Heterologous mRNA vaccine boosters induce a stronger and longer-lasting antibody response against Omicron XBB variant

Matthew Zirui Tay,[a,r] Yun Shan Goh,[a,r] Siew-Wai Fong,[a,r] Zi Wei Chang,[a] Angeline Rouers,[a] Nathan Wong,[a] Anthony Torres-Ruesta,[a] Yuling Huang,[a] Sooriya Kannan Selvam,[a] Pei Xiang Hor,[a] Chiew Yee Loh,[a] Bei Wang,[b] Siti Nazihah Mohd Salleh,[b] Eve Zi Xian Ngoh,[b] Raphael Tze Chuen Lee,[c,d] Vanessa Neo,[a] Isaac Kai Jie Kam,[a] Xuan Ying Poh,[e] Suma Rao,[e,f] Po Ying Chia,[e,f,g] Sean W. X. Ong,[e,f] Tau Hong Lee,[e,f] Clarissa Lim,[e] Jefanie Teo,[e] NCID Study Group[e,q], PRIBIVAC Cohort Study Group[a,q], Sebastian Maurer-Stroh,[a,c,d,h,i,j] Cheng-I Wang,[b] Yee-Sin Leo,[e,f,g,k,l] Raymond Tzer Pin Lin,[e,h,m] David C. Lye,[e,f,g,l] Barnaby Edward Young,[e,f,g] Lisa F. P. Ng,[a,n,o,*] and Laurent Renia[a,g,p,**]

[a]A*STAR Infectious Diseases Labs (A*STAR ID Labs), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore
[b]Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore
[c]Bioinformatics Institute (BII), Agency of Science, Technology and Research (A*STAR), Singapore, Singapore
[d]GISAID Global Data Science Initiative (GISAID), Munich, Germany
[e]National Centre for Infectious Diseases, Singapore, Singapore
[f]Department of Infectious Diseases, Tan Tock Seng Hospital, Singapore, Singapore
[g]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore
[h]National Public Health Laboratory, Singapore, Singapore
[i]Department of Biological Sciences, National University of Singapore, Singapore, Singapore
[j]Human Potential Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
[k]Saw Swee Hock School of Public Health, National University of Singapore, Singapore, Singapore
[l]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
[m]Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
[n]National Institute of Health Research, Health Protection Research Unit in Emerging and Zoonotic Infections, University of Liverpool, Liverpool, UK
[o]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, UK
[p]School of Biological Sciences, Nanyang Technological University, Singapore, Singapore

The Lancet Regional Health - Western Pacific 2023;33: 100732

Published Online 7 March 2023
https://doi.org/10.1016/j.lanwpc.2023.100732

The SARS-CoV-2 Omicron recombinant XBB subvariant was first detected in September 2022, and rapidly spread across South-East Asia, notably overtaking BA.5 to become the dominant variant in Singapore. As of December 2022, the prevalence of XBB is also increasing in the United States and Europe.[1] XBB was likely formed from a recombination between strains from the BA.2.10 and BA.2.75 lineages, with the recombination breakpoint at position 446–460.[2] XBB contains the R346T and N460K mutations which are shared with several other contemporary strains such as BQ1.1, BA2.3.20, and BA2.75. In addition, XBB carries several other sets of mutations, such as V83A, H146Q, Q183E, V445P, and F490S. Other subvariants derived from XBB harboring mutations such as H146del and D253G have now arisen as well and are rapidly spreading across the globe.

Here, we characterized the extent of evasion of XBB to vaccine-induced antibodies. To this end, we profiled a cohort of fifty-nine individuals who had taken three doses of mRNA vaccines and had no history of prior infection (Supplementary Table S1). Utilizing two complementary assays, a cell-based surface Spike-binding flow cytometric assay (SFB)[3] and a pseudovirus neutralization assay,[4] we assessed the levels of antibodies against XBB in comparison with wild type and Omicron BA.1 strains at 28 days and 180 days post-third dose.

We found that XBB displayed higher antibody evasion than Omicron BA.1. By SFB, levels of antibodies binding to the surface expressed XBB Spike at 28 days post-third dose were lower (median 35.1% binding) relative to BA.1 (median 44.8% binding) and wild type (median 50.8% binding) (Fig. 1A). The lower variant Spike binding efficacy, compared to wild type, is unlikely due to lower Spike expression as we have found similar ACE-2 binding efficacy with the variants, compared to WT (Supplemental Fig. S1). These levels also further waned by 180 days post-third dose to a median of 6.3% binding, lower than BA.1 (median 29.6% binding) and wild type (median 42.5% binding). Similarly, the mean pseudovirus neutralization IC50 for

*Corresponding author. Lisa F.P. Ng, A*STAR ID Labs, A*STAR, 8A Biomedical Grove, Immunos #05-13, Singapore 138648, Singapore.
**Corresponding author. Laurent Renia, A*STAR ID Labs, A*STAR, 8A Biomedical Grove, Immunos #05-13, Singapore 138648, Singapore.
E-mail addresses: lisa_ng@idlabs.a-star.edu.sg (L.F.P. Ng), renia_laurent@idlabs.a-star.edu.sg (L. Renia).
© 2023 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
[q]A list of authors appears in the Supplementary Appendix.
[r]These authors contributed equally to this work.

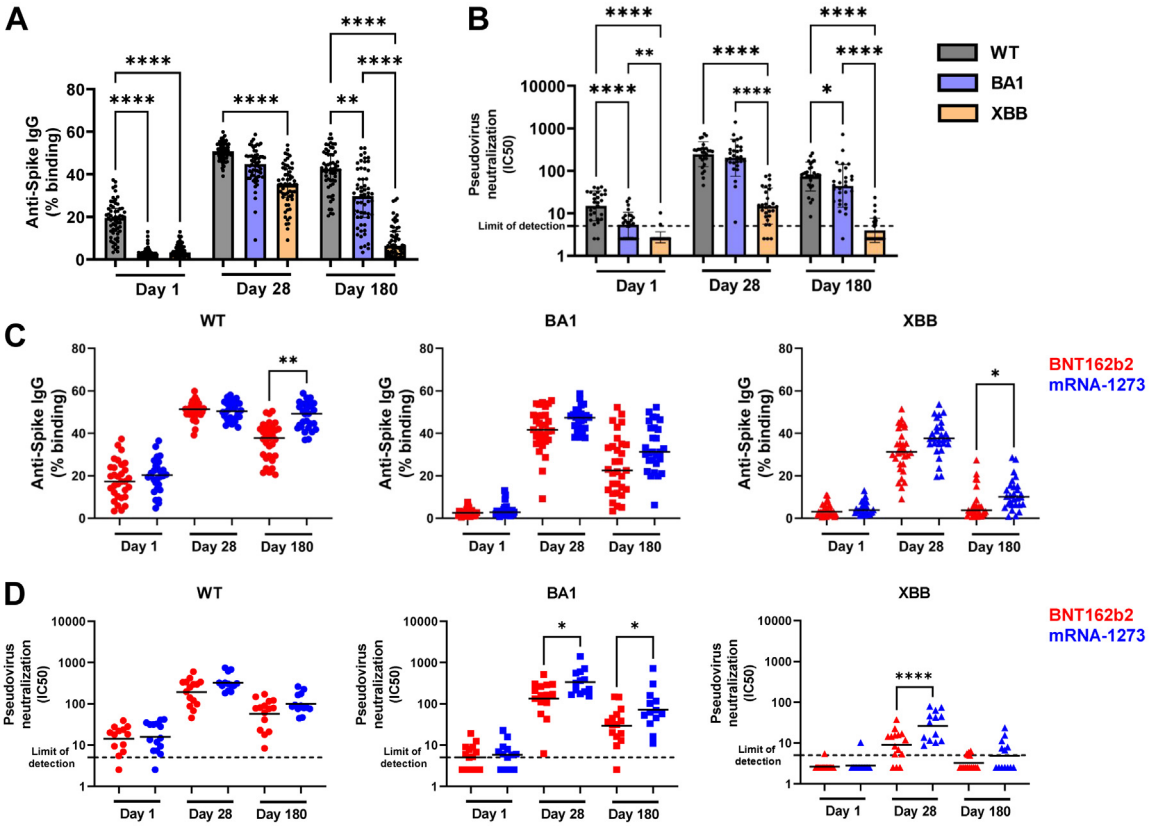

Fig. 1: (A) Anti-Spike IgG antibody binding responses were assessed in a cohort of 59 healthy individuals with no reported COVID-19 infection history who had received three doses of mRNA vaccines. Antibody responses against wild type (WT), Omicron BA.1 (BA1), and Omicron XBB (XBB) variants were assessed at the time of the third vaccine dose (day 1), as well as 28 days and 180 days subsequently. Bars and whiskers represent median values and interquartile range. Comparisons were made using Kruskal-Wallis test. (B) Pseudovirus neutralization IC50 values were assessed in a downselected group of 28 individuals from the same cohort. Bars and whiskers represent mean values and standard deviation. Comparisons were made using one-way ANOVA. (C) Anti-Spike IgG antibody responses of individuals who took the BNT162b2 monovalent vaccine for their third vaccine dose are plotted in red (n = 31), and mRNA-1273 monovalent vaccine in blue (n = 28). Bar represents the median value. (D) Pseudovirus neutralization IC50 values of individuals who took the BNT162b2 monovalent vaccine for their third vaccine dose are plotted in red (n = 15), and mRNA-1273 monovalent vaccine in blue (n = 13). IC50 = 50% inhibitory concentration. *P < 0.05. **P < 0.01. ***P < 0.001. ****P < 0.0001.

XBB at 28 days post-third dose was 22.7, which was lower than BA.1 (mean IC50 = 296) and wild type (mean IC50 = 298) (Fig. 1B). Neutralizing antibody levels further waned by 6 months post-third dose to 5.1, lower than BA.1 (mean IC50 = 86.9) and wild type (mean IC50 = 93.8).

Furthermore, we found that the vaccinees who had received the mRNA-1273 monovalent vaccine as their third dose showed higher antibody levels against XBB than those who had received the BNT162b2 monovalent vaccine. Those who received mRNA-1273 had a mean pseudovirus neutralization IC50 of 34.8 at 28 days post-third dose, which was significantly higher than those who received BNT162b2 (mean IC50 = 12.2) ($p < 0.01$) (Fig. 1D). Similarly, by SFB, levels of antibodies binding to the surface-expressed XBB Spike showed a trend toward being higher among those who received mRNA-1273 (median 37.6% binding) relative to BNT162b2 (median 31.3% binding), though this did not reach statistical significance (Fig. 1C). Furthermore, antibody levels in the mRNA-1273 vaccinees were better sustained relative to those elicited by the BNT162b2 vaccine. At 180 days post third-dose, mRNA-1273 vaccinees had a significantly higher SFB binding to XBB (mRNA-1273 median 10.1% binding; BNT162b2 median 3.8% binding) ($p < 0.05$) (Fig. 1C). A similar trend was also observed at six months for XBB pseudovirus neutralization (mRNA-1273 mean IC50 = 6.9; BNT162b2 mean IC50 < 5), though this did not reach statistical significance (Fig. 1D).

Our study shows that the recombinant Omicron XBB variant displays a higher degree of humoral immune escape relative to Omicron BA.1, confirming other studies reporting high antibody escape by Omicron XBB.[5,6] A third mRNA vaccine dose elevates antibody coverage against Omicron XBB to some degree, but

overall levels remain very low. Antibody responses against XBB also largely wane within six months post-boost. Of note, for September 2022 to January 2023, corresponding to the XBB wave in Singapore, the proportion of cases in ICU or who died due to COVID-19 in Singapore was approximately double in those without a minimum of 3 vaccine doses (0.11%) compared with those with at least 3 vaccine doses (0.063%).[7] Nevertheless, we observe that the magnitude and longevity of humoral responses are affected by vaccine dose type. In this study, the mRNA-1273 heterologous vaccine booster was more effective at eliciting anti-XBB antibodies. It remains to be seen whether a BNT162b2 booster given in the context of mRNA-1273 primary vaccination series will show a similar effect.

Further studies are required to determine how the type of a fourth vaccine dose given would affect the magnitude and longevity of antibody responses against diverse variants, and how the prior vaccine doses impact this. Additionally, it will be important to determine how modification of the vaccine strain affects breadth of antibody responses against subsequent derivative variants. Notably, a preprint has reported increased efficacy of the Omicron BA.5 bivalent vaccines against XBB,[8] although another study reported that these levels remained low overall.[9] Altogether, this also highlights the necessity of understanding the mechanisms underlying differential antibody responses elicited by different vaccine compositions.

Contributors

M.Z.T., Y.S.G. and S.-W.F.: conceptualized the study, designed and performed the experiments, analyzed data and wrote the manuscript. Z.W.C., A.R., N.W., A.T.-R., Y.H., S.K.S., P.X.H., C.Y.L., B.W., S.N.M.S., E.Z.X.N., R.T.C.L., V.N., I.K.J.K.: performed the experiments, analyzed data. X.Y.P., S.R., P.Y.C., S.W.X.O., T.H.L., C.L. and J.T.: supervised and coordinated cohort recruitment and sample collection. NCID Study Group: performed sample collection. PRIBIVAC Cohort Study Group: processed samples. S.M.-S., C.-I.W., Y.-S.L., R.T.P.L., D.C.L., B.E.Y., L.F.P.N., L.R.: conceptualized the study and reviewed the manuscript. All authors approved the final version of the manuscript.

Declaration of interests

A patent application for the SFB assay has been filed (Singapore patent #10202009679P: A Method Of Detecting Antibodies And Related Products) by Y.S.G., L.R., and L.F.P.N. All other authors declare no competing interests.

Acknowledgements

The authors would like to thank all study participants who donated blood and the healthcare workers. The authors also wish to thank the team at NCID and Tan Tock Seng Hospital for their help in subject recruitment. The authors are grateful to Professor Yee-Joo Tan, Department of Microbiology, NUS & IMCB, A*STAR, Singapore, for her kind gift of CHO-ACE2 cells for the pseudovirus neutralization work.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lanwpc.2023.100732.

References

1 World Health Organization. *Weekly epidemiological update on COVID-19-7 December 2022*; 2022. https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19—7-december-2022.
2 Focosi D, McConnell S, Casadevall A. The Omicron variant of concern: diversification and convergent evolution in spike protein, and escape from anti-Spike monoclonal antibodies. *Drug Resist Updat*. 2022;65:100882.
3 Goh YS, Ng LFP, Renia L. A flow cytometry-based assay for serological detection of anti-spike antibodies in COVID-19 patients. *STAR Protoc*. 2021;2(3):100671.
4 Poh CM, Carissimo G, Wang B, et al. Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising antibodies in COVID-19 patients. *Nat Commun*. 2020;11(1):2806.
5 Wang Q, Iketani S, Li Z, et al. Alarming antibody evasion properties of rising SARS-CoV-2 BQ and XBB subvariants. *bioRxiv*. 2022:2022.11.23.517532.
6 Zhang X, Chen L-L, Ip JD, et al. Omicron sublineage recombinant XBB evades neutralising antibodies in recipients of BNT162b2 or CoronaVac vaccines. *Lancet Microbe*. 2022.
7 Singapore Ministry of Health. *COVID-19 statistics: proportion (%) of cases ever in ICU care or died, by age and vaccination status*; 2023. https://www.moh.gov.sg/covid-19/statistics. Accessed February 2, 2023.
8 Zou J, Kurhade C, Patel S, et al. Improved neutralization of Omicron BA.4/5, BA.4.6, BA.2.75.2, BQ.1.1, and XBB.1 with bivalent BA.4/5 vaccine. *bioRxiv*. 2022:2022.11.17.516898.
9 Kurhade C, Zou J, Xia H, et al. Low neutralization of SARS-CoV-2 Omicron BA.2.75.2, BQ.1.1, and XBB.1 by parental mRNA vaccine or a BA.5-bivalent booster. *Nat Med*. 2022.