Anti-SARS-CoV-2 Omicron Antibodies Isolated from a SARS-CoV-2 Delta Semi-Immune Phage Display Library

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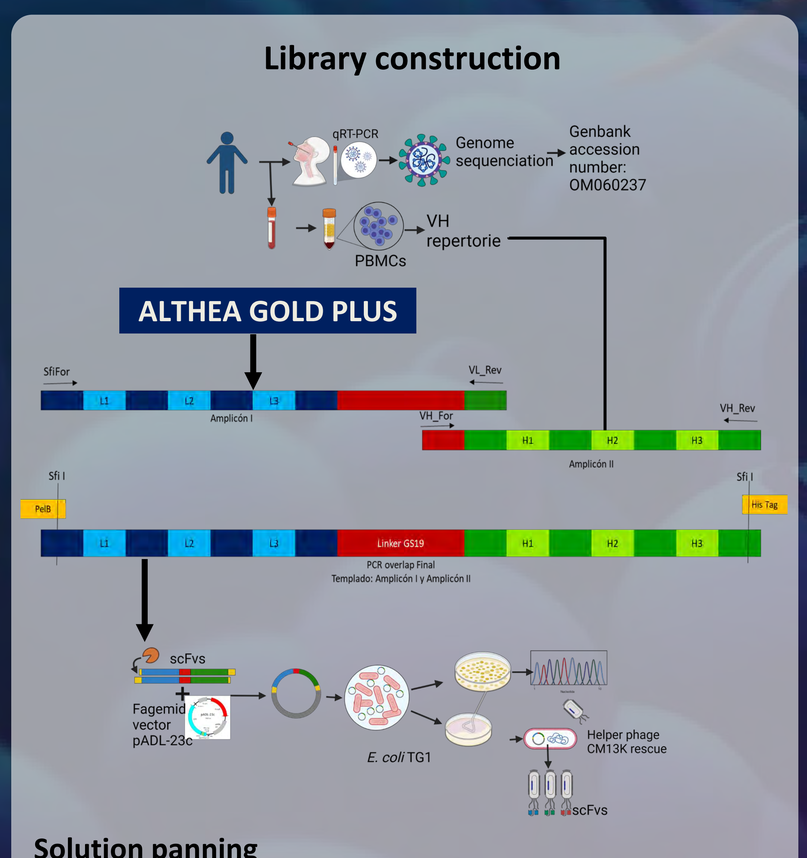
Abstract

We describe here the discovery and characterization of antibodies with potential broad SARS-CoV-2 neutralization profiles. The antibodies were obtained from a phage display library built with the VH repertoire of a convalescent COVID-19 patient who was infected with SARS-CoV-2 B.1.617.2 (Delta). The patient received a single dose of Ad5-nCoV vaccine (Convidecia™, CanSino Biologics Inc.) one month before developing COVID-19 symptoms. Four synthetic VL libraries were used as counterparts of the immune VH repertoire. After three rounds of panning with SARS-CoV-2 receptor-binding domain wildtype (RBD-WT) 34 unique scFvs, were identified, with 27 cross-reactive for the RBD-WT and RBD Delta (RBD-DT), and seven specifics for the RBD-WT. The cross-reactive scFvs were more diverse than the RBD-WT specific ones, being encoded by several IGHV genes from the IGHV1 and IGHV3 families combined with short HCDR3s. Three cross-reactive scFvs and one RBD-WT specific scFv were converted to human IgG1 (hIgG1). The four antibodies blocked the RBD-WT binding to angiotensin converting enzyme 2 (ACE2), suggesting these antibodies may neutralize the SARS-CoV-2 infection. Importantly, one of the antibodies also recognized the RBD from the B.1.1.529 (Omicron) isolate, implying that the VH repertoire of the convalescent patient would protect against SARS-CoV-2 Wildtype, Delta, and Omicron. From a practical viewpoint, the triple cross-reactive antibody provides the substrate for developing therapeutic antibodies with a broad SARS-CoV-2 neutralization profile

Library construction



Discovery campaign

Figure 1. Functional profile of the unique scFvs. Binding to RBD-WT and RBD-DT (top), competition with P5E1-A6 (middle) and RBD-WT:hACE2 blocking interaction (bottom).

Sequence features of scFvs

	Vκ				Total (%)
	VK1-39	VK3-11	VK3-20	VK4-1	
VH1-24	0	0	2	0	5.7
VH1-46	4	0	10	2	45.7
VH1-69	2	1	2	1	17.1
VH2-70	0	0	1	0	2.9
VH3-23	0	0	1	0	2.9
VH3-53	6	1	0	0	20
VH3-9	0	0	2	0	5.7
Total (%)	34.3	5.7	51.4	8.6	100

(a)

Cluster	scFv	Frequency	VL scaffold	IGHV germline gene	HCDR3 length (aa)
1	G12	1	1-39	1-69	22
2	A2	2	1-39	3-53	11
2	B1	1	1-39	3-53	12
2	A7	3	3-20	1-24	13

(b)

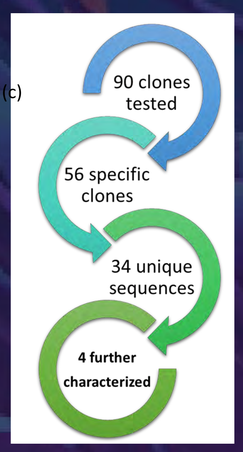


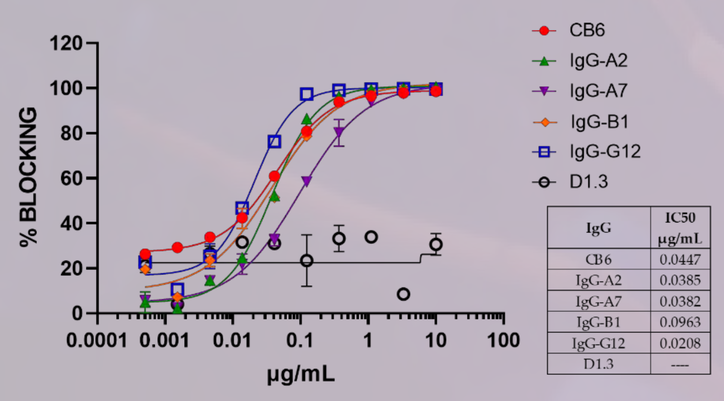
Figure 2. Sequence features of the 34 unique scFvs shown in Figure 1. (a) percentage of germline genes from the 34 unique sequences, (b) scFvs features progressed to IgG1 conversion, (c) progression of selection of clones for conversion to IgG1.

IgG developability profile

Table 1. Summary of the characteristics Developability profile of the Protein-A purified anti-SARS-CoV-2 antibodies. (a) The percent of monomer as determined by analytical SEC. (b) SDS-PAGE. Molecular weight as estimated in non-reducing (NR) and reducing (R) conditions. In the latter, the first number corresponds with the heavy chain and the second with the light chain. (c) The melting temperature (Tm) as determined by protein thermal shift assay. (d) Expression yield after four- days culture in adherents HEK 293T cells.

IgG	Monomer [a] (%)	SDS-PAGE [b]		Tm [c] (°C)	Expression Yield [d] (mg/L)
		NR (kDa)	R (kDa)		
A2	100	140	49/25	71.3	19.92
A7	100	148	52/25	68.5 (81.8)	24.76
B1	100	158	48/25	71.9	15.82
G12	100	176	50/25	71.1	19.57

RBD:hACE-2 blockade assay

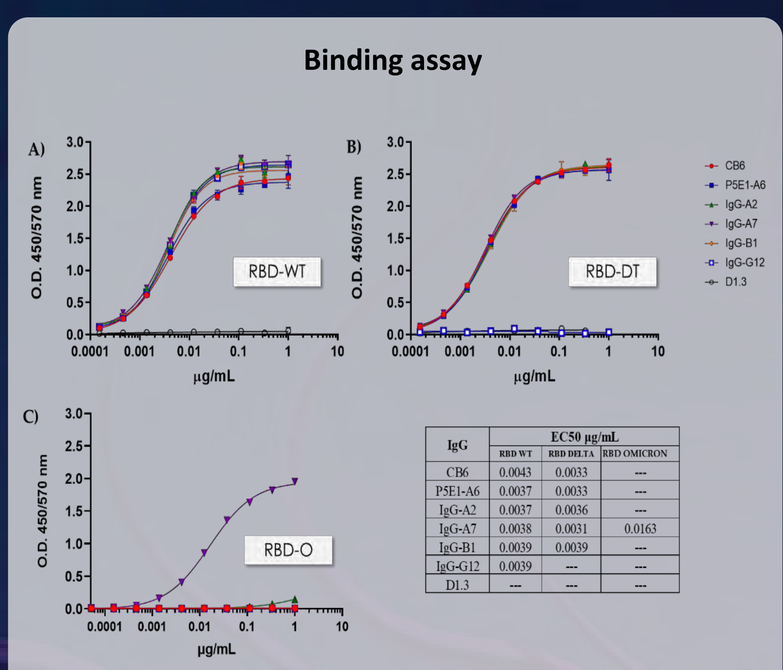


IgG	IC50 µg/mL
CB6	0.0447
IgG-A2	0.0385
IgG-A7	0.0382
IgG-B1	0.0963
IgG-G12	0.0208
D1.3	----

Figure 3. RBD:hACE-2 blockade assay. The data were fitted to a four-parameter dose–response curve in using GraphPad Prism 9.3.1., and the IC50 values were calculated

IgGs functionality

Binding assay

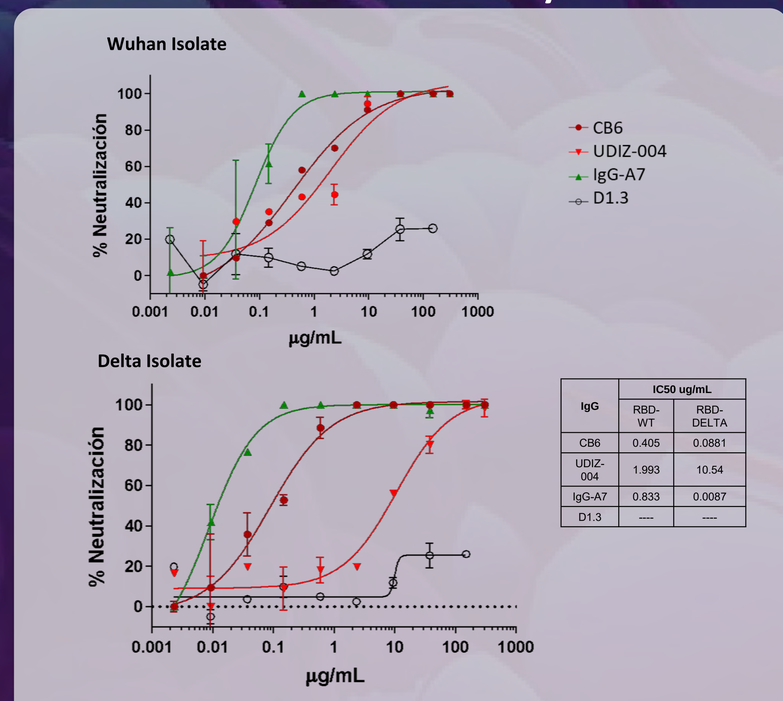


IgG	EC50 µg/mL		
	RBD WT	RBD DELTA	RBD OMICRON
CB6	0.0043	0.0033	---
P5E1-A6	0.0037	0.0033	---
IgG-A2	0.0037	0.0036	---
IgG-A7	0.0038	0.0031	0.0163
IgG-B1	0.0039	0.0039	---
IgG-G12	0.0039	---	---
D1.3	---	---	---

Figure 4. RBD:IgG binding assay. The data were fit to a four-parameter dose–response in GraphPad Prism 9.3.1. and the the EC50 values were calculated.

IgG-A7 was selected as a leader for its triple recognition against RBD-WT, Delta and Omicron

Neutralization assay



IgG	IC50 ug/mL	
	RBD-WT	RBD-DELTA
CB6	0.405	0.0881
UDIZ-004	1.993	10.54
IgG-A7	0.833	0.0087
D1.3	----	----

Figure 5. SARS-CoV-2 Neutralization assay. Plaque reduction neutralization test (PRNT) for SARS-CoV-2. The data were fit to a four-parameter dose–response in GraphPad Prism 9.3.1. and the the IC_{50} values were calculated.

Summary

- **A panel of anti-SARS CoV-2 antibodies were isolated from ALTHEA SARS-CoV-2 Libraries™ using RBD-WT as selector.**
- **90 clones were tested for binding to RBD, yielding 34 positive and unique clones.**
- **The lead molecule IgG-A2, A7, B1, G12 blockade SARS-CoV2 WT:hACE-2 interaction.**
- **IgG12-recognizes only RBD-WT, while IgG-A2, A7 and B1 recognize RBD-WT and the variant of concern Delta. Additionally, IgG-A7 recognize RBD-Omicron.**
- **IgG-A7 neutralize SARS-CoV-2 WT and Delta.**

Reference

Mendoza-Salazar, I.; Gómez-Castellano, K.M.; González-González, E.; Gamboa-Suasnavart, R.; Rodríguez-Luna, S.D.; Santiago-Casas, G.; Cortés-Paniagua, M.I.; Pérez-Tapia, S.M.; Almagro, J.C. (2022). Anti-SARS-CoV-2 Omicron Antibodies Isolated from a SARS-CoV-2 Delta Semi-Immune Phage Display Library. Antibodies, 11(1), 13. https://doi.org/10.3390/antib11010013

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IgG-A7 recognized RBD from SARS-CoV-2 Wildtype, Delta, and Omicron, neutralized SARS-CoV-2 WT and Delta in vitro, and had all the attributes to be further developed in a therapeutic antibody

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