



Broadly neutralizing antibodies to SARS-CoV-2 and other human coronaviruses

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Abstract | Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a recently emerged pathogenic human coronavirus that belongs to the sarbecovirus lineage of the genus Betacoronavirus. The ancestor strain has evolved into a number of variants of concern, with the Omicron variant of concern now having many distinct sublineages. The ongoing COVID-19 pandemic caused by SARS-CoV-2 has caused serious damage to public health and the global economy, and one strategy to combat COVID-19 has been the development of broadly neutralizing antibodies for prophylactic and therapeutic use. Many are in preclinical and clinical development, and a few have been approved for emergency use. Here we summarize neutralizing antibodies that target four key regions within the SARS-CoV-2 spike (S) protein, namely the N-terminal domain and the receptor-binding domain in the S1 subunit, and the stem helix region and the fusion peptide region in the S2 subunit. Understanding the characteristics of these broadly neutralizing antibodies will accelerate the development of new antibody therapeutics and provide guidance for the rational design of next-generation vaccines.

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<https://doi.org/10.1038/s41577-022-00784-3>

Following the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, a novel pathogenic human coronavirus (HCoV) emerged in 2019 that soon spread around the world, resulting in the COVID-19 pandemic^{1,2}. This novel virus was named ‘severe acute respiratory syndrome coronavirus 2’ (SARS-CoV-2) owing to its close sequence homology (~79.6%) with SARS-CoV^{3–6}. Compared with SARS-CoV and MERS-CoV, SARS-CoV-2 has a much lower case–fatality ratio. However, the high proportions of asymptomatic or mildly symptomatic infections caused by the original strain of SARS-CoV-2 and its ensuing variants have led to higher and more rapid transmissibility of this virus, which has resulted in serious complications for all populations of the world^{7–11}.

Coronaviruses belong to the subfamily *Coronavirinae* from the family *Coronaviridae*, and they are genotypically and serologically diversified into four major genera: alphacoronaviruses (alpha-CoVs), betacoronaviruses (beta-CoVs), gammacoronaviruses (gamma-CoVs) and deltacoronaviruses (delta-CoVs)^{5,7}. HCoVs are those coronaviruses that can infect humans. Taxonomically, historically occurring HCoV-229E and HCoV-NL63 are classified as alpha-CoVs, whereas HCoV-HKU1, HCoV-OC43, SARS-CoV, SARS-CoV-2 and MERS-CoV

are beta-CoVs. Alpha-CoVs and beta-CoVs mainly infect mammals, whereas gamma-CoVs and delta-CoVs primarily infect birds. Both SARS-CoV-2 and SARS-CoV belong to Sarbecovirus, which is a subgenus of Betacoronavirus. By contrast, MERS-CoV belongs to Merbecovirus, another subgenus of Betacoronavirus. Two other HCoVs of note, HCoV-HKU1 and HCoV-OC43, which can cause common cold-like illnesses, belong to the subgenus Embecovirus of Betacoronavirus^{7,12–14}.

HCoVs contain phosphorylated nucleocapsid (N) protein with a single-stranded genomic RNA as a core. The viral core is encapsulated by phospholipid bilayers to form spherical or pleomorphic particles 80–120 nm in size, and is characterized by the presence of the outer surface spike (S) protein^{7,8}. The S protein is composed of two subunits, S1 and S2. S1 contains an important receptor-binding domain (RBD), which is responsible for the recognition of host cell surface receptors that enable virus entry. Both SARS-CoV and SARS-CoV-2 engage angiotensin-converting enzyme 2 (ACE2), which is widely expressed by a variety of human cells, as the primary entry receptor^{15–17}. Dipeptidyl peptidase 4 (DPP4; also known as CD26) is the corresponding entry receptor for MERS-CoV^{17,18}. The S2 subunit is mainly responsible for subsequent viral fusion with and entry into the host cell. The junction of S1 and S2

Furin

A protease belonging to the proprotein convertase family that processes latent precursor proteins into biologically active products.

Transmembrane serine protease 2 (TMPRSS2)

A plasma membrane-anchored serine protease that proteolytically cleaves and activates the spike (S) glycoproteins of human coronaviruses and some other viruses.

Cathepsins

A family of proteases that are responsible for recycling cellular proteins inside the lysosomes, comprising serine, aspartate and cysteine peptidases, and that exhibit endopeptidase or exopeptidase activities.

Stem helix (SH)

A helix structure in the S2 stem region that forms part of the spike fusion machinery and is conserved among multiple betacoronaviruses.

contains a specific furin cleavage site, which is cleaved by host cell furin to facilitate virus entry into cells¹⁹. ACE2 engagement by the virus exposes the S2' cleavage site, and S2 is further cleaved into two parts at this site by transmembrane serine protease 2 (TMPRSS2) at the cell membrane surface, facilitating the process of membrane fusion between the host cell and the virus²⁰. ACE2-bound virus can also be internalized via endocytosis, and in this case, cleavage of the S2' site is mediated by cathepsins, especially cathepsin L in endosomes²¹ (FIG. 1a).

Antibodies that recognize pathogens can be categorized as neutralizing antibodies (nAbs) or non-neutralizing antibodies (non-nAbs). The difference between the two generally depends on whether the antibody binding to a specific pathogen can block cell invasion or inhibit membrane fusion after recognition (in the case of nAbs), or not block cell invasion or inhibit membrane fusion after recognition (in the case of non-nAbs)²². In general, nAbs are effective in neutralizing pathogens, reducing pathogen titres and protecting tissues or cells from infection. The neutralization activity of non-nAbs is usually undetectable^{23–25}, but they can exert their protective effects through Fc-mediated effector functions, such as antibody-dependent cell-mediated cytotoxicity, antibody-dependent cellular phagocytosis and complement-dependent cytotoxicity^{25,26}. In this Review, we focus solely on the broadly neutralizing antibodies (bnAbs) that target the neutralizing epitopes in the N-terminal domain (NTD) and the RBD of the S1

subunit, and those that target the stem helix (SH) and fusion peptide (FP) regions in the S2 subunit (FIG. 1b,c).

The NTD

The S1 subunit of the SARS-CoV-2 S protein has two important domains that are targets of monoclonal antibodies (mAbs), namely the NTD and RBD. 4A8 is one of the earliest identified nAbs targeting the NTD, with its heavy chain complementarity-determining regions (HCDRs) — HCDR1, HCDR2 and HCDR3 — interacting with NTD residues²⁷. Chi et al. defined five structural loops (N1–N5) in the NTD, with N3 and N5 mediating interaction with 4A8 (REF.²⁷). Similarly, other NTD-targeting mAbs, such as COV2-2676, COV2-2489, 4-8 and 5-24, can also recognize the epitope comprising the N1, N3 and N5 loops^{28,29}. This strongly positively charged epitope in the NTD was therefore dubbed ‘the NTD supersite’^{30,31}. However, many naturally circulating SARS-CoV-2 variants carry mutations within the NTD supersite, which could dampen the neutralization activities of these NTD supersite-recognizing mAbs. For example, a deletion of NTD amino acid residues 242–244 made 4A8, 4-8 and 5-24 almost completely lose their ability to neutralize the SARS-CoV-2 Beta variant of concern (VOC)³². Similarly, deletion of Y144 in the NTD abolished the neutralization activities of the S2M28 (FIG. 2a), S2X28 and S2X333 nAbs³³. Of note, mAbs targeting the non-supersite on the NTD have broad neutralizing potential. For example, mAb 5-7 (FIG. 2a), isolated

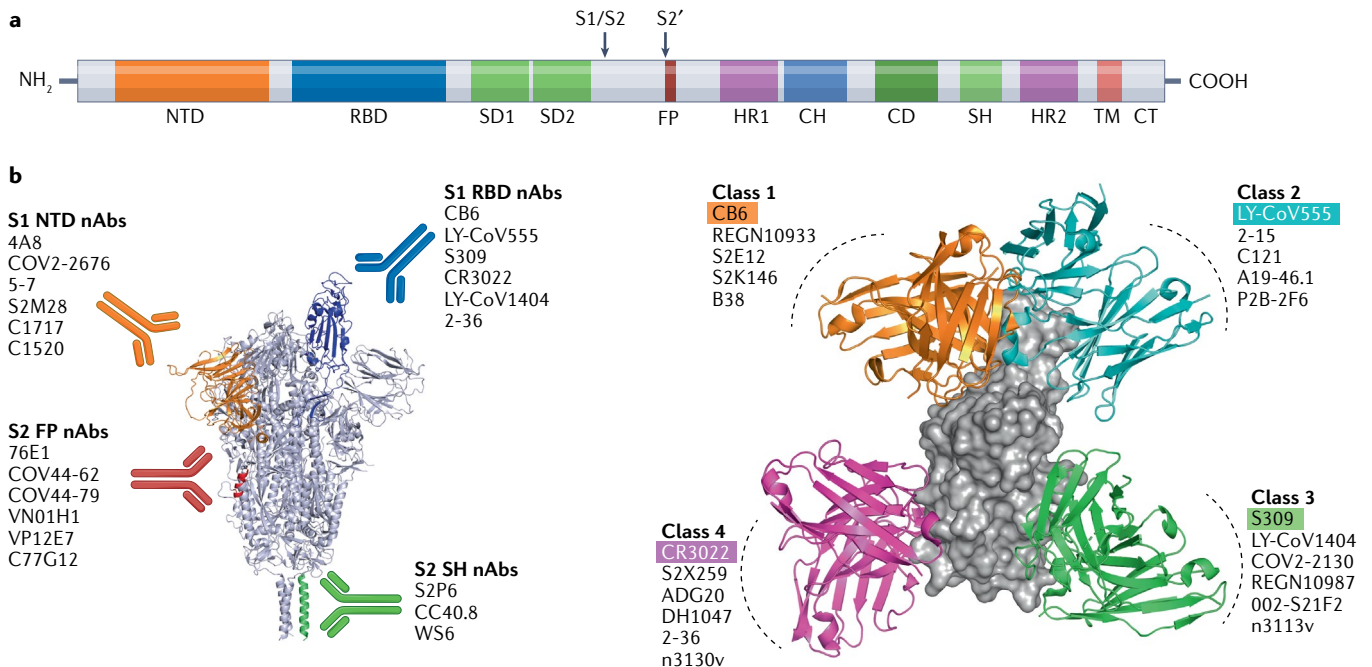


Fig. 1 | Neutralizing antibodies directed against the SARS-CoV-2 spike protein. **a** | Schematic representation of the main domains of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein. Arrows denote S1/S2 and S2' protease cleavage sites. **b** | Different groups of neutralizing antibodies (nAbs) that target the S protein. Representative nAbs targeting the S1 N-terminal domain (NTD), S1 receptor-binding domain (RBD), and S2 stem helix (SH) and S2 fusion peptide (FP) regions

are shown with the S protein depicted in the RBD ‘up’ conformation. **c** | RBD-directed nAbs can be divided into four main classes depending on the epitopes they target in the RBD of the S protein. For each class, one representative nAb bound to the RBD monomer is shown: class 1, CB6; class 2, LY-CoV555; class 3, S309; class 4, CR3022. CD, connector domain; CH, central helix; CT, cytoplasmic tail; HR, heptad repeat; SD, subdomain; TM, transmembrane domain.

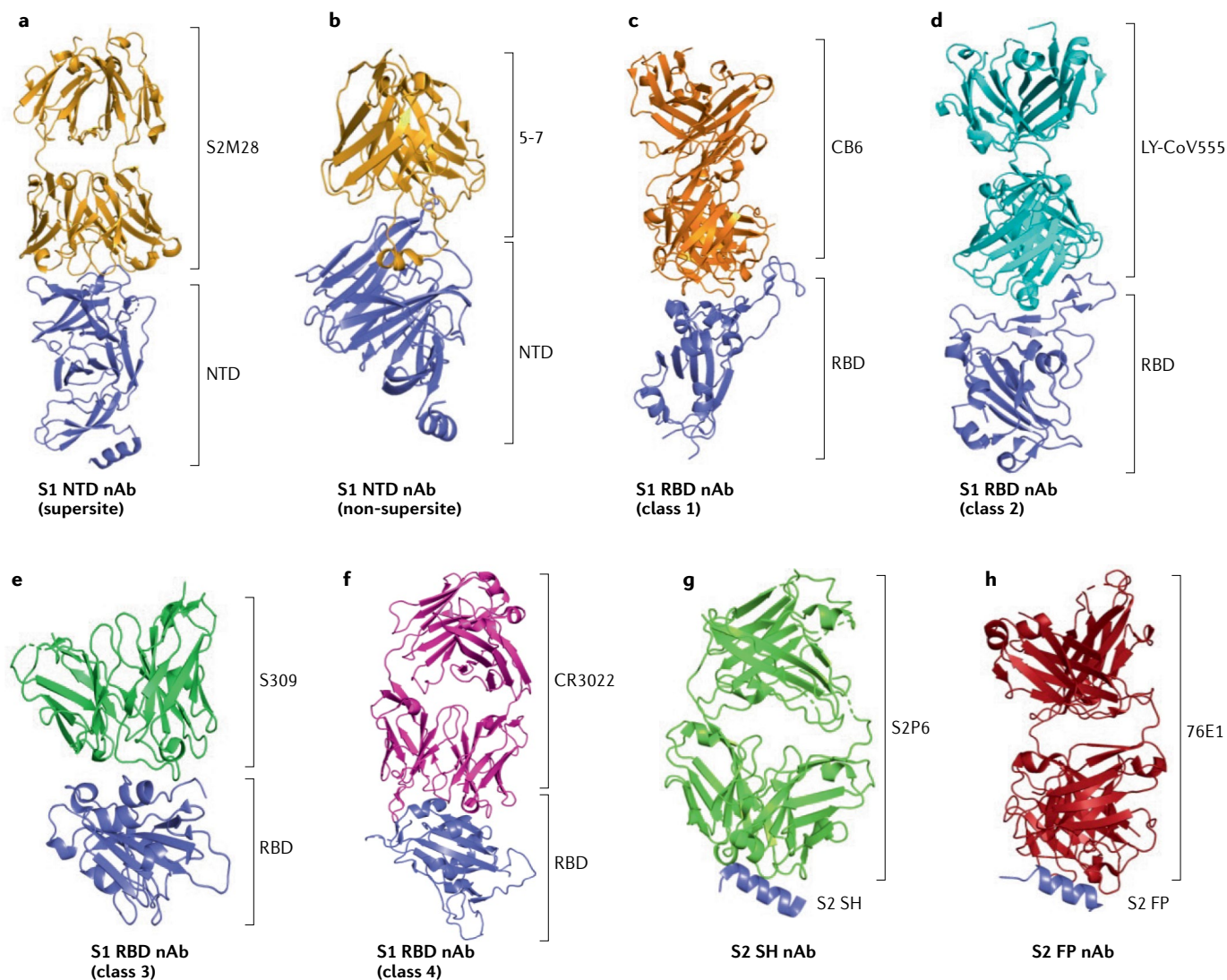


Fig. 2 | Structures of neutralizing antibodies bound to the SARS-CoV-2 spike protein. Three-dimensional modelling is used here to depict the complexes of representative neutralizing antibodies (nAbs) interacting with their targets in the S1 and S2 subunits of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein. **a** | S1 N-terminal domain (NTD) nAbs, supersite (S2M28, Protein Data Bank (PDB) ID 7LY3). **b** | S1 NTD nAbs, non-supersite (5-7, PDB ID 7RW2). **c** | S1 receptor-binding domain (RBD) nAbs, class 1 (CB6, PDB ID 7C01). **d** | S1 RBD nAbs, class 2 (LY-CoV555, PDB ID 7KMG). **e** | S1 RBD nAbs, class 3 (S309, PDB ID 7TLY). **f** | S1 RBD nAbs, class 4 (CR3022, PDB ID 7JN5). **g** | S2 stem helix (SH) nAb (S2P6, PDB ID 7RNJ). **h** | S2 fusion peptide (FP) nAb (76E1, PDB ID 7X9E).

from a patient recovering from COVID-19 (REFS.^{29,34}), retained its neutralizing potency partially against the SARS-CoV-2 Alpha and Beta VOC, as well as against the Omicron BA.1, BA.1.1 and BA.3 sublineages^{32,35,36}. Although some residues in the NTD supersite are highly mutable under selection pressure, one feasible strategy for developing NTD-targeting bnAbs is to identify 5-7-like mAbs, which bind the non-supersite on the NTD. More importantly, as the bulk of NTD-targeting antibodies did not compete with antibodies targeting other regions of the S protein, such as the RBD^{27,28,30}, combining NTD-targeting antibodies with antibodies binding non-NTD regions may be an ideal way to combat COVID-19 (see TABLE 1, Supplementary Table S1 and FIG. 2a for a summary of more NTD-targeting nAbs^{37–40}).

Fusion peptide

(FP). A conserved hydrophobic domain of a fusion protein that inserts itself into membranes during membrane fusion, which is required for the fusogenic activity of glycoproteins from divergent virus families.

The RBD

Most of the anti-SARS-CoV-2 antibodies discovered to date target the RBD, and can be further divided into different classes on the basis of their targeted epitopes. Although several different classification systems have been proposed, the most commonly referenced is that proposed by Barnes et al.⁴¹, who grouped RBD-targeting antibodies into four classes on the basis of their mode of binding to the S protein (FIG. 1c).

Class 1 RBD-targeting antibodies. The antibody-binding epitope targeted by class 1 RBD-targeting antibodies overlaps with the receptor-binding motif (RBM) in the RBD, and antibodies in this class are mostly encoded by VH3-53 and VH3-66 germ lines and recognize only the ‘up’ RBD conformation⁴¹. The substantial neutralization

Table 1 | Neutralizing antibodies targeting the N-terminal domain of the spike protein

Antibodies	Binding epitope in NTD	Mechanism of neutralization	Viruses neutralized	Refs.
4A8	Supersite	Restrains the conformational changes of the S protein	SARS-CoV-2	27
COV2-2676, COV2-2489, 5-24, BLN12	Supersite	Unknown	SARS-CoV-2	28,29,34,37,38
4-8, BLN14	Supersite	Unknown	SARS-CoV-2; Alpha VOC	29,34,37,38
5-7	Non-supersite	Restrains the conformational changes of the S protein	SARS-CoV-2; Alpha and Beta VOC; BA.1, BA.3	29,34
S2M28, S2X28, S2X333	Supersite	Prevents interaction with an auxiliary receptor, proteolytic activation or membrane fusion	SARS-CoV-2	33
C1717	Non-supersite	Prevents access to the S2' cleavage site or destabilizes S1	SARS-CoV-2; Alpha, Beta and Gamma VOC; BA.1	40
C1520, C1791	Non-supersite	Unknown	SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1	40
ADI-56479	Supersite	Inhibits the attachment of ACE2	SARS-CoV-2	39

The table provides an overview of neutralizing antibodies targeting the N-terminal domain (NTD) of the S1 subunit of the spike (S) protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and related coronaviruses. SARS-CoV-2 indicates the wild type strain. See Supplementary Table S1 for a more detailed description of each antibody. ACE2, angiotensin-converting enzyme 2; VOC, variant of concern.

mechanism of class 1 antibodies is achieved by their preventing ACE2 from binding to the S protein. During the early phase of the SARS-CoV-2 outbreak, many class 1 RBD-targeting antibodies were identified, such as CB6 and CT-P59 (REFS.^{42,43}), and some were discovered by our own group, including 1-20, 4-20 and 910-30 (REFS.^{29,44}). Many of these antibodies lost their neutralizing activities as a result of the E484K mutation seen in the Beta VOC, Gamma VOC and other SARS-CoV-2 variants^{32,45,46}. While the neutralization potency of 1-20 is not apparently affected by the E484K mutation, another common mutation, N501Y, decreases the neutralizing potency of 1-20 almost 10-fold. It is possible that the synergy of various mutants in the Beta VOC resulted in the 600-fold reduction in the neutralizing potency of 1-20 against Beta compared with wild type SARS-CoV-2 (REF.³²). CB6 (FIG. 2b) engages in polar and hydrophobic interactions with wild type SARS-CoV-2 S protein primarily through HCDR1, HCDR2 and HCDR3 (REF.⁴²), but its binding epitope is heavily mutated in the S protein of VOC such as Beta and Omicron. In particular, the critical mutation K417N eliminates the neutralizing potency of CB6 and limits its neutralizing breadth³². As a result, the presence of common mutations in the RBD, such as K417N, E484K and N501Y, to which most class 1 mAbs bind, causes most of these antibodies that show great neutralizing potency against wild type SARS-CoV-2 to lose their neutralizing abilities for variants such as Beta, Gamma and Omicron.

Nevertheless, S2E12 is one of the few class 1 mAbs that retains broad-spectrum neutralizing activity⁴⁷. Although its binding region is similar to that of the other class 1 mAbs mentioned earlier, S2E12 can still neutralize all current VOC, showing exceptional neutralization breadth^{47,48}. The cryogenic electron microscopy structure revealed that S2E12 binds the receptor-binding ridge, contacting 18 residues in the RBD. The S protein

F486 residue fills in a buried cavity lined by aromatic residues formed by its interface of heavy and light chains, while residue N487 initiates a polar interaction with S2E12 (REF.⁴⁷). Although common mutation sites, such as E484 and S477, do exist in S2E12-binding variants, they are not functional residues that interact with S2E12. Moreover, they are located at the edge of the S2E12-RBD interface, conferring considerable neutralizing activity of S2E12 against variants containing these highly frequent mutation sites⁴⁷. Another ACE2-mimic antibody, S2K146, also demonstrates sizable neutralizing breadth against SARS-CoV-2 variants and other sarbecoviruses^{49,50}. As such, comparison of S2E12 and S2K146 with other class 1 antibodies also highlights the importance of microstructure for an antibody's neutralizing performance.

Class 2 RBD-targeting antibodies. Antibodies in class 2 are similar to those in class 1 on the basis of their binding to the RBM where the RBD interacts with ACE2. Consistency also derives from the neutralizing mechanism of these antibodies, which prevents the reciprocal binding of the RBD to ACE2. Notably, mAbs in class 2 can bind both 'up' and 'down' conformations of the S protein⁴¹. For example, LY-CoV555 (FIG. 2b), which was isolated from a patient recovering from COVID-19, both bound and neutralized SARS-CoV-2 and displayed protective efficacy against SARS-CoV-2 in clinical trials⁵¹. Although the antibody neutralized SARS-CoV-2 pseudovirus very effectively (with a half-maximal inhibitory concentration (IC₅₀) of 0.004 µg ml⁻¹ (REF.⁴⁸)), LY-CoV555 lost most of its neutralization activity against the subsequently discovered VOC, which can be attributed to critical single viral mutations such as E484K and Q493R^{32,36}. As a result, the broadly neutralizing capacity of LY-CoV555 was constrained as these mutations were found to be prevalent in many SARS-CoV-2 variants.

Similarly, 2-15 (REF.²⁹), another mAb isolated from a patient with COVID-19, is also evaded as a result of the E484K and Q493R mutations, which cause an antigenic structure change^{32,36}. Although the RBD region targeted by class 1 and class 2 antibodies covers the RBM, the amino acid identity of SARS-CoV and SARS-CoV-2 is only 59% shared in the RBM and 94% shared for the rest of the RBD excluding the RBM⁵². Hence, most antibodies in class 1 and class 2 targeting this region do not have a superior broad spectrum for the inhibition of SARS-CoV and other SARS-like coronaviruses.

Class 3 RBD-targeting antibodies. Class 3 antibodies bind the outside the ACE2-binding region, and they can also bind to RBDs regardless of their ‘up’ and ‘down’ conformations⁴¹. Most class 3 antibodies, including REGN10987, COV2-2130, 2-7,1-57, A19-61.1, P2G3, S309 and LY-CoV1404, have demonstrated potent neutralizing activities against SARS-CoV-2 variants^{29,48,53–60}. S309 and LY-CoV1404 are two representative antibodies with great neutralization breadth (REFS.^{56,57}). S309 (FIG. 2b) was isolated from a patient recovering from SARS and can efficiently bind to the SARS-CoV-2 S protein without blocking the binding between ACE2 and the RBD⁵⁶. Structurally, S309 HCDR3 was shown by cryogenic electron microscopy to interact mainly with eight residues (337–344) of the RBD helix, as well as with six residues of the RBD β -sheet (356–361). In addition, the S309 light chain complementarity-determining region 1 (LCDR1) and LCDR2 directly interact with the epitope of the S protein that spans residues 440–444 (REF.⁵⁶). The epitopes recognized by S309 are the most highly conserved residues in the SARS-CoV and SARS-CoV-2 RBDs, conferring broad cross-reactivity on the S309 antibody⁵⁶. Moreover, S309 can broadly neutralize sarbecoviruses, including all currently identified SARS-CoV-2 VOC, as the epitope sequences recognized by S309 are highly conserved among sarbecoviruses^{48,56}. Interestingly, although the single mutation S371L in SARS-CoV-2 causes the loss of S309’s neutralizing potency, the detrimental effect of S371L on S309 was counteracted by the combined effect of amino acids near the point of mutation in Omicron such that S309 can still effectively neutralize Omicron (BA.1), with $IC_{50} = 0.28 \mu\text{g ml}^{-1}$ (REFS.^{48,56}).

Similarly, LY-CoV1404 can also bind to the viral RBDs, irrespective of their ‘up’ and ‘down’ conformations. However, LY-CoV1404 differs from S309 in that it binds a part of the RBD epitope that overlaps with the ACE2-binding domain⁵⁷. As the epitope bound by LY-CoV1404 is structurally closer to that recognized by class 3 antibodies, LY-CoV1404 also belongs to the class 3 antibody group⁵⁷. The neutralization mechanism of LY-CoV1404 is achieved by its preventing ACE2 from binding to the RBD⁵⁷. Of note, the epitope bound by LY-CoV1404 is also conserved in SARS-CoV-2 and its variants. Although the epitope targeted by LY-CoV1404 was associated with two high-frequency mutations at positions 439 and 501, these two mutations did not affect the binding of LY-CoV1404 to and its neutralizing potency against SARS-CoV-2 and its variants containing these mutations⁵⁷. Compared with S309, LY-CoV1404 was able to neutralize all SARS-CoV-2

VOC without much change in neutralization potency, especially against the Omicron variants⁴⁸. Among the antibodies approved for clinical trials, LY-CoV1404 was the only antibody that retained its neutralization potency against Omicron sublineages^{35,36,48,57,61,62}. Taken together, these findings suggest LY-CoV1404 is a specific and effective mAb for the treatment of COVID-19.

Recently, another class 3 mAb, named ‘SP1-77’, was obtained from a humanized mouse model (the VH1–2/V κ 1–33-rearranging mouse model). SP1-77 showed potent neutralization activity against all currently known SARS-CoV-2 variants, including the recently emerging Omicron variant BA.1 ($IC_{50} = 6.5 \text{ ng ml}^{-1}$), and its sublineages BA.2 ($IC_{50} = 33 \text{ ng ml}^{-1}$), BA.3 ($IC_{50} = 7 \text{ ng ml}^{-1}$), BA.4/BA.5 ($IC_{50} = 16 \text{ ng ml}^{-1}$) and BA.2.12.1 ($IC_{50} = 8 \text{ ng ml}^{-1}$)⁶³. Interestingly, SP1-77 does not block the RBD–ACE2 binding interaction or viral endocytosis, but instead mediates virus neutralization by preventing shedding of S1, which blocks membrane fusion⁶³. This study provides insight into how a non-ACE2 blocking antibody can also potentially neutralize SARS-CoV-2 infection.

Class 4 RBD-targeting antibodies. The epitope recognized by class 4 antibodies is highly conserved in the RBD, and these antibodies bind to the RBD, but do not directly block ACE2–RBD binding. This epitope has also been described as a cryptic region, which is consistent with the well-described cryptic epitope recognized by the CR3022 antibody (FIG. 2b), an antibody isolated from a patient who had recovered from SARS-CoV infection^{41,64}. The epitope targeted by class 4 antibodies is conserved by up to 86% in SARS-CoV and SARS-CoV-2, and CR3022 is thus able to effectively bind to both coronaviruses⁶⁴. Owing to a glycosylation site on N370 within the targeted epitope in SARS-CoV, CR3022 binds to SARS-CoV with greater affinity than to SARS-CoV-2. Of note, CR3022 can bind to SARS-CoV-2 only when at least two RBDs are in the ‘up’ conformation, which partly explains its lower binding affinity for SARS-CoV-2 and poor neutralizing potency against this virus ($IC_{50} > 400 \mu\text{g ml}^{-1}$)⁶⁴.

The 2-36 antibody identified by our group competes with CR3022 for binding to the SARS-CoV-2 RBD, and is therefore classified as a class 4 antibody^{29,65}. Similarly to CR3022, 2-36 also recognizes and binds the RBD in the ‘up’ conformation. Cryogenic electron microscopy data revealed that HCDR3 of 2-36 forms the majority of interactions by recognizing loops on the RBD spanning residues 369–385, whereas HCDR1 and LCDR2 of the antibody interact to a lesser extent with the RBD⁶⁵. The interaction between 2-36 and the SARS-CoV-2 RBD is dependent mainly on hydrophobic effects, as HCDR3 of 2-36 contains a large number of hydrophobic amino acids. Compared with CR3022, 2-36 can more effectively neutralize SARS-CoV, SARS-CoV-2 and related sarbecoviruses that use ACE2 as the entry receptor, with $IC_{50} < 0.1 \mu\text{g ml}^{-1}$ in both authentic virus and pseudovirus assays⁶⁵. 2-36 retained its neutralization potency against the SARS-CoV-2 Alpha, Beta, Gamma and Delta VOC⁶⁵, and retained partial activity against Omicron BA.1, with $IC_{50} \sim 1 \mu\text{g ml}^{-1}$ (REFS.^{35,36}). Taken together, these findings indicate that 2-36, as a bnAb to SARS-CoV-2 and related

sarbecoviruses, could be a specific drug candidate for the treatment or prevention of COVID-19 after proper engineering, and its binding epitope is a promising target for the development of pan-sarbecovirus vaccines.

Another class 4 antibody, S2X259, can also broadly neutralize SARS-CoV-2 and related sarbecoviruses⁶⁶. A very recent study demonstrated that S2X259 lost its neutralizing activity against SARS-CoV-2 strains containing the G504D mutant⁶⁶. Fortunately, the G504D substitution is rarely observed in SARS-CoV-2 strains, with a mutant rate below 0.002%⁶⁶. This antibody can neutralize not only many currently circulating SARS-CoV-2 variants and SARS-CoV but also a panel of SARS-related sarbecoviruses. Other known class 4 antibodies, such as ADG20, DH1047, COVA1-16 and H014, also demonstrate neutralizing breadth against SARS-CoV-2, SARS-CoV and other relevant sarbecoviruses^{67–71}. This suggests that the epitope recognized by class 4 antibodies is an ideal target for the development of bnAbs.

In summary, RBD-targeting antibodies in class 1 and class 2 will probably lose their neutralizing abilities with the emergence of the next major SARS-CoV-2 variant that carries new mutations in the RBM; thus, their neutralizing breadth is limited. By contrast, antibodies in class 3 and class 4 that bind highly conserved epitopes hold promise as candidates for neutralizing SARS-CoV-2 variants and other SARS-like coronaviruses. This suggests that selecting such conserved epitopes for vaccine design may elicit potent broad-spectrum antibodies that could help to overcome the current COVID-19 pandemic. In addition, it is clear that epitopes of RBD-targeting antibodies in class 1 and class 2 overlap with the ACE2 footprint on the RBD, and these antibodies achieve neutralizing activities by directly blocking the interaction between the RBD and ACE2. However, the major class 3 and class 4 antibodies do not show such explicit neutralization mechanisms, and their mechanisms of neutralization need to be further explored. Studies of antibodies such as SP1-77 may provide insight into the neutralizing mechanisms of other, similar RBD-binding (but non-RBM-targeting) antibodies (See TABLE 2, Supplementary Table S2 and FIG. 2b for a summary of more RBD-targeting nAbs^{58–62,72–81}).

The S2 SH region

The SARS-CoV-2 S protein is composed of S1 and S2 subunits. Most SARS-CoV-2 nAbs target the neutralizing epitopes in the NTD and RBD in the S1 subunit. However, such epitopes are likely to succumb to selective pressure, increasing the likelihood of immune escape by virus mutants. By contrast, the neutralizing epitopes in the S2 subunit are more conserved than those in the S1 subunit⁸². Therefore, nAbs targeting the S2 epitopes would have a greater probability of being broad-spectrum nAbs to SARS-CoV-2 and other HCoV-229E. For example, S2P6 (FIG. 2c), which was isolated from a patient recovering from COVID-19, could broadly neutralize all beta-CoVs by targeting the S2 subunit⁸³. Further study revealed that the epitope bound by this antibody is located in the S2 SH region that spans 14 residues (1146–1159), which is conserved across beta-CoVs. Moreover, the S2 SH region interacts

with the S2P6 antibody's HCDR1, HCDR2 and HCDR3, as well as with its LCDR1 and LCDR3, mainly through the formation of hydrophobic interactions and hydrogen bonds. Single-substitution analysis revealed that mutations at positions 1148, 1151–1153 and 1155–1156 abolished S2P6 binding affinity, suggesting that these are critical residues for S2P6 binding⁸³. Unlike the neutralization mechanism of antibodies targeting S1, S2P6 can inhibit SARS-CoV-2 infection by preventing S protein-mediated fusion of viral and cellular membrane⁸³. The Fc effector functions of S2P6 also play a critical role in fighting coronavirus infection in vivo⁸³. Several studies reported that S2P6 can broadly neutralize beta-CoVs, including SARS-CoV-2 and SARS-like viruses, which belong to the subgenus Sarbecovirus, MERS-CoV, which belongs to subgenus Merbecovirus, and HCoV-HKU1 and HCoV-OC43, which belong to the subgenus Embecovirus. S2P6 shows variable IC₅₀ of 1.4 µg ml⁻¹ for SARS-CoV-2, 2.4 µg ml⁻¹ for SARS-CoV, 17.1 µg ml⁻¹ for MERS-CoV and 1.3 µg ml⁻¹ for HCoV-OC43 (REF.⁸³). Although S2P6 does not show as great neutralizing potency as some RBD-targeting antibodies, it still has the potential to become a very effective antibody drug for the treatment of COVID-19 or diseases caused by other coronaviruses owing to its broad-spectrum neutralizing properties.

Another antibody targeting the S2 SH region, CC40.8, was isolated from a patient with COVID-19 (REF.⁸⁴). It binds to residues from 1140 to 1164, and was found to broadly bind and neutralize beta-CoVs⁸⁴. An in vivo study in hamsters confirmed that CC40.8 mediates effective protection against SARS-CoV-2 infection⁸⁴. WS6, an antibody isolated from an mRNA-immunized mouse, also binds the SARS-CoV-2 S2 SH region that spans residues 1143–1159 (REF.⁸⁵). Mechanistically, WS6 can neutralize SARS-CoV-2 by inhibiting the membrane fusion process following virus contact with ACE2 (REF.⁸⁵). As expected, WS6 can also broadly neutralize beta-CoVs, and pseudovirus neutralization experiments showed its neutralizing potency against all SARS-CoV-2 VOC, with IC₅₀ ranging from 2.46 to 26.52 µg ml⁻¹ (REF.⁸⁵). The three bnAbs mentioned here all target the S2 SH epitope, highlighting the importance of finding and developing bnAbs that recognize these conserved epitopes.

The S2 FPs

Apart from the SH region mentioned earlier, S2 FPs are also highly conserved among all coronavirus genera, suggesting that broad-spectrum antibodies could be found by targeting this epitope^{86–88}. Some recently identified antibodies to this epitope have excellent broadly neutralizing activity against alpha-CoVs, beta-CoVs and even some gamma-CoVs and delta-CoVs^{86,87}. For example, COV44-62 and COV44-79, which were both isolated from patients recovering from COVID-19, can bind the S2 FP region through recognition of the 'RSFIEDLLF' motif. Interestingly, these antibodies do not compete with S2P6, the aforementioned S2 SH-targeting antibody, for binding to the SARS-CoV-2 S protein⁸⁶, suggesting the possibility of combining S2 SH and S2 FP recognition in a bispecific antibody. Crystal structure analysis revealed that COV44-62

Table 2 | Neutralizing antibodies targeting the receptor-binding domain of the spike protein

Antibodies	Binding epitope in RBD	Mechanism of neutralization	Viruses neutralized	Refs.
1-20, 4-20, 910-30, CB1	Class 1	Blocks the interaction between the RBD and ACE2	SARS-CoV-2	29,42,44
CB6, CC12.3	Class 1	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha and Delta VOC	42,48,67,72
REGN10933	Class 1	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha and Delta VOC; BA.2.75	48,53,62
CT-P59	Class 1	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC	43,48
A23-58.1, S2E12	Class 1	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1, BA.2, BA.2.75	47–49,58,81
S2K146	Class 1	Blocks the interaction between the RBD and ACE2	Sarbecoviruses (SARS-CoV; SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1, BA.2, BA.3, BA.4/5, BA.2.75)	49,50,80,81
B38	Class 1	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Beta VOC	73
2-15, LY-CoV555, C121, C144	Class 2	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha VOC	29,32,48,51,74
COV2-2196	Class 2	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1, BA.2, BA.3, BA.2.75	48,54,61,62,80
A19-46.1	Class 2	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha, Beta and Gamma VOC	48,58
P2B-2F6	Class 2	Blocks the interaction between the RBD and ACE2	SARS-CoV-2	77
S309	Class 3	Leads to trimeric S protein crosslinking, causes steric hindrance or aggregation of virions	Sarbecoviruses (SARS-CoV; SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1, BA.2, BA.3, BA.4/5, BA.2.75)	36,48,56,61,62
LY-CoV1404	Class 3	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1, BA.2, BA.3, BA.4/5, BA.2.75	36,48,57,61,62
COV2-2130	Class 3	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1, BA.2, BA.3, BA.4/5, BA.2.75	48,54,61,62,80
REGN10987	Class 3	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1, BA.2, BA.4/5	48,53,61,62
SP1-77	Class 3	Prevents the shedding of S1 and blocks membrane fusion	SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1, BA.2, BA.3, BA.4/5	63
A19-61.1	Class 3	Causes steric hindrance between the RBD and ACE2	SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1	48,58
1-57	Class 3	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha, Beta and Gamma VOC	29,32,46,55
2-7	Class 3	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha, Beta and Gamma VOC; BA.1, BA.2, BA.4/5	29,32,46,55,61
002-S21F2	Class 3	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1, BA.2	76
P2G3	Class 3	Blocks the interaction between the RBD and ACE2	SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1, BA.2, BA.4/5	59,60
n3113v	Class 3	Inhibits SARS-CoV-2 S protein-mediated membrane fusion	SARS-CoV-2; Alpha, Beta, Gamma, Delta and Omicron VOC	78,79
CR3022	Class 4	Unknown	SARS-CoV	64
2-36	Class 4	Causes steric hindrance between the RBD and ACE2	Sarbecoviruses (SARS-CoV; SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1)	29,36,65
S2X259	Class 4	Blocks the interaction between the RBD and ACE2	Sarbecoviruses (SARS-CoV; SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1)	50,66
ADG20	Class 4	Competes with ACE2 for RBD binding	Sarbecoviruses (SARS-CoV; SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1)	67,68
DH1047	Class 4	Unknown	Sarbecoviruses (SARS-CoV; SARS-CoV-2; Alpha, Beta, Gamma and Delta VOC; BA.1)	61,69

Table 2 (cont.) | Neutralizing antibodies targeting the receptor-binding domain of the spike protein

Antibodies	Binding epitope in RBD	Mechanism of neutralization	Viruses neutralized	Refs.
COVA1-16	Class 4	Causes steric hindrance between the RBD and ACE2	Sarbecoviruses (SARS-CoV; SARS-CoV-2)	70
H014	Class 4	Prevents attachment of SARS-CoV-2 to ACE2	Sarbecoviruses (SARS-CoV; SARS-CoV-2; Beta VOC)	71
EY6A	Class 4	Interferes with ACE2 attachment	SARS-CoV-2	75
n3130v	Class 4	Induces S protein trimer to adopt unstable 'up' states	SARS-CoV-2; Alpha, Beta, Gamma, Delta and Omicron VOC	78

The table provides an overview of neutralizing antibodies targeting the receptor-binding domain (RBD) of the spike (S) protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and related coronaviruses. 'SARS-CoV-2' indicates the wild-type strain. See Supplementary Table S2 for a more detailed description of each antibody. ACE2, angiotensin-converting enzyme 2; VOC, variant of concern.

interacts with S2, that spans residues 814–824, mainly through hydrogen bonds, salt bridges and hydrophobic interactions within HCDR1, HCDR2, HCDR3, LCDR1 and LCDR3. Similarly, COV44-79 utilizes HCDR1, HCDR2, HCDR3 and LCDR3 to bind SARS-CoV-2 and interacts with S2 residues 812–823 (REF.⁸⁶). Of note, both antibodies broadly neutralize beta-CoVs, but show some differences in activity against MERS-CoV. The neutralizing activity of COV44-62 against MERS-CoV could be detected, whereas that of COV44-79 could not. These antibodies can also act against the more distantly evolved alpha-CoVs, including HCoV-NL63 and HCoV-229E, although they are less potent at neutralizing these viruses, with $IC_{50} \sim 10 \mu\text{g ml}^{-1}$ (REF.⁸⁶). Peptide alanine scanning revealed that E819, D820, L822 and F823 in the S2 FP region are crucial for COV44-62 binding, whereas R815, E819, D820 and F823 are crucial for COV44-79 binding⁸⁶. More importantly, the entire FP sequence is highly conserved among the four coronavirus genera⁸⁶, giving antibodies targeting this region an extremely broad ability to neutralize SARS-CoV-2 and numerous other coronaviruses.

Another panel of nAbs targeting S2 FP — VN01H1, VP12E7 and C77G12 — were recently identified⁸⁷. Among them, VN01H1 and VP12E7 can neutralize all alpha-CoV and beta-CoV pseudoviruses, including HCoV-NL63, HCoV-229E, SARS-CoV, SARS-CoV-2 and MERS-CoV; C77G12 exhibited higher neutralization potency than VN01H1 and VP12E7, but only against beta-CoVs⁸⁷. Crystal structures showed that all three antibodies concealed the R815 residue at the interface when interacting with the S protein. Furthermore, R815 is not only a conserved residue in the S protein but is also the site of cleavage of S2' by TMPRSS2, suggesting that these three antibodies neutralize SARS-CoV-2 by preventing S2' cleavage, thereby inhibiting subsequent membrane fusion⁸⁷. Intriguingly, a potent synergistic neutralization activity was found when C77G12 was combined with S2E12, a nAb in RBD class 1, implying that the FP is most likely a cryptic epitope that is normally inaccessible, but when the RBD interacts with ACE2 or ACE2-mimic antibodies, the exposed epitope is more accessibly bound by FP-targeting antibodies⁸⁷.

More recently, 76E1 (FIG. 2d) was identified as another S2 FP-targeting nAb that shows extraordinary neutralizing breadth, including for alpha-CoVs, beta-CoVs

and a few gamma-CoVs and delta-CoVs⁸⁸. It has been proved that 76E1 can potentially neutralize SARS-CoV-2 both in vitro and in vivo⁸⁸. Like C77G12, a synergistic effect of 76E1 can be observed when the S protein contacts some RBD-targeting nAbs as well as ACE2 (REF.⁸⁸). Taken together, these findings show that S2-directed antibodies target prominently conserved epitopes and exhibit the broadest neutralizing spectrum to date. Therefore, they can guide the design of bnAbs to highly variable SARS-CoV-2 variants and even other HCoVs (see TABLE 3, Supplementary Table S3 and FIGS. 2c,d for a summary of the S2-targeting nAbs mentioned above).

Conclusion

The engineering of already discovered antibodies can be used to improve their performance, including their neutralizing potency and breadth. One approach is to construct libraries containing a large number of different VH/VL sequences by phage or yeast display methods to yield antibodies with better performance^{89–92}. Another approach is to try different formats of antibodies, such as nanobodies. In contrast to human IgG, camelid antibodies (also known as nanobodies or VHHS), lack a light chain and are composed of two identical heavy chains. The nanobody is the smallest antigen-binding fragment (~15 kDa) discovered to date^{93–95}. Owing to their smaller size, nanobodies can bind more cryptic epitopes that are not easily accessible. Together with their high tissue penetration ability, they can also be formulated as an inhalable atomized powder^{78,96}.

Other effective approaches to improve antibody function include engineering them into bispecific or multispecific antibodies^{97–100}, which can bind to multiple epitopes simultaneously and synergistically. Combining two or more bnAbs targeting different epitopes as therapeutics (for example, different RBD class antibodies) or combining RBD-targeting antibodies with NTD-targeting and/or S2-targeting antibodies might be a feasible strategy against COVID-19. Some antibody cocktails, such as S2E12 and C77G12, 76E1 and CB6, and COV2-2196 and COV2-2130, have already been explored^{54,87,88}, but more combinations need to be tested in clinical trials. Furthermore, some ACE2-targeting antibodies have been reported to confer protection in animal models against infection by SARS-CoV-2 and other SARS-like coronaviruses by competing with

Table 3 | Neutralizing antibodies targeting the S2 subunit of the spike protein

Antibodies	Binding epitope in S2 subunit	Mechanism of neutralization	Viruses neutralized	Refs.
S2P6	S2 stem helix	Inhibits membrane fusion	Beta-CoVs (sarbecoviruses, merbecoviruses and embecoviruses)	83
CC40.8	S2 stem helix	Inhibits membrane fusion	Beta-CoVs (sarbecoviruses, HCoV-HKU1)	84
WS6	S2 stem helix	Inhibits membrane fusion	Beta-CoVs (sarbecoviruses)	85
COV44-79	S2 fusion peptide	Inhibits membrane fusion	Alpha-CoVs and beta-CoVs (except MERS-CoV)	86
COV44-62, VN01H1, VP12E7, 76E1	S2 fusion peptide	Inhibits membrane fusion	Alpha-CoVs and beta-CoVs	86–88
C77G12	S2 fusion peptide	Inhibits membrane fusion	Beta-CoVs	87

The table provides an overview of neutralizing antibodies targeting the S2 subunit of the spike (S) protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and related coronaviruses. See Supplementary Table S3 for a more detailed description of each antibody. alpha-CoV, alphacoronavirus; beta-CoV, betacoronavirus; HCoV, human coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus.

the S protein for receptor binding^{101,102}, and these antibodies could be used in combination with the S protein-targeting antibodies. Therefore, we can take advantage of the synergistic enhancement between antibodies to make them exert better neutralization and protective effects in vivo.

The emergence of numerous SARS-CoV-2 variants calls for the generation of bnAbs as a therapy for COVID-19. In this Review, we have described some representative nAbs that bind the NTD and RBD in the S1 subunit and the SH and FP regions in the S2 subunit of SARS-CoV-2 and have summarized more known nAbs in TABLES 1–3. Overall, antibodies in RBD class 1 and class 2, as well as those targeting the NTD supersite, are more likely to lose their neutralizing activities as the viral epitopes they target are more prone to mutate. By contrast, antibodies targeting the more conserved S2 epitopes are able to exert an incredibly broad neutralization spectrum against HCoVs, including alpha-CoVs and beta-CoVs. However, despite their having a broader neutralization spectrum, it is important to point out that the S2 antibodies are much less potent than RBD-targeting antibodies. Therefore, these findings put great emphasis on balancing the

breadth and potency of nAbs when one is selecting nAbs as candidates for antibody therapy for COVID-19. On the other hand, comprehending the characteristics of these bnAbs could provide guidance for devising more effective vaccines. Consistent with others^{103–105}, we stress the importance of focusing on conserved viral epitopes for the development of broad-spectrum antibody therapies, as well as for vaccine design. In general, the immunogens with conserved epitopes — such as the FP and SH regions in the S2 subunit^{83,86–88} — are unable to elicit potent nAb responses, possibly owing to their inappropriate conformation and/or low immunogenicity. Therefore, vaccines containing different conformations of these immunogens should be tested in combination with a highly potent adjuvant, such as the STING agonist-based adjuvant CF501 (REF¹⁰⁶). Another feasible strategy for the design of next-generation vaccines would be heterologous or multivalent immunization with S proteins from different HCoVs, which might induce the host immune system to generate bnAbs to highly conserved viral epitopes present in these coronaviruses.

Published online 27 September 2022

STING agonist

A modulator of stimulator of interferon genes (STING) that can facilitate the phosphorylation of the transcription factor interferon regulatory factor 3 (IRF3), resulting in an increase in the expression of type I interferon genes, through the binding of STING to cyclic GMP–AMP (cGAMP).

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Acknowledgements

This work was sponsored by the National Natural Science Foundation of China (32270142) and the Shanghai Rising-Star Program (22QA1408800) to P.W. X.Z. acknowledges support from the International Postdoctoral Exchange Fellowship Program (Talent-Introduction Program, YJ20220071).

Author contributions

P.W. and S.J. conceived ideas. Y.C. X.Z. and P.W. wrote the article. H. Zhou, H. Zhu and S.J. reviewed and edited the manuscript. Y.C. created the tables and H. Zhou prepared the figures with suggestions from P.W. and S.J. All authors reviewed and approved the manuscript before submission.

Competing interests

P.W. has filed patent applications for antibodies 4-8,5-24, 5-7,1-20, 4-20, 910-30, 2-15, 2-7,1-57 and 2-36. The other authors declare no competing interests.

Peer review information

Nature Reviews Immunology thanks S. Liu, Y. Wang and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Supplementary information

The online version contains supplementary material available at <https://doi.org/10.1038/s41577-022-00784-3>.

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