nature research

Peer Review File

Manuscript Title: Potent Neutralizing Antibodies Directed to Multiple Epitopes on SARS-CoV-2 Spike

Editorial Notes:

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referee #1 (Remarks to the Author):

This is an outstanding manuscript that compliments similar manuscript now available on Bioarchive or coming out in competitor journals. The manuscript re-enforces the emerging observation that most or all neutralizing antibodies target the top of the S protein, perhaps because access to the stalk regions is limited by dense packing of the spikes on the virion, and shows that the NTD can be neutralizing. The structures provided add further insight for vaccine developers as well as those engineering or selecting antibodies for passive immunization.
I only have suggestions for text modifications.
1. Figure 1 and Figure 2b: It would be helpful to see a plots between RBD-bind and pseudovirus, and also pseudovirus vs live-virus neutralization in supplementary material to understand the relationship between these assays, especially if the distinction between RBD, NTD, and other are indicated in these plots.
2. Would 2-4 or 4.8 engage with both arms to a single trimer?
3. The VH, DH, and JH, and light-chain VJ sources of the 2-4, 4-8, 2-43 could be made clear in the text.
4. Extended Data Fig. 2 could be improved by somehow making clear if genetic biases (IGHV3-30 and IGKV3-20) associated with RBD or NTD binding.
5. Similarly, not clear if these particular gene contributed to high-potency antibodies, or what the structural bases for these biases might be.
6. It is unfortunate that most publications of this nature do not provide sequence information on the antibodies they have discovered.

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This manuscript describes the most complete characterization of human monoclonal antibodies isolated from covid-19 patients that I have seen so far. The paper shows that the neutralizing antibodies target the top of the spike, and not only the RBD. They provide structural data by single-particle cryo-EM on the mode of binding of three of these neutralizing antibodies: 1- One mAb binding the RBD (mAb 2-4) does so in a closed conformation of the spike (S) protein trimer at an overall resolution of 3.25 Å. 2 - Another important and novel antibody (mAb 4-8) targets the N-terminal domain (NTD), and apparently does not interfere with the RBD adopting the “up” or “down” conformations, the former required to bind the ACE2 receptor. The paper provides a cryo-EM structure at 3.9Å of mAb 4-8 complex with the trimeric spike. 3 - The third antibody characterized also binds at the top of the spike, in a region between the RBD and the NTD. Oddly enough, this antibody (mAb 2-43) did not show binding to soluble trimeric S on an ELISA test, but did bind intact S on membranes. The authors were able to obtain a moderate resolution (7.8Å) cryo-EM structure of its complex with the soluble trimeric S. Competition experiments, together with the structural data, allowed a very useful map of the antigenic regions of the spike. The structural data are of good quality, and the results are very important given the current covid-19

epidemy.
There is one issue that the authors need to address: they used the spike construct that led to the first cryo-EM structure of the SARS-CoV-2 protein (Wrapp et al, Science 2020), which has a double proline mutation that had been identified previously, in studies with MERS-CoV and SATS-CoV-1, which apparently stabilizes the spike in its pre-fusion conformation. Yet, a recent report posted in bioRxiv (https://doi.org/10.1101/2020.05.16.099317) describes the cryo-EM structure of detergent-solubilized full-length S protein with no mutations. This preprint reports that the double proline mutation induces a change at the top of the spike such that the NTD adopts a different conformation with respect to the rest, and that there is also higher mobility of the RBD. It is therefore important that the authors look at the binding to a spike that does not have the double-proline mutation, as the alternative conformations of the NTD may affect the epitope of 2-43. Using a version of the spike without the double proline mutation could probably result in a higher resolution structure as well.
Minor issues
Line 85: Does "VL" in this sequence stands for both λ and κ genes for the light chain?
Figure 3a: The legend does not explain the double arrows and the letter code used underneath the checkerboard heat maps. After reading the text, it becomes clear that the letters denote the various clusters observed, but the Figure could be made more standalone.
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This study has several merits that justify publication in Nature. First, the collection of antibodies described is both diverse and comprehensive. The Authors comment on the individual differences of the response and the importance of donor selection. Second, this study identifies, for the first time, the NTD as the target of several potent neutralizing antibodies, a finding that is relevant for vaccine design. Third, and again for the first time in the context of SARS-CoV-2, this study identifies quaternary epitopes as targets of potent neutralizing antibodies.

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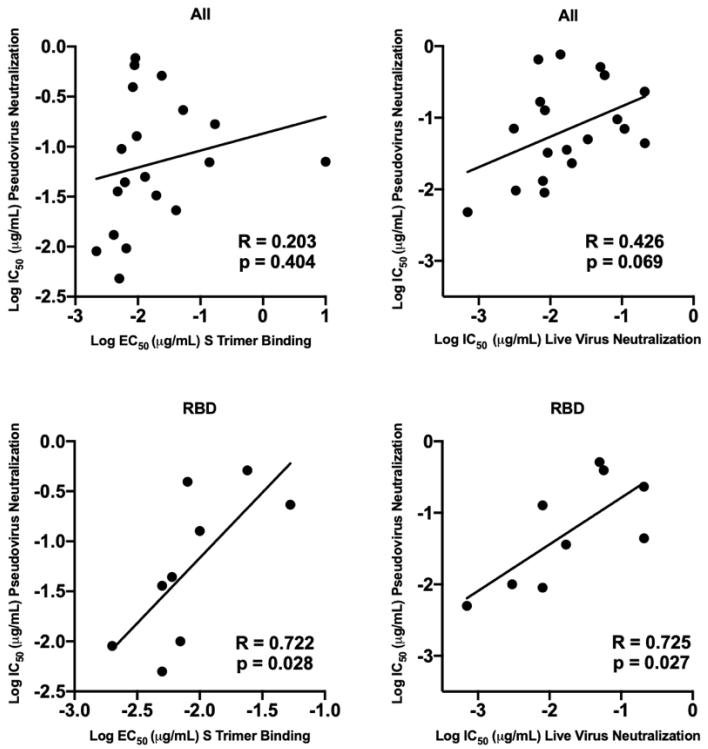

2. Would 2-4 or 4.8 engage with both arms to a single trimer? We are also interested in answering this question. However, the answer would require another series of cryo-EM studies, which are beyond the scope of this initial report.

3. The VH, DH, and JH, and light-chain VJ sources of the 2-4, 4-8, 2-43 could be made clear in the text. There are numerous technical details on antibody gene usage that are important. There is simply no room in the current manuscript to cover these fine details. We have deposited in GenBank the gene sequences for antibodies characterized in the current report. The requested information will be

available to interested readers in 48 hours. The accession numbers will be provided when ready.

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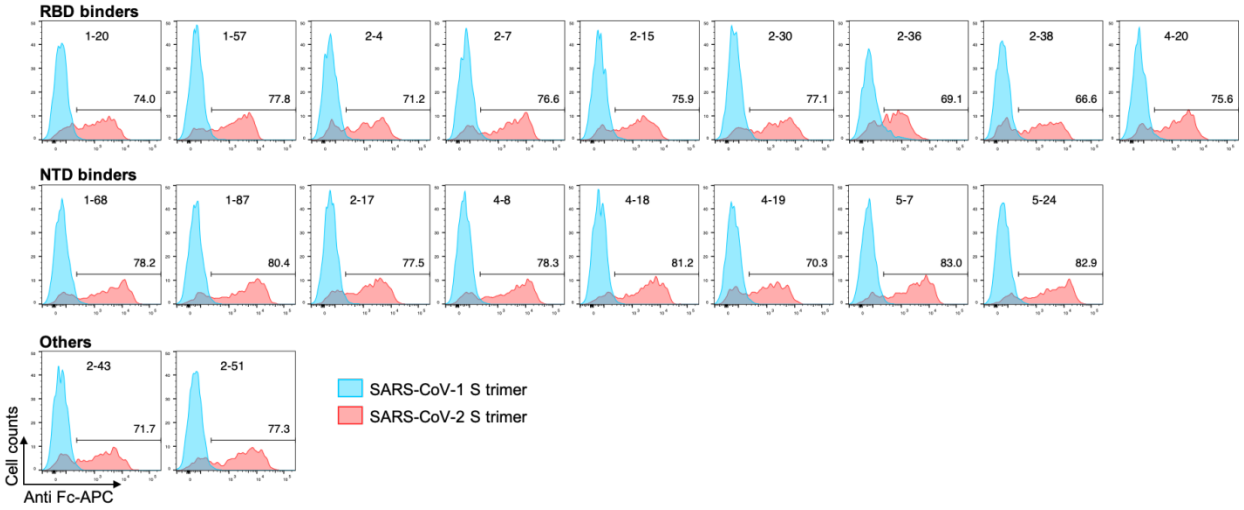
however, that our 2-43 competition experiments already utilized full-length spike on the cell surface, and all of our key neutralizing monoclonal antibodies bind to that S trimer without the two proline mutations. Those results are pasted below and have been added as Extended Data Fig. 6a. We also note that in the virus neutralization assays, the pseudovirus and the authentic virus possess spikes without mutations. Nonetheless, we do agree that it would be valuable in the long run to obtain structural information using the authentic, unmodified S trimer. But that's not achievable without spending months to do so.



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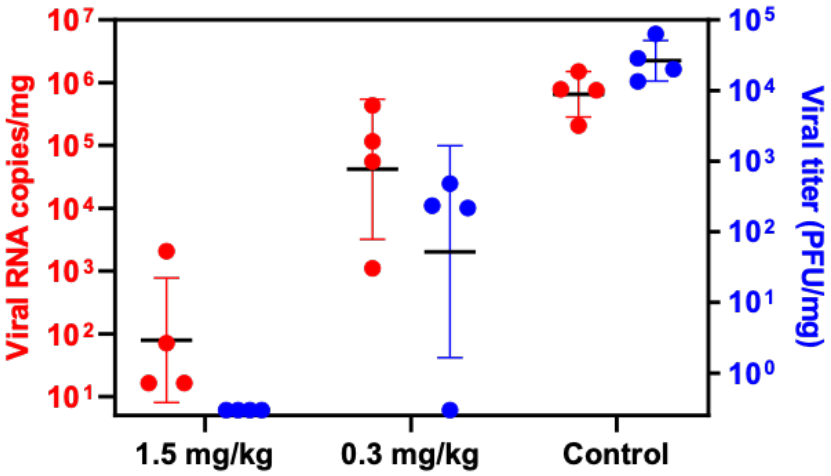
Minor comments:

The Authors may comment on the mechanism of neutralization and on the high frequency of non-neutralizing antibodies. We have already commented on the RBD-directed antibodies. Most of them are direct competitive inhibitors of receptor binding. At this point, we do not have an explanation for how NTD-directed mAbs neutralize, as stated in the text. As implied by the text and by Extended Data Table 2, about half of the S-directed mAbs are non-neutralizing. There are obviously multiple explanations for this: low affinity, epitope location, occlusion of epitope on the virion, falling off at endosomal pH, etc. However, given that we are only beginning to address this question experimentally, we are loathed to speculate, especially given the length restriction on the manuscript.

The Authors may consider analyzing the antibodies for their capacity to elicit effector function such as ADCC or ADCP. We will, of course, address these functional properties of our key mAbs, but that too is beyond the scope of this initial report.

In addition to the changes mentioned above, we have trimmed the text at various places and eliminated non-essential statements. We have also updated the cryo-EM structure on mAb 2-43 with one at a higher resolution. Once again, the antibody is found to recognize a quaternary epitope. However, the higher resolution structures show that the antibody is primarily binding to two RBDs on separate protomers. The text has been modified to reflect the new conclusion. In addition, the Extended Data Fig. 8 panels on 2-4 Fab-trimer structures have been updated as well.

Finally, as the editor has suggested, we have added a very small figure (below and new Fig. 5) and a brief corresponding text to summarize the first in vivo experiment showing the protective effect of our most potent mAb, 2-15, in the hamster model of SARS-CoV-2 infection.


Viral RNA copies/mg
10^7
10^6
10^5
10^4
10^3
10^2
10^1
Viral titer (PFU/mg)
10^5
10^4
10^3
10^2
10^1
10^0
1.5 mg/kg
0.3 mg/kg
Control