Current development of severe acute respiratory syndrome coronavirus 2 neutralizing antibodies (Review)

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Abstract. The coronavirus disease 2019 pandemic due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seriously affected global public health security. Studies on vaccines, neutralizing antibodies (NAbs) and small molecule antiviral drugs are currently ongoing. In particular, NAbs have emerged as promising therapeutic agents due to their well-defined mechanism, high specificity, superior safety profile, ease of large-scale production and simultaneous application for both prevention and treatment of viral infection. Numerous NAb therapeutics have entered the clinical research stages, demonstrating promising therapeutic and preventive effects. These agents have been used for outbreak prevention and control under urgent authorization processes. The present review summarizes the molecular targets of SARS-CoV-2-associated NAbs and screening and identification techniques for NAb development. Moreover, the current shortcomings and challenges that persist with the use of NAbs are discussed. The aim of the present review is to offer a reference for the development of NAbs for any future emergent infectious diseases, including SARS-CoV-2.

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Abbreviations: ADE, antibody-dependent enhancement; ORF, open reading frame; ACE2, angiotensin converting enzyme 2; CDR, complementarity-determining region; CDRH3, CDR 3 of antibody heavy chains; CTD, C-terminal domain; COVID-19, coronavirus disease 2019; FcγR, IgG Fc receptor; FP, fusion peptide; HR, heptagonal repeat; mAb, monoclonal antibody; Nb, nanobody; NAb, neutralizing antibody; NTD, N-terminal domain; R&D, research and development; RBD, receptor binding domain; RBM, receptor-binding motif; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sgRNA, subgenomic RNA; TMPRSS2, transmembrane protease serine 2; VHH, variable domain heavy-chain

Key words: severe acute respiratory syndrome coronavirus 2, neutralizing antibodies, receptor binding domain, nanobody, target therapy

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in a global pandemic of coronavirus disease 2019 (COVID-19). Although infection typically starts with flu-like symptoms (1), patients can also be asymptomatic and may go on to have a mild to severe disease course (2). Mechanistically, COVID-19 is characterized by an important burden of inflammation mainly in the respiratory system, although it can also affect other organ systems (3,4). Previous studies have assessed the association between hemogram indices and COVID-19 pathogenesis, improving the prognostic judgement and patient management of this disease (5,6). Nevertheless, a requirement for safe prevention and treatment methods for COVID-19 remains. Efforts for this are currently ongoing, including the development of numerous types of vaccines, attempts to establish active herd immunity, exploration of the efficacy of existing drugs and the development of novel small-molecule drugs targeting viral or host proteins to inhibit the replication of viruses (7-9). The majority of patients with SARS-CoV-2 infections typically exhibit an antibody response 5-15 days after the occurrence of symptoms, peaking at 21-28 days, before declining (10,11). Given the time it takes to develop immunity, this is a dangerous period for individuals who are at high risk of exposure, who are immunocompromised, or who are unable to acquire antibodies from

active immunization. Thus, prompt passive immunization is urgently required (12).

The earliest attempt at passive immunization was through convalescent plasma infusion, which provides neutralizing antibodies (NAbs), which can alleviate the inflammatory burden (13). Convalescent plasma is plasma donated by individuals previously infected with infectious diseases, who have produced protective NAbs 14-28 days after infection (14). Although early clinical trials on convalescent plasma transfusion reported promising results, its shortcomings were not negligible. For the treatment to work effectively, patients should ideally receive an infusion during the early stages of infection, whilst being monitored to avoid risks associated with blood transfusions, such as hyperinflammatory immune reactions and transfusion-associated circulatory overload (15). Moreover, the screening and monitoring of plasma donors is labor and resource-intensive. There is also a lack of a universally accepted standard for the infusion volume. Recent clinical trials have reported that convalescent plasma transfusion does not significantly reduce mortality, as its neutralization capacity declines after viral mutations (16,17). However, the total antibody titer following human COVID-19 immunoglobulin intravenous injection was previously reported to be three times higher compared with that of convalescent plasma, suggesting this treatment to be more effective. pH4 is manufactured from already inactivated, filtered and purified convalescent plasma of patients infected with SARS-CoV-2. It contains high purity and high titer SARS-CoV-2 NAbs, which have been reported to effectively neutralize SARS-CoV-2 *in vivo*. A number of clinical trials have reported that the intravenous injection of human immunoglobulin can abridge the duration of positive PCR confirmation and inflammation, as evidenced by computed tomography data in patients (18,19). According to the diagnostic and treatment guidelines in China, intravenous immunoglobulins can be used in an emergency for patients with rapid severe disease progression (20). Furthermore, another study has previously proposed the potential application of pH4 for patients with SARS-CoV-2 infection with low immunoglobin M levels (21).

However, one limitation of convalescent plasma and intravenous immunoglobulin is the requirement to recruit blood donors with high titers of NAbs against the pathogens of interest to maintain a stable, sufficient supply. Identification of single B cells that produce virus-specific neutralizing monoclonal antibodies (mAbs) from these donors can potentially be used to circumvent this limitation. The immunoglobulin gene expressed in B cells can be cloned and expressed to produce high titers of neutralizing monoclonal antibodies (22). Large-scale preparations of specifically targeting mAbs that are of high purity with potent neutralizing activity can render them powerful tools for the prevention and treatment of infectious diseases (23). For SARS-CoV-2, specific B cells have been obtained from patients recovered from COVID-19 to produce large quantities of humanized mAbs through numerous methods, including mouse hybridoma fusion, single B cell sorting, phage display and transgenic mice and antibody screening technologies (24-26). A number of NAbs against SARS-CoV-2 have been developed. According to randomized double-blinded controlled clinical trials, the Food and Drug Administration has previously approved the emergency use of certain NAb drugs for the treatment of SARS-CoV-2 such as bamlanivimab, etesevimab, casirivmab and imdevimab (27,28). Furthermore, The State Drug Administration of China has authorized Amubarvimab and Romlusevimab cocktail therapies, which were designed for treating adults or young individuals (12-17 years old, weight ≥40 kg) with mild and common types of SARS-CoV-2 infection who are at high risk of developing severe type infections (29). This treatment strategy is now serving a pivotal role in the clinical treatment process in Chinese hospitals to reduce incidence of serious adverse events (30). Compared with convalescent plasma, these types of NAbs confer a number of distinct advantages. The final selection of the most effective candidate antibody can be evaluated, with the IC_{50} value typically on nanomolar or picomolar levels, and the dose can be evaluated more accurately (31). Furthermore, the probability of the antibody-dependent enhancement (ADE) phenomenon associated with this type of NAbs, wherein non-neutralizing antibodies facilitate the entry or replication of a pathogen, thereby potentiating infection rather than providing protection, is considerably lower (32).

New variants continue to emerge, such as the ο variants, which are notably more transmissible compared with those of the original strain, and have negatively impacted the health and normal life of the global population (33). Mutations that produce mutant forms of the spike (S) protein have resulted in the reduction or disappearance of the efficacy of vaccines and NAbs, increasing the demand for the development of next generation vaccines and antibody drugs (34). Nanobodies (Nbs) are heavy chain antibodies, which have been previously reported to be found in camelids and cartilaginous fish, such as sharks (35). They form the smallest known complete functional structure that can target virus antigens. Numerous characteristics of Nbs, such as their small sizes, high specificity, stability, ease of production, potent penetration and low immunogenicity, render them able to recognize antibody epitopes that cannot be readily recognized by conventional antibodies (36). This would in turn increase the diversity of potential targets and binding ability of antibodies, providing a broader and more targeted choice for the research and development (R&D) of next generation antibody drugs with wide clinical implications.

In the present review (Fig. 1), the current application status of NAbs is discussed from the perspective of the epitope features in SARS-CoV-2, the current preparation techniques available and the latest research status of traditional NAbs and Nbs, including persisting challenges and future prospects.

2. Structural basis and process of SARS-CoV-2 infection

Structure of SARS-CoV-2 transcriptome. There are currently four known genera of coronaviruses, specifically α, β, γ and δ. β coronaviruses can be classified into subgenera A, B, C and D (37). SARS-CoV-2 is a member of the B subgenus of β coronaviruses. SARS-CoV-2 is a single-stranded, positive-sense RNA virus that can directly guide protein synthesis upon entering the cell to replicate itself, by generating negative strands using RNA polymerase (38). The RNA sequence of SARS-CoV-2 contains 29,891 nucleotides, encoding 9,860 amino acids with a GC percentage of ~38%.

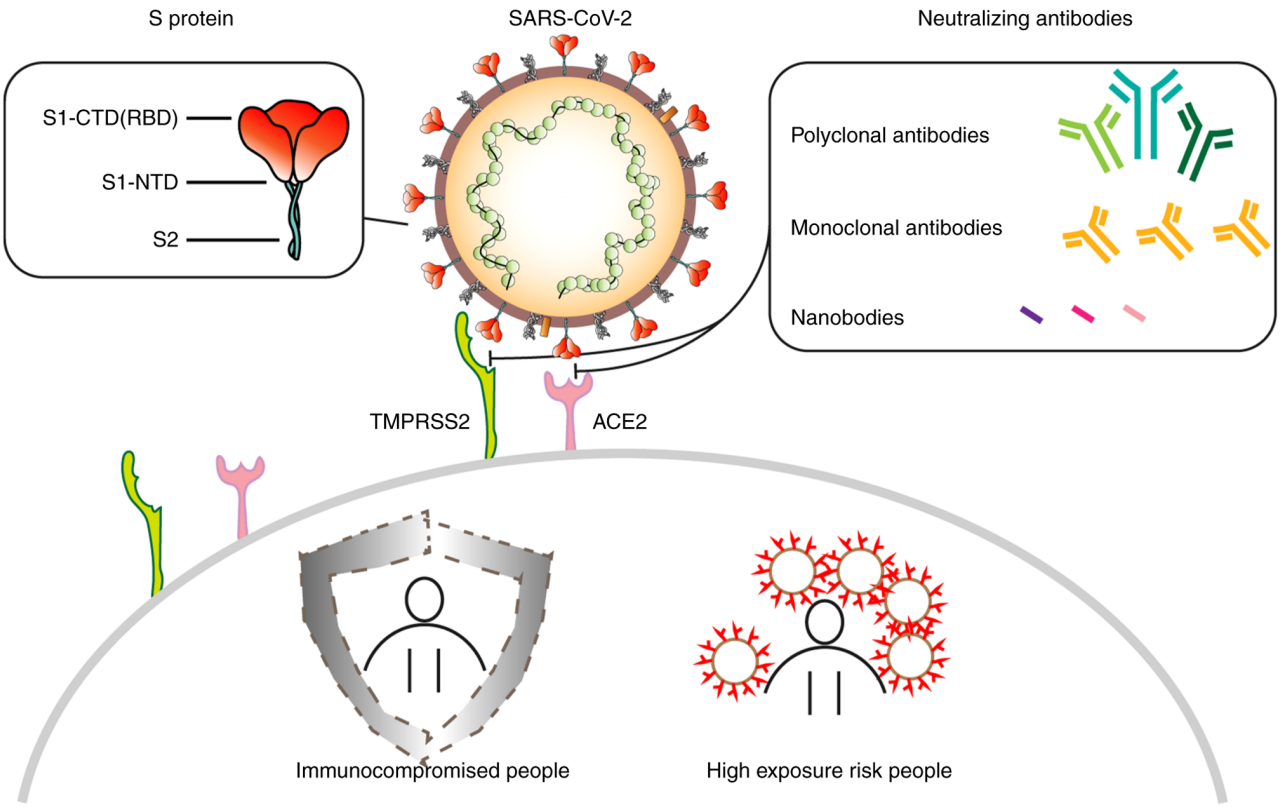

Figure 1. Schematic outline of the present review. Structure of SARS-CoV-2, including the S protein and its receptors for cell entry ACE2 and TMPRSS2. Classification of neutralizing antibodies including polyclonal antibodies, monoclonal antibodies and nanobodies and the common populations for whom neutralizing antibodies are used, including immunocompromised individuals and those with a high risk of exposure. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ACE2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane protease serine 2; S protein, spike protein; NTD, N-terminal domain; RBD, receptor binding domain; CTD, C-terminal domain.

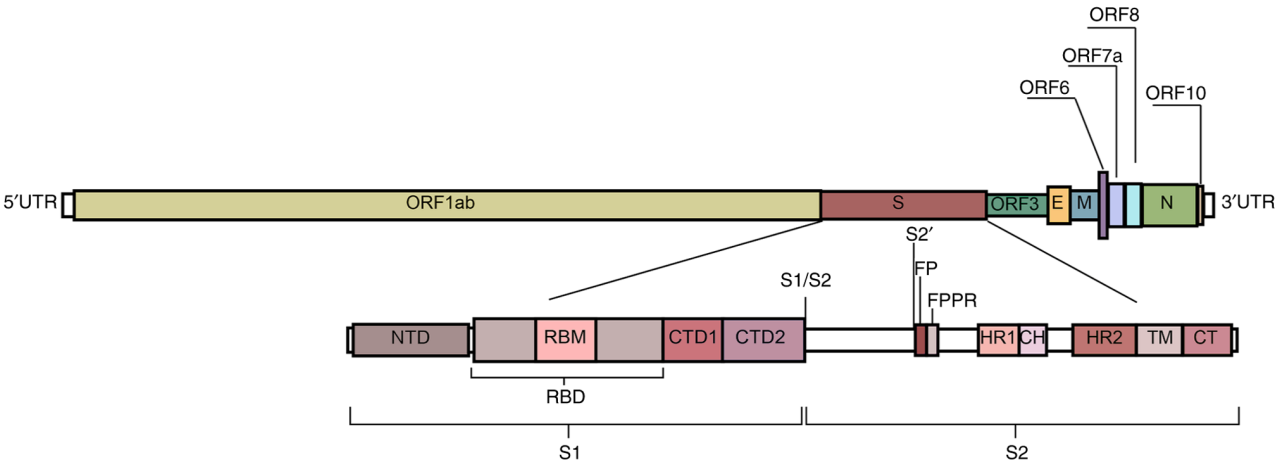

Figure 2. Specific genome structure of severe acute respiratory syndrome coronavirus 2. The ORFs are responsible for the non-structural protein encoding. The S protein can be divided into two segments. S1 consists of the CTD, NTD and RBD. RBM forms a part of the RBD for angiotensin-converting enzyme 2 binding. FP, FPPR, HR1, CH, HR2, TM and CT constitute the majority of S2. ORF, open reading frame; CTD, C-terminal domain; NTD, N-terminal domain; RBD, receptor binding domain; RBM, receptor-binding motif; FP, fusion peptide; FPPR, FP proximal domain; HR, heptagonal repeat; CH, central helix; TM, transmembrane segment; CT, cytoplasmic tail; E, envelope; M, membrane; N, nucleocapsid; S protein, spike protein; UTR, untranslated region.

Furthermore, there is a 5' cap-like structure and a 3' poly-A tail on its genome (Fig. 2) (39). In total, two overlapping open reading frames (ORFs), namely ORF1a and ORF1b, encode 16 non-structural proteins (40). The -1 frameshift between ORF1a and ORF1b contributes to the production of polypeptide 1a and a larger polypeptide 1ab. For genome amplification, SARS-CoV-2 viruses generate antisense RNAs as templates for creating the sense genomic RNA (gRNA) and subgenomic RNA (sgRNA). gRNA together with the structural proteins expressed contributes to producing the viral offspring. The shorter sgRNA has the role of expressing the four types of conserved structural proteins in coronaviruses, namely

S protein, membrane protein, envelope protein and nucleocapsid protein, as well as six auxiliary proteins (3, 6, 7a, 7b, 8 and 10) (41).

S proteins. The S protein is a type I fusion protein that mediates viral entry into targeted cells. It is a major target of post-infection NAbs and the main focus of numerous therapy and vaccine studies. The S protein forms a surface-exposed trimer on the viral particles and consists of a long extracellular region, a transmembrane region and an intramembrane region (42). On the surfaces of targeted cells, the S protein binds with angiotensin converting enzyme 2 (ACE2) before undergoing structural transformations to induce the fusion of the viral and cell membranes. Each S protein contains 1,273 amino acids, including an N-terminal signal peptide, a receptor-binding part S1 and a fusion part S2. The S protein has been previously studied using cryoelectron microscopy (Cryo-EM), which reported two structural states (43,44). While in the closed state, three receptor binding domains (RBDs) of the S protein are locked in the 'down' conformation, whereas during the open state, there is one RBD in the 'up' conformation. The RBDs form the key region necessary for SARS-CoV-2 interactions with ACE2, where its open state is the prerequisite for virus-cell membrane fusion (44,45).

S1 subunit. The S1 subunit has four domains, namely A, B, C and D, with domains A and B being responsible for receptor binding. The structure of domain A consists of a galectin-like β-fold, whereas domain B has a reverse parallel β-fold structure. There is an extended loop of domain B at the end of the virion that is structurally different depending on the species of the β-coronavirus, known as the hypervariable region (46). Domains C and D on the C-terminus constitute discrete segments of the primary protein sequence and are directly linked to the stem core of the S2 subunit to form a β-fold structure. The entire S1 subunit is connected by a ring covering the surface of S2 (47).

From the perspective of its linear peptide structure, S1 consists of the N-terminal domain (NTD), RBD and the C-terminal domain (CTD). Due to the swing of the S protein on the viral membrane with a 40 main angle of inclination, the perceived primary point of interaction with the epithelium is the NTD, which can be targeted by numerous powerful Nabs (48-50). The NTD of the SARS-CoV-2 S protein can form a ribbon structure that is analogous to that of human galectin and is located at residues 14-305. This mediates weak and reversible interactions with superficial glycans, such as sialic acid, through low-affinity hydrogen bonds, is crucial for the virus to attach to and navigate along the cell surface, a process referred to as 'viral surfing'. Subsequently, numerous critical residues of the NTD combine with sialic acid (51,52), forming a flat surface to strengthen the primary interaction between SARS-CoV-2 and targeted cells, consolidating the infection (53). The RBD is located at residues 336-525 of the S protein, which has two domains: A central structure consisting of five parallel β-sheets and an extended loop known as the receptor-binding motif (RBM) at residues 437-508. This extended loop serves to surround the edge of the central structure and interacts with ACE2 (31). In the down state, the receptor-binding motif (RBM) is partially obstructed, limiting its engagement with ACE2. Upon RBD's transition to the up state, the RBM becomes accessible to interact with ACE2, facilitating viral entry (54,55). At residues 528-685 of the S protein is the CTD, which mainly consists of a β-structure. CTD1 serves to 'sense' changes in its neighboring sites, whilst CTD2 is vital for membrane fusion with the entire rearranged S protein (46,56).

S2 subunit. The S2 subunit promotes membrane fusion and binds the S protein onto the host cell membrane. It is highly conserved among coronaviruses and contains important regions for promoting fusion with target cells (45). Upon RBD's engagement with the receptor, the fusion peptide (FP) is inserted into the cell membrane, which then triggers the unfolding of the heptad repeat 1 (HR1) domain and the folding back of the HR2 domain. This sequence of events leads to the domains coming together, causing the membranes to bend towards each other and facilitating membrane fusion, thus enabling viral entry (57). In particular, the FP proximal region in S2 appears to serve a supporting role in clamping the RBD and stabilizing the closed conformation of the S protein (46).

Viral entry into the cell. For all enveloped viruses, membrane fusion is the critical initial phase for entry into the targeted cell and the establishment of infection. There is a high dynamic barrier when the two membranes approach each other, where the free energy required to overcome the kinetic barrier comes from the rearrangement of the fusion protein encoded by the virus, specifically by changing from the basal variable conformational state to the stable state upon fusion. Subsequently, through two protein cleavage events, the S protein transforms into a state that can readily transition into a low-energy state. The first cleavage occurs at the boundary between S1 and S2, commonly known as the S1/S2 site. A four-amino acid residue Arg-Arg-Ala-Arg sequence is present at the S1/S2 site, which is cleaved by the protease furin (58). The prefusion S protein trimer fluctuates between closed and open conformations. It has been previously hypothesized that the near-universal expression of furin-like protease may have a role in enhancing the cellular and tissue tropism of SARS-CoV-2, thus enhancing its infectivity and/or modifying its pathogenicity. The second cleavage event occurs at the S2' site. This cleavage site is only accessible after the initial S1/S2 cleavage and RBD-ACE2 binding. The differential access route to SARS-CoV-2 results in the S2' site being cleaved by distinct proteases. The cleavage of S2' site occurs on the cell surface and is mediated by transmembrane protease serine 2 (TMPRSS2). In the absence of TMPRSS2 or when the likelihood of encountering TMPRSS2 diminishes on the cell surface, the virus-ACE2 complex will be internalized by lectin-mediated endocytosis into the endolysosome, where the S2' site is cleaved by cathepsins, particularly cathepsin L (59). In both of these access routes, S2' cleavage releases the structural constraints on the FP, whilst the dissociation of S1 from S2 results in a drastic change in the conformation of the S2 subunit, particularly in HR1, driving the FP into the cell membrane. This forms a fusion pore through which viral RNA is delivered into the cytosol of the target cell.

Moreover, SARS-CoV-2 can be cleaved by serine endonuclease protein convertase 1, trypsin and trypsin-like integral

membrane serine peptidase, all of which can readily recognize and cleave the S1/S2 site. The S protein is cleavable by a broader range of proteases compared with the SARS-CoV-related viruses, which is a critical contributor to its ease of entrance into targeted cells through the ACE2 pathway and infectiousness (60).

3. Current status of preparation methods and development of NAbs

Polyclonal antibodies (pAbs). pAbs are prepared by the direct injection of antigens into animals for immunization, followed by serum collection and purification (61). Compared with monoclonal antibodies, they possess a higher affinity for target antigens as they can target multiple binding sites on a single antigen. Moreover, due to the inherent diversity of pAbs, they tend to be more resistant to the polymorphism of target antigens, retaining activity despite antigen glycosylation or other post-translational modifications (62). pAb therapeutics are associated with abbreviated production timelines and reduced manufacturing expenses relative to monoclonal antibody counterparts (63). However, due to the large divergence among batches of pAb therapeutics and the rapid development of more effective vaccines and monoclonal antibody therapy, the popularity of pAb drugs has gradually waned. At present, to the best of our knowledge, there have only been a small number of studies on the use of pAb against COVID-19.

A horse pAb therapy was previously reported to be safe and potent for treating SARS-CoV-2 (64). The efficiency of this antibody against RBD was reported to be ~50 times greater than that of normal convalescent plasma, and the resultant drug INM 005 (COVID-19) derived from this horse pAb has been approved for SARS-CoV-2 treatment in Argentina (65). Moreover, another previous study used the RBD region of the S protein to immunize pigs, which then produced polyclonal NAbs that do not interact with human Fc receptors, avoiding potential ADE effects (66). A purified polyclonal IgG fraction drug, XAV-19, was also reported to be able to neutralize the original Wuhan strain and subsequent variants, including the γ and δ variants. XAV-19 was also well tolerated in hospitalized patients with moderate COVID-associated pneumonia who required low-flow oxygenation, according to results from clinical trials (67,68).

mAbs

Production technologies. Commonly used monoclonal antibody screening technologies include traditional murine hybridoma fusion, transgenic mouse technology, single B cell isolation, cloning and phage display (69). The conventional murine hybridoma method yields antibodies derived from mice, which are susceptible to human anti-mouse antibody responses, limiting their clinical applicability. Advances in transgenic mouse technology have enabled the direct production of fully humanized antibodies without the need for subsequent humanization steps (70). This method entails genetically integrating human antibody genes into the mouse genome, followed by immunization of these transgenic mice to elicit fully human antibodies. These antibodies mature *in vivo*, exhibiting high affinity and specificity, thus establishing this platform as the preferred and auspicious route for antibody-based pharmaceutical development (71). Single B-cell sorting is a technique used to isolate individual B cells capable of producing specific NAbs from recovered individuals. The genes encoding the antibody are sequenced to allow the recombinant expression and purification of the required mAbs. This method has emerged as a cornerstone for the expedited development of anti-SARS-CoV-2 NAbs, as it allows for the rapid and high-throughput generation of human antibodies from peripheral blood mononuclear cells, while maintaining the native pairing of heavy and light chains (72). Phage display is a method that involves cloning the entire repertoire of genes from the variable regions of human antibodies and inserting them into phages harboring the coat protein gene. This results in the display of exogenous genes on the phage surface as fusion proteins, creating an antibody library. Screening this phage library with target proteins facilitates the rapid isolation of antibodies with high affinity for the desired target (73,74). Phage display technology outperforms the conventional hybridoma method in terms of speed, efficiency, and simplicity. Furthermore, it can be coupled with Nb technology to generate novel Nb variants characterized by reduced molecular weight, enhanced stability and increased neutralizing capacity (75).

RBD NAbs. RBD NAbs are considered to be the most abundant and potent of the SARS-CoV-2 Nabs (76,77). A prior investigation into the humoral immune response to SARS-CoV-2 infection showed that antibodies blocking the interaction between RBD and ACE2 led to a decrease in viral RNA expression to levels below detection, suggesting a crucial role for RBD-specific neutralizing antibodies in the response to SARS-CoV-2 infection (78). A number of RBD antibody categorization systems have been proposed, with the one devised by Barnes *et al* (76) being the most widely accepted. The approach employs a classification system comprising four categories, which are delineated by the structural characteristics and binding sites of the NAbs (Fig. 3). Class 1 RBD NAbs are characterized by their immunoglobulin heavy chain variable region (IGHV) of 3-53 or 3-66 genetic origin, short complementarity-determining region (CDR)3 of antibody heavy chains (CDRH3) of <15 residues in length, RBD 'up'-state binding and ACE2 blocking capability. C105 (79), LY-CoV016 (27), B38 (80), CB6 (81) and CT-P59 (31), 1-20, 4-20 (49), 910-30 (82), S2E12 (83) and S2K146 (84) are representative examples of Class 1 RBD NAbs. They predominantly target or overlap with the ACE2 binding site at the RBM, competing with ACE2 for binding at this site. However, these antibodies do not bind adjacent RBDs (85,86).

Class 2 RBD antibodies are also able to bind to the ACE2 binding site by recognizing both the 'up'- and 'down'-state RBDs, with added specificity to neighboring RBDs. Unlike the Class 1 IGHV3-53 antibodies, Class 2 antibodies have CDRH3 loops that are >15 residues. The prominence of Class 2 antibodies in the RBD-targeting fraction of plasma may be partially attributed to their binding capacity to both the 'up' and 'down' states of RBD. They are typically produced by germline genes, including variable heavy-chain (VH) 1-2, VH1-69 and VH3-53 (87). C135, C110 (87), C144, C002, C104, C119, C121 (76), DH1041, DH1042 and DH1043 (88) belong to this class of RBD NAbs. These antibodies not only directly obstruct ACE2 engagement but can also bridge adjacent 'down'

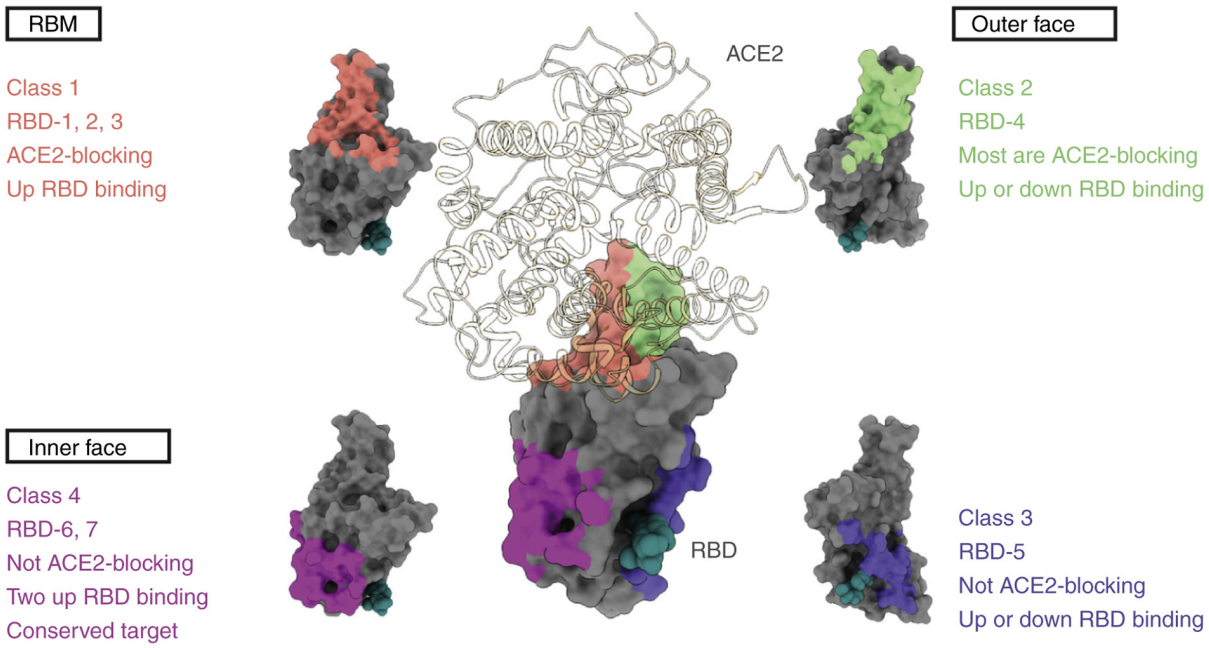

Figure 3. Classification of binding sites of RBD NAbs. The binding sites of RBD NAbs can be divided into four classes. Using high-throughput surface plasmon resonance and cryoelectron microscopy structure determination, seven RBD-binding subsites were discovered. RBD 1, 2 and 3 (orange) are correspond to Class 1, which bind with the RBM region. RBD 4 (green) and 5 (blue) correspond to Class 2 and 3 binding to the outer face of RBD, respectively. RBD 6 and 7 (purple) lie parallel to Class 4 and bind to the inner face. The interaction with ACE2 and the status of the RBD of each group of antibodies are shown in the figure. The structure of RBD and ACE2 was cited from the review of Gruell *et al* (167) (PDB: 6VXX). NAb, neutralizing antibody; RBD, receptor binding domain; ACE2, angiotensin-converting enzyme 2.

state RBDs, locking S proteins in a 'closed' pre-fusion state to inhibit S-ACE2 engagement. Class 1 and Class 2 NAbs do not cross-neutralize viruses due to the limited conservation of RBM structures in different β-coronavirus species. By contrast, a number of SARS-CoV-2 NAbs that can identify conserved RBD epitopes further away from the ACE2 engaging site have been reported to exhibit considerable cross-neutralizing ability. Such non-RBM-targeting RBD antibodies are categorized into structural classes 3 or 4. Class 3 RBD NAbs can identify both the 'up' state and 'down' state RBDs but do not inhibit ACE2 binding, whilst Class 4 RBD NAbs do not recognize the 'down' RBD conformation. For example, CR3022 is a weak Class 4 cross-reactive neutralizing antibody and is one of the most broad-spectrum coronavirus mAbs identified to date (89,90). The cryptic site targeted by CR3022 shares 86% similarity among SARS and SARS-CoV-2 (91). Furthermore, the Class 3 antibody S309, which was first identified in a blood sample from a patient with SARS in 2003, can inhibit a range of associated coronaviruses, including SARS-CoV-2. S309 recognizes a proteoglycan epitope accessible in both the 'up' and 'down' conformations of RBD but differs in the mode of binding to RBM on SARS-CoV-2 (25). In addition, the IgG1κ mAb sotrovimab was developed from the S309 antibody and was previously approved under emergency use authorization for mild-to-moderate COVID-19 cases in patients aged ≥12 years and weighing >40 kg because of its ability to reduce the risk of disease progression (92). However, since sotrovimab only targets a single viral antigenic epitope, resistance can readily develop in patients (93).

Other antibodies, such as DH1047 (94), ADI-56046, ADI-55689 (95) and ADG-2 (96), can block the interaction between ACE2 and S to inhibit SARS-CoV, SARS-CoV-2 and Bat SARS-like coronavirus infection. Specifically, DH1047 binds the SARS-CoV-2 RBD at an epitope outside of the N-terminal end of the RBM, unlike other known non-cross-neutralizing antibodies. Furthermore, the epitopes targeted by the ADG-2 antibody overlap but differ from DH1047. They are linked by rotation around the longitudinal axis of fragment antigen-binding (Fab), with the ADI-56046, ADI-55689 and ADG-2 antibodies preferring the ACE2 engaging region of RBD. These cross-neutralizing antibodies contact S proteins by an angle of proximity to the viral surface in a relatively horizontal manner (96), which indicates the importance of a highly conserved recognition pattern and horizontal angle of proximity for cross-neutralizing activity (95,97).

Using high-throughput surface plasmon resonance analysis and Cryo-EM structure determination, RBD antibodies can be classified into seven different 'communities' ranging from RBD-1 to RBD-7 according to their binding epitopes, giving rise to another categorization system (98). This categorization system offers a more detailed landscape of RBD NAbs, which can be used to complement the four aforementioned classes. Possessing non-overlapping epitopes and considerable potency, the neutralizing effects of RBD-1 to -4 clusters are highly susceptible to deletions and mutations in emerging SARS-CoV-2 variants. By contrast, RBD-5 to -7 antibodies are generally less potent, but the epitopes they target are highly conserved and therefore more resistant to mutations. Therefore, combining and/or engineering these antibodies into multivalent formulas can produce mutagenesis-resistant NAb therapeutic cocktails (98).

NTD NAbs. The NTD is an essential locus for the development of vaccines (49). NTD-targeting NAbs account for

Table I. NTD antibodies and their neutralizing capacity.

NTD antibody (coding VH gene)	Neutralizing mechanism	Binding site	(Refs.)
COV2-2676 (VH1-69), COV2-2489 (VH4-39)	Inhibits post-attachment steps	Supersite	(168)
4A8 (VH1-24)	Limits the structural shifts of the S protein	Supersite	(100)
S2X333 (VH3-33), S2M28 (VH3-33), S2L28 (VH3-21), S2X28 (VH3-30)	Blocks membrane fusion, Fc-mediated effector functions promote Ab-dependent cytophagy and Ab-dependent cytotoxicity *in vivo*	Supersite	(99)
FC05 (VH1-24)	Restricts conformational transition of RBD and blocks membrane fusion	Supersite	(106)
CM25 (VH1-24)	Not reported	Supersite	(169)
DH1050.1 (VH1-24)	Not reported	Supersite	(88)
4-8 (VH1-69), 2-17 (VH1-69), 5-24 (VH3-33)	Not reported	Supersite	(49)
ADI-56479 (not mentioned)	Binds an epitope on adjacent S trimers which decreases their density	Supersite	(107)
5-7 (VH1-46)	Limits the structural shifts of the S protein	Non-supersite	(170)
P008_056 (VH3-21)	Blocks access to the ACE2 engaging site	Non-supersite	(171)
C1520 (VH3-48), C1565 (VH3-48)	Prevents the cleavage of the S2' site or destabilizes S1	Non-supersite	(172)

VH, variable heavy-chain; Ab, antibody; Fc, fragment crystallizable; S protein, spike protein; ACE2, angiotensin converting enzyme 2; NTD, N-terminal domain; RBD, receptor binding domain.

5-20% of all S protein-targeting mAbs derived from memory B cells of patients with SARS-CoV-2 infections (99). The first NTD-targeting NAb, 4A8, was reported by Chi *et al* (100). Instead of blocking the S-ACE2 binding, the majority of NTD NAbs impede the fusion state change of the S protein (101). A number of NTD NAbs, such as S2L28, S2M28 and S2X333, have been reported to obstruct the TMPRSS2-independent infection pathway, a noteworthy pathway in S-protein-mediated infection of human lung cells (99,102). In general, NTD NAbs possess a common structure and genetic origin, use a similar set of VH genes and bind to the S protein in a similar manner (103). Notable examples of NTD-targeting NAbs include 1-68, 1-87, 2-51 (49), DH1049, DH1050.1, DH1050.2 (88) and 4A8 (48), which are all generated from the VH1-24 gene segment. In particular, the IGHV1-24 expression in B cells is ~10 times higher in patients with COVID-19 (5-8%) (85,104) compared with that in healthy individuals (0.4-0.8%) (105), underscoring the importance of the N-terminal domain (NTD)-targeting NAbs in the immune response to SARS-CoV-2. Consistent with the genetic and structural features of NTD NAbs are their binding sites. The NTD NAbs previously isolated by McCallum *et al* (99) were all reported to target a specific site (site 1). Other previous studies have also identified the same binding mechanism and subsequently named it the 'antigenic supersite' (99,103). The NTD is highly glycosylated, but site 1 has the largest non-glycosyl-modified surface on the NTD domain. A list of antibodies targeting the NTD antigenic supersite and their genetic origins are summarized in Table I.

Although NAbs generally achieved effective neutralization against the original 2019 strain of SARS-CoV-2, with the emergence of variants of concerns (VOCs), the NTD region has been reported to be highly variable. Antibodies targeting the supersite of NTD appeared to be especially susceptible to potency loss, with a large number losing their affinity to α, β and γ VOCs. However, numerous NTD NAbs, such as 5-7, C1520 and C1565, remain capable of binding other sites, thereby remaining effective even for the o variant BA.1. However, NTD NAbs combined with RBD antibodies have been proposed to be a more effective therapeutic cocktail for treating the variants. The combination of FC05 with H014, HB27 and P17 (106), as well as the combination of ADI-56479 with ADI-56443 (107), are both examples of combining NTD-targeting mAbs with RBD-targeting mAbs, and have been reported to result in lower virus escape compared with either class of NAbs when used alone. This combination was also proposed to reduce S protein mutations that lead to neutralization escape.

S2 NAbs. In a study investigating SARS-CoV-2-related IgG antibodies, the majority of healthy individuals who had not been exposed to SARS-CoV-2 exhibited the presence of IgG antibodies targeting the S2 subunit in their sera (108). Numerous studies have previously suggested that S2-targeting antibody responses against SARS-CoV-2 are associated with superior outcomes for patients and broader neutralization, suggesting the crucial protective effect of this type of antibodies (109-111). S2 is more conserved compared with S1 and shows 63-98% sequence similarity among the same protein from seven different human coronaviruses (112). S2 contains numerous conserved antigenic sites, including the stem helix, the FP and the hinge region. S2 NAbs tend to inhibit the formation of the six-helix bundle structures by HR1 or HR2, thereby blocking membrane fusion and viral entry. Certain S2 antibodies are summarized in Table II. Nevertheless, S2 NAbs appear to rely on the Fc mechanism for protection *in vivo*, although combination with the Fc has been reported

Table II. S2 antibodies and their neutralizing capacity.

S2 antibody	Neutralizing mechanism	Binding site	(Refs.)
S2P6	Inhibits membrane fusion	Stem helix	(173)
CC40.8	Inhibits membrane fusion	Stem helix	(174)
WS6	Inhibits membrane fusion and post-viral attachment	Stem helix	(175)
CV3-25	Inhibits membrane fusion	Stem helix	(176)
COV44-79, COV44-62	Inhibits membrane fusion	Fusion peptide	(177)
C77G12, VN01H1	Inhibits membrane fusion	Fusion peptide	(178)
76E1	Inhibits membrane fusion	Fusion peptide	(179)
3A3, RAY53	Inhibits membrane fusion	Hinge region	(112)

S, spike protein.

to be a promising strategy in the development of antibody drugs (113).

Nbs. Heavy-chain-only antibodies (HCAbs) in camelids include two constant structural domains CH2 and CH3, a hinge region and a variable domain heavy-chain (VHH), and retain complete antigen binding capability (Fig. 4A) (114). In cartilaginous fish, their immunoglobulin new antigen receptors (NARs) consist of a homodimer of five constant domains and a variable domain (V-NAR) (Fig. 4A) (115). Recombinantly expressed VHH and V-NAR domains exhibit remarkable structural stability under extreme temperature and pH conditions, and their antigen-binding capabilities are comparable to those of HCAbs (116). These fragments represent the minimal functional units necessary for antigen targeting. Due to their low molecular weight (<15 kDa), VHH and V-NAR are also classed as Nbs. The modularity and small size of Nbs allow them to be readily linked to other molecules, rendering them optimal for generating bispecific or multispecific antibodies with ideal affinity or effectiveness (117). Furthermore, VHH-72 is an Nb that has been previously generated by immunizing camelids with SARS-CoV and middle East respiratory syndrome coronavirus RBDs. After the attachment of human IgG Fc to induce bivalency, it was reported to be able to neutralize the SARS-CoV-2 pseudovirus *in vitro* and can be expressed in transiently transfected ExpiCHO cells (101).

VHHs consist of four conserved sequence regions that surround three highly variable CDRs, whilst V-NARs possess two CDRs (CDR1 and CDR3) (118,119) (Fig. 4B). CDR3 is the primary binding domain responsible for 60-80% of antigen interactions (120). The SARS-CoV-2 spike protein exhibits an average spacing of 25 nm, which is not optimal for the 5-10 nm range required for efficient B-cell response activation. Consequently, this larger spacing results in inadequate stimulation of B cells and complement recruitment, leading to a less efficient and transient neutralizing antibody response (121). Compared with the poor diversity in CDR loop lengths in conventional antibodies, VHH and V-NAR possess long protruding CDR3 loops that allow them to access more occluded antigenic epitopes. Due to their low molecular weight and weak cohesive interactions between monomers, Nbs can be readily concatenated through genetic engineering to form multimers. When designed as therapeutic agents, this property allows for the creation of multivalent and bispecific antibodies, enhancing their binding capabilities to antigens (122).

Immunization of animals with specific antigens is the first step in Nb production (Fig. 4C). Multiple immunizations are typically administered to stimulate the immune system in the animal into producing antibodies against specific antigens. After isolating the B lymphocytes from the blood of immunized animals, molecular biology techniques, such as reverse transcription PCR, are used to amplify the antibody genes from the B cells. Specific screening methods, such as phage display or yeast surface display technology, are then used to select Nb genes with high affinity from the amplified antibody gene library, which are subsequently cloned into the expression vectors. These vectors are transformed into suitable host cells, such as *Escherichia coli* or mammalian cells, for recombinant protein expression. Nbs are then purified from cell culture supernatants or cell lysates using a range of purification methods, such as affinity chromatography, ion exchange chromatography and gel filtration. The purified Nbs undergo numerous bioactivity assays, cell-based assays and animal experiments, to verify their affinity and specificity. Depending on the requirements, further modification and optimization of the Nbs may be performed to enhance their stability and affinity (123).

Although VHHs are not of human origin, they exhibit low immunogenicity because of the substantial sequence identity with the human VH gene family III (124). However, due to the evolutionary distance between sharks and humans, V-NARs from sharks exhibit minimal sequence identity with the human VH and variable region light chain structural domains (~30%). Therefore, humanization of V-NARs would be desirable prior to clinical application, which at present is the routine practice. However, in fully humanized VHH, conserved hydrophilic amino acids in framework region (FR)2 are changed, leading to unsatisfactory solubility and stability. Therefore, partial humanization is typically performed. Traditionally, there are two main approaches for humanization, CDR grafting and resurfacing. CDR grafting is a process in which the CDR region is directly grafted from a heterologous antibody to human FRs (125). Resurfacing is the replacement of exposed FR residues on the surface of non-human antibodies with corresponding residues of human antibody FRs, minimizing the immunogenicity of the antibody in humans (126). Humanized

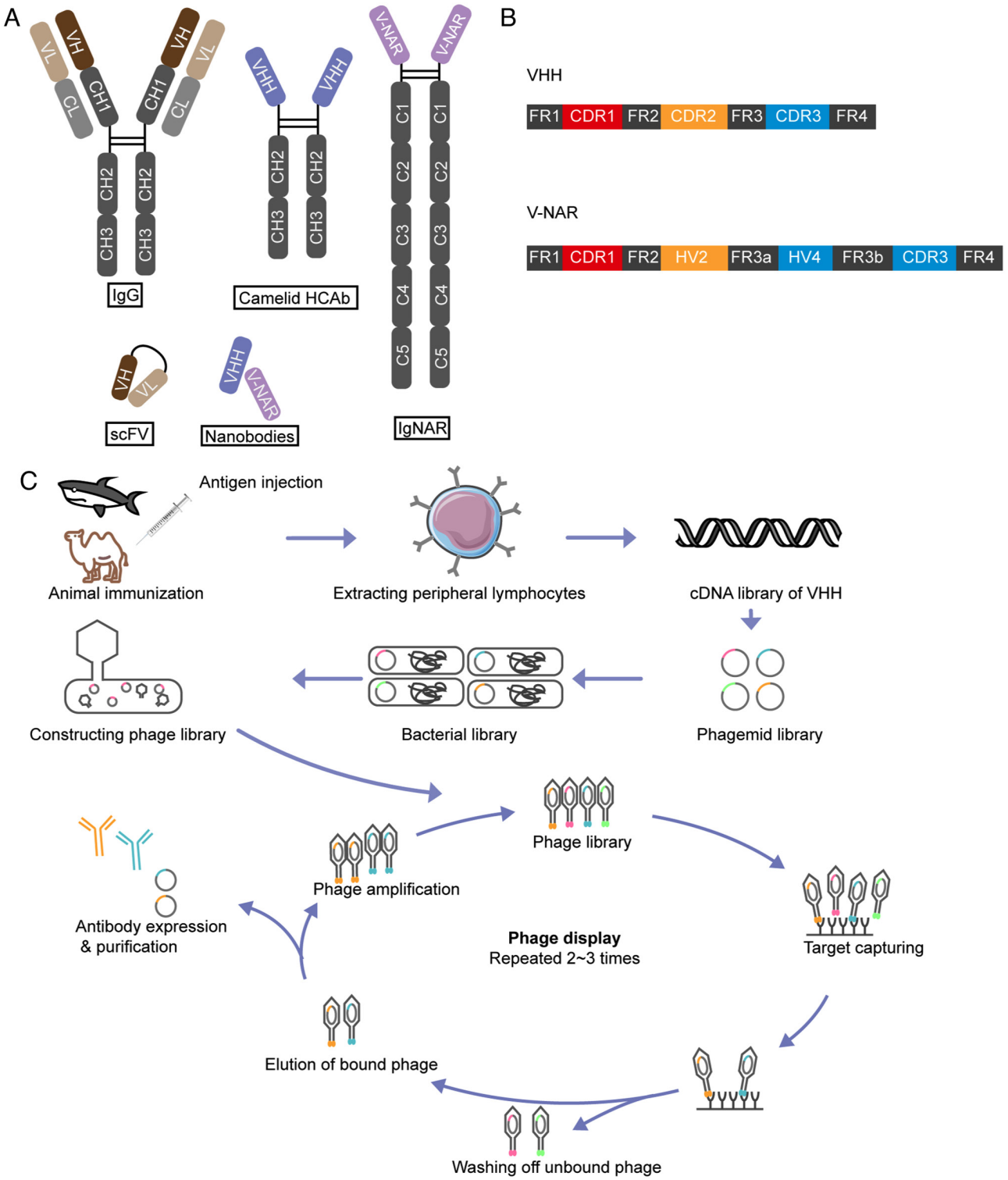

Figure 4. Nb and phage display. (A) Due to the high cost and large size of monoclonal antibodies, a number of small-sized antigen-engaging fragments have been developed, such as scFv fragments and Nbs including VHHs and V-NARs, which are produce more easily compared with full length antibodies. (B) Structure of VHH and V-NAR. There are three CDRs in VHH, while there are two CDRs and two HVs in V-NAR. (C) A flow chart of phage display technology used to produce Nbs. After immunizing animals, antibodies *in vivo* undergo maturation by the immune system. B lymphocytes are subsequently isolated from peripheral blood for RNA extraction. Reverse transcription is employed to synthesize cDNA, which serves as a template for PCR amplification to generate a variety of Nb gene fragments. These fragments are then cloned into vectors and then fused with phages to create phage libraries, facilitating the display and screening of Nb candidates. Nb, nanobody; scFv, single-chain variable fragment; VHH, variable domain heavy-chain; NAR, new antigen receptors; CDR, complementarity-determining region; HVs, hypervariable loops; cDNA, complimentary DNA; FR, framework region; HCAb, heavy-chain-only antibody; CH, constant heavy-chain; VH, variable heavy-chain; CL, constant light-chain; VL, variable light-chain.

antibodies can also be produced directly through the immunization of humanized mice. Moreover, it is theoretically possible to directly extract antibody gene clones from humans and combine them with Nb. A full Nb library was constructed

by grafting the CDR sequences from a natural antibody repertoire derived from healthy donor blood onto human IGHV gene framework regions. A total of 18 unique single-domain antibodies were isolated from the library, which demonstrated efficient and specific binding to the SARS-CoV-2 RBD and were categorized into three competitive groups (127). Among the isolated antibodies, n3088 and n3130 were found to bind to a concealed epitope of the SARS-CoV-2 RBD and exhibited significant neutralizing activity.

Due to their stability during long-term storage, VHHs serve as a viable therapeutic option for future epidemic responses (128). Their favorable biophysical attributes facilitate large-scale production via prokaryotic expression systems in a matter of weeks, which allows for their swift deployment in an emergency situation (129). A Nb termed Nb6 has been previously reported to stabilize two adjacent RBDs in the 'down' state after binding to S proteins, which may then reorganize the second and third binding sites of Nb6 to maintain the closed conformation, causing the RBD to detach from ACE2. Moreover, the affinity and neutralizing capacity of Nb6 was reported to be enhanced further after dimerization and trimerization, where the trimerized version of Nb6 showed picomolar-range neutralization and femtomolar-range affinity for the SARS-CoV-2 RBD. Efficient neutralization was even maintained after nebulization, lyophilization and thermal processing (130).

The majority of mAbs must be generated in mammalian cells and require intravenous injection. In comparison, Nbs are produced in bacteria or yeast and can be delivered to the lungs by inhalation, which can confer significant advantages for SARS-CoV-2 treatment. Possible benefits include low systemic exposure, rapid onset of action and high concentrations at the lesion site (131). After immunization of alpacas with SARS-CoV-2 S protein, Xiang *et al* (132) identified a large number of Nbs with high affinity to S protein RBD by using a custom-designed Nb platform technology. Through further screening, purification and testing, a number of neutralizing Nbs with exceptional antiviral ability were identified. Among them, Nb21 was reported to bind to RBD with picomolar-range affinity and displayed neutralizing ability against viruses. Upon formation into multivalent antibodies, their antiviral capabilities are further enhanced. In particular, Nb21 was reported to be highly thermally stable and retained the same antiviral capacity after lyophilization and nebulization. An inhalable nebulizer based on this antibody, Pittsburgh inhalable Nb-21, was reported to be effective against severe SARS-CoV-2 infection in hamsters in an *in vitro* viral infection assay (133). Nbs, such as NIH-CoVnb-112, Nb11-59, bn03, 2-3-Fc, Nb22, RBD-1-2G, pan-Sarbecovirus Nbs, TP17, TP86, R14 and S43 have all also been demonstrated to exert positive neutralizing effects against SARS-CoV-2 following respiratory administration (134-141).

4. Challenges and current countermeasures

ADE following infection. Not all antibodies against viruses are protective (142). Antibodies against the dengue virus protein expressed by the dengue fever virus can provoke severe reactions mediated by the Fc receptor, namely the ADE of infection (143). ADE is frequently detected in monocytes, macrophages and B cells and is typically mediated by IgG Fc receptors (FcγRs) or complement receptors (144,145). Typically, the ADE of viral infections uses the FcγR. The Fab domains of antibodies with the ADE phenotype bind to viral particles and the Fc domains bind to cells containing FcγR, allowing the virus to enter the host cell through the FcγR, circumventing the involvement of specific receptors (146). For SARS-CoV-2, two forms of ADE mechanisms have been identified. The first form acts through RBD antibodies inducing ADE through FcγRs (147). MW05, the first infection-enhancing SARS-CoV-2 antibody reported, was demonstrated to contribute to ADE by crosslinking its Fc region with FcγRIIB (148). The second ADE pathway is induced by NTD antibodies changing the conformation of the S protein and inducing the open state of the RBD, thereby promoting its binding to ACE2 (149). The ACE2 receptor has also been reported to be partially responsible for ADE (150).

ADE has been reported to impact the degranulation of mast cells, contributing to heart damage or multisystem inflammatory syndrome (151,152). Due to the complex nature of the immune system *in vivo*, whether complement-dependent cytotoxicity and/or antibody-dependent cell-mediated cytotoxicity occurs during ADE will likely depend on the balance of virus removal and infection augmentation. ADE risk has been previously associated with the concentration of antibodies (153). The balance between neutralizing and deleterious antibodies in the body favors the neutralization of SARS-CoV-2 (154). *In vivo* experiments prior to clinical trials are necessary for avoiding ADE. Furthermore, non-neutralizing antibodies have been reported to be mostly responsible for ADE. Focusing on the RBM or other highly neutralizing sites may mitigate these drawbacks. Likewise, engineering antibodies to avoid contact with FcRs or using Nbs without a Fc region may also reduce these side effects. Additionally, studies have identified associations between ADE and specific epitopes on the RBD and NTD of SARS-CoV-2, providing crucial insight for the development of safe and effective Nabs (88,155).

Viral variants escaping neutralizing antibodies. Neutralizing antibody development is challenged by the continuous emergence of mutant variants (156). As an RNA virus, SARS-CoV-2 undergoes constant mutation during replication and under the pressure of antibody selection. The first prevalent S protein mutation that received worldwide attention was D614G, a site that does not come into direct contact with any RBD antibodies, and therefore had no significant impact on antibody neutralization activity. By contrast, the ο variant, which was first detected at the end of 2021, rapidly replaced the δ variant as the major epidemic strain worldwide due to its high transmissibility. The ο variants with enhanced resistance to NAbs also challenged vaccination and infection-induced immunity, rendering therapeutic mAbs ineffective (157). Continued mutational evolution of the virus has led to the emergence of a wide range of variants with greater growth advantage, which evaded almost all current neutralizing antibody drugs and vaccinated or convalescent plasma, as represented by the XBB strain, BQ 1.1 strain and CH 1.1 strain, subvariants of the ο variant of the SARS-CoV-2 (158). Breakthrough infections with the BA.2 and BA.5 subvariants of SARS-CoV-2 have reduced the diversity of NAb binding sites and increased the proportion of non-NAb clones. This, in turn, has increased the

selective pressure on the humoral immune response, fostering the convergent evolution of RBD (158,159). Subsequent analysis uncovered that the o variant harbors 15 mutation sites within the RBD, which confer the ability to circumvent antibody neutralization. Among them, the K417, E484, G446 and S371 mutations mediated the binding inhibition of Class 1-4 antibodies, respectively. Specifically, Class 4 antibodies were rendered ineffective against the variants, leading to a marked reduction in the plasma neutralization capacity among recovered and vaccinated individuals (84,160).

Antibodies against conserved epitopes are promising in dealing with the ever-emerging variants. Starr *et al* (83) previously identified a broad-spectrum neutralizing antibody referred to as S2H97, which binds to a previously unidentified hidden epitope on RBD, causing a conformational change in the RBD, thereby preventing the binding of ACE2. This antibody was reported to retain neutralizing activity against a wide range of strains of Sarbecovirus, including SARS-CoV and SARS-CoV-2. Antibodies which interact with the same binding site as S2H97 could not be found in the blood of convalescent individuals, which is most likely due to the fact that these epitopes are not easily accessible and therefore cannot sufficiently trigger an immune response (83).

Through biological computing technologies, antibodies with superior neutralizing potency can be designed and produced. An antibody named AI-1028, which is a modified S2H97 with an improved neutralization capacity compared with S2H97, was previously reported to be capable of broadly neutralizing Sarbecovirus, including the emerging o subvariants XBB, BQ.1.1 and BA.2.3.20 (161).

It is of strategic importance to predict the direction of virus evolution to anticipate possible mutant strains in advance. Cao *et al* (162) developed a computational model for predicting the trend of mutational evolution in SARS-CoV-2 using a high-throughput deep mutation scanning method. Specifically, mutations in BA.5 and BA.2 that may escape existing herd immunity were analyzed, identified and validated.

The simultaneous use of multiple antibodies that target distinct epitopes are known as cocktail therapeutic regimens, which presents potential for disease treatment and advancement of novel antibodies. In cases where existing antibody products exhibit diminished ability to neutralize viral variants, it would appear logical to modify them using engineering techniques, such as site mutation, creating bispecific or multispecific antibodies, bivalent or multivalent constructs (163,164). Furthermore, the combination of soluble cytokine receptors or exosomes with antiviral Nbs has increased effectiveness. c19s130Fc is a bispecific therapeutic that hinders both the IL-6 signaling pathway and the SARS-CoV-2 RBD. It is created by fusing a soluble cytokine receptor with an antiviral Nb, enabling it to block viral entry and dampen the inflammatory response induced by the virus simultaneously (165). In addition, researchers have engineered exosomes with a S-protein-targeting Nb and human IFN-β bound to MFG-E8, creating a dual-action system. The Nbs on the exosome surface bind to the SARS-CoV-2 S protein, blocking its entry into host cells, while the encapsulated IFN-β is delivered to infected cells to trigger antiviral responses and boost the expression of interferon-stimulated genes (166). In summary, further exploration of antibody engineering is justified to improve the neutralization capacity of available antibodies.

Future perspectives. Novel NAbs that can target different parts of SARS-CoV-2 proteins, including the S protein, are constantly being discovered. It is likely that highly effective novel antibodies will be screened in the future for treating patients with COVID-19, especially during the early stages of the disease. Combination therapy using multiple different NAbs may improve treatment efficacy and reduce the likelihood of resistance. Furthermore, the development of long-acting NAbs may reduce the required frequency of administration and improve patient compliance. This may involve modifications in the engineering process of the antibody to extend its half-life in the body. NAbs can be used not only for treatment but also for pre- or post-exposure prophylaxis, particularly in high-risk groups, such as healthcare workers and the elderly. Broad-spectrum neutralizing antibodies, such as the SA55 and SA58 antibody combination, can be administered as a nasal spray to establish rapid short-acting prophylaxis in the respiratory tract. These antibodies can also be injected during the initial stages of infection to provide medium-to-long-term prophylaxis, which is particularly suitable for the protection of high-risk healthcare workers, patients with compromised immune systems who cannot be vaccinated and the elderly (162). Therefore, investigations into enhanced delivery methods will likely contribute to the advancement and use of NAbs. Nebulized inhalation and nasal dripping have also demonstrated promising outcomes in animal models (138-140). Further research into the mechanism of NAbs to understand how they interact with the different regions of the virus may be helpful in designing more effective antibodies and vaccines. Reducing the cost of producing NAbs by optimizing the production process would make them more widely available and economically viable. To aid end, research institutions and companies worldwide should strengthen their collaboration and share resources and data to accelerate the R&D of neutralizing antibodies. Moreover, large-scale clinical trials are warranted to assess the safety and efficacy of neutralizing antibodies in different populations. Governments and regulatory agencies should also provide policy support to accelerate the R&D and approval processes of NAbs, whilst ensuring product quality and safety.

5. Conclusion

The present review summarized the current research progress on antibodies against SARS-CoV-2 by considering the target sites of antibodies, the SARS-CoV-2 invasion mechanism, as well as the preparation methods, structural properties, mechanisms of action and clinical applications of different NAbs. Following the analysis of a broad body of research, a number of conclusions can be drawn. Firstly, NAbs serve a role in the prevention and treatment of SARS-CoV-2. These antibodies are capable of recognizing and binding to key parts of SARS-CoV-2, preventing viral invasion into host cells and viral replication. Screening and evaluation of SARS-CoV-2 NAbs is another required step for optimization.

Through the screening of a large number of candidate antibodies, antibodies with high neutralizing activity have been identified, providing a foundation for subsequent research and applications. Additionally, evaluating these antibodies for numerous parameters, such as affinity, stability and safety may ensure their effectiveness and safety in clinical applications. Furthermore, a series of notable advancements have been made in the practical clinical application of SARS-CoV-2 NAbs, especially in the elderly, immunosuppressed or critically ill patients. Moreover, NAbs can be used as an emergency treatment to reduce viral replication and disease severity. For individuals at high risk of exposure to SARS-CoV-2, such as healthcare workers, NAbs can be used as a preventive treatment. NAbs can also be combined with vaccines as a 'passive immunization' strategy to provide immediate protection whilst awaiting the establishment of the 'active immunization' response induced by the vaccine. For patients who continue to experience long-term symptoms after recovery, NAbs may also help alleviate symptoms and improve quality of life. In summary, the research and application of SARS-CoV-2 NAbs not only provide a notable therapeutic tool for understanding the COVID-19 pandemic, but also hold potential preventive and therapeutic value for future outbreaks. With increased understanding and the advancement of technology, the clinical application of NAbs will likely become more widespread, making a greater contribution to global public health security.

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Competing interests

The authors declare that they have no competing interests.

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