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Short communication

# Interferon alpha inhibits replication of a live-attenuated porcine reproductive and respiratory syndrome virus vaccine preventing development of an adaptive immune response in swine

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ABSTRACT

Type I interferons, such as interferon alpha (IFN-α), contribute to innate antiviral immunity by promoting production of antiviral mediators and are also involved in promoting an adaptive immune response. Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most devastating and costly viruses to the swine industry world-wide and has been shown to induce a meager IFN-α response. Previously we administered porcine IFN-α using a replication-defective adenovirus vector (Ad5-IFN-α) at the time of challenge with virulent PRRSV and demonstrated an increase in the number of virus-specific IFNγ secreting cells, indicating that the presence of IFN-α at the time of infection can alter the adaptive immune responses to PRRSV. In the current experiment, we explored the use of IFN-α as an adjuvant administered with live-attenuated PRRSV vaccine as a method to enhance immune response to the vaccine. Unlike the previous studies with fully virulent virus, one injection of the Ad5-IFN-α abolished replication of the vaccine virus and as a result there was no detectible adaptive immune response. Although IFN-α did not have the desired adjuvant effect, the results further highlight the use of IFN-α as a treatment for PRRSV infection.

## 1. Introduction

The host response to virus infection begins almost immediately with recognition of pathogen-associated molecular patterns by pattern recognitions receptors on host cells leading to the production of antiviral mediators, particularly interferons. Type I interferons, including interferon alpha (IFN-α), play a significant role in the antiviral immune response by stimulating the production of antiviral proteins that inhibit viral replication in host cells (Bonjardim, 2005; Katze et al., 2002). In addition, IFN-α serves as a link between innate and adaptive immunity by stimulating dendritic cells, which then activate expansion of antigen-specific T cells. Collectively, the innate and adapative response are responsible for clearing the virus and preventing future infections (Belardelli and Gresser, 1996; Bonjardim, 2005).

Porcine reproductive and respiratory syndrome virus (PRRSV) is an RNA virus that is a major world-wide pathogen of swine causing pneumonia and reproductive failure and predisposing pigs to secondary infections associated with the porcine respiratory disease complex (Rossow, 1998). One of the characteristics of PRRSV infection is that it does not generate a timely and robust immune response (Murtaugh et al., 2002). PRRSV infection induces a meager IFN-α response, which contributes to the inadequate immune response to the virus and vaccines and the persistence of PRRSV in the host (Albina et al., 1998; Loving et al., 2007; Miller et al., 2004).

Previous swine studies investigating the effects of IFN-α on the development of PRRSV disease and immunity demonstrated that pigs inoculated with a non-replicating adenovirus (Ad5) that delivers porcine IFN-α had lower febrile responses, decreased lung lesions, delayed viremia and decrease viral load in the sera in pigs challenged with a field strain of PRRSV (Brockmeier et al., 2009, 2012). In addition, an increase in the number of virus-specific IFN-γ secreting cells, as well as an altered cytokine profile in the lung 14 days post-infection, were detected in the PRRSV-infected pigs receiving Ad5-IFN-α, indicating that the presence of IFN-α at the time of infection can alter innate and adaptive immune responses to PRRSV. The current study was designed to determine whether administration of Ad5-IFN-α with attenuated PRRSV vaccine would enhance the immune response to vaccination and subsequently improve efficacy of the vaccine.

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**Table 1**
Experimental design.

| Group | Primary Vaccine | Boost |
|---|---|---|
| Group 1 | LAPV | LAPV |
| Group 2 | Ad5-pIFN-α + LAPV | LAPV |
| Group 3 | Ad5-pIFN-α | LAPV |
| Group 4 | None | None |

LAPV = Live-attenuated PRRSV vaccine [Ingelvac PRRS® ATP (Boehringer Ingelheim Vetmedica)].
Ad5-IFN-α = replication-defective human adenovirus type 5 IFN-α vector.

## 2. Materials & methods

### 2.1. Viruses

A recombinant, replication-defective human adenovirus type 5 was used to deliver porcine IFN-α (Chinsangaram et al., 2003). Pigs were given $5 \times 10^9$ plaque-forming units of the adenovirus in a 1 ml volume intramuscularly (IM) in the neck. The live-attenuated PRRSV vaccine, Ingelvac PRRS® ATP (Boehringer Ingelheim Vetmedica), was administered IM per manufacturer's instructions. PRRSV strain JA142, the parent strain to Ingelvac PRRS® ATP vaccine, was used in the ELISpot assays.

### 2.2. Swine experiment

Forty, 4-week-old pigs were divided into 4 groups of 10 pigs each that were housed in separate isolation rooms and given the following treatments (Table 1): Group 1 pigs received live-attenuated PRRSV vaccine (LAPV) only; group 2 pigs received both the adenovirus expressing IFN-α and live-attenuated PRRSV vaccine (Ad-IFN-α + LAPV) simultaneously, on opposite sides of the neck; group 3 pigs received the adenovirus expressing IFN-α only (Ad-IFN-α); and group 4 pigs were non-treated controls (None). On days 0, 1, 3, 5, 7, 14, 21 and 28 relative to vaccination, blood was collected from all pigs for serum isolation to measure PRRSV, IFN-α, and PRRSV-specific antibody levels. On day 21 after vaccination blood was also collected for peripheral blood mononuclear cell (PBMC) isolation to perform IFNγ ELISpot assays. Pigs in groups 1 and 2 were boosted with Ingelvac PRRS® ATP on day 49 after the first vaccination to determine if there would be a difference in the response to repeat vaccine exposure in these groups. Pigs in group 3 were also given Ingelvac PRRS® ATP at this time as a control group receiving vaccine for the first time. Blood was collected on day 49, 53, and 58 to collect serum for virus titration and to measure antibody response and on days 49 and 58 for PBMC isolation to perform IFN-γ ELISpot assays.

### 2.3. PRRSV isolation and titration

Virus isolation was performed by adding 50 µl of serum or lung lavage to a monolayer of MARC-145 cells in 1 well of a 24-well plate. Each well was examined for cytopathic effect (CPE) and assessed as positive or negative daily for one week of culture. Titration was completed by preparing 10-fold serial dilutions of each positive sample and adding 50 µl of each dilution to 4 wells of a monolayer of MARC-145 cells in a 96-well plate. The Reed and Muench method was used to calculate the 50% end point titer (Reed and Muench, 1938).

### 2.4. Humoral immune response

To evaluate seroconversion to PRRSV, ELISA S/P ratios were generated on collected serum samples using the HerdCheck® PRRS ELISA 2XR (IDEXX Laboratories) according to manufacturer's instructions.

### 2.5. Cell-mediated immune response

To assess peripheral PRRSV-specific IFN-γ secretory cells (ISC), whole blood was collected into BD Vacutainer® CPT tubes with sodium citrate and the PBMC collected according to the manufacturer's recommendations. PBMCs were washed once with RPMI-1640 (Invitrogen), passed through a 40 µm screen filter, washed a second time and enumerated. An ELISpot assay to enumerate ISC was performed following manufacturer's protocol (R & D Systems, Minneapolis, MN). PBMC were seeded $5 \times 10^5$ cells per well, and appropriate wells were treated in triplicate with live PRRSV at an MOI = 0.05 (strain JA142), control sham MARC medium, or pokeweed mitogen (PWM, 1ug/ml). Plates were incubated for 18 h at 37° C in 5% $CO_2$ and developed as recommended. After drying, plates were scanned, spots enumerated and quality checked using CTL-ImmunoSpot® S5 UV Analyzer and ImmunoSpot software.

### 2.6. IFN-α ELISA

IFN-α levels in the sera were determined by enzyme-linked immunosorbent assay (ELISA) using reagents from PBL Assay Science: Anti-pig IFN-α monoclonal antibodies (MAb) Clone F17 (27105-1) and Clone K9 conjugated to horseradish peroxidase (27100-1), recombinant porcine IFN-α (17100-1). The protocol was followed as previously described (Diaz de Arce et al., 1992; Lee et al., 2004). Briefly, 96-well Immulon-2 plates were coated with 3 µg F17 MAb. Samples and standards were set up in duplicate and plates were washed prior to the addition of peroxidase-conjugated K9 (300 ng/ml). To develop, TMB substrate (Life Technologies, SB02) was added to each well. The optical density was measured at 450 nm on a SpectraMax M5. IFN-α concentrations were calculated on the basis of a standard curve.

### 2.7. Statistics

Data were compared using a two-way analysis of variance with multiple comparisons and Tukey's correction at a significance level of $P < 0.05$ using GraphPad Prism 7.01 software.

## 3. Results and discussion

### 3.1. Pigs given Ad5-IFN-α had increased levels of IFN-α in their serum

Similar to what has been reported in previous studies (Brockmeier et al., 2009, 2012), pigs in groups given the Ad5-IFN-α, regardless of LAPV administration, had IFN-α levels between 15 and 120 ng/ml in their serum by day one post-administration (mean 45.1 and 69.1 ng/ml for groups Ad5-IFN-α + LAPV and Ad5-IFN-α, respectively) (Fig. 1). Serum IFN-α levels had declined to about one tenth the day one levels by day three post-administration. Little to no IFN-α was detected in the sera from pigs given LAPV alone or non-treated control pigs.

### 3.2. IFN-α inhibited LAPV viremia

Virus was isolated from the sera of all pigs from the LAPV-only group from at least one blood sample taken between days 5 and 21 after primary vaccination (Fig. 2). No virus was isolated from the sera from any of the pigs in the Ad5-IFN-α + LAPV group through day 28 after primary vaccination, an indication that IFN-α inhibited viral replication in these pigs. This is in contrast to previous results with this same IFN-α vector and virulent wild-type PRRSV, where viremia was delayed and reduced but not eliminated (Brockmeier et al., 2009, 2012). No virus was isolated from the sera of any of the pigs in the Ad5-IFN-α group or from non-treated controls.