**CORONAVIRUS**

**Structural basis for neutralization of SARS-CoV-2 and SARS-CoV by a potent therapeutic antibody**

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The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in an unprecedented public health crisis. There are no approved vaccines or therapeutics for treating COVID-19. Here we report a humanized monoclonal antibody, H014, that efficiently neutralizes SARS-CoV-2 and SARS-CoV pseudoviruses as well as authentic SARS-CoV-2 at nanomolar concentrations by engaging the spike (S) receptor binding domain (RBD). H014 administration reduced SARS-CoV-2 titers in infected lungs and prevented pulmonary pathology in a human angiotensin-converting enzyme 2 mouse model. Cryo-electron microscopy characterization of the SARS-CoV-2 S trimer in complex with the H014 Fab fragment unveiled a previously uncharacterized conformational epitope, which was only accessible when the RBD was in an open conformation. Biochemical, cellular, virological, and structural studies demonstrated that H014 prevents attachment of SARS-CoV-2 to its host cell receptors. Epitope analysis of available neutralizing antibodies against SARS-CoV and SARS-CoV-2 uncovered broad cross-protective epitopes. Our results highlight a key role for antibody-based therapeutic interventions in the treatment of COVID-19.

The receptor-binding domain (RBD) followed by virus-host membrane fusion (1, 2). Abrogation of this crucial role played by the S protein in the establishment of an infection is the main goal of neutralizing antibodies and the focus of therapeutic interventions as well as vaccine design (3–6). Several previously characterized SARS-CoV-neutralizing antibodies (NAbs) were demonstrated to exhibit very limited neutralization activities against SARS-CoV-2 (7–9). Among these, CR3022, a weakly neutralizing antibody against SARS-CoV, is tight binding, but non-neutralizing for SARS-CoV-2, indicative of possible conformational differences in the neutralizing epitopes (9). More recent studies have reported two SARS-CoV-neutralizing antibodies, 47D11 and S309, that have been shown to neutralize SARS-CoV-2 as well as SARS-CoV-2 RBD and SARS-CoV RBD, respectively (10, 11), suggesting that broad cross-neutralizing epitopes exist within lineage B. Convalescent plasma containing SARS-CoV-2 NAbs have been shown to confer clear protection in COVID-19 patients (12, 13), yet gaps in our knowledge concerning the immunogenic features and key epitopes of SARS-CoV-2 have hampered the development of effective immunotherapeutics against the virus.

The RBDs of SARS-CoV and SARS-CoV-2 have an amino acid sequence identity of around 75%, raising the possibility that RBD-targeting cross-neutralizing NAbs could possibly be identified. Using a phage display technique, we constructed an antibody library that was generated from RNAs extracted from peripheral lymphocytes of mice immunized with recombinant SARS-CoV RBD. SARS-CoV-2 RBD was used as the target for screening the phage antibody library for potential hits. Antibodies that showed tight binding for SARS-CoV-2 RBD were further propagated as chimeric antibodies and tested for neutralizing activities with a vesicular stomatitis virus (VSV)–based pseudotyping system (fig. S1) (14). Among the antibodies tested, clone 014, which showed potent neutralizing activity against SARS-CoV-2 pseudovirus, was humanized and named H014. To evaluate the binding affinities, we monitored real-time association and dissociation of H014 binding to either SARS-CoV-2 RBD or SARS-CoV RBD using the OCTET system (Fortebio). Both H014 immunoglobulin G (IgG) and Fab fragments exhibited tight binding to both RBDs with comparable binding affinities at subnanomolar concentrations for SARS-CoV-2 RBD and SARS-CoV RBD, respectively (Fig. 1A and fig. S2). Pseudovirus neutralization assays revealed that H014 has potent neutralizing activities: a 50% neutralizing concentration value (IC50) of 3 nM and 1 nM against SARS-CoV-2 and SARS-CoV pseudoviruses, respectively (Fig. 1B). Plaque-reduction neutralization test (PRNT) conducted against an authentic SARS-CoV-2 strain (BetaCoV/Beijing/AMMS01/2020) verified the neutralizing activities with an IC50 of 38 nM, 10-fold lower than those observed in the pseudotyping system (Fig. 1C). We next sought to assess in vivo protection efficacy of H014 in our previously established hACE2 humanized mouse model that was sensitized to SARS-CoV-2 infection (15). In this model, as a result of hACE2 expression on lung cells, SARS-CoV-2 gains entry into the lungs and replicates as in a human disease, exhibiting lung pathology at 5 days post-infection (dpi). hACE2-humanized mice were treated by intraperitoneal injection of H014 at 50 mg per kilogram of body weight either 4 hours after (one dose, therapeutic) or 12 hours before and 4 hours after (two doses, prophylactic plus therapeutic) intranasal infection with 5 × 104 plaque-forming units (PFU) of SARS-CoV-2 (BetaCoV/Beijing /AMMS01/2020). All challenged animals were sacrificed at day 5. Whereas the viral loads in the lungs of the phosphate-buffered saline (PBS) group (control) increased rapidly to ~107 RNA copies/g at day 5 (Fig. 1D), in the prophylactic and prophylactic plus therapeutic groups, H014 treatment resulted in a ~10-fold and 100-fold reduction of viral titters in the lungs at day 5, respectively (Fig. 1D). Lung pathology analysis showed that SARS-CoV-2 caused mild interstitial pneumonia characterized by inflammatory cell infiltration, alveolar septal thickening, and distinctive vascular system injury upon PBS treatment. By contrast, no obvious lesions of alveolar epithelial cells or focal hemorrhage were observed in the lung sections from mice that received H014 treatment (Fig. 1E), indicative of a potential therapeutic role for H014 in treating COVID-19.

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The overall structure of SARS-CoV-2 S trimer resembles that of SARS-CoV and other coronaviruses. Each monomer of the S protein is composed of two functional subunits. The S1 subunit binds the host cell receptor, whereas the S2 subunit mediates fusion of the viral membrane with the host cell membrane (2, 16). The four domains within S1 include N-terminal domain (NTD), RBD, and two subdomains (SD1 and SD2), the latter of which are positioned adjacent to the S1 and S2 cleavage site. Hinge-like movements of the RBD give rise to two distinct conformational states referred to as "close" and "open," where close corresponds to the receptor-inaccessible state and open corresponds to the receptor-accessible state, which is reported to be metastable (17–20). Cryo–electron microscopy (cryo-EM) characterization of the stabilized SARS-CoV-2 S ectodomain in complex with the H014 Fab fragment revealed that the complex adopts three distinct conformational states, corresponding to one RBD open + two RBDS closed (state 1), two RBDS open + one RBD closed (state 2), and all three open RBDS (state 3) (Fig. 2A). The structure of the completely closed (state 4) SARS-CoV-2 S trimer without any Fab bound was also observed during three-dimensional (3D) classification of the cryo-EM data, albeit in the presence of excessive Fab, suggesting that the binding sites of H014 are exposed through protein “breathing” followed by a stochastic RBD movement (Fig. 2A). We determined asymmetric cryo-EM reconstructions of the four states at 3.4 to 3.6 Å (figs. S3 to S6 and table S1). However, the electron potential maps for the binding interface between RBD and H014 are relatively weak, owing to conformational heterogeneity. To solve this problem, we performed focusing classification and refinement by using a “block-based” reconstruction approach to further improve the local resolution up to 3.9 Å, enabling reliable analysis of the interaction mode (figs. S5 and S6). Detailed analysis of the interactions between H014 and S was done using the binding interface structure.

H014 recognizes a conformational epitope on one side of the open RBD, only involving protein–protein contacts, distinct from the receptor-binding motif (RBM) (Fig. 2B). The H044 paratope constitutes all six complementary-determining region (CDR) loops (CDRL1 to -3 and CDRH1 to -3) and the unusual heavy-chain framework (HF-R, residues 58 to 65) that forges tight interactions with the RBD, resulting in a buried area of ~1000 Å² (Fig. 2C). Variable domains of the light chain and heavy chain contribute ~32 and 68% of the buried surface area, respectively, through hydrophobic and hydrophilic contacts. The H014 epitope is composed of 21 residues, primarily located in the α2-β2-η2 (residues 368 to 386), δ3 (residues 405 to 408 and 411 to 413), α4 (residue 439), and η4 (residues 503) regions, which construct a cavity on one side of the RBD (Fig. 2, B and D, and fig. S7). The 12-residue-long CDRH3 inserts into this cavity, and the hydrophobic residue (YDPTVYM)–enriched CDRH3 contacts the η3 and edge of the five-stranded β-sheet (P2) region of the RBD (Fig. 2D). Tight binding between the RBD and H014 is primarily due to extensive hydrophobic interactions contributed by two patches: one formed by F54 from CDRH2; Y101 from CDRH3; and A411, P412, and Y508 of the RBD, and the other composed of Y49 from CDR2; P103, Y104, and Y105 from CDRH3; and V407, V503, and Y508 of the RBD (Fig. 2E and table S2). Additionally, hydrophilic contacts from CDRH1 and HF-R further enhance the RBD-H014 interactions, leading to an extremely high binding affinity at subnanomolar concentration at temperatures of 25° or 37°C (Fig.
Fig. 2. Cryo-EM structures of the SARS-CoV-2 S trimer in complex with H014. (A) Orthogonal views of SARS-CoV-2 S trimer with three RBDs in the closed state (left), one RBD in the open state and complexed with one H014 Fab (middle), two RBDs in the open state and each complexed with one H014 Fab. NTD, N-terminal domain. All structures are presented as molecular surfaces with different colors for each S monomer (cyan, violet, and yellow), and the H014 Fab light (hot pink) and heavy (purple-blue) chains. (B) Cartoon representations of the structure of SARS-CoV-2 RBD in complex with H014 Fab with the same color scheme as in (A). Residues that constitute the H014 epitope and the RBM are shown as spheres and colored in green and blue, respectively. The overlapped residues between the H014 epitope and the RBM are shown in red. (C and D) Interactions between the H014 and SARS-CoV-2 RBD. The CDRs of the H014 that interact with SARS-CoV-2 RBD are displayed as thick tubes over the cyan surface of the RBD (C). The H014 epitope is shown as a cartoon representation over the surface of the RBD (D). (E) Details of the interactions between the H014 and SARS-CoV-2 RBD. Some residues involved in the formation of hydrophobic patches and hydrogen bonds are shown as sticks and labeled. Color scheme is the same as in (A). Abbreviations for the amino acid residues: A, Ala; D, Asp; F, Phe; G, Gly; K, Lys; L, Leu; N, Asn; P, Pro; Q, Gln; S, Ser; T, Thr; V, Val; and Y, Tyr.

2E and fig. S8). Residues that constitute the epitope are mostly conserved, with three single-site mutants (R406I, N439K, and V503F) in this region among currently circulating SARS-CoV-2 strains reported (fig. S9). In addition, a number of SARS-CoV-2 isolates bear a common mutation, V367F, in the RBD (21), which lies adjacent to the major epitope patch α2-β2-β2. Constructs of the recombinant RBD harboring point mutations of the above-mentioned residues and other reported substitutions exhibited an indistinguishable binding affinity for H014 (fig. S10), suggesting that H014 may exhibit broad neutralization activities against SARS-CoV-2 strains currently circulating worldwide. Of the 21 residues in the H014 epitope, 17 (81%) are identical between SARS-CoV-2 and SARS-CoV (fig. S9), which explains the cross-reactivity and comparable binding affinities.

To investigate whether H014 interferes with the binding of RBDS of SARS-CoV-2 or SARS-CoV to ACE2, we performed competitive binding assays at both protein and cellular levels. The enzyme-linked immunosorbent assay (ELISA) indicated that H014 competed with recombinant ACE2 for binding to RBDS of SARS-CoV-2 and SARS-CoV with EC_{50} values of 0.7 and 5 nM, respectively (Fig. 3A). Additionally, H014 efficiently blocked both the attachment of SARS-CoV-2 RBD to ACE2-expressing 293T cells and the binding of recombinant ACE2 to SARS-CoV-2 S expressing 293T cells (Fig. 3B). To verify its potential full occlusion on trimeric S, we conducted two sets of surface plasmon resonance (SPR) assays by exposing the trimeric S first to H014 and then to ACE2 or vice versa. As expected, binding of H014 completely blocked the attachment of ACE2 to trimeric S. Moreover, ACE2 could be displaced from trimeric S and replaced by H014 (Fig. 3C). To further verify these results in a cell-based viral infection model, we performed real-time reverse transcription–quantitative polymerase chain reaction (RT-PCR) analysis to quantify the amount of virus remaining on the host cell surface, which was exposed to antibodies before or after attachment of virus to cells at 4°C. H014 efficiently prevented attachment of SARS-CoV-2 to the cell surface in a dose-dependent manner, and the viral particles that had already bound to the cell surface could be partially stripped by H014 (Fig. 3D). Superimposition of the structure of the H014-SARS-CoV-2 trimeric S complex over the ACE2–SARS-CoV-2 RBD complex structure revealed clashes between the ACE2 and H014, arising from an overlap of the regions belonging to the binding sites located at the apical helix (n4) of the RBD (Fig. 3E). This observation differs substantially from those of most known SARS RBDS–targeted antibody complexes, where the antibodies directly recognize the RBM (22–25). Thus, the ability of H014 to prevent SARS-CoV-2 from attaching to host cells can be attributed to steric clashes with ACE2.

Similar to the RBM, the H014 epitope is only accessible in the open state, indicative of a role akin to that of the RBM—invoking dynamic interferences in interactions with host cells. Our structures, together with previously reported coronavirus S structures, not including human coronavirus HKU1 (20), have observed the breathing of the S1 subunit, which mediates the transition between “closed” and “open” conformation (Fig. 4A) (2, 9, 20). In contrast to the hinge-like movement of the RBDS observed in most structures, the conformational transition from “closed” to “open” observed in our structures mainly involves two steps of rotations: (i) counterclockwise movement of SD1 by ~25° encircling the hinge point (at residue 320); (ii) counterclockwise rotation by ~60° of the RBD itself (Fig. 4B). These conformational rotations of the SD1 and RBDS at proximal points relay an amplified alteration at the distal end, leading to opening up of adequate space for the binding of H014 or ACE2 (Fig. 4B). Ambiguously, the special conformational transition observed in our complex structures results from the engagement of H014 or a synergistic movement of the SD1 and RBD.

Humoral immunity is essential for protection against coronavirus infections, and passive immunization of convalescent plasma has been...
ACE2, and RBD, are presented as cartoons. The epitope, RBM, and the overlapped binding region of ACE2 trimer is shown in ribbon representation. Inset is a zoomed-in view of the interactions of the RBD, H014, and upon binding to SARS-CoV-2 S. H014 and ACE2 are represented as the molecular surface; SARS-CoV-2 S represent mean ± SD. Experiments were performed in duplicates. (D) Amount of virus on the cell surface, as detected by RT-PCR. Preattachment mode: Incubate SARS-CoV-2 with ACE2 (lower) RBD protein was coated on 96-well plates; recombinant ACE2 and serial dilutions of H014 were then added for competitive binding to SARS-CoV-2 or SARS-CoV RBD. Values are the mean ± SD. Experiments were performed in triplicate. (B) Blocking of SARS-CoV-2 RBD binding to 293T-ACE2 cells by H014 (upper). Recombinant SARS-CoV-2 RBD protein and serially diluted H014 were incubated with ACE2-expressing 293T cells (293T-ACE2) and tested for binding of H014 to 293T-ACE2 cells. Competitive binding of H014 and ACE2 to SARS-CoV-2-S cells (lower). Recombinant ACE2 and serially diluted H014 were incubated with 293T cells expressing SARS-CoV-2 spike protein (SARS-CoV-2-S) and tested for binding of H014 to SARS-CoV-2-S cells. Bovine serum albumin was used as a negative control (NC). Values are mean ± SD. Experiments were performed in triplicate. (C) BIAcore SPR kinetics of competitive binding of H014 and ACE2 to SARS-CoV-2 S trimer. For both panels, SARS-CoV-2 S trimer was loaded onto the sensor. In the upper panel, H014 was first injected, followed by ACE2, whereas in the lower panel, ACE2 was injected first and then H014. The control groups are depicted by black curves. (D) Amount of virus on the cell surface, as detected by RT-PCR. Preattachment mode: Incubate SARS-CoV-2 and H014 first, then add the mixture into cells (left); postattachment mode: Uncubate SARS-CoV-2 and cells first, then add H014 into virus-cell mixtures (right). High concentrations of H014 prevented attachment of SARS-CoV-2 to the cell surface when SARS-CoV-2 was exposed to H014 before cell attachment. Values represent mean ± SD. Experiments were performed in duplicates. (E) Clashes between H014 Fab and ACE2 upon binding to SARS-CoV-2 S. H014 and ACE2 are represented as the molecular surface; SARS-CoV-2 S trimer is shown in ribbon representation. Inset is a zoomed-in view of the interactions of the RBD, H014, and ACE2 and the clashed region (oval ellipse) between H014 and ACE2. The H014 Fab light and heavy chains, ACE2, and RBD, are presented as cartoons. The epitope, RBM, and the overlapped binding region of ACE2 and H014 on RBD are highlighted in green, blue, and red, respectively.

Fig. 3. Mechanism of neutralization of H014. (A) Competitive binding assays by ELISA. Recombinant SARS-CoV-2 (upper) or SARS-CoV (lower) RBD protein was coated on 96-well plates; recombinant ACE2 and serial dilutions of H014 were then added for competitive binding to SARS-CoV-2 or SARS-CoV RBD. Values are the mean ± SD. Experiments were performed in triplicate. (B) Blocking of SARS-CoV-2 RBD binding to 293T-ACE2 cells by H014 (upper). Recombinant SARS-CoV-2 RBD protein and serially diluted H014 were incubated with ACE2-expressing 293T cells (293T-ACE2) and tested for binding of H014 to 293T-ACE2 cells. Competitive binding of H014 and ACE2 to SARS-CoV-2-S cells (lower). Recombinant ACE2 and serially diluted H014 were incubated with 293T cells expressing SARS-CoV-2 spike protein (SARS-CoV-2-S) and tested for binding of H014 to SARS-CoV-2-S cells. Bovine serum albumin was used as a negative control (NC). Values are mean ± SD. Experiments were performed in triplicate. (C) BIAcore SPR kinetics of competitive binding of H014 and ACE2 to SARS-CoV-2 S trimer. For both panels, SARS-CoV-2 S trimer was loaded onto the sensor. In the upper panel, H014 was first injected, followed by ACE2, whereas in the lower panel, ACE2 was injected first and then H014. The control groups are depicted by black curves. (D) Amount of virus on the cell surface, as detected by RT-PCR. Preattachment mode: Incubate SARS-CoV-2 and H014 first, then add the mixture into cells (left); postattachment mode: Uncubate SARS-CoV-2 and cells first, then add H014 into virus-cell mixtures (right). High concentrations of H014 prevented attachment of SARS-CoV-2 to the cell surface when SARS-CoV-2 was exposed to H014 before cell attachment. Values represent mean ± SD. Experiments were performed in triplicate. (E) Clashes between H014 Fab and ACE2 upon binding to SARS-CoV-2 S. H014 and ACE2 are represented as the molecular surface; SARS-CoV-2 S trimer is shown in ribbon representation. Inset is a zoomed-in view of the interactions of the RBD, H014, and ACE2 and the clashed region (oval ellipse) between H014 and ACE2. The H014 Fab light and heavy chains, ACE2, and RBD, are presented as cartoons. The epitope, RBM, and the overlapped binding region of ACE2 and H014 on RBD are highlighted in green, blue, and red, respectively.
Fig. 4. Breathing of the S1 subunit and epitopes of neutralizing antibodies. (A) H014 can only interact with the “open” RBD, whereas the “closed” RBD is inaccessible to H014. The open RBD and RBD-bound H014 are depicted in lighter colors corresponding to the protein chain that they belong to. Color scheme is the same as in Fig. 2A. (B) Structural rearrangements of the S1 subunit of SARS-CoV-2 transition from the closed state to the open state. SD1, subdomain 1; SD2, subdomain 2; RBD, RBD (closed state) from adjacent monomer, SD1, SD2, NTD, RBD, and RBD are colored in pale green, light orange, cyan, blue and yellow, respectively. The red dot indicates the hinge point. The angles between the RBD and SD1 are labeled. (C) Epitope location analysis of neutralizing antibodies on SARS-CoV and SARS-CoV-2 S trimers. The S trimer structures with one RBD open and two RBD closed from SARS-CoV and SARS-CoV-2 were used to show individual epitope information, which is highlighted in green. The accessible and inaccessible states are encircled and marked by green ticks and red crosses. (D) Footprints of the seven mAbs on RBDs of SARS-CoV (left) and SARS-CoV-2 (right). RBDs are rendered as molecular surfaces in light blue. Footprints of different mAbs are highlighted in different colors as labeled in the graph. Epitopes recognized by the indicated antibodies are labeled in blue (non-overlapped), yellow (overlapped once), and red (overlapped twice).
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A steric block to SARS-CoV-2

In response to infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the immune system makes antibodies, many of which target the spike protein, a key player in host cell entry. Antibodies that potently neutralize the virus hold promise as therapeutics and could inform vaccine design. Lv et al. report a humanized monoclonal antibody that protected against SARS-CoV-2 in a mouse model. The cryo-electron microscopy structure, together with biochemical, cellular, and virological studies, showed that the antibody acts by binding to the receptor-binding domain of the spike and blocking its attachment to the host receptor.

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