Anti-SARS-CoV-2 Omicron antibodies isolated from a SARS-CoV-2 Delta semi-immune phage display library

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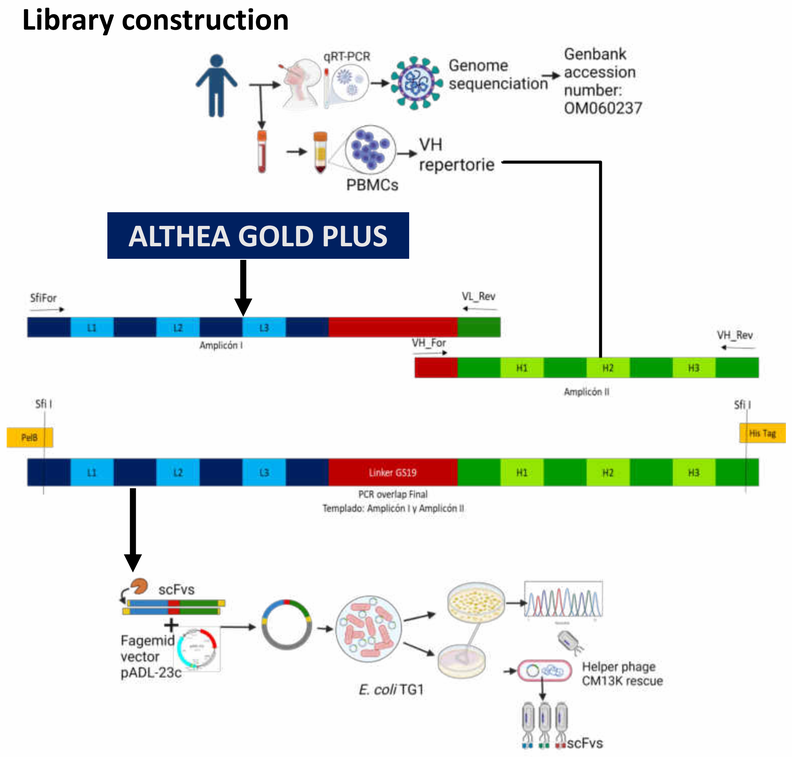
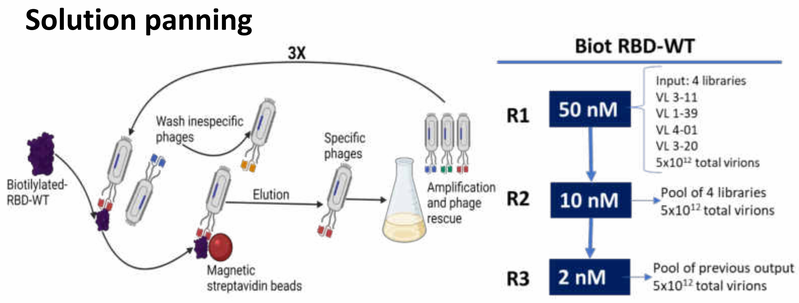
Abstract

This report describes 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). Importantly, one of the antibodies also recognized the RBD from the B.1.1.529 (Omicron) isolate (RBD-O) called IgG-A7.
This antibody neutralized SARS-CoV-2 WT, Delta and Omicron virus, and their physicochemical characteristics suggest it could be an optimal candidate for developing therapeutic drugs with a broad SARS-CoV-2 neutralization profile.

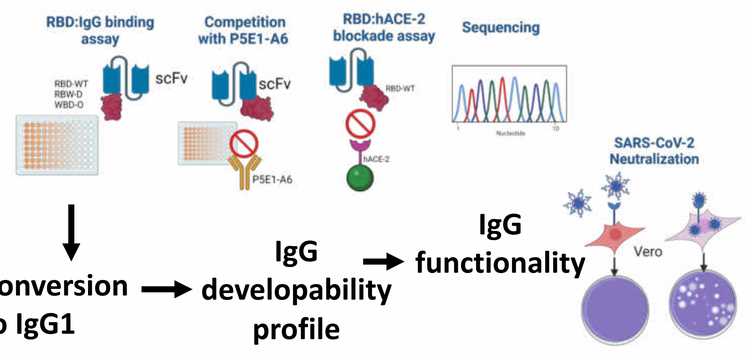
Library construction



Solution panning



Screening for functional clones



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Discovery campaign

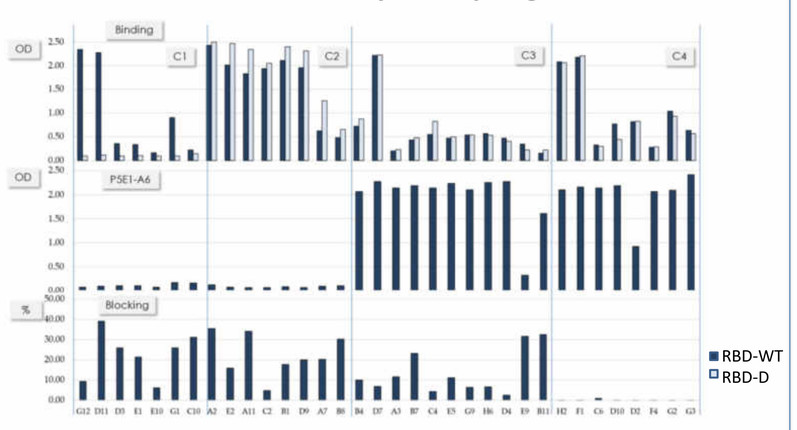


Figure 1. Functional profile of the unique scFvs. Binding to RBD-WT and RBD-DT (top), competition with P5E1-A6 (middle) and RBD-WT:hACE2 blocking interaction (bottom).

Sequence features of scFvs

(a)

	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

(b)

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

(c)

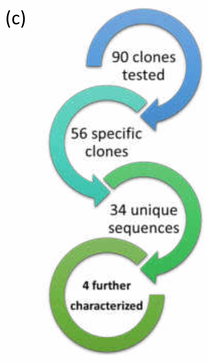


Figure 2. Sequence features of the 34 unique scFvs shown in Figure 1. (a) percentage of germline genes from the 34 unique sequences, (b) scFvs features progressed to IgG1 conversion, (c) progression of selection of clones for conversion to IgG1.

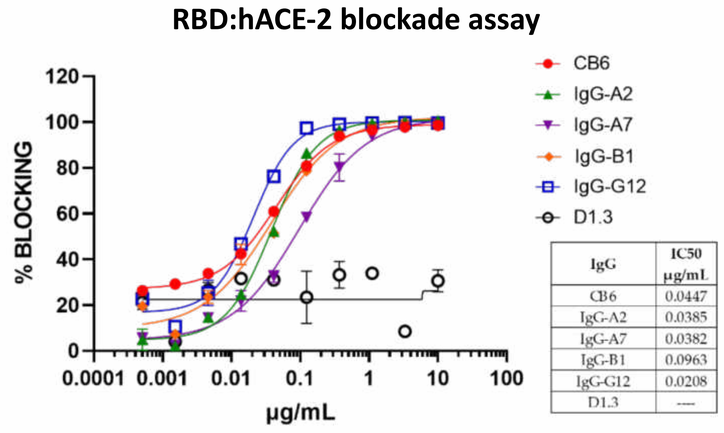
IgG developability profile

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

IgGs functionality

RBD:hACE-2 blockade assay

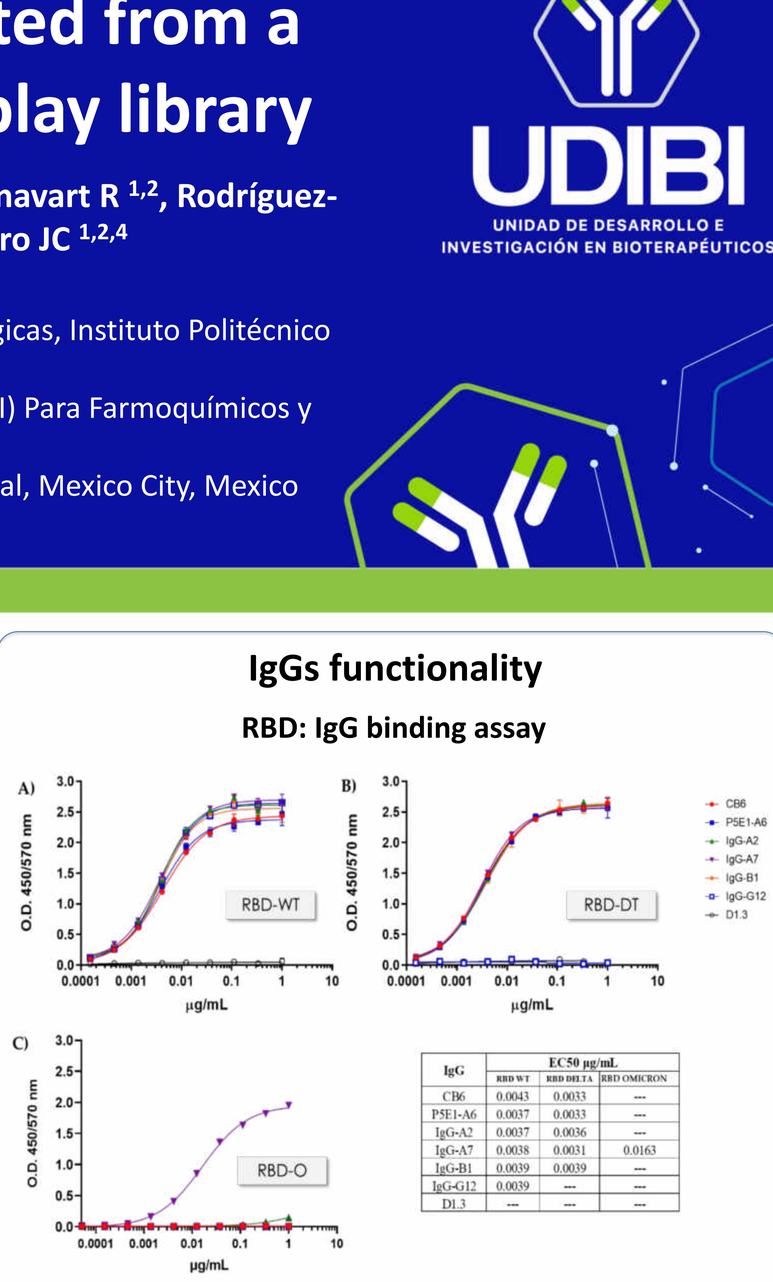


IgG	IC50 µg/mL
CB6	0.0447
IgG-A2	0.0385
IgG-A7	0.0382
IgG-B1	0.0963
IgG-G12	0.0208
D1.3	----

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

RBD: IgG binding assay

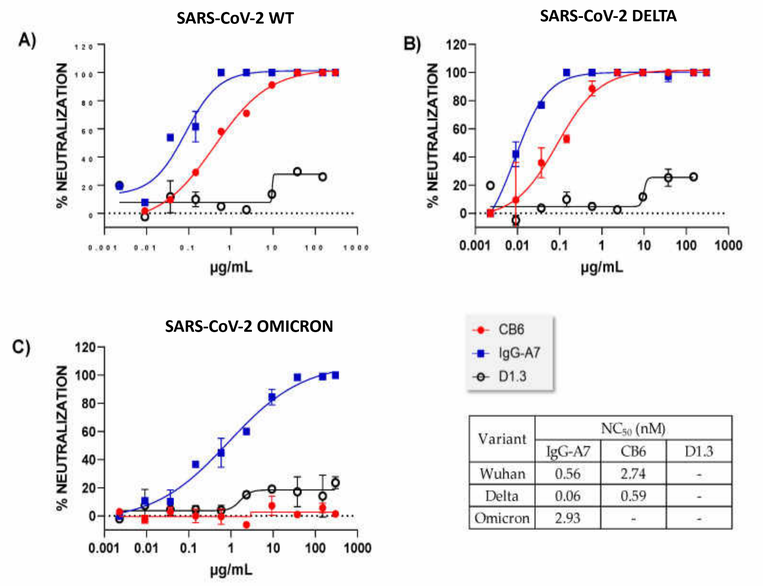


IgG	EC50 µg/mL		
	RBD WT	RBD DELTA	RBD OMICRON
CB6	0.0043	0.0033	---
P5E1-A6	0.0037	0.0033	---
IgG-A2	0.0037	0.0036	---
IgG-A7	0.0038	0.0031	0.0163
IgG-B1	0.0039	0.0039	---
IgG-G12	0.0039	---	---
D1.3	---	---	---

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



Variant	NC_{50} (nM)		
	IgG-A7	CB6	D1.3
Wuhan	0.56	2.74	-
Delta	0.06	0.59	-
Omicron	2.93	-	-

Figure 5. SARS-CoV-2 Neutralization assay. The data were fit to a four-parameter dose–response in GraphPad Prism 9.3.1. and the the NC_{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.**
- **IgG-G12-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, Delta and Omicron.**

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.

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CONCLUSION: IgG-A7 neutralized SARS-CoV-2 WT, Delta and Omicron by the RBD recognition, furthermore, had all the attributes of a good candidate to be developed in a therapeutic antibody

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