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Heterologous prime–boost vaccination with adenoviral vector and protein nanoparticles induces both Th1 and Th2 responses against Middle East respiratory syndrome coronavirus

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ABSTRACT

The Middle East respiratory syndrome coronavirus (MERS-CoV) is a highly pathogenic and zoonotic virus with a fatality rate in humans of over 35%. Although several vaccine candidates have been developed, there is still no clinically available vaccine for MERS-CoV. In this study, we developed two types of MERS-CoV vaccines: a recombinant adenovirus serotype 5 encoding the MERS-CoV spike gene (Ad5/MERS) and spike protein nanoparticles formulated with aluminum (alum) adjuvant. Next, we tested a heterologous prime–boost vaccine strategy, which compared priming with Ad5/MERS and boosting with spike protein nanoparticles and vice versa, with homologous prime–boost vaccination comprising priming and boosting with either spike protein nanoparticles or Ad5/MERS. Although both types of vaccine could induce specific immunoglobulin G against MERS-CoV, neutralizing antibodies against MERS-CoV were induced only by heterologous prime–boost immunization and homologous immunization with spike protein nanoparticles. Interestingly, Th1 cell activation was induced by immunization schedules including Ad5/MERS, but not by those including only spike protein nanoparticles. Heterologous prime–boost vaccination regimens including Ad5/MERS elicited simultaneous Th1 and Th2 responses, but homologous prime–boost regimens did not. Thus, heterologous prime–boost may induce longer-lasting immune responses against MERS-CoV because of an appropriate balance of Th1/Th2 responses. However, both heterologous prime–boost and homologous spike protein nanoparticles vaccinations could provide protection from MERS-CoV challenge in mice. Our results demonstrate that heterologous immunization by priming with Ad5/MERS and boosting with spike protein nanoparticles could be an efficient prophylactic strategy against MERS-CoV infection.

1. Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic beta coronavirus that can infect several kinds of animals including humans, camels, and bats [1]. It is known to cause severe respiratory symptoms and to have a high mortality rate [1]. The key receptor for MERS-CoV infection, dipeptidyl peptidase 4 (DPP4), is widely distributed on human endothelial and epithelial cells [2]. Except for cases in Korea in 2015, most infections with MERS-CoV (82%) have occurred in the kingdom of Saudi Arabia. The total number of laboratory-confirmed cases of MERS-CoV infection is 2040 with 712 deaths related to MERS-CoV infection since September 2012. Thus, the human mortality rate of MERS-CoV infection is approximately 35% [3].

The genome of MERS-CoV is single-stranded RNA that encodes 10 proteins including two replicase polyproteins (open reading frames [ORF], 1ab and 1a), three structural proteins (E, N, and

Abbreviations: MERS-CoV, Middle East respiratory syndrome coronavirus; DPP4, Dipeptidyl peptidase 4; RBD, Receptor binding domain; ORF, Open reading frame; Ad5/MERS, Adenovirus 5 expressing MERS-CoV spike protein.

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M), a surface glycoprotein (S, spike), which comprises S1 and S2, and five nonstructural proteins (ORF 3, 4a, 4b, and 5) [4]. The main viral protein is the spike protein, which binds to the cell surface receptor DPP4 during the viral entry stage via the receptor-binding domain (RBD) of spike subunit S1 [5]. Because the spike protein is the most immunogenic structural protein [6,7], the final goal of most current studies of MERS-CoV vaccines is to elicit neutralizing antibodies against this specific MERS-CoV spike protein.

Although several approaches to developing a MERS-CoV vaccine have been reported, there is no clinically approved vaccine for MERS-CoV. Previous studies have investigated viral vector-based vaccines [8–12], subunit vaccines [13–17], and DNA vaccines [18,19]. Of these, vaccination using viral vectors or DNA immunization successfully generated neutralizing antibodies and protected against infection [12]. However, safety concerns about DNA vaccines and their weak induction of neutralizing antibodies plus the possibility of reduced efficacy of viral vector vaccines because of preexisting immunity against the viral vectors induced by repeated immunization cannot be ignored. Although protein subunit vaccines can induce neutralizing antibody, they usually elicit a lower level of cellular immune response which has close association with rapid viral clearance when infection occurs. In addition, subunit vaccines could not induce enough immune responses in host, resulting in failure to make long-term memory of antigen [20].

Therefore, we used a heterologous prime–boost immunization strategy combining recombinant adenovirus serotype 5 delivering MERS-CoV spike protein gene (Ad5/MERS) and MERS spike protein nanoparticles, because both types of vaccine have been shown to be safe in human trials. The results of this study showed that this heterologous prime–boost immunization strategy induced good humoral and cellular immune responses including neutralizing antibodies and activation of Th1 cells against MERS-CoV, and could protect mice against MERS-CoV infection. Therefore, this combined immunization with recombinant Ad5/MERS and spike protein nanoparticles may avoid the hurdles of preexisting antibody induced by repeated viral vector immunization and weak Th1 cell responses induced by protein subunit immunization.

2. Methods

2.1. Supporting information (SI) for Materials and methods

See the Supplemental data for Materials and Methods for details regarding Cell, Virus preparation and titration, MERS spike protein nanoparticles, SDS-PAGE and Immunoblot analysis, Recombinant Ad5, Electron microscopy, Enzyme-linked immunosorbent assay (ELISA), Plaque reduction neutralization test (PRNT), MERS-CoV infection, and Statistical analysis.

2.2. Mice

Six-week-old female specific-pathogen-free BALB/c mice were purchased from Dae Han Bio Link Co., Ltd., (Chungcheongbuk-do, Korea). Mouse experiments were performed in accordance with the relevant ethical guidelines and regulations established by the Korean Association for Laboratory Animals [21]. All mice were housed in specific-pathogen-free conditions with a standard light cycle (12 h light/dark) and maintained according to protocols approved by the Institutional Animal Care and Use Committee, Sungsim Campus, Catholic University of Korea. All mice were fed a normal fat (5%) diet (Harlan Laboratories, Livermore, CA, USA) and sterile water. Mice were randomly allocated to groups of six and immunized three times as indicated (Table 1).

2.3. Virus preparation and titration

MERS-CoV was provided by the Korean Centers for Disease Control and Prevention (National Control Number 1-001-MER-IS-2015001). All experimental procedures were performed in the Biosafety Level 3 facility of the Korea Zoonosis Research Institute at Chonbuk National University. The virus was passaged and titered on Vero E6 cells.

2.4. MERS spike protein nanoparticles

Spodoptera frugiperda Sf9 insect cells were obtained from the American Type Culture Collection and maintained in Insect-XPRESSTM medium. The MERS-CoV spike protein sequence was referred from NCBI reference sequence (Genbank accession No. AGN70962), and the nucleotide sequence was codon optimized for optimal expression in insect cells. Full length spike gene was cloned into pBacPAK8 baculovirus transfer vector. MERS-CoV spike proteins were produced in Sf9 cells infected with recombinant baculovirus. Spike proteins were purified using a combination of anion exchange and glucose affinity chromatography.

2.5. Recombinant adenovirus 5 expressing MERS spike protein gene (Ad5/MERS) and human DPP4 (Ad5/hDPP4)

Recombinant adenoviruses encoding the MERS spike protein and human DPP4 were purchased from Sirion Biotech (London, UK). The detailed production protocol is in *Supporting Information (SI)*.

2.6. Vaccination and serum collection

Groups of mice were immunized using heterologous (different vaccine candidates for priming and boosting) or homologous (the same vaccine candidate for priming and boosting) prime–boost immunization. The detailed immunization schedules and grouping are shown in Table 1 and Fig. 2A.

2.7. Enzyme-linked immunospot (EliSpot) assay

Mouse splenocytes were collected and isolated after mice were euthanized. Then, 3×10^6 splenocytes were seeded into wells of an EliSpot plate for detection of IFN-γ secreting T cells. To stimulate the splenocytes, 1.6 μg/well of MERS-CoV spike-specific peptide

Table 1
Detailed information about each vaccination protocol.

Group	Prime (1st vaccination)	1st Boost (2nd vaccination)	2nd Boost (3rd vaccination)	Abbreviation
PBS	PBS	PBS	PBS	Control groups
Ad5/GFP	Ad/GFP	Ad/GFP	Ad/GFP	
Spike protein	Spike protein	Spike protein	Spike protein	Homologous prime–boost groups
Ad5/MERS	Ad5/MERS	Ad5/MERS	Ad5/MERS	
Ad5/MERS–spike protein	Ad5/MERS	Spike protein	Spike protein	Heterologous prime–boost groups
Spike protein–Ad5/MERS	Spike protein	Ad5/MERS	Ad5/MERS	

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