BROAD ANTIBODIES TO FUNCTIONALLY CONSTRAINED REGIONS OF SARS-COV-2 SPIKE

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BACKGROUND

While remarkable and rapid progress was made in fighting the SARS-CoV-2 pandemic with vaccines and therapeutic antibodies, these approaches were quickly compromised by viral evolution. To achieve durable protection against SARS-CoV-2, novel mAbs need to show breadth and potency across VOCs and target epitopes that are more constrained.

METHODS

Isolation of spike-specific memory B cells from participant in the Hospitalized or Ambulatory Adults with Respiratory Viral Infections (HAARVI) Study (Dr. Helen Chu, UW) with a Delta infection post-vaccination

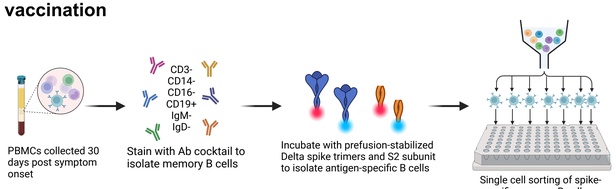
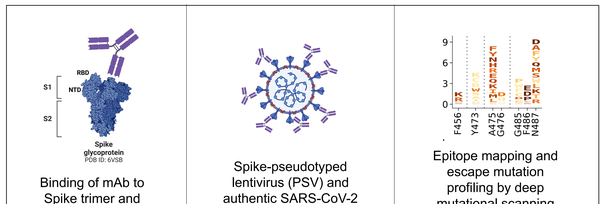


Figure generated with BioRender.com

Functional Characterization of mAbs



ACKNOWLEDGEMENTS

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REFERENCES

B. Dadonaite et al., bioRxiv 10.1101/2022.10.13.512056 (2022).
T. N. Starr et al., Cell 182, 1295-1310 e1220 (2020).

Two novel Spike-specific mAbs targeting a functionally constrained region of RBD and a rare, conserved region in SD1 show breadth and potency across SARS-CoV-2 VOCs including XBB and BQ.1.1.

RESULTS

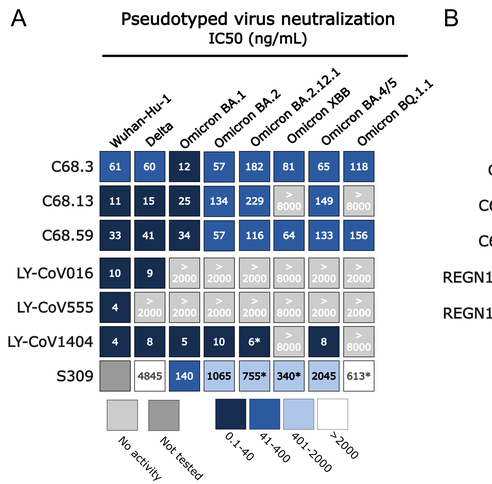


Figure 2. C68 mAbs show broad and potent neutralization against PSV and authentic SARS-CoV-2 viruses. Neutralization of spike-PSVs(A) and authentic SARS-CoV-2 viruses (B) by mAbs is shown. IC50 values (ng/mL) are the average of three independent experiments (A) or two independent experiments (B). Darker shade = lower IC50 = higher potency. Light grey boxes denote mAbs with no measured activity in the dilution range of the assay.

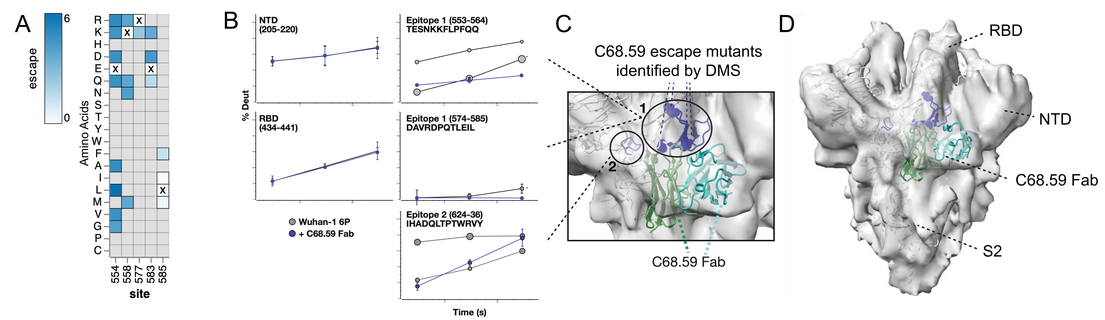


Figure 3. Mapping C68.59 epitope and neutralization escape mutations in SD1 by DMS and structural methods. (A) Heatmap showing spike-pseudotyped lentivirus escape at key sites identified by DMS (method described in Dadonaite 2022). Darker blue = more escape. Residues marked with an X are wildtype amino acids at that site in Omicron BA.2. (B-D) Structural mapping of the C68.59 epitope by HDX-MS and cryo-EM. (B) By HDX-MS, adjacent peptide segments 553-564, 574-585, and 624-636 showed increase in local ordering and quenching of conformational sampling when C68.59 Fab was bound to S6P trimeric spike. Bubble plots show the uptake rates of the two distinct populations at the unbound epitope sites (grey), with the size of the bubble indicating the population fraction. (C-D) Cryo-EM reconstruction of C68.59 Fab bound to spike (PDB: 7SBP). The zoomed view of the epitope (C) and the overall structure of the C68.59 Fab bound to S6P spike (D). Binding site peptides that showed protection by HDX-MS (residues 553-568, 575-585, 624-636) are colored blue in the cartoon structure. Residues exhibiting escape mutations by DMS (A) are noted.

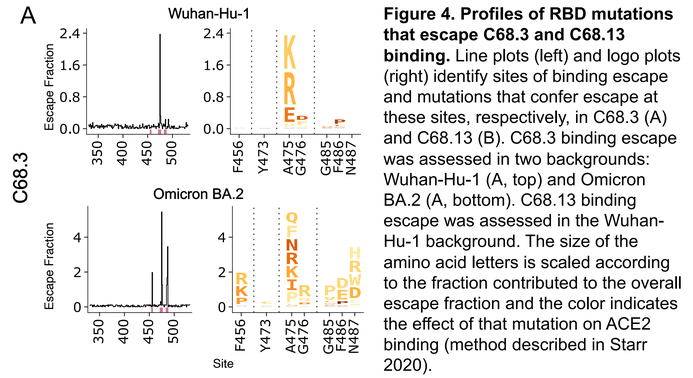


Figure 4. Profiles of RBD mutations that escape C68.3 and C68.13 binding. Line plots (left) and logo plots (right) identify sites of binding escape and mutations that confer escape at these sites, respectively, in C68.3 (A) and C68.13 (B). C68.3 binding escape was assessed in two backgrounds: Wuhan-Hu-1 (A, top) and Omicron BA.2 (A, bottom). C68.13 binding escape was assessed in the Wuhan-Hu-1 background. The size of the amino acid letters is scaled according to the fraction contributed to the overall escape fraction and the color indicates the effect of that mutation on ACE2 binding (method described in Starr 2020).

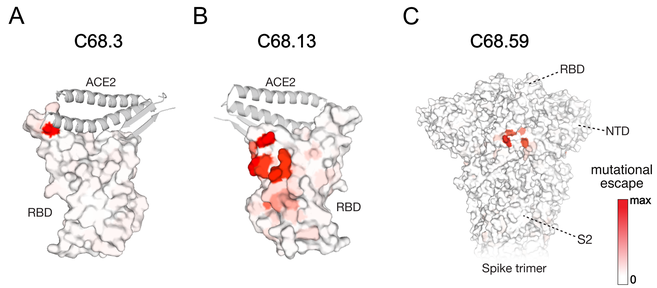


Figure 5. Epitopes of C68 mAbs defined by mutational escape profiles using DMS. Regions with the highest mutational escape scores, indicative of mAb binding residues, are mapped in red onto RBD bound to ACE2 (A-B, PDB:6M0J) or Spike trimer (C, PDB:68R8) structures.

FUTURE DIRECTIONS

- *In vivo* assessment of protective/therapeutic efficacy in mice
- Evaluate neutralization potency in combination
- Track affinity maturation of these mAbs in later timepoints
- Investigation of C68.59 neutralization mechanism
- Validation of escape mutations from DMS

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