Omicron breakthrough infections in wild-type SARS-CoV-2 vaccinees elicit high levels of neutralizing antibodies against pangolin coronavirus GX_P2V

Lihua Song[1], Shanshan Lu[1], Shengdong Luo[2], Bingke Bai[2], Zhenping Fan[2], Bixia Hong[1], Wen Xu[2], Hongbin Ma[2], Wei-Wei Chen[2], Huahao Fan[1], and Yigang Tong[1]

[1]Beijing University of Chemical Technology College of Life Science and Technology
[2]5th Medical Center of Chinese PLA General Hospital

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Abstract

Omicron BF.7 became the predominant SARS-CoV-2 variant in Beijing after the adjustment of COVID-19 response strategies in December 2022. The ability of antibodies elicited by BF.7 infection to cross-react with SARS-CoV-2-like viruses is unknown. This study aimed to investigate the cross-reactive neutralizing antibodies against SARS-CoV-2-related pangolin coronavirus GX_P2V in sera from vaccinated and/or SARS-CoV-2 infected individuals. All vaccinated individuals who recovered from Omicron BF.7 breakthrough infections exhibited substantially higher levels of neutralizing antibodies against GX_P2V among collected subject populations (geometric mean titer [GMT] = 362). Uninfected individuals who received four-mixed-dose vaccines also demonstrated higher levels of neutralizing antibodies (GMT = 44) against GX_P2V than those who received two- or three-dose vaccines and those who recovered from wild type SARS-CoV-2. This finding highlights the significance of prior and hybrid booster vaccinations with wild-type SARS-CoV-2 vaccines in generating potent cross-protective immunity against future spillovers of SARS-CoV-2-like viruses.

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[1]Beijing Advanced Innovation Center for Soft Matter Science and Engineering, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing, China;[2]Department of Infectious Diseases, Fifth Medical Center of Chinese PLA General Hospital, Beijing, China

Correspondence

Lihua Song, Yigang Tong and Huahao Fan, Beijing Advanced Innovation Center for Soft Matter Science and Engineering, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing, China.

Email: Lihua Song, songlihua@gmail.com, Yigang Tong, tong.yigang@gmail.com and fanhuahao@mail.buct.edu.cn

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KEYWORDS

COVID-19, Omicron BF.7, pangolin coronavirus GX_P2V, cross-protective immunity

1 INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2), has persisted for more than three years[1], and numerous variants have emerged globally. In early December 2022, China lifted its COVID-zero policy, followed shortly by the quick spread of the SARS-CoV-2 Omicron variant across most parts of the country.[2] Although vaccination with wild-type SARS-CoV-2 vaccines did not appear to provide significant protection against Omicron in Beijing, most people only experienced influenza-like symptoms. By February 2023, only sporadic COVID cases were reported each day, indicating that herd immunity against Omicron variants was achieved in a relatively short period in China. However, concerns remain regarding the effectiveness of this immunity against future spillovers of SARS-CoV-2-like viruses.

To address this issue, we analyzed the neutralizing antibodies in sera from wild-type SARS-CoV-2 vaccine recipients who recovered from Omicron BF.7 variant infection by performing viral neutralizing assays with a SARS-CoV-2-related pangolin coronavirus GX_P2V[3]. The differences between SARS-CoV-2 and GX_P2V primarily lie in the spike glycoprotein (S), a primary target for neutralizing antibody (Figure S1). However, compared to SARS-CoV-2, the spike protein of pangolin-CoV GX_P2V has a comparable binding affinity to human ACE2 but has a distinct receptor binding domain (RBD)[4] (Figure 1A), making it a practical model for testing neutralizing antibodies against unpredictable SARS-CoV-2 related viruses.

2 MATERIALS AND METHODS

2.1 Cell culture

BGM cells purchased from the American Type Culture Collection (ATCC, USA) were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA) at 37°C in a 5% CO2 incubator. Mycoplasma testing was performed, and cell cultures were confirmed to be negative.

2.2 Patients and sample collection

Serum samples were collected from the subsequent cohorts: healthy individuals uninfected prior to the global COVID pandemic; convalescents of wild-type SARS-CoV-2 with qPCR-negative results 2-8 weeks post-diagnosis in 2020; recipients of two-dose inactivated vaccines (2-3 weeks post-second vaccination), three-

dose inactivated vaccines (3-5 months post-third vaccination), and four-mixed-dose vaccines (three-dose inactivated plus one-dose of alternative vaccine types, 2-4 weeks post-fourth vaccination). These vaccinated participants had no history of SARS-CoV-2 infection, as confirmed by regular qPCR screening tests. Serum samples from both vaccinated and unvaccinated individuals infected with the Omicron BF.7 variant were obtained when the subjects were tested qPCR-negative 2-9 weeks and 3-5 weeks post-diagnosis, respectively. Sera from golden hamsters infected twice with GX_P2V served as positive controls.

2.3 Neutralizing antibody assays

Serum samples from participants were initially 10-fold diluted and subsequently 3-fold serially diluted. The diluted serum samples were then combined with an equal volume of GX_P2V (100 $TCID_{50}$, GenBank accession number MW532698) incubated at 37 in a 5% CO_2 incubator for 2 hours. Following incubation, 100 μL of the virus-serum mixture was transferred into 96-well plates and combined with an equal volume of BGM cells (3×10^4 cells/well). The inoculated plates were incubated continuously at 37 in a 5% CO_2 incubator for an additional 5 days, after which the cytopathic effect (CPE) of the virus were observed using a light microscope. GX_P2V neutralization titers were calculated using the Reed-Muench equation and expressed as the sample dilution at which a 50% neutralization titer (NT_{50}) was achieved.

2.4 Statistical analysis

Statistical analyses were performed using GraphPad Prism 9 software. The differences between two groups were analyzed by Student's t-test (paired two-tailed). P-values less than 0.05 ($P< 0.05$) were considered statistically significant.

3 RESULTS AND DISCUSSION

A total of 52 infected patients and 32 uninfected population participated in this study. Descriptive characteristics for the study population are presented in Table 1 and Table 2. We assayed the neutralizing activity against pangolin-CoV GX_P2V in serum samples obtained from 27 participants who had been vaccinated and boosted but recovered from SARS-CoV-2 BF.7 variant. Sera from patients who did not receive any vaccination but recovered from wild-type SARS-CoV-2 strain or Omicron infections, and sera from individuals who received two, three and four mixed vaccinations but had no history of SARS-CoV-2 infection, were used as controls. Sera from GX_P2V-infected golden hamsters were used as positive controls.

The geometric mean titers (GMTs) of neutralizing antibodies against GX_P2V in each group are presented in Table S1 and Figure 1B. Notably, regardless of the vaccine types, all vaccinated individuals who recovered from Omicron BF.7 infections had substantially higher GMTs of neutralizing antibodies against GX_P2V compared to cohorts of wild-type SARS-CoV-2 convalescents, uninfected two-dose vaccinees, uninfected three-dose vaccinees, uninfected four-mixed-dose vaccinees, and unvaccinated Omicron convalescents by factors of 45.3, 27.8, 60.3, 8.2, and 12.9, respectively (Figure 1B). Thus, Omicron breakthrough infections in wild-type SARS-CoV-2 vaccinees elicited superior cross-neutralizing antibodies against GX_P2V, a SARS-CoV-2 related virus.

Moreover, compared with unvaccinated individuals recovered from the Omicron BF.7 variant infection, the GMT was lower by a factor of 3.5, 2.2, 4.7 in wild-type SARS-CoV-2 convalescents, uninfected recipients of two-dose and three-dose inactivated vaccines, respectively. The GMT of uninfected participants but with four-mixed-dose vaccines was also higher by a factor of 5.5, 3.4, 7.3 than in wild-type SARS-CoV-2 convalescents, uninfected recipients of two-dose and three-dose inactivated vaccines, respectively. The results showed the effectiveness of mixed booster vaccinations and hybrid immunity protection against SARS-CoV-2-like virus infection.

In conclusion, our finding highlights the significance of prior vaccinations with wild-type SARS-CoV-2 vaccines in generating potent cross-protective immunity against breakthrough infections and supports reports of pan-SARS-CoV-2 neutralizing epitopes in the wild-type SARS-CoV-2 RBD.[5,6] Additionally, our results further support GX_P2V as a promising live vaccine candidate against SARS-CoV-2.[7] Overall, our study suggests that the general population in China has likely developed superior immunity to prevent future

breaks of SARS-CoV-2-like viruses after the Omicron epidemic. Further research is necessary to corroborate these findings and to evaluate the long-term efficacy of such immunity in the population.

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AUTHOR CONTRIBUTIONS

Study concept and design: Lihua Song, Yigang Tong; *Data acquisition* : Shanshan Lu, Shengdong Luo, Wen Xu, Bixia Hong, Wen Xu, Hongbin Ma; *Analysis and interpretation of data* : Huahao Fan, Bingke Bai, Zhenping Fan, Hongbin Ma, Weiwei Chen; *Manuscript write-up* : Lihua Song, Shanshan Lu. Revision and approval of the final draft by all authors

CONFLICT OF INTERESTS

No potential conflict of interest was reported by the authors.

Ethics statement

This study was approved by the ethics committee (EC) of the Fifth Medical Center, General Hospital of Chinese PLA (approval number: 2020027D).

DATA AVAILABILITY STATEMENT

The data in this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-733.

2. Pan Y, Wang L, Feng Z, et al. Characterisation of SARS-CoV-2 variants in Beijing during 2022: an epidemiological and phylogenetic analysis. Lancet. 2023;401(10377):664-672.

3. Lam TT, Jia N, Zhang YW, et al. Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. Nature. 2020;583(7815):282-285.

4. Zhang S, Qiao S, Yu J, et al. Bat and pangolin coronavirus spike glycoprotein structures provide insights into SARS-CoV-2 evolution. Nat Commun. 2021;12(1):1607.

5. Liu Z, Zhou J, Wang X, et al. A pan-sarbecovirus vaccine based on RBD of SARS-CoV-2 original strain elicits potent neutralizing antibodies against XBB in non-human primates. Proc Natl Acad Sci U S A. 2023;120(11):e2221713120.

6. Qi H, Liu B, Wang X, Zhang L. The humoral response and antibodies against SARS-CoV-2 infection. Nat Immunol. 2022;23(7):1008-1020.

7. Lu S, Luo S, Liu C, et al. Induction of significant neutralizing antibodies against SARS-CoV-2 by a highly attenuated pangolin coronavirus variant with a 104nt deletion at the 3'-UTR. Emerg Microbes Infect. 2023;12(1):2151383.

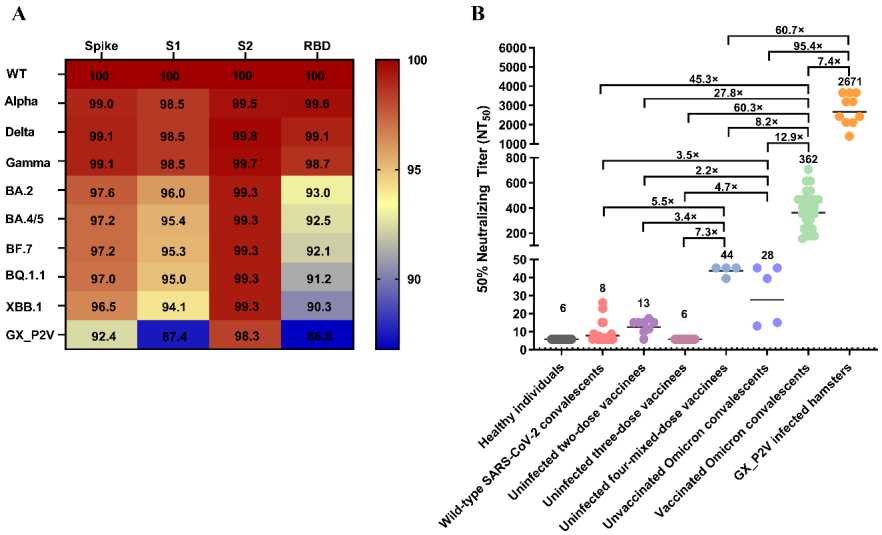

FIGURE 1 Neutralizing antibody responses to pangolin coronavirus GX_P2V.

(A) Amino acids sequences of the GX_P2V and SARS-CoV-2 variants were aligned based on the whole spike protein, S1, S2, and RBD. The percent identities of the aligned sequences were calculated, and a heatmap was generated using GraphPad Prism 9 with SARS-CoV-2 as the reference sequence. (B) Neutralizing antibody responses against pangolin coronavirus GX_P2V were assessed and expressed as neutralizing titer 50 (NT_{50}). The numbers over the different colors of dots in each group represent geometric mean titers (GMTs). GMTs for each group were displayed as horizontal black lines. Ratios between GMTs of two different groups are indicated. Individual samples of various groups are represented by different colors of dots.

TABLE 1 Characteristics of the infected population

Variable	**Wild-type SARS-CoV-2 convalescents**	**Unvaccinated Omicron convalescents**	**Vaccinated Omicron convalescents**
Number of participants	20	5	27
Age (years), median (range)	53 (17-79)	40 (34-43)	29 (25-54)
Sex at birth, female	10 (50%)	3 (60%)	19 (70%)
COVID vaccination history			
Two-dose inactivated vaccines	0	N/A	1 (3.7%)
Three-dose inactivated vaccines	0	N/A	18 (66.7%)
Two-dose Ad5-nCoV (adenovirus vector vaccine)	0	N/A	6 (22.2%)
Three-dose Recombinant Fusion Protein vaccines	0	N/A	1 (3.7%)
Two-dose inactivated vaccines plus one-dose Ad5-nCoV	0	N/A	1 (3.7%)
COVID disease severity			

Asymptomatic	0	1 (20%)	0
Mild	4 (20%)	4 (80%)	19 (70%)
Moderate	9 (45%)	0	8 (30%)
Severe	4 (20%)	0	0
Critical	3 (15%)	0	0
Time between last vaccination and confirmed infection	N/A	N/A	6-22 months
Time between infection and serum sampling when SARS-CoV-2 negative	2-8 weeks	3-5 weeks	2-9 weeks
Method of SARS-CoV-2 testing			
Quantitative PCR	20 (100%)	1 (20%)	5 (19%)
Rapid antigen detection	0	4 (80%)	22 (81%)

Note: Data are median (range), or n (%).

TABLE 2 Characteristics of the uninfected population

Variable	**Healthy individuals**	**Uninfected two-dose vaccinees**	**Uninfected three-dose vaccinees**	**Uninfected four-mixed-dose vaccinees**
Number of participants	10	8	10	4
Age (years), median (range)	41 (33-63)	35 (32-50)	/	31 (28-51)
Sex at birth, female	7 (70%)	7 (88%)	/	2 (50%)
COVID vaccination history				
Inactivated vaccines	0	8 (100%)	10 (100%)	0
Ad5-nCOV (adenovirus vector vaccine)	0	0	0	0
dNS1-RBD	0	0	0	0
Recombinant Fusion Protein	0	0	0	0
Three-dose inactivated vaccines plus one-dose dNS1-RBD	0	0	0	1 (25%)

Three-dose inactivated vaccines plus one-dose Ad5-nCOV	0	0	0	2 (50%)
Three-dose inactivated vaccines plus one-dose Recombinant Fusion Protein	0	0	0	1 (25%)
Time between last vaccination and serum sampling	N/A	2-3 weeks	3-5 months	2-4 weeks

Note: Data are median (range), or n (%).