Antibody-mediated disruption of the SARS-CoV-2 spike glycoprotein

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Abstract

The CR3022 antibody, selected from a group of SARS-CoV-1 monoclonal antibodies for its ability to cross-react with SARS-CoV-2, has been examined for its ability to bind to the ectodomain of the SARS-CoV-2 spike glycoprotein. Using electron cryo-microscopy we show that antibody binding requires rearrangements in the S1 domain that result in dissociation of the spike.

Full Text

The spike (S) membrane glycoproteins of SARS-CoV-2, like those of other coronaviruses, are responsible for receptor binding and membrane fusion at the beginning of virus replication¹. They are also the antigens recognised by antibodies that neutralise virus infectivity. Because of the urgency of the pandemic and the similarities between SARS-CoV-1 and SARS-CoV-2², monoclonal antibodies prepared from convalescent patients at the time of the SARS-CoV-1 outbreak³ have been tested for their reactivities with SARS-CoV-2 S. Among them CR3022 was found to target the receptor-binding domain (RBD) of the spike⁴, which, together with the N-terminal domain (NTD), forms the main antigenic surface of the S1 domain. S from SARS-CoV-1 and SARS-CoV-2 share about 77% amino acid identity and bind the same receptor, ACE2². A recent crystal structure⁵ of the complex of the SARS-CoV-2 RBD and the Fab of CR3022 indicates that the antibody binds to a conserved region of the RBD, which does not overlap with the receptor-binding surface. Trimeric S on the virus surface is thought to adopt two main conformations: closed, in which much of the RBD surface is buried inside the trimer and unable to bind the receptor ACE2, and open, in which one RBD protrudes from the trimer and can bind the receptor^{6,7}. Neither of these conformations is able to accommodate the binding of CR3022. To investigate how CR3022 interacts with intact S of SARS-CoV-2 we have used binding and neutralisation assays and studied the structure of the complex by electron cryo-microscopy. Our data indicate that CR3022 binding accompanies dissociation of the S trimer. We also find that CR3022 does not neutralise SARS-CoV-2.

We expressed CR3022 Fab and the stabilised, trimeric ectodomain of the SARS-CoV-2 S in human cells and performed biolayer interferometry to investigate Fab binding to immobilised trimers. Analysis of CR3022 binding provides a dissociation constant (K_d) of 80 nM towards the full trimeric spike (**Fig. S1**). This value is similar to those reported before in experiments measuring Fab binding to isolated RBD^{4,5}.

We used cryo-EM to determine the molecular basis of S recognition by CR3022 (**Fig. 1**). The single particle reconstructions generated a map with a global resolution of 3.7 Å resolution (**Fig. S2**) and enabled the description of all the domains of S1 and unbiased building of the Fab and RBD. The high resolution of the data is partly accounted for by the formation, over time, of an adventitious but stable dimer of two CR3022/S1 complexes (**Fig. S2**). The reconstruction shows that the CR3022 Fab binds an epitope on the RBD that is not accessible in either the open or closed forms of trimeric S. These data are consistent with the results from the crystal structure of CR3022 Fab bound to the spike RBD⁵. Binding is made possible by the dissociation of the trimeric S into monomers presumably driven by the binding

energy of the Fab. Dissociation also results in the S2 domain having no interaction surface with S1 so the two remain being covalently tethered but rotationally independent of each other. The S2 domain is not visualised in the reconstruction.

The structure of the S1 domain, to which the Fab is bound, has undergone significant rearrangements (**Fig. 1**). The RBD has rotated by about 30 degrees, the adjacent intermediate domain by somewhat less and the entire protein has extended to accommodate the Fab (**Fig. 1**). The overall interaction area between the Fab and RBD is 930 Å², of which 590 Å² and 340 Å² are contributed by the interaction between the CDRs of the heavy and light chains respectively. A second, smaller interface is observed between the VL chain of the Fab with the NTD and accounts for an additional 270 Å² of interaction surface.

The most substantive component of the interface between CR3022 and the RBD involves the heavy chain of the Fab interacting with one short loop of the RBD (residues 368:370) and a second longer loop (residues 374:386) (**Fig. 2a&b**). The interface of these two loops presents a strongly hydrophobic interaction to the Fab flanked (top and bottom in **Fig. 2b**) by electrostatic interactions. The light chain of the Fab makes two smaller loop interactions; residues Phe-429 and Thr-430 of the RBD make hydrogen bond interactions (**Fig. 2c**) while Ile-39 and Trp-56 make hydrophobic interactions (**Fig. 2d**). Finally, a loop on the NTD (residues 42:45) makes a series of potential hydrogen bond interactions with the VL chain of CR3022 as well as a hydrophobic contact between Val-44 of NTD with the aliphatic moiety of the side-chain of an arginine from the Fab (**Fig. 2e**).

There are two reasons for Fab binding requiring the breakdown of the S trimer. First, the conformation in which S1 binds to the Fab cannot be accommodated in either the closed or open forms of the trimer. Second, the way the Fab binds to the RBD/NTD would generate considerable steric clashes within the trimeric S (**Fig. 1**). To look at the timescale of CR3022-S1 complex formation we collected data sets at different times after mixing: 60 seconds, 5 minutes, and 40 hours. There was no evidence in any of the samples of trimeric S bound to Fab (**Fig. S2**), suggesting that the binding event rapidly captures a transient, probably poorly populated, species of S that is able to bind Fab, resulting in the disruption of the SARS-CoV-2 S trimer. Whether the trimer is permanently dissociated with this S construct, that does not have a basic cleavage site between the S1 and S2 chains, is not accessible from either our structural or biophysical studies. However, biologically, when S cleavage has resulted in the formation of S1 and S2 chains, CR3022 binding would be expected to lead at least to release of S1 as an S1-Fab complex, from membrane-associated S2.

To further characterise CR3022 we carried out plaque reduction neutralisation assays. Even though the experimental design compensates for the substantially weaker binding of CR3022 Fab to SARS-CoV-2 than to SARS-CoV-1, in terms of fraction bound, our data show no sign that CR3022 neutralises SARS-CoV-2 (**Fig. S3**). While this result is in agreement with data reported previously for CR3022 binding to SARS-CoV-2⁵ it is in contrast to data with SARS-CoV-1 and perhaps surprising given the molecular mechanism described here. We suggest that a potential explanation for these observations relates to the

kinetics of the binding of CR3022 to the two different viruses. Mechanistically the “off” rate for SARS-CoV-2 suggests a short half-life for the S-CR3022 Fab complex that could account for the failure of the Fab to neutralize the virus.

Declarations

Author Contributions

A.G.W., D.J.B., S.H., R.H., S.R.M., C.R. performed research, collected and analysed data; A.G.W., D.J.B., P.B.R., J.J.S., S.J.G. conceived and designed research and wrote the paper.

Conflict Statement

We have no conflicts of interest to declare.

Data Availability

Maps and models have been deposited in the Electron Microscopy Data Bank, <http://www.ebi.ac.uk/pdbe/emdb/> (Accession numbers XXX and XXX). Models have been deposited in the Protein Data Bank, <https://www.ebi.ac.uk/pdbe/> (PDB ID codes XXX and XXX). [Accession numbers will be available before publication].

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Figures

Figure 1

Structure of CR3022 Fab bound to S in space-filling representation. Column on the left shows the native S, while that on the right shows S with CR3022 Fab bound. The top row shows a monomer of S1 without and with CR3022. The individual subunits are labelled and coloured according to the bar diagram at the bottom of the figure. The second row shows a full S1/S2 trimer in the same orientation as the top panel with one monomer coloured according to the top panel with the other S subunits in grey. Where part of a

coloured subunit is obscured by a grey subunit the surface is mottled; where there is a steric clash the surface of the coloured subunit is hatched. The third and fourth rows are orthogonal views of the top two rows with the axes of the trimers vertical. The S2 chain associated with the coloured S1 chain is shown in red.

Figure 2

Interactions between CR3022 Fab and SARS-CoV-2 spike protein. (A) Ribbon representation of the interactions between CR3022 Fab (with light chain in yellow and heavy chain in green) and the S1 domain of SARS-CoV-2 spike (coloured according to Fig. 1). (B) Zoom view of the interaction between the Fab heavy chain, shown as a surface representation coloured according to surface potential, and, a worm-and-side-chain representation of two loops of RBD (residues 379-386 and 368-370) shown in pink. There is a notable hydrophobic component in the centre of the interaction with a number of electrostatic interactions on the outside. (C-E) Interactions between CR3022 light chain (yellow) with RBD (C and D) and NTD (E).

Supplementary Files

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