Vaccine 30 (2012) 5246–5252

Contents lists available at SciVerse ScienceDirect

Vaccine

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

Enhancing immune responses to inactivated *porcine parvovirus* oil emulsion vaccine by co-inoculating porcine transfer factor in mice

Rui-ning Wang[a,1], Ya-bin Wang[a,1], Jing-wei Geng[a], Dong-hui Guo[a], Fang Liu[b], Hong-ying Chen[b], Hong-ying Zhang[b], Bao-an Cui[a], Zhan-yong Wei[b,*]

[a] *The College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, Henan 450002, PR China*
[b] *Key Laboratory for Animal-derived Food Safety of Henan Province, Zhenzhou, Henan 450002, PR China*

ARTICLE INFO

Article history:
Received 28 December 2011
Received in revised form 15 May 2012
Accepted 29 May 2012
Available online 13 June 2012

Keywords:
Inactivated PPV oil-emulsion vaccine
Transfer factor
Cellular immune responses
Humoral immune response

ABSTRACT

Inactivated *porcine parvovirus* (PPV) vaccines are available commercially and widely used in the breeding herds. However, inactivated PPV vaccines have deficiencies in induction of specific cellular immune response. Transfer factor (TF) is a material that obtained from the leukocytes, and is a novel immune-stimulatory reagent that as a modulator of the immune system. In this study, the immunogenicity of PPV oil emulsion vaccine and the immuno-regulatory activities of TF were investigated. The inactivated PPV oil emulsion vaccines with or without TF were inoculated into BALB/c mice by subcutaneous injection. Then humoral and cellular immune responses were evaluated by indirect enzyme-linked immunosorbent assays (ELISA), fluorescence-activated cell sorter analyses (FACS). The results showed that the PPV specific immune responses could be evoked in mice by inoculating with PPV oil emulsion vaccine alone or by co-inoculation with TF. The cellular immune response levels in the co-inoculation groups were higher than those groups receiving the PPV oil emulsion vaccine alone, with the phenomena of higher level of IFN-γ, a little IL-6 and a trace of IL-4 in serum, and a vigorous T-cell response. However, there was no significant difference in antibody titers between TF synergy inactivated vaccine and the inactivated vaccine group ($P > 0.05$). In conclusion, these results suggest that TF possess better cellular immune-enhancing capability and would be exploited into an effective immune-adjuvant for inactivated vaccines.

1. Introduction

Porcine parvovirus (PPV) is one of mainly pathogens responsible for reproductive failure, characterized by stillbirth, mummification, embryonic death, infertility and delayed return to oestrus [1]. PPV is capable of transplacental infection during gestation and spreading between fetuses, the main characteristics of PPV existence in pigs exhibit the extremely durable and highly infection, besides its role in post-weaning multi-systemic wasting syndrome, and porcine respiratory disease complex [2,3]. These diseases become endemic in many pig-producing countries, with a majority of herds being persistently infected for several years, which resulted in critical economic losses to swine industry [4].

At present there is no effective treatment against PPV, several studies have pointed out that vaccination is an effective tool for controlling of this disease [4,5]. Therefore, the development of an effective vaccine against PPV infection in animals is warranted. There are several types of vaccines against PPV including attenuated vaccine, inactivated vaccine, subunit vaccine and DNA vaccine, but they are likely to be less reactogenic and immunogenic [6–8]. It has been verified that subunit vaccines elicit only moderate levels of protection, and the live attenuated vaccine has been using for several years in some countries, but it is expensive, causes side effects, and may revert to a pathogenic strain [6–8], so inactivated vaccines are the most common type of PPV vaccines used in animals [9,10]. However, inactivated PPV vaccines have deficiencies of specific cellular immune efficacy and failure to increase immunogenicity of weak antigen, and cannot effectively control and prevent PPV through humoral immunity. Various efforts have been made to enhance cellular immune responses of inactivated PPV vaccines, such as the co-stimulator reagents [11,12].

Transfer factor (TF) contains many molecules, some act in an antigen specific manner (MW, ca. 5000 Da), whereas smaller molecules (<3500 Da) have immune modulating activities [13,14]. TF forms the core of body immune system's intelligence network by storing information about immune system encounters with bacteria and viruses. Since the discovery of TF by Sherwood Lawrence over 40 years ago, many clinical reports have established that TF could be used as an immuno-modulator in illnesses where a characteristic common trait is an inappropriate or deficient cell mediated

* Corresponding author. Tel.: +86 0 371 63554361.
E-mail address: weizhanyong@henau.edu.cn (Z.-y. Wei).
[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.vaccine.2012.05.077

immunity [15]. Several studies mentioned that TF can be used as a preventive tool that could transfer immunity prior to the infection [16]. A number of clinical experiments indicated that TF has the capability to modulate the immune system [17].

It is well known that TF has immuno-regulation function, but the immune effects of TF synergy inactivated PPV vaccine had not been investigated, thus, in present study, we have investigated whether TF could enhance the immune response of inactivated PPV vaccine. The effect of TF synergy PPV oil emulsion vaccines on sera antibody tiers, T-lymphocyte ratio and sera secreting IFN-γ, IL-6 and IL-4 were compared in BALB/c mice, and the immune protective tests were compared too. The aim of our work was to confirm the immune enhancement of TF on inactivated PPV vaccine and offer theoretical evidence for exploiting new adjuvant of PPV vaccine.

2. Materials and methods

2.1. Experimental animals

Six-week-old BALB/c mice were purchased from Henan Province Laboratory Animal Management Committee (STXK (Yu) 2010–0002) in China, all mice have similar body weight (about 20 g per mice), and were provided with pathogen-free water and food. The mice fed a particular diet for one week before the first inoculation. Prior to experiment, PPV antibodies of all mice were confirmed to be negative by using ELISA method.

2.2. TF preparation

TF was prepared as described by Li and made some advance modifications [18]. In brief, the frozen pig spleens were taken turns through mechanically crashing, centrifuged, filtrated and ultrafiltration, then non-specific TF was prepared and the quality was examined. All of laboratory tests were in line with China Biologicals Regulations. Polypeptides concentration was determined by biuret reaction [19]. The concentration of polypeptide was 6.83 mg/ml. The safety of TF was proved by injecting it subcutaneously into the specific pathogen-free guinea pigs (GPs) and mice, all the GPs and mice injected with TF were remain healthy during the entire period, without any local reactions or alterations in normal behavior.

2.3. Experimental design

Two kinds of vaccines were used in current study. One is the commercial oil emulsion vaccine (batch number, 1 009 006 – 2), purchased from China animal husbandry industry co., Ltd, which named vaccine-1 (vacc.1) in this study. Another is HN-4 PPV oil emulsion vaccine which had been prepared in our laboratory, named vaccine-2 (vacc.2). The inactivated virus contained 512 haemagglutination units (HAU ml^{-1}) was as the vaccine antigen.

One hundred and twenty BALB/c mice were randomly divided into six groups, and housed separately. Each group included the same number of male and female mice. The immunization schedule is summarized in Table 1. Each vaccine was added in a total volume of 0.2 ml per mice in vaccine only group, and the co-inoculating groups were added 0.2 ml vaccine combined with 0.2 ml TF. Vaccines and TF were injected simultaneously but separately at different sites on the mice. Two weeks after the first injection, the second injection was offered at the same dosage as before. Blood samples were taken weekly from the retro-orbital plexus in the test tubes with heparin at day 0, 7, 14, 21, 28, 35 and 42 respectively.

Table 1
Immunization schedule for the different groups.

Design	Inculation	Dose per mouse
Group I	Vacc.1[b]	0.2 ml
Group II	Vacc.1 – TF[c]	0.2 + 0.2 ml
Group III	Vacc.2[d]	0.2 ml
Group IV	Vacc.2 – TF[e]	0.2 + 0.2 ml
Group V	TF[a]	0.2 ml
Group VI	NS[f]	0.2 ml

[a] TF: transfer factor.
[b] Vacc.1: HN-4 PPV inactivated vaccine.
[c] Vacc.1-TF: HN-4 PPV inactivated vaccine and transfer factor.
[d] Vacc.2: commercial PPV inactivated vaccine.
[e] Vacc.2-TF: commercial PPV inactivated vaccine and transfer factor.
[f] NS: normal saline.

2.4. Serum ELISA antibody assay

Blood samples were allowed to clot at 37 °C for 2 h, then serum was collected and inactivated at 56 °C for 30 min. ELISA was used to analyze PPV specific antibodies according the reports [20]. Briefly, 96-well plates (Costar) were coated with purified HN-4 PPV (5 μg/ml, prepared by our laboratory) overnight at 4 °C. The plates were washed three times and blocked with 1% bovine serum albumin buffered solution for 1 h at 37 °C. The diluted sera samples were added and then incubated for 2 h at 37 °C. After three washes with PBS-T (phosphate buffered saline-tween 20), 100 μl horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (sigma) was added to each well. After incubation at room temperature for 1 h, the plates were washed and 100 μl of substrate consisting of 0.05 M citrate buffer (pH 4.0), 2-20-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) and thirty percent hydrogen peroxide was added to each wells. After incubation for 10 min at room temperature in the dark, the reaction was stopped with five percent sodium dodecyl sulfate. Reactions were read at an absorbance of 630 nm using an ELISA plate reader (Bio-Rad). The values of mouse serum from experimental groups were considered positive when they were over or equal to 2.1 times of the values of the control groups. Values < 0.05 were not included.

2.5. Lymphocyte ratio of peripheral blood mononuclear cell (PBMC) analysis

Blood were collected for determination of lymphocyte subpopulation. Briefly, the blood were washed with cold PBS and cell surface staining was carried out using the following monoclonal antibodies (mAbs): fluorescein isothiocyanate (FITC)-labeled anti-mouse CD3 antibody, phycoerythrin (PE)-labeled anti-mouse CD4 antibody, PE-labeled anti-mouse CD8 antibody (all from PharMingen). For double cytofluorometric analysis, 30 μl blood were incubated with 5 μl of FITC-labeled anti-mouse CD3 and 5 μl of PE-labeled anti-mouse CD4 mAb, another samples containing 30 μl blood were incubated with 5 μl of FITC-labeled anti-mouse CD3 and 5 μl of PE-labeled anti-mouse CD8 mAb, 5 μl of mouse FITC and 5 μl of mouse PE monoclonal antibody as isotype controls. All incubations were carried out at 4 °C for 20 min. 150 μl Opticyle C was added to each tube at room temperature for 10 min, then centrifuged at 1500 r/min for 10 min and the deposits were washed with 0.1 M PBS (pH 7.2), containing zero point three percent bovine serum albumin (BSA). Then analyzed by cytofluorometry (Coulter Epics XL, CoulTER & BeckMAN, USA), and the data were analyzed with Cellquest V3.3 software.

2.6. Evaluation of cytokine levels

Mouse sera secreting cytokines of interferon-gamma (IFN-γ), interleukin 6 (IL-6) and interleukin 4 (IL-4) were assayed using the