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Comparison of protection provided by type 1 and type 2 porcine reproductive and respiratory syndrome field viruses against homologous and heterologous challenge

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ABSTRACT

The objective of this study was to compare protection provided by type 1 and type 2 porcine reproductive and respiratory syndrome virus (PRRSV) against homologous and heterologous challenge based on clinical, virological, immunological, and pathological analysis. At 3 and 8 weeks of age, pigs were inoculated intranasally with either 3 mL of tissue culture fluid containing 10^5 $TCID_{50}$/mL of type 1 PRRSV or 3 mL of tissue culture fluid containing 10^5 $TCID_{50}$/mL of type 2 PRRSV. The homologous challenges resulted in a significant boost of the neutralizing antibodies (NA) and interferon-γ secreting cells (IFN-γ-SC) compared to heterologous challenges. The reduction of secondary challenging PRRSV viremia coincided with the appearance of homologous PRRSV-specific NA and IFN-γ-SC. Homologous challenge reduced the severity of lung lesions and levels of PRRSV viremia significantly in pigs in comparison with heterologous challenge. The differences in homologous and heterologous NA and IFN-γ-SC response may explain the differences in protection against homologous and heterologous challenge between type 1 and type 2 PRRSV. Primary challenge (immunization) with type 1 PRRSV provided protection against the secondary homologous challenge with type 1 PRRSV but failed to provide protection against the secondary heterologous challenge of type 2 PRRSV. Primary challenge with type 2 PRRSV provided protection against both the secondary homologous challenge with type 2 PRRSV and the secondary heterologous challenge with type 1 PRRSV.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the most economically devastating disease for global swine production leading to huge economic losses due to reproductive failure in sows and respiratory disease in growing pigs (Zimmerman et al., 2012). The etiological agent, PRRS virus (PRRSV), is a single, positive-stranded RNA virus and classified in the genus *Arterivirus*, family *Arteriviridae*, order *Nidovirales*, together with lactate dehydrogenase virus, simian hemorrhagic fever virus and equine arteritis virus (Snijder et al., 2013). PRRSV isolates from all over the world can be divided into two major genotypes with distinct genetic and antigenic differences: type 1 (European) and type 2 (North American) genotype (Allende et al., 1999; Murtaugh et al., 2010).

In Korea, type 2 and type 1 PRRSV were first identified in 1995 and 2005, respectively (Kweon et al., 1994; Nam et al., 2009). Cross-protection between two genotypes is a clinical issue because of co-circulation of both genotypes. With regards to cross-protection, homologous challenge with either genotype showed complete protection but heterologous challenge failed to provide protection (Lager et al., 1997, 1999; Shibata et al., 2000). In addition, cross-protection of PRRSV modified live vaccine (MLV) against other genotype provides inconsistent results. PRRSV MLV provides incomplete protection against other genotype of PRRSV (van Woensel et al., 1998; Kim et al., 2015). However, type 2 PRRSV MLV provides cross-protection against type 1 PRRSV challenge (Park et al., 2015).

Diagnostic testing has shown that sequential co-infection with both genotypes is more common than concurrent co-infection with both genotypes in co-infected pig farms based on analysis of PRRSV genotypes by real-time polymerase chain reaction (PCR) in serum samples collected at different ages (C. Chae, personal observation). Sequential co-infection with both genotypes within the same pig farm raised the possibility that immunity induced by

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prior infection of one genotype can provide cross-protection against the other genotype. Growing pigs can be exposed to single or dual genotypes of PRRSV at various growing and finishing periods. However, the clinical consequence of sequential exposure of two genotypes in growing-finishing pigs at various periods is not currently known. Moreover, there have been no reports on the natural cross-protection of respiratory disease between the two genotypes. The objective of this study was to compare protection provided by type 1 and type 2 PRRSV against homologous and heterologous challenge based on the clinical, virological, immunological, and pathological outcomes.

2. Materials and methods

2.1. PRRSV inocula

Type 1 (SNUVR090485, pan-European subtype 1) and type 2 (SNUVR090851, lineage 1) PRRSVs were used as inocula. The SNUVR090485 virus was isolated from lung samples from an aborted fetus and a weaned pig in a 1000-sow herd in southwestern Kyounggi Province (Han et al., 2012). The SNUVR090851 virus was isolated from lung samples from different newly weaned pigs and from lymph node samples from an aborted fetus in a 1000-sow herd in Chungcheung Providence in 2009 (Han et al., 2013a). The SNUVR090485 and SNU090851 share 59% and 62% nucleotide identity for open reading frame (ORF) 5 and ORF7, respectively.

2.2. Experimental design

A total of 70 colostrum-fed, cross-bred, conventional piglets were purchased at 14 days of age from a commercial PRRSV-free farm. All piglets were negative for PRRSV according to routine serological testing. All piglets were negative for type 1 and type 2 PRRSV viremia by real-time polymerase chain reaction (PCR) as previously described (Wasilk et al., 2004).

The experiment was repeated twice. In each experiment, thirty five piglets were moved to a research facility, housed each group in separate rooms, and assigned into 7 groups (5 pigs in each group) using the random number generation function (Excel, Microsoft Corporation, Redmond, Washington, USA) as follows: primary challenge of type 1 PRRSV followed by secondary challenge of type 1 PRRSV (Ch1/Ch1), primary challenge of type 1 PRRSV followed by secondary challenge of type 2 PRRSV (CH1/Ch2), primary challenge of type 2 PRRSV followed by secondary challenge of type 1 PRRSV (Ch2/Ch1), primary challenge of type 2 PRRSV followed by secondary challenge of type 2 PRRSV (Ch2/Ch2), no primary challenge of PRRSV followed by secondary challenge of type 1 PRRSV (UnCh/Ch1), no primary challenge of PRRSV followed by secondary challenge of type 2 PRRSV (UnCh/Ch2), and no primary and secondary challenge of PRRSV (UnCh/UnCh) (Table 1).

The time of post primary challenge was indicated as days post primary challenge (dppc) and the time of post secondary challenge as days post secondary challenge (dpsc). At 21 (0 dppc), and 56 (0 dpsc) days of age, the pigs were inoculated intranasally either 3 mL of tissue culture fluid containing 10^5 $TCID_{50}$/mL of type 1 PRRSV (SNUVR090485 strain, second passage in alveolar macrophages) or 3 mL of tissue culture fluid containing 10^5 $TCID_{50}$/mL of type 2 PRRSV (SNUVR090851 strain, second passage in MARC-145 cells). Blood samples were collected from each pig by jugular venipuncture for post primary challenge at 0, 7, 14, 21, 28 and 35 dppc and for post secondary challenge at 0 (same as 35 dppc), 3, 7 and 14 dpsc. Pigs were sedated by an intravenous injection of sodium pentobarbital and then euthanized by electrocution at 14 dpsc as previously described (Beaver et al., 2001). Tissues were collected from each pig at necropsy. All of the methods were approved by the Seoul National University Institutional Animal Care and Use, and Ethics Committee.

2.3. Clinical observation

Following infection of PRRSV, the pigs were monitored weekly for physical condition and scored daily for clinical respiratory disease severity using scores ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) (Halbur et al., 1995). Observers were blinded to vaccination status. Stress was induced daily by pig handler by holding the pig under his arm and taking the rectal temperature (Halbur et al., 1996). Rectal thermometer (Digital Fever Thermometer, Becton-Dickinson, Franklin Lakes, NJ, USA) was lubricated and inserted approximately 6–7 cm into the rectum and readings were taken when the thermometer beeped (Thoresen et al., 2001). Rectal temperatures were recorded daily at the same time by same personnel.

2.4. Quantification of PRRSV RNA

RNA was extracted from serum samples using the QIAamp Viral RNA Mini Kit (QIAGEN Ltd, Crawley, UK). Within the highly conserved ORF 7 region and 3′ untranslated region (UTR) of the genome of both virus types, forward primer for type 1 PRRSV 5′-GTGAATGGCCGCGATTG-3′ (nucleotide no. 14997-15013) and reverse primer 5′-CGGTCACATGGTTCCTGC-3′ (nucleotide no. 15093-15110) were selected. The forward primer for type 2 PRRSV is 5′-GTGGTGAATGGCACTGATTG-3′ (nucleotide no. 15308-15327) and reverse primer is 5′-CCCCACACGGTCGCC′-3′ (nucleotide no. 15358-15372). Two type-specific TaqMan probes were that the type 1 PRRSV specific probe 5′-TCACCTATTCAATTAGGGCG-3′ (nucleotide no. 15023-15042) was labeled with FAM attached to the 5′ terminus (reporter) and a non-fluorescent quencher (NFQ) and minor groove binder (MGB) at its 3′ end (quencher), whereas the type 2 PRRSV specific probe 5′-TCCTCTAAGTCACCTATTCAAT-TAGGGCGA-3′ (nucleotide no. 15344-15373) was 5′ labeled with

Table 1
Experimental design, microscopic lung lesion score, and in situ hybridization of PRRSV-positive cells at 14 days post challenge.

Groups	PRRSV challenge		Lung lesion score	PRRSV-positive cells within lung lesion	
	Primary (21 days)	Secondary (56 days)		Type 1	Type 2
Ch1/Ch1	Type 1	Type 1	$0.54 \pm 0.25^{\ddagger,\S}$	$9.96 \pm 8.42^{\dagger}$	0 ± 0
Ch1/Ch2	Type 1	Type 2	$2.70 \pm 0.52^{*}$	0 ± 0	$40.04 \pm 7.21^{*}$
Ch2/Ch1	Type 2	Type 1	$0.92 \pm 0.50^{\ddagger}$	$15.75 \pm 9.75^{\dagger}$	0 ± 0
Ch2/Ch2	Type 2	Type 2	$1.67 \pm 0.70^{\dagger}$	0 ± 0	$26.79 \pm 5.33^{\dagger}$
UnCh/Ch1	None	Type 1	$1.81 \pm 0.78^{\dagger}$	$27.13 \pm 4.40^{*}$	0 ± 0
UnCh/Ch2	None	Type 2	$2.90 \pm 0.81^{*}$	0 ± 0	$37.83 \pm 10.60^{*}$
UnCh/UnCh	None	None	$0.38 \pm 0.25^{\S}$	0 ± 0	0 ± 0

Symbols (*, †, ‡, and §) indicate statistically significant differences ($P < 0.05$) among groups.