EFFICACY OF AN INACTIVATED PRRSV VACCINE

Induction of virus-neutralizing antibodies and partial virological protection upon challenge

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1. INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, single-stranded, positive-sense RNA virus belonging to the family *Arteriviridae*, grouped into the order *Nidovirales*, together with the *Coronaviridae* and the *Roniviridae*.[1,2] *In vivo* PRRSV has a predilection for porcine macrophages that express porcine sialoadhesin.[3,4] *In vitro* porcine alveolar macrophages (PAM), some cultivated peripheral blood monocytes and the non-macrophage African green monkey kidney cell line MA-104, and cells derived from MA-104 (Marc-145 and CL-2621) support PRRSV infection[3–5]. Two PRRSV receptors have already been identified on PAM. The glycosaminoglycan heparan sulfate is a PRRSV receptor that is involved in PRRSV attachment[6] and porcine sialoadhesin is essential for both PRRSV attachment and internalization.[4] PRRSV attachment to porcine sialoadhesin on PAM is mediated by sialic acids potentially present on the viral glycoproteins.[7]

PRRSV infection is characterized by reproductive failures in sows and respiratory problems in pigs of all ages.[1,8,9] PRRSV causes major economical losses in swine farms. Vaccination of both sows and young piglets is frequently performed to prevent this disease, however there are some problems associated with the currently used vaccines. Inactivated vaccines are safe to use in sows, becuase these vaccines do not induce reproductive failure, but their capacity to induce a protective immunity against challenge with wild-type virus has been questioned, especially in naive pigs.[10–12] Attenuated live vaccines have been proven to be effective in inducing protective immunity upon challenge with virulent PRRSV.[13,14] However, they only protect against virus-induced disease if the challenge virus is genetically and antigenically similar to the vaccine

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virus.[13,15] Only some degree of protection against heterologous strains is observed.[16] Further, there are concerns about the safety of these attenuated vaccines. Reversion of vaccine virus to virulence has been shown to occur causing major problems.[17] They can themselves spread, change genetically and be the cause of reproductive disorders. Due to the highly variable nature of RNA viruses and more specific of PRRSV, one of the major challenges of future vaccine research is to make vaccines that are safe to use and either are capable of inducing protective immunity toward the antigenically heterogenous array of viruses that are circulating, or can be quickly adapted to new circulating virus strains that are antigenically different.

Development of inactivated vaccines that are capable of inducing neutralizing antibodies would be one good strategy, as (1) the presence of neutralizing antibodies was previously shown to protect towards challenge and virus-induced disease,[18–20] (2) inactivated vaccines cannot induce disease by themselves and are thus safe to use, and (3) inactivated vaccines can rapidly be adapted to new circulating virus variants.

In this study, we wanted to investigate if neutralizing antibodies can be induced in pigs upon vaccination with an inactivated vaccine, and if vaccinated pigs were virologically protected towards challenge with wild-type PRRSV.

2. MATERIALS AND METHODS

2.1. Vaccine and Challenge Virus

Three different inactivated vaccines were used in the experiments: one based on a commercial, European type attenuated vaccine virus, one based on Marc-145 grown Lelystad virus (5th passage) and one based on porcine alveolar macrophage grown Lelystad virus (13th passage). Viruses were concentrated and semipurified by ultracentrifugation at 100,000 x *g* for 3 hours through a 30% sucrose cushion in an SW41 Ti rotor (Beckman Coulter Inc.). Virus was then inactivated with beta-propiolactone and formulated in a water/oil emulsion so that each 2 ml dose of vaccine contained an equivalent of $10^{8.0}$ $TCID_{50}$.

2.2. Pigs and Experimental Design

A total of 26 pigs were obtained from PRRSV naive sows at the age of 4 weeks. The pigs were randomly divided into 4 groups and housed in isolation units with HEPA filtered air. The designation of the groups and the experimental design is shown in Table 1. At 6 and 10 weeks of age, the pigs were vaccinated intramuscularly with the designated vaccine. Four weeks after the booster vaccination, all animals were challenged intranasally with $10^{6.0}$ $TCID_{50}$ (2 ml) of the virulent Lelystad virus strain.

Table 1. Experimental design.

Group	Number of pigs	Vaccination	Inactivated vaccine		Challenge virus
			Virus	Cell line	
A	6	No			Lelystad virus
B	12	Yes	European type attenuated vaccine	Marc-145 cells	Lelystad virus
C	4	Yes	Lelystad virus	Marc-145 cells	Lelystad virus
D	4	Yes	Lelystad virus	Porcine alveolar macrophage	Lelystad virus

2.3. Serological Examinations and Virus Titrations of Serum Samples

Starting from the first vaccination, serum was collected weekly to detect virus specific antibodies with immunoperoxidase monolayer assay (IPMA) and virus neutralizing (VN) antibodies with serum neutralization (SN) test on Marc-145 cells as described previously.[19] At 0, 3, 7, 10, 14, 21 and 28 days after challenge, serum was collected for IPMA, SN and for virus isolation.

3. RESULTS

3.1. Immunoperoxidase Monolayer Assay

None of the vaccines induced IPMA antibodies after the first immunization. Following the booster vaccination, IPMA antibodies were detected in most of the animals vaccinated with inactivated attenuated vaccine (Fig. 1). Animals vaccinated with inactivated Lelystad virus grown in Marc-145 and porcine alveolar macrophage grown had respectively low and low to undetectable levels of IPMA antibodies (Fig. 1). Upon challenge, a more rapid antibody response was observed in vaccinated animals, indicating that memory was induced.

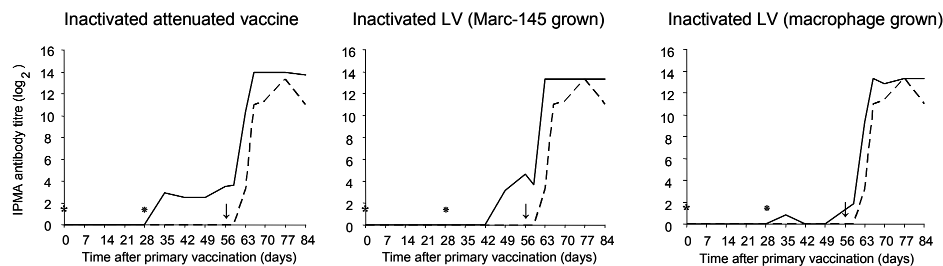

Figure 1. Course of IPMA antibody titers in pigs vaccinated twice (* *) and challenged (↓) eight weeks later with PRRSV (Lelystad) (mean, —) and non-vaccinated control pigs (mean, - -).

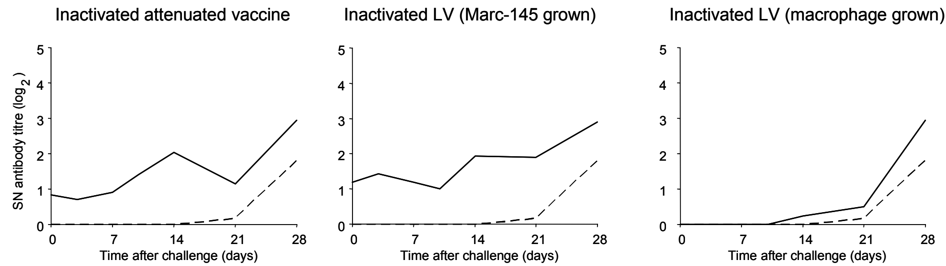

Figure 2. Course of virus neutralizing (VN) antibodies in vaccinated pigs (mean, —) and non-vaccinated control pigs (mean, - -) challenged with PRRSV (Lelystad).

3.2. Serum Neutralization Assay

None of the vaccines induced serum neutralizing (SN) antibodies after the first immunization. At the time of challenge, SN antibodies were present only in animals vaccinated with inactivated attenuated virus and Marc-145 grown Lelystad virus (Fig. 2). Upon challenge, a more rapid neutralizing antibody response was observed in vaccinated animals, indicating that memory was induced. Although vaccination not always induces neutralizing antibodies in all pigs, it is observed that vaccination enhances neutralizing antibodies upon challenge.

3.3. Viremia

Upon challenge with Lelystad virus, viremia was observed in all control animals. In 2 animals vaccinated with inactivated attenuated virus, no viremia was detected, while in the others a clear reduction in the levels and duration of viremia was observed (95% reduction at 10 d postchallenge; absolute values). Vaccination with inactivated Marc-145 and macrophage grown Lelystad virus had only a small effect on the levels of viremia, but reduced the duration of viremia (Fig. 3).

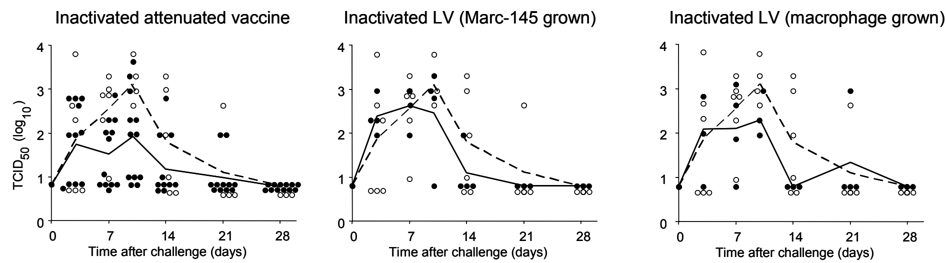

Figure 3. Virus titers in serum of vaccinated pigs (individual, ● ; mean, —) and non-vaccinated pigs (individual, ○ ; mean, - -) upon challenge with PRRSV (Lelystad).

4. CONCLUSIONS

In this study, it was shown that an inactivated vaccine can induce virus-neutralizing antibodies that results in a strong to partial virological protection upon challenge. However, differences were observed in efficacy, depending on the virus strain and the cells used to make the vaccine. Because the capacity of an inactivated vaccine to induce neutralizing antibodies is most likely correlated with the conservation of neutralizing epitopes during inactivation, we will evaluate the antigenic structure of the virus upon different inactivation methods.

5. ACKNOWLEDGMENTS

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