anti-mouse IgG_{2a} Alexa Fluor 594 antibody (1:500, Invitrogen) and a goat anti-mouse IgG_1 Alexa Fluor 488 antibody (1:500, Invitrogen). To reduce the background signal, 10% negative goat serum was included for blocking during each step. Cell nuclei were stained with Hoechst (10 μg/ml, Hoechst 33342, Invitrogen) for 5 min at room temperature (RT). To confirm the specificity of each antibody, negative isotype-specific control antibodies were used: 13D12 against gD of PRV (IgG_1), 1C11 against gB of PrV (IgG_{2a}), at the same dilution as the primary antibodies (Nauwynck and Pensaert, 1995). Each isolate was examined on tissues obtained from three different pigs, and means were calculated. Analysis was performed in a minimum of 20 sections with 5 fields per section. PRRSV-positive cells were counted within regions of interest (ROIs) including the epithelium and the lamina propria.

Download English Version:

https://daneshyari.com/en/article/2466496

Download Persian Version:

https://daneshyari.com/article/2466496

Daneshyari.com