Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

DNA vaccine encoding Middle East respiratory syndrome coronavirus S1 protein induces protective immune responses in mice

Hang Chi [a], Xuexing Zheng [a,b], Xiwen Wang [a], Chong Wang [a], Hualei Wang [a,c], Weiwei Gai [a], Stanley Perlman [d], Songtao Yang [a,c,*], Jincun Zhao [e,*], Xianzhu Xia [a,c,*]

[a] Key Laboratory of Jilin Province for Zoonosis Prevention and Control, Institute of Military Veterinary, Academy of Military Medical Science, Changchun, China
[b] School of Public Health, Shandong University, Jinan, China
[c] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, China
[d] Department of Microbiology, University of Iowa, Iowa City, IA, USA
[e] State Key Laboratory of Respiratory Diseases, Guangzhou Institute of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

ARTICLE INFO

Article history:
Received 10 June 2016
Received in revised form 13 February 2017
Accepted 28 February 2017
Available online xxxx

Keywords:
MERS-CoV
DNA vaccine
Spike protein

ABSTRACT

The Middle East respiratory syndrome coronavirus (MERS-CoV), is an emerging pathogen that continues to cause outbreaks in the Arabian peninsula and in travelers from this region, raising the concern that a global pandemic could occur. Here, we show that a DNA vaccine encoding the first 725 amino acids (S1) of MERS-CoV spike (S) protein induces antigen-specific humoral and cellular immune responses in mice. With three immunizations, high titers of neutralizing antibodies (up to 1: 10^4) were generated without adjuvant. DNA vaccination with the MERS-CoV S1 gene markedly increased the frequencies of antigen-specific $CD4^+$ and $CD8^+$ T cells secreting IFN-γ and other cytokines. Both pcDNA3.1-S1 DNA vaccine immunization and passive transfer of immune serum from pcDNA3.1-S1 vaccinated mice protected Ad5-hDPP4-transduced mice from MERS-CoV challenge. These results demonstrate that a DNA vaccine encoding MERS-CoV S1 protein induces strong protective immune responses against MERS-CoV infection.

1. Introduction

Middle East respiratory syndrome (MERS)-coronavirus (MERS-CoV), an emerging zoonotic virus, is the causative agent of MERS. MERS-CoV was first identified in Saudi Arabia in 2012 and MERS cases have been reported in 27 countries since then [1,2]. As of February 10, 2017, 1905 laboratory-confirmed cases, including 677 deaths related to MERS-CoV, had been reported to WHO (~36% mortality). Several family clusters and nosocomial clusters cases have been reported, revealing the human-to-human transmissibility of MERS-CoV, and raising the concern of a MERS-CoV global pandemic [3–5]. Currently, no licensed therapeutic or vaccine is available, which highlights the need for efficient vaccines against MERS-CoV.

To date, several vaccine candidates have been developed, such as viral vector-based recombinants [6–11], subunit vaccines [12–19], DNA vaccines [20], DNA prime/protein-boost vaccines [21] and a reverse genetics-constructed recombinant coronavirus vaccine [22]. Among them, DNA vaccines present a range of unique advantages such as proper antigen protein folding, rapid design and production, cost-effectiveness, and stability at non-refrigerated temperatures for convenient storage and shipping [23]. Furthermore, it has been reported that DNA vaccines can induce both humoral and cellular immune responses against MERS-CoV and SARS-CoV infection [20,24,25].

MERS-CoV is the first lineage of *Betacoronavirus* known to infect humans [26]. The genome of MERS-CoV encodes four structural proteins – spike (S), envelope (E), membrane (M) and nucleocapsid (N) [27]. The S protein, a class I fusion protein forming protruding spikes on the virus surface, is composed of an N-terminal S1 subunit and a C-terminal S2 subunit [28]. It has been reported that MERS-CoV binds to host cell receptor dipeptidyl peptidase 4 (DPP4) through an independently folded receptor binding domain (RBD) localized within the S1 subunit [29,30]. Moreover, S protein has been identified as the most immunogenic antigen of MERS-CoV. It plays an important role in the induction of neutralizing antibody and anti-viral T-cell responses [28]. Thus, S protein is

* Corresponding authors at: Department of Virology, Institute of Military Veterinary, Academy of Military Medical Sciences, 666 Liuying West Road, Changchun, Jilin 130012, China (S. Yang and X. Xia). State Key Laboratory of Respiratory Diseases, Guangzhou Institute of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China (J. Zhao).

E-mail addresses: yst62041@163.com (S. Yang), zhaojincun@gird.cn (J. Zhao), xiaxzh@cae.cn (X. Xia).

http://dx.doi.org/10.1016/j.vaccine.2017.02.063
0264-410X/

the major target for current vaccines development to protect against MERS [8,10,28]. However, previous studies have demonstrated that vaccines based on full-length S potentially induce harmful side effects caused by non-neutralizing epitopes [27,31]. In contrast, RBD protein-based subunit vaccines are able to induce both neutralizing antibody and anti-viral T-cell responses against MERS-CoV infection, with the additional superiority of safety [28]. Nevertheless, to improve the immunogenicity of these subunit vaccines, it has been found necessary to use an appropriate adjuvant or even adjuvant combinations, or immune enhancers (e.g., human IgG Fc), and optimized delivery routes and doses [12–17]. An ideal MERS vaccine should induce potent neutralizing antibody response without inducing harmful immune effects such as virus-enhancing antibody or immunopathology [28,32]. Based on the established background and our previous research results, we selected S1 protein as the target for our DNA vaccine development.

In the present study, we designed and constructed a DNA vaccine encoding the S1 subunit of MERS-CoV (pcDNA3.1-S1), and evaluated antigen-specific humoral and cellular immune responses induced by this DNA vaccine in mice. Further, we investigated the protective efficacy of pcDNA3.1-S1 DNA vaccine in an Ad5-hDPP4-transduced mouse model following MERS-CoV challenge. Vaccinated mice and mice receiving immune serum before infection were found to have significantly decreased virus loads in their lungs.

2. Material and methods

2.1. Mice, virus and cells

Six-to eight-week-old specific pathogen-free female BALB/c mice were purchased from the Changchun Institute of Biological Products Co., Ltd (Changchun, China) or the National Cancer Institute and Jackson Laboratories (Maine, USA). The EMC/2012 strain of MERS-CoV (passage 8, designated MERS-CoV) was kindly provided by Bart Haagmans and Ron Fouchier (Erasmus Medical Center, Rotterdam, The Netherlands). Vero 81 cells (derived from African Green monkey kidney) [ATCC No. CCL81] were grown in DMEM (Gibco, San Diego, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, San Diego, CA, USA). MERS-CoV EMC/2012 was passaged once in Vero 81 cells and titrated by plaque assay in the same cell line.

2.2. Construction of the recombinant plasmids expressing MERS-CoV spike protein

The gene sequence encoding amino acid 1-1353 (S) of the spike protein of the Al-Hasa_15_2013 strain of MERS-CoV (GenBank accession No. KF600645.1) was synthesized by Sangon Biotech Company (Shanghai, China). The synthetic full-length S, SΔCD (S without the entire cytoplasmic domain), and S1 fragment were respectively subcloned into the mammalian expression vector pcDNA3.1 (+) (Invitrogen, San Diego, CA, USA) to generate recombinant plasmid pcDNA3.1-S, pcDNA3.1-SΔCD, and pcDNA3.1-S1 (Fig. 1A). The recombinant plasmid was then amplified in *Escherichia coli* HST08 (TaKaRa, Dalian, China) and purified using the Endo-Free Plasmid Maxi Kit (QIAGEN GmbH, Shanghai, China). The recombinant plasmid was dissolved in PBS at a final concentration of 1 μg/μL for *in vitro* transfection and *in vivo* animal immunization.

2.3. Western blot analysis of spike protein expression in vitro

A 6-well plate was seeded with 293T cells which were grown to 80–90% confluence. Cells were respectively transfected with the recombinant plasmids and pcDNA3.1 empty vector using Lipofectamine 3000 Transfection Reagent (Invitrogen, San Diego, CA, USA) according to the manufacturer's instructions. Cells were harvested at 48 h post-transfection. Cell lysates were prepared using RIPA Lysis buffer (Solarbio LIFE SCIENCES, Beijing, China) according to the manufacturer's instructions, then separated on an 12% polyacrylamide gel and transferred onto a 0.45 μm nitrocellulose blotting membrane (GE Healthcare Life Sciences, Freiburg, Germany) for Western blotting analysis using mouse anti-MERS-S1 mono-

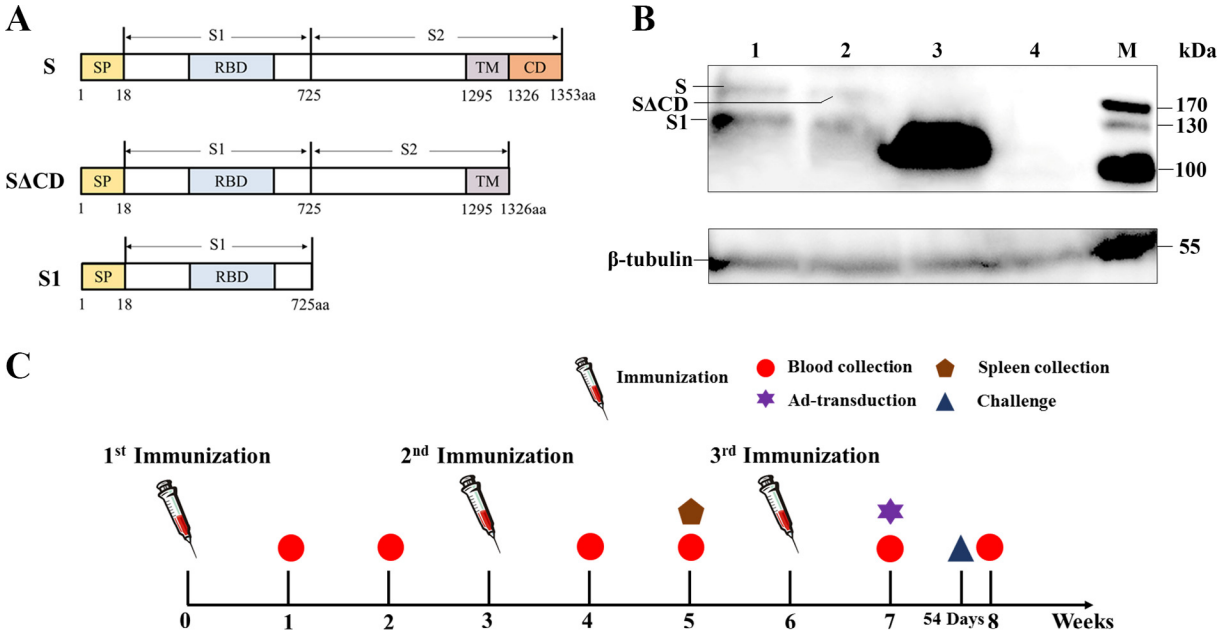

Fig. 1. Construction and verification of DNA vaccine. Schematic diagrams of the construction of DNA vaccines encoding different fragments of MERS-CoV spike protein (A). Western blot analyses of MERS-CoV spike protein expression *in vitro*. Lysates from pcDNA3.1-S, pcDNA3.1-SΔCD, pcDNA3.1-S1 transfected 293T cells (lane 1–3) and lysates from pcDNA3.1-Empty transfected 293T cells (lane 4) were incubated with mouse anti-MERS-S1 monoclonal antibodies and mouse anti-β-tubulin monoclonal antibodies (B). The schematic of the experiment (C).

Please cite this article in press as: Chi H et al. DNA vaccine encoding Middle East respiratory syndrome coronavirus S1 protein induces protective immune responses in mice. Vaccine (2017), http://dx.doi.org/10.1016/j.vaccine.2017.02.063