Vaccine 29 (2011) 7960–7965

Contents lists available at SciVerse ScienceDirect

Vaccine

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

CpG oligodeoxynucleotide and montanide ISA 206 adjuvant combination augments the immune responses of a recombinant FMDV vaccine in cattle

Jiling Ren[a,c], Liang Yang[b], Haifei Xu[b], Yongsheng Zhang[b], Min Wan[b], Guoying Liu[d], Lixia Zhao[d], Liying Wang[b,**], Yongli Yu[a,*]

[a] Department of Immunology, Norman Bethune College of Medicine, Jilin University, Changchun 130021, China
[b] Department of Molecular Biology, Norman Bethune College of Medicine, Jilin University, Changchun 130021, China
[c] Department of Parasitology, Basic Medical College, Tianjin Medical University, Tianjin 300070, China
[d] Inner Mongolia Jinyu group Baoling Bio-pharmaceutical Corporation, Huhehaote, Inner Monglia 013300, China

ARTICLE INFO

Article history:
Received 20 January 2011
Received in revised form 26 July 2011
Accepted 15 August 2011
Available online 26 August 2011

Keywords:
CpG ODN
FMDV
Vaccine
Cattle

ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals. To prevent the spread of FMDV, inactivated virus vaccines are used to immunize animals in developing countries. However, there are safety concerns. In addition, it is difficult to distinguish the vaccinated animals from those naturally infected ones. In our lab, we have developed a recombinant FMDV vaccine named A7. A7 contained multiple B cell and T cell epitopes, which reside in a capsid protein (VP1) of FMDV. To enhance its immunogenicity, A7 was formulated with CpG ODN RW03 in combination with Montanide ISA 206 (ISA), and the resultant vaccine (A7 + ISA + CpG ODN RW03) was used to immunize mice and cattle. It was found that CpG ODN RW03 and ISA combination could facilitate A7 to induce a vigorous and long-lasting specific antibody response in mice and cattle. After FMDV challenge, 80% (4/5) of the calves immunized with A7 + ISA + CpG ODN RW03 were protected, which was superior to those immunized with A7 + ISA (25%, 1/4) or inactivated FMDV vaccine (50%, 2/4). These findings suggest that CpG ODN RW03 could be used with Montanide ISA 206 as a potent adjuvant for recombinant FMDV in cattle.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals and is endemic in many regions in Africa, Asia and South America. The outbreaks of FMD will reduce livestock productivity and restrict access to international markets for livestock and livestock products [1]. To prevent the spread of the virus, inactivated FMDV vaccines are used to immunize animals in developing countries, which are proved to be effective. However, there are risks of inactivating the virus incompletely, and of virus escaping from vaccine production plants [2]. Moreover, it is difficult to distinguish the vaccinated animals from those naturally infected ones. To overcome these problems, we have developed a recombinant FMDV vaccine named A7. A7 contained multiple B cell and T cell epitopes, which reside in a capsid protein (VP1) of FMDV. When combined with Montanide ISA 206 (ISA), A7 could induce neutralizing antibodies in cattle. However, the duration of the induced antibodies was short (unpublished data).

In recent decade, multiple studies have shown that synthetic oligodeoxynucleotide containing unmethylated CpG dinucleotide (CpG ODN) is potent adjuvant due to its capability of activating B cells, inhibiting apoptosis of B cells and inducing the maturation and differentiation of dendritic cells after recognizing toll like receptor 9 [3–5]. With the deep going of the research, it has been realized that optimal CpG ODN sequence varies from species to species [6]. Currently, most of CpG ODNs have been tested as adjuvants in the mouse model and human clinical trials [7,8]. This heralds the way for use of species specific CpG ODN as adjuvants for animal vaccines. To develop CpG ODN adjuvant of cattle use, CpG ODN 2006, a prototype B type CpG ODN in human, was able to stimulate proliferation of bovine peripheral blood mononuclear cells (PBMC) and secretion of IFN-γ, IL-6, IL-12 [9,10]. When immunized with alum-adjuvanted rickettsial major surface protein 2 (MSP2) in cattle, CpG ODN 2006 promoted strong MSP2-specific T lymphocyte proliferation, IFN-γ production, and IgG responses [11]. CpG ODN 2007 and CpG ODN 2135, with sequences similar to CpG ODN 2006, were also tested for their adjuvant activity in cattle [9]. The addition of CpG ODN 2007 to Mycobacterium bovis culture filtrate protein (CFP) formulated with Emulsigen (Em) significantly enhanced cell-mediated responses and elevated antibody responses in cattle [12]. Immunization of cattle with a recombinant Theileria parva protein plus Em and CpG ODN 2007 significantly enhanced the induction

* Corresponding author. Tel.: +86 431 85647872.
** Co-corresponding author. Tel.: +86 431 85619369.
E-mail addresses: wlying@jlu.edu.cn (L. Wang), ylyu@jlu.edu.cn (Y. Yu).

0264-410X/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2011.08.072

of CD4+ cytotoxic T-lymphocyte response [13]. Being formulated in alum, CpG ODN 2135 significantly enhanced the efficacy of bovine herpesvirus 1 glycoprotein D (BHV-1 tgD) in reducing the duration of virus shedding after viral challenge and increasing IgG2:IgG1 ratio in calves [14].

Oil-based adjuvants are currently being used in variety of vaccines in animals. Several reports indicated that, addition of CpG ODN to oil-based adjuvants could contribute to a synergistic immunostimualtory effect. Inclusion of CpG ODN 1826 in a subunit Malaria vaccine plus Montanide ISA 51 could induce high levels of IgG2a and IgG 2b antibodies and reduce the parasite burden in infected mice [7]. In cattle, bovine herpesvirus 1 glycoprotein D (tgD) formulated with Em and CpG ODN 2007 produced a Th1/Th2 balanced immune response, higher levels of virus neutralizing antibodies, and greater protection after BHV-1 challenge compared to tgD adjuvanted with either Em or CpG ODN alone [15]. Oil-based adjuvant at reduced dose in combination with CpG ODN 2007 attenuated the tissue damage while not compromising the magnitude of the immune response in sheep [16]. In this study, to develop an efficient FMDV vaccine, a novel CpG ODN, designated as CpG ODN RW03, was found and tested for its adjuvant activity in enhancing the immunogenicity of a recombinant FMDV vaccine formulated in Montanide ISA 206 (ISA) in mice and cattle.

2. Materials and methods

2.1. ODNs

The ODNs used in this study were synthesized in TAKARA Biotech Company (Dalian, China). The following CpG ODNs were used in this study: CpG ODN 2006 (5′-TCGTCGTTTTGTCGTTTTGTCGTT-3′), CpG ODN RW03 (5′-TCGCGAACGTTCGCCCGATCGTCGGTA-3′) and GC-ODN (5′-TGCAGCTTGCTGCTTGCTGCTTC-3′). GC-ODN is used as the non-CpG ODN control, in which there is no CG dinucleotide. All ODNs were phosphorothioate-modified. They were diluted in PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) and tested no detectable endotoxin by using Limulus amebocyte lysate assay (Associates of Cape Cod, Inc.).

2.2. Animals

All animal care and use procedures were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and with the approval of the Scientific Investigation Board of Science & Technology of Jilin Province.

6–8-week-old female BALB/c mice were provided by Animal Center of Norman Bethune Medical College, Jilin University. Mongolia cattle were provided by Baoling Bio-pharmaceutical Corporation. Twenty 8–10-month-old healthy calves were housed in a high-security containment unit of the company. These calves had never been vaccinated against FMDV and they were sero-negative to FMDV before immunization. They were randomly divided into four groups (five animals each). The average weight of the calves was 150 kg. All calves were housed within the same pen throughout the experiment.

2.3. Splenocyte isolation and proliferation assay

Portions of spleen were collected aseptically from the cattle. Spleen tissue was minced in a sterile Petri plate. The erythrocytes were lysed in lysis buffer (10 mM $KHCO_3$, 150 mM NH_4Cl, 10 mM EDTA, pH 7.4). The viability of the cells was 95–100% as determined by trypan blue exclusion. The cells were cultured at 37 °C in a 5% CO_2 humidified incubator and maintained in Iscove's Modified Dulbecco's Medium (IMDM, GIBCO) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS, GIBCO) and antibiotics (100 IU of penicillin/ml and 100 IU of streptomycin/ml).

Typically, bovine splenocytes (2×10^5 per well) were cultured with or without ODNs in 96-well U-bottom plate (Costar). ConA (Sigma) at 5 μg/ml was used as a control. The cells were cultured for 54 h, and followed by adding with 0.5 μCi/well ^{3}H-thymidine (New England Nuclear, Boston, MA) for addition 18 h. Cells were harvested on glass fiber filters and detected in a scintillation counter. The cell proliferation was expressed as mean cpm (counts per minute) from triplet wells ± standard deviations. Stimulation indices (SI) were calculated as the quotient of the mean cpm of the treated samples over the mean cpm of the media control.

2.4. Vaccines and viruses

The commercially available FMDV vaccine was used as a positive control in this study (Baoling Bio-pharmaceutical Corporation). It is a bivalent inactivated vaccine containing FMDV type Asia I and type O. Its adjuvant is Montanide ISA 206. FMDV Asia I/Jiangsu/China/2005 strain (GenBank accession number EF149009.1) was propagated in BHK-21 cell line, which was isolated and preserved in Baoling Bio-pharmaceutical Corporation.

2.5. Immunization in mice

Mice in four groups (six mice each) were injected once intramuscularly in the posterior limb with one of the following formulations: (1) Placebo (PBS); (2) A7 with Montanide ISA 206 (ISA); (3) A7 with 5 μg CpG ODN RW03 and ISA; (4) 5 μg CpG ODN RW03. A7 was administrated in 50 μg per mouse. All tested mice were bled before immunization (day 0) and then were immunized. The blood samples were also obtained on days 7, 14, 21, 28, 42, 56, 70, 84, 112, 175 and 196 post-immunization. Sera were separated by centrifugation at 4000 rpm for 10 min and stored at −20 °C till tested by ELISA.

2.6. Immunization in cattle and challenge study

Twenty calves were randomly divided into four groups (five animals each). They were injected intramuscularly in the neck with one of the following formulations: (1) Placebo (PBS); (2) inactivated FMDV vaccine; (3) A7 with ISA; (4) A7 with 500 μg CpG ODN RW03 and ISA. A7 was administrated in 2 mg per cattle. All immunized cattle were bled before immunization (day 0) and then were immunized. The blood samples were collected on days 14, 28, 56 and 84 post-immunization. Sera were separated by centrifugation at 4000 rpm for 10 min and stored at −20 °C till tested by virus neutralization. According to the descriptions by standard protocol of OIE (http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.05_FMD.pdf), all vaccinated cattle were challenged by intradermal inoculation of 10,000 bovine infectious doses (BID_{50}) of Asia I/Jiangsu/China/2005 at two sites in the tongue on day 84 post-immunization. The cattle were carefully examined in the mouth, and feet every day for ten days after challenge. Animals that showed lesions at sites other than the tongue were considered non-protected.

2.7. ELISA

Serum IgG antibody in mice was determined by ELISA. Briefly, all wells in 96-well plates were coated with appropriately diluted inactivated FMDV (100 μl) in carbonate/bicarbonate buffer (PH 9.6) and incubated overnight at 4 °C and blocked using 200 μl of PBS supplemented with 5% (v/v) FBS by incubation at 37 °C for 1 h after washing. The test sera and control sera were subjected to serial 2-fold dilutions (100 μl) with PBS-Tween 20 (PBS containing 0.05% Tween 20, v/v; PBST) as a diluent and incubated at 37 °C for 1 h. After