EBioMedicine 2 (2015) 1438–1446

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Article

Recombinant Receptor Binding Domain Protein Induces Partial Protective Immunity in Rhesus Macaques Against Middle East Respiratory Syndrome Coronavirus Challenge☆

Jiaming Lan [a,d,1], Yanfeng Yao [b,1], Yao Deng [a,1], Hong Chen [a], Guangwen Lu [c], Wen Wang [a], Linlin Bao [b], Wei Deng [b], Qiang Wei [b], George F. Gao [c], Chuan Qin [b,*], Wenjie Tan [a,**]

[a] *Key Laboratory of Medical Virology, Ministry of Health, National Institute for Viral Disease Control and Prevention, China CDC, Beijing 102206, China*
[b] *Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences (CAMS) & Comparative Medicine Center, Peking Union Medical Collage (PUMC), Key Laboratory of Human Disease Comparative Medicine, Ministry of Health, Beijing, China*
[c] *CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China*
[d] *Department of Pathogenic Biology, Hebei Medical University, Shijiazhuang 050017, China*

ARTICLE INFO

Article history:
Received 24 June 2015
Received in revised form 16 August 2015
Accepted 17 August 2015
Available online 18 August 2015

Keywords:
Immunity
RBD
MERS-CoV
Vaccine
Protection
Rhesus macaque

ABSTRACT

Background: Development an effective vaccine against Middle East respiratory syndrome coronavirus (MERS-CoV) is urgent and limited information is available on vaccination in nonhuman primate (NHP) model. We herein report of evaluating a recombinant receptor-binding domain (rRBD) protein vaccine in a rhesus macaque model.
Methods: Nine monkeys were randomly assigned to high-dose, low-dose and mock groups,which were immunized with different doses of rRBD plus alum adjuvant or adjuvant alone at different time points (0, 8, 25 weeks). Immunological analysis was conducted after each immunisation. Monkeys were challenged with MERS-CoV at 14 days after the final immunisation followed by observation for clinical signs and chest X-rays. Nasal, oropharyngeal and rectal swabs were also collected for analyses. Monkeys were euthanized 3 days after challenge and multiple specimens from tissues were collected for pathological, virological and immunological tests.
Conclusion: Robust and sustained immunological responses (including neutralisation antibody) were elicited by the rRBD vaccination. Besides, rRBD vaccination alleviated pneumonia with evidence of reduced tissue impairment and clinical manifestation in monkeys. Furthermore, the rRBD vaccine decreased viral load of lung, trachea and oropharyngeal swabs of monkeys. These data in NHP paves a way for further development of an effective human vaccine against MERS-CoV infection.
© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The Middle East respiratory syndrome coronavirus (MERS-CoV) is the only lineage C *betacoronavirus* known to infect humans (Annan et al., 2013; Anthony et al., 2013; van Boheemen et al., 2012). Similarly to severe acute respiratory syndrome coronavirus (SARS-CoV), MERS-CoV infection can result in acute respiratory distress syndrome and organ dysfunction, including progressive renal function impairment (Zaki et al., 2012). According to the World Health Organization, by the end of 12 August 2015, a total of 1401 cases had been laboratory confirmed, with at least 500 deaths following MERS-CoV infection. Among them, 186 MERS-CoV infection cases, including 36 deaths, had been reported by the Republic of Korea (http://www.who.int/csr/don/12-august-2015-mers-saudi-arabia/en/). These recent clustered cases firstly sprang up outside the Arabian Peninsula, indicating the potential human-to-human transmission of MERS-CoV. To date, no specific antiviral drug exists for MERS-CoV infection and supportive treatment is the mainstay of management (Zumla et al., 2015). Ribavirin and interferon alfa-2b exhibited potential in a rhesus macaque model (Falzarano et al., 2013a, b), but in a retrospective cohort study, ribavirin and interferon alfa-2a therapy was associated with significantly improved survival at 14 days, but not at 28 days in patients with severe MERS-CoV infection (Omrani et al., 2014). Besides, specific peptide fusion inhibitors of MERS-CoV (Lu et al., 2014), convalescent sera from recovered patient and human monoclonal neutralising antibodies (Jiang et al., 2014; Tang et al., 2014; Ying et al., 2014) provided a novel approach to MERS-CoV treatment. However, more data are needed from animal studies and carefully done clinical studies (Zumla et al., 2015). Therefore, developing a prophylactic vaccine against MERS-CoV infection remains a priority (Papaneri et al., 2015).

☆ One sentence summary: Protective immunity of the MERS-CoV RBD vaccine in monkeys.

* Correspondence to: C. Qin, Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences, No. 5, Panjiayuan Nanli, Chaoyang District, Beijing, China.
** Correspondence to: W. Tan, Key Laboratory of Medical Virology, Ministry of Health, National Institute for Viral Disease Control and Prevention, China CDC, 155 Changbai Road, ChangPing District, Beijing 102206, China.
E-mail addresses: qinchuan@pumc.edu.cn (C. Qin), tanwj28@163.com (W. Tan).
[1] Co-first authors.

http://dx.doi.org/10.1016/j.ebiom.2015.08.031
2352-3964/© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Considerable evidence has proved that recombinant receptor binding domain (rRBD)-based subunit vaccine is a promising candidate vaccine against the SARS-CoV infection. As rRBD of SARS-CoV Spike protein induced strong neutralisation antibody and long-term protective immunity in rabbits and mice and completely protected immunized mice from SARS-CoV infection (Zhu et al., 2013). Furthermore, high titres of neutralisation antibodies in non-human primates (NHP) were induced by vaccination with the rRBD of SARS-CoV (Wang et al., 2012). Experience using rRBD-based subunit vaccines against SARS could inform the design of a rRBD-based MERS vaccine. Several human neutralising antibodies targeting the RBD of the MERS-CoV spike protein, have been identified from the naïve-antibody library (Tang et al., 2014; Ying et al., 2014), suggesting that RBD contains epitopes that can induce nAbs and therefore may represent a target antigen against MERS-CoV. Our group and others have confirmed rRBD protein induced strong neutralising antibody responses against MERS-CoV infection in mice and rabbits (Du et al., 2013; Lan et al., 2014; Ma et al., 2014; Mou et al., 2013; Zhang et al., 2015). Although the rRBD subunit vaccine is a highly potent neutraliser of antibodies and T-cell immune responses, no formulation has been tested on a higher animal model with MERS-CoV challenge to verify its prophylactic efficacy (Gretebeck and Subbarao, 2015). Recently, MERS-CoV infection and disease animal models have been developed (Agrawal et al., 2015; de Wit et al., 2013a,b; Falzarano et al., 2014; Munster et al., 2013; Pascal et al., 2015; Yao et al., 2013; Zhao et al., 2014), including a rhesus macaque model of naturally permissive MERS-CoV disease (de Wit et al., 2013a,b; Munster et al., 2013; Yao et al., 2013). We herein evaluate a rRBD subunit vaccine in a rhesus macaque model, to identify a prophylactic approach that could be used in humans to prevent MERS-CoV infection.

2. Methods

2.1. Ethics Statement

Animal studies were conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals of the People's Republic of China. The study protocol was approved by the Committee on the Ethics of Animal Experiments of the Chinese Centre for Disease Control and Prevention. The approved registration number is 20140609015. To comply with 3R (reduction, replacement, refinement) animal experiment principles, a total of 9 rhesus macaques were used.

During experiments, all procedures were performed under ethyl ether anaesthesia, and every effort was made to minimise suffering. Following inoculation with MERS-CoV, all experiments were conducted within an animal biosafety level 3 (ABSL-3) facilities.

2.2. Virus and Cell Culture

MERS-CoV strain (hCoV-EMC/2012) was kindly provided by Professor Fouchier (Erasmus Medical Centre, Netherlands). MERS-CoV seed stocks were propagated in Vero cells (ATCC Number: CCL-81) without mycoplasma contamination maintained in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) supplemented with 10% foetal bovine serum (FBS), 100 international units (IU)/mL penicillin, and 100 μg/mL streptomycin, cultured at 37 °C in 5% CO_2. Seed stocks were diluted to the desired titre and used to determine the MERS-CoV 50% tissue culture infection dose ($TCID_{50}$), and for neutralising antibody and virus inoculation assays.

2.3. Vaccine Formulation

MERS-CoV rRBD protein, containing a 240-amino-acid fragment spanning residues 367–606, was prepared using a baculovirus expression vector system and purified through a HisTrap HP and Superdex 200 column (GE Healthcare, Bucks, UK) as described previously (Lan et al., 2014; Lu et al., 2013). The rRBD protein was admixed with aluminium hydroxide (alum) adjuvant 1 day before immunisation.

2.4. Animal Immunisation and MERS-CoV Challenge

Nine monkeys were randomly assigned to high-dose (H), low-dose (L), and mock (M) groups (Table 1). High-dose animals were primed with 200 μg rRBD and boosted with 100-μg rRBD admixed with 1 mg of alum adjuvant. The three low-dose animals were primed with 50 μg rRBD and boosted with 25 μg rRBD admixed with 1 mg of alum adjuvant; control animals were immunised with PBS with 1 mg of alum adjuvant. Animals received their second immunisation 8 weeks subsequent to their first. To assess long-term immunological responses, the interval between the second and third immunisations was 17 weeks. Each vaccine formulation was administered intramuscularly (i.m.). Two weeks after each immunisation, animals were bled periodically to obtain serum and peripheral blood mononuclear cells (PBMCs) for immunological analysis.

One week after the final immunisation, animals were transferred to an ABSL-3 laboratory. Following an adaptive phase of 1 week, nine immunised monkeys were anaesthetised and inoculated intratracheally with MERS-CoV of the hCoV-EMC strain at a dosage of 6.5×10^7 $TCID_{50}$, diluted in 1-mL DMEM. After the challenge with MERS-CoV, monkeys were observed twice daily, with detailed recording of clinical signs and symptoms, morbidity and mortality, including the nature, onset, severity and duration of all gross or visible changes. Chest X-rays were performed 1 day pre- and 1 and 3 days post-inoculation (dpi) with MERS-CoV. Nasal, oropharyngeal and rectal swabs were collected at the same time points. Three days after challenge with MERS-CoV, monkeys were euthanized and tissue specimens, including lungs, trachea, spleen and kidney, were collected for various pathological, virological, and immunological tests. The schematic of the vaccination schedule and biological specimen collection timeline are displayed in Fig. 1.

2.5. IgG Antibody Analyses Using Enzyme-linked Immunosorbent Assay (ELISA)

Animals were bled periodically throughout the study (Fig. 1) to derive serum for antibody detection. Endpoint anti-RBD IgG antibody titres were determined by ELISA as described previously (Lan et al., 2014).

2.6. Pseudovirus Neutralisation Assay

A MERS-CoV pseudovirus system was conducted within biosafety level-2 facilities as reported previously (Lan et al., 2014). Relative light units were determined immediately using a Gaomax luminometer (Promega). All experiments were performed in triplicate. The pseudovirus inhibition (PI) rate was calculated as follows:

$$(\text{Relative luciferase units of mock sera} - \text{Relative luciferase units of immune serum for a given dilution}) / \text{Relative luciferase units of mock sera}.$$

2.7. Neutralising Antibody to hCoV-EMC

The virus neutralisation assay was conducted on Vero Cells. Briefly, sera were diluted twofold and mixed with 50 μL of 2×10^3 $TCID_{50}$/mL virus, incubated at 37 °C for 1 h in quadruplicate. Thereafter, 100-μL virus-serum mixture was added to Vero cells previously seeded at 1.5×10^4/well. Inoculated plates were incubated in a CO_2 incubator at 37 °C for 3 days, following which the cytopathic effects (CPEs) of the virus were observed microscopically at 40× magnification. All tests were repeated twice independently.

Download English Version:

https://daneshyari.com/en/article/2121232

Download Persian Version:

https://daneshyari.com/article/2121232

Daneshyari.com