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Attempts to enhance cross-protection against porcine reproductive and respiratory syndrome viruses using chimeric viruses containing structural genes from two antigenically distinct strains

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ABSTRACT

Due to significant antigenic variations between field isolates of porcine reproductive and respiratory syndrome virus (PRRSV), suboptimal cross-protection between different viruses impedes the effective control of PRRS via vaccination. Our previous study showed that chimeric viruses containing mixed structural genes from two distinct strains (VR2332 and JA142) of PRRSV were highly susceptible to the viral neutralizing activity of antisera generated against both parental strains. In this study, three chimeric viruses (JAP5, JAP56 and JAP2–6) were constructed by replacing ORF5, ORFs 5 and 6, and ORFs 2-6 of VR2332 with the corresponding genes of JA142, respectively, and their ability to confer cross-protection against challenge with the VR2332 and JA142 strains was evaluated *in vivo*. A total of 114 pigs were divided into 6 groups, and each group was intramuscularly injected with one of the 3 chimeric viruses ($n = 16$ pigs per group), VR2332 ($n = 24$), JA142 ($n = 24$), or sham inoculum ($n = 18$). At 44 days post-inoculation (dpi), these pigs were further divided into 15 groups ($n = 6$ or 8 pigs per group) and intranasally challenged with VR2332, JA142, or sham inoculum. All pigs inoculated with one of the chimeric viruses prior to challenge had lower viremia levels than the challenge control pigs. Prior inoculation with JAP56 markedly decreased viremia to nearly undetectable levels in pigs challenged with either VR2332 or JA142. These results suggest that chimeric viruses harboring mixed structural genes from two distinct PRRSV strains can provide protection against both donor viruses.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive failure in sows, including increased stillbirths, mummification, and early or delayed return to heat, and semen abnormality in boars. PRRSV also causes respiratory illness, anorexia, and pyrexia in pigs of all ages [1–4]. PRRSV was first isolated in the Netherlands in 1991 and designated the Lelystad virus (LV) [5]. PRRSV was subsequently isolated in the United States in 1992 and designated ATCC VR-2332 [6]. PRRS is currently the most important viral disease of pigs and causes enormous economic losses to the swine industry worldwide [7–9]. The annual economic loss to the US swine industry due to PRRS has been estimated to be US$ 500–660 million [10,11].

PRRSV belongs to the family *Arteriviridae*, which includes equine arteritis virus (EAV), simian hemorrhagic fever virus (SHFV) and lactate dehydrogenase-elevating virus (LDV) of mice [12]. Because the viruses identified in both Europe and North America are genetically distinct from one another, PRRSV is categorized into two genotypes; European (Type 1) and North American (Type 2) types. These two genotypes share less than 70% sequence homology in the entire genome [13,14]. More importantly, there is no significant cross-reactivity between the two genotypes in

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serological tests, and they are not cross-neutralized [15–21]. The PRRSV genome encodes at least 10 open reading frames (ORFs). ORF1a and ORF1b encode nonstructural proteins that are involved in virus replication. ORFs 2a, 2b (or E), 3, 4, 5a, 5, and 6 encode envelope-associated structural proteins, and ORF7 produces nucleocapsid (N) protein. GP5, which is encoded by ORF5, has been considered to be the most important protein for inducing VN antibodies [22–24]. GP3, GP4 and M protein expressed from ORFs 3, 4 and 6, respectively, have also been reported to play roles in inducing VN antibodies [25–27]. ORF5a, which consists of 153 nucleotides with a 143-nucleotide overlap with ORF5, was recently identified. Although an ORF5a protein-specific antibody was detected at 20 days after infection, the role of the ORF5a product in inducing virus-neutralizing (VN) antibodies is unclear [28]. In addition to inducing VN antibodies, GP3, GP4, GP5, M and N proteins have been reported to have T-cell epitopes that can stimulate cell-mediated immunity (CMI) [27,29,30]. Because several structural proteins of PRRSV have been reported to be associated with virus neutralization, the role of these proteins in the induction of VN antibodies could vary among different PRRSV strains.

A previous study demonstrated that chimeric viruses with combined structural genes from two antigenically distinct PRRSV strains (VR2332 and JA142) were susceptible to antisera generated against both donor strains *in vitro* [26]. Thus, the present study was conducted to explore whether such chimeric viruses can confer protection in pigs challenged with these two heterologous donor PRRS viruses.

Table 1
Design of the animal experiments.

Treatment & inoculum		Number of pigs			
Vaccination[a](0 dpi)	Challenge(44 dpi)	0 dpi	42 dpi	44 dpi	58 dpi
Sham inoculum		18	16[b]		
	VR2332			6	3
	JA142			6	3
	Sham			4	2
JAP5		16	16[b]		
	VR2332			8	4
	JA142			8	4
JAP56		16	16		
	VR2332			8	4
	JA142			8	4
JAP2-6		16	16		
	VR2332			8	4
	JA142			8	4
VR2332		24	24		
	VR2332			8	4
	JA142			8	4
	Sham			8	4
JA142		24	20[b]		
	VR2332			7	4
	JA142			7	4
	Sham			6	3

[a] The live virus inoculation was considered to be vaccination.
[b] Four pigs from the JA142-vaccinated group, two pigs from the sham-vaccinated group and two pigs from the JAP5-vaccinated group were culled for humane reasons due to bacterial infection during the study.

2. Materials and methods

2.1. Viruses and cells

Two field isolates of type 2 PRRSV, VR2332 [6] and JA142 [31], were used as reference viruses. These 2 strains share 91% nucleotide identity in ORF5 and 93% nucleotide identity in ORFs 2–7. Genetic and antigenic differences between these viruses have been thoroughly documented previously [32,33]. Three chimeric PRRS viruses, designated JAP5, JAP56 and JAP2-6, were previously constructed based on a VR2332-derived infectious clone [26]. The chimeric viruses were named based on the ORF(s) of the VR2332 infectious cDNA clone that was (were) replaced with the corresponding ORF(s) of JA142. All the viruses were propagated in MARC-145 cells, a sub-clone of the African green monkey kidney cell line MA104, which is known to be highly permissive to PRRSV [34]. MARC-145 cells were maintained in RPMI-1640 (Sigma–Aldrich Corporation, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS; Atlas Biologicals, Fort Collins, CO), 100 U/mL penicillin, 100 μg/mL streptomycin and 250 ng/mL amphotericin B (hereafter, RPMI-1640 growth medium) at 37 °C in a humidified incubator with 5% CO_2.

2.2. Animal study

A total of 114 three-week-old cross-bred pigs were purchased from a commercial operation historically known to be free of PRRSV and were randomly allocated to 6 groups housed separately in the Iowa State University Large Animal Infectious Disease Isolation Facility. After acclimation, each of five randomly selected groups was intramuscularly inoculated with JAP5, JAP56, JAP2-6, VR2332, or JA142 (2 mL of 10^3 $TCID_{50}$/mL per pig), which was considered live virus vaccination. The remaining group was inoculated with sham inoculum (RPMI-1640 growth medium). At 42 days post-inoculation (dpi), pigs in each group were randomly divided into 2 or 3 subgroups and housed separately in one of 15 rooms (Table 1). Then, each subgroup was intranasally challenged with JA142 or VR2332 (2 mL of 10^3 $TCID_{50}$/mL per pig) or with sham inoculum at 44 dpi. Two weeks after intranasal challenge (i.e., 58 dpi from the first inoculation), one half of the pigs in each subgroup were euthanized for necropsy. The remaining pigs were euthanized at 4 weeks after challenge (i.e., 72 dpi from the first inoculation). All the pigs were bled at 0, 7, 14, 21, 28, 35, 42, 48, 51, 55, 58, 65 and 72 dpi. During necropsy, the pigs were examined for any gross lesions. Lung samples were collected from each pig for histopathology and PRRSV immunohistochemistry (IHC) [35].

The animal use protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee (ISU-IACUC No. 8-07-6407-S), and the study was conducted in strict adherence to the appropriate animal care and well-being regulations.

2.3. Sample processing

An approximately 10% (w/v) homogenate of each lung tissue was generated in Earle's balanced salt solution (Sigma Aldrich Corporation, St. Louis, MO) using a Stomacher® Biomaster (Seward Laboratory System Inc., Port Saint Lucie, FL). After centrifugation at 3000*g* for 30 min at 4 °C, the supernatant was collected for testing. Blood samples collected into Vacutainer® SST™ Plus Blood Collection Tubes (BD, Franklin Lakes, NJ) were centrifuged at 2000*g* for 10 min, and the supernatants (i.e., serum) were transferred into new snap cap tubes. All the processed samples (lung tissue homogenates and sera) were stored at −80 °C.

2.4. Determination of viral titers in specimens

Viral RNA was extracted from sera and lung homogenates using a MagMAX™ 96 Viral RNA Isolation Kit (Ambion, Foster City, CA) with a Kingfisher® 96 Magnetic Particle Processor (Thermo scientific, Waltham, MA) according to the user manual. Commercial real-time RT-PCR using TaqMan® NA and EU PRRSV Reagents