**Virologica Sinica**

**Supplementary Data**

**SARS-CoV-2 breakthrough infections following inactivated vaccine vaccination induce few neutralizing antibodies against the currently emerging Omicron XBB variants**

Fan Shen a, b, 1, Chun Liang a, b, 1, Cui-Xian Yang c, Ying Lu a, b, An-Qi Li a, b, Ying Duan a, b, Mi Zhang c, Ren-Rong Tian a, Xing-Qi Dong c, Yong-Tang Zheng a, b, \*, Wei Pang a, b, \*

a Key Laboratory of Bioactive Peptides of Yunnan Province/Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences, KIZ-CUHK Joint Laboratory of Bioresources and Molecular Research in Common Diseases, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, 650223, China

b University of Chinese Academy of Sciences, Beijing, 100049, China

c Yunnan Provincial infectious disease hospital, Kunming, 650399, China

\* Corresponding author

Emails: zhengyt@mail.kiz.ac.cn. (Y.-T. Zheng), pangw@mail.kiz.ac.cn. (W Pang)

1 Fan Shen and Chun Liang contributed equally to this study.

**Supplemental materials and methods**

**Convalescent serum samples**

Convalescent serum samples from SARS-CoV-2 ancestral, Delta and Omicron BA.1 infected individuals were obtained from Yunnan Infectious Disease Hospital, Yunnan province, China. Convalescent serum samples from Omicron BA.5 infected individuals were collected from our laboratory during the Omicron BA.5 waves. All the samples were collected one week after recovery (or virion were cleared from the SARS-CoV-2-infected individuals) and inactivated at 56 °C for 1 h. The participants received none, two or three doses of inactivated vaccines BBIBP-CorV or CoronaVac, and their detailed vaccination information were listed in Supplementary Table S1 and the interval between the first and second dose was one month and the second and third doses was six months. Each variant infection was confirmed by the Yunnan Center for Disease Prevention and Control, and the infection occurred between one month and one year after the last dose of vaccine.

**Generation of SARS-CoV-2 variants pseudovirus**

The plasmids expressing D614G mutant and Omicron BA.5 spike were described in our previous study (Shen et al., 2023), and the plasmids expressing BF.7, BQ.1, BQ.1.1, XBB, XBB.1, and XBB.1.5 spikes were synthesized according to the mutation sites listed in Supplementary Table S2. SARS-CoV-2 pseudovirus was packaged using the VSV system. Briefly, HEK293T cells were cultured in basal DMEM supplemented with 10% FBS in a T75 cell flask, and spike protein expression plasmid (10 μg) was transfected to cells using the jetPRIME reagent (Polyplus) when cells density reached 80%. The next day, the cells were infected with G\*ΔG-VSV-Rluc (M.O.I. = 1) and then were washed three times at six hours after infection. After 24 hours, the supernatant was collected and stored at –80 °C.

**Pseudovirus-based neutralization**

Pseudovirus-based neutralization assays were performed as previously described (Nie et al., 2020). Briefly, HEK293T-ACE2 cells (1×104 per well) were inoculated into 96-well plates. The next day, four-fold serially diluted serum samples was mixed with SARS-CoV-2 pseudovirus (M.O.I. = 1) for one hour at 37 °C and added to the cells. After 24 hours, luciferase activity of cells was determined using the Renilla luciferase assay kit (Promega). The PVNT50 values of the serum samples were calculated in GraphPad Prism 9.5.1 software.

**Statistical analysis**

Statistical analyses were performed using Graphpad Prism 9.5.1 software. Two-tailed Wilcoxon matched-pairs signed-rank test and Mann-Whitney U test were used to compare with the PVNT50 value. *p* ≤ 0.05 was considered to be statistically significant (ns, not significant, *P* > 0.05; \*, *P* ≤ 0.05; \*\*, *P* ≤ 0.01; \*\*\*, *P* ≤ 0.001; \*\*\*\*, *P* ≤ 0.0001).

**References**

Nie, J., Li, Q., Wu, J., Zhao, C., Hao, H., Liu, H., Zhang, L., Nie, L., Qin, H., Wang, M., Lu, Q., Li, X., Sun, Q., Liu, J., Fan, C., Huang, W., Xu, M., Wang, Y, 2020. Quantification of SARS-CoV-2 neutralizing antibody by a pseudotyped virus-based assay. Nat Protoc, 15, 3699-3715.

Shen, F., Yang, C. X., Lu, Y., Zhang, M., Tian, R. R., Dong, X. Q., Li, A. Q., Zheng, Y. T., Pang, W., 2023. Significant neutralizing escapes of Omicron and its sublineages in SARS-CoV-2-infected individuals vaccinated with inactivated vaccines. J Med Virol, 95, e28516.

Figure S1

**Figure S1.** The comparison of serum neutralizing antibody titers in different doses of inactivated vaccines and different variant infection.Inancestral strain(**A**), Delta (**B**) and Omicron variant (**C**) infected serum samples, the cross-neutralizing antibody (nAb) titers against the OmicronBF.7, BQ.1, BQ.1.1, XBB, XBB.1, and XBB.1.5 variants between different doses of inactivated vaccines were compared. Meanwhile, in all examined 91 samples, the cross-nAb production against other current variants were separately compared to those against Omicron BA.5 (**D**). “I” represented a dose of inactivated vaccine. Each point represented the PVNT50 value from a serum sample. In (**A**-**C**), data were presented as geometric mean with 95% confidence interval. Mann-Whitney U test (**A**-**C**) and two-tailed Wilcoxon matched-pairs signed-rank test (**D**) were used to compare the PVNT50 values between two groups. The significant label were labeled in all figures (ns, not significant, *P* > 0.05; \*, *P* ≤ 0.05; \*\*, *P* ≤ 0.01; \*\*\*, *P* ≤ 0.001; \*\*\*\*, *P* ≤ 0.0001).

**Table S1: Demographics of SARS-CoV-2-infected convalescents**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample ID** | **Vaccine type and infected strain** | **Age** | **Gender** |
| Convalescent 1 | Unvaccinated/Ancestral strain | 43 | Male |
| Convalescent 2 | Unvaccinated/Ancestral strain | 31 | Male |
| Convalescent 3 | Unvaccinated/Ancestral strain | 22 | Male |
| Convalescent 4 | Unvaccinated/Ancestral strain | 51 | Male |
| Convalescent 5 | Unvaccinated/Ancestral strain | 28 | Male |
| Convalescent 6 | Unvaccinated/Ancestral strain | 55 | Male |
| Convalescent 7 | Unvaccinated/Ancestral strain | 36 | Male |
| Convalescent 8 | CoronaVac/CoronaVac/Ancestral strain | 38 | Male |
| Convalescent 9 | BBIBP-CorV/BBIBP-CorV/Ancestral strain | 24 | Female |
| Convalescent 10 | CoronaVac/CoronaVac/Ancestral strain | 33 | Male |
| Convalescent 11 | BBIBP-CorV/BBIBP-CorV/Ancestral strain | 53 | Male |
| Convalescent 12 | BBIBP-CorV/BBIBP-CorV/Ancestral strain | 44 | Male |
| Convalescent 13 | CoronaVac/CoronaVac/Ancestral strain | 20 | Male |
| Convalescent 14 | BBIBP-CorV/BBIBP-CorV/Ancestral strain | 21 | Male |
| Convalescent 15 | BBIBP-CorV/BBIBP-CorV/Ancestral strain | 56 | Male |
| Convalescent 16 | CoronaVac/CoronaVac/Ancestral strain | 48 | Male |
| Convalescent 17 | BBIBP-CorV/BBIBP-CorV/Ancestral strain | 51 | Male |
| Convalescent 18 | CoronaVac/CoronaVac/Ancestral strain | 26 | Male |
| Convalescent 19 | BBIBP-CorV/BBIBP-CorV/Ancestral strain | 48 | Male |
| Convalescent 20 | CoronaVac/CoronaVac/Ancestral strain | 44 | Male |
| Convalescent 21 | BBIBP-CorV/BBIBP-CorV/Ancestral strain | 46 | Male |
| Convalescent 22 | BBIBP-CorV/BBIBP-CorV/Ancestral strain | 58 | Male |
| Convalescent 23 | Unvaccinated/Delta | 10 | Male |
| Convalescent 24 | Unvaccinated/Delta | 36 | Male |
| Convalescent 25 | Unvaccinated/Delta | 53 | Male |
| Convalescent 26 | Unvaccinated/Delta | 39 | Female |
| Convalescent 27 | Unvaccinated/Delta | 35 | Male |
| Convalescent 28 | Unvaccinated/Delta | 52 | Male |
| Convalescent 29 | CoronaVac/CoronaVac/Delta | 42 | Male |
| Convalescent 30 | CoronaVac/CoronaVac/Delta | 44 | Male |
| Convalescent 31 | BBIBP-CorV/BBIBP-CorV/Delta | 29 | Male |
| Convalescent 32 | CoronaVac/CoronaVac/Delta | 29 | Male |
| Convalescent 33 | BBIBP-CorV/BBIBP-CorV/Delta | 38 | Male |
| Convalescent 34 | BBIBP-CorV/BBIBP-CorV/Delta | 25 | Male |
| Convalescent 35 | BBIBP-CorV/BBIBP-CorV/Delta | 32 | Male |
| Convalescent 36 | BBIBP-CorV/BBIBP-CorV/Delta | 39 | Male |
| Convalescent 37 | BBIBP-CorV/BBIBP-CorV/Delta | 35 | Female |
| Convalescent 38 | BBIBP-CorV/BBIBP-CorV/Delta | 54 | Male |
| Convalescent 39 | BBIBP-CorV/BBIBP-CorV/Delta | 35 | Male |
| Convalescent 40 | CoronaVac/CoronaVac/BA.1 | 53 | Female |
| Convalescent 41 | CoronaVac/CoronaVac/BA.1 | 55 | Male |
| Convalescent 42 | CoronaVac/CoronaVac/BA.1 | 34 | Male |
| Convalescent 43 | BBIBP-CorV/BBIBP-CorV/BA.1 | 31 | Male |
| Convalescent 44 | BBIBP-CorV/BBIBP-CorV/BA.1 | 30 | Male |
| Convalescent 45 | CoronaVac/CoronaVac/BA.1 | 42 | Male |
| Convalescent 46 | CoronaVac/CoronaVac/BA.1 | 31 | Male |
| Convalescent 47 | BBIBP-CorV/BBIBP-CorV/BA.1 | 37 | Male |
| Convalescent 48 | CoronaVac/CoronaVac/BA.1 | 29 | Female |
| Convalescent 49 | BBIBP-CorV/BBIBP-CorV/BA.1 | 34 | Male |
| Convalescent 50 | BBIBP-CorV/BBIBP-CorV/BA.1 | 51 | Male |
| Convalescent 51 | CoronaVac/CoronaVac/BA.1 | 36 | Female |
| Convalescent 52 | CoronaVac/CoronaVac/BBIBP-CorV/BA.1 | 19 | Female |
| Convalescent 53 | CoronaVac/CoronaVac/CoronaVac/BA.1 | 44 | Male |
| Convalescent 54 | CoronaVac/CoronaVac/BBIBP-CorV/BA.1 | 44 | Male |
| Convalescent 55 | BBIBP-CorV/BBIBP-CorV/BBIBP-CorV/BA.1 | 46 | Male |
| Convalescent 56 | CoronaVac/CoronaVac/CoronaVac/BA.1 | 30 | Female |
| Convalescent 57 | BBIBP-CorV/BBIBP-CorV/BBIBP-CorV/BA.1 | 53 | Male |
| Convalescent 58 | BBIBP-CorV/BBIBP-CorV/BBIBP-CorV/BA.1 | 35 | Male |
| Convalescent 59 | BBIBP-CorV/BBIBP-CorV/CoronaVac/BA.1 | 33 | Male |
| Convalescent 60 | BBIBP-CorV/BBIBP-CorV/CoronaVac/BA.1 | 39 | Male |
| Convalescent 61 | CoronaVac/CoronaVac/BBIBP-CorV/BA.1 | 17 | Male |
| Convalescent 62 | CoronaVac/CoronaVac/BBIBP-CorV/BA.1 | 38 | Female |
| Convalescent 63 | BBIBP-CorV/BBIBP-CorV/BBIBP-CorV/BA.1 | 26 | Female |
| Convalescent 64 | BBIBP-CorV/BBIBP-CorV/CoronaVac/BA.1 | 33 | Male |
| Convalescent 65 | BBIBP-CorV/BBIBP-CorV/BBIBP-CorV/BA.1 | 36 | Male |
| Convalescent 66 | BBIBP-CorV/BBIBP-CorV/CoronaVac/BA.1 | 31 | Male |
| Convalescent 67 | CoronaVac/CoronaVac/BBIBP-CorV/BA.1 | 49 | Male |
| Convalescent 68 | BBIBP-CorV/BBIBP-CorV/BBIBP-CorV/BA.5 variant | 31 | Female |
| Convalescent 69 | CoronaVac/CoronaVac/BBIBP-CorV/BA.5 variant | 26 | Female |
| Convalescent 70 | CoronaVac/BBIBP-CorV/BBIBP-CorV/BA.5 variant | 45 | Male |
| Convalescent 71 | BBIBP-CorV/BBIBP-CorV/BBIBP-CorV/BA.5 variant | 46 | Male |
| Convalescent 72 | BBIBP-CorV/CoronaVac/BBIBP-CorV/BA.5 variant | 27 | Female |
| Convalescent 73 | CoronaVac/CoronaVac/CoronaVac/BA.5 variant | 24 | Female |
| Convalescent 74 | BBIBP-CorV/BBIBP-CorV/BBIBP-CorV/BA.5 variant | 32 | Female |
| Convalescent 75 | BBIBP-CorV/BBIBP-CorV/BBIBP-CorV/BA.5 variant | 29 | Female |
| Convalescent 76 | BBIBP-CorV/CoronaVac/BBIBP-CorV/BA.5 variant | 26 | Female |
| Convalescent 77 | CoronaVac/BBIBP-CorV/CoronaVac/BA.5 variant | 26 | Female |
| Convalescent 78 | BBIBP-CorV/CoronaVac/CoronaVac/BA.5 variant | 28 | Female |
| Convalescent 79 | BBIBP-CorV/CoronaVac/BBIBP-CorV/BA.5 variant | 43 | Male |
| Convalescent 80 | BBIBP-CorV/CoronaVac/CoronaVac/BA.5 variant | 24 | Female |
| Convalescent 81 | CoronaVac/CoronaVac/CoronaVac/BA.5 variant | 41 | Female |
| Convalescent 82 | CoronaVac/CoronaVac/BBIBP-CorV/BA.5 variant | 22 | Female |
| Convalescent 83 | BBIBP-CorV/BBIBP-CorV/BBIBP-CorV/BA.5 variant | 22 | Male |
| Convalescent 84 | CoronaVac/CoronaVac/CoronaVac/BA.5 variant | 24 | Female |
| Convalescent 85 | CoronaVac/CoronaVac/BBIBP-CorV/BA.5 variant | 25 | Female |
| Convalescent 86 | CoronaVac/CoronaVac/CoronaVac/BA.5 variant | - | Female |
| Convalescent 87 | CoronaVac/CoronaVac/CoronaVac/BA.5 variant | - | Female |
| Convalescent 88 | BBIBP-CorV/BBIBP-CorV/BBIBP-CorV/BA.5 variant | - | Female |
| Convalescent 89 | CoronaVac/CoronaVac/BBIBP-CorV/BA.5 variant | 26 | Female |
| Convalescent 90 | BBIBP-CorV/BBIBP-CorV/BBIBP-CorV/BA.5 variant | 26 | Male |
| Convalescent 91 | CoronaVac/BBIBP-CorV/CoronaVac/BA.5 variant | 59 | Female |

**Table S2. SARS-CoV-2-S variants constructed in this study.**

|  |  |
| --- | --- |
| **SARS-CoV-2 S**  **variants** | **Mutations** |
| Omicron BA.5 | T19I, L24S, Δ25-27, Δ69-70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K |
| Omicron BF.7 | T19I, L24S, Δ25-27, Δ69-70, G142D, V213G, G339D, R346T, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K |
| Omicron BQ.1 | T19I, L24S, Δ25-27, Δ69-70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, K444T, L452R, N460K, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K |
| Omicron BQ.1.1 | T19I, L24S, Δ25-27, Δ69-70, G142D, V213G, G339D, R346T, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, K444T, L452R, N460K, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K |
| Omicron XBB | T19I, ΔL24, ΔP25, ΔP26, A27S, V83A, G142D, ΔY144, H146Q, Q183E, V213E, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486S, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K |
| Omicron XBB.1/ XBB.1.9 | T19I, ΔL24, ΔP25, ΔP26, A27S, V83A, G142D, ΔY144, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486S, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K |
| Omicron XBB.1.5/ XBB.1.9.1 | T19I, ΔL24, ΔP25, ΔP26, A27S, V83A, G142D, ΔY144, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486P, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K |