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Trends in Immunology

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REVIEWS

Review

Structural Basis of SARS-CoV-2 and SARS-CoV Antibody Interactions

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The 2019 coronavirus pandemic remains a major public health concern. Neutralizing antibodies (nAbs) represent a cutting-edge antiviral strategy. We focus here on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and SARS-CoV, and discuss current progress in antibody research against rampant SARS-CoV-2 infections. We provide a perspective on the mechanisms of SARS-CoV-2-derived nAbs, comparing these with existing SARS-CoV-derived antibodies. We offer insight into how these antibodies cross-react and cross-neutralize by analyzing available structures of spike (S) glycoprotein–antibody complexes. We also propose ways of adopting antibody-based strategies – such as cocktail antibody therapeutics against SARS-CoV-2 – to overcome the possible resistance of currently identified mutants and mitigate possible antibody-dependent enhancement (ADE) pathologies. This review provides a platform for the progression of antibody and vaccine design against SARS-CoV-2, and possibly against future coronavirus pandemics.

Highlights

Newly isolated SARS-CoV-2-derived mAbs [RBM-, N-terminal domain (NTD)-, and S2 subunit-targeting mAbs] have shown neutralizing effects and protection efficacy against SARS-CoV-2 in *in vitro* assays, animal models, and human clinical trials.

Cross-reactivity and cross-neutralization of SARS-CoV-2-derived and SARS-CoV-derived mAbs may be largely influenced by the S glycoprotein domain that is targeted.

The $S1_B$ core domain-targeting mAbs of both SARS-CoV and SARS-CoV-2 might exhibit better cross-reactivity and cross-neutralization compared with receptor-binding motif (RBM)-targeting mAbs. However, RBM-targeting mAbs might exhibit better neutralizing effects, although these effects might be virus-specific.

The current Asp614Gly and other mutations in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) S glycoprotein may increase infectivity and reduce monoclonal antibody (mAb) sensitivity. An ideal therapeutic strategy against escape mutants might lie in applying cocktail mAbs such as REGN-COV.

The Emergence of Coronaviruses and Progress in Coronavirus Research

In recent decades three highly pathogenic betacoronaviruses have emerged in humans: SARS-CoV [1,2] in 2002, Middle East respiratory syndrome coronavirus (MERS-CoV) [3] in 2012, and SARS-CoV-2 in 2019, the causative agent of the rampant coronavirus disease 2019 (COVID-19) [4,5]. Despite appreciable progress in coronavirus research, the overwhelming number of COVID-19 deaths has warranted urgent and novel intervention [6,7]. In terms of a strategy against SARS-CoV-2, lessons are being drawn from previous approaches – small-molecule inhibitors, of which remdesivir has received the most accolade, that are entering Phase II/III clinical trials (NCT04292899[i]) [8,9]. Other recent findings have included the assessment of convalescent plasma [10,11], polyclonal and monoclonal antibodies (mAbs) [12,13], as well as putative vaccines [14]. Several vaccine candidates have been rolled out for clinical trials with promising preliminary results as of August 2020, including ChAdOx1 S (Phase III: NCT04400838[ii]), Lunar-Cov19 (Phases I/II ARCT-021: NCT04480957[iii]), and adenovirus-based vaccines (Ad26.nCoV: NCT04436276[iv]) [15] and (Ad5-nCoV: NCT04341389[v]) [16,17]. Indeed, the current therapeutic race against SARS-CoV-2 might need to be multifaceted. New paradigms such as T cell-based immunotherapies [18] might be hopeful. However, of these options, we posit that nAbs present timely and safe opportunities for early intervention against viral pandemics, as noted previously for the successful control of Ebola virus [19,20] and many efforts towards HIV-1 treatments [21] and the neutralization of hepatitis E by antibodies [22,23].

nAbs hold remarkable potential for therapeutic and prophylactic applications against coronaviruses, and various avenues for antibody treatment (Tables 1, 2, and 3) are currently being explored, with a surge in research findings. Previous review articles [17,24–26] have provided a solid foundation for understanding antibody reactivity and neutralization regarding SARS-CoV-derived antibodies. Furthermore, in recent weeks, new SARS-CoV-2-derived [27–32] and human host-specific nAbs have been reported [33–35]. Early attempts to cross-

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Table 1. SARS-CoV-2-Derived RBD-Binding nAbs[a]

mAb name and source	Neutralizing activity	Targeted region in S protein	Receptor and mAb competition	Neutralizing mechanism	Protective efficacy	PDB/Refs
REGN10987 and REGN10933 Humanized and human mAb cocktail	Neutralizes SARS-2 virus and escape mutants	REGN10933: RBM of SARS-2 REGN10987: RBD/$S1_BCD$	REGN10933 competes with hACE2	Block hACE2–RBD binding ADCC and ADCP	Clinical trials	6XDG [27,45]
414-1 and 553-15 Human mAbs	Neutralize authentic SARS-2 and pseudoviruses 553-15 cross-neutralize SARS	RBD and S ectodomain of SARS-2	Compete with hACE2	Block hACE2-RBD binding	Preclinical	N/A [43]
COVA1-18, COVA2-15, and COVA1-16, COVA2-02 Human mAbs	Neutralize authentic SARS-2 and pseudoviruses COVA1-16 and COVA2-02 cross-neutralizes SARS pseudovirus	RBD and S proteins of SARS-2 and SARS	COVA1-18 and COVA2-15 compete with hACE-2	Block hACE2–RBD binding	N/A	N/A [91]
CV30 Human mAb	Neutralizes SARS-2	SARS-2 RBD but not with SARS RBD	Competes with hACE-2	Blocks hACE2 RBD binding	N/A	N/A [79]
CC12.1 and CC6.33 Human mAbs	Neutralize authentic SARS-2 and pseudoviruses CC6.33 neutralizes SARS pseudovirus	SARS-2 RBD/RBM SARS RBD	Likely compete with hACE-2 interface Do not compete with CR3022	Block hACE2–RBD binding	Protect a hamster model	6XC2 6XC3 [27,32,34,93]
H014 Humanized mAb	Neutralizes SARS and SARS-2 pseudoviruses and also authentic SARS-2 virus	SARS and SARS-2 RBD/ $S1_BCD$	Competes with hACE2	Blocks hACE2–RBD binding.	Protects a hACE mouse model	7CAH [40]
2-15, 2-7, 1-57, 1-20, and 2-4 Human mAbs	Neutralize authentic SARS-2 and pseudoviruses	SARS-2 RBD	Likely to compete with hACE2 2-15, 2-7, and 1-57 compete with each other 1-20 competes with 2-15	Block hACE2–RBD binding	2-15 protects hamsters against SARS-2 infection	2-4: 6X2Y [31]
2-43 and 2-51 Human mAbs	Neutralize authentic SARS-2 and pseudoviruses	Unknown	2-43 binding blocked by numerous RBD-directed mAbs	2-43: probably blocks hACE2–RBD binding	N/A	N/A [31]
COV2-2196, COV2-2130, COV2-2196, and COV2-2381 mAbs	Neutralize wild-type SARS-2 virus and pseudoviruses in a synergistic manner	SARS-2 RBD/RBM	Compete with hACE-2 COV2-2196 competes with COV2-2165 COV2-2196 does not compete with COV2-2130	Block hACE2–RBD binding	Protect mice and rhesus macaques against SARS-CoV-2 infection	N/A [41]
C121, C135, C144, and C105 Human mAbs	Neutralize authentic SARS-2 and pseudoviruses	SARS-2 RBD	Might compete with hACE2 Do not compete with CR3022	Block hACE2–RBD binding	Promising candidates	C105 6XCA 6XCN 6XCM [28–30,37,38,73]
COV21 Human Ab	Neutralizes authentic SARS-2 and	SARS and SARS-2	COV21 might compete with	Blocks hACE2–RBD	Promising candidate	[28,30,37,38,73]

(continued on next page)

Table 1. (continued)

mAb name and source	Neutralizing activity	Targeted region in S protein	Receptor and mAb competition	Neutralizing mechanism	Protective efficacy	PDB/Refs
	pseudoviruses	RBD/$S1_B$CD	mAbs C105, B38, S230, and hACE-2	binding		
EY6A Mouse mAb	Neutralizes wild-type SARS-2	Both SARS and SARS-2 RBD/$S1_B$CD	Competes with CR3022 Does not compete with hACE2	Might engage multiple mechanisms	Promising candidate	6ZCZ 6ZDH 6ZDG 6ZER 6ZFO [38,85]
BD-368-2, BD-218, and BD-23 Human mAbs	Neutralize authentic and pseudotyped SARS-2	SARS-2 RBD	Likely compete with hACE2	Block hACE2–RBD binding	Protect hACE2 transgenic mice	BD-23: 7BYR [39]
2M-10B11 Human mAb	Nonneutralizing	2M-10B11: RBD/$S1_B$CD	Competes with hACE2 and CR3022	N/A	2M-10B11 protects against pseudotyped virus	N/A [38,88]
CA1 and CB6 Human mAbs	Neutralize SARS-2 pseudovirus	SARS-2 RBD/RBM	Compete with hACE2 CA1 competes with CB6	Block hACE2–RBD binding	CB6-LALA protects rhesus macaques	CB6: 7C01 [42]
B38 and H4 Human mAbs	Neutralize SARS-2 Exhibit additive inhibition	SARS-2 RBD/RBM/ $S1_B$CD although at different sites	Compete with hACE2 B38 and H4 are noncompeting	Block hACE2–RBD binding	Protect hACE-2 transgenic mice	B38: 7BZ5 [29]
311mab-31B5 and 311mab-32D4 Human mAbs	Neutralize SARS-2 pseudovirus	SARS-2 RBD/RBM	Compete with hACE2	Block hACE2–RBD binding	Preclinical	N/A [35]
P2C-1F11 and P2B-2F6 Human mAbs	Neutralize authentic SARS-2 and pseudoviruses	SARS-2 RBD/RBM	Compete with hACE-2 P2B-2F6 competes with all mAbs tested	Block hACE2–RBD binding	Preclinical	P2B-2F6: 7BWJ [33]
P2A-1A10 and P2A-1B3 Human mAbs	Minimally neutralize live SARS-2 virus	SARS-2 RBD	57% reduction in ACE2 binding	Block hACE2–RBD binding	N/A	N/A [33]
P2C-1A3 and P2C-1C10 Human mAbs	Moderately neutralize SARS-2 virus	SARS-2 RBD	P2C-1A3 competes with all mAbs tested	Block hACE2–RBD binding	N/A	N/A [33]

[a]Abbreviations: Ab, antibody; ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; hACE2, human angiotensin-converting enzyme 2; mAb, monoclonal antibody; N/A, not applicable/not available; nAb, neutralizing antibody; RBD, receptor-binding domain; RBM, receptor-binding motif; SARS, SARS-CoV; SARS-2, SARS-CoV-2; SARS-S, SARS-CoV-spike; SARS-2 S, SARS-CoV-2 spike; $S1_B$CD, $S1_B$ core domain.

neutralize SARS-CoV-2 with SARS-CoV-derived antibodies did not yield ideal results [36–38]. However, most of the newly reported SARS-CoV-2-derived antibodies demonstrate a potent neutralizing effect *in vitro* and/or protection in animal models such as hamsters [27,29], mice [39,40], and rhesus macaques [41] (Tables 1 and 2). SARS-CoV-2-specific nAbs such as B38 [29], BD-368-2 [39], CA1/CB6-LALA [42], P2C-1F11/P2B-2F6/P2A-1A3 [33], 414-1 [43], ADI-55689/56046 [44], REGN-CoV-2 (Phase I/II/III: NCT04425629[vi], NCT04426695[vii], and NCT04452318[viii]) [27,45], and COVI-SHIELD [17] (Tables 1 and 2) have either entered clinical trials or are in preclinical stages. In addition, the study of camelid antibodies (**nanobodies**;

Table 2. nAbs Targeting the SARS-CoV RBD[a]

mAb name and source	Neutralizing activity	Targeted region in S protein	Receptor and mAb competition	Neutralizing mechanism	Protective efficacy	PDB/Refs
ADI-55689 and ADI-56046 Human mAbs	Potently cross-neutralize SARS, SARS-2, and bat virus WIV1	SARS, SARS-2, and bat WIV1 RBD/RBM/ S1BCD	ADI-56046 competes with hACE2 and CR3022	Block hACE2–RBD binding and induce S1 shedding	Preclinical	[44]
CR3014 (scFv)	Neutralizes SARS in cocktail with mAb CR3022;	SARS-RBD/RBM	Competes with hACE2 Does not compete with CR3022	Blocks hACE2–RBD binding	N/A	[38,92]
S109.8, S227.14, and S230.15 Human mAbs	Neutralize human, raccoon dog, and palm civet SARS strains	SARS-RBD/RBM	Compete with hACE2 S227.14 and S230.15: compete with other mAbs tested	Block hACE2–RBD binding S109.8 causes steric hindrance	Protect mice	[83]
M396 and 80R Human mAbs	M396 neutralizes human and palm civet SARS strains 80R: neutralizes live SARS	M396: SARS-RBD/RBM 80R: SARS-S	Compete with hACE2	Block hACE2–RBD binding	M396 protects mice	M396: 2DD8 and 2G75 80R: 2GHW [37,75,76]
S230 Human mAb	Neutralizes SARS isolates of human and animal origin Does not neutralize SARS-2	SARS-RBD/RBM	Competes with hACE2	Blocks hACE2–RBD binding	N/A	6NB8 6NB7 6NB6 [73]
47D11 Human mAb	Neutralizes SARS and SARS-2 pseudoviruses, and SARS-2 live virus	SARS-2 and SARS RBD/$S1_B$CD	Does not with compete with hACE2	Unknown	Phase I trials expected	N/A [64]
CR3022 Human mAb	Neutralizes live SARS and SARS-2 Synergistic effect with CR3014	SARS and SARS-2 RBD/$S1_B$CD	Does not compete with hACE2	Destabilizes and destroys the prefusion S trimer	Preclinical	6W41 [37,38,92]
SARS-VHH-72 (HCAb) Llama (camelid) mAb	Bivalent form neutralizes SARS-2 pseudovirus	SARS, SARS-2, and bat WIVI CoV RBD/$S1_B$CD	Competes with hACE2	Blocks hACE2–RBD binding Destabilizes the prefusion spike	Preclinical	6WAQ [36]
S309 Human mAb	Neutralizes SARS and SARS-2 pseudoviruses and authentic SARS-2	SARS and SARS-2 RBD/$S1_B$CD	Does not compete with hACE2	Unknown Might engage one or multiple mechanisms	Fc variant fast-tracked for clinical trials	6WPS 6WPT 6WS6 [12]

[a]Abbreviations: HCAb, heavy-chain antibody; pAb, polyclonal antibody; scFv, single-chain variable fragment.

see Glossary) is also becoming an attractive research area that has given early positive results against COVID-19 [36,46–48].

Moreover, much effort has gone into exploring host-specific antibodies. nAbs that target the host system have the unique advantage of overcoming virus mutations and might be easily repurposed for related viral outbreaks [49–52]. Furthermore, decoy strategies are being explored, such as utilizing the SARS-CoV-2 binding partner – the angiotensin-converting enzyme 2 (ACE-2) receptor – fused to human immunoglobulin (IgG), and preliminary *in vitro* and mouse studies are encouraging [34].

We discuss here the properties and mechanisms of neutralization of several antibodies. We analyze the available structures of virus S glycoprotein–antibody complexes and offer insights into

Table 3. SARS-CoV-2-Derived Non-RBD-Binding Antibodies

mAb name and source	Neutralizing activity	Region targeted in S protein	Receptor and mAb competition	Neutralizing mechanism	Protective efficacy	PDB/Refs
2-17, 5-24, and 4-8 Human mAbs	Neutralize authentic SARS-2 and pseudoviruses	SARS-2 NTD	mAbs compete	Unknown	N/A	[31]
COV57 Human pAb	Neutralizes authentic SARS-2 and pseudoviruses	SARS-1 and SARS-2 NTD, MERS S glycoprotein	Might not interrupt hACE-2 binding	Unknown	Promising candidate against SARS-2 infections	[28,30,37,38,73]
9A1	Does not neutralize authentic SARS-2 virus	SARS-2 S2 domain	May not interrupt hACE-2 Binding	N/A	2M-10B11 protects against pseudotyped virus 9A1 confers weak protection	N/A [38,88]
4A8, 1M-1D2, and 0304-3H3 Human mAbs	4A8 neutralizes both authentic and pseudotyped SARS-2 1M-1D2 and 0304-3H3 neutralize only authentic SARS-2 virus	4A8: NTD of S1 1M-1D2: S1 domain 0304-3H3: S2 domain	Do not interrupt hACE-2 binding 4A8 competes with 1M-1D2	Likely by restraining conformational change in S protein	Preclinical	4A8: 7C2L [88]
CV1 and CV35 Human mAbs	Neutralize SARS-2	Outside the RBD	N/A	Unknown	N/A	N/A [80]

cross-reactivity and cross-neutralization mechanisms of SARS-CoV-2- and SARS-CoV-derived antibodies. We also briefly touch on the possibility of **ADE** in SARS-CoV-2, and provide ways of mitigating ADE if it becomes a challenge. In addition to ADE, another emerging concern for SARS-CoV-2 therapeutic design is the emergence of more virulent escape mutants [53,54]. We focus on the residues of S glycoprotein that are recognized by antibodies, and we speculate about whether emerging SARS-CoV-2 mutations might affect antibody binding and neutralization. Lastly, we discuss existing proof-of-concept cocktail antibody therapies against SARS-CoV-2, and propose new antibody cocktails that might be used against COVID-19.

S Glycoprotein Immune Responses against SARS-CoV-2 and SARS-CoV

The S glycoprotein of coronaviruses is the primary determinant of viral tropism and plays a vital role in cell receptor binding and membrane fusion [55–57]. The S glycoprotein assembles into a trimeric form on the virion surface in a crown-like shape [58]. Upon cleavage by host proteases, the S glycoprotein is subdivided into two functionally distinct subunits: the S1 subunit that is responsible for receptor recognition, and the S2 subunit that facilitates fusion with the host cell membrane [55–57] (Figures 1–3). The S glycoproteins of both SARS-CoV-2 and SARS-CoV primarily recognize human ACE2 (hACE2) [59,60]. Given that the S glycoprotein is the major surface protein that interacts with host cells, it is a potential therapeutic target in coronavirus infections [55]. The S glycoprotein is the immunodominant target for nAbs [61–63], and comprises an N-terminal domain (NTD), a receptor-binding domain (RBD/$S1_B$), and an S2 subunit (Figures 1A and 3A). The SARS-CoV RBD [amino acids (aa) 338–506] consists of an $S1_B$ core domain

($S1_BCD$) (aa 318–424) and a receptor-binding motif (RBM) (aa 438–498) that directly engages the human receptor hACE2 [64] (Figure 1A,C). Although the N-terminal region of the RBD is referred to as the $S1_B$ core domain ($S1_BCD$) in the literature [64], it is important to clarify that the $S1_BCD$ is not an independent structural unit. Antibodies can be raised against the full-length (FL) S glycoprotein or its subdomains. Vaccination of African green monkeys with an attenuated parainfluenza virus-encoded FL-S glycoprotein of the SARS-CoV Urbani strain elicited nAbs that protected monkeys from identical SARS-CoV infection [65]. On the one hand, although the FL-S glycoprotein is highly immunogenic and induces nAb responses, it can also induce harmful immune responses in ferrets [66,67]. On the other hand, antibodies raised against epitopes of S1 (aa 485–625) or S2 (aa 1029–1192) can neutralize virus infection by SARS-CoV strains (e.g., Tor2 and Sin2774) in Vero E6 cells [68,69]. The RBD is also a significant neutralization determinant in the inactivated SARS-CoV vaccine because it induces potent nAbs that block SARS-CoV entry [14]. This was confirmed by the reduced neutralizing activity produced by depletion of RBD-specific antibodies from patient or rabbit immune sera (SARS) relative to affinity-purified anti-RBD antibodies that elicited higher-potency neutralizing activity against SARS-CoV **pseudoviruses** in 293T/ACE2 cells [70].

Impact of the Asp614Gly and Other Mutations on SARS-CoV-2 Infectivity and Antibody Design

The high propensity of viruses to adapt and develop mutations that dodge therapeutics adds further complexity to antibody design. Indeed, viral mutations can facilitate virus adaptation, potentially increasing transmissibility, worsening disease symptoms, and sometimes creating mutants that are resistant to therapeutics [53]. Two key mutations have been identified in SARS-CoV-2 S glycoprotein: Asp614Gly and Ser943Pro. The Gly614 mutation seems to cause enhanced infectivity of pseudotyped single-cycle vesicular stomatitis virus (VSV) displaying the SARS-CoV-2 S glycoprotein, relative to the Asp614 reference strain, in 293T/ACE2, 293T/ACE2-TMPRSS2, and Vero cells [53,54]. These residues might be considered in antibody therapeutics development because they might render mutant strains resistant to existing nAbs, as shown in 293T/hACE2 and Vero cells [54], and also perhaps cause ADE-related pathologies [3,71]. Nevertheless, the impact of the Asp614Gly mutation is currently a topic of debate [72]. One argument is that this mutation is not located in the RBD of the S glycoprotein [72]. Furthermore, antibodies raised against either Asp614 or Gly614 mutant glycoproteins have cross-neutralizing activity [72]. It is debatable whether there is sufficient scientific evidence to confirm that this variant will worsen COVID-19 [72]. However, new evidence suggests that the Asp614Gly strain does increase infectivity, and other sporadic mutations such as Asn234Gln, Leu452Arg, Ala475Val, and Val483Ala (most of which are in the RBD) do present marked resistance to some nAbs [54]. Specifically, Ala475Val reduced virus sensitivity to mAbs 157, 247, CB6, P2C-1F11, B38, and CA1, whereas Phe490Leu reduced sensitivity to mAbs X593, 261-262, H4, and P2B2F6 in 293T/hACE2 and Vero cells [54]. Moreover, the Val483Ala mutant became resistant to mAbs X593 and P2B-2F6, and Leu452Arg to mAbs X593 and P2B-2F6 [52]. In addition, Tyr508His/Asp614Gly + Ala435Ser, Asn439Lys, Ala831Val, Asp614Gly + Ile472Val mutations reduced virus sensitivity to mAbs H014, H00S022, B38, and X593, respectively, by more than fourfold in 293T/hACE2 and Vero cells [54]. These results demand immediate improvement of antibody and vaccine design strategies against COVID-19. Given the efficacy of the REGN-COV cocktail antibody, antibody combination/cocktail therapies may be an excellent place to start, and other upcoming cocktails have been demonstrated to neutralize SARS-CoV-2 pseudovirus particle mutant escape variants in Calu-3 cells and in Vero E6 cells [27,45].

The fight against SARS-CoV-2 using antibody therapies has been extensive, and many structures of antibody–virus subunit complexes have now been determined at high resolution [Protein Data Bank

Glossary

Antibody-dependent enhancement (ADE): a phenomenon in which antibody binding to virus particles enhances viral entry into host cells, promoting virus replication and possibly worsening disease.
Fc domain: the antibody tail region that interacts with cell-surface receptors and/or proteins of the complement system, allowing the antibody to activate the immune system.
Fc-mediated cytotoxicity: a mechanism of cell-mediated immune defense that allows effector cells of the immune system to lyze a target cell whose surface antigens are bound to specific antibodies.
Fcγ receptors (FcγR): proteins at the surface of some immune cells that contribute to the protective functions of the immune system and that can recognize antibodies attached to infected cells.
Microneutralization assay: a highly sensitive and specific assay for detecting virus-specific neutralizing antibodies (e.g., against influenza viruses) in human and animal sera.
Nanobodies: small (12–15 kDa) single-domain antibodies that selectively bind to a specific antigen.
Plaque reduction neutralization test: a method used to quantify the titer of a virus-neutralizing antibody.
Prefusion spike: the spike protein before virus fusion/attachment induces a conformational change.
Pseudoviruses: synthetic virus particles that are closely related to infectious viruses in structure and behavior but are not contagious.

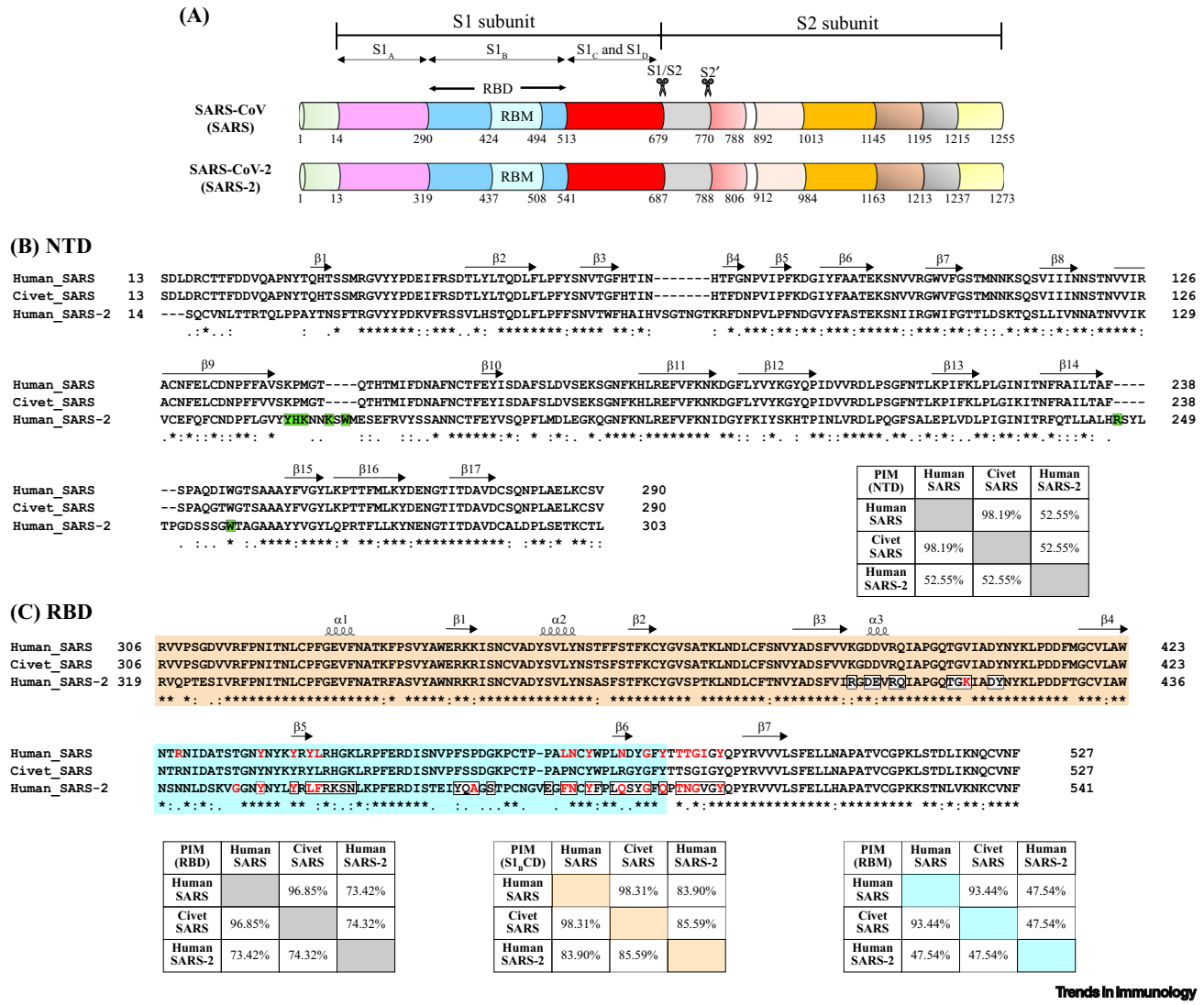


Figure 1. Sequence Comparison of SARS-CoV and SARS-CoV-2 S Glycoproteins. (A) Bar diagram comparison of the S glycoprotein. For illustration purpose, the structure-based sequence alignment of (B) the NTD (PDB 5X4S), and (C) the RBD from human SARS-CoV (PDB 5WRG), civet SARS-CoV, and human SARS-CoV-2 (PDB 6VXX) were carried out using DALI and subsequently Clustal and ESPript. Secondary structures (α for helix, and β for strand) of the NTD and RBD indicated above the sequence. The residues of S glycoprotein that interact with mAbs 4A8 and B38 are highlighted in green (interacting with the NTD in panel B) and white (interacting with the RBD in panel C), respectively. The residues of human SARS-CoV and human SARS-CoV-2 that interact with the human ACE2 receptor are shown in bold red font in panel C. The RBD sequence alignment is highlighted in orange for $S1_BCD$ and in cyan for the RBM. The percent identity matrix (PIM) for the NTD, the RBD ($S1_BCD$ and RBM), S1BCD, and the RBM are indicated in the table. Note: these calculations were performed purely for illustration purposes in this review. Abbreviations: CP, cytoplasmic tail; FP, fusion peptide; HR1/2, heptad repeat 1/2; NTD, N-terminal domain; RBD, receptor-binding domain; RBM, receptor-binding motif; SARS-CoV, severe acute respiratory syndrome coronavirus; SP, signal peptide; TM, transmembrane.

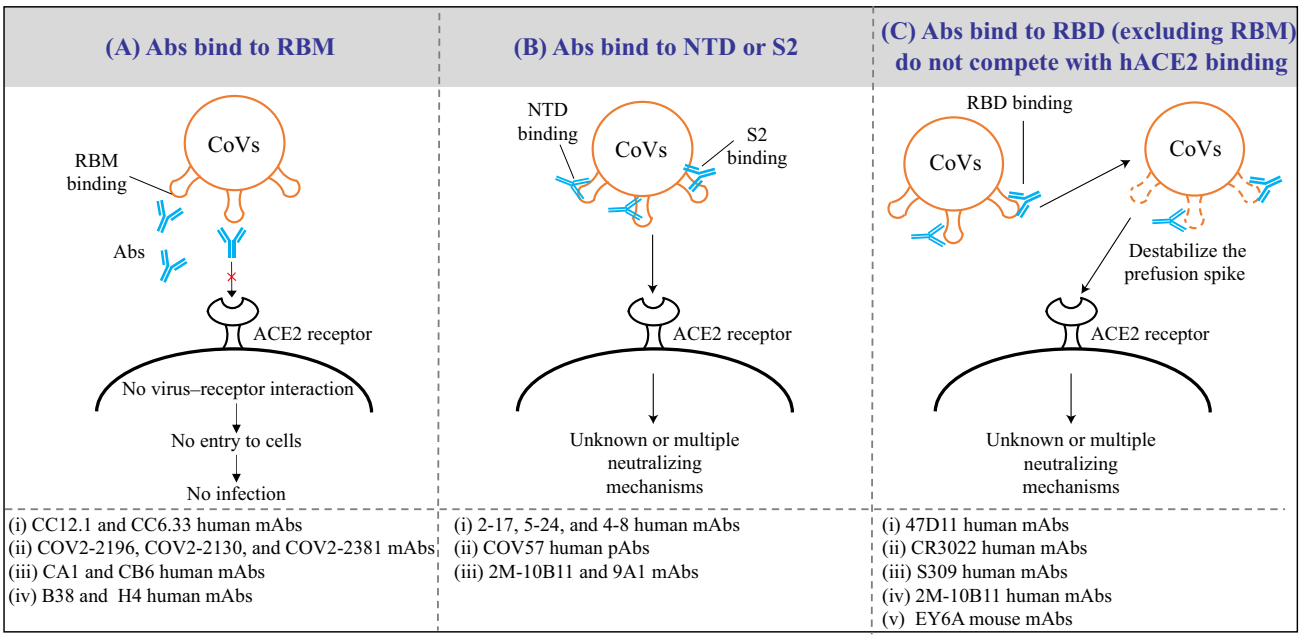
(rcsb.org) PDB: 7BYR/7BZ5/7BWJ] [29,33,39]. Therefore, we aim here to dissect, synthesize, and link many independent research findings on antibody-mediated neutralization of SARS-CoV-2.

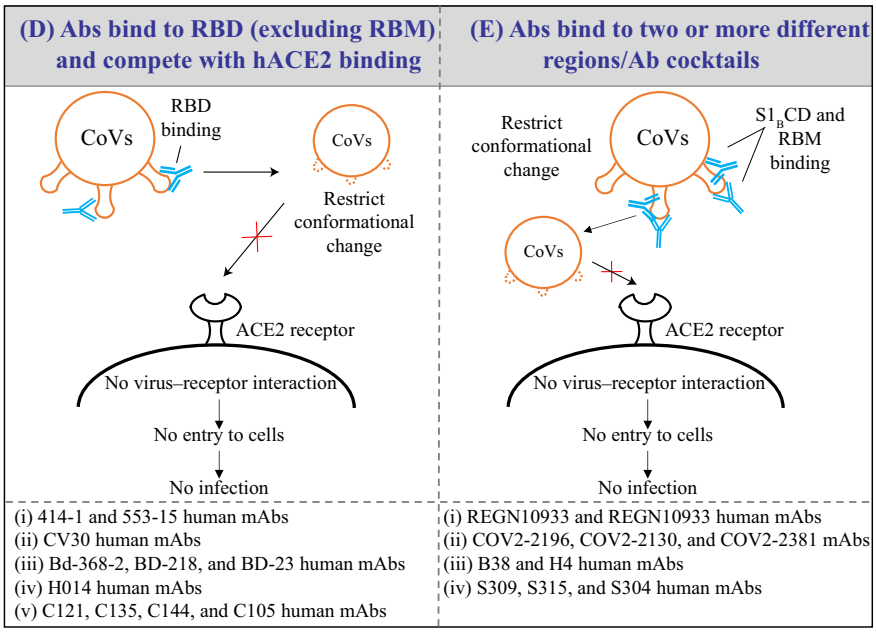
Modes of Antibody-Mediated SARS-CoV-2 Neutralization

Antibodies against coronaviruses are predominantly designed to target the S glycoprotein because of its ability to induce specific immune responses that are crucial against viral infections

Key Figure

SARS-CoV2 Antibodies Grouped Based on Targeting Region and Mechanism of Neutralization





Trends in Immunology

Figure 2. (A) Antibodies binding to the RBM of the S (spike) protein that compete for hACE2 binding and block hACE2–RBD interactions. (B,C) Antibodies that bind to (B) the NTD or S2 and (C) the RBD (excluding RBM), but do not compete with hACE2 binding. These antibodies exhibit neutralization activity against the virus via unknown or multiple mechanisms. (D) Neutralizing antibodies that bind to the RBD and compete for hACE2 binding, either by restricting conformational changes or through steric hindrance, and that prevent the virus from interacting with the receptor. (E) Single antibodies or antibody cocktails that bind to multiple epitopes could also mediate virus neutralization by

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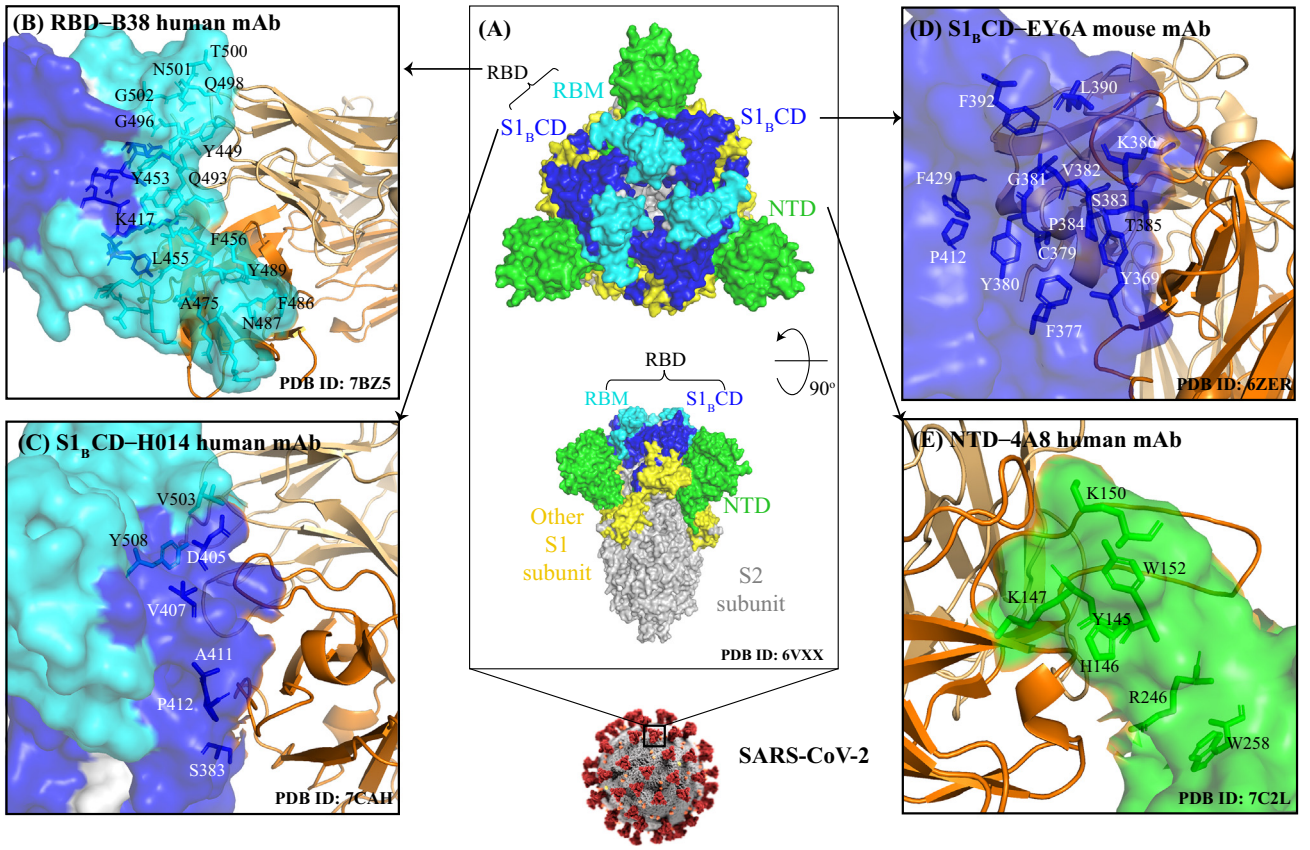

Figure 3. Mapping of Antibody-Interacting Regions in the Trimeric S (Spike) Protein of SARS-CoV-2. (A) Illustration of coronavirus SARS-COV-2 S protein in trimeric form, showing its molecular surface representation (top and side view) (PDB: 6VXX). The NTD, $S1_BCD$, the RBM, other S1 subunit, and the S2 subunit are colored in green, blue, cyan, yellow, and grey respectively. Molecular surface representation of neutralizing antibodies (B) B38 human mAb (PDB: 7BZ5), (C) H014 humanized mAb (PDB: 7CAH), and (D) EY6A mouse mAb (PDB: 6ZER) targeting the RBD ($S1_BCD$ and/or RBM) of S protein. (E) Molecular surface representation of human mAb 4A8 (PDB: 7C2L) interacting with the NTD of S protein. Panels B–E depict the S glycoprotein amino acids interacting with neutralizing antibodies (represented by sticks). The heavy and light chains of the indicated antibodies are represented in orange and light orange in the cartoon, respectively. For clarity, only one monomer of the trimer is shown. The SARS-CoV-2 virion ultrastructure was created by the Centers for Disease Control and Prevention (CDC) (Alissa Eckert, MS, and Dan Higgins, MAMS). Abbreviations: mAb, monoclonal antibody; NTD, N-terminal domain PDB, Protein Data Bank; RBM, receptor-binding motif; $S1_BCD$, $S1_B$ core domain; SARS-CoV-2 severe acute respiratory syndrome coronavirus 2.

[61–63]. The S glycoprotein conformational state and the domain targeted by an antibody seem to determine antibody cross-reactivity and cross-neutralization between SARS-CoV-2 and SARS-CoV [40,73]. Despite the similarity between the SARS-CoV-2 RBD and SARS-CoV RBD structures, the domains have different electrostatic surface potential maps [37,74], perhaps accounting for the differential S glycoprotein immunogenicity observed so far [33,36–38].

Neutralization via Direct Blocking of the hACE2–RBD interaction

Most nAbs bind directly to the RBM [27,29,40,45], which results in direct blocking of virus–receptor interactions (Figure 2A). Although nAbs that target the RBM of SARS-CoV-2 and SARS-CoV tend to have higher potency than non-RBM-targeting antibodies [75,76,84], the

restricting conformational changes in S protein. Abbreviations: Abs, antibodies; CoVs, coronaviruses; hACE2, human angiotensin-converting enzyme 2; mAbs, monoclonal antibodies; NTD, N-terminal domain; pAbs, polyclonal antibodies; RBD, receptor-binding domain; RBM, receptor-binding motif; $S1_BCD$, $S1_B$ core domain; S2, subunit 2.

RBM-targeting nAbs are often virus-specific and generally bind poorly or show limited cross-neutralization [77]. This might reflect the poorly conserved nature of the S glycoprotein RBMs of the two viruses (Figure 1A,C). S glycoprotein sequence comparison of SARS-CoV-2 and SARS-CoV shows that $S1_B$CD has higher sequence conservation than the RBM (85% versus ~50% identity; Figure 1C) [78].

SARS-CoV-2-Derived nAbs

Human anti-SARS-CoV-2 mAbs 311mab-31B5 and 311mab-32D4 [35] (Table 1) specifically bind to the RBD, most likely at the hACE2 receptor-binding interface [35]. Both mAbs can neutralize SARS-CoV-2 pseudoviruses [35] but have not been tested against SARS-CoV. Thus, further structural information will be necessary to elucidate the neutralizing mechanism of these two antibodies.

In addition, six SARS-CoV-2-specific mAbs – P2C-1F11, P2C-1A3, P2B-2F6, P2A-1A10, P2A-1B3, and P2C-1C10 (Table 1) – interfere with hACE2 binding to the SARS-CoV-2 S glycoprotein *in vitro* [33]. P2C-1F11 and P2B-2F6 antibodies target the RBD with high binding affinities. Both antibodies efficiently block hACE2 binding with almost 100% efficiency and exhibit potent neutralizing activities against SARS-CoV-2 live and pseudoviruses [33]. These results point to a correlation between mAb neutralizing activity and hACE2 receptor competition; however, none of these mAbs cross-react with or cross-neutralize SARS-CoV, possibly because they recognize the poorly conserved RBM, although this remains to be tested [33,64]. Accordingly, the crystal structure of the P2B-2F6–RBD (PDB: 7BWJ) complex reveals residue interactions mostly with the RBM [33]. Of 12 RBM contacts, only four residues (Asn448, Gly447, Tyr 449, and Asn450) are conserved in SARS-CoV [33,64]. Despite the strong competition of P2B-2F6 with hACE2 for RBD binding, the only common residues recognized by P2B-2F6 and hACE2 are Gly446 and Tyr449. This is surprising considering the strong competition observed for the RBD binding [33]. However, the similarly high binding affinities of P2B-2F6–RBD and hACE2–RBD suggest that they compete for the RBD interaction [33].

Similarly, other SARS-CoV-2-derived antibodies have been shown to elicit neutralization by occupying the RBM and precluding hACE2 engagement: MAbs CV30 (in HEK293T-hACE2 cells) [79] and CC12.1 (pseudovirus in HeLa-hACE2 cells) [32] strongly neutralize SARS-CoV-2 *in vitro*, but do not cross-react or cross-neutralize SARS-CoV, confirming a similar pattern to P2C-1F11 and P2B-2F6 [33].

Thus, SARS-CoV-2-derived RBM-targeting antibodies might be virus-specific, and hence might confer less neutralizing activity and protection against SARS-CoV and other coronavirus strains.

Cocktail Antibodies as Putative Alternative Immunotherapies against COVID-19

One of the most efficacious nAbs (REGN10987, REGN10933) against SARS-CoV-2 has highly potent neutralization efficacy in HEK293T, Calu-3, and Vero cells [29]. The REGN10933 binding site extensively overlaps the RBM and precludes hACE2 binding, whereas REGN10987 binds away from the RBM, and has little to no overlap with the hACE2 binding site (Figure 2E) (PDB: 6XDG) [29]. Thus, a combination of REGN10987 and REGN10933 antibodies is being used to form the REGN-COV cocktail against COVID-19 in Phase II/III clinical trials [29]. These are proof-of-concept trials for putative combination therapies.

To corroborate this phenomenon, other SARS-CoV-2-derived antibodies (414-1 and 553-15, Figure 2D [43]; COV2-2196 and COV2-2130, Figure 2E [41]) as well as B38 (PDB: 7BZ5, Figure 3A) and H4 [29], that probably occupy noncompeting epitope regions of the RBD, have

demonstrated similar synergistic neutralizing effects [29,41,43]. Specifically, a cocktail intraperitoneal (i.p.) injection of COV2-2196 + COV2-2130 antibodies protected SARS-CoV-2-infected wild-type female BALB/c mice from weight loss and reduced viral burden and inflammation in the lungs, as evidenced from the reduced expression of chemokine and cytokine genes compared with the untreated group [41]. Another combination, the B38 + H4 antibody cocktail injected i.p., protected hACE2 transgenic mice against SARS-CoV-2 infection at 3 days post-infection (dpi) relative to the control group, which exhibited pneumonia [29]. Moreover, single residues – Phe486, Asn487, Lys444, or Gly447 – were essential for COV2-2196 binding, as supported by the reduced binding of the Phe486Ala, Asn487Ala, Lys444Ala and Gly447Arg mutants [41]. Thus, a mutant strain of the virus might easily evade COV2-2196 alone if any of these positions are mutated [29]. In addition, passive transfer of potent hACE2-blocking mAbs (COV2-2196 or COV2-2381) as monotherapy protected rhesus macaques from SARS-CoV-2 infection and, unlike controls, no infectious virus was detected in the lungs at 2 dpi [41]. Of note, the mAb 553-15 was reported to enhance the neutralizing efficacy of many other antibodies and should be considered in a cocktail with other untested mAbs [43].

Other hACE-2-blocking antibodies with potent neutralization and protection efficacy include nAbs 2-15 [31], BD-368-2 [39], and CB6-LALA [42]. Respectively, these antibodies have been reported to protect hamsters (4 dpi), mice (5 dpi), and rhesus macaques (4 dpi) against SARS-CoV-2 infection [31,39,42]. Despite its potent neutralizing activity, mAb2-15 did not neutralize three mutants, Lys455Arg, Ala475Arg, and Gly502Arg, suggesting that mAb2-15 might function better if combined with other antibodies. The CB6-LALA antibody is an engineered **Fc domain** form of mAb CB6 in an attempt to minimize **Fc-mediated cytotoxicity** [17,42]. Nanobodies [46] lacking the Fc domain can neutralize viruses and mitigate ADE [80] that is currently of concern for vaccine and antibody design against SARS-CoV-2 [17,82]. This is evidenced by the potent neutralizing activities of a typical nanobody (ALX-0171) against respiratory syncytial virus (RSV) in respiratory epithelial cells, and this is currently being tested in Phase II clinical trials [82]. Although not yet observed with SARS-CoV-2 [81], perhaps by way of caution, researchers might consider routinely adopting strategies to mitigate possible ADE, perhaps by considering cocktail therapy where, for example, two nAbs are applied simultaneously or by ensuring adequate optimization of antibody concentrations to achieve complete neutralization of the virus [27,29,41,45]. **Fcγ receptor (FcγR)** engineering is another technique that might help to mitigate possible ADE because *in vitro* modeling of ADE has attributed increased pathogenesis to FcγR-mediated viral entry [80].

Overall, the newly isolated SARS-CoV-2 S glycoprotein-derived mAbs that directly target the RBM might offer hopes of protection against COVID-19 and might potentially perform best when used as a cocktail, although cross-reactivity and neutralization of SARS-CoV might be less effective by virtue of poor RBM sequence identity. However, these possibilities warrant further robust investigation.

SARS-CoV-Derived nAbs

Many highly potent SARS-CoV nAbs that target the hACE2 binding site on the RBD, such as S230.15, cross-react with and cross-neutralize other CoV strains, including human (Urbani, GZ02, CUHK-W1), raccoon dog (A031G), and palm civet (HC/SZ/61/03) strains in *in vitro* assays and/or in BALB/c mice (2 dpi) [84]. Most of these antibodies recognize an epitope in the RBM (Leu443, Thr487), validated with Leu443Arg and Thr487Ser virus escape mutants [83], as supported by plaque reduction assays in Vero E6 cells (Table 2) [75]. The same is true for mAb S230 (Tyr408, Tyr442, Leu443, and Tyr475) (PDB: 6NB7), also substantiated with the Leu443Arg escape mutant in Vero E6 cells [73]. Likewise, the human mAb m396 (Table 2)

cross-reacts with and neutralizes multiple human (GD03, Urbani, Tor2) and palm civet (SZ3, SZ16) strains of SARS-CoV in Vero E6 cells by recognizing a 21-residue epitope region [37,77] (PDB: 2DD8).

However, none of these mAbs (m396, S230, S230.15) cross-react with or cross-neutralize SARS-CoV-2 [36–38]. Of the four residues recognized by S230, there are two variations in SARS-CoV-2 RBM: Leu455 replaces Tyr442 in SARS-CoV, and Leu443 – a key residue for virus neutralization – is replaced by Phe456; these findings are also consistent for mAb S230.15 [84]. Accordingly, for mAb m396, extensive mapping of SARS-CoV–Fab fragment complexes [76,77] (PDB: 2DD8) onto the aligned RBD sequences of SARS-CoV-2 and SARS-CoV [37] showed that, of the seven residue variations, three are in the hACE2-binding interface: Arg426 to Asn439, Tyr484 to Gln 498, and Thr487 to Asn501 (SARS-CoV S glycoprotein to SARS-CoV-2 S glycoprotein numbering) [37]. This might explain the nonreactivity towards SARS-CoV-2 RBD.

Other antibodies such as CR3014 and 80R [37] (Table 2) do not cross-react with or cross-neutralize SARS-CoV-2. A similar epitope mapping analysis with 80R (PDB: 2GHW) revealed that five of 16 residue variations are hACE2-binding residues: Arg426 to Asn439, Tyr484 to Gln 498, Thr487 to Asn501, Asn479 to Gln493, and Leu472 to Phe486 [37]. These results might provide a logical structural basis for the lack of cross-reactivity and neutralization of both m396 and 80R, perhaps owing to the poor RBM sequence identity (Figure 1C) between SARS-CoV-2 and SARS-CoV S glycoproteins.

Overall, the structural and functional characterization of SARS-CoV Abs suggests that relying on existing SARS-CoV antibodies might not provide ideal therapeutic results; by contrast, targeting the SARS-CoV-2 RBD might represent a more promising therapeutic strategy.

nAbs Indirectly Overlapping with the RBM: Binding to the RBD $S1_BCD$ Region

mAbs in this group bind to epitopes in the RBD that are distal from the RBM, and block S glycoprotein binding to the host receptor (Figure 3C) [49,85,90]. They thus induce neutralization via a mechanism that is dependent on inhibiting hACE2–RBD binding and a pre-to-post-fusion conformational change in the S glycoprotein [84]. These properties provide broad reactivity and neutralization, making these nAbs promising candidates for achieving synergistic effects when combined with other nAbs [27,36,44,45]. This unique phenomenon has been demonstrated for the SARS-VHH-72 [36,85] and ADI-56046 [44] nAbs (Table 2).

Although SARS-VHH-72 binds to the $S1_BCD$ (PDB: 6WAQ) of the RBD, it also prevents hACE2 binding to the RBD of both SARS-CoV and SARS-CoV-2 S glycoproteins by trapping the RBD in the 'up' conformation [36]. This disrupts the S glycoprotein dynamics resulting in the neutralization of both SARS-CoV-2 and SARS-CoV pseudoviruses in HEK293S and Vero E6 cells [36]. The SARS-VHH-72 antibody, like ADI-56046, can cross-react with the SARS-like WIV1-CoV from *Rhinolophus sinicus* bats in HEK293S and Vero E6 cells [36] by recognizing a conserved epitope [36]. Of eight residue contacts of SARS-VHH-72, only Asn439 in the SARS-CoV-2 RBD replaces the Arg426 in SARS-CoV [36]. This conservation of residues might likely explain the conserved binding, although this remains to be demonstrated. ADI-56046 also neutralizes SARS-CoV-2, SARS-CoV, and bat coronavirus WIV1 in Vero E6 and HeLa-ACE2 cells, perhaps via a similar mechanism to SARS-VHH-72 [36,44].

Similarly to SARS-VHH-72 and ADI-56046, other nAbs such as CC6.33 [32], H014 (Figure 3C) [40], and COV21 [30] seem to recognize the $S1_BCD$ of both SARS-CoV-2 and SARS-CoV and neutralize both viruses, exerting a protective effect in hamsters (7 dpi) and humanized hACE2

mice (5 dpi), as well as demonstrating neutralization activity in HEK293T/hACE2 cells, respectively [30,32,40]. Although the binding of COV21 to the S glycoprotein resembles the binding of SARS-CoV S230 (PDB: 6NB7) [73], the binding interface also overlaps with sites for other highly potent nAbs such as B38 (Figure 3B) [29] and C105 [28], suggesting a similar mechanism of neutralization.

The above analysis suggests that the $S1_B$CD antigenic surface exhibits extensive conservation among SARS-like coronaviruses, as revealed by sequence alignment (Figure 1C). It is therefore tempting to speculate that $S1_B$CD-targeting antibodies might provide broad and potent neutralizing activity against coronaviruses. We argue that these should be tested alongside RBM or NTD-targeting antibodies in cocktail combinations.

Neutralization Independent of hACE2 Receptor Blocking

The SARS-CoV S glycoprotein-derived human mAb 47D11 can neutralize SARS-CoV-2 authentic and pseudoviruses in Vero E6 and HEK293T cells [64]. Others have reported that mAb S309, another human mAb designed against SARS-CoV S glycoprotein, can potently neutralize both SARS-CoV and SARS-CoV-2 authentic and pseudoviruses in Vero E6 cells by recognizing the RBD [12]. Both 47D11 and S309 bind to the $S1_B$CD of the two viruses with similar affinities but do not compete with hACE2 binding to the RBD [12,64]. This property is also exhibited by the highly potent SARS-CoV-2-derived mAb EY6A (Figure 3D) (PDB: 6ZCZ/6ZDH/6ZDG/6ZER/6ZFO) [85]. EY6A binds tightly to the $S1_B$CD of the S glycoprotein [85]. Residues within the footprints of EY6A are key to stabilizing the **prefusion spike** [13,36,85,87]. There is currently no structural information for mAb 47D11. However, the cryo-electron microscopy (cryo-EM) reconstruction of the SARS-CoV-2-S–S309-Fab [12] complex (PDB: 6WPS/6WPT/6WS6) and the crystal structure of the SARS-CoV-2-S–EY6A-Fab complex [85] suggest one or multiple mechanisms of neutralization, including S-trimer crosslinking or spike prefusion destabilization [36,38]. These neutralizing mechanism(s) might also apply to 47D11, although this remains to be tested [12,64] (Table 2). On a positive note, S309 Fc variants with increased half-life and effector functions have been fast-tracked for clinical trials against COVID-19 [12].

Unlike 47D11 and S309, other antibodies have shown discrepancies in neutralization results. Specifically, the SARS-CoV-specific human mAb, CR3022 (Table 2) that is used as a control antibody in many studies [40,44,85,88] – targets the RBD (PDB: 6W41) and neutralizes SARS-CoV potently [89,90], but shows no neutralizing activity against SARS-CoV-2, even though it binds tightly to the RBD [38,84]. However, others have reported that CR3022 neutralizes SARS-CoV-2 [89] by destabilizing and destroying the prefusion S glycoprotein trimer [89]. The latter study suggested that the **microneutralization assay** mode of assessment might have contributed to the observed nonneutralizing effect of CR3022 on SARS-CoV-2 [90] because a **plaque reduction neutralization test** showed a positive neutralization of SARS-CoV-2 by CR3022 [89]. Hence, to avoid similar discrepancies researchers should consider using different virus neutralization methods to validate their results.

The crystal structure of SARS-CoV RBD with CR3022 (PDB: 6W41), superimposed with that of SARS-CoV-2 RBD with hACE2 (PDB: 6LZG), shows that CR3022 occupies the $S1_B$CD [38,90]. The crystal structure shows that, of the 28 residues in the CR3022 epitope, 24 are conserved between SARS-CoV-2 and SARS-CoV [90]. The high sequence conservation of the $S1_B$CD might explain the cross-reactivity and perhaps neutralization potential of CR3022, but this remains to be investigated [90,91]. An interaction study with 10 different variants of SARS-CoV shows that the mAb CR3022 bound to the RBD of all isolates from human and civet cat. By contrast, mAb CR3014 (which likely recognizes the RBM) did not bind to variants of SARS coronavirus strains

such as GD03T0013, the Asn479Ser mutant, or the civet cat SARS-CoV-like isolate SZ3 (which bears RBM mutations) [92]. Similarly, structural analysis has shown that, of the 22 residue interactions of mAb S309 (PDB: 6WPS/6WPT/6WS6), that potently neutralizes both SARS-CoV and SARS-CoV-2 pseudoviruses, 17 of these residues are conserved [12]. We posit that this conservation of residues likely accounts for the observed cross-reactivity and neutralization, and certainly merits further testing. Of note, a cocktail of S309 with weaker nAbs (e.g., S315 or S304) significantly enhanced the neutralization of SARS-CoV-2, lending further support to the notion that antibody cocktail therapy might be therapeutically effective against COVID-19.

Collectively, these groups of antibodies utilize unknown mechanisms to neutralize either SARS-CoV-2 or SARS-CoV without necessarily blocking virus–receptor engagement. However, these mechanisms might result in lower neutralization efficiency [32]. Consequently, these groups of antibodies might require co-binding for effective neutralization [64,84], and might be tested as part of antibody cocktails.

NTD- and S2-Targeting SARS-CoV-2 nAbs

In addition to the most common antibody groups identified as the RBD-targeting antibodies, recent studies have demonstrated that NTD- and S2-targeting antibodies are also elicited in COVID-19 patients and might be effective nAbs. Specifically, nAbs 4A8 (PDB: 7C2L) [88], COV57 [30], 2-17, 5-24, and 4-8 [31] target the NTD of SARS-CoV-2 S protein (Figure 1B), whereas mAbs 9A1 and 0304-3h3 [88] target the S2 region (Figure 1A) [64]. All these antibodies neutralized authentic SARS-CoV-2 virus, with the exception of 9A1 that is nonneutralizing [88]. Of note, COV57 is a SARS-CoV-2-derived antibody that, almost for the first time, recognized the MERS S protein in ELISA assays – a special property observed so far [30].

Overall, non-RBD binding antibodies might also neutralize SARS-CoV-2 without interrupting virus–receptor engagement, although the neutralizing effect of these antibodies might be less than that of RBD-binding antibodies, and therefore might require co-binding antibodies in a cocktail to exert a potent therapeutic effect. Further future testing is thus eagerly awaited.

Concluding Remarks

nAbs may be an alternative source of treatment against COVID-19. Unfortunately, the poor cross-neutralizing efficacy of SARS-CoV-derived antibodies against SARS-CoV-2 has required additional input to generate new antibodies and improve existing ones. Thus, the shift in attention towards producing SARS-CoV-2-specific antibodies that have demonstrated higher neutralizing potential is timely and imperative. Antibodies such as REGN-COV, BD-23, CB6-LALA, SARS-VHH-72, S309, 47D11, 311 mAb-31B5, and 311 mAb-32D4 appear to be particularly promising for combating the COVID-19 pandemic in view of their potent *in vitro* neutralizing activities and/or *in vivo* protection efficacies in animal models. Current structural and sequence comparison-based analyses have attempted to summarize the various possible mechanistic reasons why most SARS-CoV-2 and SARS-CoV-derived antibodies do not cross-react and/or cross-neutralize. We have offered some insights into what types of antibodies might cross-react and cross-neutralize SARS-CoV-2 and SARS-CoV, and these should be further addressed experimentally. We have also provided a perspective on the impact of the current Asp614Gly and other mutations on the neutralizing effect of current antibodies (see Outstanding Questions). We have considered a platform to easily identify and choose antibodies that might be tested in a cocktail against COVID-19 to overcome escape mutant strains. For example, promising cocktails might include REGN-COV, 414-1 + 553-15, COV2-2196 + COV2-2130, CR3022 + CR3014, or B38 + H4. The prospect of combining mAbs 553-15 and S309 with other antibodies

Outstanding Questions

What is the Achilles' heel for blocking SARS-CoV-2? Many targets have been proposed for the control of SARS-CoV-2 infections; namely virus-specific targets such as the dominant S glycoprotein and its subunits, the replication complex such as the RNA-dependent RNA polymerase, and human proteases that are required for virus S glycoprotein processing such as transmembrane serine protease 2.

What is the driving force behind the Asp614Gly and other mutations in the SARS-CoV-2 S glycoprotein? Will these mutations worsen COVID-19 symptoms? Further investigations will be necessary to better understand the impact of the identified mutations on disease.

What are the clinical correlates of current vaccine and antibody treatments of COVID-19? Will ADE of disease arise with these vaccines and antibodies? Careful studies will be necessary to understand whether ADE pathologies might be associated with vaccine and antibody candidates against SARS-CoV-2.

Do all recovered COVID-19 patients produce protective immune responses? How long do these immune responses last? Retrospective investigations will be essential to better understand the immune paradigm in COVID-19 patients.

Will virus-like particles (VLPs) of SARS-CoV-2 induce more potent protective neutralizing antibodies than isolated S glycoprotein or its subunits? VLPs are safer to use because they do not contain genetic material. VLPs present antigenic epitopes akin to an actual virion; they can stimulate both humoral and cellular immune responses that can offer antiviral protection. Hence, the recent effort to establish the VLP platform for SARS-CoV-2 is a key initiative.

What is the half-life of current therapeutic antibodies? Will treated patients or individuals given antibodies as prophylactics require multiple administrations? How often should subsequent administrations be given to ensure adequate protection? What is the antibody dosage? Extensive retrospective studies will be necessary to elucidate these parameters.

in a cocktail is particularly attractive because these mAbs demonstrate a potent synergistic neutralizing effect with many of the other antibodies [12,43]. Moreover, mAb CR3022 might be combined with mAbs COV21, C105, or B38 in a cocktail because CR3022 does not appear to compete with these three antibodies for binding to the SARS-CoV-2 S glycoprotein, and therefore might offer synergistic neutralizing effects [28,29]. Similarly, the potent NTD-binding nAb 4A8 might also be considered in a cocktail with RBD-binding antibodies because 4A8 binding to the NTD leaves the RBD region of the S glycoprotein free for co-binding antibodies that might offer additive neutralizing effects. Of note, in addition to cocktail antibody therapies, a cocktail with other antiviral drugs such as remdesivir might be therapeutically explored against COVID-19. Moving forward, because ADE of COVID-19 cannot be reliably predicted after vaccination or antibody treatment, careful analysis of safety will need to be conducted in humans (see Outstanding Questions).

Will positive *in vitro* neutralization assays and animal models mirror human trials?

Finally, as and when they become available, will vaccines be applicable for every individual? This is relevant because older and/or immunocompromised persons might respond poorly or present adverse reactions to vaccine immunization.

Overall, the alarming number of COVID-19 deaths is disheartening and calls for immediate public health interventions. Nevertheless, the above discussion suggests that there is hope of combating COVID-19 in view of the many vaccines, antibodies targeting the S glycoprotein, and small-molecule candidates that are currently being tested in clinical trials and in preclinical research. We therefore expect that vaccines or antibody therapeutics might become available sooner rather than later, although we need to be vigilant for emerging mutations in the S glycoprotein that might thwart current therapeutic efforts in the future.

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Resources

[i] https://clinicaltrials.gov/ct2/show/NCT04292899
[ii] https://clinicaltrials.gov/ct2/show/NCT04400838
[iii] https://clinicaltrials.gov/ct2/show/NCT04480957
[iv] https://clinicaltrials.gov/ct2/show/NCT04436276
[v] https://clinicaltrials.gov/ct2/show/NCT04341389
[vi] https://clinicaltrials.gov/ct2/show/NCT04425629
[vii] https://clinicaltrials.gov/ct2/show/NCT04426695
[viii] https://clinicaltrials.gov/ct2/show/NCT04452318

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