Virologica Sinica 37 (2022) 248–255

Contents lists available at ScienceDirect

Virologica Sinica

journal homepage: www.keaipublishing.com/en/journals/virologica-sinica
www.virosin.org

Research Article

Immunogenicity of a recombinant VSV-Vectored SARS-CoV vaccine induced robust immunity in rhesus monkeys after single-dose immunization

Dan Shan [a,1], Xiaoyan Tang [a,1], Renqiang Liu [a,1], Dan Pan [a], Xijun Wang [a], Jinying Ge [a], Zhiyuan Wen [a,*], Zhigao Bu [a,b,*]

[a] State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, 150069, China
[b] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, 225127, China

ARTICLE INFO

Keywords:
Severe acute respiratory syndrome coronavirus (SARS-CoV)
Vesicular stomatitis virus (VSV)
Vaccine
Immunization

ABSTRACT

Severe acute respiratory syndrome (SARS) is a highly contagious zoonotic disease caused by SARS coronavirus (SARS-CoV). Since its outbreak in Guangdong Province of China in 2002, SARS has caused 8096 infections and 774 deaths by December 31st, 2003. Although there have been no more SARS cases reported in human populations since 2004, the recent emergence of a novel coronavirus disease (COVID-19) indicates the potential of the recurrence of SARS and other coronavirus disease among humans. Thus, developing a rapid response SARS vaccine to provide protection for human populations is still needed. Spike (S) protein of SARS-CoV can induce neutralizing antibodies, which is a pivotal immunogenic antigen for vaccine development. Here we constructed a recombinant chimeric vesicular stomatitis virus (VSV) VSVΔG-SARS, in which the glycoprotein (*G*) gene is replaced with the SARS-CoV *S* gene. VSVΔG-SARS maintains the bullet-like shape of the native VSV, with the heterogeneous S protein incorporated into its surface instead of G protein. The results of safety trials revealed that VSVΔG-SARS is safe and effective in mice at a dose of 1×10^6 $TCID_{50}$. More importantly, only a single-dose immunization of 2×10^7 $TCID_{50}$ can provide high-level neutralizing antibodies and robust T cell responses to non-human primate animal models. Thus, our data indicate that VSVΔG-SARS can be used as a rapid response vaccine candidate. Our study on the recombinant VSV-vectored SARS-CoV vaccines can accumulate experience and provide a foundation for the new coronavirus disease in the future.

1. Introduction

Severe acute respiratory syndrome (SARS) is a highly contagious zoonotic disease caused by SARS coronavirus (SARS-CoV), which belongs to the family *Coronaviridae*, genus *β-coronavirus* (Guan et al., 2003). Since its initial identification in Guangdong Province of China in November 2002, SARS-CoV has resulted in 8096 infections and 774 deaths (mortality rate: ~10%) in 29 countries by December 31st, 2003 (Drosten et al., 2003; Ksiazek et al., 2003; WHO, 2004). With no more SARS-CoV infected cases reported, World Health Organization (WHO) has declared that SARS-CoV is eradicated in human populations in 2004 (WHO, 2004). However, the COVID-19 pneumonia outbreak underlines the importance of the prevention and control of SARS-CoV and the SARS-like CoVs.

The SARS-CoV spike (S) protein is one of the most immunogenic antigens for vaccine development, which induces neutralization antibodies to block virus-binding and stimulates host immune responses against infections (Du et al., 2009). A variety of S protein-specific vaccines have been proven effective in animal models, such as inactivated vaccines, DNA vaccines and virus-like particles (VLPs) vaccines (Woo et al., 2005; Qin et al., 2006; Liu et al., 2011). However, some of these strategies demand multiple doses to elicit favorable immune responses. And some strategies have other disadvantages, including uncertain expenditure costs and the possible requirement for appropriate vaccine adjuvants (Graham et al., 2013). Once SARS break out again, a rapid response vaccine is urgently needed.

Vesicular stomatitis virus (VSV), which causes a self-limited disease in humans and many other species, has been used as a desired attenuated vector to develop commercial vaccines (Lawson et al., 1995). Multiple recombinant VSV-based vaccines can induce rapid robust immune responses in animal models, such as Ebola, Marburg and Influenza

* Corresponding authors. State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, 150069, China.
E-mail addresses: wenzhiyuan@caas.cn (Z. Wen), buzhigao@caas.cn (Z. Bu).
[1] Dan Shan, Xiaoyan Tang and Renqiang Liu contributed equally to this work.

https://doi.org/10.1016/j.virs.2022.01.002
Received 23 February 2021; Accepted 4 August 2021
Available online 12 January 2022

vaccines. More importantly, VSV-based vaccines can be used as promising post-exposure prophylaxis against high-risk virus infections (Ryder et al. 2015; Coller et al., 2017; Marzi et al., 2018; Monath et al., 2019). VSV-based vaccine expressing the Ebola virus (EBOV) glycoprotein, has successfully completed a phase III clinical trial during the West African EBOV epidemic (Gsell et al., 2017; Henao-Restrepo et al., 2017). A VSV-based chimeric virus expressing Middle East respiratory syndrome coronavirus (MERS-CoV) spike (S) protein was constructed by our lab, which has been proven to rapidly induce good immune response in rhesus monkeys (Liu et al., 2018). During the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, a recombinant replication-competent VSV-based vaccine candidate expressing the SARS-CoV-2 S protein was constructed and proved to protect against severe SARS-CoV-2 infection, supporting its further development as a vaccine (Case et al., 2020; Yahalom-Ronen et al., 2020). Though some similar studies about VSV-vectored vaccines against SARS-CoV-2 have been performed in different kinds of experimental animals, taking significant differences between SARS-CoV and SARS-CoV-2 into consideration, it is necessary to develop recombinant VSV-vectored vaccines especially for SARS-CoV.

Here, we constructed a chimeric recombinant VSV (designated as VSVΔG-SARS), in which the VSV glycoprotein (*G*) gene was replaced with the spike (*S*) gene of SARS-CoV. Only a single-dose immunization with VSVΔG-SARS generated high-level S protein-specific neutralizing antibodies and cellular immune responses in rhesus monkeys. Overall, our data show that VSVΔG-SARS can protect against SARS-CoV infection, supporting its further development as a vaccine. This study paves the way for further development of a VSV-vectored CoVs vaccine.

2. Materials and methods

2.1. Cell lines and plasmids

Hamster Syrian kidney cells (BSR cells), BSR-T7/5 and Vero E6 cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin (Invitrogen, Oregon, USA) at 37 °C in a 5% CO_2 incubator.

2.2. Recombinant virus recovery

The VSVΔG-eGFP pseudo-virus system described before was modified to construct the recombinant virus VSVΔG-SARS and VSVΔG-eGFP-SARS (Liu et al., 2018). SARS-CoV *S* gene (GenBank accession No. AAP13441.1) was chemically synthesized and cloned into the pVSVΔG-eGFP plasmid, which contains VSV full-length genomic cDNA without *G* gene. The final plasmid was designated as pVSVΔG-eGFP-SARS. The pVSVΔG-SARS plasmid was also prepared, in which the *eGFP* gene was deleted from the pVSVΔG-eGFP-SARS plasmid. The pVSVΔG-eGFP plasmid or the pVSVΔG-eGFP-SARS plasmid, together with plasmids encoding the N, P, and L proteins were transfected into BSR-T7/5 cells. At 96 h (h) post-transfection, the supernatant was collected and serially passaged on Vero E6 cells, until obvious green fluorescence or cytopathic effect (CPE) was observed. The rescued viruses were plaque purified and further passaged to generate viral stocks. The viral titer of the recombinant VSV viral stock was as high as 2×10^7 $TCID_{50}$/mL.

2.3. Replication kinetics

Multi-step growth experiments were conducted to evaluate the kinetics of the recombinant viruses. Confluent monolayers of Vero E6 in 6-well plates were respectively infected with native VSV, VSVΔG-SARS or VSVΔG-eGFP-SARS at an MOI = 0.01. The supernatant was harvested at different time intervals (0, 12, 24, 36, 48, 60, 72, 84 and 96 h) post infection. Supernatants of each time were separately collected and preserved at −80 °C. The viral titers were determined by the index of the highest dilution titer (Tissue culture infective dose, $TCID_{50}$/mL).

2.4. Indirect immunofluorescence and western blot assay

For Western blot assay, infected Vero E6 cells were collected and lysed at 72 h post-infection with the recombinant viruses at an MOI = 1. Samples were electrophoresed in SDS-10% polyacrylamide gels, and the proteins were transferred onto nitrocellulose membranes. Western blot detection was performed with mouse anti-S serum at a dilution of 1:1000 (Invitrogen, Oregon, USA) and HRP-labeled goat anti-mouse IgG at a dilution of 1:3000 (Genscript, Nanjing, China). The bands were visualized with Azure C300–C500 (Cycloud, Beijing, China).

For indirect immunofluorescence assay, Vero E6 cells were planted onto 24-well plates and infected with the recombinant viruses at an MOI = 1. At 36 h post-infection, the infected cells were fixed and subjected to indirect immunofluorescence with mouse anti-S serum as the primary antibody at a dilution of 1:100 (Invitrogen, Oregon, USA), and Alexa Fluor 568-conjugated goat anti-mouse IgG as the secondary antibody at a dilution of 1:2000 (Invitrogen, Oregon, USA). Cell nuclei were stained with Hoechst 33342 (Invitrogen, Oregon, USA). The Staining cells were analyzed with LSM880-ZEISS confocal laser scanning microscopy with fast Airyscan (Carl Zeiss, Germany).

2.5. Cell transfection and infection

BSR cells were seeded at 24 well plate. Lipofectamine2000 (Invitrogen, Oregon, USA) was used for transfection of the BSR cells with the cloned vectors pCAGGs-hACE2-Flag and pCAGGs-Flag according to the manual instructions. After 24 h post transfection, pCAGGs-transfected BSR cells and human ACE2-transfected BSR cells were infected with VSVΔG-eGFP-SARS at an MOI = 0.05. At 36 h post-infection, the infected cells were stained with anti-Flag antibodies (Genscript, Nanjing, China) and Hoechst 33342 and observed with inverted fluorescence microscope.

2.6. Immunoelectron microscopy

Immunoelectron microscopy method was conducted to show the morphology of the recombinant viruses as described (Ge et al., 2011). The high-speed (10,000×*g*) centrifuged recombinant viruses were adsorbed for 10 min by flotation onto a freshly discharged 200 mesh palladium carbon-coated nickel grids (Electron Microscopy Sciences, Hatfield, UK). For immunoelectron microscopy, mouse anti-SARS S antibody at a dilution of 1:100 (Invitrogen, Oregon, USA) was used as primary antibody, and 10 nm gold particle-conjugated goat anti-mouse IgG (Sigma-Aldrich, St. Louis, USA) was used as the secondary antibody. Viral particles were examined under a model H7500 transmission electron microscope (Hitachi High Technologies, Schaumburg, JPN) at 80 kV. Images were obtained by using an XR100 digital camera system (Advanced Microscopy Techniques, Danvers, USA).

2.7. Animal immunization

For mouse immunizations, sixteen 6-week-old female Balb/c mice were randomly divided into two groups. In group 1, intramuscular (*i.m*) immunizations were performed with VSVΔG-SARS at a dose of 1×10^6 $TCID_{50}$ in 0.1 mL via hind limb muscle injection. In group 2, *i.m* immunizations were performed with 0.1 mL PBS as a control. The mice received the booster dose injection three weeks post the prime immunization. Blood samples were collected at 21 days after the prime and boost immunizations.

For monkey immunizations, five 2-year-old male rhesus monkeys were intramuscularly immunized with VSVΔG-SARS at a dose of 2×10^7 $TCID_{50}$ via hind limb muscle injection under anesthesia. All monkeys were housed in separate cages in a Biosafety level-3 laboratory, and supplied with a specialized monkey puffed diet, various fresh fruits and

adequate drinking water. Blood samples were collected at day 0, 10, 21, 28, and 42 post-immunizations.

2.8. Neutralization assays

SARS-CoV neutralization assay was adapted from our previously published protocol (Liu et al., 2017). Briefly, mouse or monkey serum samples were heat-inactivated at 56 °C for 30 min before use. Vero E6 cells were seeded in 96-well plates and grown into confluent monolayer overnight. Serum samples were two-fold serially diluted in DMEM (from 1:2 to $1:2^8$), VSVΔG-eGFP-SARS were diluted to 1×10^2 $TCID_{50}$ per well and added on top of serum dilutions. The virus-serum mix was incubated at 37 °C for 2 h. The infected cells with virus-serum mixtures were scored for the inhibition of expressing GFP at 36 h post-infection. The titer of neutralizing antibody is defined as the reciprocal of the highest dilution, at which the percentage of GFP expression is less than or equal to 50%.

2.9. ELISA

The level of antibodies against SARS-CoV S protein was measured using an enzyme-linked immunosorbent assay (ELISA) as described before (Kong et al., 2012). Briefly, Vero E6 cells were infected with recombinant Newcastle disease virus expressing SARS-CoV S protein at an MOI = 0.1. The clarified ultrasonicated supernatant after detergent extraction was collected after 48 h post-infection and coated in 96-well ELISA plates overnight at 4 °C. The wells were washed and blocked for 1 h at 37 °C with PBST containing 5% skim milk. Next, the mouse or monkey serum samples were 2-fold serially diluted and incubated at 37 °C for 1 h. The plates were then washed three times with PBST, and anti-mouse IgG, IgG1 or IgG2a-peroxidase antibody (Southernbiotech, AL, USA) or anti-monkey IgG-peroxidase antibody (Southernbiotech, AL, USA) at a dilution of 1:5000 was added. After 1 h of incubation 37 °C, the plates were washed five times with PBST, and the color reaction was developed with 3,3′,5,5′-Tetramethylbenzidine (TMB) liquid substrate (Sigma, USA). The developing process was stopped after 15 min at 37 °C with 2 mol/L H_2SO_4, and the plates were read at OD450 with a Model 680 microplate reader (Bio-Rad). A standard curve was generated by coating mouse IgG at known concentrations, and a linear equation was built based on the IgG concentration and their OD values. The concentration of SARS-specific IgG in each sample was tested in duplicate and the mean absorbance was calculated according to the linear equation.

2.10. ELISPOT

Enzyme-linked immune-spot (ELISPOT) assay was carried out as described before (Liu et al., 2018). A synthetic overlapping peptide pool spans the whole SARS-CoV S protein, which consisted of 249 peptides (15-mers with 10 amino acids overlapped, designed for CD^{8+} T cells) (Supplementary Table S1). Briefly, peripheral blood mononuclear cells (PBMCs) were separated from monkey blood at 10 days after immunization. Millipore 96-well HTS HA sterile plates (Millipore, USA) were pre-coated with purified mouse anti-human IFN-γ (BD Pharmingen, San Diego, USA), and blocked with RPMI 1640 medium with 10% FBS. Each 15-mers peptide from the whole SARS-CoV S protein synthetic overlapping peptide pool was diluted and added into the well. A total of 5×10^5 monkey PBMCs from the same group were pooled, added into the well and mixed with peptides. The monkey PBMCs were added with irrelevant peptides as negative control, and added 10 ng/mL phorbol myristate acetate (PMA) (Sigma, USA) with 1 μmol/L Ionomycin (Sigma, USA) as positive control (PMA+iono). After incubation at 37 °C for 24 h, the plates were vigorously washed with PBS, and incubated with biotin-labeled mouse anti-human IFN-γ at a dilution of 1:3000 (BD Pharmingen, San Diego, USA) for 1 h. After the final wash with PBS, AEC substrate (BD Pharmingen, San Diego, USA) was added to develop spots. The number of spots was counted by ElISPOT Reader (AID, Germany) and analyzed by GraphPad Prism 6 (GraphPad Software, CA, USA).

2.11. Statistical analysis

Statistical analyses were performed using one-way ANOVA with Bonferroni's multiple comparison tests. All P value < 0.05 was considered statistically significant. ***, $P < 0.001$. **, $P < 0.01$. *, $P < 0.05$.

3. Results

3.1. Construction of VSVΔG-SARS and VSVΔG-eGFP-SARS and in vitro characterization of the viruses

As expected, the recombinant VSVΔG-SARS and VSVΔG-eGFP-SARS were successfully generated and rescued by using an established VSV reverse genetics system, in which the SARS-CoV spike (*S*) gene was inserted between the *M* and *L* gene instead of the VSV *G* gene (Fig. 1A). EGFP expression was observed in VSVΔG-eGFP-SARS-infected Vero E6 cells (Fig. 1C). Indirect confocal immunofluorescence staining and Western blot confirmed the expression of the S protein in VSVΔG-eGFP-SARS and VSVΔG-SARS-infected Vero E6 cells, which indicated the incorporation of S protein into the viral particles (Fig. 1B and C).

VSVΔG-SARS and VSVΔG-eGFP-SARS grew to similar growth kinetic in Vero E6 cells, whose peak titers both reached $1 \times 10^{6.7}$ $TCID_{50}$/mL at 72 h post-infection (Fig. 2). The recombinant viruses showed delayed growth kinetics and lower peak titer as compared with that of VSV. RT-PCR and IFA results demonstrated the stable expression of SARS-CoV *S* gene after 10 passages of VSVΔG-SARS and VSVΔG-eGFP-SARS in Vero E6 cells (Supplementary Figure S1).

3.2. VSVΔG-SARS and VSVΔG-eGFP-SARS can infect hACE2-Transfected BSR cells

The results of electron microscopy assay showed that S protein was successfully expressed on the surface of VSVΔG-SARS and VSVΔG-eGFP-SARS particles (Fig. 3, white arrows indicating S protein). Immunoelectron microscopy clearly showed that the recombinant VSVs were bound with gold-labeled anti-S antibodies. These data confirmed the efficient incorporation of S protein into VSVΔG-SARS and VSVΔG-eGFP-SARS.

Since VSVΔG-SARS and VSVΔG-eGFP-SARS have SARS-CoV S protein instead of VSV G protein, their infection should solely depend on the SARS-CoV receptor, human ACE2 (hACE2). A SARS-CoV non-permissive cell line, hamster Syrian kidney cells (BSR cells), were transfected with pCAGGs-FLAG or pCAGGs-ACE2-FLAG (a eukaryotic plasmid encoding human ACE2 protein) and infected with VSVΔG-eGFP-SARS. Results showed that VSVΔG-eGFP-SARS couldn't infect pCAGGs-FLAG-transfected BSR cells, while hACE2-FLAG-transfected BSR cells were infected by the recombinant virus (Fig. 4), which indicated that the recombinant VSVs use hACE2 as receptors to infect cells. We also observed that syncytia formation in VSVΔG-eGFP-SARS and VSVΔG-SARS infected Vero E6 cells (Figs. 1C and 4), which indicated that the recombinant viruses rely on S protein fusion mechanism to mediate viral entry.

3.3. VSVΔG-SARS induces significant SARS S-Specific IgG and neutralizing antibodies in mice

The safety and immunogenicity of VSVΔG-SARS were evaluated in Balb/c mice at first. Mice were intramuscularly inoculated with 1×10^5 $TCID_{50}$ VSVΔG-SARS and daily monitored. All the mice were healthy and did not show any sign of sickness. There was no significant difference between the experimental and the PBS control group mice in body weight gain (Fig. 5A). SARS-CoV neutralizing antibodies and S protein-specific IgG antibodies were detected after the first dose injection, and the

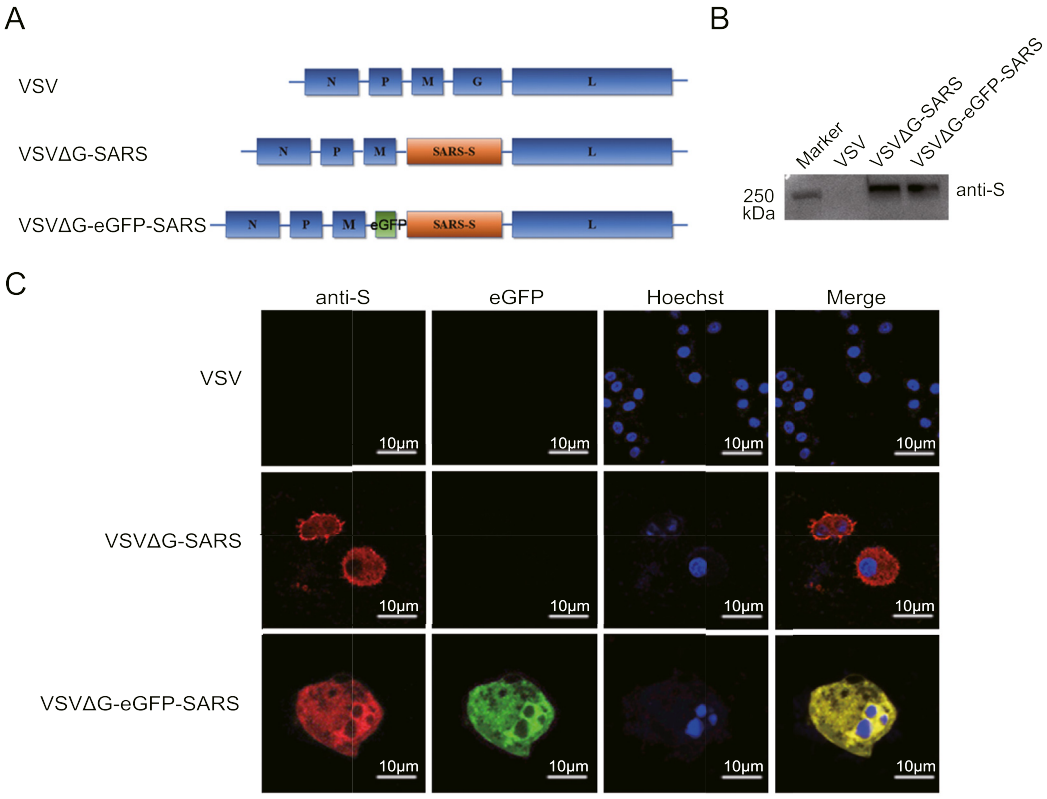

Fig. 1. Generation of VSVΔG-SARS and VSVΔG-eGFP-SARS and expression of S protein. **(A)** Schematic representation of the genome structure in native VSV and the recombinant viruses. **(B)** Western blot analysis of S protein expression in recombinant VSVs-infected Vero E6 cells. **(C)** Indirect immunofluorescence staining of S protein expression in VSVΔG-SARS and VSVΔG-eGFP-SARS infected Vero E6 cells.

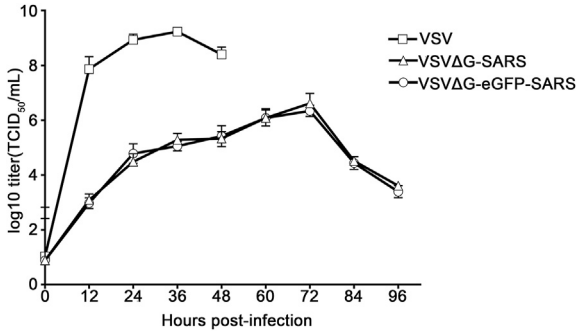

Fig. 2. Multistep growth properties of recombinant viruses in Vero E6 cells. Vero E6 cells planted on 6-well plate were respectively infected with native VSV, VSVΔG-SARS or VSVΔG-eGFP-SARS at an MOI = 0.01, the supernatant was harvested from 0 h to 96 h at 12 h intervals and flash frozen in −80 °C. The titer was expressed as the index of the highest dilution titer (Tissue culture infective dose, $TCID_{50}$). Data were shown as mean ± SD.

antibody levels rose significantly after the booster dose injection (Fig. 5B and 5C-III). Furthermore, VSVΔG-SARS induced high-level specific IgG1 (Th2) (Fig. 5C–I) and IgG2a (Th1) antibodies (Fig. 5C–II) after the second dose.

3.4. Single-dose intramuscular immunization of VSVΔG-SARS induces significant SARS-CoV specific humoral and T cell responses in rhesus monkeys

To further evaluate the immunogenicity of the VSVΔG-SARS vaccine in non-human primates, the immunization experiment was performed in rhesus monkeys to monitor their humoral and cellular responses (Fig. 6). The rhesus monkeys were received single dose of 2×10^7 $TCID_{50}$ of VSVΔG-SARS via the intramuscular (*i.m*) route. SARS-CoV neutralizing antibodies and S protein-specific IgG were detected 10-day after immunization, which respectively reached their peak level at day 21 and day 28 (Fig. 6A and B). Moreover, the ELISPOT assay was performed to further evaluate the T cell response in immunized monkeys. The PBMCs from the immunized monkeys were stimulated with a 15-peptide from an overlapping SARS-CoV S peptide pool, which consisted of 249 peptides designed for $CD8^+$ T cells. Results showed that immunized rhesus monkeys produced the IFN-γ secreting T cells specific to the S protein peptide stimulation (Fig. 6C). There are six relatively concentrated peptide groups in the S protein synthetic overlapping peptide pool, which can produce most dominant spots in ELISPOT assay. The six most 'dominant' peptides (Fig. 6C) were selected from six peptide groups to list in Table 1, which induced the highest level of IFN-γ (400 spots/well on average). These peptides were speculated to contain S protein specific $CD8^+$ T cell epitopes in rhesus monkeys.

4. Discussion

SARS has been a highly threatening zoonotic disease. Although no more SARS cases have been reported in human populations since 2004, given the zoonotic nature and emergence possibility of SARS-CoV or SARS-like CoVs, developing a fast-response vaccine is urgently needed. Here we used the VSV reverse genetics system to successfully generate a recombinant chimeric virus VSVΔG-SARS as a candidate vaccine, in which the glycoprotein (*G*) gene of VSV is replaced with the spike (*S*) gene of SARS-CoV. The VSVΔG-SARS maintains the bullet-like shape of the native VSV, and can use S protein on its surface to bind with human ACE2 to infect host cells. Furthermore, the recombinant chimeric virus VSVΔG-SARS can induce high-level neutralizing antibodies and robust T cell responses in rhesus monkeys after a single-dose immunization, which

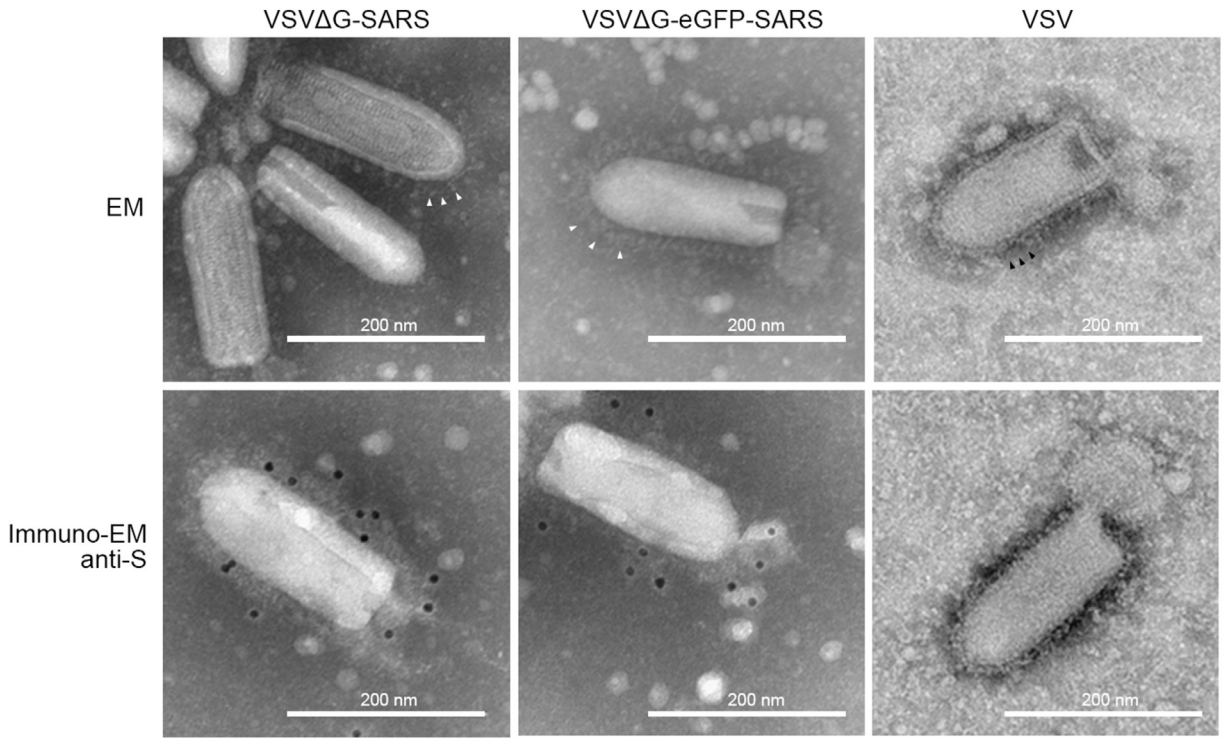

Fig. 3. SARS-CoV S protein is incorporated into the surface of the recombinant viral particles. The results of electron and immunoelectron microscopy showed that S protein is efficiently incorporated into the viral particles. White arrows indicate S protein and black arrows indicate VSV G protein.

indicates the potential of VSVΔG-SARS as a rapid response vaccine candidate for human use.

To date, several SARS candidate vaccines have been reported, including inactivated virus vaccines, live-attenuated virus vaccines, subunit vaccines, DNA vaccines and recombinant viral vector vaccines (Bukreyev et al., 2004; He et al., 2004; Zhao et al., 2004; Li et al., 2005; Ge et al., 2006; Graham et al., 2012; Fett et al., 2013). Although these attempts are all worth the efforts, there is a crucial need for an effective response vaccine for the fast spread of diseases caused by SARS-CoV or SARS-like CoVs. Vesicular stomatitis virus (VSV) is a desired attenuated vector for developing commercial vaccines. First, VSV-vectored vaccines can trigger a rapid immune response, but DNA vaccines and subunit vaccines always need to immunize for several times (Zhao et al., 2004; Chen et al., 2005; Li et al., 2005). Our results showed a single-dose VSVΔG-SARS immunization could induce significant neutralizing antibodies and T cell responses in rhesus monkeys. Second, VSV can reach high titers in multiple cell lines, and be easily prepared in large quantity. VSVΔG-SARS grows to high titers in Vero E6, which indicates that its manufacture is technically and economically feasible in developing countries. Although an adenovirus-based vaccine described before was shown to induce SARS-CoV-specific immune responses in the monkeys, costly helper cell lines and high-dose immunizations do not promise its nice application (Gao et al., 2003). Thus, VSVΔG-SARS should be more practicable for applications among human populations for its highly-effectiveness and convenience, especially in the developing countries.

Recently, SARS-CoV-2, related to SARS-CoV, has resulted in a global pandemic and shutdown of economies around the world (Zhou et al., 2020; Zhu et al., 2020). Some recent studies on the recombinant VSV-vectored vaccines for SARS-CoV-2 were reported (Case et al., 2020; Yahalom-Ronen et al., 2020). The study of VSV-ΔG-MERS in our lab showed that intranasal administration did do better than intramuscular injection in monkeys (Liu et al., 2018). However, the advantage of intranasal administration is not obvious in our study, thus these data

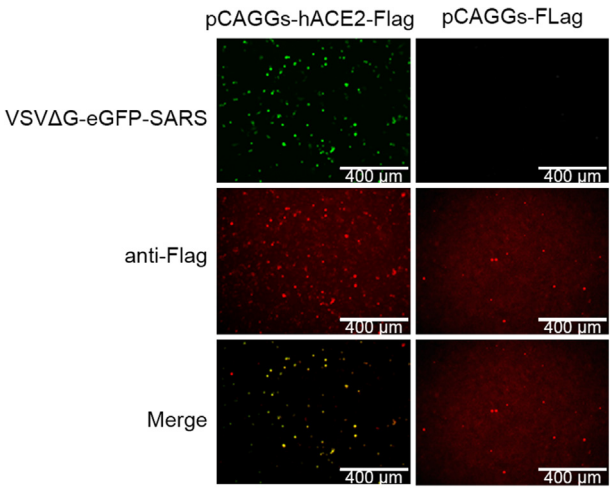

Fig. 4. VSVΔG-eGFP-SARS utilize human ACE2 (hACE2) as a functional receptor. pCAGGs-Flag transfected BSR cells and pCAGGs-hACE2-Flag transfected BSR cells were infected with VSVΔG-eGFP-SARS. At 36 h post-infection, the infected cells were observed with inverted fluorescence microscope.

were not shown in our paper. We deduced that viral stability and the binding ability of receptors in experimental animals could be the reason for that. Systematic studies are needed to study the immunology on VSV-vectored vaccines of different major viral antigen proteins. Our study is just the beginning, in-depth studies are needed to investigate the immunogenicity of VSV-vectored vaccines against coronaviruses. Thus, developing a rapid response SARS vaccine can be an effective means to provide the protection for high-risk populations, which still have deep enlightenment and reference meaning for prevention and control of emerging coronaviruses in the future.

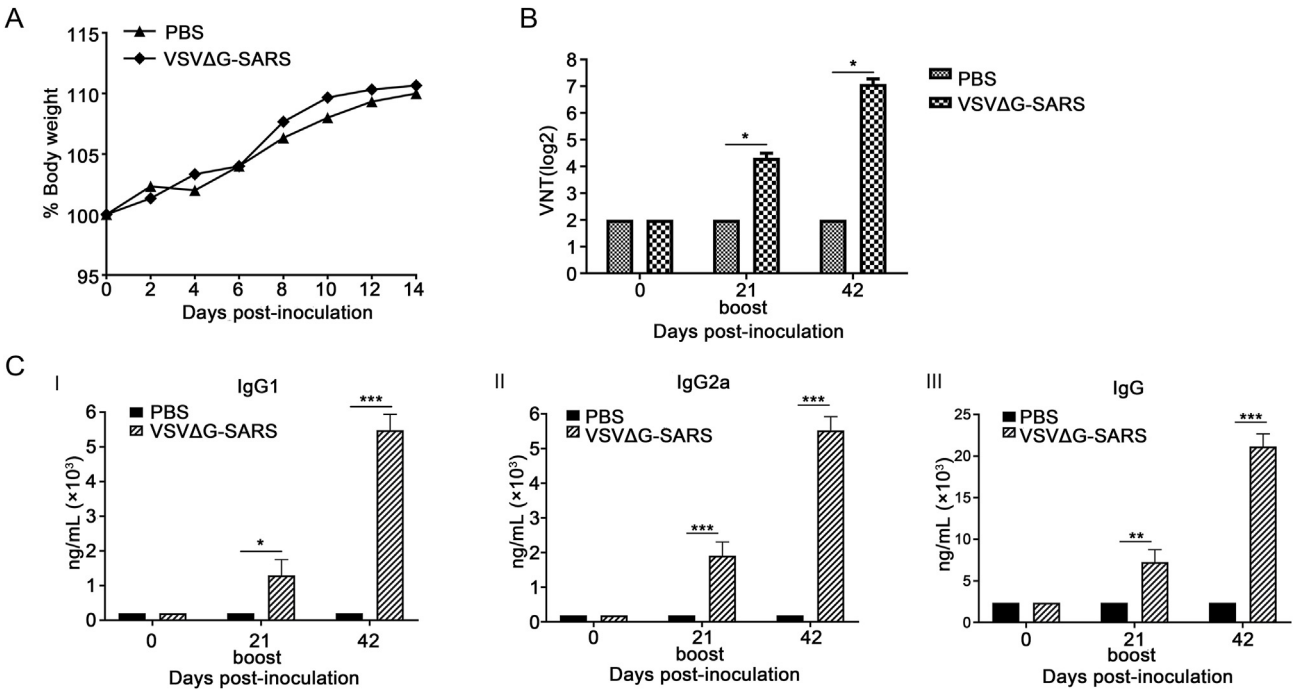

Fig. 5. Humoral responses of VSVΔG-SARS-immunized mice. Sixteen mice were intramuscularly immunized with 1×10^5 $TCID_{50}$ VSVΔG-SARS or PBS in the same volume. Each vaccinated mouse was daily monitored and weighed during the observation period **(A)**. At 3 weeks after the initial inoculation, all mice were given the booster dose. Neutralizing antibody **(B)** and S-specific IgG (**C**) titers were detected by neutralization and ELISA assays. Data were shown as mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

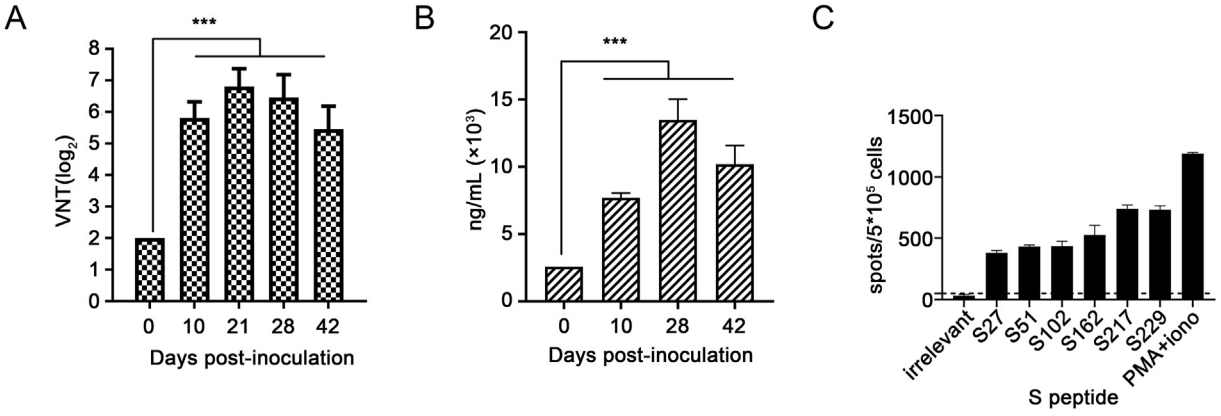

Fig. 6. Humoral immune responses and T cell responses of rhesus monkeys to VSVΔG-SARS vaccination. Monkeys were immunized with 2×10^7 $TCID_{50}$ of the recombinant virus intramuscularly. Blood samples were collected at the indicated time-points. Neutralizing antibody (**A**) and serum IgG (**B**) titers were determined by neutralization and ELISA assays. **(C)** ELISPOT assay was performed to evaluate T cell responses in rhesus monkeys. PBMCs from immunized monkeys were stimulated with SARS-CoV S peptide from an overlapping peptide pool, which contained 249 peptides designed for CD^{8+} T cells. The data of six selected peptides were shown for presentation, which produced most spots in ELISPOT assay. The monkey PBMCs were added with irrelevant peptides as negative control, and added with PMA and ionomycin (PMA+iono) as positive control.

Table 1
T cell reactive most 'dominant' S protein peptides in monkeys vaccinated with VSVΔG-SARS.

Peptide[a]	Peptide sequence	Amino acid position
S27	ELCDNPFFAVSKPMG	131–145
S51	AYFVGYLKPTTFMLK	251–265
S102	APATVCGPKLSTDLI	506–520
S162	NKVTLADAGFMKQYG	806–820
S217	GTSWFITQRNFFSPQ	1081–1095
S229	HTSPDVDLGDISGIN	1141–1155

[a] Sequences were based on the *S* gene of SARS-CoV (isolate Urbani, GenBank accession No. AAP13441.1).

Data availability

All the data generated during the current study are included in the manuscript.

Ethics statement

The whole study was approved by the Committee on the Ethics of Animal Experiments of the Harbin Veterinary Research Institute (HVRI), Chinese Academy of Agricultural Sciences (CAAS) (approval number 2020-01-01JiPi). All animal experiments were performed in strict accordance with the regulations for the Institutional Animal Care and

Ethics Committee (IACEC) of Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences.

Author contributions

Dan Shan: Data curation, Formal analysis, Methodology, Writing. Xiaoyan Tang: Data curation, Formal analysis, Methodology, Writing. Renqiang Liu: Data curation, Formal analysis, Funding acquisition, Methodology, Writing. Dan Pan: Data curation, Formal analysis, Methodology. Xijun Wang: Formal analysis, Methodology. Jinying Ge: Data curation, Funding acquisition, Methodology. Zhiyuan Wen: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing – review & editing. Zhigao Bu: Conceptualization, Methodology, Project administration, Writing – review & editing.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

We thank Prof. Bernard Moss (University of Tennessee Health Science Center) for providing the VSVΔG-eGFP pseudovirus system. This study was supported by grants from the National Key Research and Development Program of China (2017YFD0501804).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2022.01.002.

References

Bukreyev, A., Lamirande, E.W., Buchholz, U.J., Vogel, L.N., Elkins, W.R., St Claire, M., Murphy, B.R., Subbarao, K., Collins, P.L., 2004. Mucosal immunisation of African green monkeys (Cercopithecus aethiops) with an attenuated parainfluenza virus expressing the SARS coronavirus spike protein for the prevention of SARS. Lancet 363, 2122–2127.

Case, J.B., Rothlauf, P.W., Chen, R.E., Kafai, N.M., Fox, J.M., Smith, B.K., Shrihari, S., McCune, B.T., Harvey, I.B., Keeler, S.P., Bloyet, L.M., Zhao, H., Ma, M., Adams, L.J., Winkler, E.S., Holtzman, M.J., Fremont, D.H., Whelan, S.P.J., Diamond, M.S., 2020. Replication-competent vesicular stomatitis virus vaccine vector protects against SARS-CoV-2-mediated pathogenesis in mice. Cell Host Microbe 28, 465–474 e464.

Chen, Z., Zhang, L., Qin, C., Ba, L., Yi, C.E., Zhang, F., Wei, Q., He, T., Yu, W., Yu, J., Gao, H., Tu, X., Gettie, A., Farzan, M., Yuen, K.Y., Ho, D.D., 2005. Recombinant modified vaccinia virus Ankara expressing the spike glycoprotein of severe acute respiratory syndrome coronavirus induces protective neutralizing antibodies primarily targeting the receptor binding region. J. Virol. 79, 2678–2688.

Coller, B., Blue, J., Das, R., Dubey, S., Finelli, L., Gupta, S., Helmond, F., Grant-Klein, R., Liu, K., Simon, J., Troth, S., VanRheenen, S., Waterbury, J., Wivel, A., Wolf, J., Heppner, D., Kemp, T., Nichols, R., Monath, T., 2017. Clinical development of a recombinant Ebola vaccine in the midst of an unprecedented epidemic. Vaccine 35, 4465–4469.

Drosten, C., Gunther, S., Preiser, W., van der Werf, S., Brodt, H.R., Becker, S., Rabenau, H., Panning, M., Kolesnikova, L., Fouchier, R.A., Berger, A., Burguiere, A.M., Cinatl, J., Eickmann, M., Escriou, N., Grywna, K., Kramme, S., Manuguerra, J.C., Muller, S., Rickerts, V., Sturmer, M., Vieth, S., Klenk, H.D., Osterhaus, A.D., Schmitz, H., Doerr, H.W., 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N. Engl. J. Med. 348, 1967–1976.

Du, L., He, Y., Zhou, Y., Liu, S., Zheng, B., Jiang, S., 2009. The spike protein of SARS-CoV–a target for vaccine and therapeutic development. Nat. Rev. Microbiol. 7, 226–236.

Fett, C., DeDiego, M.L., Regla-Nava, J.A., Enjuanes, L., Perlman, S., 2013. Complete protection against severe acute respiratory syndrome coronavirus-mediated lethal respiratory disease in aged mice by immunization with a mouse-adapted virus lacking E protein. J. Virol. 87, 6551–6559.

Gao, W., Tamin, A., Soloff, A., D'Aiuto, L., Nwanegbo, E., Robbins, P.D., Bellini, W.J., Barratt-Boyes, S., Gambotto, A., 2003. Effects of a SARS-associated coronavirus vaccine in monkeys. Lancet 362, 1895–1896.

Ge, J., Wang, X., Tao, L., Wen, Z., Feng, N., Yang, S., Xia, X., Yang, C., Chen, H., Bu, Z., 2011. Newcastle disease virus-vectored rabies vaccine is safe, highly immunogenic, and provides long-lasting protection in dogs and cats. J. Virol. 85, 8241–8252.

Ge, J., Wen, Z., Wang, X., Hu, S., Liu, Y., Kong, X., Chen, H., Bu, Z., 2006. Generating vesicular stomatitis virus pseudotype bearing the severe acute respiratory syndrome coronavirus spike envelope glycoprotein for rapid and safe neutralization test or cell-entry assay. Ann. N. Y. Acad. Sci. 1081, 246–248.

Graham, R., Donaldson, E., Baric, R., 2013. A decade after SARS: strategies for controlling emerging coronaviruses. Nat. Rev. Microbiol. 11, 836–848.

Graham, R.L., Becker, M.M., Eckerle, L.D., Bolles, M., Denison, M.R., Baric, R.S., 2012. A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease. Nat. Med. 18, 1820–1826.

Gsell, P.S., Camacho, A., Kucharski, A.J., Watson, C.H., Bagayoko, A., Nadlaou, S.D., Dean, N.E., Diallo, A., Diallo, A., Honora, D.A., Doumbia, M., Enwere, G., Higgs, E.S., Mauget, T., Mory, D., Riveros, X., Oumar, F.T., Fallah, M., Toure, A., Vicari, A.S., Longini, I.M., Edmunds, W.J., Henao-Restrepo, A.M., Kieny, M.P., Keita, S., 2017. Ring vaccination with rVSV-ZEBOV under expanded access in response to an outbreak of Ebola virus disease in Guinea, 2016: an operational and vaccine safety report. Lancet Infect. Dis. 17, 1276–1284.

Guan, Y., Zheng, B., He, Y., Liu, X., Zhuang, Z., Cheung, C., Luo, S., Li, P., Zhang, L., Guan, Y., Butt, K., Wong, K., Chan, K., Lim, W., Shortridge, K., Yuen, K., Peiris, J., Poon, L., 2003. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science (New York, N.Y.) 302, 276–278.

He, Y., Zhou, Y., Siddiqui, P., Jiang, S., 2004. Inactivated SARS-CoV vaccine elicits high titers of spike protein-specific antibodies that block receptor binding and virus entry. Biochem. Biophys. Res. Commun. 325, 445–452.

Henao-Restrepo, A.M., Camacho, A., Longini, I.M., Watson, C.H., Edmunds, W.J., Egger, M., Carroll, M.W., Dean, N.E., Diatta, I., Doumbia, M., Draguez, B., Duraffour, S., Enwere, G., Grais, R., Gunther, S., Gsell, P.S., Hossmann, S., Watle, S.V., Konde, M.K., Keita, S., Kone, S., Kuisma, E., Levine, M.M., Mandal, S., Mauget, T., Norheim, G., Riveros, X., Soumah, A., Trelle, S., Vicari, A.S., Rottingen, J.A., Kieny, M.P., 2017. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ca Suffit!). Lancet 389, 505–518.

Kong, D., Wen, Z., Su, H., Ge, J., Chen, W., Wang, X., Wu, C., Yang, C., Chen, H., Bu, Z., 2012. Newcastle disease virus-vectored Nipah encephalitis vaccines induce B and T cell responses in mice and long-lasting neutralizing antibodies in pigs. Virology 432, 327–335.

Ksiazek, T., Erdman, D., Goldsmith, C., Zaki, S., Peret, T., Emery, S., Tong, S., Urbani, C., Comer, J., Lim, W., Rollin, P., Dowell, S., Ling, A., Humphrey, C., Shieh, W., Guarner, J., Paddock, C., Rota, P., Fields, B., DeRisi, J., Yang, J., Cox, N., Hughes, J., LeDuc, J., Bellini, W., Anderson, L., 2003. A novel coronavirus associated with severe acute respiratory syndrome. N. Engl. J. Med. 348, 1953–1966.

Lawson, N., Stillman, E., Whitt, M., Rose, J., 1995. Recombinant vesicular stomatitis viruses from DNA. In: Proceedings of the National Academy of Sciences of the United States of America, vol. 92, pp. 4477–4481.

Li, F., Li, W., Farzan, M., Harrison, S.C., 2005. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science 309, 1864–1868.

Liu, R., Wang, J., Shao, Y., Wang, X., Zhang, H., Shuai, L., Ge, J., Wen, Z., Bu, Z., 2018. A recombinant VSV-vectored MERS-CoV vaccine induces neutralizing antibody and T cell responses in rhesus monkeys after single dose immunization. Antivir. Res. 150, 30–38.

Liu, R.Q., Ge, J.Y., Wang, J.L., Shao, Y., Zhang, H.L., Wang, J.L., Wen, Z.Y., Bu, Z.G., 2017. Newcastle disease virus-based MERS-CoV candidate vaccine elicits high-level and lasting neutralizing antibodies in Bactrian camels. J. Integr. Agric. 16, 2264–2273.

Liu, Y., Massare, M., Barnard, D., Kort, T., Nathan, M., Wang, L., Smith, G., 2011. Chimeric severe acute respiratory syndrome coronavirus (SARS-CoV) S glycoprotein and influenza matrix 1 efficiently form virus-like particles (VLPs) that protect mice against challenge with SARS-CoV. Vaccine 29, 6606–6613.

Marzi, A., Menicucci, A., Engelmann, F., Callison, J., Horne, E., Feldmann, F., Jankeel, A., Feldmann, H., Messaoudi, I., 2018. Protection against Marburg virus using a recombinant VSV-vaccine depends on T and B cell activation. Front. Immunol. 9, 3071.

Monath, T., Fast, P., Modjarrad, K., Clarke, D., Martin, B., Fusco, J., Nichols, R., Heppner, D., Simon, J., Dubey, S., Troth, S., Wolf, J., Singh, V., Coller, B., Robertson, J., 2019. rVSVΔG-ZEBOV-GP (also designated V920) recombinant vesicular stomatitis virus pseudotyped with Ebola Zaire Glycoprotein: standardized template with key considerations for a risk/benefit assessment. Vaccine X 1, 100009.

Qin, E., Shi, H., Tang, L., Wang, C., Chang, G., Ding, Z., Zhao, K., Wang, J., Chen, Z., Yu, M., Si, B., Liu, J., Wu, D., Cheng, X., Yang, B., Peng, W., Meng, Q., Liu, B., Han, W., Yin, X., Duan, H., Zhan, D., Tian, L., Li, S., Wu, J., Tan, G., Li, Y., Li, Y., Liu, Y., Liu, H., Lv, F., Zhang, Y., Kong, X., Fan, B., Jiang, T., Xu, S., Wang, X., Li, C., Wu, X., Deng, Y., Zhao, M., Zhu, Q., 2006. Immunogenicity and protective efficacy in monkeys of purified inactivated Vero-cell SARS vaccine. Vaccine 24, 1028–1034.

Ryder, A., Nachbagauer, R., Buonocore, L., Palese, P., Krammer, F., Rose, J., 2015. Vaccination with vesicular stomatitis virus-vectored chimeric hemagglutinins protects mice against divergent influenza virus challenge strains. J. Virol. 90, 2544–2550.

WHO, 2004. WHO Guidelines for the Global Surveillance of Severe Acute Respiratory Syndrome. https://www.who.int/publications/i/item/who-guidelines-for-the-global-surveillance-of-severe-acute-respiratory-syndrome-(-sars). (Accessed 23 February 2021).

Woo, P., Lau, S., Tsoi, H., Chen, Z., Wong, B., Zhang, L., Chan, J., Wong, L., He, W., Ma, C., Chan, K., Ho, D., Yuen, K., 2005. SARS coronavirus spike polypeptide DNA vaccine priming with recombinant spike polypeptide from Escherichia coli as booster induces high titer of neutralizing antibody against SARS coronavirus. Vaccine 23, 4959–4968.

Yahalom-Ronen, Y., Tamir, H., Melamed, S., Politi, B., Shifman, O., Achdout, H., Vitner, E.B., Israeli, O., Milrot, E., Stein, D., Cohen-Gihon, I., Lazar, S., Gutman, H., Glinert, I., Cherry, L., Vagima, Y., Lazar, S., Weiss, S., Ben-Shmuel, A., Avraham, R., Puni, R., Lupu, E., Bar-David, E., Sittner, A., Erez, N., Zichel, R., Mamroud, E., Mazor, O., Levy, H., Laskar, O., Yitzhaki, S., Shapira, S.C., Zvi, A., Beth-Din, A.,

Paran, N., Israely, T., 2020. A single dose of recombinant VSV-G-spike vaccine provides protection against SARS-CoV-2 challenge. Nat. Commun. 11, 6402.
Zhao, P., Ke, J.S., Qin, Z.L., Ren, H., Zhao, L.J., Yu, J.G., Gao, J., Zhu, S.Y., Qi, Z.T., 2004. DNA vaccine of SARS-Cov S gene induces antibody response in mice. Acta Biochim. Biophys. Sin. 36, 37–41.
Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L., Chen, H.D., Chen, J., Luo, Y., Guo, H., Jiang, R.D., Liu, M.Q., Chen, Y., Shen, X.R., Wang, X., Zheng, X.S., Zhao, K., Chen, Q.J., Deng, F., Liu, L.L., Yan, B., Zhan, F.X., Wang, Y.Y., Xiao, G.F., Shi, Z.L., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273.
Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G.F., Tan, W., China Novel Coronavirus, I., Research, T., 2020. A novel coronavirus from patients with pneumonia in China, 2019. N. Engl. J. Med. 382, 727–733.