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Development of a broadly protective modified-live virus vaccine candidate against porcine reproductive and respiratory syndrome virus

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ABSTRACT

Modified-live virus (MLV) vaccines are widely used to protect pigs against porcine reproductive and respiratory syndrome virus (PRRSV). However, current MLV vaccines do not confer adequate levels of heterologous protection, presumably due to the substantial genetic diversity of PRRSV isolates circulating in the field. To overcome this genetic variation challenge, we recently generated a synthetic PRRSV strain containing a consensus genomic sequence of PRRSV-2. We demonstrated that our synthetic PRRSV strain confers unprecedented levels of heterologous protection. However, the synthetic PRRSV strain at passage 1 (hereafter designated CON-P1) is highly virulent and therefore, is not suitable to be used as a vaccine in pigs. In the present study, we attenuated CON-P1 by continuously passaging the virus in MARC-145 cells, a non-natural host cell line. Using a young pig model, we demonstrated that the synthetic virus at passages 90 and 122 (designated as CON-P90 and CON-P122, respectively) were fully attenuated, as evidenced by the significantly reduced viral loads in serum and tissues and the absence of lung lesion in the infected pigs. Most importantly, CON-P90 confers similar levels of heterologous protection as its parental strain CON-P1. Taken together, the results indicate that CON-P90 is an excellent candidate for the formulation of next generation of PRRSV MLV vaccines with improved levels of heterologous protection.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most important viral pathogens currently affecting swine production worldwide. The virus infects pigs of all ages; however, it causes more severe clinical manifestations when infecting young pigs and pregnant sows (reviewed in [1]). According to the recently updated and amended taxonomy, PRRSV belongs to genus *Porartevirus*, family *Arteriviride*, and order *Nidovirales* [2]. There are two species of PRRSV: PRRSV-1 and PRRSV-2. These two species share approximately 65% sequence identity [3,4]. The genetic variation among PRRSV isolates within each species is substantial. Phylogenetically, PRRSV-2 isolates are classified into nine lineages, with the average genetic distances between two lineages varying from 11 to 18% [5]. PRRSV-1 isolates are classified into four subtypes, with the subtype 1 is further divided into 13 clades [6]. Multiple factors have been hypothesized to be the driving forces for the remarkable diversity of PRRSV [7].

Multiple types of PRRSV vaccines (e.g. modified live virus (MLV) vaccines, killed virus (KV) vaccines and subunit vaccines) are commercially available; of these, MLV vaccines are considered the most effective (reviewed in [8]). All PRRSV MLV vaccines currently licensed for clinical applications are produced by successive passaging of naturally occurring PRRSV strains on non-natural host cell lines such as MARC-145 cells (Review in [9]) or on a recombinant cell line stably expressing porcine CD163, a key receptor for PRRSV infection [10,11]. One major limitation of the current MLV vaccines is that they do not provide optimal levels of heterologous protection against divergent PRRSV isolates circulating in the field, presumably due to the substantial genetic variation of PRRSV (reviewed in [8,12]).

To overcome the genetic variation challenge, we recently generated a fully synthetic PRRSV strain containing a consensus genome sequence deduced from a set of 59 non-redundant full-genome sequences of PRRSV-2. We demonstrated that the synthetic consensus virus at passage 1 (hereafter designated CON-P1), while highly virulent, can provide unprecedented levels of heterologous protection to the convalescent animals [13]. In the present study, we describe the attenuation and evaluation of the protective efficacy of the attenuated PRRSV-CON.

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2. Material and methods

2.1. Cells, viruses and antibodies

MARC-145 cells, a monkey kidney cell line [14], were used for propagation and titrations of PRRSV. The synthetic CON-P1 was described previously [13]. PRRSV strain MN184C was kindly provided by Dr. K.S. Faaberg, from the U.S. National Animal Disease Center.

2.2. Virus attenuation

CON-P1 was attenuated by successively passaging the virus in MARC-145 cells. Briefly, CON-P1 virus stock was diluted in Dulbecco's Modified Eagle's Medium (DMEM) to the ratio of 1:1000 and inoculated to a monolayer of MARC-145 cells plated in a T-25 flask 48 h earlier. After 1 h of adsorption, the virus inoculum was removed and the cell monolayer was replenished with fresh DMEM containing 2% FBS. The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO2. Once early cytopathic effect (CPE) was visible (approximately 48 h post-infection), culture supernatant containing virus was harvested and clarified by centrifugation at 2000*g* for 10 min. The culture supernatant (designated CON-P2) was divided to 0.5 mL aliquots and stored at −80 °C. This procedure was repeated for a total of 122 times.

2.3. Multiple-step growth curve and plaque assay

Multiple-step growth curve and plaque assay were performed in MARC-145 cells as previously described [15,16].

2.4. Genome sequencing

Complete genomic sequences of CON-P90 and CON-P122 were determined by Next Generation Sequencing using Illumina sequencing technologies as described previously [17].

2.5. Pig experiments

Pig experiments conducted in this study were approved by the University of Nebraska-Lincoln (UNL) Institutional Animal Care and Use Committee protocol number 930. All pigs used in these experiments were PRRSV-seronegative and were accommodated in biosecurity level 2 (BSL-2) animal research facilities. The first experiment was to evaluate the levels of attenuation of CON-P90 and CON-P122 respectively. For this experiment, a total of 24 three-week old pigs were randomly assigned into four groups of six pigs. After one week of acclimation, pigs in group 1 were inoculated intramuscularly with 2 mL DMEM medium to serve as a normal control. Pigs in the remaining groups were separately inoculated intramuscularly with $10^{5.0}$ $TCID_{50}$ of CON-P1, CON-P90 or CON-P122. Blood samples were collected at various time-points post-infection (p.i.), and serum samples were extracted and stored at −80 °C for evaluation of viremia levels and seroconversion. At day 14 p.i., all pigs were humanely sacrificed and necropsied. Microscopic lung lesions were examined by a board-certified pathologist in a blind manner as described previously [18]. During necropsy, samples of tonsil and inguinal lymph node (LN) were collected for quantification of viral load in tissues.

The second experiment was to evaluate the levels of heterologous protection of CON-P90 when compared to CON-P1. For this experiment, a total of 18 three-week-old pigs were randomly divided into three groups of six pigs. After one week of acclimation, pigs in group 1 were injected intramuscularly with DMEM medium to serve as a normal control. Pigs in group 2 and 3 were vaccinated by intramuscular inoculation with $10^{5.0}TCID_{50}$ of CON-P1 or CON-P90, respectively. Blood samples were collected periodically for isolation of plasma and peripheral blood mononuclear cell (PBMC). At day 56 post vaccination (p.v.), all pigs including control group were challenged intramuscularly with heterologous PRRSV strain MN184C at a dose of $10^{5.0}$ $TCID_{50}$ per pig. At day 14 post-challenge (p.c.) all pigs were humanely sacrificed and necropsied. Samples of tonsil and inguinal LN were collected for quantification of viral loads in tissues.

2.6. Quantification of viral loads

Viral loads in serum and tissues were measured by real-time reverse transcription PCR (RT-PCR). For the first experiment, viral loads were measured by a commercial RT-PCR kit (Tetracore Inc., Rockville, MD). For the second experiment, viral loads were measured by using two different RT-PCR kits: the commercial RT-PCR kit (Tetracore, Rockville, MD) that detects total viral RNA resulting from primary and challenge infection, and a differential RT-PCR kit [13] that selectively detects viral RNA from challenge infection. Viral loads in serum were reported as $\log_{10}$ copies per mL whereas viral loads in tissues were reported as $\log_{10}$ copy per μg of total RNA used in the RT-PCR reaction. For statistical purposes, samples that had no detectable levels of viral RNA were assigned a value of 0.

2.7. Measurements of immune responses

IFN-α Porcine ProcartaPlex Simplex Kit (ThermoFisher Scientific) was used for quantification of the concentrations of IFN-α in plasma samples. The serum-virus neutralization (SVN) assay was performed as previously described [19] using plasma rather than serum. Results were expressed as the $\log_2$ of the reciprocal of the highest dilution that showed a ≥90% reduction in the number of fluorescent foci presenting in the control wells. The frequencies of IFN-γ secreting cells (IFN-γ SCs) in peripheral blood mononuclear cells (PBMCs) were measured by using an IFN-γ Elispot assay as previously described [20]. For this assay, PBMCs were stimulated with either CON-P1 or MN184C at the dose of 0.1 $TCID_{50}$ per cell.

2.8. Statistical analysis

Viremia data were analyzed by two-way analysis of variance (ANOVA) while viral loads in tissues were analyzed by one-way ANOVA. Tukey's multiple comparisons test was used for comparison among treatments. Lung microscopic scores were analyzed by Kruskal-Wallis test, followed by Dunn's multiple comparisons test. All statistical analysis was done in GraphPad Prism 7.0 (GraphPad Software, Inc).

3. Results

3.1. In vitro growth properties of CON-P90 and CON-P122

CON-P1 was successively passaged in MARC-145 cells for a total of 122 passages. We then characterized the *in vitro* growth properties of the virus at passages 90 and 120 (designated CON-P90 and CON-P122, respectively). CON-P90 and CON-P122 replicated more efficiently in MARC-145 cells than CON-P1 (Fig. 1A). There was no difference in growth kinetics between CON-P90 and CON-P122. Furthermore, plaque assay results consistently showed that CON-P90 and CON-P122 produced larger plaques than CON-P1 (Fig. 1B). Together, the data indicate that CON-P90 and CON-P122 were well adapted for replication in MARC-145 cells.

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