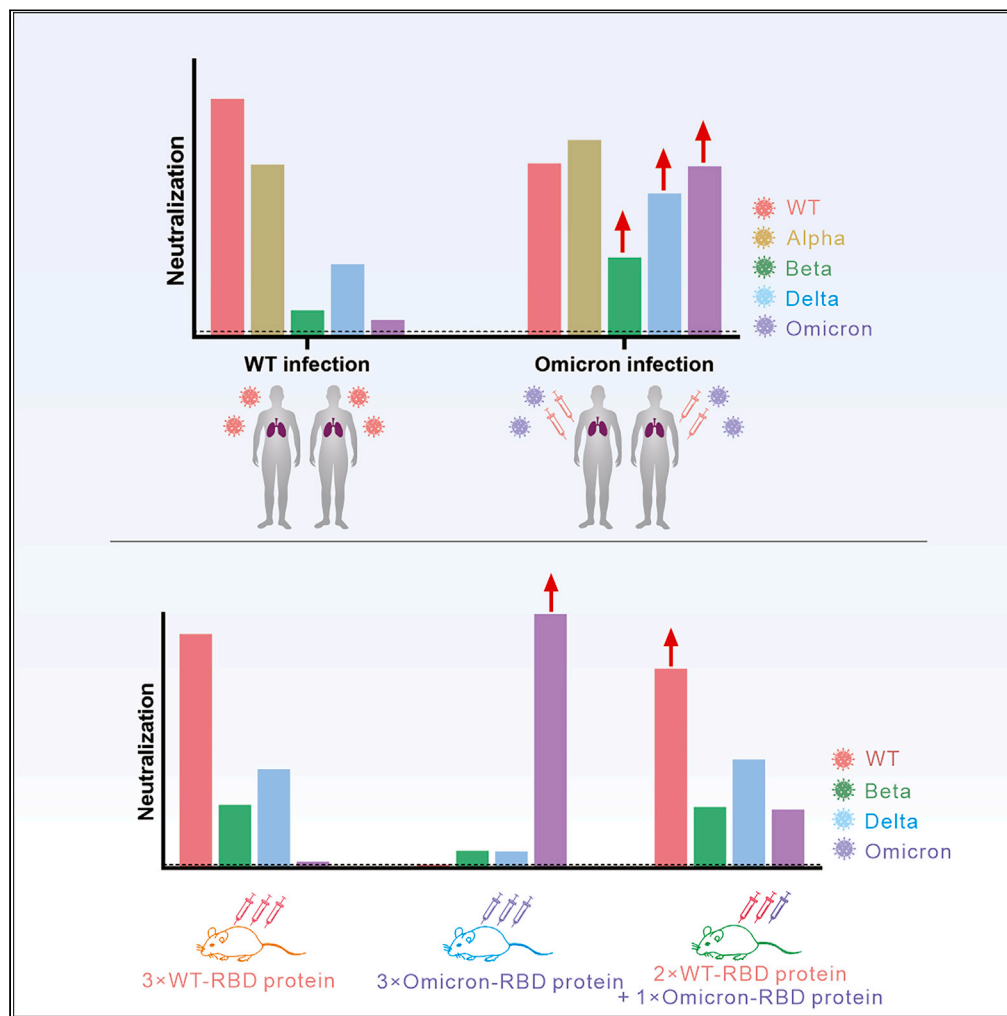


Article

Extensive neutralization against SARS-CoV-2 variants elicited by Omicron-specific subunit vaccine as a heterologous booster



Pai Peng,
Chengqian Feng,
Jie Hu, ..., Ni Tang,
Xiao-ping Tang,
Ailong Huang

tangxp@gzhmu.edu (X.-p.T.)
ahuang@cqmu.edu.cn (A.H.)

Highlights

Post-vaccination SARS-CoV-2 infection can elicit higher and broader immune response

A heterologous booster with the Omicron-RBD protein elicits a 9-fold increased NAb

Heterologous boosting with Omicron enhance the potency and breadth of immune response



Article

Extensive neutralization against SARS-CoV-2 variants elicited by Omicron-specific subunit vaccine as a heterologous booster

Pai Peng,^{1,5} Chengqian Feng,^{2,5} Jie Hu,^{1,5} Changlong He,^{1,3,5} Haijun Deng,^{1,5} Qinghong Fan,² Jin Xiang,¹ Guofang Tang,² Meng-ling Jiang,² Fengyu Hu,² Feng Li,^{2,4} Kai Wang,¹ Ni Tang,¹ Xiao-ping Tang,^{2,4,*} and Ailong Huang^{1,6,*}

SUMMARY

To overcome the increased risk of SARS-CoV-2 reinfection or post-vaccination infection caused by the Omicron variant, Omicron-specific vaccines were considered a potential strategy. We reported the increased magnitude and breadth of antibody response against VOCs elicited by post-vaccination Delta and Omicron infection, compared to WT infection without vaccination. Then, in mouse models, three doses of Omicron-RBD immunization elicited comparable neutralizing antibody (NAb) titers with three doses of WT-RBD immunization, but the neutralizing activity was not cross-active. By contrast, a heterologous Omicron-RBD booster following two doses of WT-RBD immunization increased the NAb titers against Omicron by 9-folds than the homologous WT-RBD booster. Moreover, it retains neutralization against both WT and current VOCs. Results suggest that Omicron-specific subunit booster shows its advantages in the immune protection from both WT and current VOCs and that SARS-CoV-2 vaccines including two or more virus lineages might improve the NAb response.

INTRODUCTION

Since the coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began in 2019, it had experienced several waves driven by variants of this virus. At present, five variants including Alpha, Beta, Gamma, Delta, and Omicron were designated as “variants of concern” (VOCs). As a dominant strain, even though the pandemic of Delta had lasted over one year, it has been replaced swiftly by Omicron (B.1.1.529) causing a new round of pandemic within a short time due to its rapid spread with plenty of mutations.¹ Compared to the SARS-CoV-2 ancestral wild-type (WT) virus, more than 30 mutations have been accumulated in the spike (S) protein of the Omicron variant, especially 15 of those occur on the receptor-binding domain (RBD), which is not only the vital binding site to the host receptor angiotensin-converting enzyme 2 (ACE2) for the entry of SARS-CoV-2 but also the key target of neutralizing antibodies produced by immune responses and therapeutic antibodies.^{2–4}

Due to the lack of effective drugs treating SARS-CoV-2 infection, acquired immunity induced by vaccines or natural infection has been a major protection strategy. Spike mutation has been well documented to be correlated with its infectivity alteration and immune evasion in infection cases caused by several variants.^{5–9} The neutralizing activity of sera against Omicron has been suggested for an extensive reduction in convalescents or vaccinees who received various types of SARS-CoV-2 vaccines.^{6,10,11} Reduced neutralization elicited by infection or vaccination shows that Omicron has an increased risk of SARS-CoV-2 reinfection or post-vaccination infection. Throughout this article, post-vaccination infection as a term is used to describe SARS-CoV-2 infections in fully vaccinated individuals. In 31220 Norwegian households, the secondary attack rate caused by Omicron was 25.1% (95% CI, 24.4%–25.9%).¹² A study based on the Qatar national database suggests that the effective protection of the previous infection against reinfection with the Omicron variant was approximately 60%, which is lower than alpha, beta, and delta variants (at approximately 90%).¹³ The vaccine effectiveness against Omicron after two BNT162b2 doses was 65.5% (95% confidence interval [CI], 63.9 to 67.0) at 2 to 4 weeks, and dropped to 8.8% (95% CI, 7.0 to 10.5) at 25 or more weeks.¹⁴ Currently, an Omicron sub-variant BA.2 shows a faster spread and a similar resistance to immunity

¹Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Institute for Viral Hepatitis, Department of Infectious Diseases, the Second Affiliated Hospital, Chongqing Medical University, Yixue Yuan Road No.1, Chongqing 400016, China

²Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, China

³Department of Laboratory Medicine, People's Hospital of Jiulongpo District, Chongqing, China

⁴Guangzhou Laboratory, Guangzhou, China

⁵These authors contributed equally

⁶Lead contact

*Correspondence: tangxp@gzhmu.edu (X.-p.T.), ahuang@cqmu.edu.cn (A.H.) <https://doi.org/10.1016/j.isci.2022.105465>



with a high rate of post-vaccination infection.^{15–17} After breaking through prior immune protection by vaccines based on the ancestral wild-type (WT) virus, how the immune response elicited by the post-vaccination Omicron infection needs to be delineated. With post-vaccination infection as a common situation in the real world, it will give hints for the further development of protective vaccines and vaccination strategies.

Due to the continuous appearance of SARS-CoV-2 variants, the reduced efficiency of existing vaccines accelerates the need for new vaccine strategies. Boosters following the primary vaccination series showed their potential efficiency of promoting high neutralizing activity¹⁸ and reducing symptomatic SARS-CoV-2 infection,¹⁹ but booster shots displayed failure in the post-vaccination infection of some SARS-CoV-2 VOCs.²⁰ Moreover, simply additional boosters might finitely improve immune protection. A fourth-dose booster using the same vaccine could not generate higher antibody titers than the three doses of vaccination and shows low prevention against mild or asymptomatic Omicron infections and post-vaccination infections.²¹ As another candidate strategy, boosting with heterologous types of vaccines, that are different from the prime vaccine, has been proven to be a safe and efficient immune response.^{22–24} Given the correlation of the immune escape of the Omicron variant with its great number of mutations, Omicron-specific vaccines have been proposed. Omicron-specific mRNA vaccine booster could induce a neutralizing response to Omicron itself but fail to previous VOCs.²⁵ As a booster with Omicron-matched DNA vaccines, increased width of the immune response has been observed.²⁶ However, in macaque models, vaccination with Omicron-specific boosters does not increase neutralizing antibody (NAb) titers against Omicron and remain the equivalent levels of B cell response.²⁷ Of note, both two types of vaccines were designed according to full-length spike proteins. Considering the key role of SARS-CoV-2 RBD as the target of neutralizing antibodies, it has an important significance to study how the Omicron-specific immune response plays alone and cooperates with previous doses of SARS-CoV-2 wild-type RBD (WT-RBD) immunization. Here, we report the Omicron-specific immunogenicity and cross-neutralization activity induced by Omicron-specific RBD subunit proteins in mouse models to highlight the need for the next generation of SARS-CoV-2 vaccines with specific antigens of circulating virus strains.

RESULTS

The immune response elicited by post-vaccination Omicron infections

In this study, twenty persons infected with Omicron after full vaccination were recruited, while 20 Delta-infected individuals who were fully vaccinated and 13 individuals who were previously infected with WT but unvaccinated were matched according to age, sex, and the time of sample collection (Table 1). Post-vaccination Delta infections occurred 2.5–5 months (median 4.1 months) after the last dose of vaccines, while post-vaccination Omicron infections occurred 3.4–6.6 months (median 5.2 months) after the last dose of vaccines. Serum samples were collected at 3–4 time points within 50 days.a.o/d.a.a. Their anti-WT-RBD IgG-binding antibodies and neutralizing antibodies were determined by enzyme-linked immunosorbent assay (ELISA) and the pseudotype-based neutralizing assay. Within the acute phase of SARS-CoV-2 infection, anti-WT-RBD IgG levels of most sera in all three cohorts gradually raised to peak, and then remained relatively stable (Figures 1A and S1). As expected, due to the lack of the pre-existing immune protection from vaccination, lower IgG titers in the WT-infected cohort were observed at the early stages of viral infection than that in post-vaccination Omicron or Delta infection. With the accumulation of antibody levels, anti-WT-RBD IgG-binding antibodies in these three cohorts reached comparable levels at the late stages of acute infection.

Moreover, the neutralization ability of post-vaccination infection has been observed at different time points (Figures 1B and S2). As exemplified by the third time point (18 days.a.o/d.a.a, IQR: 16–23 days.a.o/d.a.a), NAb titers of sera from the WT cohort against four VOCs (Alpha, Beta, Delta, and Omicron) are lower than the NAb titers against WT itself with 1-, 8-, 3-, and 13-fold decrease, respectively. In the Delta cohort, compared to the NAb titer against Delta itself, NAb titers of sera against WT, Alpha, Beta, and Omicron are decreased by 1.5-, 1.4-, 4.9-, and 6.0-, respectively. In the Omicron cohort, compared to the NAb titer against Omicron itself, NAb titers of sera against WT, Alpha, Beta, and Delta are decreased by 1.0-, 0.9-, 2.1-, and 1.2-folds, respectively. The consistent relationship of the fold change of NAb levels among VOCs was also presented at other time points (Figure S2). The minor fold change indicated the effective cross-neutralization elicited by post-vaccination infections.

We further compared the neutralization activity of these three cohorts against each SARS-CoV-2 strain (Figure 1C). Even though NAb titers against WT (geometric mean titers, GMTs = 1452 and 1131) induced

Table 1. Clinical characteristics of 53 individuals experienced with SARS-CoV-2 wild-type infection, post-vaccination Delta or Omicron infection in this study

Characteristics	COVID-19 cases				p values		
	Total (n = 53)	Wild-type (n = 13)	Delta (n = 20)	Omicron (n = 20)	W vs. D	W vs. O	D vs. O
Age (mean ± SD)	41.6 ± 13.2	45.8 ± 14.9	36.5 ± 9.7	44.0 ± 14.0	0.041	0.102	0.102
Sex							
Male (n, %)	34 (64.2%)	5 (38.5%)	13 (65.0%)	16 (80.0%)	0.169	0.027	0.480
Female (n, %)	19 (35.8%)	8 (61.5%)	7 (35.0%)	4 (20.0%)			
BMI	23.8 (21.0–25.5)	–	22.6 (19.9–25.3)	23.8 (21.7–25.5)	–	–	0.425
Onset to admission, median days (median, IQR)	2 (1–3)	4 (3–7)	1 (0–3)	1 (1–2)	0.003	0.000	0.525
Onset to qPCR negative for SARS-CoV-2 (median, IQR)	16 (13–20)	16 (12–20)	14 (12–17)	19 (16–23)	0.349	0.168	0.003
Duration of hospitalization (median, IQR)	21 (17–26)	21 (20–26)	18 (15–32)	21 (18–24)	0.542	0.551	0.422
Vaccination doses							
Unvaccinated (n, %)	13 (24.5%)	13 (100.0%)	0 (0.0%)	0 (0.0%)	–	–	0.231
≥ 3 doses (n, %)	3 (5.7%)	–	0 (0.0%)	3 (15.0%)			
<3 dose (n, %)	37 (69.8%)	–	20 (100.0%)	17 (85.0%)			
Vaccination type							
Unvaccinated (n, %)	13 (24.5%)	13 (100.0%)	0 (0.0%)	0 (0.0%)	–	–	1.000
mRNA (n, %)	6 (11.3%)	–	3 (15.0%)	3 (15.0%)			
Inactivated vaccine (n, %)	29 (54.7%)	–	14 (70.0%)	15 (75.0%)			
Co-inoculation	1 (1.9%)	–	0 (0.0%)	1 (5.0%)			
Unknown (n, %)	4 (7.5%)	–	3 (15.0%)	1 (5.0%)			
Months of last vaccination to the first sampling (median, IQR)	4.2 (2.6–6)	–	4.1 (2.5–5)	5.2 (3.4–6.6)	–	–	0.082
Samples collected during follow-up (n, %)							
≤ 7 days.a.o	44 (23.5%)	5 (13.2%)	15 (20.8%)	24 (31.2%)	0.131	0.009	0.362
8–14 days.a.o	46 (24.6%)	8 (21.1%)	22 (30.6%)	16 (20.8%)			
15–21 days.a.o	32 (17.1%)	9 (23.7%)	12 (16.7%)	11 (14.3%)			
22–28 days.a.o	22 (11.8%)	4 (10.5%)	8 (11.1%)	10 (13.0%)			
29–35 days.a.o	23 (12.3%)	4 (10.5%)	12 (16.7%)	7 (9.1%)			
36–42 days.a.o	6 (3.2%)	4 (10.5%)	1 (1.4%)	1 (1.3%)			
≥ 43 days.a.o	6 (3.2%)	4 (10.5%)	2 (2.8%)	0 (0.0%)			

d.a.o, days after symptom onset; IQR, interquartile range.

respectively by post-vaccination Delta and Omicron infection did not exceed NAb levels in the WT cohort (GMT = 1551), sera from two post-vaccination infection cohorts displayed decreased neutralizing resistance against SARS-CoV-2 variants Beta, Delta, and Omicron: NAb GMTs against Beta variant were 451 and 520 in the post-vaccination Delta and Omicron infection vs GMT = 175 in the WT-infected group ($p < 0.01$ for both variants); NAb GMTs against Delta variant were 2219 and 935 in the post-vaccination Delta and Omicron infection vs GMT = 475 in the WT-infected group ($p < 0.001$ for Delta, $p < 0.05$ or Omicron); NAb GMTs against Omicron variant were 369 and 1112 in the post-vaccination Delta and Omicron infection vs GMT = 112 in the WT-infected group ($p < 0.001$ for both variants).

During the follow-up visit (Figures 1D–1F, S3), within 7 days.a.o/d.a.a, NAb levels produced by WT infection were lower than that of post-vaccination infections, which is in concert with levels of anti-WT-RBD-binding

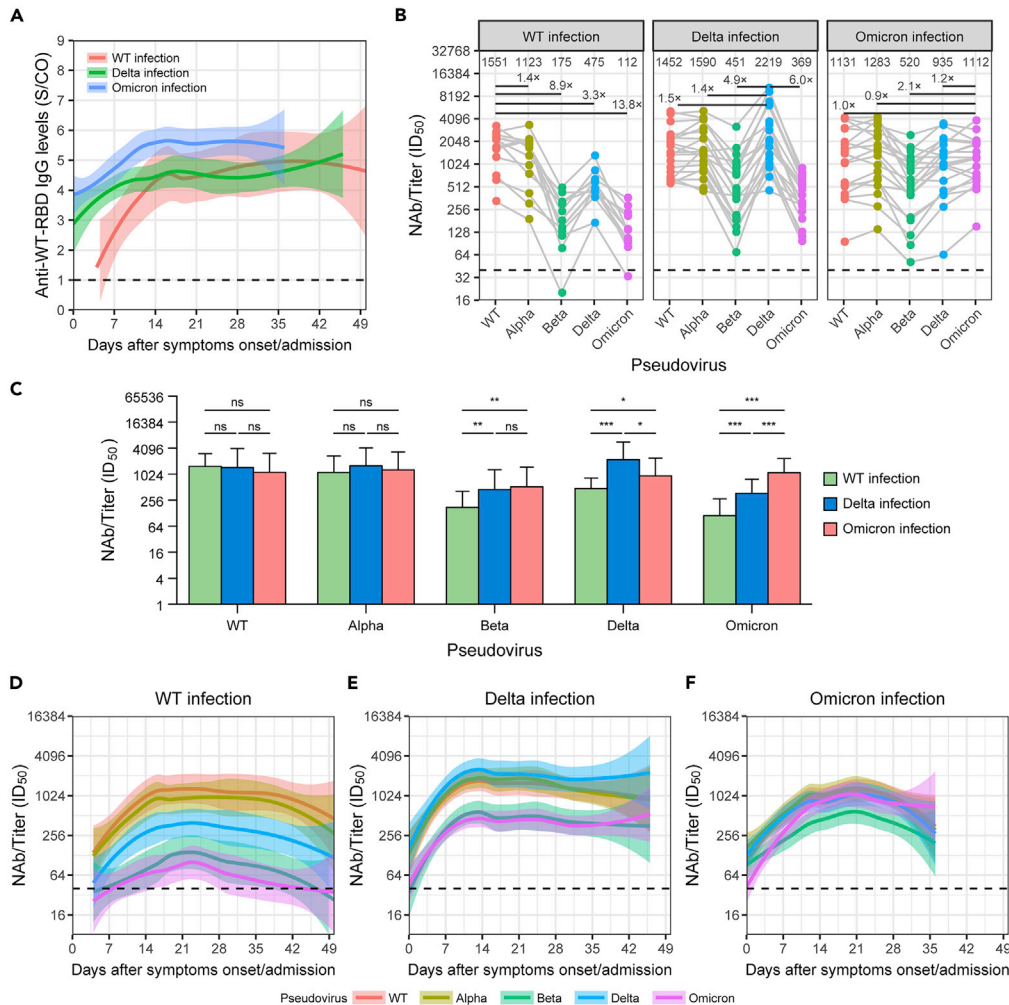


Figure 1. The characteristics of immune response elicited by the post-vaccination Omicron infection

(A) Dynamic change of anti-WT-RBD IgG-binding antibodies in three cohorts: post-vaccination Omicron infection or post-vaccination Delta infection (n = 20 for each cohort, sampled at 4 time points within 46 days after symptom onset/admission), and WT infection without prior vaccination (sampled at 3 different time points within 50 days after symptom onset/admission).

(B) Pseudovirus-based neutralizing assays were performed to test the neutralizing activity of the sera against WT, Alpha, Beta, Delta, and Omicron variants from three cohorts at the time point 3 (18 days, IQR:16–23 days).

(C) Bar graph comparing neutralizing antibody (NAb) titers of these three cohorts against different SARS-CoV-2 pseudoviruses.

(D–E) Longitudinal observation of NAb titers of individuals who experienced WT infection (C), Delta (D), and Omicron (E) post-vaccination infection against WT, Alpha, Beta, Delta, and Omicron pseudovirus. A, D–F: A locally weighted regression (LOESS) model was used to draw the kinetics of neutralizing antibody by R software. The lines show the mean value expected from the LOESS model, and the ribbons indicate the 95% confidence interval. Statistical data analysis was performed using GraphPad Prism software. The half-maximal inhibitory dose (ID₅₀) was calculated as NAb titers. The values above points indicate the geometric mean titers (GMTs). The threshold of ID₅₀ detection was 1:40. Statistical significance labels: n.s. indicates no significant differences, * indicates p < 0.05, ** indicates p < 0.01 and *** indicates p < 0.001.

antibodies. Between WT-infected and Omicron-infected cohorts presented similar dynamic changes of antibody levels: they reached to the peak within 14–21 days.a.o/d.a.a, and then declined gradually. However, in the Delta-infected cohort, antibody levels remained stable or even rose after reaching to the peak. However, The WT-infected cohort showed various degrees of neutralizing resistance to SARS-CoV-2 VOCs, especially to Beta and Omicron. In the Delta-infected and Omicron-infected cohorts, high neutralizing

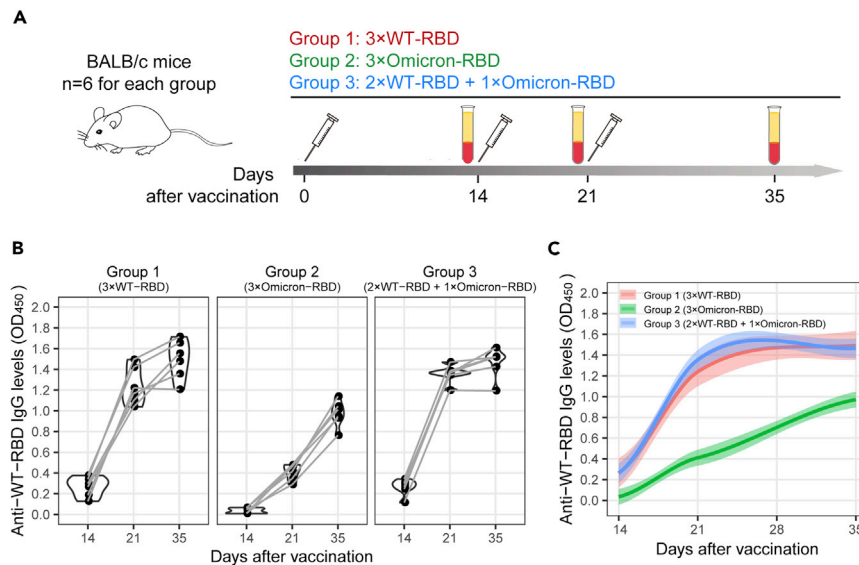


Figure 2. Anti-SARS-CoV-2 RBD antibody IgG levels elicited by Omicron-specific booster in mouse models

(A) Schematic of BALB/c mice immunization programs. 6 mice for each group were intramuscularly injected with the indicated recombinant proteins: Group 1 was immunized with three doses of recombinant WT-RBD proteins; Group 2 was immunized with three doses of recombinant Omicron-RBD proteins; Group 3 was immunized with two doses of recombinant WT-RBD proteins and boosted with one dose of recombinant Omicron-RBD protein.

(B) Dynamic change of anti-WT-RBD IgG-binding antibodies in three groups. Serum samples were collected three times within 35 days after the first dose of immunization according to panel A. 1:16000 diluted sera were determined by ELISA. Each point represents a single mouse.

(C) The fitted curve of the dynamic change of anti-WT-RBD IgG-binding antibodies during the full doses of immunization. Colorful areas showed the 95% confidence intervals.

activity against themselves has been seen due to specific immune responses induced by post-vaccination infection. Taken together, compared with WT infection, post-vaccination infection especially by Omicron could induce a wide range and high levels of the humoral immune response to Omicron and other VOCs.

Omicron-specific humoral response induced by recombinant SARS-CoV-2 RBD subunit proteins in mouse models

Widespread neutralizing activity of post-vaccination Omicron or Delta infection against WT and other variants has been seen based on the exposure of two antigens. Then, mouse models were used to evaluate whether previous vaccination or Omicron-specific immune response has the major contribution to the increased protection. BALB/c mice were distributed into 3 groups and immunized with two doses of recombinant SARS-CoV-2 RBD subunit proteins at a 2-week interval plus one booster one week after the second dose (Figure 2A): Group 1 was immunized with 3 doses of WT-based RBD recombinant proteins (WT-RBD); Group 2 was immunized with 3 doses of Omicron-based RBD recombinant proteins (Omicron-RBD); Group 3 was immunized with 2 doses of WT-RBD plus one dose of Omicron-RBD. Another group was immunized by the adjuvant (Cat: KX0210042, Biodragon, China) as controls (data were not shown).

Mice sera were collected and determined their IgG binding antibody levels and neutralizing effects on different SARS-CoV-2 strains. Among all these groups, immunization at a two-week interval induced the growth of anti-WT-RBD IgG-binding antibody levels (Figure 2B). The additional third dose boosted IgG antibody levels, which is consistent with that of the three clinical cohorts we tested above. After two-dose immunization with WT-RBD, boosting by either WT-RBD in Group 1 or Omicron-RBD in Group 3 as the third dose produced comparable levels of anti-WT-RBD IgG. Of note, the lower anti-WT-RBD IgG levels have been shown in these sera elicited by three doses of Omicron-specific immunization in Group 2 (Figure 2C). It suggests partial cross-recognition of antibody response elicited by Omicron.

Two-week interval with boosting by the third-dose immunization led to a 26-fold increase (GMTs from 138 to 3688) of NAbs against WT in Group 1 (Figure 3A), while a 30-fold increase (GMTs from 183 to 5525) of

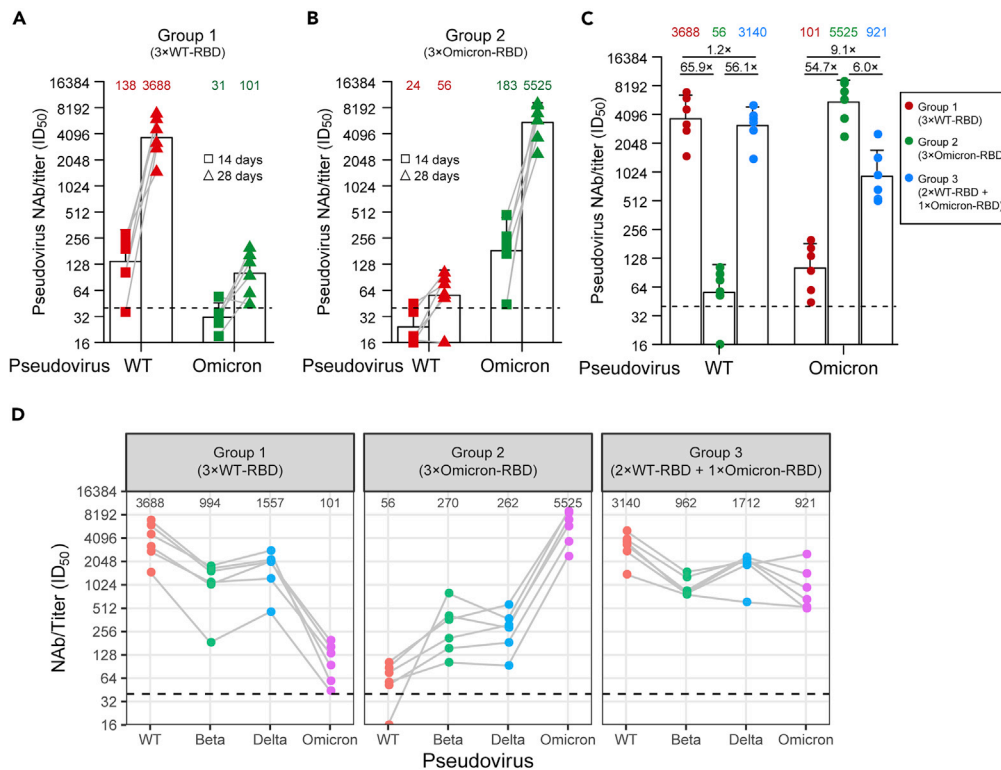


Figure 3. Omicron-specific neutralizing antibody response in mouse models

(A-B) Sera were collected and performed the pseudotype-based neutralizing assay to determine their neutralizing capacity to WT or Omicron pseudoviruses at 14 days (squares) and 28 days (triangles) after the first dose of immunization by WT-RBD (A) or Omicron-RBD (B) recombinant subunit proteins.

(C) NAb titers of sera against WT or Omicron from 3 groups collected 28 days after the first dose of immunization.

(D) Cross-neutralization of mice sera of 3 groups against WT, Beta, Delta, and Omicron variants at 28 days after the first dose of immunization. Statistical data analysis was performed using GraphPad Prism version 8.0 software. Data on dot-bar plots are shown as GMT \pm SEM with individual data points in plots. The values above points indicate the GMTs. The threshold of ID₅₀ detection was 1:40.

NAb titers against the Omicron variant in Group 2 (Figure 3B). Omicron-specific NAb titers against Omicron in Group 2 (GMT = 5525) are comparable with WT-induced immune response (GMT = 3688) to WT itself in Group 1. It suggests that the immunogenicity of Omicron does not change. In contrast, sera from only WT-immunized mice in Group 1 or only Omicron-immunized mice in Group 2 do not neutralize with each other, except for itself. However, it is noteworthy that Omicron-specific boosting following two-dose WT-RBD immunization can raise NAb titers against Omicron by 9-folds (Figure 3C). Subsequently, we tested the cross-neutralization ability of these mouse sera among three groups 28 days after the first-dose immunization (Figure 3D). In Group 1 and Group 3, both only WT-RBD immunization and the Omicron-RBD immunization as a booster after prime vaccination with two doses of WT-RBD showed the similar neutralizing capacity against WT, Beta, and Delta pseudoviruses (with GMTs of 3688 vs 3140 against WT, 994 vs 962 against Beta, 1557 vs 1712 against Delta, respectively). However, simplex 3-dose WT-RBD immunization has weak neutralization against Omicron, while Omicron-RBD as a heterologous booster followed two doses of WT-RBD immunization displayed a high neutralization potency for Omicron. In Group 2, mice administrated with 3 doses of Omicron-RBD displayed low neutralization against WT, Beta, and Delta (GMTs were 56, 270, and 262, respectively). As NAb titers are verified to be correlated with effective protection,²⁸ the data presented here suggest that Omicron-specific boosting will help to improve the immune protection from Omicron and that a heterologous booster with the Omicron strain after the prime vaccination with the WT strain retains the immune protection against other different SARS-CoV-2 variants (WT, Beta, and Delta). That is to say, in our mouse models, Omicron-specific recombinant subunit protein as a heterologous booster can enhance the breadth and potency of neutralization against SARS-CoV-2 variants.

DISCUSSION

Widespread immune escape of SARS-CoV-2 variants in COVID-19 convalescents and vaccinees has been reported extensively. Furthermore, the fast transmission of Omicron and a surge of Omicron-infected cases indicated the high risk of reinfection and post-vaccination infection.^{20,29} In another RNA virus infecting the respiratory tract, influenza studies have a plentiful of clinical experience about vaccine efficiency. Authors reported that individuals with prior viral infection can be elicited to enhanced antibody responses to inactivated influenza vaccine. It suggests that immunological memory induced by the previous infection is important to enhance protective antibody titers.³⁰ In the post-vaccination Delta infection after full vaccination, 31-fold higher neutralizing antibody titers against the SARS-CoV-2 Delta variant than vaccinees without infection were observed.³¹ To evaluate the effect elicited by Omicron on immune response based on prior vaccination, we compared the characteristics of humoral immune response elicited by post-vaccination Delta or Omicron infection and WT infection without any record of vaccination. In the early period of infection, anti-WT-RBD IgG-binding antibodies accumulated over time. In two post-vaccination infection cohorts, the initial levels of binding antibodies were higher than in the WT-infected group. It indicates that the immune memory elicited by the previous vaccination based on the ancestral WT strain rapidly produces more specific anti-WT-RBD antibodies. When IgG titers reached the peak, the comparable levels in these three groups may suggest the limited antibody recognition induced by Omicron or Delta infection.

The kinetics of NABs in these three cohorts was in accordance with the basic principles of SARS-CoV-2 antibody responses: Individuals' NAb titers in these three cohorts reached to the peak within 14–21 days.a.o/d.a.a. However, NAb levels declined gradually in the WT-infected and Omicron-infected cohorts, while in the Delta-infected cohort antibody levels remained stable or even rose after reaching to the peak. It may be due to the similar spike epitopes between SARS-CoV-2 WT virus and the Delta variant, but significant differences between WT and Omicron. Immune memory against WT elicited by the post-vaccination infection in the Delta-infected cohort led to more epitope recognition than in the Omicron-infected cohort.^{32,33}

In both the WT-infected cohort and the post-vaccination Delta-infected cohort, higher neutralization resistance of Beta and Omicron variants has been seen. It is largely due to the pivotal alteration of their shared mutations in RBD, which is consistent with previous reports.³⁴ Meanwhile, the completely different mutations of other areas of spike proteins between Beta and Omicron indicate the divergent mechanism of immune escape. Even so, compared to the previous infection with the ancestral strain WT, the neutralization ability against Beta, Delta, and Omicron from individuals infected with Delta or Omicron after breaking through the early immune protection generated by vaccines is significantly increased. Neutralization activity against WT and Alpha was still kept in two post-vaccination infections. It is worth noting that in our study cohorts, most of individuals were vaccinated with SARS-CoV-2-inactivated vaccines. It has been known that neutralizing antibodies elicited by SARS-CoV-2-inactivated vaccines persisted for 6 months after the second dose. Because the post-vaccination infection in our study happened 3.4–6.6 months (median 5.2 months) after the last dose of vaccine, it implied the low neutralization activity elicited by vaccines when infections happened. Nevertheless, recent studies also reported consistent results that vaccination based on mRNA or viral vector vaccines followed by post-vaccination Omicron infection improved cross-neutralization of VOCs,^{35,36} while the neutralizing capacity of the unvaccinated individuals, which is triggered by Omicron, does not cross-neutralize other variants. These results suggest that prior immunity induced by vaccines will be beneficial to overcome the high neutralization resistance of Omicron.³⁷

Considering extensive neutralization observed in post-vaccination Omicron infection, we sought to understand the respective contribution of prior vaccination and Omicron-specific immunogenicity to this to establish more efficient immune protection. Therefore, we used immunized animals with recombinant WT-RBD and Omicron-RBD proteins to exhibit the immune response elicited by Omicron-specific booster and heterologous antigens. At first, we determined anti-WT-RBD IgG-binding antibody levels based on these three different vaccination strategies. After finishing the two-dose primary series, a booster by either WT-RBD or Omicron-RBD showed a parallel dynamic change in antibody levels. These results were consistent with what we observed in the clinical data above. As expected according to previous studies,³⁸ three doses of Omicron-RBD immunization presented lower antibody levels over time, which suggested limited cross-activity of Omicron-specific antibody response to WT-RBD. With the appearance of Omicron, multiple COVID-19 tests based on molecular methods or antigens showed potential impacts due to SARS-CoV-2 genetic mutations. Even so, the recognition of Omicron-specific binding antibody to WT-RBD in our results indicated that serological test for Omicron based on the epitope of ancestral WT strain is still available.

Moreover, tests of blood antibodies targeting the specific epitope of the Omicron variant may be applicable for distinguish Omicron from other VOCs.³⁹

Compared to NAb titers against WT pseudovirus produced by 3 doses of WT-RBD, 3 doses of Omicron-RBD can induce comparable NAb titer against Omicron pseudovirus itself. It indicates that in our mouse models, Omicron-RBD has similar immunogenicity to WT-RBD. Furthermore, the Omicron-RBD booster following two doses of WT-RBD can induce 9-fold higher levels of NAb against Omicron pseudovirus than the WT-RBD booster, but two boosters induced comparable NAb titers against WT pseudovirus. It suggests that Omicron-specific immune response mainly increases its neutralization activity against this variant itself and retains the similar neutralization activity against WT, compared to the immune response elicited by WT. In other words, we showed that Omicron-RBD as a heterologous booster following primary series could expand the breadth and enhance the magnitude of immune protection against the SARS-CoV-2 WT strain and circulating variants, which is also consistent with what we observed above in post-vaccination Omicron infection. In the influenza virus, it also suggested that if the antigenicity of the virus strains used for vaccines is not well matched to the circulating virus strains, the vaccine effectiveness decreases sharply.⁴⁰

Through the mouse models, our study has seen that variant-specific boosters have the potential to be a kind of potent vaccine candidate to improve the efficiency of acquired immunity. However, by contrast, as another type of vaccine, Omicron-specific mRNA vaccines used as boosters showed various efficiencies. Omicron-mRNA booster 9 months post prime vaccination in macaques has not displayed significantly different NAb titers and B cells response,²⁷ while Omicron-mRNA vaccine as a heterologous booster at over 3.5 months post prime vaccination elicited 10- to 20-fold higher neutralizing titers than WT-mRNA booster in mouse models.⁴¹ This could be due to different immunization intervals and antigen epitopes. The time interval between vaccination and infection has been shown significant correlation with the potency of Omicron-neutralizing antibodies. In our animal models, there is only a 7-day interval between the primary series (two doses) and the booster. So, the correlation of protection efficiency and time interval of SARS-CoV-2 variant vaccines as boosters should be further estimated. On the other hand, of note, immunization by Omicron-RBD recombinant subunit proteins in our study shows the potential advantage that producing more NAb to specially target against Omicron variant, while mRNA vaccine targeting full-length spike protein may produce more irrelevant antibodies, instead of targeting Omicron RBD.⁴²

Our results indicate that heterologous antigens with various epitopes, which is different from single antigen as we have been vaccinated, may help to improve the magnitude and breadth of NAb activity.^{43,44} Except for the booster vaccination strategies, a “bivalent” lipid nanoparticle (LNP) mRNA vaccine containing both Omicron and Delta RBD-LNP in half dose has been observed cross-neutralization against WT and three SARS-CoV-2 variants.⁴⁵ Multivalent vaccines could be alternative for the future development of SARS-CoV-2 vaccines and vaccination programs.

Collectively, our data provide hints that the current booster vaccinations using WT-RBD protein or WT-S mRNA vaccine may be less efficient in preventing infections with the Omicron variant. Our results from the observational study of the clinical cohorts to the validation of mouse models support the hypothesis that an additional boost vaccination with recombinant Omicron-RBD subunit protein could increase the humoral immune response against both WT and current VOCs.

Limitations of the study

There are several limitations to our study. Due to limited participants with post-vaccination Omicron or Delta infection being included in our study, the correlation of clinical characteristics with antibody response cannot be analyzed. Unvaccinated individuals who were infected with Omicron had not been recruited, but Omicron-specific immune response was observed in mouse models. Intramuscular injection in mouse models is not completely equivalent to natural infection; hamster models could be used for virus challenge for further study.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- [KEY RESOURCES TABLE](#)
- [RESOURCE AVAILABILITY](#)

- Lead contact
- Materials availability
- Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
 - Patients and samples
 - Mouse models and study design
- **METHOD DETAILS**
 - ELISA
 - Production and titration of SARS-CoV-2 pseudoviruses
 - Pseudovirus neutralization assay
 - Ethics statement
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.105465>.

ACKNOWLEDGMENTS

We thank Prof. Ai-shun Jin (Chongqing Medical University, Chongqing, China) for the constructive suggestion about the design of mouse models. We acknowledge funding support from the National Natural Science Foundation of China [grant no. U20A20392], the 111 Project [grant no. D20028], the Emergency Project from the Science & Technology Commission of Chongqing [grant no. cstc2020jscx-fyzx0053]; National Natural Science Foundation of China [grant no. 82102361], China Postdoctoral Science Foundation [grant no. 2021M693924], Natural Science Foundation of Chongqing, China [grant no. cstc2021jcyj-bshX0115 and no. CSTB2022NSCQ-BHX0685], Chongqing Postdoctoral Science Special Foundation [grant no. 2010010005216630 and no. 2021XM3075] and the Research Fund Program of the Key Laboratory of Molecular Biology for Infectious Diseases, CQMU [grant no. 202105]; Emergency Key Program of Guangzhou Laboratory [grant no. EKPG21-29 and EKPG21-31], the Science and Technology Research Program of Chongqing Municipal Education Commission (Grant no. KJQN202200466) and Zhongnanshan Medical Foundation of Guangdong Province [grant no. ZNSA-2021004] for sample collection and processing. Funders had no role in study design, data collection, data analyses, interpretation, or writing of reports.

AUTHOR CONTRIBUTIONS

A.H., X.T., N.T., K.W., F.L., F.Y., and P.P. developed the conceptual ideas and designed the study. P.P., J.H., H.D., J.X., and C.H. performed the experiments, analysis, and interpretation of data. C.F. provided essential assistance through experiments. Q.F., G.T., and M.J. were responsible for sample collection. All authors provided scientific expertise and the interpretation of data for the work. P.P. drafted the manuscript. All authors contributed to the critical revision of the manuscript for important intellectual content. All authors reviewed and approved the final version of the report.

DECLARATION OF INTERESTS

The authors declare no conflicts of interest.

Received: May 4, 2022

Revised: August 9, 2022

Accepted: October 26, 2022

Published: November 18, 2022

REFERENCES

1. Viana, R., Moyo, S., Amoako, D.G., Tegally, H., Scheepers, C., Althaus, C.L., Anyaneji, U.J., Bester, P.A., Boni, M.F., Chand, M., et al. (2022). Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. *Nature* 603, 679–686.
2. Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T.S., Herrler, G., Wu, N.-H., Nitsche, A., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271–280.e8.
3. Walls, A.C., Park, Y.-J., Tortorici, M.A., Wall, A., McGuire, A.T., and Veeler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 183, 281–292.e286.
4. Shi, R., Shan, C., Duan, X., Chen, Z., Liu, P., Song, J., Song, T., Bi, X., Han, C., Wu, L., et al. (2020). A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. *Nature* 584, 120–124.

5. Tseng, H.F., Ackerson, B.K., Luo, Y., Sy, L.S., Talarico, C.A., Tian, Y., Bruxvoort, K.J., Tubert, J.E., Florea, A., Ku, J.H., et al. (2022). Effectiveness of mRNA-1273 against SARS-CoV-2 omicron and delta variants. *Nat. Med.* **28**, 1063–1071.
6. Dejnirattisai, W., Huo, J., Zhou, D., Zahradnik, J., Supasa, P., Liu, C., Duyvesteyn, H.M.E., Ginn, H.M., Mentzer, A.J., Tuekprakhon, A., et al. (2022). SARS-CoV-2 Omicron-B. 1.1. 529 leads to widespread escape from neutralizing antibody responses. *Cell* **185**, 467–484.e15.
7. Cui, Z., Liu, P., Wang, N., Wang, L., Fan, K., Zhu, Q., Wang, K., Chen, R., Feng, R., Jia, Z., et al. (2022). Structural and functional characterizations of infectivity and immune evasion of SARS-CoV-2 Omicron. *Cell* **185**, 860–871.e13.
8. Cele, S., Jackson, L., Khoury, D.S., Khan, K., Moyo-Gwete, T., Tegally, H., San, J.E., Cromer, D., Scheepers, C., Amoako, D.G., et al. (2022). Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. *Nature* **602**, 654–656.
9. Carreño, J.M., Alshammary, H., Tcheou, J., Singh, G., Raskin, A.J., Kawabata, H., Sominsky, L.A., Clark, J.J., Adelsberg, D.C., Bielak, D.A., et al. (2022). Activity of convalescent and vaccine serum against SARS-CoV-2 Omicron. *Nature* **602**, 682–688.
10. Zhao, X., Li, D., Ruan, W., Chen, Z., Zhang, R., Zheng, A., Qiao, S., Zheng, X., Zhao, Y., Dai, L., et al. (2022). Effects of a prolonged booster interval on neutralization of Omicron variant. *N. Engl. J. Med.* **386**, 894–896.
11. Planas, D., Saunders, N., Maes, P., Guivel-Benhassine, F., Planchais, C., Buchrieser, J., Bolland, W.-H., Porrot, F., Staropoli, I., Lemoine, F., et al. (2022). Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. *Nature* **602**, 671–675.
12. Jørgensen, S.B., Nygård, K., Kacelnik, O., and Telle, K. (2022). Secondary attack rates for Omicron and Delta variants of SARS-CoV-2 in Norwegian households. *JAMA* **327**, 1610–1611.
13. Altarawneh, H.N., Chemaitelly, H., Hasan, M.R., Ayoub, H.H., Qassim, S., AlMukdad, S., Coyle, P., Yassine, H.M., Al-Khatib, H.A., Benslimane, F.M., et al. (2022). Protection against the omicron variant from previous SARS-CoV-2 infection. *N. Engl. J. Med.* **386**, 1288–1290.
14. Andrews, N., Stowe, J., Kirsebom, F., Toffa, S., Rickeard, T., Gallagher, E., Gower, C., Kall, M., Groves, N., O’Connell, A.M., et al. (2022). Covid-19 vaccine effectiveness against the Omicron (B. 1.1. 529) variant. *N. Engl. J. Med.* **386**, 1532–1546.
15. Yu, J., Collier, A.-r.Y., Rowe, M., Mardas, F., Ventura, J.D., Wan, H., Miller, J., Powers, O., Chung, B., Siamatu, M., et al. (2022). Neutralization of the SARS-CoV-2 omicron BA. 1 and BA. 2 variants. *N. Engl. J. Med.* **386**, 1579–1580.
16. Lyngse, F.P., Kirkeby, C.T., Denwood, M., Christiansen, L.E., Mølbak, K., Møller, C.H., Skov, R.L., Krause, T.G., Rasmussen, M., and Sieber, R.N.J.M. (2022). Transmission of SARS-CoV-2 omicron VOC subvariants BA. 1 and BA. 2: evidence from Danish households. Preprint at medRxiv.
17. Iketani, S., Liu, L., Guo, Y., Liu, L., Chan, J.F.-W., Huang, Y., Wang, M., Luo, Y., Yu, J., Chu, H., et al. (2022). Antibody evasion properties of SARS-CoV-2 Omicron sublineages. *Nature* **604**, 553–556.
18. Nemet, I., Kliker, L., Lustig, Y., Zuckerman, N., Erster, O., Cohen, C., Kreiss, Y., Alroy-Preis, S., Regev-Yochay, G., Mendelson, E., and Mandelboim, M. (2022). Third BNT162b2 vaccination neutralization of SARS-CoV-2 Omicron infection. *N. Engl. J. Med.* **386**, 492–494.
19. Accorsj, E.K., Britton, A., Fleming-Dutra, K.E., Smith, Z.R., Shang, N., Derado, G., Miller, J., Schrag, S.J., and Verani, J.R. (2022). Association between 3 doses of mRNA COVID-19 vaccine and symptomatic infection caused by the SARS-CoV-2 Omicron and Delta variants. *JAMA* **327**, 639–651.
20. Kuhlmann, C., Mayer, C.K., Claassen, M., Maponga, T., Burgers, W.A., Keeton, R., Riou, C., Sutherland, A.D., Suliman, T., Shaw, M.L., and Preiser, W. (2022). Breakthrough infections with SARS-CoV-2 omicron despite mRNA vaccine booster dose. *Lancet* **399**, 625–626.
21. Regev-Yochay, G., Gonen, T., Gilboa, M., Mandelboim, M., Indenbaum, V., Amit, S., Meltzer, L., Asraf, K., Cohen, C., Fluss, R., et al. (2022). Efficacy of a fourth dose of Covid-19 mRNA vaccine against omicron. *N. Engl. J. Med.* **386**, 1377–1380. <https://doi.org/10.1056/NEJMc2202542>.
22. Zhang, J., He, Q., An, C., Mao, Q., Gao, F., Bian, L., Wu, X., Wang, Q., Liu, P., Song, L., et al. (2021). Boosting with heterologous vaccines effectively improves protective immune responses of the inactivated SARS-CoV-2 vaccine. *Emerg. Microbes Infect.* **10**, 1598–1608.
23. Atmar, R.L., Lyke, K.E., Deming, M.E., Jackson, L.A., Branche, A.R., El Sahly, H.M., Rostad, C.A., Martin, J.M., Johnston, C., Rupp, R.E., et al. (2022). Homologous and heterologous Covid-19 booster vaccinations. *N. Engl. J. Med.* **386**, 1046–1057.
24. Wang, Z., Zhao, Z., Cui, T., Huang, M., Liu, S., Su, X., Li, G., Song, T., Li, W., Zhong, N., et al. (2022). Heterologous boosting with third dose of coronavirus disease recombinant subunit vaccine increases neutralizing antibodies and T cell immunity against different severe acute respiratory syndrome coronavirus 2 variants. *Emerg. Microbes Infect.* **11**, 829–840. <https://doi.org/10.1080/22221751.2022.2048969>.
25. Ying, B., Scheaffer, S.M., Whitener, B., Liang, C.-Y., Dmytrenko, O., Mackin, S., Wu, K., Lee, D., Avena, L.E., Chong, Z., et al. (2022). Boosting with variant-matched or historical mRNA vaccines protects against Omicron infection in mice. *Cell* **185**, 1572–1587.e11.
26. Jia, L., Zhou, Y., Li, S., Zhang, Y., Yan, D., Wang, W., Zhang, W., Wan, Y., and Qiu, C. (2022). Omicron booster in ancestral strain vaccinated mice augments protective immunities against both delta and omicron variants. Preprint at bioRxiv **13**. <https://doi.org/10.3389/fimmu.2022.897879>.
27. Gagne, M., Molina, J.I., Foulds, K.E., Andrew, S.F., Flynn, B.J., Werner, A.P., Wagner, D.A., Teng, I.-T., Lin, B.C., Moore, C., et al. (2022). mRNA-1273 or mRNA-Omicron boost in vaccinated macaques elicits similar B cell expansion, neutralizing responses, and protection from Omicron. *Cell* **185**, 1556–1571.e18.
28. Feng, S., Phillips, D.J., White, T., Sayal, H., Aley, P.K., Bibi, S., Dold, C., Fuskova, M., Gilbert, S.C., Hirsch, I., et al. (2021). Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nat. Med.* **27**, 2032–2040.
29. Pulliam, J.R.C., van Schalkwyk, C., Govender, N., von Gottberg, A., Cohen, C., Groome, M.J., Dushoff, J., Mlisana, K., and Moultrie, H. (2022). Increased risk of SARS-CoV-2 reinfection associated with emergence of Omicron in South Africa. *Science* **376**, eabn4947.
30. Auladell, M., Phuong, H.V.M., Mai, L.T.Q., Tseng, Y.-Y., Carolan, L., Wilks, S., Thai, P.Q., Price, D., Duong, N.T., Hang, N.L.K., et al. (2022). Influenza virus infection history shapes antibody responses to influenza vaccination. *Nat. Med.* **28**, 363–372.
31. Collier, A.-r.Y., Brown, C.M., McMahan, K.A., Yu, J., Liu, J., Jacob-Dolan, C., Chandrashekar, A., Tierney, D., Ansel, J.L., Rowe, M., et al. (2022). Characterization of immune responses in fully vaccinated individuals after breakthrough infection with the SARS-CoV-2 delta variant. *Sci. Transl. Med.* **14**, eabn6150.
32. Kumar, S., Thambiraja, T.S., Karuppanan, K., and Subramaniam, G. (2022). Omicron and Delta variant of SARS-CoV-2: a comparative computational study of spike protein. *J. Med. Virol.* **94**, 1641–1649.
33. Socher, E., Heger, L., Paulsen, F., Zunke, F., and Arnold, P. (2022). Molecular dynamics simulations of the delta and omicron SARS-CoV-2 spike–ACE2 complexes reveal distinct changes between both variants. *Comput. Struct. Biotechnol. J.* **20**, 1168–1176.
34. Garcia-Beltran, W.F., Lam, E.C., St Denis, K., Nitido, A.D., Garcia, Z.H., Hauser, B.M., Feldman, J., Pavlovic, M.N., Gregory, D.J., Poznansky, M.C., et al. (2021). Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell* **184**, 2323–2383.e79.
35. Rössler, A., Knabl, L., von Laer, D., and Kimpel, J. (2022). Neutralization profile after recovery from SARS-CoV-2 omicron infection. *N. Engl. J. Med.* **386**, 1764–1766. <https://doi.org/10.1056/NEJMc2201607>.
36. Richardson, S.I., Madzorera, V.S., Spencer, H., Manamela, N.P., van der Mescht, M.A., Lambson, B.E., Oosthuisen, B., Ayres, F., Makhado, Z., Moyo-Gwete, T.J.C.h., and

- microbe. (2022). SARS-CoV-2 Omicron triggers cross-reactive neutralization and Fc effector functions in previously vaccinated, but not unvaccinated, individuals. *Cell Host Microbe*.
37. Miyamoto, S., Arashiro, T., Adachi, Y., Moriyama, S., Kinoshita, H., Kanno, T., Saito, S., Katano, H., Iida, S., Ainai, A., et al. (2022). Vaccination-infection interval determines cross-neutralization potency to SARS-CoV-2 Omicron after breakthrough infection by other variants. *Med* 3, 249–261.e4.
 38. Zhou, W., He, P., Li, J., Liu, H., Shi, M., Yu, J., and Wei, H. (2022). Steep decline in binding capability of SARS-CoV-2 omicron variant (B.1.1.529) RBD to the antibodies in early COVID-19 convalescent sera and inactivated vaccine sera. *Viruses* 14, 335.
 39. Fernandes, Q., Inchakalody, V.P., Merhi, M., Mestiri, S., Taib, N., Moustafa Abo El-Ella, D., Bedhiafi, T., Raza, A., Al-Zaidan, L., Mohsen, M.O., et al. (2022). Emerging COVID-19 variants and their impact on SARS-CoV-2 diagnosis, therapeutics and vaccines. *Ann. Med.* 54, 524–540.
 40. Krammer, F. (2019). The human antibody response to influenza A virus infection and vaccination. *Nat. Rev. Immunol.* 19, 383–397.
 41. Fang, Z., Peng, L., Filler, R., Suzuki, K., McNamara, A., Lin, Q., Renauer, P.A., Yang, L., Menasche, B., Sanchez, A., et al. (2022). Omicron-specific mRNA vaccination alone and as a heterologous booster against SARS-CoV-2. *Nat. Commun.* 13, 3250.
 42. Tai, W., Zhang, X., Drelich, A., Shi, J., Hsu, J.C., Luchsinger, L., Hillyer, C.D., Tseng, C.-T.K., Jiang, S., and Du, L. (2020). A novel receptor-binding domain (RBD)-based mRNA vaccine against SARS-CoV-2. *Cell Res.* 30, 932–935.
 43. He, C., Yang, J., He, X., Hong, W., Lei, H., Chen, Z., Shen, G., Yang, L., Li, J., Wang, Z., et al. (2021). A bivalent recombinant vaccine targeting the S1 protein induces neutralizing antibodies against both SARS-CoV-2 variants and wild-type of the virus. *MedComm* 2, 430–441.
 44. Wang, J., and therapy, t. (2021). A bivalent recombinant vaccine: a promising strategy against both SARS-CoV-2 variants and wild type of the virus. *Signal Transduct. Target. Ther.* 6, 278.
 45. Lee, I.-J., Sun, C.-P., Wu, P.-Y., Lan, Y.-H., Wang, I.-H., Liu, W.-C., Tseng, S.-C., Tsung, S.-I., Chou, Y.-C., and Kumari, M.J.B. (2022). Omicron-specific mRNA vaccine induced potent neutralizing antibody against Omicron but not other SARS-CoV-2 variants. Preprint at [BioRxiv](https://www.biorxiv.org/).
 46. Hu, J., Gao, Q., He, C., Huang, A., Tang, N., and Wang, K. (2020). Development of cell-based pseudovirus entry assay to identify potential viral entry inhibitors and neutralizing antibodies against SARS-CoV-2. *Genes Dis.* 7, 551–557. <https://doi.org/10.1016/j.gendis.2020.07.006>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Goat anti-Human IgG (H + L) Secondary Antibody	Thermo fisher	Cat# A18808; RRID:AB_2535585
Goat Anti-Mouse IgG H&L (HRP)	Abcam	Cat# ab6789; RRID:AB_955439
Goat Anti-Human IgG Fc (HRP)	Abcam	Cat# ab97225; RRID:AB_10680850
Bacterial and virus strains		
E.coli DH5 α	Thermo fisher	Cat# 18265017
Chemicals, peptides, and recombinant proteins		
WT-RBD protein	Okaybio	Cat# K1516
Omicron-RBD protein	Sino Biological	Cat# 40592-V08H121
TMB (3,3',5,5'-tetramethylbenzidine) substrate	Beyotime	Cat# P0209-500mL
Lipofectamine 3000	Invitrogen	Cat# L3000001
Lysis buffer	Promega	Cat# A8261
Luciferase Assay Reagent	Promega	Cat# E1500
QuickAntibody™-Mouse 3W adjuvant	Biodragon	Cat# KX0210042
Biological samples		
Human blood samples	Guangzhou Medical University	N/A
Critical commercial assays		
TRIZOL™ Plus RNA Purification Kit	Invitrogen	Cat# 12183555
SuperScript™ III Platinum™ SYBR™ Green	Thermo fisher	Cat# 11736051
Experimental models: Cell lines		
HEK293T	ATCC	CRL-3216
BALB/c mice	the Laboratory Animal Center of Chongqing Medical University	SCXK (YU) 2018-0003
Software and algorithms		
GraphPad Prism v8.0	GraphPad	https://www.graphpad.com/
R software	The R Foundation	https://www.r-project.org
Other		
Microplate reader	Biotek	N/A
GloMax® Discover Microplate Reader	Promega	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ailong Huang (ahuang@cqmu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Patients and samples

We enrolled 53 patients who had been identified to be previously infected with SARS-CoV-2 at the Eighth People's Hospital of Guangzhou from January 2020 to January 2022. Thereinto, 20 patients (aged 30 to 58 years) infected with the SARS-CoV-2 Omicron virus after vaccination with the target antigen derived from the SARS-CoV-2 WT strain were included in our study. Among them, all individuals received SARS-CoV-2 vaccines with at least 2 doses. The post-vaccination infection happened 3.4–6.6 months (median 5.2 months) after the last dose of vaccine. According to age, sex, the time of sample collection after symptom onset/admission, and the interval between the last dose of vaccination and symptom onset/admission, 13 patients infected with the SARS-CoV-2 WT virus strain and 20 individuals infected with the SARS-CoV-2 Delta virus after vaccination were matched. All infections were confirmed by RT-qPCR and sequenced to identify the genotype. All participants did not report by themselves or were not recorded any known or suspected exposure to other SARS-CoV-2 strains. The collection of all samples obtained consent from subjects according to the protocols approved by the Ethics Review Board of the Eighth People's Hospital of Guangzhou Institutional Review Board. Blood samples were collected at four different time points: the time point 1 is 2 days (interquartile range, IQR: 1–5 days after symptom onset) (d.a.o)/admission (d.a.a); the time point 2 is 9 days.a.o/d.a.a (IQR:7–13 days.a.o/d.a.a); the time point 3 is 18 days.a.o/d.a.a (16–23 days.a.o/d.a.a); the time point 4 is 30 days.a.o/d.a.a (IQR: 26–36 days.a.o/d.a.a). Peripheral blood was isolated for sera within 2 h after collection according to the following steps: (1) Peripheral blood samples were heat incubated for inactivation at 56°C in a water bath for 30 min; (2) Centrifuge at 3000 rpm for 15 min, followed by transferring the supernatant to new tubes; (3) Store at –80°C for further use.

Mouse models and study design

Eight-week-old female BALB/c mice (6 mice per group) were provided by the Laboratory Animal Center of Chongqing Medical University (SCXK (YU) 2018–0003). Recombinant SARS-CoV-2 wild-type RBD (WT-RBD) protein (Cat: K1516, Okaybio, China) and SARS-CoV-2 Omicron RBD (Omicron-RBD) protein (Cat: 40592-V08H121, Sinobiological, China) as antigens for immunization were diluted with PBS, then mixed with an equal volume of QuickAntibody™-Mouse 3W adjuvant and completely emulsified by syringes. Each mouse was intramuscularly injected with a total of 3 doses of 100µL antigen/adjuvant mixture with a 14-day interval and a 7-day interval, respectively. Serum samples were collected from tail tips before each vaccination and 28 days after the first injection. We measured the antibody titers by Enzyme-linked immunosorbent assay (ELISA) and pseudovirus neutralization assay.

To compare the effects of different booster strategies on the immune response to SARS-CoV-2, four groups were distributed: Group 1 was immunized with three doses of the recombinant WT-RBD protein; Group 2 was immunized with three doses of the recombinant Omicron-RBD protein; Group 3 was immunized with two doses of recombinant WT-RBD protein and boosted with one dose of recombinant Omicron-RBD protein. Another group was immunized by the adjuvant with the same doses as the control (data were not shown).

METHOD DETAILS

ELISA

The recombinant RBD protein derived from WT were coated on the 96-well microtiter plates (100ng/well) at 4°C overnight. After blocking with 5% skim milk and 2% BSA in PBST for 2 h at room temperature, the sera of enrolled patients were diluted and added to these plates, then incubated at 37°C for 1 h. After washing, wells were incubated with goat anti-mouse/human IgG-Horseradish peroxidase (HRP) antibody (1:10000 dilution) for 1 h at 37°C. After washing, TMB (3,3',5,5'-tetramethylbenzidine) substrate was added to each well. Then, plates were incubated for 15 min at 37°C for color development. Reactions were stopped with stop solution, and the absorbance was determined at 450 nm using a microplate reader (Biotek, USA).

Production and titration of SARS-CoV-2 pseudoviruses

The preparation of SARS-CoV-2 spike-protein pseudovirus was carried out as previously described,⁴⁶ with some modifications. In brief, plasmids of pNL4-3.luc.R-E– and recombinant SARS-CoV-2 spike (D614G) or its variants (Alpha, Beta, Delta, and Omicron) were co-transfected into HEK293T cells using Lipofectamine 3000. After transfection for 48 h, supernatants containing pseudotyped viruses were harvested, centrifuged, filtered through 0.45 µm filters (Millipore, USA), and stored at –80°C. These pseudoviruses were

estimated using RT-qPCR by determining the number of viral RNA genomes per mL of viral stock solution, with primers targeted to the LTR (Geraerts M, et al., BMC Biotechnol. 2006). Briefly, viral RNAs were extracted using TRIzol (Invitrogen, Rockville, MD, USA), and then, the RNAs were amplified using the TaqMan One-Step RT-PCR Master Mix Reagents. A known quantity of pNL4-3. Luc. R-E- vector was used to generate standard curves. The harvested pseudoviruses were adjusted to the same titers (copies/mL) for the following experiment.

Pseudovirus neutralization assay

SARS-CoV-2 pseudoviruses (Alpha, Beta, Delta, Omicron or D614G), equivalent to 3.8×10^4 vector genomes, were incubated respectively with the same amount of serum samples (with serial dilutions of 1:40, 160, 640, 2560) from patients or mice for 1 h at 37°C. Then, the mixture was added to the 96-well plates seeded with 293T-hACE2 cells. After the 8-h infection, cells were supplemented with fresh medium. Then, they were lysed by 30 μ L lysis buffer (Promega, Madison, WI, USA) at 72 h post-infection to measure relative light unit (RLU) with luciferase assay reagent (Promega, Madison, WI, USA) according to the product instruction. Neutralization inhibition rate was calculated using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). The titers of neutralizing antibodies were calculated as 50% inhibitory dose (ID50).

Ethics statement

The observation study of clinical cohorts was approved by the Ethics Committees of Guangzhou Eighth People's Hospital, Guangzhou Medical University (Approval number: 202202214). Animal studies were approved and conducted in compliance with the Committee on the Ethics of Animal Experiments of the Institutional Animal Care and Use Committee at the Laboratory Animal Center of Chongqing Medical University.

QUANTIFICATION AND STATISTICAL ANALYSIS

Neutralization inhibition rate was calculated using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). One-way ANOVA was used to estimate the differences in neutralization antibody levels at the three different clinical cohorts. p-value less than 0.05 was considered statistically significant, and p values less than 0.001, 0.01 and 0.05 were marked as ***, ** and * respectively. The fitted curves were drawn by using the locally weighted regression (LOESS) method. Statistical analysis was performed using R software, version 3.6.0.