Immune escape by SARS-CoV-2 Omicron variant and structural basis of its effective neutralization by a broad neutralizing human antibody VacW-209

Dear Editor,

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its variants is still a pandemic raging across the world (Supplementary information, Fig. S1a, b). A new variant, Omicron, was first detected in South Africa and got dominant in many regions.1 Omicron has been classified as a variant of concern by the World Health Organization (WHO), whose spike carried more than 30 mutations (Supplementary information, Fig. S1c).1,2

To make a comprehensive evaluation of the susceptibility of Omicron, we summarized plasma samples from 19 convalescent individuals infected with the wild-type (WT) virus, and measured their neutralizing activities against the WT, Beta, Delta, Mu, C.1.2, and Omicron (Supplementary information, Figs. S1d and S2). The Omicron showed more serious resistance to neutralizing antibodies (nAbs) than other variants including Beta and Mu (45.6-fold, 9.6-fold, and 15.4-fold, respectively, compared with that against the WT), the latter two of which largely escaped the antibody neutralization prior to the Omicron pandemic.3,4 More seriously, some plasma (3/19) lost their neutralizing activities against Omicron (Supplementary information, Fig. S1e).

To study the mechanism of antibody escape, we analyzed 12 published nAbs binding to the receptor binding domain (RBD) of SARS-CoV-2 with clear structural information.5,6 We used these nAbs to mimic the polyclonal antibodies in plasma to explore what kind of nAbs were mostly affected by the mutations. The Omicron decreased or abolished the neutralizing and binding nAbs to mimic the polyclonal antibodies in plasma to explore SARS-CoV-2 with clear structural information.5,6 We used these published nAbs binding to the receptor binding domain (RBD) of against Omicron (Supplementary information, Fig. S1e).

Seriously, some plasma (3/19) lost their neutralizing activities against virus escape in the future. The Mu and C.1.2 variants were identified post the global pandemic of Delta. Mutations in the spike proteins of Delta, Mu, and C.1.2 caused different resistances to the neutralization by polyclonal plasma and monoclonal nAbs.9,10 To define the structural basis of the broadly neutralizing activity of VacW-209, we resolved the cryo-electron microscopy (cryo-EM) structures of the antigen-binding fragment (Fab) of VacW-209 complexed with the spike proteins of SARS-CoV-2 WT, Delta, Mu, C.1.2, or Omicron. Five cryo-EM structures of immune complexes at 2.98–3.45 Å revealed nearly identical binding modes of VacW-209 (Fig. 1c–g,
Supplementary information, Figs. S9–S13 and Table S1). Three VacW-209 Fabs bind to a completely opened spike with three "up" RBDs. We then performed the focus refinement of regions of Fab-bound RBDs of these five structures (Fig. 1h–l). High-resolution structures revealed that the binding epitope of VacW-209 completely evaded the key RBD mutations in Delta, Mu, C.1.2, and rarely overlapped with mutations in Omicron. The footprints of VacW-209 on WT-RBD and Omicron-RBD are slightly different, and three mutations in Omicron (K417N, S373P, and S375F) are involved in the nAb–RBD interaction (Fig. 1m, n).

We next analyzed the interaction details of VacW-209 binding to WT and Omicron spikes and revealed that VacW-209 mainly used its long heavy loop at complementarity determining region (HCDR) 3 to mediate spike recognition. In general, longer HCDR3
may mediate the recognition of nAbs to target some conserved epitopes, which often reside at structurally deeper or cryptic regions in viral antigens,\textsuperscript{11,12} The identification of Vac-W-209 with long HCDR3 partly demonstrated the ability of SARS-CoV-2 inactivated vaccine to induce bnAbs against variants. Besides, the light chain CDR (LCDR) 2 and D34 from LCDR1 are also involved in nAb-RBD interactions (Fig. 1o–r). For WT-RBD, residues 371, 379, 408, 414, and 415 form an interaction network to Vac-W-209 containing 11 hydrogen bonds and 2 salt bridges (Fig. 1o, p). The heavy chain R106 (R106H) inserts its long side chain into the pocket formed by RBD aa. 371–385, which contains three key mutations of Omicron (S371L/S373P/S375F) (Supplementary information, Fig. S14a–c). Although Vac-W-209 showed a decreased neutralization against Omicron (Fig. 1a), our structural analysis showed that the mutations surrounding aa. 371–385 loop seemed not to obviously affect the binding of Vac-W-209 since the S373P and S375F build three new hydrogen bonds with R106H (Fig. 1q). Other Omicron mutations are not involved in the hydrogen bond interactions, and there are a total of 12 hydrogen bonds and 1 salt bridge formed (Fig. 1q, r), which are comparable to that in WT. We further found that the binding of Vac-W-209 to Omicron RBD need a slight conformational change of 371–385 loop (Supplementary information, Fig. S14d, e), which may partly account for the reduced neutralization of Vac-W-209 against Omicron.

Finally, we compared the binding mode of Vac-W-209 to several nAbs of Class 1–4 and defined a new binding mode of Vac-W-209 which bind to an epitope between Class 1 and Class 4, yet not overlapping with that of Class 2 or Class 3 (Supplementary information, Fig. S15a). Despite some minor differences in details, the binding of Vac-W-209 to RBDs of WT, Delta, Mu, C.1.2, and Omicron are all mediated by the long HCDR3 (in particular R106, Y116, and D119), LCDR2 (in particular Y51, N55, and S58), and LCDR1 residue D34 (Supplementary information, Fig. S15b–f). We also explored the potential binding sites of Vac-W-209 on other variant RBDs as well as SARS-CoV RBD based on the binding characterization revealed in the WT-S2P:Vac-W-209 (Supplementary information, Fig. S15g–k). The epitope of Vac-W-209, which is mainly comprised of aa. 376–385 and 405–416, nearly excludes all of above RBD mutations and is highly conserved between SARS-CoV-2 and SARS-CoV with only three amino acid substitutions (A372T, P384A, and E406D) between SARS-CoV-2 and SARS-CoV (Supplementary information, Fig. S15h). The similar binding mode of Vac-W-209 was also found in some previously reported nAbs including C118, CO22, SX235, and SX2X59\textsuperscript{13–15} (Fig. 1s). Available structural information revealed that the aforementioned four nAbs and Vac-W-209 shared lots of epitope residues located in conserved RBD aa. 376–385 and 405–416, while with diverse coverage of key mutations of Omicron (Fig. 1t; Supplementary information, Fig. S16a–e). Of these mutations, S371L, S373P, S375F, K417N, N501Y, and Y505H were structurally close to or involved in the binding epitopes of Vac-W-209-like nAbs. In the head-to-head comparison, although CO22 and SX235 showed significant reductions of neutralization against Omicron, these Vac-W-209-like nAbs generally maintained effectively neutralizing and binding activities to various SARS-CoV-2 variants and even SARS-CoV (Supplementary information, Fig. S16f and G17). The molecular mechanism underlying why these similar nAbs display diverse neutralizing activities need to be elucidated in the future.

In conclusion, Vac-W-209 identifies a highly conserved epitope on the RBDs among SARS-CoV-2 variants overlapping with the ACE2-binding site, which explains its potent neutralization. Vac-W-209 could strongly compete with Class 4 nAbs, indicating the potential cross-neutralization against sarbecoviruses. These Vac-W-209-like nAbs shared a similar antibody response to both SARS-CoV-2 and SARS-CoV, highlighting a key target for the universal vaccine design. As this binding epitope is highly conserved in different variants, vaccine design pursuant to this epitope feature may induce more bnAbs and benefit for the development of broad-spectrum COVID-19 vaccines. Vac-W-209, alone or in combination with S309, could also be used as countermeasure against SARS-CoV-2 variants including Omicron and even other forthcoming sarbecoviruses in the future.

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DATA AVAILABILITY

Coordinates have been deposited in the Protein Data Bank under accession code 7WPS (Omicron-S6P:Vac-W-209). The corresponding density maps have been deposited in the Electron Microscopy Data Bank under accession numbers EMD-32674 (Omicron-S6P:Vac-W-209) and EMD-32669 (Omicron-S6P-Vac-W-209, local refinement).

REFERENCES

1. Liu, L. et al. Nature https://doi.org/10.1038/s41586-021-04388-0 (2021).
2. Cameroni, E. et al. Nature https://doi.org/10.1038/s41586-021-04386-2 (2021).
3. Lucas, C. et al. Nature 600, 523–529 (2021).
4. Uriu, K. et al. N. Engl. J. Med. 385, 2397–2399 (2021).
5. Barnes, C. O. et al. Nature 588, 682–687 (2020).
6. Cheng, L. et al. Cell Discov. 7, 112 (2021).
7. Cao, Y. et al. Nature https://doi.org/10.1038/s41586-021-04385-3 (2021).
8. Pinto, D. et al. Nature 583, 290–295 (2020).
9. Tada, T. et al. Cell Rep. 38, 110237 (2021).
10. Liu, C. et al. Cell 184, 4220–4236 (2021).
11. Eckert, D. C. et al. Nature 489, 526–532 (2012).
12. Walker, L. M. et al. Nature 477, 466–470 (2011).
13. Starr, T. N. et al. Nature 597, 97–102 (2021).
14. Tortorici, M. A. et al. Nature 597, 103–108 (2021).
15. Jette, C. A. et al. Cell Rep. 36, 109760 (2021).

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AUTHOR CONTRIBUTIONS

Z.Z., N.X., S.L., and B.J. conceived and designed the study. B.J., Q.Z., H.G., Q.F., T.L., S. Song, and H.S. performed all experiments and analyzed data together with S. Shen,
X.Z., W.X., L.C., and B.Z. Z.Z., N.X., S.L., B.J., and Q.Z. participated in discussion of the results and wrote the paper. All authors read and approved this version of paper.

COMPETING INTERESTS
The authors declare no competing interests.

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