PNAS

Supplementary Information for

A pan-sarbecovirus vaccine based on RBD of SARS-CoV-2 original strain elicits potent neutralizing antibodies against XBB in NHPs

Zezhong Liu[1,#,*], Jie Zhou[1,#], Xinling Wang[1,#], Wei Xu[1], Zheng Teng[2], Hongyou Chen[2], Min Chen[2], Guangxu Zhang[1], Yuanzhou Wang[1], Jinghe Huang[1], Qian Wang[1], Shibo Jiang[1,*], Lu Lu[1,*]

[1] MOE/NHC/CAMS Key Laboratory of Medical Molecular Virology, Shanghai Institute of Infectious Disease and Biosecurity, School of Basic Medical Sciences, Biosafety Level 3 Laboratory, Department of Pharmacology, School of Pharmacy, Shanghai Medical College, Fudan University, Shanghai 200032, China.

[2] Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China.

[#] These authors contributed equally.

* Lu Lu, Shibo Jiang and Zezhong Liu

Email: lul@fudan.edu.cn (L.L.), shibojiang@fudan.edu.cn (S.J.), zzliu17@fudan.edu.cn (Z.L.)

This file includes:

Materials and Methods

Cell lines

HEK-293T cells, Expi-293F cells, Vero-E6 cells and Huh-7 cells were obtained from the American Type Culture Collection (ATCC). Dulbecco's Modified Eagle's Medium (DMEM) was used to culture HEK-293T cells, Vero-E6 cells and Huh-7 cells with supplementation of 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C, 5% CO_2. 293T II medium (Sino Biological) was used to culture Expi-293F cells at 37 °C in 5% CO_2 with shaking at 100 rpm.

Serum samples

Sera from immunized rhesus macaques were from our previous studies (1). A total of six rhesus macaques were assigned into two groups, and each group had three animals. Macaques in the first group were immunized with 100 μg RBD-Fc protein formulated with 400 μg CF501. 100 μg RBD-Fc protein formulated with an equal volume of Alum were used to immunize macaques in the second group. Immunizations were conducted on days 0, 21 and 115, and sera were collected as previously described.

Protein expression

RBD expression was performed as previously reported (2). The plasmid of Pfuse-SARS-CoV-2-BA.2.2-RBD, Pfuse-SARS-CoV-2-BA.2.9-RBD, Pfuse-SARS-CoV-2-BA.2.12.1-RBD, Pfuse-

SARS-CoV-2-BA.5-RBD, Pfuse-SARS-CoV-2-BA.2.75-RBD, Pfuse-SARS-CoV-2-BF.7-RBD, Pfuse-SARS-CoV-2-BQ.1.1-RBD or Pfuse-SARS-CoV-2-XBB-RBD was separately transfected into Expi-293F cells using EZ Trans transfection reagent (Life iLAB Bio-Technology, China). After 6 days, suspensions were harvested, and the RBDs were purified using Ni-NTA (Qiagen). The purified proteins were identified by SDS-PAGE. All proteins were stored at -80 °C.

ELISA

Enzyme-linked immunosorbent assay (ELISA) was performed as previously described(1). Briefly, a concentration of 1 μg/ml RBD was coated onto the ELISA plates overnight at 4 °C. Next, the ELISA plates were blocked using a blocking buffer (PBS containing 5% BSA). Sera were serially diluted and applied to the ELISA plates for incubation at 37 °C for 45 min. After that, the plates were washed using PBST 5 times. HRP-conjugated goat anti-monkey IgG (Abcam, UK) (1:10,000) was added into the plates and further incubated for 45 min at 37°C. Finally, the reactions were visualized by adding 3,3',5,5'-tetramethylbenzidine (TMB), and H_2SO_4 was used to stop the reaction. Absorbance at 450 nm (A450) was read by using a microplate reader (InfiniteM200PRO, Switzerland). Endpoint titers were defined as the highest dilutions that showed A450>2.1-fold of background values.

Pseudovirus production

The production of pseudoviruses was performed as previously described (1). Briefly, the plasmids of pNL4-3.Luc.R−E and PcDNA3.1-BA.2.2-S/ PcDNA3.1-BA.2.9-S/ PcDNA3.1-BA.2.12.1-S/ PcDNA3.1-BA.5-S/ PcDNA3.1-BA.2.75-S/ PcDNA3.1-BF.7-S/ PcDNA3.1-BQ.1.1-S/ and PcDNA3.1-XBB-S were co-transfected into HEK293T cells using the Vigofect transfect reagent. After 12 h, fresh DMEM containing 10% FBS was used to replace supernatants. Finally, supernatants were collected after further culture for 48 h and stored at -80 °C.

Pseudovirus neutralization assay

The pseudovirus neutralization assay was conducted as described previously (1). Briefly, Huh-7 cells were used as the target cells. A total of 1×10^4 cells/well were seeded in a 96-well plate. After 8 h, serially diluted sera were incubated with pseudovirus for 30 min. The mixture of sera and pseudovirus was applied into Huh-7 cells. After 60 h, luciferase activities were detected using the Firefly Luciferase Assay Kit (Promega, USA). NT50 is defined as the dilution of sera that shows 50% of the luminescence units of viral control. Once the titer was below 1:100, the titer was then recorded as 1:50.

Authentic virus neutralization assay

Vero-E6 cells were used as the target cells for live Omicron BA.2.2 infection. 1×10^4 Vero-E6 cells/well were seeded in a 96-well plate. Diluted sera from immunized rhesus macaques were first mixed with live Omicron BA.2.2 for 30 min. Mixtures of virus and serum were added into the Vero-E6 cells. After 48 h, supernatants were collected to conduct the RT-qPCR assay. RT-qPCR was conducted to quantify SARS-CoV-2 viral mRNA level by using the One-Step PrimeScrip RT-PCR Kit (Takara, Japan). Cells were used to perform the immunofluorescence assay. First, the cells were treated with paraformaldehyde and 0.1% Triton X. After blocking with 3% BSA, cells were incubated with mouse anti-SARS-CoV-2 N antibody (1:1000) for 30 min at 37 °C. Finally, after washing with PBS 5 times, cells were added to Alexa Fluor donkey anti-mouse IgG 488-labeled antibody (Thermo). Fluorescence microscopy was used to capture SARS-CoV-2 N protein.

References

1. Z. Liu *et al.*, A novel STING agonist-adjuvanted pan-sarbecovirus vaccine elicits potent and durable neutralizing antibody and T cell responses in mice, rabbits and NHPs. *Cell Res* **32**, 269-287 (2022).
2. Z. Liu *et al.*, RBD-Fc-based COVID-19 vaccine candidate induces highly potent SARS-CoV-2 neutralizing antibody response. *Signal Transduct Target Ther* **5**, 282 (2020).