Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Wang G-L, Wang Z-Y, Duan L-J, et al. Susceptibility of circulating SARS-CoV-2 variants to neutralization. N Engl J Med. DOI: 10.1056/NEJMc2103022

Supplemental Appendix

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Materials and Methods

Ethical Approval

The study was approved by the Institutional Review Board of the Academy of Military Medical Sciences (IRB number: AF/SC-08/02.60). All patients and vaccinees provided written informed consent.

Serum samples

Convalescent sera (n = 34) from COVID-19 patients were collected in our previous cross-sectional study of antibody and T cell response[1]. We collected serum samples from the patients between July and September 2020, about five months after infection. The serum samples of the patients included in this study were pre-selected as the age and sex of patients were matched with the vaccinee who received two different inactivated COVID-19 vaccine—BBIBP-CorV and CoronaVac.

Sera for the BBIBP-CorV vaccine was obtained from the staff received two doses of immunization administered 14-28 days apart at the Dezhou Center for Disease Control and Prevention in Dezhou City, Shandong Province, China. Samples tested against the WT and other three variants were collected approximately 55 days (about 20 days after second dose immunization).

Sera for the CoronaVac vaccine was obtained from the healthcare workers who received two doses of immunization administered 14-28 days apart at the Ningjin County Community Health Servers Center in Dezhou City, Shandong Province, China. Samples tested against the WT and other three variants were collected approximately 29 days after the first dose

immunization (14 days after second dose immunization).

Pseudovirus neutralization assay (pVNT)

The VSV-based SARS-CoV-2 pseudovirus bearing WT, D614G, B.1.1.7, and B.1.351 variants Spike protein were provided by the Professor Wei-Jin Huang from the Institute for Biological Product Control, National Institutes for Food and Drug Control, Beijing, China, and the pseudoviruses were generated as previously described[2,3]. The SARS-CoV-2 pseudovirus based neutralization assay was performed as described previously[4]. In brief, Huh7 cells were seeded in 96-well plates (200,000 cells/well) and incubated for approximately 24 hrs until 90%–100% confluent. Serial 3-fold diluted serum, starting at 1:30, were incubated with 650 $TCID_{50}$ of the pseudovirus for 1 hour at 37°C. DMEM was used as the negative control. The supernatant was then removed, and luciferase substrate was added to each well, followed by incubation for 2 minutes in darkness at room temperature. Luciferase activity was then measured using GloMax 96 Microplate Luminometer (Promega). Half-maximal inhibitory concentrations (IC_{50}) of the serum samples were determined by luciferase activity 48 hrs after exposure to the virus-serum mixture with a three-parameter non-linear regression inhibitor curve in GraphPad Prism 8.4.1 (GraphPad Software). Titers were determined as the serum dilution that inhibited 50% virus infection (IC_{50}). Samples that do not neutralize at the limit of detection at 50% (IC_{50}<30) are assigned a value of 10 for geometric mean calculations and was considered seronegative. Healthy control serum collected pre-pandemic of COVID-19 was included as a negative control and showed no detectable neutralization (IC_{50}<30).

Statistical analysis

Kruskal-Wallis test with FDR method was used for multiple group comparisons. Multiple testing correction was subsequently applied to each comparison using the Benjamini-Hochberg (BH) procedure with a false discovery rate (FDR or q) at 5%. All statistical analyses were performed using GraphPad Prism (version 8.4.2, La Jolla, California, USA), and all statistical tests were 2-sided with a significance level of 0.05.

Table S1. Characteristics of study subjects.

Characteristics	COVID-19 patients	BBIBP-CorV recipients	CoronaVac recipients
No. of participants	34	25	25
Age (median, range)	44.0 (23.0-64.0)	41.0 (25.0-59.0)	38.0 (23.0-58.0)
Sex			
Male (%)	11 (32.4)	9 (36.0)	9 (36.0)
Female (%)	23 (67.6)	16 (64.0)	16 (64.0)
Time interval to blood sampling (Median day, IQR)	158.0 (149.5-164.5)	55.0 (55.0-56.0)	29.0 (29.0-40.0)
Disease severity			
Severe	5 (12.8)	NA	NA
Non-severe	29 (87.2)	NA	NA

COVID-19, coronavirus disease 2019; IQR, interquartile range; NA, not applicable.

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Author contributions

MJM conceived the study. ZYW, QCM, MDJ, and JC collected serum samples. GLW, MYW, DLJ, and LY performed experiment; GLW, LJD, YL, and WJH analyzed the data; MJM drafted the manuscript. All authors reviewed and approved the final manuscript.

Declaration of interests

We declare no competing interests.

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