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Immunogenicity of Vaccine Encoding Spike Protein against Middle East Respiratory Syndrome Coronavirus in Mammalian Model

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Abstract

The Middle East respiratory syndrome coronavirus (MERS-CoV) represents a major threat to human health worldwide. No licensed MERS-CoV vaccines or therapeutics were developed. So, the aim of the current study is the generation and immunological evaluation of DNA vaccine candidate against MERS-CoV. The spike gene was selected to generate the DNA vaccine, which encodes the spike protein of coronaviruses that plays a pivotal role in viral entry into host cells and serves as a primary target for host immune responses. Moreover, the antibody responses post-immunization with spike-DNA fragment and inactivated coronaviruses vaccines was explored, using a microneutralization assay with severe acute respiratory syndrome (SARS-CoV-2), and the log2 antibody titers at different time points (0, 2, 4, 6, and 8 weeks) post-vaccination was measured. The inactivated MERS-CoV and pCDNA3.1-S-MERS-CoV vaccines maintained steady log2 antibody titers, showing no specific response to SARS-CoV-2 in the control group (PBS). Microneutralization against MERS-CoV showed no significant antibody titers for Inactivated SARS-CoV-2, suggesting no cross-reactivity. Sustained antibody titers for Inactivated MERS-CoV indicate vaccine-induced stability. This study sheds light on antibody responses induced by these vaccines against MERS-CoV. These insights are crucial for optimizing vaccine strategies, particularly in the context of the evolving MERS-CoV pandemic.

Keywords: Vaccine, spike, MERS-CoV, SARS-CoV-2, antibodies

1. Introduction

The emergence of human coronaviruses as significant public health threats has sparked intense research into understanding their molecular and evolutionary characteristics. Viral attachment and entry into cells were processed by spike protein (S) which has a central role in viral pathogenesis.

Human coronaviruses (HCoVs) have been a subject of immense global concern due to their potential to cause severe respiratory illnesses and outbreaks [1]. The S protein of HCoVs plays a pivotal role in eliciting host immune responses. Understanding its evolutionary dynamics is crucial for deciphering viral adaptation, transmission, and pathogenesis amid ongoing public health threats [2]. Comparing the S protein in various human coronaviruses offers understandings into genetic variations, amino acid mutations, viral fitness, host specificity, and potential cross-protection against emerging variants [3, 4]

Molecular and serological studies propose that the zoonotic source for MERS-CoV so far is dromedary camels, but the origin of MERS-CoV is still under research [5-7] Human-to-human transmission noted in outbreaks in South Korea, Jeddah, and Riyadh clusters [8-10]. Due to the identity of MERS-CoV sequences obtained from the patient and the contacted camel support the role of camel as a reservoir for virus transmission to human [11, 12] MERS-CoV and SARS-CoV-1 outbreaks in 2012 and 2002-2003, respectively, highlighted the urgency of studying the molecular characteristics and identifying genetic changes of HCoVs, especially in the S protein. The COVID-19 pandemic which emerged by SARS-CoV-2 emphasized the need to understand the S protein evolutions and implications for vaccine development and viral pathogenesis [13].

Continuous interaction with the host immune system drives the emergence of genetic variants, affecting viral replication, transmission, and immune evasion. Selective pressures on circulating HCoVs can lead to beneficial spike protein mutations, influencing viral tropism, disease severity, and vaccine and antiviral efficacy

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[14].The evolution dynamics of the spike protein in human coronaviruses have garnered significant attention, particularly in the context of understanding viral pathogenesis and designing effective vaccines. Mutations and variations in the spike protein can influence viral transmissibility, tissue tropism, and virulence, shaping the course of infection and disease severity [15].

This study focuses on the intricate evolutionary dynamics of the spike protein in human coronaviruses, focusing on insights that can profoundly impact the understanding of viral pathogenesis and guide the development of vaccines. Through a comprehensive exploration of antibody responses post-immunization with DNA spike and inactivated coronaviruses vaccines, to shed light on the adaptive changes and antibody interactions that occur within the spike protein. This knowledge is paramount for enhancing the efficacy and breadth of vaccines against emerging and re-emerging coronaviruses, particularly in the backdrop of the ongoing COVID-19 pandemic.

2. Materials and methods

2.1. Cells and Viruses

Vero-E6 cells from the stocks at the Center of Scientific Excellence for Influenza Viruses (CSEIV), National Research Center (NRC), were cultured in Dulbecco modified Eagle medium (DMEM) (Lonza, Switzerland) containing 10% fetal bovine serum (FBS), and 1% antibiotic antimycotic mixture (Lonza). Two viruses were used in this study; MERS-CoV/Egypt/camel/NC 270/2014 and SARS -CoV-2 Egypt/NRC-03/2020 that were isolated in CSEIV and propagated in Vero-E6 cells.

2.2. Viral RNA extraction

RNA of both MERS-CoV/Egypt/camel/NRC 270/2014, GenBank accession no. KJ477103 and SARS-CoV-2 Egypt/NRC-03/2020, GISAID accession # EPI_ISL_43020 were extracted according to the manufacturer instructions using viral RNA Mini Kit (Viral RNA MiniKit; Qiagen, Germany).

2.3. Spike fragment RT-PCR for coronaviruses

Complementary DNA (cDNA) synthesis was synthesized from extracted MERS-CoV RNAs using Superscript IV Kit (Invitrogen) according to the manufacturer's instructions. The spike genes of both viruses were then amplified using in-house designed primers. PCR reaction was performed in a 50 µl total reaction volume of 25 µl of 2X RT-PCR Master Mix, 10 µl of extracted viral RNA, 1.5 µl of each forward and reverse primers (10 µM), 0.5 µl enzyme mix and 11.5 µl of sterile distilled water. Thermal cycling involved 50°C for 15 min, followed by 98°C for 2 min and then 40 cycles of 98°C for 10 s, 55°C for 10 s, 72 °C for 1 min, then final extension for 5 min at 72°C. The PCR amplicons were visualized by electrophoresis in 1% agarose gel with gene ruler 1 Kb DNA ladder.

2.4. Extraction and purification of PCR amplicons from Agarose gel

Appropriate-sized DNA fragments were excised from the gel and purified using the QIAquick gel extraction kit (QIAGEN, Hilden, Germany). Briefly, the gel slice was dissolved by incubating at 50°C for 10 min in QG Buffer. After dissolution, isopropanol was added, and the lysate was applied to a QIAquick column, followed by centrifugation. The column was washed and then centrifuged again to remove any remaining liquid. The eluted DNA was collected in 30 µl of elution buffer (EB). DNA concentration was measured at 260 nm using a NanoDrop2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and sample purity was assessed by calculating the OD260/OD280 ratio.

2.5. Construction of DNA full-length S-plasmid vector vaccine

Enzymatic digestion and ligation for spike gene of MERS-CoV and plasmid vector pcDNA3.1 (negative) mammalian expression vector (Invitrogen), according to the manufacturer's instructions, the underwent enzymatic digestion by KPN1 and Not1 restriction enzymes from NEB cutter and New England Bio labs tools according to manufacturer's instructions in order to form sticky ends for subsequent ligation by T4 DNA Ligase (Promega), forming DNA pcDNA3.1 (negative)-S construct. pcDNA3.1 (negative)-S was transformed to TOP 10 competent E. coli (Thermo scientific, Carlsbad, California, USA), by addition of 5 µl of ligation reaction product to 50 µl vial of TOP 10 competent cells then incubated on ice for 30 min and 30 s in 42°C water bath, then placed on ice with addition of 250 µl of Super Optimal Culture medium, and then incubated in a shaking incubator at 37°C for exactly 1 h at 225 rpm. The mixture was inoculated on Luria–Bertani agar plate and incubated at 37°C overnight. Ampicillin resistance-based selection of colonies was done followed by large-scale plasmid isolation (Maxiprep) using Plasmid Maxi Kit (Qiagen) according to the manufacturer's instructions.

The plasmid mini-preparations were performed according to manufacture protocol of QIAprep® Spin Miniprep Kit. Briefly, selection for the positive colonies from agar plate by using a clean and sterile pipette tip then put the tip in a falcon tube that have containing 3 ml of LB media with selectable marker ampicillin, then incubate overnight at 37°C overnight.

A volume of 1.5 ml of bacterial culture was further pelleted by centrifugation at >8000 rpm for 3 min at room temperature, bacterial pellets were resuspended in 250 µl of buffer P1. A 250 µl of buffer P2 was then added and mixed thoroughly by inverting the tube 4-6 times until the solution becomes clear. Subsequently, 350 µl Buffer N3 was added and mixed then centrifuged for 10 min at 13,000 rpm.

Afterwards, 800 µl of supernatant was added to the column, centrifuged for 30–60 sec, The column was washed by adding 0.75 ml Buffer PE and centrifuged for 30–60 sec, then centrifuged for 1 min at 13,000 rpm,. To elute DNA, 50 µl Buffer EB was added and centrifuged for 1 min at 13,000 rpm.

2.6. Preparation of inactivated MERS-CoV and SARS-CoV2 coronaviruses (positive control) vaccine

The two viruses were propagated in Vero-E6 cells and concentrated using Amicon Ultra-15 system (Amicon, USA), briefly 15 ml of propagated viruses were inactivated using 0.1% formaldehyde. The 15 ml viruses were centrifuged at 4500 rpm at 4ºC for 20 min. The concentrated viral proteins in 300 µl PBS were determined using Nanodrop. Inactivated viruses were mixed with Adjuplex adjuvant (Sigma Aldrich, Germany) by mixing 1 part of Adjuplex to 4 parts of prepared antigen concentrated virus (v/v).

2.7. DNA and inactivated vaccines immunization to BALB/c mice

Female BALB/c mice (6- to 8-week-old) were obtained from the Animal House at NRC (ethical approval # 16/247). Five groups (5 mice/ group) were injected intramuscularly with 100 µg with two injections divided between the two thighs; 50 µg/each mixed with 20% Adjuplex adjuvant. The groups were pcDNA3.1-S-MERS-CoV, pcDNA3.1-S-SARS-CoV-2 genes, inactivated MERS-CoV, inactivated SARS-CoV-2 and a group as a negative control injected with sterile 1x PBS. All the animals received booster immunizations after 2 weeks. Serum samples were collected at 0, 2, 4, 6 and 8 weeks from immunization. All mice sera were separated and stored at -20 ºC until used.

2.8. Neutralizing capacity of the prepared constructs using micro-neutralization (MN) assay

The MN was performed as described previously using Vero-E6 cell monolayers. Briefly, serial 2-fold dilutions of the mice sera starting with a dilution of 1:10 were mixed with equal volumes of 100 $TCID_{50}$/ml of MERS-CoV and SARS-CoV-2. The mix were incubated for 1 h at 37°C, 35 µl of virus-sera mixture were inoculated to Vero-E6 cells in 96-well plates and incubated for 1 h. The inoculums were aspirated and the plates were then incubated for 72 h at 37°C in a humidified incubator with 5% CO_2. A virus back-titration was performed without immune serum to confirm the virus titer. The highest serum dilution that completely protected the cells from cytopathic effect (CPE) was investigated as the MN titer.

2.9. Statistical analysis

GraphPad-Prism software version 5 was used to analyze the data.

3. Results

Selection and genetic characteristics of MERS-CoV

The MERS-CoV has a genetic diversity. The phylogenetic tree was performed based on the nucleotide variations of the spike genes. MERS-CoV is classified into 3 major clades A, B, and C as shown in (Fig. 1). Clade A contains first strains were isolated from Saudi Arabia and Jordan in 2012. Clade B is the common clade that circulated in Arabian Peninsula and responsible for human infections. Clade C comprises MERS-CoV strains from Africa. We selected the strain for this study from clade C which is dominant strain in Egypt, to investigate the genetic relationship between Egyptian MERS-CoVs and other MERS-CoV. Consequently, spike gene phylogenetic analysis was performed using MEGA-X and the obtained findings showed that the studied strains fell into a cluster distinct from other MERS-CoVs circulated in Africa (Kenya, Ethiopia, Nigeria, Burkina Faso, and Morocco). Multiple alignment of the sequences used within the three clades of MERS-CoV revealed no variations, deletions and/or insertions in receptor binding domain (Fig. 2).

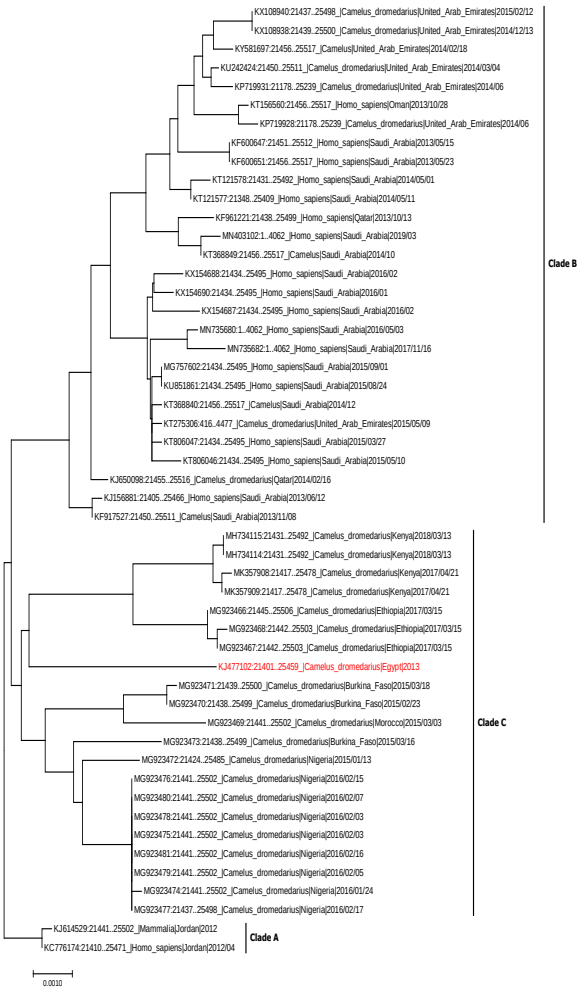

Fig 1: Phylogenetic tree of MERS-CoV spike nucleotide sequences**.**

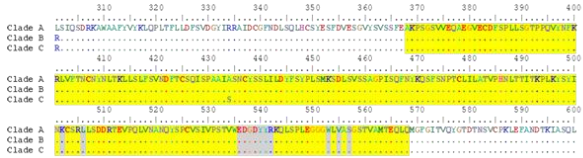

Fig.2: Amino-acid sequence alignment of spike protein among MERS-CoV clades. Human_MERS-CoV_Spike_S1_RBD (yellow highlight), receptor binding site (gray highlight).

Phylogenetic analysis was performed using the neighbor-joining algorithm with the Kimura two-parameter model with 1000 replications bootstrap. MEGA X was used in evolutionary analyses. The virus isolate used in this study in red color.

3.1. Construction of DNA vaccine candidates against MERS-CoV

Plasmid DNA was extracted after positive clones were selected and cultured. Spike gene was amplified using F-Spike-EcoR1 (GATATTGAATTCgccac cATGATACACTCAGTGTTTCTACTG), R-Spike-KpNI (TTATTAGGTACCTTAATG AACATGAAC CTTATGCGGC) and then ligated with the plasmid fragment that was digested using the same enzyme. Positive clones in the transformed plates were selected randomly. Plasmid mini-prep was conducted to select the positive colonies. The nucleotide sequence of the recombinant gene encoding Ag85A/MPT64 had no mutations, and the gene was correctly inserted into the vector.

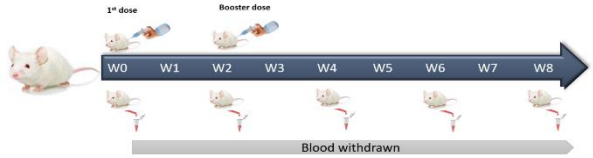

Fig.3. Timeline of immunization of DNA ad inactivated vaccines in mice. 3.2. Immune response of MERS-CoV spike DNA vaccine in mice

The antibody response was assessed in mice post immunization with DNA-spike MERS-CoV vaccine constructs and inactivated coronaviruses candidate vaccines, focusing on the evolutionary dynamics of the spike protein against human coronaviruses. The log2 antibody titers was measured using a microneutralization assay with MERS-CoV. The antibody titers were recorded at various time points post-vaccination (0, 2, 4, 6, and 8 weeks) (Fig 3). 4.

Discussion

Middle East respiratory syndrome coronavirus (MERS-CoV) represent emerging threats to public health worldwide. Development of candidate vaccines for zoonotic viruses is critical for controlling continual outbreaks of these viruses in humans and animals. MERS-CoV was first discovered in Saudi Arabian patient suffering from a cute pneumonia and renal failure on June 13, 2012, [16]. In this study, a candidate vaccine was developed to investigate its ability to elicit potent immune responses against MERS-CoV infection and to study the cross reactivity with SARS-CoV-2 vaccine.

MN results against MERS-CoV showed that, the log2 antibody titers for Inactivated SARS-CoV-2 showed no antibody titer throughout the entire duration of the study (0 to 8 weeks' post-vaccination) (Fig 5). Similarly, the log2 antibody titers for Inactivated MERS-CoV were consistently at week 0 and remained relatively stable at 20-160 from weeks 2 to 6 post-vaccination. At week 8 post-vaccination, the log2 antibody titers for Inactivated MERS-CoV showed a slight decrease, ranging from 0 to 80 (Fig.4).

The log2 antibody titers for pCDNA-S-SARS-CoV-2 and pCDNA-S-MERS-CoV remained at a value of 0 across all time points (0 to 8 weeks) post-vaccination, indicating no antibody response for the DNA spike coronaviruses vaccines. Control-PBS also showed log2 antibody titers of 0 at all-time points, confirming the absence of a specific antibody response in the negative control group.

These results provide insights into the antibody responses induced by the respective vaccines against MERS-CoV and SARS-CoV-2, informing the study on the evolutionary dynamics of the spike protein in human coronaviruses.

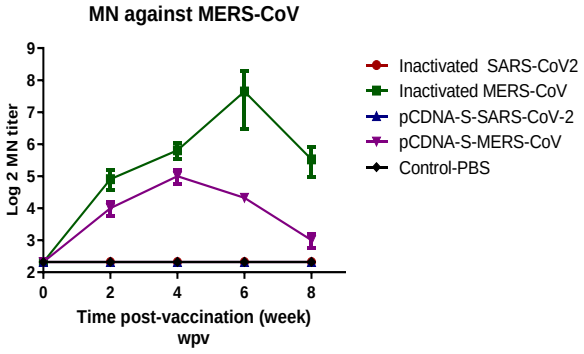

Fig. 4. The log2 antibody titers at different time points post-vaccination for MERS-CoV, using MN assay for mice sera vaccinated with DNA vaccines of spike genes and inactivated vaccines.

According to the World Health Organization (WHO), No vaccine or specific treatment for MERS is currently available, however there are several vaccines for MERS in development. Additionally, several MERS vaccines have undergone Phase 1 trials, but no vaccines are yet approved for human use [17,18]. There are different platforms to produce vaccines for MERS-CoV include those based on DNAs, proteins, nanoparticles or virus-like particles (VLPs), viral vectors, and live-attenuated viruses. Most of these vaccines are designed using the viral S protein, including its fragments, to protect animals against MERS-CoV infection [18-20].

DNA vaccine platforms are rapidly responded to emerging infectious diseases, so in the current study extensively examined the antibody responses in mice following immunization with DNA spike vaccine against MERS-CoV comparing with inactivated vaccine.

DNA-spike vaccine was found to protect non-human primates from MERS-CoV-induced disease by reducing viral replication in their lungs [21].

The inactivated MERS-CoV vaccine, pCDNA3.1-S-MERS-CoV vaccine, and the control (PBS) groups demonstrated consistent log2 antibody titers at all examined time points, suggesting an absence of a specific antibody response against the SARS-CoV-2 virus under study. Microneutralization results against MERS-CoV shed light on the absence of significant antibody titers in response to Inactivated SARS-CoV-2, suggesting a lack of cross-reactivity between the two coronaviruses. Additionally, the stable antibody titers for Inactivated MERS-CoV indicate the effectiveness and stability of the vaccine-induced response over the study period.

5. Conclusions

Our findings provide critical insights into the antibody responses induced by the respective vaccines against MERS-CoV. These insights hold substantial implications for the ongoing efforts towards the development and optimization of effective vaccines against emerging and re-emerging human coronaviruses.

Declarations:

Ethics approval and consent to participate

All experiments including infectious viruses were performed a biosafety level 3 cabinets at the National Research Centre (NRC), Egypt. All animal experiments were conducted in accordance with the Medical Research Ethics Committee (MREC) of the NRC, Egypt (approval code: 19–274).

Consent for publication

Not applicable

Availability of data and materials

All data and materials are available inside the manuscript and on demand.

Competing interests

There are no conflicts to declare

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Authors' contributions

Mohamed E. Abo Sherif, was responsible for the lab work, Noura M. Abo Shama, participate in animal work; Sara H. Mahmoud, was the direct supervisor in the lab., Mohamed A. El-Desouky, and Demiana H. Hanna, were participate in the suggested project and wright and analysed the data, Mohamed A. Ali, who funded the work and wright and analysed the data.

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