VHH DIMER P559-R45L NEUTRALIZES SARS-CoV-2 BY STABILIZING SPIKE IN UP CONFORMATION

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BACKGROUND

- SARS-CoV-2 Omicron subvariants are highly resistant to vaccine-induced immunity and therapeutic monoclonal antibodies.
- We previously reported anti-SARS-CoV-2 spike alpaca nanobodies (VHHs) P86 and P17 that potently neutralize the wild type and VOCs from Alpha to Omicron BA.1 and BA.2, but not Omicron subvariants after that (eg; BA.4/5).

METHODS

- We developed VHH trimers and heterodimers based on the structural and computational analysis of spike-immunized alpaca VHH library.
- We tested representative VHHs against SARS-CoV-2 spike by pseudovirus assays and generated VHH heterodimers.
- We further obtained Cryo-EM structure of Spike trimer and VHH monomer or heterodimer.

RESULTS

- First, we generated series of P86 VHH mutants to counteract L452R mutation in Delta or Omicron BA.5 subvariants and found that P86 R45L is most potent against D614G with an IC_{50} of 0.03 μg/mL (Fig. 1, A-D).
- By panning with the Delta spike, we also identified that homo-trimer of VHH P599 neutralizes SARS-CoV-2 Delta and Omicron BA.5 variants with an IC_{50} of 0.077 and 0.54 μg/mL, respectively (Fig. 1A, E-G).

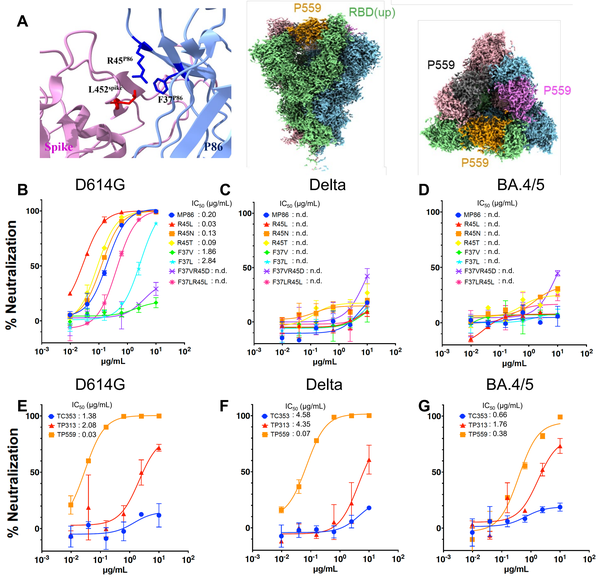

Fig. 1 MP86 R45L and TP559 showed highest neutralization activities against D614G.
(A) Spike L452 interacts with F37 and R45 of MP86 (P86 monomer). Neutralizing activities of various F37/R45 mutants of MP 86 against D614G (B), Delta (C) and BA.4/5 variants (D). Neutralizing activities of TC353, TP313 and TP559 derived from concentrated VHH library with the Delta spike against D614G (E), Delta (F) and BA.4/5 variants (G).

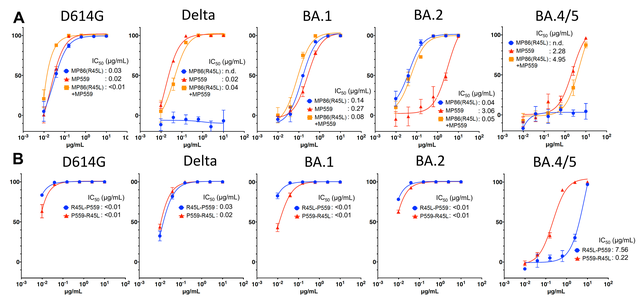

Fig. 2 VHH heterodimer P559-R45L effectively neutralizes BA.4/5 spike pseudovirus.
(A) Neutralizing activities of MP86(R45L) (MP86(R45L) monomer), MP559 (P559 monomer) and mixture of MP86(R45L) and MP559 against D614G, Delta and Omicron subvariants. (B) Neutralizing activities of VHH heterodimers, P559-R45L and R45L-P559 against D614G, Delta and Omicron variants.

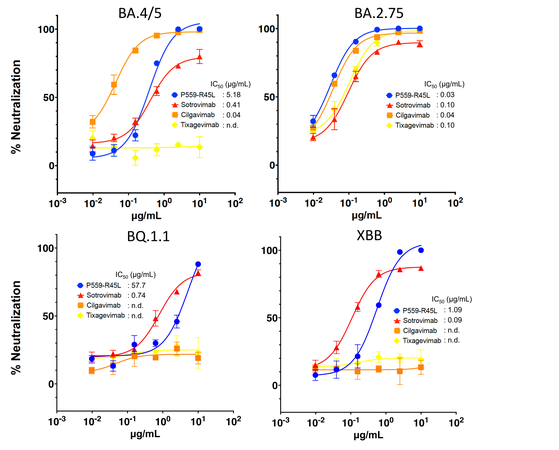

Fig. 3 Neutralizing activities against Omicron subvariants, BA4/5, BA.2.75, BQ.1.1 and XBB. Neutralizing activities of P559-R45L against Omicron subvariants compared with those of therapeutic antibodies including Sotrovimab, Cilgavimab and Tixagevimab.

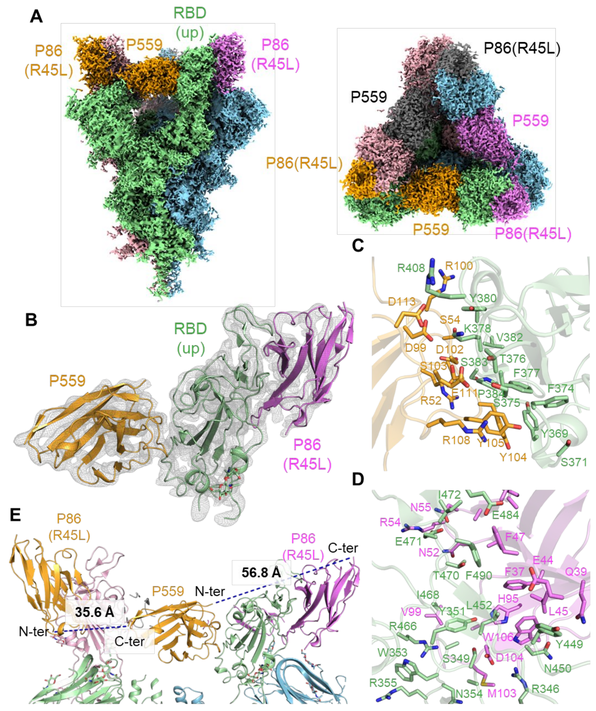

Fig. 4. CryoEM structure of spike protein complexed with P559-P86(R45L) heterodimer.
(**A**) Two orthogonal views of the final sharpened map: side view (left panel) and top view (right panel). The map regions corresponding to each protomer in spike protein are colored in green, cyan, and pink, respectively. The map regions corresponding to each VHH heterodimer are colored in yellow, magenta, and gray, respectively. (**B**) The locally refined raw map around one RBD with the atomic model. (**C**) Close-up view of RBD-P559 interface. (**D**) Close-up view of RBD-P86(R45L) interface. (**E**) Distance between N- and C-terminus of the two VHHs.

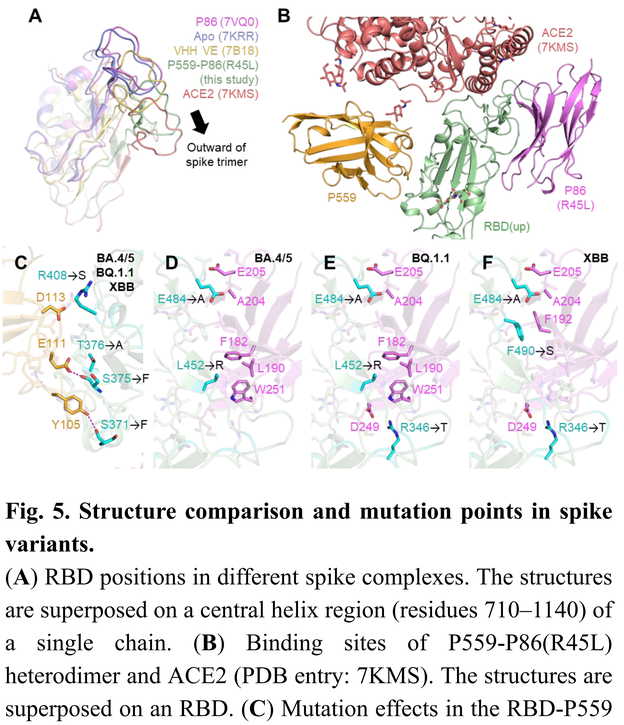

Fig. 5. Structure comparison and mutation points in spike variants.
(**A**) RBD positions in different spike complexes. The structures are superposed on a central helix region (residues 710–1140) of a single chain. (**B**) Binding sites of P559-P86(R45L) heterodimer and ACE2 (PDB entry: 7KMS). The structures are superposed on an RBD. (**C**) Mutation effects in the RBD-P559 interface. Mutation points in BA.4/5, BQ.1.1, and XBB variants are shown in cyan (all mutations are common to these variants), and the related residues of P559 are highlighted. (**D–F**) Mutation effects in the RBD-P86(R45L) interface. Mutation points in BA.4/5 (D), BQ.1.1 (E), and XBB (F) variants are shown in cyan, and the related residues of R45L are highlighted.

DISCUSSION

Three molecules of heterodimerized VHH P559-R45L bound to different epitopes of SARS-CoV-2 spike stabilized spike timer in the up conformation. Mutations including S371F, S375F, T376A, R408S, R346T, L452R, E484A, F490S, found in recent Omicron subvariants, BQ.1.1 and XBB, could be involved in the resistance of neutralizing activity of P559-R45L.

CONCLUSION

We developed VHH heterodimer P559-R45L potently neutralizes Omicron subvariants through unique structural interaction.

Acknowledgements

This study was supported in part by AMED Research Program on Emerging and Re-emerging Infectious Diseases (20fk0108268, 20fk0108517 to ATK; JP20fk0108414); AMED Research Program on HIV/AIDS (21fk0410034 to ATK); JSPS KAKENHI Grant-in-Aid for Scientific Research C (19K07591, 22K07085 to KS); and Joint Usage/Research Center program of Institute for Frontier Life and Medical Sciences, Kyoto University (to ATK). KYOTO industrial Support Organization 21, the subsidies (Sangakukou no Mori) to COGNANO Inc., and donations from POPURI Pharmacy Co., Ltd. (Kyoto, Japan). We thank the staff of COGNANO Inc., Kaori Yurugi, Naomi Tsutsui, and Seishiro Yamamoto for their experimental support.