SARS-CoV-2 neutralizing antibodies discovered across three individuals following vaccination and infection show diverse epitopes across virus spike protein

Natalie Castellana Thiago Lima Stefano Bonissone Richard Carson
Qiulong Huang Mar De la Cruz Brinley Moorman Anand Patel

Abterra Biosciences, San Diego, CA

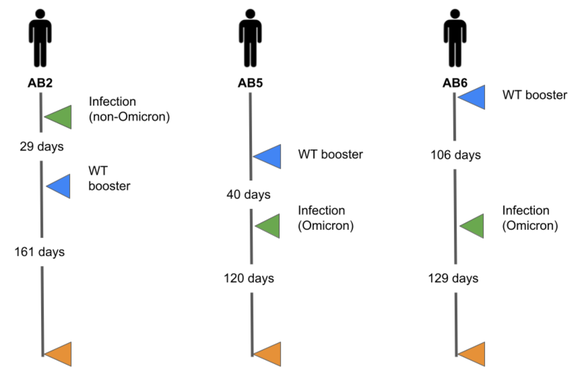
Abstract

The immune system of an individual can produce trillions of unique antibody proteins. Identifying the antibodies selected for secretion in serum is key to understanding the human immune response to disease and crucial for future antibody therapeutic development. Mass spectrometry (MS) can be used to analyze serum antibodies that are reactive to a target; however, recovering complete antibody sequences from MS requires a comprehensive database of candidate antibodies.

Alicanto® is a platform to identify high affinity antibodies present in patient serum by combining next-generation sequencing of the B cell receptor (BCR) repertoire with MS measurements of antibody proteins. In this study, we use Alicanto to identify SARS-CoV-2 antibodies from three donors who were vaccinated and naturally infected with SARS-CoV-2.

Experimental set up

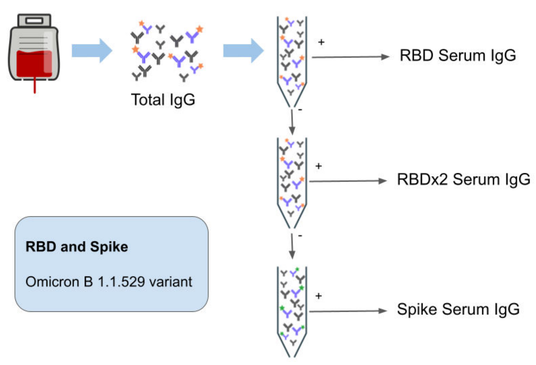
Whole blood was collected from three donors who had been vaccinated and infected with SARS-CoV-2.



Timeline of exposure to SARS-CoV-2 antigens of three donors.

Blood was collected 3+ months from the most recent infection/vaccination. Donor AB2 was only exposed to wild-type vaccines and a non-omicron natural infection. Donors AB5 and AB6 received wild-type vaccines and suspected omicron natural infections.

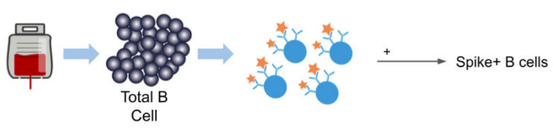
Proteomic Analysis



Strategy to purify receptor binding domain (RBD)-specific antibodies and non-RBD Spike-specific antibodies from serum.

The antibodies were reduced, alkylated with iodoacetic acid and digested with multiple proteases to generate peptides that cover the entire variable region. Peptides were analyzed by nanoLC-MS/MS with an Ultimate 300 Nano HPLC system interfaced to a Thermo Fisher Orbitrap Eclipse.

Immune Repertoire Sequencing



Strategy to enrich Spike-reactive B cells from total B cells.

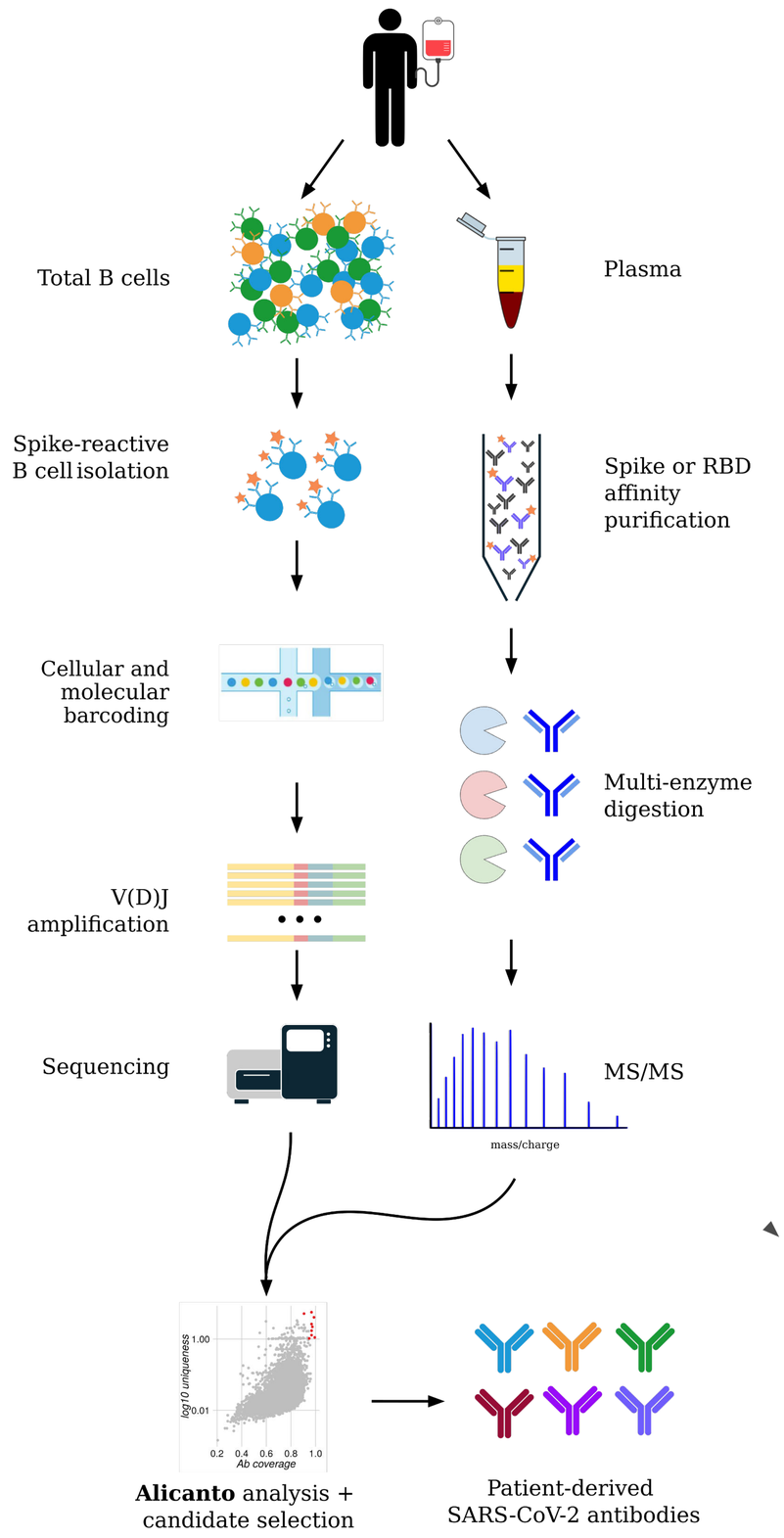
From whole blood, total B cells were collected, and enriched for cells that bind Spike-trimer coated beads. The Omicron variant B.1.1.529 was used for the enrichment. The enriched cells were then analyzed using the 10X Genomics Single Cell VDJ workflow and sequenced. In this study, we define a **clone** as a group of sequences that share the same CDR3.

About Abterra Biosciences

Check out our antibody related services:

- Alicanto®: antibody discovery directly from serum.
- Valens: monoclonal sequencing from protein service.
- Reptor: immune repertoire sequencing and analysis.

Alicanto

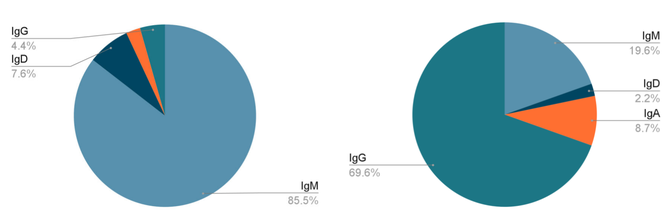


The Alicanto workflow uses both circulating B cells and serum antibodies to identify SARS-CoV-2 antibodies derived from the donors. Alicanto overlays the mass spectrometry measurements of serum antibodies onto the BCR repertoire. A subset of antibodies in the repertoire have evidence of being secreted in serum.

Alicanto Analysis

Donor	# Sequences	#Clones	#RBD clones in serum	#RBDx2 clones in serum	#Spike clones in serum
AB2	30,917	30,506	2	0	0
AB5	45,426	44,754	59	10	22
AB6	32,511	31,823	13	6	3

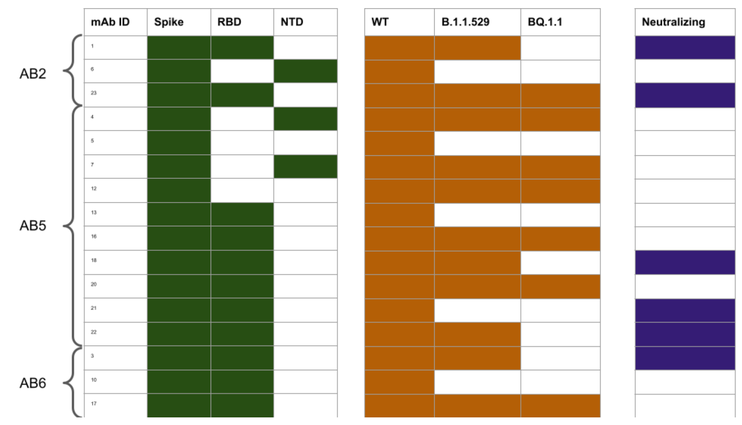
Table of heavy chain sequences identified in the BCR repertoire and the number of sequences found in the proteomic analysis. Clones are defined by unique CDR3 amino acid sequence.



Above Left: BCR repertoire isotype distribution, Above Right: Serum antibody isotypes in the BCR repertoire. The BCR repertoire predominantly expressed IgM antibodies (> 85%). The antibodies detected in serum all appeared as IgG isotype in serum, but when matched to the BCR repertoire the corresponding repertoire sequence may be expressing the antibody in a different isotype. Close to 20% of the antibodies detected in serum appeared as IgM in the BCR repertoire.

Alicanto Antibody Characterization

The antibodies present in serum are most relevant to understanding immune response to disease. We selected 24 antibodies across the donors to express recombinantly and characterize. First we tested reactivity in ELISA to Spike trimer, RBD, and N-terminal domain (NTD) for three SARS-CoV-2 variants, wild-type (WT), Omicron B.1.1.529, and Omicron BQ.1.1.

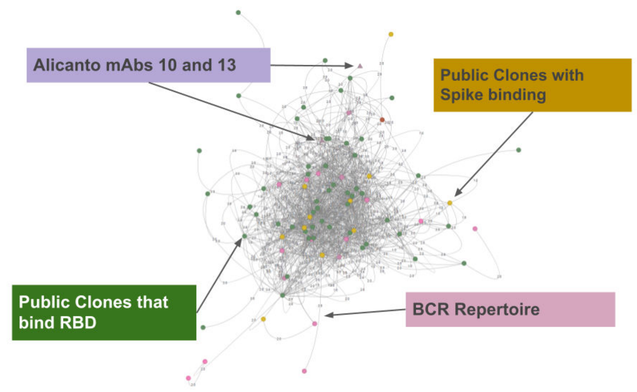


Antibodies derived from AB2 and AB5 showed binding to RBD and NTD. Two antibodies (5 and 12) showed binding to Spike trimer, but not to any subdomain. 11 Antibodies showed cross-reactivity to Omicron B.1.1.529, including antibodies 1 and 23 which were derived from donor AB2 who had never been exposed to an Omicron variant of the virus. In addition, 7 antibodies showed cross-reactivity to Omicron variant BQ.1.1, which evolved after the specimens were collected for this study.

We further tested the antibodies for neutralization using an in vitro ACE2 inhibition ELISA. Inhibition was measured against only Omicron B.1.1.529. 6 of the antibodies showed neutralization.

Public SARS-COV-2 Clones

Clones that are highly similar across individuals are called public clones, and may indicate convergent immune responses. Public clones to SARS-CoV-2 have been previously reported, and known Spike and RBD binding antibodies have been collected in the CoV-AbDab. We compared our BCR repertoire clones to the public clones requiring 80% sequence similarity of CDR3 and the same V gene. We identified 338 of our clones that cluster with public clones in CoV-AbDab.



An example cluster of clones including chracterized monoclonals 10 and 13, the BCR repertoire, and CoV-AbDab clones. Each node is a clone and an edge is drawn between nodes if are at least 80% similar and share the same V gene.

Conclusion

- *Only a subset of antigen-specific antibodies are secreted in serum.* Among over 100,000 antigen specific clones in the BCR repertoire, less than 1% are detected in serum after antigen-purification. This suggests that B cell repertoire in blood is a poor proxy for antibodies detected in serum.
- *Serum IgGs may appears as a different isotype in the B cell repertoire.* About 30% of clones identified in serum appear in the BCR repertoire as a non-IgG isotype, suggesting the B cell lineage underwent class switching.
- *Convergent clones appear across patients.* For 338 clones, we found similar clones in the Cov-AbDab suggesting convergent immune response to the virus. In addition, mAbs 10 and 13 were derived from different donors in this study and appear in a clone cluster with public sequences.