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

Running title:

Neutralization elicited by Omicron-specific subunit vaccine booster

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Abstract

With the development of COVID-19, even though increased global vaccination coverage, a super variant of SARS-CoV-2, Omicron, carrying a great number of mutations, has been verified its strong capacity of immune escape. An increased risk of SARS-CoV-2 reinfection or breakthrough infection should be concerned. We analyzed the humoral immune response of Omicron breakthrough infection and found its cross-neutralization against VOCs. We established mouse models to verify whether Omicron-specific RBD subunit boost immune response by immunizing Omicron-RBD recombinant proteins. The results suggest that an additional boost vaccination with Omicron-RBD protein could increase humoral immune response against both WT and current VOCs.

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 dominate strain, the pandemic of Delta has lasted over one year. However, Omicron (B.1.1.529) caused a new round of pandemic since November 2021 due to its rapid spread with a great deal of mutations. More than 30 mutations have been accumulated in the spike (S) protein of Omicron variant, especially 15 of those occurs on 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 response and therapeutics antibodies. It has been proved to be correlated to its infectivity alteration and immune evasion[1-3]. The neutralizing activity of Omicron by sera from not only convalescents but also vaccinees who received two doses of Oxford/AstraZeneca (AZD1222) or the Pfizer BioNtech (BNT16b2) vaccines has been suggested a widespread reduction[3]. It shows that Omicron has an increased risk of SARS-CoV-2 reinfection or breakthrough infection. After breaking through previous immune protection by vaccine based on the ancestral wild-type (WT) virus, how the immune

response elicited by Omicron breakthrough infection is needs to be delineated. It will give hints for further vaccine and vaccination strategies.

Reduced efficiency of existing vaccines accelerates the need of new vaccine strategies. Boosting with heterologous vaccines as one of the candidates, it has been proved to be safe and improve immune response[4,5]. At present, some vaccination program with heterologous vaccines have been approved in some countries. With immune escape of Omicron variant, Omicron-specific vaccines has been proposed. Omicron-specific vaccine could induce neutralizing response to Omicron itself but fail to previous VOCs[6]. As a booster with Omicron-matched DNA vaccines, increased width of immune response has been observed[7]. However, in macaque models, vaccination with Omicron-specific boosters do not increase neutralizing antibody (NAb) titers against Omicron and remain the equivalent levels of B cell response[8]. In consideration of the key role of SARS-CoV-2 RBD, it has a significance to verify whether Omicron-specific RBD subunit boost immune response after previous WT-RBD doses.

Results

In this study, twenty persons who were infected with Omicron or Delta after vaccination in each cohort were recruited, while 13 individuals previously infected with WT and unvaccinated were matched as control cohort according

to ages, sex and the time of sample collection of other two groups. Sera samples were collected at 3-4 time points within 50 days post symptom onset and their neutralizing antibodies were determined by the pseudotype-based neutralizing assay. During the follow-up visit, the range of NAb geometric mean titers (GMTs) in the WT cohort is 779-1152 against WT, 356-457 against Delta, 56-91 against Omicron; the range of NAb GMTs in the Delta cohort is 1191-1519 against WT, 1566-3251 against Delta, 282-479 against Omicron within four time points; the range of NAb GMT in the Omicron cohort is 151-2123 against WT, 360-1516 against Delta, 144-1834 against Omicron within four time points. Compared to the WT cohort, NAb titers of Delta and Omicron cohorts presents extremely high levels against WT, Delta and Omicron (Figure 1A-C). Furthermore, we observed the cross-neutralization ability of breakthrough infection. In the WT cohort, NAb titers of sera against four VOCs (Alpha, Beta, Delta and Omicron) are decreased by 1.5 folds, 5.7 folds, 2.2 folds and 15.6 folds, respectively (Figure 1D). In the Delta cohort, except for high NAb titers against Delta itself, NAb titers of sera against WT, Alpha, Beta and Omicron are decreased by 1.5 folds, 1.4 folds, 5.1 folds and 6.8 folds, respectively (Figure 1E). In the Omicron cohort, except for high NAb titers against Omicron itself, NAb titers of sera against WT, Alpha, Beta and Delta are decreased by 0.8 folds, 0.8 folds, 1.8 folds and 1.1 folds, respectively (Figure 1F). Taken together, compared with WT infection, breakthrough

infection, especially by Omicron could induce wide range of the humoral immune response to Omicron and other VOCs.

Higher and more extensive neutralizing activity of Omicron or Delta breakthrough infection has been seen on the basis of the exposure of two antigens. Then, we used mouse models to evaluate whether previously ancestral vaccination or Omicron-specific immune response has the major contribution to the increased protection. BALB/c mice were distributed into 4 groups and immunized by two doses of SARS-CoV-2 RBD recombinant proteins subunit with 2-week interval plus one booster one week after the second dose (Figure 2A): Group1 immunized with 3 doses of WT-based RBD recombinant proteins (WT-RBD), Group2 immunized with 3 doses of Omicron-RBD proteins, Group3 immunized with 2 doses of WT-RBD plus one dose of Omicron-RBD and Group4 immunized by the adjuvant (Cat# KX0210042, Biodragon, China) as the control.

Mice sera were collected and performed the pseudotype-based neutralizing assay to determine their neutralizing effect on different VOCs at 14 days and 28 days after the first dose of immunization. Two-week interval with boosting by the third dose lead to 26-fold increase (from 138 to 3688) of NAbs against WT in Group1, while 30-fold increase (from 183 to 5525) of NAb titers against Omicron variant in Group2(Figure 2B-C). Omicron-specific humoral response

could produce comparable level of NAbs against Omicron itself with WT-induced immune response to WT itself. In contrast, sera from only WT-immunized mice in Group1 or only Omicron-immunized mice in Group2 do not neutralize other VOCs, except for itself (Figure 2D). However, it is noteworthy that Omicron-specific boosting after two-dose WT-RBD immunization can raise NAbs against Omicron by 9 folds. Subsequently, we tested cross-neutralization of these mice among three groups (Figure 2E). In Group1 only WT-RBD immunization showed high neutralizing capacity against WT itself and Delta, but weak neutralizing capacity against Omicron and Beta variants (3.7- and 2.4-fold decline, respectively). Likewise, in Group2, mice administrated with 3-dose of Omicron-RBD generated extremely high NAbs against Omicron variant itself but fail to neutralize other tested VOCs. Interestingly, in Group3, the Omicron-specific shot boost NAb titers not only against Omicron, but also against WT, Beta and Delta variants with GMT 921, 3140, 962 and 1712, respectively. As NAb titers are found to be correlates of protection[9], the data presented here suggest that Omicron-specific boosting will help to improve the immune protection from Omicron and that immunization by heterologous antigens will be beneficial to obtain wider protection against different SARS-CoV-2 variants.

Discussion

When the new round of COVID-19 wave caused by Omicron variant, global average coverage of SARS-CoV-2 vaccines is estimated at 17%, while the vaccination coverage of some countries reaches to 78%[10]. Even so, the fast transmission of Omicron and a surge of Omicron infected cases indicated the high risk of reinfection and breakthrough infection[11,12]. At first, we analyzed the characteristics of humoral immune response elicited by Omicron variant under the background of the breakthrough infection. Compared to the previous infection with the ancestral strain WT, NAb titers against several variants from individuals infected with Delta or Omicron after breaking through the early immune protection generated by vaccines is significantly higher and wider. Recent research reported the consistent result that vaccination followed by breakthrough Omicron infection improved cross-neutralization of VOCs[12,13], while neutralizing capacity of the unvaccinated individuals, which is triggered by Omicron, do not cross-neutralize other variants.

To establish more efficient immune protection from Omicron by vaccine strategies, heterologous antigens with various epitopes, which is different from single antigen as we have been vaccinated, may contribute to increase the height and width of NAb activity. 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[14]. Therefore, we used immunized animal with WT-RBD and Omicron-RBD recombinant proteins to exhibit immune response elicited by Omicron-specific

booster and heterologous antigens. Compared to NAb titers against WT pseudovirus produced by 3-dose of WT-RBD, Omicron-RBD alone can induce comparable NAbs against Omicron pseudovirus itself. It indicates that in mice models, Omicron-RBD has the similar immunogenicity with WT-RBD. Furthermore, Omicron-RBD booster following two-dose of WT-RBD can induce 9-fold higher levels of NAbs against Omicron pseudovirus than three-dose WT-RBD. However, Omicron-mRNA boost in vaccinated macaques has not displayed significantly different NAb titers and B cells response[8]. This could be due to different immunization intervals and antigen epitopes. In our animal models, there is only 7-day interval between primary series (two doses) and booster. Moreover, Omicron-RBD could produce more NAbs to specially target against Omicron variant, while mRNA vaccine targeting full-length spike protein may produce more irrelevant antibodies, instead of targeting Omicron RBD. We show Omicron-RBD boost following primary series could produce wider protection against the SARS-CoV-2 WT strain and circulating variants, which is consistent with what we observed in Omicron breakthrough infection.

Collectively, our data provides 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 support the hypothesis that an additional boost vaccination with Omicron-RBD protein could increase humoral immune response against both WT and current VOCs.

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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. and C.H. performed the experiments and statistical analysis. C.F. provided the 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 critical revision of the manuscript for important intellectual content. All authors reviewed and approved the final version of the report.

Conflict of Interest: The authors declare no conflicts of interest.

Materials and Methods

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. Thirteen patients infected with the SARS-COV-2 wild-type virus strain, 20 individuals infected with the SARS-COV-2 Delta virus after vaccination, and 20 patients infected with the SARS-COV-2 omicron virus after vaccination were included in our study. All infection were confirmed by using q-PCR and sequence to identify the genotype. The collection of all samples obtained the consent from subjects according to the protocols approved by the Ethics Review Board of the Eighth People's Hospital of Guangzhou Institutional Review Board. plasma was isolated from blood samples within 2h after collection according to the following steps: (1) Patient sera were heat incubated for inactivation at 56 ℃ in water bath for 30 min.; (2) centrifugation at 3000 rpm for 15 min, followed by transferring to new tubes; (3) Store at -80℃ for further use.

Mouse models and study design

8-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 WT-RBD and Omicron-RBD proteins as antigens for immunization were diluted with PBS, then mixed with an equal volume of QuickAntibody™-Mouse 3W adjuvant (Cat# KX0210042, Biodragon, China) and completely emulsified by a syringe. Each mouse was intramuscularly

injected with 100 μl the antigen/adjuvant mixture. Serum samples were collected from tail tips before each vaccination and at 28 days after first injection, then measure the antibody titers by ELISA and pseudovirus neutralization assay.

Pseudovirus neutralization assay

For the neutralization assay, 50μL pseudoviruses of SARS-CoV-2 (Alpha, Beta, Delta, Omincron, D614G), equivalent to 3.8×10^4 vector genomes, were incubated with serial dilutions of sera samples (dilutions of 1:40, 160, 640, 2560) from patients or mice for 1h at 37℃, then added the mixture into the 96-well plates seeded with 293T-ACE2 cells(1.6×10^4 cells/well). The cells were refreshed with DMEM medium 8 h post-infection. Cells were lysed by 30 μl lysis buffer (Promega, Madison, WI, USA) at 72 h post-infection to measure RLU with luciferase assay reagent (Promega, Madison, WI, USA) according to the product instruction. Neutralization inhibition rate was calculated using GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA, USA). The titers of neutralizing antibodies were calculated as 50% inhibitory dose (ID50).

Ethics statement

Animal studies were approved by 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.

FIGURE LEGEND

Fig.1 The effect of the Omicron variant on viral infectivity and immune escape. **A-C** Pseudovirus-based neutralizing assays were performed to test neutralizing activity of the sera against WT, Delta and Omicron variants from Omicron (a) breakthrough infection and Delta (b) breakthrough infection (n=20 for each group, sampled at 4 time points within around 50 days after symptom onset). Sera from 13 individuals infected with the ancestral strain WT (c) were matched as controls (sampled at 3 time points within 50 days after symptom onset). **D-F** Cross-neutralizing titers of individuals experienced Omicron (d) and Delta (e) breakthrough infection, and WT infection(f) against WT, Alpha, Beta, Delta and Omicron. Statistical data analysis was performed using GraphPad Prism version 8.0 software. The half-maximal inhibitory dose (ID_{50}) was calculated as NAb titers. The threshold of ID_{50} detection was 1:40.

Fig. 2 Humoral immune response elicited by Omicron-specific booster. A Schematic of BALB/c mice immunization programs. 6 mice for each group were intramuscularly injected with the indicated recombinant proteins or the adjuvant as control. **B-C** Sera were collected and performed the pseudotype-based neutralizing assay to determine their neutralizing capacity to WT or Omicron at 14 days (B) and 28 days (C) after the first dose of immunization. **D** NAbs titers of sera from 3 groups collected at 28 days after the first dose of immunization. **E** 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 is shown as geometric mean titers (GMT) ± SEM with individual data points in plots.

Figure 1

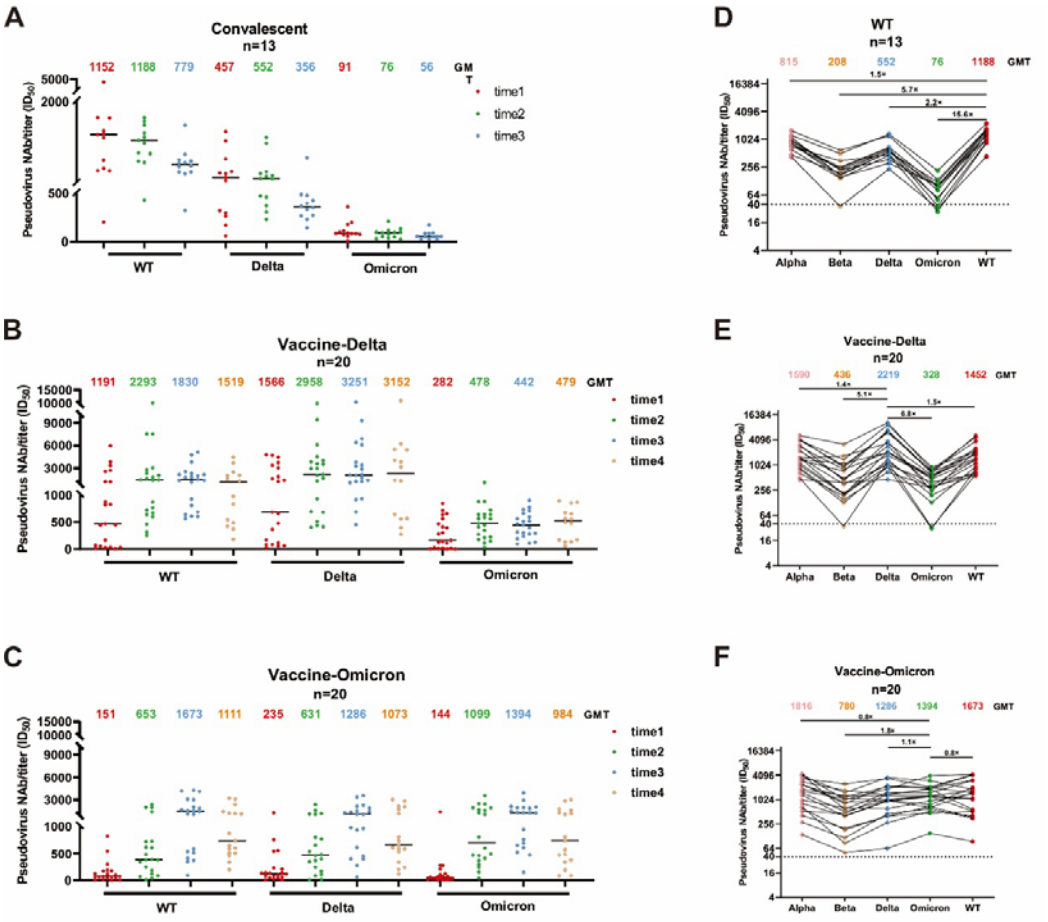
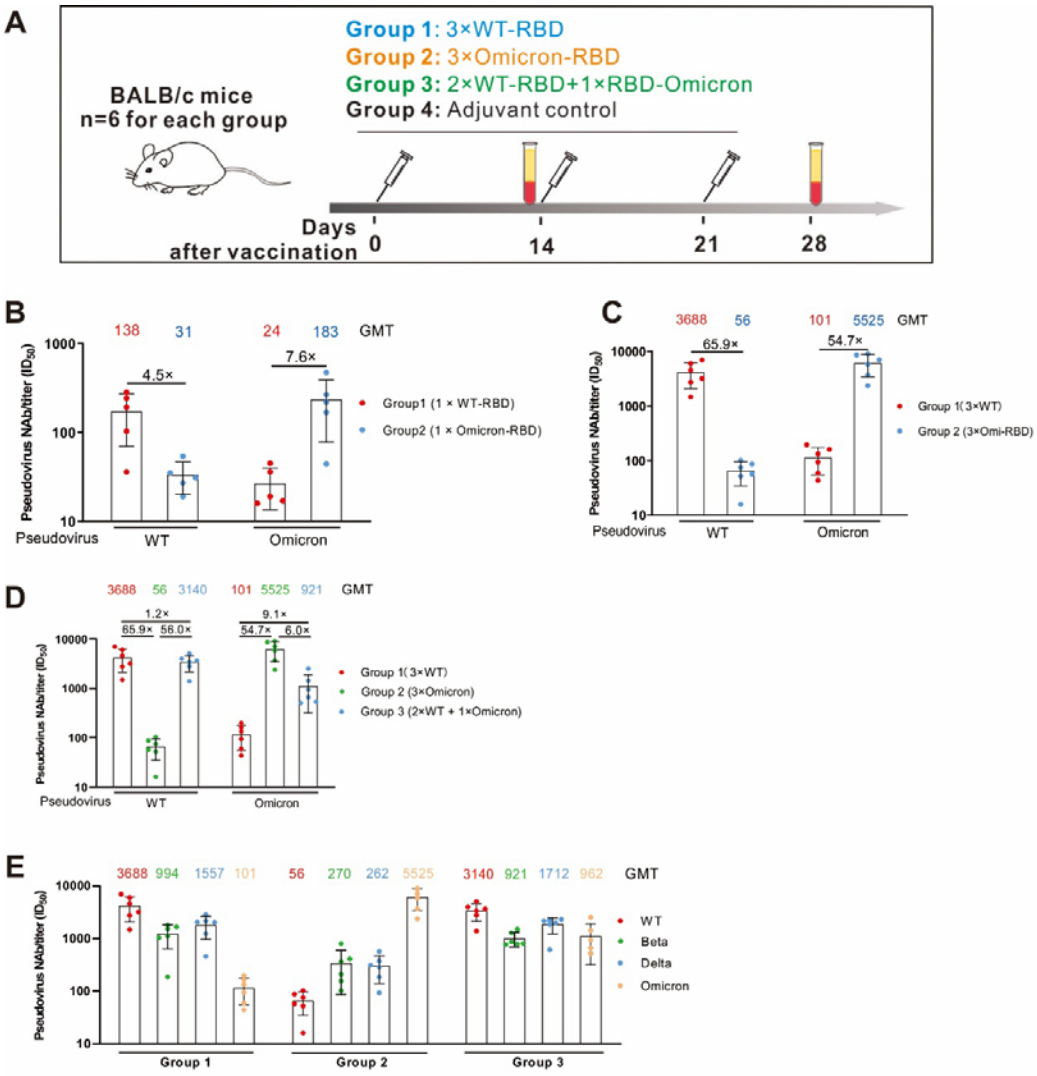

A
Convalescent
n=13
1152 1188 779 457 552 356 91 76 56 GMT
Pseudovirus NAb/titer (ID50)
5000
2000
500
0
time1
time2
time3
WT
Delta
Omicron
B
Vaccine-Delta
n=20
1191 2293 1830 1519 1566 2958 3251 3152 282 478 442 479 GMT
Pseudovirus NAb/titer (ID50)
15000
10000
9000
6000
3000
1000
500
0
time1
time2
time3
time4
WT
Delta
Omicron
C
Vaccine-Omicron
n=20
151 653 1673 1111 235 631 1286 1073 144 1099 1394 984 GMT
Pseudovirus NAb/titer (ID50)
15000
10000
9000
6000
3000
1000
500
0
time1
time2
time3
time4
WT
Delta
Omicron
D
WT
n=13
815 208 552 76 1188 GMT
1.5×
5.7×
2.2×
15.6×
Pseudovirus NAb/titer (ID50)
16384
4096
1024
256
64
40
16
4
Alpha
Beta
Delta
Omicron
WT
E
Vaccine-Delta
n=20
1590 436 2219 328 1452 GMT
1.4×
5.1×
6.8×
1.5×
Pseudovirus NAb/titer (ID50)
16384
4096
1024
256
64
40
16
4
Alpha
Beta
Delta
Omicron
WT
F
Vaccine-Omicron
n=20
1816 780 1286 1394 1673 GMT
0.8×
1.8×
1.1×
0.8×
Pseudovirus NAb/titer (ID50)
16384
4096
1024
256
64
40
16
4
Alpha
Beta
Delta
Omicron
WT

Figure 2


A
Group 1: 3×WT-RBD
Group 2: 3×Omicron-RBD
Group 3: 2×WT-RBD+1×RBD-Omicron
Group 4: Adjuvant control
BALB/c mice
n=6 for each group
Days
after vaccination
0
14
21
28
B
138 31 24 183 GMT
4.5×
7.6×
Pseudovirus NAb titer (ID50)
Group1 (1 × WT-RBD)
Group2 (1 × Omicron-RBD)
Pseudovirus
WT
Omicron
C
3688 56 101 5525 GMT
65.9×
54.7×
Group 1(3×WT)
Group 2 (3×Omi-RBD)
D
3688 56 3140 101 5525 921 GMT
1.2×
9.1×
65.9× 56.0×
54.7× 6.0×
Group 1(3×WT)
Group 2 (3×Omicron)
Group 3 (2×WT + 1×Omicron)
E
3688 994 1557 101 56 270 262 5525 3140 921 1712 962 GMT
WT
Beta
Delta
Omicron
Group 1
Group 2
Group 3

Reference

1. Tseng, H.F., *et al.* Effectiveness of mRNA-1273 against SARS-CoV-2 Omicron and Delta variants. *Nat. Med.* (2022).
2. Dejnirattisai, W., *et al.* SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. *Cell* **185**(2022).
3. Cui, Z., *et al.* Structural and functional characterizations of infectivity and immune evasion of SARS-CoV-2 Omicron. *Cell* **185**, 860-871.e813 (2022).
4. Zhang, J., *et al.* Boosting with heterologous vaccines effectively improves protective immune responses of the inactivated SARS-CoV-2 vaccine. *Emerging microbes & infections* **10**, 1598-1608 (2021).
5. Atmar, R.L., *et al.* Homologous and Heterologous Covid-19 Booster Vaccinations. *The New England journal of medicine* (2022).
6. Ying, B., *et al.* Boosting with Omicron-matched or historical mRNA vaccines increases neutralizing antibody responses and protection against B.1.1.529 infection in mice. *bioRxiv*, 2022.2002.2007.479419 (2022).
7. Jia, L., *et al.* Omicron booster in ancestral strain vaccinated mice augments protective immunities against both the Delta and Omicron variants. *bioRxiv*, 2022.2002.2019.481110 (2022).
8. Gagne, M., *et al.* mRNA-1273 or mRNA-Omicron boost in vaccinated macaques elicits comparable B cell expansion, neutralizing antibodies and protection against Omicron. *bioRxiv*, 2022.2002.2003.479037 (2022).

9. Feng, S., *et al.* Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nat Med* **27,** 2032–2040 (2021).

10. McIntyre, P.B., *et al.* COVID-19 vaccine strategies must focus on severe disease and global equity. *Lancet (London, England)* **399**, 406-410 (2022).

11. Pulliam, J.R.C., *et al.* Increased risk of SARS-CoV-2 reinfection associated with emergence of the Omicron variant in South Africa. *medRxiv*, 2021.2011.2011.21266068 (2021).

12. Kuhlmann, C., *et al.* Breakthrough infections with SARS-CoV-2 omicron despite mRNA vaccine booster dose. *Lancet (London, England)* **399**, 625-626 (2022).

13. Rössler, A., Knabl, L., Laer, D.v. & Kimpel, J. Neutralization profile of Omicron variant convalescent individuals. *medRxiv*, 2022.2002.2001.22270263 (2022).

14. Richardson, S., *et al.* SARS-CoV-2 Omicron triggers cross-reactive neutralization and Fc effector functions in previously vaccinated, but not unvaccinated individuals. (medRxiv, 2022).

15. Lee, I.-J., *et al.* Omicron-specific mRNA vaccine induced potent neutralizing antibody against Omicron but not other SARS-CoV-2 variants. *bioRxiv*, 2022.2001.2031.478406 (2022).