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Augmented immune responses in pigs immunized with an inactivated porcine reproductive and respiratory syndrome virus containing the deglycosylated glycoprotein 5 under field conditions

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Purpose: Porcine reproductive and respiratory syndrome virus (PRRSV) leads to major economic losses in the swine industry. Vaccination is the most effective method to control the disease by PRRSV.

Materials and Methods: In this study, the efficacy of a glycoprotein (GP) 5-modified inactivated vaccine was investigated in pigs. The study was performed in three farms: farm A, which was porcine reproductive and respiratory syndrome (PRRS)–negative, farm B (PRRS-active), which showed clinical signs of PRRS but had not used vaccines, and farm C (PRRS-stable), which had a history of endemic PRRS over the past years, but showed no more clinical signs after periodic administration of modified live virus vaccine.

Results: The inactivated vaccine induced great enhancement in serum neutralizing antibody titer, which was sufficient to protect pigs from further infections of PRRSV in a farm where pre-existing virus was circulating.

Conclusion: These results indicated that vaccination with the inactivated vaccine composed of viruses possessing deglycosylated GP5 would provide enhanced protection to pigs from farms suffering from endemic PRRSV.

Keywords: Porcine reproductive and respiratory syndrome virus, Inactivated vaccines, Neutralization test

Introduction

The porcine reproductive and respiratory syndrome virus (PRRSV) is a swine pathogen that generates major concerns as it causes significant economic losses in the swine industry worldwide [1]. The most realistic method to control PPRSV infection is through maintenance of herd immunity by vaccination. Currently, there are two types of commercially available porcine reproductive and respiratory syndrome (PPRS) vaccines: inactivated vaccine and modified live virus (MLV) vaccine. The MLV vaccination can be given at any stage of pig production, is generally programmed as one shot to pigs at nursery and growing stage, and as two shots to gilts and sows before breeding. While a major advantage of inactivated vaccines is its safety, their efficacy is relatively lower than MLV vaccine. It has been demonstrated that pigs vaccinated with the inactivated virus require more than two shots to reach the same enhanced immune

responses as those immunized with the MLV vaccine [2].

A major concern of the MLV vaccine is its safety. Indeed, vaccine-derived PRRSV can be detected in both the serum and fecal samples of MLV vaccinated pigs due to viral shedding, which may lead to PRRSV outbreaks through pig-to-pig transmission [3]. The MLV strain can easily regain virulence and convert to a mutant strain by recombination with field strains [4]. In addition, the protective efficacy of the MLV vaccine is generally genotype-specific. For example, MLV vaccines consisting of the North American (NA) genotype can only confer protection against NA strains, not European strains.

The commercially available inactivated vaccines are considered ineffective in protection pigs against PRRSV, even when pigs are exposed by a homologous strain [5,6]. The inactivated vaccine has been used in PRRSV-positive farms for suppressing additional damage by pre-existing virus such as field strains or vaccine-like strains. The inactivated vaccine is also advantageous as a therapeutic vaccine as it can be used on infected pregnant sows and piglets.

Numerous studies have reported on the development of effective PRRSV vaccines that improved immunogenicity and protection using techniques such as DNA vaccine, subunit vaccine, virus-like particle, and vectored vaccine [7-10]. It is critical to evaluate whether an immunogenicity-enforced inactivated vaccine can augment immune response in pigs under field conditions, thereby providing a vaccine that carries maximum benefit. In a previous study, deglycosylation of glycoprotein (GP) 5, which contains major neutralizing epitopes, in the inactivated PRRSV vaccine was demonstrated to be successful in inducing neutralizing antibody responses as protective level [11]. The aim of this study was to investigate the ability of an inactivated vaccine with the deglycosylated PRRSV in pigs reared under farm conditions. The vaccine strain used in this study was a chimeric virus consisting of structural proteins of the dominant field virus found in Korea for assessment of its application under the Korean field condition [12].

Materials and Methods

For the animal trial, three farms were selected based on the following criteria; herd size, history of PRRS outbreak, herd immunity, vaccination, and current status of PRRSV infection (Table 1). The animal experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee of Konkuk University. All pigs were kept on a similar single site-rearing system. Farm A was declared PRRSV free over the past 2 years, and had not used PRRSV vaccine. Farm B showed some clinical signs of PRRS, but refrained from vaccination due to concerns regarding side effects of commercial vaccines. Farm C had a history of endemic PRRS over the past year, but exhibited no obvious clinical signs after periodic administration of the MLV vaccine.

Table 1. Summary of farms that participated in this study

Farm	Herd size	No. of pigs	Vaccination	Pre-existing PRRSV
A	1,500	15	-	-
B	2,500	15	-	+ (LMY, VR2385)
C	4,500	15	MLV[a]	+ (VR2332[b])

PRRSV, porcine reproductive and respiratory syndrome virus; MLV, modified live virus.
[a]The 15 pigs using in this study were not vaccinated with MLV vaccine.
[b]The parental strain of MLV vaccine.

The inactivated vaccine strain used for this study, K418/GP5DM, lacked two potential N-glycosylation sites on GP5 [12]. The virus was inactivated using binary ethylenimine, and was mixed with the Montanide IMS1313 VG adjuvant (SEPPIC, Paris, France) in a 7:3 ratio. One dose of the inactivated vaccine contained 10^8 $TCID_{50}$ of K418/GP5DM.

Forty-five 3-week-old piglets were individually identified by ear-tagging. Fifteen piglets in each farm were assigned to two groups: vaccinated (12 heads) and non-vaccinated control (3 heads). Inactivated vaccine was administered to pigs in the vaccinated groups via intramuscular injection in the neck, followed by a booster vaccination 3 weeks later. Pigs in the control groups were mock vaccinated with phosphate buffered saline. All pigs were kept under regular management practices of each farm except for farm C, where the pigs in the experimental groups were not vaccinated with the MLV vaccine. Serum samples were collected at 0, and 7 weeks post-first vaccination for neutralization tests.

The polymerase chain reaction (PCR) and sequence analysis was performed with serum samples as previously described [13]. The GP5 region of PRRSV were amplified by reverse transcription PCR. The nucleotide sequences of the amplified PCR products were determined using Sanger sequencing.

The neutralization test was performed as previously described [14]. Briefly, 100 μL of the two-fold serially diluted sera were mixed with 300 $TCID_{50}$/100 μL of the K418/GP5DM vaccine strain, and kept at 4°C for 48 hours. This was combined with 50 μL of the guinea pig complement (Life Technologies, Gaithersburg, MD, USA), and was incubated at 37°C for 1 hour. The reaction mixture was added to a monolayer of

MARC-145 cells and incubated at 37°C for 5 days. The reciprocal of the highest serum dilution factor that induced a cytopathogenic effect was determined to be the neutralizing antibody titer of the sample. Neutralizing antibody titer was analyzed using the Mann-Whitney U test. A value of $p<0.05$ was considered statistically significant.

Results

No abnormal condition was observed in any of the vaccinated pigs. Growth performance of the vaccinated pigs was markedly improved, when it was compared to the non-vaccinated group at the farm B, where a PRRSV outbreak occurred. Sequence analysis revealed that the pre-existing PRRSVs in the farm B were the LMY, a strain of virus found predominantly in Korea, and the VR2385 that is a highly virulent NA strain and shares 95% of nucleotide sequence identity of full-genome with the LMY. In the farm C, the VR2332 strain, which was used as MLV vaccine, has been detected due to the vaccine-derived virus spreading from vaccinated pigs. These results indicate that pigs in the farm C used in this study were infected with the vaccine-derived virus although without MLV vaccination. All pigs used in this study were tested negative for porcine circovirus type 2 until the end of the experimental period using PCR (data not shown).

Prior to vaccinations, titer of neutralizing antibodies was measured to be negligible in all pigs across the three farms (Fig. 1). Vaccination with the inactivated K418/GP5DM virus elicited a neutralizing antibody titer of less than 1:4 in the farm A, which was PRRSV-free. At the farm C, administration of the inactivated vaccine slightly increased neutralizing antibody titer in both the vaccinated pigs and non-vaccinated pigs, indicating that the inactivated K418/GP5DM vaccine can accelerate the immune response. At the farm B, the neutralizing antibody titer was markedly enhanced as protection level in vaccinated pigs, but not in non-vaccinated pigs. This result indicates that the neutralizing antibody titers of experimental pigs in the farm B were highly affected by the inactivated vaccine, even though the pre-existing virus was LMY, a parental strain of the K418/GP5DM vaccine.

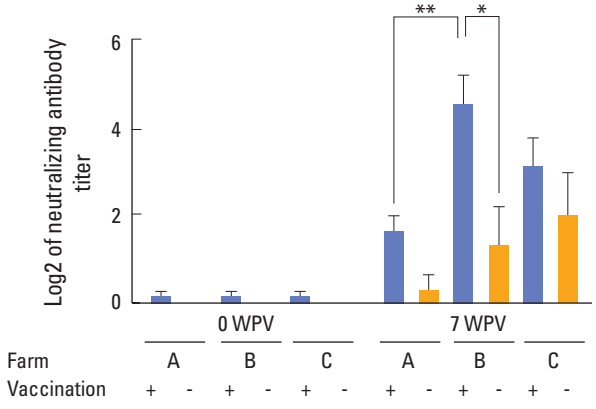

Fig. 1. Serum neutralizing antibody titer of pigs from three different farms post-vaccination with the inactivated vaccine. Farm A was porcine reproductive and respiratory syndrome–negative; farm B showed clinical signs of porcine reproductive and respiratory syndrome virus, but had not used modified live virus (MLV) vaccine; farm C had used periodic MLV vaccine in pigs since 1 week of age. WPV, weeks post-vaccination. Significant difference (*$p<0.05$ and **$p<0.01$).

Discussion

Vaccination is recognized as the most practical way to control PRRSV because it may suppress an endemic outbreak by maintaining a stable level of herd immunity, and subsequently protect pigs from new infections. Improved immunogenicity of an inactivated PRRSV vaccine through modification of the GP5 has been demonstrated to be a more efficacious alternative to the current vaccines [11,15,16]. The inactivated vaccine has been preferred over the MLV vaccine despite defects of efficacy, since it could be used as a therapeutic to immunize pregnant sows and piglets that were infected with the virus [17].

In previous studies, the inactivated vaccine failed to reduce viremia, viral shedding, and clinical lesions in the lungs of PRRSV-negative pigs [18,19]. However, it improved growth performance such as farrowing rate and health status of litters from PRRSV-infected sows [20]. These results indicate that the inactivated vaccine is more effective in farms with circulating PRRSV or farms vaccinated with MLV vaccine compared with efficacy of the inactivated vaccine in PRRSV-free farms. In the present study, usage of the inactivated vaccine with deglycosylated GP5 showed great enhancement of neutralizing antibody titer, which was enough to protect pigs from further infections in a farm where pre-existing virus was circulating. Similar to previous observations, growth performance was improved in vaccinated pigs at the farm B, where circulating PRRSV field strain was present [21].

Concentration of the K418/GP5DM antigen used in this study was 10^8 $TCID_{50}$. The neutralizing antibody response of pigs that were given 10^8 $TCID_{50}$ of the inactivated virus was enhanced compared to those that were given a lower concentration of the antigen (unpublished data).

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In summary, the mutant strain of PRRSV with exposed neutralizing epitopes, K418/GP5DM was selected as an inactivated virus vaccine candidate due to similarities in its structural proteins with the dominant field strains in Korea. This immunogenicity-enforced vaccine may not generate any immediate immune responses that are beneficial to clinically healthy pigs, but has shown significant impact on immune responses in pigs infected with field virus or vaccine-derived virus. This type of inactivated vaccine may therefore have the potential to be used as emergency vaccine for PRRSV-infected pigs in order to improve their growth performance and health status within a short period of time.

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