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Enhanced protective efficacy and reduced viral load of foot-and-mouth disease DNA vaccine with co-stimulatory molecules as the molecular adjuvants

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Abstract

To improve efficacy of DNA vaccination, various approaches have been developed, including the use of plasmid expressing co-stimulatory molecules as molecular adjuvants. In this study, we investigated whether co-inoculation of a construct expressing either 4-1BBL or OX40L as the molecular adjuvant with FMDV DNA vaccine, pcD-VP1, can increase immune responses and protective efficacies. Compared to the group immunized with pcD-VP1 alone, the co-inoculation of either molecular adjuvant induced a higher ratio of IgG2a/IgG1, higher levels of expression of IFN-γ in $CD4^+$ and $CD8^+$ T cells and antigen-specific CTL responses, and more importantly provided an enhanced protection against the live FMDV challenge in animals. Concurrently, 4-1BBL as the molecular adjuvant dramatically reduced the viral loads of FMDV in vivo after the challenge. Together, the results demonstrate that co-stimulatory molecules 4-1BBL and OX40L can enhance the antigen-specific cell-mediated responses elicited by VP1 DNA vaccine and provide an enhanced protective efficacy with the reduced viral loads.

Keywords: FMDV DNA vaccine; Protection; Viral load; Co-stimulatory molecules; Neutralizing antibody; in vivo CTL; Cell-mediated immune responses; 4-1BBL; OX40L

1. Introduction

Foot-and-mouth disease (FMD) is a disease caused by FMD virus (FMDV) which is belongs to the genus Aphthovirus in the family Picornaviridae (Pereira, 1981) and mainly infects cloven hoofed animals appearance of vesicles on the feet and mouth. FMD is an important problem world wide, especially in many developing countries, the outbreak of FMD results in great loss of economy in those countries (Sobrino and Domingo, 2001; Grubman and Baxt, 2004). The use of killed FMDV in oil emulsion as vaccines for livestock industry represents an effective strategy to prevent the viral infections (Brown, 1992). Protective immunity to FMDV is not yet fully understood. However, the high level of neutralizing antibodies is highly effective in controlling disease and viral transmission after immunization of a killed FMDV vaccine. In spite of such vaccination and generation of neutralizing antibodies, persistent infections are detectable in those vaccinated animals (Alexandersen et al., 2002). Although cell-mediated immunities (CMI) including antigen-specific CD4+ and CD8+ T cell responses are induced upon FMDV infections (Collen, 1994; Childerstone et al., 1999; Garcia-Briones et al., 2004; McCullough and Sobrino, 2004), their roles in protection against FMDV remains largely unknown, although some reports describe at least to contribute partial protection (Sanz-Parra et al., 1999; Garcia-Briones et al., 2004). Moreover, the residual live FMDV were detected and persisted in the farm animals after they were vaccinated, suggesting an inefficiency of the inacti-

Abbreviations: RT-PCR, reverse transcriptase PCR; GAPDH, glyseraldehyde-3-phosphate dehydrogenase; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; PMS, phenazine methosulfate; CFSE, carboxyfluorescein succinimidyl ester; TCID50, 50% of tissue culture infectious dose

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vated FMDV vaccine in oil adjuvant (McVicar and Sutmoller, 1969).

DNA-based vaccination is an alternative method which has been used successfully in raising protective immune responses against various pathogenic agents including HIV-1, influenza, parasites, and tumor cells (Fynan et al., 1993; Ulmer et al., 1993; Wang et al., 1993; Wang et al., 1995; Srivastava and Liu, 2003) in small animal models. On the other hand, it could effective induce both humoral and cellular immune responses in vivo. Although DNA vaccination is limited in that it often generates only weak immune response when used alone in large animals, this could be augmented by introducing of optimal adjuvants (Tacket et al., 1999; Boyer et al., 2000; Jin et al., 2004).

Adjuvants are widely used in various vaccine formulations for the enhancement of immune responses. Among the adjuvants, expressing constructs containing genes encoding cytokines or co-stimulatory molecules have been considered as molecular adjuvants and can be used to enhance immune effects of DNA vaccine (Svanholm et al., 1997; Kim et al., 1998).

Within the co-stimulatory molecules, OX40L and 4-1BBL are belong to tumor necrosis factor (TNF) receptor family and found to be expressed on the professional antigen presenting cell (APC). Their functions are believed to activate $CD4^+$ T cell functions, enhance $CD8^+$ CTL activity, and promote memory T cells. The use of OX40L and 4-1BBL proteins or expressing genes as adjuvants for vaccinations has been reported previously (Guinn et al., 1999; Gramaglia et al., 2000; Ishii et al., 2003; Serghides et al., 2005; Du et al., 2007), however, their roles in protection and clearance of viral infections are not completely clear.

In this study, we attempted to use a eukaryotic construct encoding either OX40L or 4-1BBL as a molecular adjuvant to co-inoculate with FMDV VP1 DNA vaccine and evaluated their roles in the immune efficacy and clearance of viral infection in a rodent viral challenge model. The results showed that both humoral and cellular immunities have been significantly enhanced in animals after the addition of either adjuvant with the pcD-VP1. The enhanced cell-mediated responses were apparently correlated with the levels of protection induced by FMDV DNA vaccines. The results demonstrated that the co-inoculation of OX40L or 4-1BBL construct could significantly improve the protective efficacy of FMD DNA vaccine against FMDV infection.

2. Materials and methods

2.1. Animal and cell

Female Balb/c mice aged 6–8 weeks and guinea pigs weighing at 400–500 g were purchased from Animal Institute of Chinese Medical Academy (Beijing, China) and were maintained under a clear air condition with pathogen-free food and water. The Hela cell line was cultured in DMEM (GibcoBRL, NY, USA) supplemented with 10% of fetal bovine serum and penicillin–streptomycin (GibcoBRL, NY, USA) in a humidified 37 °C incubator under 5% CO_2.

2.2. FMDV vaccine and antigen

The killed FMDV vaccine (chemically inactivated FMDV and emulsified in mineral oil) was obtained from Jinyu Group Corp. (Huhhot, Inner Mongolia, China) and used as a positive control vaccine in both antibody and viral challenge studies. 146S antigen, a chemically inactivated FMDV and purified by sucrose grading at Jinyu Group Corp., was quantified by the Bradford micro-assay kit (Bio-Rad, USA) and used for the specific antigens for ELISA, T cell proliferations and intracellular staining. FMDV VP1 peptide of a T cell epitope (aa133-147, SSKYGDTSTNNVRGD) was synthesized by GL Biochem Co., Ltd. (Shanghai, China) and used for in vivo CTL assay.

2.3. Antibodies and fluorescent dye

Fluorescent conjugated rat anti-mouse monoclonal antibodies including anti-IL-4-PE, anti-IFN-γ-FITC, anti-CD4-FITC, anti-CD4-PE, anti-CD8-PE and isotype controls were purchased from BD PharMingen (San Diego, CA). CFSE was obtained from Molecular Probes (Eugene, OR).

2.4. Plasmid constructions

The plasmid pcD-VP1 encoding for the FMDV VP1 protein was constructed in our lab and used as the DNA vaccine previously (Jin et al., 2004). The gene of 4-1BBL or OX40L was respectively cloned into the downstream of a CMV promoter and hCG-β leader sequence of a provax vector constructed previously in this lab (Tu Yixian et al., 2005), and designated as provax-4-1BBL or provax-OX40L used as the molecular adjuvants (Du et al., 2007).

2.5. Immunization

All the plasmids were maxi-prepared by alkaline method and purified by PEG8000 precipitation (Sambrook et al., 1989), subsequently diluted in saline solution. For vaccination, the mice were randomly divided into 6 groups, 15 animals per group, and guinea pigs were randomly divided into 6 groups and 5 animals per group. They were immunized intramuscularly with pcD-VP1 alone or co-immunized with the respective molecular adjuvant listed in Table 1 on days 0, 14 and 28. pcD-VP1 plus provax vector were also used to eliminate the so-called "sparing effect" from the vector. The mice and guinea pigs were prebled and bled on day 7 after the third immunization.

2.6. Detection of anti-FMDV antibody

ELISA was performed following a previously published procedure (Jin et al., 2005). 96-Well plates were coated with 2 μg/ml 146S antigen per well. The serum total IgG, IgG1 and IgG2a isotypes antibody titers were defined as the highest dilution that gave an above 2:1 ratio between testing serum and the naive negative control.