Striking Similarities between CDRs in Some mAbs That Neutralize COVID-19

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ABSTRACT: Four of five different monoclonal antibodies (mAbs) that have been crystallized in complex with the receptor binding domain (RBD) of the SARS-CoV-2 spike protein (S) have remarkably similar primary and secondary loop structures at the heavy chain complementarity-determining regions (HCDR) 1 and 2. All these reports give a structural basis for the deceptively difficult problem of accurate peptidomimetic loop mimic design.

KEYWORDS: SARS-CoV-2, spike protein, monoclonal antibody, complementarity-determining region

One of the natural processes that biomedicinal chemistry does not come close to emulating is the generation of antibodies to almost any antigen. Combinations of a series of loops to bind new and totally foreign substrates is impressive. However, it is almost shocking when Abs derived from different individuals arrive at almost the same answer, indicating there are only a limited number of solutions to this problem that may be selected from a vast array of possibilities.

Coronaviruses project spike proteins to bind a cell surface receptor on the host (frequently angiotensin converting enzyme 2, ACE2). There are mAbs that bind the SARS-CoV-2 spike protein (S) without neutralizing the virus, but recently the first reports of mAb’s that do block COVID-19 are emerging. For instance, a group from The Netherlands found 47D111 that binds S and impairs syncytia formation associated with fusion of the virus and host cell, i.e. function of other S-protein fragment formed after proteolytic cleavage, S2, but not in a way that blocks RBD-AE2. This research did not involve crystallographic analyses, but there are suddenly data for at least three mAbs that similarly block the COVID-19 infectivity that have been structurally characterized at high resolution. These structures include mAbs called P2B-2F6, CB6, and B38 (Figure 1). Selected data to compare structures of the three featured mAbs are in Table 1.

Competitive binding experiments featuring disruption of ACE2-RBD showed P2B-2F6 to be a good virus-blocker relative to most other mAbs tested; it could neutralize live cells bearing SARS-CoV-2 S protein with the IC50 as 0.41 μg/mL. P2B-2F6 primarily uses three loops to achieve RBD binding (Figure 1a; loops in red), where the two on the heavy chain (green) are most important. It emerges that P2B-2F6 is an outlier in this series of five mAb, the only one to bind RBD side-on relative to its interaction with ACE2. The other four mAbs are similar to each other.

P2B-2F6 was isolated from the blood of COVID-19 patients, whereas a Nature paper describes a more direct approach to find mAbs that bind S and suppress infectivity. Specifically, the procedure comprised affinity selection using S as bait for specific memory B-cells from a COVID-19 patient, amplification, variable-region sequencing of IgG mAbs in a single B cell, then FACS sorting to further select mAbs’s that block binding of RBD to hACE2 expressed on HEK293T cells; CB6 emerged from that process.

Three rhesus macaque monkeys were challenged with an infectious dose of the virus and then treated intraperitonially with CB6 on days 1–3 post infection (slightly modified form; 50 mg/kg). This experiment resulted in approximately three log viral titer reduction immediately after administration. For another cohort (also n = 3), a single dose of CB6 before SARS-CoV-2 challenge protected the monkeys from viral infection such that only minimal virus levels were detected (collected via throat swabs), indicating a powerful prophylactic effect. Post mortem pathological analyses from both the therapeutic and

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Comparison of P2B-2F6, CB6, and B38 could just have coincidently shown a similarity between two of three members of the series. Whereas P2B-2F6 is a "wing-man" insofar as it binds S1 side-on relative to the ACE2-RBD contact patch (overlaid structures in Figure 2a), relatively, binding of CB6 and B38 to RBD occurs "head-on" to directly obstruct complexation with ACE2. Overlays Figure 2b and c visually explain why CB6 and B38 can block binding of the spike protein to ACE2 in vivo to dampen effects of viral challenge, though this is not quite so clear from the overlays in Figure 2a featuring P2B-2F6. After this, however, comes a surprise.

While this article was in preparation, another paper appeared, which describes two more mAbs that bind S1: CC12.1 and CC12.3 (Kd 17 and 14 nM, respectively).5 Remarkably, these mAb-RBD complexes have similar overall structures to those derived from CB6 and B38. The four structurally similar complexes (from CB6, B38, CC12.1, and CC12.3) use similar residues to bind the RBD epitope (Table 2). In fact, there is a strikingly close correspondence between the interface residues in HCDR1 and 2 for these structures.

Figure 3 focuses on the CB6, B38, CC12.1, and CC12.3 HCDRs (this graphic does not involve P2B-2F6 because it is clearly different). HCDR1s and HCDR2s from the four Abs' overlay closely, as might be expected from the sequence correspondences shown in Table 2. Structural similarities between the loops contacting the RBD and the amino acids that comprise those loops is close. It is amazing to researchers (like us) who deal with mAbs less than experts in the protein chemistry involving loops at protein interfaces has far to develop to answer questions like these.

Table 1. Key Data for Three Structurally Characterized mAbs’s That Bind SARS-CoV-2 S RBD

| mAb   | PDB | resolution (Å) | Kd (nM) | IC50 (μg/mL) |
|-------|-----|----------------|---------|--------------|
| P2B-2F6 | 7BWJ | 2.85           | 5.14    | 0.41         |
| CB6   | 7C01 | 2.88           | 2.49 ± 1.65 | 0.056 ± 0.007 (ND<sub>o</sub>) |
| B38   | 7BZ5 | 1.84           | 70.1     | 0.177        |

“From surface plasmon resonance.” From BLI.
Table 2. Residues the Five mAbs Use to Contact SARS-CoV-2 S RBD as Specified in the Three Citations

| mAb  | CDR     | Residues                  | CDR     | Residues       |
|------|---------|---------------------------|---------|----------------|
| CB6  | GFTVSSNY| YSGGSTF                   | RVLPYMYGYLDLY | SISRY          |
| B38  | GFIVSSNY| YSGGSTY                   | REAYGMD  | QGIGSYY        |
| CC12.1| GLTVSSNY| YSGGSTF                   | RDDLWYGGLDV | QGIGSYY        |
| CC12.3| GFTVSSNY| YSGGSTF                   | RDFDPFYGFDY | SVSSY          |
| P2B-2F6 |        | GIVVVPAAGRR                | GYNY     |                |

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Notes
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ABBREVIATIONS
hmAb, human monoclonal antibody; FACS, fluorescence-activated cell sorting

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Figure 2. (a) P2B-2F6 (7BWJ) overlaid with ACE2-RBD (PDB code: 6M0J). (b) CB6 (7C01) overlaid with ACE2-RBD. (c) B38 (7BZ5) overlaid with ACE2/RBD complex.

Figure 3. Overlaid HCDR loops 1–3 from CB6 (red), B38 (blue), CC12.1 (magenta), and CC12.3 (cyan).

Figure 4. Overlaid HCDR loops 1–3 from CB6 (red), B38 (blue), CC12.1 (magenta), and CC12.3 (cyan).
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