SARS-CoV-2 Neutralizing Antibodies Discovered Fast: A Novel Blocking Assay to Speed to the Finish

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Abstract

We used recombinant receptor binding domain (RBD) protein to immunize a cohort of wild-type mice and discovered antibodies using AbTheneum™ technology. AbTheneum™ output gives full-length sequences paired with binding activity against the antigen for thousands of cells simultaneously. A novel blocking assay using recombinant angiotensin-converting enzyme 2 (ACE2) was used to identify blocking antibodies during the discovery process. All antibody sequences were clustered by sequence similarity. A diverse set of blocking and non-blocking antibodies were reconstructed and validated using a pseudovirus assay with the widely prevalent D614G mutant of SARS-CoV-2. Neutralization activity was confirmed for 13 antibodies out of 15 blocking antibodies reconstructed. Two non-blocking antibodies that showed no blocking were reconstructed and had no or low neutralization activity in the pseudovirus assay. Positive controls from published sources were reconstructed and assayed alongside SCT antibodies. Eight SCT antibodies have lower IC50s than than controls. The entire process took 2.5 months from lymphocytes to neutralization assay results.

Blocking Assay Setup

It has been published by several studies that SARS-CoV-2 infects cells by the RBD on the S1 subunit of the spike protein binding with ACE2 (Figure 1). Many therapeutic efforts to target SARS-CoV-2 have focused on blocking this interaction.

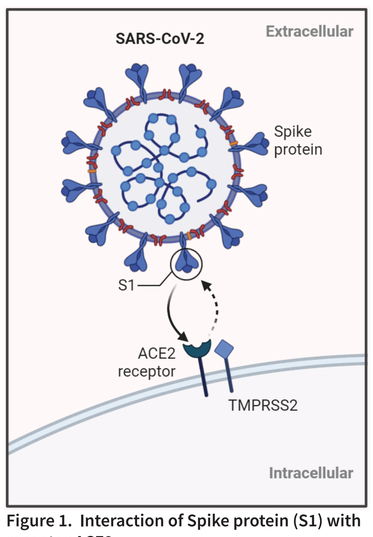

Figure 1. Interaction of Spike protein (S1) with receptor ACE2.

A differentiating feature in AbTheneum™ is to generate antibody microarrays for thousands of antibodies (Figure 2). This allows all antibodies to be assayed simultaneously. A suite of assays, called SimulScreen™, can be done on these antibody microarrays.

For this work, we have developed a novel blocking assay to be included as a part of SimulScreen™ options using recombinant receptor proteins.

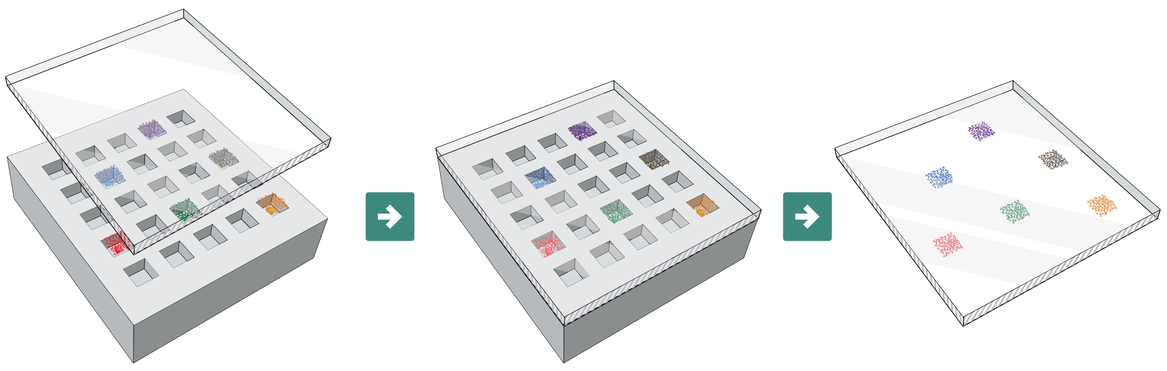

Figure 2. Generation of antibody microarray from antibody secreting cells in AbTheneum™ workflow. Antibody microarrays are assayed by SimulScreen assays such as affinity ranking, cross-reactivity, and specificity.

Figure 3 is a schematic of the novel blocking assay. First, we generated two antibody microarrays. Slide 1 identifies all secreted antibodies that bind RBD and also allow ACE2 to bind. Positive signal on Slide 1 means that the antibody secreted by the cell binds to RBD at a non-overlapping epitope with ACE2. Slide 2 identifies all secreted antibodies that bind to RBD. Positive signal on Slide 2 means the antibody secreted by the cell binds to RBD. By aligning the signals from Slides 1 and 2, we find antibodies from wells that have positive signal on Slide 2 and negative signal on Slide 1. These antibodies have an overlapping epitope with the ACE2 binding site and potential to neutralize the virus. We define antibodies with overlapping epitopes as “blocking” antibodies.

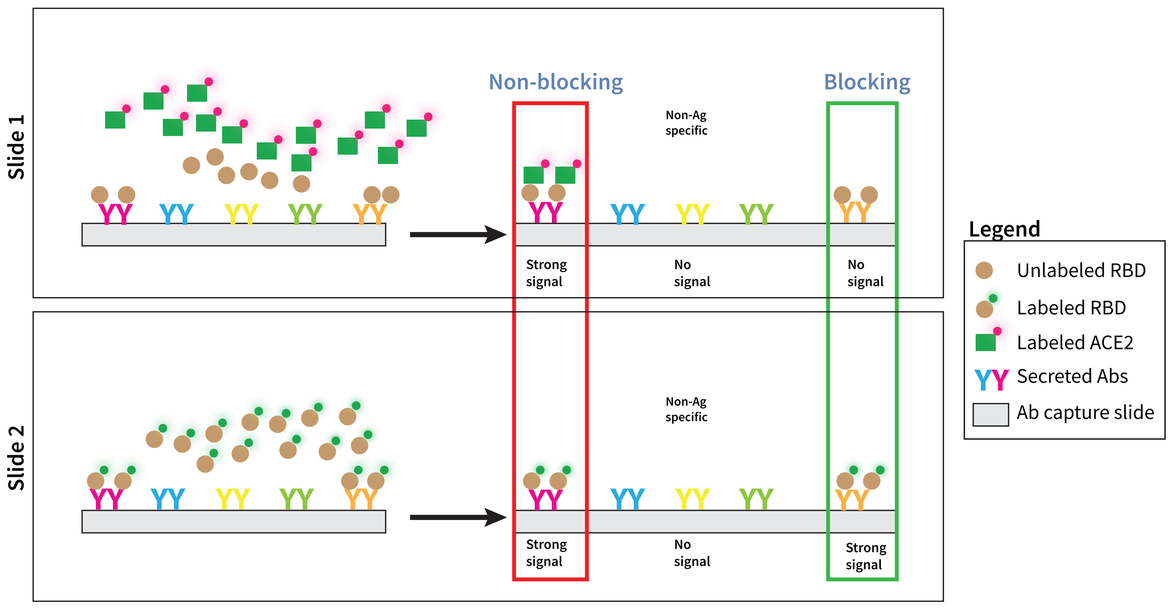

Figure 3. Novel blocking assay setup for in-line blocking assessment in the AbTheneum™ discovery workflow.

Discovery Campaign

Four wild-type mice were immunized with recombinant RBD protein (ACROBiosystems) and lymph nodes harvested after 4 week rapid immunization. Plasma cells were isolated and 20,000 cells were put into AbTheneum™ workflow. After screening captured antibodies for blocking activity (Figure 3), all cells were lysed and mRNA captured and sequenced en masse. AbTheneum™ informatics aligned blocking assay data with full length sequence pairs.

Results & Lead Selection

Over 1500 RBD-specific antibody sequences were recovered and clustered by sequence similarity. We propose that it is highly likely that similar sequence families bind to overlapping if not identical epitope of the target. We further analyzed the sequence of the discovered antibodies to compare other members within a related sequence family for blocking and non-blocking activity. A sequence family in this analysis was defined a group of sequences that has less than 10 amino acid changes from each other.

We found that similar sequence families showed similar activity in the blocking assay. In Figure 4, related clusters are shown with each antibody’s signal cutout for Slide 1 and Slide 2, labeled S1 and S2. In our assay, antibodies showing blocking activity have an overlapping epitope with the RBD-ACE2 interaction. Our sequence analysis also confirms that similar antibodies also share this overlapping epitope (or non-overlapping in the case of non-blockers).
17 diverse antibodies were selected for recombinant reconstruction and expression, maximizing sequence diversity. 2 antibodies did not express. The remaining 15 had 13 show blocking activity (shown in pink in Figure 4) in the novel SimulScreen™ blocking assay and 2 with non-blocking (shown in blue in Figure 4).

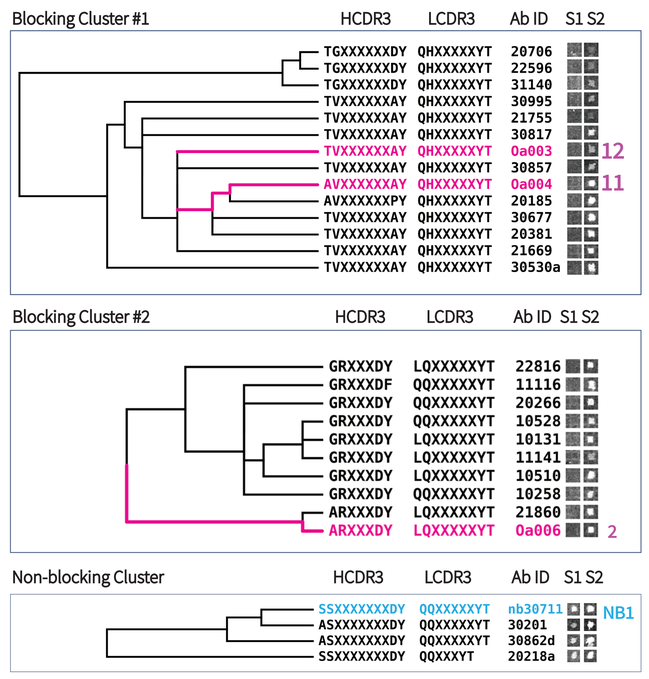

Figure 4. Three sequence clusters shown with CDR3 sequences of H and L chain pairs (full-length VH-VL was recovered), Ab ID, and Slide 1 and Slide 2 signals (S1, S2 respectively). Clusters have less than 10 amino acid changes between each other.

Validation by Pseudovirus Assay

Reconstructed antibodies were tested using SARS-CoV-2 reporter virus particles (RVPs) by Integral Molecular. The RVPs have a modified genome that expresses luciferase upon cellular infection. Luciferase was quantified using Renilla-GLO system (Promega) on the GloMax Navigator. We define antibodies that inhibit the RVPs as “neutralizing” antibodies (not to be confused with “blocking” antibodies as defined previously). We tested neutralization of the widespread D614G mutant pseudovirus construct. Published antibodies CA1 and CB6 recovered from convalescent patients (Shi et al.) were reconstructed and run as positive controls alongside SCT antibodies. Eight SCT antibodies out of the 15 blocking antibodies performed better than published controls (Figure 5). The two nonblocking antibodies showed low and no potency, as shown in Figure 5, labeled NBAb 1 and 2.

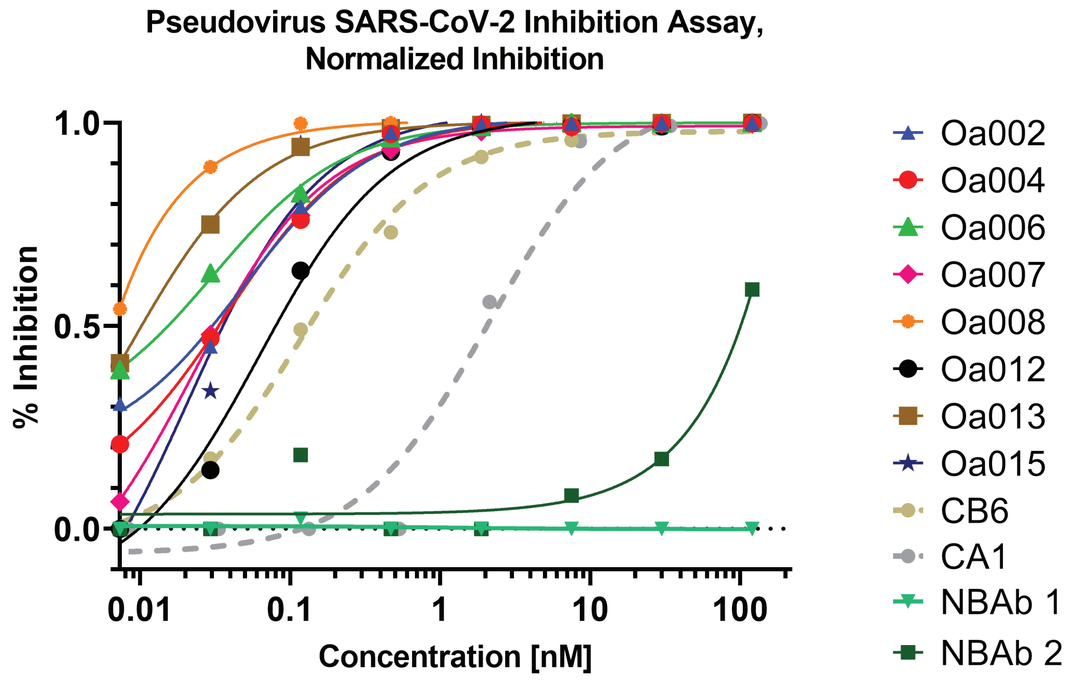

Figure 5. Pseudovirus neutralization of D614G mutant of SARS-CoV-2 for top 8 antibodies and 2 non-blocking antibodies.

Conclusions

We have discovered a set of potent neutralizing antibodies that perform better than published controls from convalescent patients. We have also demonstrated that our novel SimulScreen™ blocking assay is useful for determining blocking antibodies during the discovery process. The data shows a high correlation with blocking activity from the blocking assay and neutralization of the virus using RVPs. We further conclude that the SimulScreen™ blocking assay would be useful for other targets where a recombinant receptor can be synthesized. The SimulScreen™ assays are performed in-line during discovery, which saves downstream time.

References

Shi, R., Shan, C., Duan, X. et al. A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. Nature **584**, 120–124 (2020).

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