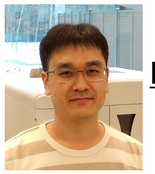
Neutralization Profiles of Monoclonal Antibodies Derived from Ancestral SARS-CoV-2 Receptor Binding Domain



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BACKGROUND

SARS-CoV-2 has evolved rapidly into many genetic lineages with different antigenic properties (https://nextstrain.org/) since the first human infections were reported in December, 2019. Depending on the risk to global public health, five of SARS-CoV-2 variants (Alpha, Beta, Gamma, Delta, and Omicron) have been named as variants of concern (VOCs) by the World Health Organization. These VOCs not only circulated at high levels globally, but also accumulated numerous mutations in the viral surface glycoprotein – spike, the major antigen targeted by vaccines and antibodies. Many mutations acquired are heavily concentrated in the receptor binding domain (RBD) of spike, which not only increases the binding affinity for host cell receptor angiotensin-converting enzyme 2 (ACE2) for viral entry but also changes viral antigenicity enabling them to escape recognition by convalescent sera and vaccine-elicited polyclonal antibodies. Ongoing efforts are focused on generating and selecting broadly neutralizing monoclonal antibodies (mAbs) for vaccine development against evolving SARS-CoV-2 variants.

METHODS

Monoclonal antibody (mAb) generation and selection

Mouse hybridomas were generated by immunizing mice with baculovirus-expressed recombinant receptor binding domain (RBD) of the original SARS-CoV-2 Wuhan-Hu-1 strain. Five mAb clones (17A7, 17B10, 2G5, 3A6, and 20B5) showed not only high affinity to the original RBD but also high neutralizing activity against pseudovirus expressing the Wuhan-Hu-1 spike protein. These five clones were further evaluated in live virus-based microneutralization assay in an animal biosafety level 3 (ABSL3) facility.

Live virus-based microneutralization (MN) assay

Serially diluted mouse mAbs were pre-incubated with 100 $TCID_{50}$/well of live virus at room temperature for 1 h. The virus-mAb mixtures were then added to 96-well tissue culture plates pre-seeded with Vero E6 cells. After incubation at 37°C, 5% CO2 for 2 days, virus-infected cells were detected using SARS-CoV-2 nucleocapsid specific antibodies. The % virus infection as compared to the wells that contained virus only were plotted by nonlinear regression to determine IC_{50} of mAbs for each virus using GraphPad Prism.

Passive transfer and challenge

Hemizygous B6.Cg-Tg(K18-ACE2)2Prlmn/J (K18-hACE2) transgenic mice were used at 8-12 weeks old for passive transfer and challenge in the ABSL3 lab. Age-matched K18-hACE2 female and male adult mice (approximately 1:1 ratio) were injected intraperitoneally with 0.1 ml/mouse of selected mAbs or isotype control. Recipient mice were then challenged intranasally with a lethal dose of wild type SARS-CoV-2 and variants. Infected mice were monitored daily for body weight (BW) and mortality for 10 days post infection (dpi). All surviving mice were humanely euthanized in the end of the experiments.

RESULTS

Figure 1. Microneutralization profile against wild type SARS-CoV-2.

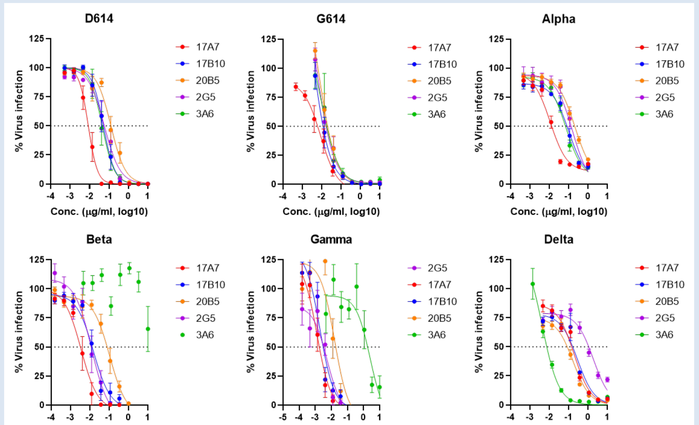

Figure 2. Passive transfer and challenge against VOCs.

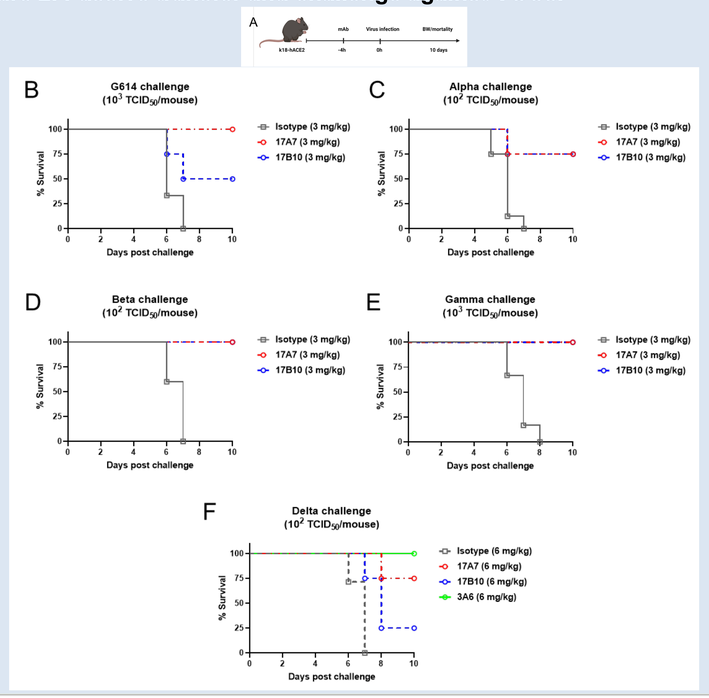

Figure 3. Microneutralization against Omicron subvariants.

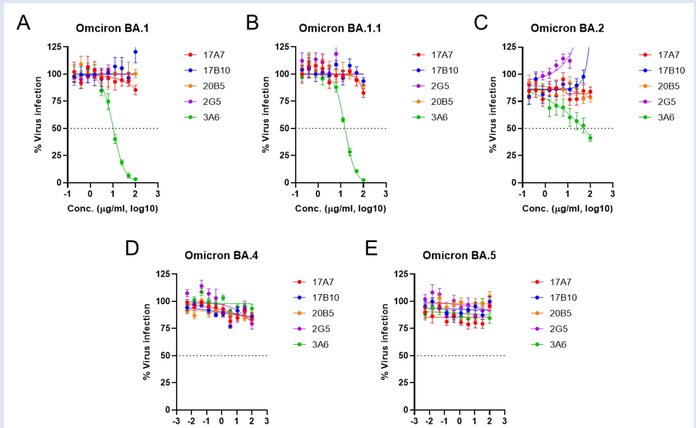

Table 1. Microneutralization IC_{50} of mAbs against wild type live SARS-CoV-2 isolates.

Virus & Lineage		Microneutralization IC_{50} (μg/ml)				
		2G5	3A6	17A7	17B10	20B5
WA/1 (614D) (A)		0.0570	0.0443	0.0085	0.0512	0.1255
NY (614G) (B.1.3)		0.0204	0.0201	0.0047	0.0134	0.0238
Alpha (B.1.1.7)		0.0118	0.0076	0.0039	0.0117	0.0175
B.1.1.298		0.0037	0.0022	0.0025	0.0028	0.0080
B.1.222		0.0407	0.0441	0.0149	0.0440	0.1049
Epsilon (B.1.429)		0.0195	0.0105	0.0038	0.0116	0.0354
B.1.5		0.0462	0.0612	0.0291	0.0656	0.1352
Lambda (C.37)		0.0021	0.0011	0.0009	0.0013	0.0048
Beta (B.1.351)		0.0101	>100	0.0025	0.0053	0.0226
Gamma (P.1)		0.0030	1.5959	0.0002	0.0014	0.0067
Zeta (P.2)		0.0444	5.5271	0.0130	0.0380	0.0457
R.1		0.0100	0.3920	0.0086	0.0114	0.0118
Kappa (B.1.617.1)		0.0132	0.0891	0.0051	0.0124	0.0146
Delta (B.1.617.2)		0.8881	0.0073	0.1249	0.1358	0.0701
Omicron (B.1.1.529)	BA.1	>100	10.4954	>100	>100	>100
	BA.1.1		15.4882			
	BA.2		>100			
	BA.4					
	BA.5					

SUMMARY

The results demonstrate that several RBD-reactive mAbs are highly potent at cross-neutralizing a wide range of live infectious SARS-CoV-2 variants. Although no mAbs extend the cross-neutralization to the latest Omicron subvariants, they can serve useful tools for characterizing SARS-CoV-2 variants and related vaccines.

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