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Transdermal delivery of plasmid encoding truncated nucleocapsid protein enhanced PRRSV-specific immune responses

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ABSTRACT

Background: Porcine Reproductive and Respiratory Syndrome virus (PRRSV) induces several immunomodulatory mechanisms that resulted in delayed and ineffective anti-viral immune responses. Recently, it has been shown that intradermal immunization of plasmid encoding truncated nucleocapsid protein (pORF7t) could reduce PRRSV-induced immunomodulatory activities and enhances anti-PRRSV immunity in vaccinated pigs. However, intradermal immunization may not be practical for farm setting. Currently, there are several transdermal delivery systems available in the market, although they were not originally designed for plasmid delivery.
Objectives: To investigate the potential use of a transdermal delivery system for delivering of pORF7t and its immunological outcomes.
Method: The immunomodulatory effects induced by transdermal delivery of pORF7t were compared with intradermal immunization in an experimental pig model. In addition, immunomodulatory effects of the DNA vaccine were determined in the fattening pigs kept in a PRRSV-positive farm environment, and in the experimental pigs receiving heterologous prime-boost, pORF7t-modified live vaccine (MLV) immunization.
Result: The patterns of PRRSV-specific cellular responses induced by transdermal and intradermal immunizations of pORF7t were similar. Interestingly, the pigs transdermally immunized with pORF7t exhibited higher number of PRRSV-specific $CD8^+IFN\text{-}\gamma^+$ cells. Pigs immunized with pORF7t and kept at PRRSV-positive environment exhibited enhanced PRRSV-specific IFN-γ^+ production, reduced numbers of regulatory T lymphocytes (Tregs) and lower lung scores at the end of the finishing period. In the heterologous prime-boost experiment, priming with pORF7t prior to MLV vaccination resulted in significantly higher numbers of $CD3^+IFN\text{-}\gamma^+$ subpopulations, lower numbers of PRRSV-specific $CD3^+IL\text{-}10^+$ cells and Tregs, and rapid antibody responses in immunized pigs.
Conclusion: Transdermal immunization with pORF7t reduced PRRRSV-induced immunomodulatory effects and enhanced long-term PRRSV-specific cellular responses in vaccinated pigs. Furthermore, heterologous DNA-MLV prime-boost immunization significantly improved the quality of PRRSV-specific cellular and humoral immunity. The information could benefit the future development of PRRSV management and control strategies.

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1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is currently one of the most important swine pathogens causing significant economic losses worldwide. Porcine Reproductive and Respiratory Syndrome virus (PRRSV) induces several immunomodulatory mechanisms that resulted in delayed and ineffective antiviral immune responses [1–5]. Several PRRSV-induced immune evasion mechanisms were identified and recently reviewed elsewhere [6]. Among these mechanisms, inductions of interleukin-10 (IL-10) and regulatory T lymphocytes (Tregs) are believed to be responsible for the unique immunological outcomes following PRRSV infections [6–11]. Among the PRRSV proteins, nucleocapsid (N) protein, a highly conserved non-glycosylated protein, [12–14] possesses several negative immunomodulatory properties including induction of IL-10 and subsequent development of PRRSV-specific Tregs [15,16]. Interestingly, earlier report also indicated that N protein contains immunodominant T cell epitopes [17]. However, induction N-specific antibodies may contribute to antibody-dependent enhancement (ADE) of infection [18].

It has been previously proposed that reduction of PRRSV-induced negative immunomodulatory effects should restore proper establishment anti-PRRSV immunity and better viral control in infected pigs [19]. Recently, we demonstrated that intradermal immunization of plasmid encoding truncated nucleocapsid protein (pORF7t), designed to specifically induce cellular immunity against the PRRSV N protein, could positively modulate the anti-PRRSV immunity. The vaccine-induced immunomodulatory effects included improvement in PRRSV-specific IFN-γ production, reduction of PRRSV-specific Tregs, and enhanced viral clearance, without induction of antibody responses in the vaccinated-challenged pigs [20]. The finding indicated the potential use of the novel DNA vaccine in PRRSV management and control strategies. However, one of the issues raised from the field was the practicality of intradermal injection, which is laborious and time consuming. There is a need to find a more practical method for delivering of the plasmid in the commercial farm setting. Currently, there are several commercially available needle-free injection systems for vaccine delivery in pigs [21–25]. In addition, needle-free injection system has been successfully used for delivering of plasmid in pigs [26] and dogs [27] with superior immunological outcomes, compared to the conventional delivery method.

In this work, the potential use of needle-free transdermal delivery system for pORF7t immunization was investigated in comparison to the intradermal immunization. In addition, immunomodulatory effects of the plasmid in the fattening pigs kept in a PRRSV-positive farm environment, and in the experimental pigs receiving heterologous prime-boost, pORF7t-modified live vaccine (MLV), immunization were investigated.

2. Materials and methods

2.1. Viruses and cells

The US genotype, Thai PRRSV strain 01NP1 (passage no. 15), isolated from PRRSV-infected pigs [28] was provided by the Chulalongkorn University-Veterinary Diagnostic Laboratory (CU-VDL). The virus was cultured and titrated in MARC-145 cells as previously described [29], and stored at −80 °C until needed.

2.2. Plasmids

The genes encoding PRRSV-N protein from the Thai PRRSV, 01NP1 strain (ORF7) or truncated PRRSV N protein (ORF7t) were subcloned into pMASIA expression vector (a gift from Dr. S. van Drunen Little-van den Hurk, Vaccine and Infectious Diseases Organization, University of Saskatchewan, Canada). The plasmids were referred to pORF7 or pORF7t, respectively. Details of cloning procedure, *in vitro* characterization, and plasmid amplification were described in the earlier report [15].

2.3. Antibody and secondary conjugates

Treg staining system: The direct conjugated monoclonal antibody (mAb), anti-swine CD25-PE (PGBL25A, IgG1), was a gift from Dr. J. A. Roth (Iowa State University, Ames, IA, USA). Anti-swine CD25 mAb (K231.3B2, IgG1) was purchased from AbD Serotec (Kidlington, UK). Anti-swine CD4-FITC (74-12-4, IgG2b) conjugate was purchased from BD Biosciences (San Diego, CA, USA). Biotinylated anti-swine CD4 mAb (74-12-4, IgG2b) was purchased from SouthernBiotech (Birmingham, AL, USA). Anti-human Foxp3-APC conjugate (236A/E7, IgG1) was purchased from eBioscience (San Diego, CA, USA). Goat anti-mouse IgG1-FITC was purchased from AbD Serotec. Streptavidin-PE was purchased from BioLegend (San Diego, CA, USA).

IFN-γ and IL-10 staining system: Anti-swine CD4-FITC (74-12-4, IgG2b) was purchased from SouthenBiotech. Anti-swine CD8-PE (76-2-11, IgG2b), and biotinylated anti-swine IFN-γ mAb (P2C11, IgG2a) conjugates were purchased from BD Biosciences. Anti-swine CD3-FITC mAb (BB23-8E6, IgG2b) conjugate was purchased from SouthernBiotech. Anti-swine IL-10 mAb (945A4C437B1, IgG1) was purchased from Biosource (Camarillo, CA, USA). Streptavidin-PETR, goat anti-mouse IgG1-Alexaflur 647 and IgG1 isotype control were purchased from Invitrogen (Carlsbad, CA, USA).

2.4. Animal experiment

All animal studies were conducted under the approval of Chulalongkorn University Animal Care and Use Committee, Chulalongkorn University; Animal Use Protocol No. 0931054 (Experiment 1, 2) and No. 1431086 (Experiment 3).

2.4.1. Experiment 1

Four-week-old, PRRSV-seronegative, crossbred pigs (8 pigs/group) were immunized once with 500 μg of pORF7t on day 0 (d0). Intradermal (ID) immunization was performed using the earlier described protocol [20]. Transdermal (TD) immunization was performed, at the neck area, using the Derma-Vac™ NF transdermal vaccination system (Merial Ltd., USA), using 100 pound per square inch (psi) air pressure, at a volume of 500 μl per dose. The control groups receiving null plasmid (pMASIA), either intradermally or transdermally, were included in the study. The pigs were kept in a PRRSV-negative commercial farm throughout the experiment. Immunological responses in the experimental groups were monitored every 2 weeks until d56. The pigs (5 pigs/group) were randomly selected and subjected to blood collection at each time point.

2.4.2. Experiment 2

The study was conducted in a PRRSV-positive production system with known PRRSV serological status located in central Thailand. Routinely, weaning pigs were produced and kept in a PRRSV-free unit 9 weeks old, when they were moved to a PRRSV-positive finisher site. Four-week-old, PRRSV-seronegative, crossbred pigs (30 pigs/group) were immunized twice with 500 μg of pORF7t on d0 and d21 using transdermal delivery system. The control group receiving null plasmid (pMASIA) or PBS transdermally, were included in the study. The pigs were kept at the finisher until 25 weeks old. Blood samples were collected from randomly selected pigs (5 pigs/group) on day 0, 21, 35, 49, 84, 112 and 147 for immunological and serological studies. Lung scores of the experimental