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Novel chimeric virus-like particles vaccine displaying MERS-CoV receptor-binding domain induce specific humoral and cellular immune response in mice

Chong Wang [a, b, 1], Xuexing Zheng [c, 1], Weiwei Gai [a, d, 1], Gary Wong [e], Hualei Wang [a, f], Hongli Jin [a], Na Feng [a, f], Yongkun Zhao [a, f], Weijiao Zhang [a, d], Nan Li [a], Guoxing Zhao [a], Junfu Li [e], Jinghua Yan [e], Yuwei Gao [a, f], Guixue Hu [g, ***], Songtao Yang [a, f, **], Xianzhu Xia [a, f, *]

[a] Key Laboratory of Jilin Province for Zoonosis Prevention and Control, Institute of Military Veterinary, Academy of Military Medical Sciences, Changchun, China
[b] State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China
[c] School of Public Health, Shandong University, Jinan, China
[d] College of Veterinary Medicine, Jilin University, Changchun, China
[e] CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China
[f] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, China
[g] College of Animal Science and Technology, Jilin Agricultural University, Changchun, China

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ABSTRACT

Middle East respiratory syndrome coronavirus (MERS-CoV) has continued spreading since its emergence in 2012 with a mortality rate of 35.6%, and is a potential pandemic threat. Prophylactics and therapies are urgently needed to address this public health problem. We report here the efficacy of a vaccine consisting of chimeric virus-like particles (VLP) expressing the receptor binding domain (RBD) of MERS-CoV. In this study, a fusion of the canine parvovirus (CPV) VP2 structural protein gene with the RBD of MERS-CoV can self-assemble into chimeric, spherical VLP (sVLP). sVLP retained certain parvovirus characteristics, such as the ability to agglutinate pig erythrocytes, and structural morphology similar to CPV virions. Immunization with sVLP induced RBD-specific humoral and cellular immune responses in mice. sVLP-specific antisera from these animals were able to prevent pseudotyped MERS-CoV entry into susceptible cells, with neutralizing antibody titers reaching 1: 320. IFN-γ, IL-4 and IL-2 secreting cells induced by the RBD were detected in the splenocytes of vaccinated mice by ELISpot. Furthermore, mice inoculated with sVLP or an adjuvanted sVLP vaccine elicited T-helper 1 (Th1) and T-helper 2 (Th2) cell-mediated immunity. Our study demonstrates that sVLP displaying the RBD of MERS-CoV are promising prophylactic candidates against MERS-CoV in a potential outbreak situation.

1. Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV) infections can cause severe and possibly lethal acute respiratory disease in humans. A total of 630 deaths from 1769 cases of MERS-CoV were reported between September 2012 to June 2016, a mortality rate of 35.6%. Of considerable concern, imported cases of MERS have been being reported in countries worldwide, which raise concerns of a potential pandemic. Vaccination is the best means to control and prevent disease, but a licensed prophylaxis is not currently available against MERS-CoV.

* Corresponding author. Department of Virology, Institute of Military Veterinary, Academy of Military Medical Sciences, 666 Liuying West Road, Changchun, Jilin, 130012, China.
** Corresponding author. Department of Virology, Institute of Military Veterinary, Academy of Military Medical Sciences, 666 Liuying West Road, Changchun, Jilin, 130012, China.
*** Corresponding author.
E-mail addresses: yst62041@163.com (S. Yang), xiaxzh@cae.cn (X. Xia).
[1] These authors contributed equally to this paper.

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The surface of MERS-CoV particles is composed of the spike (S) glycoprotein, which plays a crucial step in the viral life cycle by interacting with the dipeptidyl peptidase 4 (DPP4) molecules on the target cell to initiate viral docking and entry. Studies have shown that the receptor-binding domain (RBD) is a major antigenic determinant for the induction of neutralizing antibodies (Du et al., 2013a, 2013b; Mou et al., 2013). Therefore, a candidate expressing the RBD of MERS-CoV is a plausible design for an effective vaccine.

Virus-like particles (VLP) are an excellent platform for epitope presentation because they have been shown to efficiently interact with antigen-presenting cells and display heterologous epitopes at high density (Barcena and Blanco, 2013). Parvovirus-like particles expressing the major structural protein VP2 in insect cells are very stable and highly immunogenic (Lopez de Turiso et al., 1992; Martinez et al., 1992). Moreover, canine parvovirus (CPV) does not cause disease in human, therefore parvovirus-like particles is a safe expression platform. Parvovirus VLP has been successfully used for the expression of foreign antigens and induction of robust B-, T-cell responses (Miyamura et al., 1994; Casal et al., 1999; Lo-Man et al., 1998; Xu et al., 2014; Rueda et al., 1999).

In this study, we evaluated the efficacy of a chimeric CPV VLP expressing the RBD of MERS-CoV. The structural integrity of these VLP was confirmed by electron microscopy and the immunogenicity of parvovirus-like VLP was evaluated in a mouse model of infection.

2. Methods

2.1. Construction of recombinant baculovirus

Nucleotides encoding spike glycoprotein residues 367–606 of the MERS-CoV RBD (GenBank accession no. KF600645) were codon-optimized for the highest expression levels possible in insect cells and biochemically synthesized (Sangon Biotech, China). Three repeat flexible linkers $(Gly_4Ser)_3$ were cloned to the C-terminus of RBD. The RBD and linkers were subcloned into the *SalI-NotI* sites of pFastBac1-VP2, to generate pFastBac1-RBD-VP2. pFastBac1-RBD-VP2 was then transformed into *E. coli* DH10Bac cells, and recombinant bacmids containing the RBD insert were confirmed by PCR.

Recombinant baculoviruses were generated by recombinant bacmids transfected into Sf9 insect cells in the presence of the Liposome 2000 transfection reagent, following the Bac-to-Bac Expression Systems manual (Invitrogen, USA). Supernatants containing recombinant baculovirus were harvested 5 days after transfection as viral stocks, which are designated Bac-RBD-VP2. The titers of these baculovirus stocks were determined using a rapid titration kit (BacPakBaculovirus Rapid Titer Kit; Clontech, USA).

2.2. Indirect immunofluorescence assay

Expression of RBD-VP2 protein was confirmed by indirect immunofluorescence as previously described (Feng et al., 2014). Briefly, Sf9 insect cells were maintained at 27 °C in TNM-FH medium supplemented with 10% FBS and infected with Bac-RBD-VP2 at a multiplicity of infection (MOI) of 3. Control cells were infected with baculovirus pFastBac1. At 48 h post infection, the culture plates were fixed with 4% paraformaldehyde at room temperature for 20 min, washed with PBS-0.05% Tween 20 (PBST), and then incubated with mouse anti-MERS-S polyclonal antibody or mouse anti-VP2 monoclonal antibody containing 1% bovine serum albumin at 37 °C for 1 h. After washing with PBST, the cells were stained with FITC-tagged goat anti-mouse IgG and Evans Blue (diluted 1:500 in PBST) at 37 °C for 1 h. After washing, the cells were examined under a fluorescent microscope.

2.3. Preparation and purification of chimeric VLP

Sf9 insect cells were maintained as suspension cultures in serum-free SF900II medium (Life technologies, USA) at 27 °C, with shaking at 120 rpm. Sf9 cells were infected with recombinant baculovirus at a MOI of 3. Cells were harvested 96 h after infection, washed with PBS, and lysed with 25 mM bicarbonate solution on ice for 10 min. After centrifugation at 12,000 × g for 10 min, the cell pellet is removed and purified chimeric VLP was obtained by a quarter volume of saturated ammonium sulfate precipitation for 20 min on ice, and then subsequently centrifugated at 12,000 g for 20 min. The viral pellet is resuspended in PBS and dialyzed overnight.

2.4. Western blot

Samples of purified sVLP, purified recombinant RBD proteins, CPV VLP and lysate from pFastBac1 infected cells were transferred onto a polyvinylidene fluoride (PVDF) membrane (Immobilin-P, Millipore, USA) after SDS-PAGE for Western blotting with mouse anti-RBD or -VP2 monoclonal antibodies.

2.5. Characterization by electron and immunoelectron microscopy

sVLP were loaded onto grids, kept at room temperature for 5 min, stained with 1% sodium phosphotungstate, and then examined directly under a transmission electron microscope (TEM). For immunoelectron microscopy (IEM), the sVLP were loaded onto formvar-coated grids after removal of excess sample solution and incubated with mouse anti-RBD monoclonal antibody. After washing with PBS, the grids were stained with gold-tagged goat anti-mouse IgG antibody (Sigma-Aldrich, SaintLouis, MO, USA). After another wash with PBS, the grids were observed under the TEM.

2.6. Hemagglutination (HA) test

HA tests were carried out as previously described (Feng et al., 2014). Briefly, 25 μL of 2-fold serial dilutions of sVLP were made in PBS, and then an additional 25 μL of PBS was added to each well. 50 μL of 1% (V/V) pig erythrocytes were then added to each well for 1 h at 4 °C. The HA titer was determined by the highest dilution of the sVLP that agglutinated the erythrocytes.

2.7. Immunization studies

Thirty-two BALB/c mice (6-week-old, female) were randomized into four groups. All groups were vaccinated intramuscularly (IM) in the gastrocnemius muscle. Mice in group 2 were given 10 μg of sVLP; mice in group 3 were given 10 μg of sVLP and mixed with 50 μg Alum adjuvant (Thermo, USA) per animal; mice in group 4 were given 10 μg of sVLP and mixed with 50 μg polyriboinosinic acid [poly(I:C)] adjuvant (Sigma-Aldrich, Saint Louis, MO, USA), and the mice in group 1 were given PBS as control. Identical vaccinations were then repeated at 14-days after the initial administration for all groups. Blood samples were obtained from the orbital vein of mice on days 14 and 28 post-vaccination.

2.8. RBD-specific antibody measurement in the sera of mice

RBD-specific IgG antibodies from the sera of immunized and control mice were measured by indirect ELISA as described previously (Wang et al., 2016). Briefly, 96-well microtiter plates (Corning Costar, USA) were pre-coated with 100 μL of purified RBD antigen at a final concentration of 1 μg/mL and incubated overnight. After the plates were blocked with skimmed milk for 2 h at 37 °C, 100 μL of 2-