Veterinary Microbiology 164 (2013) 253–260

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Toll-like receptor ligands enhance the protective effects of vaccination against porcine reproductive and respiratory syndrome virus in swine

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ARTICLE INFO

Article history:
Received 2 October 2012
Received in revised form 5 February 2013
Accepted 15 February 2013

Keywords:
PRRSV
Killed virus (KV) vaccine
TLR ligands
Adjuvants

ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is mainly responsible for the heavy economic losses in pig industry in the world. Current vaccination strategies provide only a limited protection. Previous studies have demonstrated the immunostimulatory adjuvant effects of Toll-like receptor (TLR) ligands, synthetic double-stranded RNA polyriboinosinic polyribocytidylic [poly(I:C)], lipoteichoic acid (LTA) and CL097 in humans and animals. To study the effects of these compounds on the induction of PRRSV-specific immune responses, mice were immunized subcutaneously with killed virus (KV) antigens incorporating pairs of TLR ligands. It was found that poly(I:C) and CL097 induced the higher IFN-γ levels and PRRSV-specific antibodies, comparing with that KV with or without LTA in mice. Piglets were vaccinated with the KV mixed with poly(I:C) or CL097 and the protective effects of the vaccination were evaluated. The results showed that PRRSV-specific antibodies and T lymphocyte proliferation levels in KV mixed with poly(I:C) or CL097 groups were higher than those in KV group. Following challenge with PRRSV, pigs inoculated with KV mixed with poly(I:C) or CL097 showed lighter clinical signs, lower viremia and less pathological lesion of lungs, as compared to those of KV and challenge control groups. It indicated that co-administration of poly(I:C) and CL097 with killed PRRSV vaccine conferred higher protection against PRRSV challenge. TLR3 and TLR7/8 ligands are promising adjuvant candidates for the development of novel vaccines against PRRSV.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first identified in the late 1980s in North America and Europe (Collins et al., 1992; Wensvoort et al., 1991), and subsequently spread worldwide. In May 2006, a highly pathogenic infectious disease attacked swine in the central region of China, with high morbidity and mortality in some outbreaks (Li et al., 2007; Tian et al., 2007; Zhou et al., 2008b). Currently, PRRS still causes serious health problems in swine, resulting in significant economic losses to the swine industry. Currently available vaccines against the disease include live attenuated vaccines and killed virus (KV) vaccines (Guo et al., 2011; Quan et al., 2010). Live attenuated vaccine is well recognized for its protective efficacy against PRRSV that are genetically homologous to the vaccine virus. It is of concern, however, for its immunogenicity, cross protective efficacy and safety. KV vaccine, on the other hand, is well known for its safety, but it only confers limited protection. Therefore, changes to the mode of vaccination or the use of more powerful immune adjuvant may help to enhance the efficacy of current killed virus (KV) vaccine.

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http://dx.doi.org/10.1016/j.vetmic.2013.02.016

Adjuvant can stimulate innate immunity by interacting with specialized pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) (Akira et al., 2006; Kawai and Akira, 2009) and nucleotide-binding oligomerization domain receptors (Li et al., 2008a). Ligation of the TLRs by their specific ligands results in conformational changes in the receptors, leading to downstream signal transduction that primarily involves MyD88- and TRIF-dependent pathways (Akira and Takeda, 2004; Beutler et al., 2006). TLR is triggered by a distinct set of microbial compounds. For example, macrophage-activating lipoprotein 2 (MALP2) and lipoteichoic acid (LTA) trigger TLR2 (Lahiri et al., 2008). TLR3 recognizes double-stranded RNA of viral origin and synthetic double-stranded RNA, polyriboinosinic polyribocytidylic acid, poly(I:C) (Matsumoto et al., 2004). Single-stranded RNA, CL097 is recognized by TLR7 and TLR8. They have been evaluated as adjuvant for vaccine development HIV (Salem et al., 2006; San Roman et al., 2012; Stahl-Hennig et al., 2009; Stevceva, 2011; Trumpfheller et al., 2008), HBV (Salem et al., 2006; Stahl-Hennig et al., 2009; Trumpfheller et al., 2008) and FMDV (Cao et al., 2012). However, the adjuvant effectiveness of TLR ligands is not completed understood in PRRSV. Our previous animal experiment results showed that TLR2, TLR3 and TLR7 correlated with PRRSV infection. Here the ability of adjuvant effectiveness of poly(I:C), LTA and CL097 on killed PRRSV was initially examined in mice and the protective efficacy was examined in swine. It was found that co-injection with poly(I:C) or CL097 significantly promoted immune responses to killed PRRSV, and induced increased protective efficiency against PRRSV challenge in swine.

2. Materials and methods

2.1. Ethics statement

All experiments were approved by the Animal Care and Ethics Committee of Nanjing Agricultural University (permit number IACECNAU20111105) and followed the Guiding Principles for Biomedical Research Involving Animals. Animals were kept in cages and provided with food and water *ad libitum*.

2.2. Vaccines

An HP-PRRSV isolate SY0608 (passaged in MARC-145 cells 10 times) (GenBank no. HQ315835) was isolated from pigs in the Jiangsu province of China in 2006. It was characterized by a genomic marker with a 30-amino acid (aa) deletion in the nonstructural protein 2 (NSP2)-coding region and identified as a highly pathogenic Northern American type PRRSV (Li et al., 2007). The titer of the virus stock determined by cytopathic effects (CPEs) in MRAC-145 cells was $10^{5.87}$ $TCID_{50}$/ml. Inactivated vaccine was prepared by treating the virus with 0.1% β-propiolactone (Ferak Berlin Gmbh, Germany) at 4 °C for 8 h, then at 37 °C for 2 h. The vaccine was tested for its inability to infect MRAC-145 cells. The protein content of the vaccines was determined with Pierce BCA Protein Assay Kit (Thermo, USA).

2.3. Immunization of BALB/c mice

One hundred female BALB/c mice (provided by the Animal Center of Nanjing General Hospital, Nanjing, China) were divided equally into ten groups. TLR ligands including LTA, poly(I:C) and CL097 were purchased from Invivogen (San Diego, CA). For subcutaneous (s.c.) immunization, LTA, poly(I:C) and CL097 were mixed as 0.1 μg, 25 μg and 2 μg per doses, respectively. Briefly, the killed virus (1 μg) and TLR ligands were mixed with 20 μg of DOTAP liposomal transfection reagent (Applygen Technologies Inc., China). Two weeks later mice received booster injection with the same vaccines.

2.4. Immunization of pigs

Twenty, 6-week-old healthy crossbred piglets free of PRRSV and porcine circovirus type2 (PCV2) infection were randomly divided into four groups with 5 pigs per group. Group A was co-immunized with PRRS KV vaccine and 100 μg of poly(I:C) coated with 80 μg DOTAP. Group B was co-immunized with PRRS KV vaccine and 8 μg CL097 coated with 80 μg DOTAP. Group C was immunized with PRRS KV vaccine. Group D was immunized with Dulbecco's modified Eagle medium (DMEM) (Gibco-Invitrogen). Immunization was carried out by intramuscular injection. All formulations were delivered in a total volume of 2 ml. Two weeks later, pigs received booster injection with the same vaccines. At 14 and 28 days post first vaccination, the blood samples were collected for the detection of PRRSV specific antibodies using indirect enzyme-linked immunosorbent assay (iELISA). Meanwhile, the blood samples were collected for cytokine assays to determine the levels of IFN-γ and IL-4. All groups were challenged at 28 dpi with $2 \times 10^{5.0}$ $TCID_{50}$ PRRSV SY0608 strain, which was the homologous strain as the vaccination. And then the animals were monitored for 21 days. Rectal temperatures and clinical signs were observed daily. The blood samples were collected from all animals at 7, 14 and 21 days post challenge for the detection of PRRSV or anti-PRRSV antibody. At the end of experiment, all pigs were euthanized for pathological detection.

2.5. PRRSV-specific antibody examination

Purified-PRRSV antigen coated 96-well plates were used to perform indirect enzyme-linked immunosorbent assay (ELISA) for the titration of antibodies present in mice and pig sera, while the wells coated with purified uninfected MARC-145 cell antigens were used as negative controls. Briefly, 96-well micro titer plates were coated with 10 μg/ml of purified PRRSV SY0608 overnight at 4 °C. The plates were washed and blocked by adding 200 μl per well of 1% of bovine serum albumin in PBS-Tween and incubated for 1 h at 37 °C. Mice and swine serum samples were detected using a single dilution (1:100). The plates were then washed and treated with HRP-goat anti-mouse IgG or HRP-goat anti-pigs IgG according to the manufacturer's instructions (Boster, China). The OD was read at 450 nm. Sera, giving a ratio value higher than 2.1 were considered positive.