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Neutralizing monoclonal antibodies against highly pathogenic coronaviruses

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The pandemic of Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome 2 coronavirus (SARS-CoV-2) is a continuing worldwide threat to human health and social economy. Historically, SARS-CoV-2 follows SARS and MERS as the third coronavirus spreading across borders and continents, but far more dangerous with long-lasting symptomatic consequences. The current situation is strong evidence that coronaviruses will continue to be pathogens of consequence in the future, thus calling for the development of neutralizing antibody-based prophylactics and therapeutics for prevention and treatment of COVID-19 and other human coronavirus diseases. This review summarized the progresses of developing neutralizing monoclonal antibodies against infection of SARS-CoV-2, SARS-CoV, and MERS-CoV, and discussed their potential applications in prevention and treatment of COVID-19 and other human coronavirus diseases.

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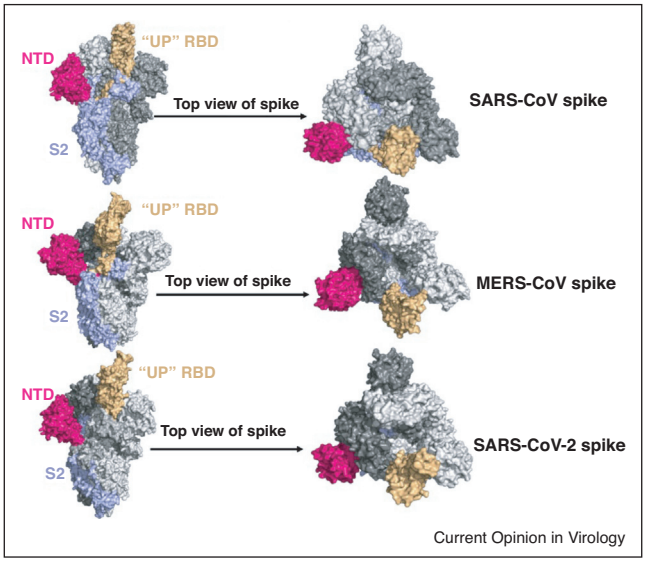
Introduction

By the time SARS-CoV-2 (originally named 2019-nCoV by WHO) (https://www.who.int/emergencies/diseases/novel-coronavirus-2019) first emerged in late 2019 [1], seven human coronaviruses, including SARS-CoV in 2002/2003 (https://www.who.int/publications/m/item/summary-of-probable-sars-cases-with-onset-of-illness-from-1-november-2002-to-31-july-2003) and MERS-CoV in 2012 (https://www.who.int/emergencies/disease-outbreak-news/item/2021-DON317), had caused the outbreaks of severe coronavirus diseases a worldwide. However, COVID-19, caused by SARS-CoV-2 infection, has posed more serious threat to public health, social stability and economy development. Presently, many vaccines against COVID-19 are in the clinical trials (https://clinicaltrials.gov/ct2/results?term=vaccine&cond=Covid19&age_v=&gndr=&type=&rslt=&phase=2&phase=3&Search=Apply), and some have already applied for and obtained emergency use authorization. Cases of side effects after vaccination have been reported. This means that safety and efficacy, particularly in view of the growing number of mutant strains diverging from wild type [2••], and length of immunization still need further study with more data. Beyond vaccine development, antibody cocktails have shown some efficacy against viral mutants [2••]. Fully human antibodies can accurately and efficiently identify antigens with few side effects in humans. Some neutralizing monoclonal antibodies (NMAbs) have also entered clinical trials (https://clinicaltrials.gov/ct2/results?term=antibody&cond=Covid19&age_v=&gndr=&type=&rslt=&Search=Apply). In view of the importance of NMAbs in the prevention and treatment of coronavirus diseases, this review summarizes the progresses of developing NMAbs against SARS-CoV, MERS-CoV, and SARS-CoV-2, providing scientific knowledge about these NMAbs to combat the current COVID-19 pandemic and future emerging and re-emerging coronavirus diseases.

Key targets of coronavirus NMAbs

The coronavirus spike (S) glycoprotein is the primary immunogenic target for the design of neutralizing antibodies. The trimeric S protein is a type I fusion transmembrane protein which mediates virus binding to corresponding receptors and finally entry into host cells. In the case of SARS-CoV and SARS-CoV-2, they recognize the same receptor angiotensin-converting enzyme 2 (ACE2), whereas MERS-CoV S protein binds to dipeptidyl peptidase-4 (DPP4). The S protein trimer comprises three copies of an S1 subunit that contains the N-terminal domain (NTD) and receptor binding domain (RBD) and three copies of S2 [3–6,7••,8]. The RBD has two conformational states, the closed 'down' state, which hides the receptor-binding regions, and the open 'up' state, which

Figure 1



The crystal structure of S glycoproteins with one receptor-binding domain (RBD); up conformation of three coronaviruses that cause severe symptoms. The order of crystal structures is SARS-CoV S, PDB: 6vyb; (5x5f) MERS-CoV S, PDB: 5x5f and SARS-CoV-2 S, PDB: 7kj5, respectively. In one S glycoprotein monomer, N-terminal domain (NTD) is shown in purple, RBD is shown in earth yellow, and S2 is shown in wathet blue. The other two are shown in gray.

exposes the determinants of receptor binding (Figure 1). Finally, the S2 subunit mediates the fusion of coronavirus and host cell membrane [9••,10].

NMAbs against SARS-CoV

Human NMAbs against SARS-CoV

NMAbs identified by screening of antibody libraries

As the SARS outbreak during 2002/2003, some fully human-derived NMAbs targeting the RBD were identified from nonimmune phage libraries of human antibodies [11–16], such as 80R, CR3014, and m396 (Figure 2a) (Table 1). The S protein of SARS-CoV continued to mutate during transmission, but researchers found that CR3014 did not neutralize all mutant strains. However, researchers also discovered that the combination of CR3022 and CR3014, now known as an antibody cocktail, could effectively neutralize multiple mutant strains [17]. B1 is the first S2-targeting mAb screened from an antibody library of SARS-CoV convalescent patients [18] (Table 1).

NMAbs identified by use of Epstein–Barr virus (EBV) transformation technology

Similar to the use of hybridoma technology, researchers used EBV to infect antibody-secreting B cells in order to construct immortal cell lines that stably express antibodies. In this way, a pool of human NMAbs was screened out, such as S3.1 and S230.15 [16,19] (Table 1).

NMAbs identified from transgenic mice

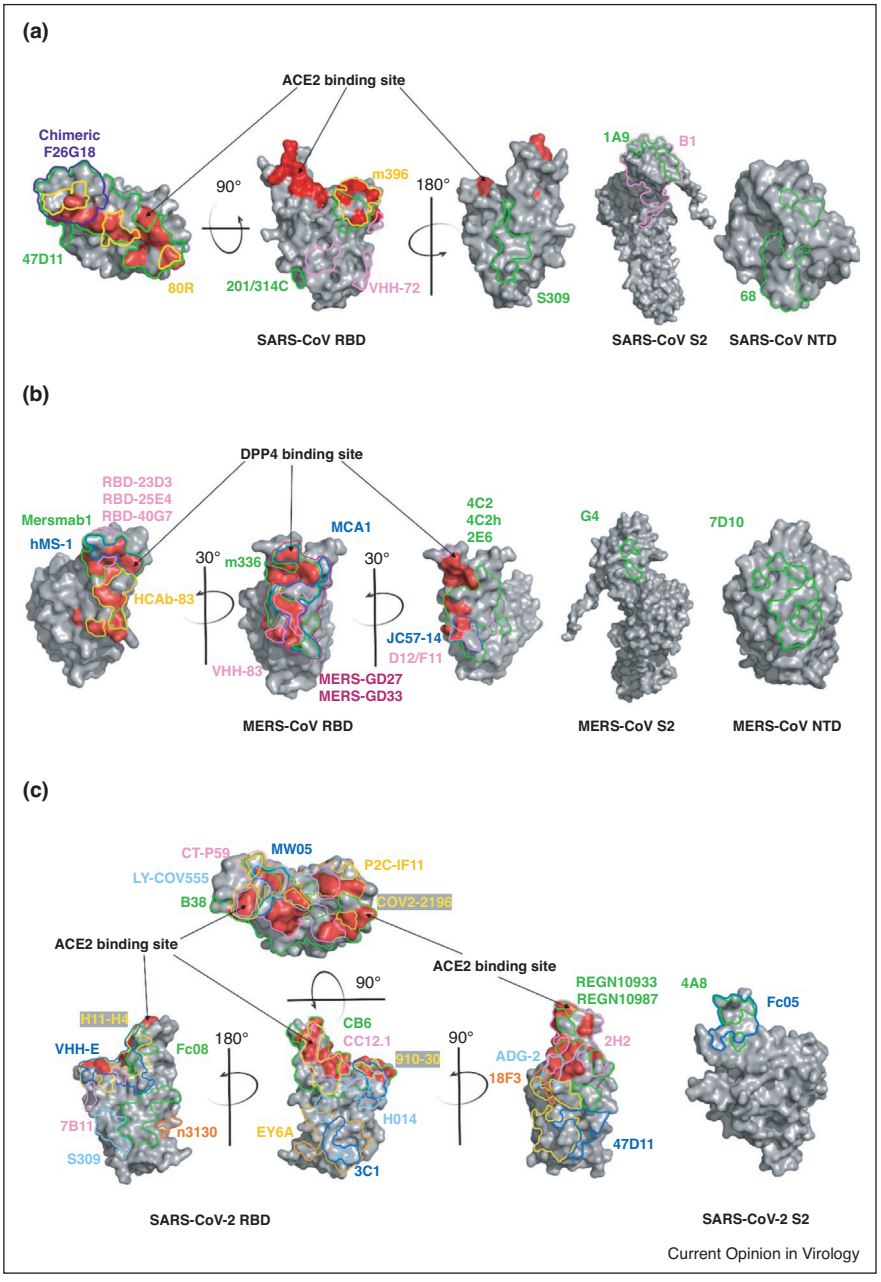
Fully humanized NMAbs have been developed from the human immunoglobulin G (IgG) transgenic mouse, XenoMouse®, immunized with the SARS-CoV S protein [20]. The NMAbs 68 and 201 targeting the NTD and RBD, respectively, identified from the immunized transgenic mice. Mice receiving 40 mg/kg of either NMAb before SARS-CoV challenge were completely protected [21] (Table 1).

NMAbs against SARS-CoV from other sources

NMAbs identified by use of hybridoma technology

Owing to limited human trials, the development of animal immunization and hybridoma technology has substantially enriched SARS-CoV antibody research. A large number of animal-derived NMAbs were screened out, such as F26G18, and the corresponding chimeric antibodies were obtained by antibody humanization. These chimeric NMAbs were shown to target RBD and exert antiviral effects by inhibiting ACE2 binding to RBD [22–24]. Similarly, many NMAbs with strong neutralizing activity against SARS-CoV were identified, including 1A5, 2C5, and 341C, all targeting RBD [25,26]. To explore effective targets, researchers immunized mice with different regions of the S protein as antigens and obtained S34 and S84 with correspondingly different targets [27]. The mutation of the S2 region was much slower, compared to S1, resulting in the development of more

Figure 2



Binding interface of neutralizing monoclonal antibodies on SARS-CoV, MERS-CoV and SARS-CoV-2 S glycoproteins. The binding sites of neutralizing antibodies with S proteins of **(a)** SARS-CoV, **(b)** MERS-CoV and **(c)** SARS-CoV-2 are indicated on the NTD, S2 and 'up' RBD. Arrow points to red area, the site where RBD binds to the receptor. Multiple colors were used to represent different antibodies. PDBs of crystal structure were shown as follows: SARS-CoV RBD S2 PDB: 2ajf; SARS-CoV NTD PDB: 5x4s; MERS-CoV RBD S2 PDB: 4kqz; MERS-CoV NTD PDB: 6pxh; SARS-CoV-2 RBD PDB: 6m0j; SARS-CoV-2 NTD PDB: 7l2c.

broad-spectrum S2-targeting antibodies against SARS-CoV mutant strains [28,29]. Accordingly, researchers immunized mice with S2 as the antigen and screened a number of NMAbs targeting S2, among which 1A9 was the most potent [30–32].

NMAbs against MERS-CoV

Human NMAbs against MERS-CoV

NMAbs identified by screening of antibody libraries

NMAbs m336, m337, and m338 that were identified from a phage-displayed Fab library from healthy donors

Table 1

NMAbs against highly pathogenic coronaviruses

Name of NMAb	Type	Source	Preparation	Target	Mechanisms of neutralization	Developing stage	Refs
NMAbs against SARS-CoV							
80R	scFv	Human	Non-immune phage libraries of human antibodies	RBD	Competition with ACE2 in binding with RBD	Preclinical	[11,12]
CR3014	scFv	Human	Non-immune phage libraries of human antibodies	RBD	Competition with ACE2 in binding with RBD	Preclinical	[13,14]
CR3022	scFv	Human	A scFv phage display library generated from cells of a convalescent SARS patient	RBD	Blocking conformational changes of S proteins	Preclinical	[17]
m396	Fab	Human	Antibody library derived from cells of healthy volunteers	RBD	Competition with ACE2 in binding with RBD	Preclinical	[15,16]
B1	scFv	Human	A scFv phage display library generated from cells of a convalescent SARS patient	S2	–	Preclinical	[18]
S3.1	IgG	Human	Epstein-Barr virus transformation of human B cells of a convalescent SARS patient	S	–	Preclinical	[19]
S230.15	IgG	Human	Epstein-Barr virus transformation of human B cells of a convalescent SARS patient	RBD	Competition with ACE2 in binding with RBD	Preclinical	[16]
68	IgG	Human	Transgenic mice	NTD	–	Preclinical	[21]
201	IgG	Human	Transgenic mice	RBD	Competition with ACE2 in binding with RBD	Preclinical	[21]
F26G18	IgG	Mouse	Animal immunization and hybridoma technology	RBD	Competition with ACE2 in binding with RBD	Preclinical	[22–24]
1A5 2C5	IgG	Mouse	Animal immunization and hybridoma technology	RBD	Competition with ACE2 in binding with RBD	Preclinical	[25]
341C	IgG	Mouse	Animal immunization and hybridoma technology	RBD	Competition with ACE2 in binding with RBD	Preclinical	[26]
S34 S84	IgG	Mouse	Animal immunization and hybridoma technology	548 to 567 of S protein	–	Preclinical	[27]
1A9	IgG	Mouse	Animal immunization and hybridoma technology	S2	–	Preclinical	[30–32]
NMAbs against MERS-CoV							
m336 m337 m338	Fab	Human	A phage-displayed antibody Fab library generated from B cells of healthy donors	RBD	Competition with DPP4 in binding with RBD	Preclinical	[33,34]
3B11	scFv	Human	A non-immune phages-displayed scFv library	RBD	Blocking the binding of DPP4 and RBD	Preclinical	[35]
MERS-4 MERS-27	scFv	Human	A non-immune yeast-displayed scFv library	RBD	Competition with DPP4 in binding with RBD	Preclinical	[36]
LCA60	IgG	Human	Epstein-Barr virus transformation of B cells of a convalescent SARS patient	RBD	Interfering with the binding of RBD to cell receptor DPP4	Preclinical	[38]
MCA1	Fab	Human	A phage-displayed antibody library from a MERS-CoV survivor	RBD	Interfering with the binding of RBD to cell receptor DPP4	Preclinical	[37]
CDC2-C2	IgG	Human	Antibody gene cloning of memory B cells from a MERS patient	RBD	Interfering with the binding of RBD to cell receptor DPP4	Preclinical	[41]
MERS-GD27	IgG	Human	Antibody gene cloning of memory B cells from convalescent MERS patient	RBD	Interfering with the binding of RBD to cell receptor DPP4	Preclinical	[42,43]
REGN3051 REGN3048	IgG	Human	Transgenic mice	RBD	Blocking the binding of RBD to DPP4	Preclinical	[39]
7.7g6 1.6f9 1.2g5 4.6e10	IgG	chimeric	Transgenic mice	RBD	Interfering with the binding of RBD to cell receptor DPP4	Preclinical	[40]
1.6c7 3.5g6	IgG	chimeric	Transgenic mice	S2	Preventing comformational changes in the S2 subunit	Preclinical	[40]
Mersmab1	IgG	Mouse	Animal immunization and hybridoma technology	RBD	Blocking the binding of RBD to DPP4	Preclinical	[45,47]

Table 1 (*Continued*)

Name of NMAb	Type	Source	Preparation	Target	Mechanisms of neutralization	Developing stage	Refs
4C2 2E6	IgG	Mouse	Animal immunization and hybridoma technology	RBD	Interfering with the binding of RBD to cell receptor DPP4	Preclinical	[46]
D12 F11	IgG	Mouse	Animal immunization and hybridoma technology	RBD	Interfering with the binding of RBD to cell receptor DPP4	Preclinical	[49]
G2	IgG	Mouse	Animal immunization and hybridoma technology	NTD	–	Preclinical	[49]
G4	IgG	Mouse	Animal immunization and hybridoma technology	S2	Inhibition of membrane fusion	Preclinical	[49,50]
5F9	IgG	Mouse	Animal immunization and hybridoma technology	NTD	Precluding the conformational changes required for membrane fusion	Preclinical	[52]
7D10	IgG	Mouse	Animal immunization and hybridoma technology	NTD	Interfering with the binding of RBD to cell receptor DPP4 and precluding the conformational changes required for membrane fusion	Preclinical	[51]
RBD-23D3 RBD-25E4 RBD-40G7	IgG	Mouse	Animal immunization and hybridoma technology	RBD	Blocking the binding of RBD to DPP4	Preclinical	[48]
JC57-14	IgG	Macaques	Animal immunization and gene cloning	RBD	Blocking the binding of RBD to DPP4	Preclinical	[41]
JC57-13 FIB-H1	IgG	Macaques	Animal immunization and gene cloning	Non-RBD regions of S1	–	Preclinical	[41]
VHH-83	HCAbs	Camel	VHH complementary DNA library	RBD	Interfering with the binding of RBD to cell receptor DPP4	Preclinical	[61]
NbMS10	HCAbs	Llama	A VHH phage display library	RBD	Interfering with the binding of RBD to cell receptor DPP4	Preclinical	[62]
VHH-55	HCAbs	Llama	A VHH phage display library	RBD	Interfering with the binding of RBD to cell receptor DPP4	Preclinical	[63•]
NMAbs against SARS-CoV-2							
ab1	scFv	Human	Non-immune phage libraries of human antibodies	RBD	Competition with ACE2 in binding with RBD	Preclinical	[64•]
rRBD-15	Fab	Human	A synthetic human Fab antibody library AB1	RBD	Competition with ACE2 in binding with RBD	Preclinical	[65]
n3130	HCAbs	Human	A fully human phage displayed single-domain antibody library of healthy adult donors	S1	Non-competition with ACE2 in binding with RBD	Preclinical	[67••]
5A6	Fab	Human	A highly diverse naïve human Fab library	RBD	Blocking the binding of RBD to ACE2	Preclinical	[66]
CT-P59	IgG	Human	A scFv phage display library generated from cells of a convalescent SARS patient	RBD	Competition with ACE2 in binding with RBD	Clinical	[68•]
910-30	Fab	Human	A yeast-displayed Fab library generated from cells of a COVID-19 convalescent patient	RBD	Competition with ACE2 in binding with RBD	Preclinical	[70]
2B11 1E10	IgG	Human	Phage-display immune libraries constructed from the pooled PBMCs of COVID-19 convalescent patients	RBD	Blocking the binding of RBD to ACE2	Preclinical	[69]
ADI-55689 ADI-55993 ADI-56000 ADI-55688 ADI-56046 ADI-56010 ADI-55690 ADI-55951	IgG	Human	A yeast-displayed library generated from cells of SARS-infected patients	RBD	Blocking receptor attachment and inducing S1 shedding	Preclinical	[71•]
ADG-2	IgG	Human	Engineered antibody	RBD	Interfering with the binding of RBD to ACE2	Preclinical	[72]
S309	IgG	Human	Epstein-Barr virus transformation of human B cells of SARS-infected patients	RBD	S trimer cross-linking, steric hindrance or aggregation of virions	Preclinical	[73•]

Table 1 (*Continued*)

Name of NMAb	Type	Source	Preparation	Target	Mechanisms of neutralization	Developing stage	Refs
BD-368-2	IgG	Human	High-throughput single-cell RNA and VDJ sequencing of convalescent COVID-19 patients' B cells	RBD	Competition with ACE2 in binding with RBD	Preclinical	[74]
CB6	IgG	Human	Antibody gene cloning of B cells from a COVID-19 convalescent patient	RBD	Competition with ACE2 in binding with RBD	Preclinical	[79]
B38 H4	IgG	Human	Antibody gene cloning of B cells from a COVID-19 convalescent patient	RBD	Competition with ACE2 in binding with RBD	Preclinical	[86••]
COV2-2196 COV2-2130	IgG	Human	Antibody gene cloning of B cells from COVID-19 patients	RBD	Blocking the binding of RBD to ACE2	Preclinical	[87,88]
REGN10933 REGN10987	IgG	Human	Antibody gene cloning of B cells from transgenic mice and SARS-CoV-2-infected patients	RBD	blocking the binding of ACE2 to the RBD	Clinical	[90,92,93••]
P2C-1F11 P2B-2F6	IgG	Human	Antibody gene cloning of B cells from a COVID-19 patient	RBD	Competition with ACE2 in binding with RBD	Preclinical	[82]
CC12.1	IgG	Human	Antibody gene cloning of B cells from COVID-19 patients	RBD	Blocking the binding of RBD to ACE2	Preclinical	[75]
COVA1-18 COVA2-15 COVA1-16 COVA2-02	IgG	Human	Antibody gene cloning of B cells fromCOVID-19 patients	RBD	Competition with ACE2 in binding with RBD	Preclinical	[94,100]
S2E12 S2M11	IgG	Human	Antibody gene cloning of B cells from COVID-19 patients	RBD	Competition with ACE2 in binding with RBD	Preclinical	[89]
CV07-209	IgG	Human	Antibody gene cloning of B cells from of COVID-19 patients	RBD	Blocking the binding of RBD to ACE2	Preclinical	[83]
C1A-B12	IgG	Human	Antibody gene cloning of B cells from of COVID-19 patients	RBD	Blocking the binding of RBD to ACE2	Preclinical	[80]
A19-46.1 A19-61.1 A23-58.1 B1-182.1	IgG	Human	B cell sorting of COVID-19 patients and V(D)J sequencing	RBD	Blocking the binding of RBD to ACE2	Preclinical	[101]
DH1047	IgG	Human	B cell sorting of SARS patients and V(D)J sequencing	RBD	Interfering with the binding of RBD to ACE2	Preclinical	[102,104]
CV2-75 CV1-30	IgG	Human	B cell sorting of COVID-19 patients and V(D)J sequencing	RBD	Blocking the binding of RBD to ACE2	Preclinical	[103]
2-15	IgG	Human	single-cell 5′-mRNA and V(D)J sequencing of COVID-19 patients' B cells	RBD	Interfering with the binding of RBD to cell receptor ACE2	Preclinical	[78••]
2-17 5-24 4-8	IgG	Human	single-cell 5′-mRNA and V(D)J sequencing of COVID-19 patients' B cells	NTD	–	Preclinical	[78••]
4A8	IgG	Human	Antibody gene cloning of B cells from of COVID-19 patients	NTD	Altering the conformation of S protein	Preclinical	[76•]
MW05 MW07	IgG	Human	Antibody gene cloning of B cells from a COVID-19 convalescent patient	RBD	Blocking the binding of RBD to ACE2	Preclinical	[95]
311mab-31B5 311mab-32D4	IgG	Human	Antibody gene cloning of B cells from a COVID-19 convalescent patient	RBD	Blocking the binding of RBD to ACE2	Preclinical	[96]
C121 C144 C135	IgG	Human	Antibody gene cloning of B cells fromof COVID-19 patients	RBD	Interfering with the binding of RBD to cell receptor ACE2	Preclinical	[97]
CV30	IgG	Human	Antibody gene cloning of B cells from a COVID-19 patient	RBD	Blocking the binding of RBD to ACE2	Preclinical	[98,99•]
EY6A	IgG	Human	Antibody gene cloning of B cells froma COVID-19 convalescent patient	RBD	Altering the pre-fusion conformation of S protein	Preclinical	[106]
LY-CoV555	IgG	Human	Antibody gene cloning of B cells from a COVID-19 patient	RBD	Interfering with the binding of RBD to cell receptor ACE2	Clinical	[84••,85]
S2X259	IgG	Human	Antibody gene cloning of B cells from a COVID-19 patient	RBD	Blocked binding of the RBD to ACE2	Preclinical	[105]
S2H13 S2H14	IgG	Human	Antibody gene cloning of B cells of COVID-19 patients	RBD	Blocking the binding of ACE2 and RBD	Preclinical	[81]

Table 1 (*Continued*)

Name of NMAb	Type	Source	Preparation	Target	Mechanisms of neutralization	Developing stage	Refs
2H2 3C1	IgG	Mouse	Animal immunization and hybridoma technology	RBD	Blocking the binding of RBD to ACE2	Preclinical	[107]
7B11	IgG	Mouse	Animal immunization and hybridoma technology	RBD	Blocking the binding of RBD to ACE2	Preclinical	[109•]
18F3	IgG	Mouse	Animal immunization and hybridoma technology	RBD	Non-competition with ACE2 in binding with RBD	Preclinical	[109•]
7D6 6D6	IgG	Mouse	Animal immunization and hybridoma technology	RBD	Non-competition with ACE2 in binding with RBD	Preclinical	[108]
H014	IgG	humanized	A phage-display scFv library generated from mice immunized with SARS-CoV RBD	RBD	Blocking the binding of ACE2 and RBD through steric hindrance	Preclinical	[110]
47D11	IgG	chimeric	Transgenic mice	RBD	–	Preclinical	[111•]
VHH-72	HCAbs	llama	A phage display library generated from cells of immune camels	RBD	Blocking the binding of ACE2 and RBD through steric hindrance	Preclinical	[63•]
3F11	HCAbs	camel	A phage display library generated from cells of nonimmune camels	RBD	blocking the binding of ACE2 to the RBD	Preclinical	[112]
H11	HCAbs	camel	A naive llama phage display antibody library	RBD	blocking the binding of ACE2 to the RBD	Preclinical	[113]
NIH-CoVnb-112	HCAbs	llama	A phage display library generated from cells of immunized llama	RBD	Blocking the binding of ACE2 and RBD	Preclinical	[114]
W25	HCAbs	alpaca	A VHH *E. coli* displayed antibody library	RBD	Competition with ACE2 in binding with RBD	Preclinical	[115]
Ty1	HCAbs	alpaca	A phage display library generated from cells of alpaca	RBD	Competition with ACE2 in binding with RBD	Preclinical	[117]
VHH E	HCAbs	camel	A phage display library generated from cells of camel	RBD	Competition with ACE2 in binding with RBD	Preclinical	[116••]

showed potent antiviral activity against MERS-CoV pseudovirus [33,34]. The 3B11 was screened from a nonimmune phage-displayed single chain fragment variable (scFv) library [35]. In addition, MERS-4 and MERS-27 were identified from a yeast-displayed scFv library from healthy donors [36]. These antibodies all targeted the RBD and inhibited viral invasion by blocking the binding between RBD and DPP4 (Figure 2b). Originating from MERS-CoV-infected patients, MCA1 is an RBD-targeting NMAb screened from a phage display library [37].

NMAbs identified by use of EBV transformation technology

In addition to constructing phage libraries, immortalized B cell-based EBV infection has also been performed in antibody studies. For MERS-CoV, LCA60 was screened in this way [38].

NMAbs identified from transgenic mice

REGN3051 and REGN3048 are fully humanized NMAbs screened from transgenic mice [39] (Table 1). A group of chimeric antibodies were also screened from transgenic mice [40]. Among them, 7.7g6, 1.6f9, 1.2g5 and 4.6e10 target RBD, while 1.6c7 and 3.5g6 target S2 to prevent viral invasion by inhibiting the conformational change of S2 [40] (Table 1).

NMAbs identified by use of gene cloning technology

Many NMAbs, such as CDC2-C2 [41] and MERS-GD27 [42,43], have also been obtained using a fast and efficient method known as cloning and expressing antibody genes [44].

NMAbs against MERS-CoV from other sources

NMAbs identified by use of hybridoma technology

A large number of mouse-derived antibodies have been screened. Among of them, Mersmab1 [45], 4C2 and 2E6 were screened for targeting RBD and subsequently produced humanized antibodies that showed potent antiviral activity *in vitro* and *in vivo* [46,47]. RBD-23D3, RBD-25E4, and RBD-40G7, all targeting RBD, were identified with high cross-neutralizing activity among mutant isolates [48]. NMAbs D12 and F11 targeting RBD, G2 targeting NTD, and G4 targeting S2 subunit were all identified by immunized mice [49,50]. Screened by hybridoma technology, 5F9 and 7D10 are murine NMAbs targeting the NTD [51,52] (Table 1). In addition to murine-derived antibodies, researchers have obtained neutralizing antibodies from immunized animals of other species. For example, JC57-14, targeting RBD, JC57-14 and FIB-H1, targeting non-RBD regions of S1, were screened from macaques [41]. Furthermore, JC57-14 could protect DPP4-transgenic mice against MERS-CoV infection [41].

Single domain antibodies (sdAbs) identified by screening of antibody libraries

In addition to conventional antibodies, heavy-chain-only antibodies (HCAbs) produced by camelids contain a single-variable domain (VHH), instead of two variable regions on the heavy and light chains, respectively, of conventional IgG antibodies that affords the equivalent effect [53]. VHH shows affinities and specificities for antigens comparable to conventional antibodies. VHHs can be easily constructed into multivalent formats and show higher thermo-stability and chemo-stability, compared to most other antibodies [54–59]. VHHs are also less susceptible to steric hindrance during binding [60]. For MERS-CoV, VHH-83, NbMS10 and VHH-55 were screened from antibody libraries of immunized camels [61,62,63•] (Table 1).

NMAbs against SARS-CoV-2

Human NMAbs against SARS-CoV-2

NMAbs identified by screening of antibody libraries

Ab1, rRBD-15 and 5A6 were screened from nonimmune antibody libraries of healthy humans and showed strong neutralizing activity against SARS-CoV-2 *in vitro* or *in vivo* [64•,65,66]. In addition, to solve the immunogenicity problem of heterologous single-domain antibodies, researchers constructed a fully human single-domain antibody phage-displayed library by modifying healthy human heavy chains to obtain soluble and highly stable single-domain antibodies [67••], and a pool of NMAbs against SARS-CoV-2 was identified. Among of them, n3130 had the most potency in targeting SARS-CoV-2 S1 [67••]. However, it did not effectively inhibit the binding of RBD to receptor ACE2.

CT-P59, screened from a patient antibody library [68•], showed good therapeutic efficacy against SARS-CoV-2 infection *in vitro* and *in vivo* and was used in clinical trials. Similarly, 910-30 and 2B11 were identified from convalescent patient-derived yeast and phage display libraries, respectively [69,70]. Notably, a number of cross-reactive NMAbs (like ADI-55689) against SARS-CoV and SARS-CoV-2 were identified from yeast-displayed libraries established with B cells of SARS convalescent patients based on the genome similarity between SARS-CoV and SARS-CoV-2 [71•]. Through genetic mutations, diversity was introduced into the heavy and light chain variable genes of ADI-55688, ADI55689 and ADI-56046, and three highly active antibodies were identified, among which, ADG-2 showed broad-spectrum neutralizing activity against clade 1 sarbecoviruses [72].

NMAbs identified from EBV transformed memory B cells of a recovered SARS patient

S309 was identified from EBV-transformed memory B cells of a recovered patients who was infected by SARS-CoV in 2003 and showed strong cross-neutralizing activity against both SARS-CoV and SARS-CoV-2 [73•].

NMAbs screened by gene cloning and sequencing techniques

Antibody gene cloning and sequencing technologies for identification of SARS-CoV-2 NMAbs from B cells sorted from COVID-19 patients are being used more frequently, and several high-throughput screening methods have been established [74,75], considerably reducing the time required for antibody development and enriching antibody diversity. These NMAbs showed strong neutralizing activity *in vitro* or *in vivo*. Most of them target the RBD in S1 subunit, and their mechanism of action is summarized in Table 1. Also, NMAbs targeting SARS-CoV-2 NTD, for example, 4A8 and 4–8, were isolated in this way [76•,77,78••]. A large group of RBD-targeting NMAbs, including BD-368-2, P2C-1F11, CB6, S2H13 and C1A-B12, could interfere with the binding of RBD to the receptor ACE2, showing strong neutralizing activity *in vitro* [74,79–82]. CB6 showed potent *in vivo* efficacy, protecting rhesus macaques against SARS-CoV-2 infection in both prophylactic and treatment settings [79]. CC12.1 exhibited the most potent *in vitro* neutralizing activity and completely protected Syrian hamsters against the challenge of a Washington strain (USA-WA1/2020) *in vivo* [75]. CV07-209 could reduce lung pathology in a COVID-19 hamster model [83]. LY-CoV555 protected against SARS-CoV-2 infection in nonhuman primates and showed potent neutralization effect and good safety profiles in clinical trials [84••,85] (Table 1). Notably, B38 and H4 target different neutralizing epitopes in RBD [86••]. No competition takes place between the two NMAbs; therefore, the combination results in an ideal cocktail candidate for COVID-19 therapy, which is also effective in preventing escape mutations. Such antibody pairs are not uncommon in SARS-CoV-2 antibody studies, and their combination has shown better neutralizing activity compared to the use of each compound alone. Examples are COV2-2196/COV2-2130 [87,88], S2M11/S2E12 [89] and REGN10933/REGN10987 (REGN-CoV2) [90,91••,92] (Figure 2c). Further, REGN-CoV2 has shown neutralization effect and safety in clinical trials [93••] (Table 1). Similarly, researchers screened a large set of NMAbs with different targets against SARS-CoV-2 [94]. Among them, COVA1-18 and COVA2-15 showed the strongest antiviral activity [94]. Many NMAbs, such as MW05 [95], 311mab-31B5/311mab-32D4 [96], C121 [97] and CV30 [98,99•] were identified from the sorted SARS-CoV-2 RBD-specific, IgG class-switched memory B cell of COVID-19 convalescent patients using antibody gene cloning technology. They have shown neutralizing activity against SARS-CoV-2 *in vitro* and *in vivo* through competition with ACE2 in binding with RBD (Table 1). It was found that epitopes of some NMAbs are relatively conservative in sequence (e.g. DH1047, A19-46.1, S2X259 and CV1-30), and these NMAbs show cross-neutralizing activity against SARS-CoV-2 variants and other sarbecoviruses [100,111•,102–105]. Like these NMAbs, EY6A targets a conserved footprint in RBD that

is distinct from receptor binding motifs, and it inhibits viral invasion by altering the pre-fusion conformation of S proteins [106]. Moreover, it showed cross-reactivity against SARS-CoV S1 protein [106].

NMAbs against SARS-CoV-2 from other sources

NMAbs identified by use of hybridoma technology

2H2 and 3C1 were identified by using animal immunization and hybridoma technology. Because the two NMAbs target different epitopes in SARS-CoV-2 RBD, they can be used in combination, that is, a cocktail therapy (Figure 2c). Their combination exhibited more potent neutralizing activity against authentic SARS-CoV-2 infection *in vitro* [107]. Similarly, 7D6 and 6D6 were identified from mice immunized with SARS-CoV-2 S protein, and SARS-CoV-2/SARS-CoV S protein/MERS-CoV RBD, respectively, showing cross-neutralizing activity against SARS-CoV and SARS-CoV-2 as well as its variants [108]. 7B11 and 18F3, SARS-CoV neutralizing mAbs by targeting different neutralizing epitopes in RBD of SARS-CoV S protein, were identified from mice immunized with SARS-CoV S-RBD [109•].

NMAbs identified by screening of antibody libraries

H014, a humanized SARS-CoV-2 NMAb, was originally identified from a phage display antibody library generated from RNAs of the peripheral lymphocytes of SARS-CoV RBD-immunized mice. It exhibited potent neutralizing activity against SARS-CoV-2 infection *in vitro* by blocking RBD-ACE2 binding through steric hindrance [110].

NMAbs identified from transgenic mice

47D11, a chimeric antibody with human variable region and rat constant region, was identified from transgenic mice, showing cross-neutralizing reactivity against SARS-CoV and SARS-CoV-2 [111•].

***SdAbs** identified by screening of antibody libraries*

3F11 was identified from a phage display library from nonimmune camel and was expressed by fusion with human IgG Fc fragment in order to overcome the limitations of sdAbs [58,112]. H11 was also identified from a naive llama phage display antibody library. Researchers obtained H11-H4 and H11-D4 with more affinity for SARS-CoV-2 RBD by random mutation of H11, both exhibiting strong antiviral activity *in vitro* [113].

More commonly, camels are immunized to obtain sdAbs. VHH-72, was identified from a phage display library of a llama immunized with SARS-CoV and MERS-CoV S proteins multiple times showed cross-neutralizing activity against pseudotyped SARS-CoV, MERS-CoV and SARS-CoV-2 [63•]. NIH-CoVnb-112 was isolated from an immune llama phage display library [114]. W25 was identified from a VHH *Escherichia coli* (*E. coli*) — displayed antibody library of immune alpaca. It showed potent neutralizing activity against the D614G isolate, whether monomer or dimer [115]. Another sdAb that exhibited strong neutralizing activity in multimeric form is VHH E (Figure 2c), which was screened from an immune camel phage display library [116••]. The trimeric VHH EEE inhibits both SARS-CoV-2 pseudovirus and authentic virus infection. The combination of VHH E and VHH V, targeting different sites of the RBD, is effective in preventing escape mutations, whereas multimers could not [116••]. In a similar method, Ty1 was screened from an alpaca phage display library [117]. In addition, a large number of nanobodies have been screened as candidate drugs for the treatment of COVID-19 [118–120].

Conclusion and prospects

Coronaviruses constitute a large group in nature, and genome sequence analysis shows that many coronaviruses are highly homologous to SARS-CoV, MERS-CoV or SARS-CoV-2 [121]. Therefore, coronaviruses may continue to threaten human health. Rapid development of therapeutic and prophylactic drugs is essential, both for coronaviruses that have already emerged to infect humans and for those that may emerge in the future. With the development of high-throughput screening technology for antibodies, the cycle time for antibody development is shortening. Antibody drugs could be the antiviral drug of choice based on their advantages of high targeting and low side effects. Moreover, different species of coronaviruses have conserved loci between their genomes, and it may be possible to design and screen antibodies with broad-spectrum antiviral activity based on these loci. Many studies on the mechanism of NMAbs with cross-neutralizing activity against SARS-CoV-2 variants and other sarbecoviruses have shown that the targets of these NMAbs are relatively conservative [85,100–103,105]. In a recent study, 41 RBD-directed NMAbs were classified into seven antibody communities with distinct footprints and competition profiles [122]. A number of NMAb cocktails consist of NNAbs from different RBD-directed antibody communities showed enhanced neutralizing potency. However, the potency of some NNAbs in the combinations is compromised by emerging SARS-CoV-2 variants. Improving the neutralizing activity of these NMAbs through other means (e.g. mutation and multimeric forms) greatly enhance their application prospects [72,122]. Therefore, in addition to the combination strategy, the *in vitro* modification of antibodies is also crucial to improve the neutralizing activity of the antibody drugs. Of course, the acceleration of antibody drug formation, the miniaturization of effective antibody molecules and the improvement of *in vivo* longevity are expected.

Conflict of interest statement

Nothing declared.

Data availability

No data was used for the research described in the article.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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