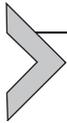




Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Antibody-mediated immunity to SARS-CoV-2 spike

John M. Errico^a, Lucas J. Adams^a, and Daved H. Fremont^{a,b,c,*}

^aDepartment of Pathology & Immunology, Washington University School of Medicine, St Louis, MO, United States

^bDepartment of Molecular Microbiology, Washington University School of Medicine, St Louis, MO, United States

^cDepartment of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St Louis, MO, United States

*Corresponding author: e-mail address: fremont@wustl.edu

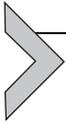
Contents

1. The SARS-CoV-2 pandemic	2
2. Overview of SARS-CoV-2	3
2.1 SARS-CoV-2 virion	3
2.2 SARS-CoV-2 spike protein structure and function	3
2.3 Polyclonal antibody responses to SARS-CoV-2 spike protein	7
2.4 Evasion of antibody-mediated immunity by SARS-CoV-2 variants	10
3. Monoclonal antibodies targeting the receptor-binding domain of spike	12
3.1 Receptor-binding domain structure and function	13
3.2 RBM epitopes and antibodies	13
3.3 Non-RBM RBD epitopes and antibodies	22
4. Monoclonal antibodies to the N-terminal domain of spike	28
4.1 NTD structure and function	28
4.2 NTD epitopes and antibodies	31
5. Monoclonal antibodies targeting S2	40
5.1 S2 structure and function	40
5.2 S2 epitopes and antibodies	43
6. Conclusions	46
Acknowledgments	46
Disclosures	47
References	47

Abstract

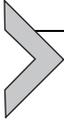
Despite effective spike-based vaccines and monoclonal antibodies, the SARS-CoV-2 pandemic continues more than two and a half years post-onset. Relentless investigation has outlined a causative dynamic between host-derived antibodies and reciprocal viral subversion. Integration of this paradigm into the architecture of next generation antiviral strategies, predicated on a foundational understanding of the virology and immunology of SARS-CoV-2, will be critical for success. This review aims to serve as a primer on

the immunity endowed by antibodies targeting SARS-CoV-2 spike protein through a structural perspective. We begin by introducing the structure and function of spike, polyclonal immunity to SARS-CoV-2 spike, and the emergence of major SARS-CoV-2 variants that evade immunity. The remainder of the article comprises an in-depth dissection of all major epitopes on SARS-CoV-2 spike in molecular detail, with emphasis on the origins, neutralizing potency, mechanisms of action, cross-reactivity, and variant resistance of representative monoclonal antibodies to each epitope.



1. The SARS-CoV-2 pandemic

In late 2019, a novel coronavirus related to severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in the city of Wuhan in China (Zhou, Yang, et al., 2020; Zhu, Zhang, et al., 2020). In the following years, the novel coronavirus, now termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the respiratory illness COVID-19 (Huang, Wang, et al., 2020; Wang, Hu, et al., 2020), has spread to every country on earth, causing greater than 460 million infections and 6 million deaths worldwide at the time of writing (Dong, Du, & Gardner, 2020; Holshue et al., 2020), although the true toll is likely higher (Wang, Li, et al., 2020). Though genetically similar (Lu et al., 2020; Wu, Peng, et al., 2020; Wu, Zhao, Yu, et al., 2020; Zhou, Yang, et al., 2020), SARS-CoV-2 has caused exponentially greater morbidity and mortality than SARS-CoV despite a lower fatality rate ($\sim 2\%$ versus $\sim 10\%$ for SARS-CoV) largely because, unlike SARS-CoV, transmission can occur prior to the onset of symptoms and via asymptomatic carriers (Bai et al., 2020; Gandhi, Yokoe, & Havlir, 2020). The global response to this threat has been unprecedented. mRNA vaccines developed by BioNTech/Pfizer and Moderna display excellent prophylactic efficacy and were approved by regulatory agencies after only 1 year, becoming desperately needed tools in preventing infection and transmission of SARS-CoV-2, and death from COVID-19 (Baden et al., 2021; Polack et al., 2020). Monoclonal antibodies isolated from infected patients were developed into therapeutic cocktails to treat patients unable to be vaccinated, or in cases of breakthrough infection. At the same time, transmission bottlenecks and immunological pressure resulted in the emergence of mutated SARS-CoV-2 variants which evade antibody-mediated immunity, leading to a protracted arms race between countermeasure design and attritive viral evolution with no clear resolution.



2. Overview of SARS-CoV-2

2.1 SARS-CoV-2 virion

SARS-CoV-2 is an enveloped RNA virus belonging to the *Sarbecovirus* subgenus of β -coronaviruses in family Coronaviridae. A \sim 30 kb positive-sense RNA genome encodes numerous open reading frames, including coding sequences for 4 structural proteins: Spike protein (spike, S), Membrane protein, Envelope protein, and Nucleocapsid protein (Naqvi et al., 2020; Wu, Zhao, Yu, et al., 2020). Nucleocapsid is responsible for packaging the viral genome inside a lipid envelope containing spike, membrane, and envelope proteins, that assemble together into roughly spherical virions with an average diameter of 80–90 nm (Ke et al., 2020; Yao et al., 2020). Infectious particles produced by host lung cells are exhaled in respiratory droplets that facilitate airborne spread to nearby recipients (Li, Qian, et al., 2021; Liu, Ning, et al., 2020; Sia et al., 2020; van Doremalen et al., 2020). The most prominent feature of coronavirus virions are the large spike molecules which extend outwards from transmembrane anchors in the viral envelope through long connecting stalks to give the eponymous crown appearance (Ke et al., 2020; Yao et al., 2020).

2.2 SARS-CoV-2 spike protein structure and function

The SARS-CoV-2 spike glycoprotein (spike, S) is a highly glycosylated class I viral fusion protein displayed as a trimer on the viral surface with each 1273 amino acid long protomer divided into two major subunits, S1 and S2 (Walls et al., 2020; Wrapp et al., 2020) (Fig. 1A). S1, which begins after a 13-residue long signal peptide (Huang, Yang, Xu, Xu, & Liu, 2020), forms the apical N-terminal portion of the mature molecule (residues 14–685), and encodes 4 distinct subdomains that form a V shape characteristic of β -coronaviruses (Fig. 1B) (Kirchdoerfer et al., 2018; Walls, Tortorici, Bosch, et al., 2016; Walls et al., 2020). The receptor binding domain (RBD, also called S^B, residues T333 to P527), lies at the apex of the spike protomer, and encodes the receptor-binding motif (RBM) at its tip, which plays a critical role in binding angiotensin-converting enzyme II (ACE2) on target host cells as the initiating step in viral entry (Hoffmann et al., 2020; Lan et al., 2020; Yan et al., 2020) (Fig. 1B and C). The N-terminal domain (NTD, also called S^A, residues Q14 to S305,) folds as a galectin-like antiparallel beta-sandwich and sits at the edges of the triangular spike trimer, similar

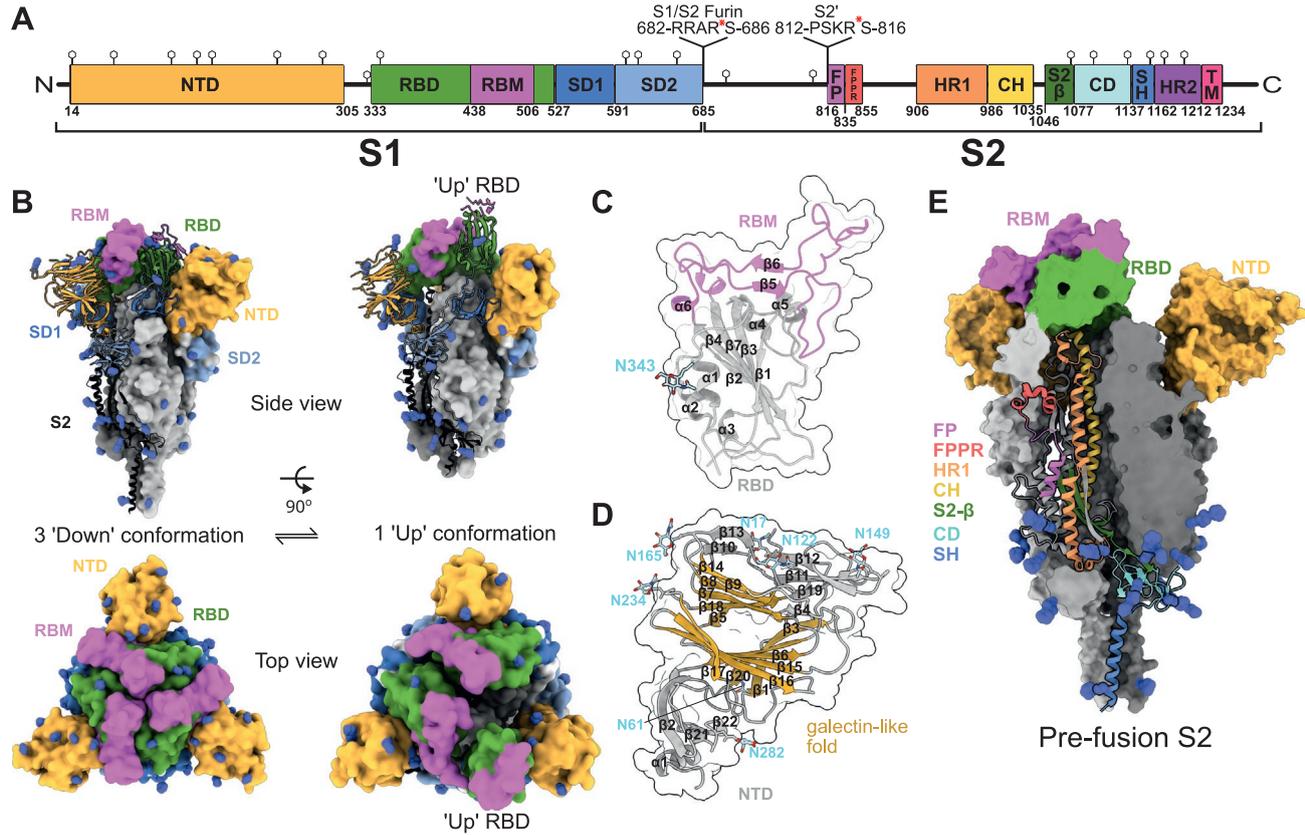


Fig. 1 See figure legend on opposite page.

to other coronaviruses (Li, 2016; Zhang, Xiao, Cai, & Chen, 2021) (Fig. 1D). Notably, the RBD is conformationally dynamic, sampling two discrete states via hinge motion; the “Down” conformation, where the RBD is compacted against the rest of the spike molecule beneath it and contacting the NTD of an adjacent spike protomer, and the “Up” conformation, a metastable configuration with the RBD orienting vertically up and away from the spike, positioning the RBM at the highest, most envelope distal point of the molecule (Henderson et al., 2020; Walls et al., 2020; Wrapp, Wang, et al., 2020) (Fig. 1B). This conformational transition is critical for spike function, as RBDs in the “down” conformation are incapable of binding to ACE2 due to steric constraints (Gui et al., 2017; Henderson et al., 2020; Walls et al., 2020). As spike is a trimer, there are 4 possible RBD conformational configurations; the majority of studies indicate that the “one up, two down” and “all three down” configurations tend to predominate (Henderson et al., 2020; Walls et al., 2020; Wrapp, Wang, et al., 2020), although the “two up” and “three up” conformations have also been observed less frequently (Barnes et al., 2020; Ke et al., 2020). Following the RBD, there are two additional subdomains termed SD1 and SD2 (also called CTD1 and CTD2) that modulate hinge movements (and thus

Fig. 1 Overview of SARS-CoV-2 spike structure. (A) Linear diagram showing the domains and subdomains of SARS-CoV-2 spike with residue numbers listed below each segment. Black hexagons indicate the location of N-linked glycans. S1/S2 furin cleavage motif and S2' dibasic cleavage motif are listed above their respective sites. NTD, N-terminal domain; RBD, receptor binding domain; RBM, receptor binding motif; SD1, subdomain 1; SD2- subdomain 2; FP, fusion peptide; FPPR, fusion peptide proximal region; HR1, heptad repeat 1; CH, central helix; CD, connector domain; SH, stem helix; HR2, heptad repeat 2; TM, transmembrane anchor. (B) Structures of the S1 portion of SARS-CoV-2 spike in both the all “down” configuration (PDB: 6XR8) and the “one up” configuration (PDB: 6VSB). One monomer of the trimer is displayed as a cartoon ribbon, while the other 2 are shown as surface representations. Subdomains of S1 are colored as in (A). N-linked glycans are shown as blue spheres. (C) Close up of the RBD (PDB: 6W41). RBM motif is colored as in (A); N343 glycan shown as ball and stick representation with carbon atoms colored light blue, oxygen atoms red and nitrogen atoms dark blue. Secondary structural elements are labeled as previously described (Lan et al., 2020). (D) Close up of the NTD (PDB: 7B62). The galectin-like fold is shown in gold, with glycans shown in ball and stick representation. Secondary structural elements identified via DSSP are labeled. (E) Pre-fusion structure of S2 (PDBnn6XR8). One monomer of S2 is displayed as a cartoon ribbon and colored according to (A). The remaining two S2 protomers are rendered as gray surfaces. S1 cap is shown as a clipped surface representation and colored according to (A). N-linked glycans are shown as blue spheres. All structural depictions were generated using UCSF ChimeraX (Goddard et al., 2018).

conformational switching) of the RBD and contribute to trimer stability (Benton et al., 2020; Cai et al., 2020; Henderson et al., 2020; Zhou, Tsybovsky, et al., 2020) (Fig. 1A and B).

The S2 portion of the molecule begins at residue S686, immediately following a furin cleavage motif unique among sarbecoviruses to SARS-CoV-2, which is processed during spike biosynthesis, severing linkage between S1 and S2 (Hoffmann, Kleine-Weber, Schroeder, et al., 2020). Downstream, a conserved dibasic cleavage site (S2') can be proteolytically processed by TMPRSS2 on host cell plasma membranes after ACE2 binding (or Cathepsins in endosomal compartments, if the virus is internalized), immediately followed by a hydrophobic fusion peptide (Hoffmann, Kleine-Weber, Schroeder, et al., 2020; Ou et al., 2020). The majority of S2 after the fusion peptide is composed of several helical domains including two heptad repeats and a connector domain that form the fusion machinery responsible for mediating fusion of viral and host cell lipid membranes (Benton et al., 2020; Cai et al., 2020; Xia et al., 2020) (Fig. 1A and E).

Upon inhalation of respiratory droplets containing SARS-CoV-2, the virus localizes to target cells in the nasal passages and lung, recruited by attachment factors such as heparin sulfates and lectins which bind N-linked glycans on spike (Amraei et al., 2021; Clausen et al., 2020; Lempp et al., 2021). "Up" conformation RBDs are then able to bind to ACE2 on target cells, at which point TMPRSS2 is able to cleave the S2' dibasic motif (Benton et al., 2020; Gobeil et al., 2021; Yu et al., 2022). ACE2 binding to an "Up" RBD on a spike trimer is followed by conformational changes which reduce interactions between S1 and S2, leading to S1 dissociation from S2, thus "unsheathing" the fusion apparatus for accessibility to the now juxtaposed target cell membrane (Benton et al., 2020; Cai et al., 2020; Wrapp, Wang, et al., 2020). Fusion begins when S2 undergoes a dramatic conformational shift as the spring-loaded HR1 domains launch the fusion peptide into the opposing target cell membrane before folding back to contact HR2, localized near the viral envelope, thus dragging the opposing membranes into contact, leading to fusion, and completing viral entry (Jackson, Farzan, Chen, & Choe, 2022; Tortorici & Veesler, 2019).

Given its critical role in SARS-CoV-2 infection and exposure on the surface of the virion, it is not surprising that spike is the major target of protective host antibody responses. During viral entry, spike is most vulnerable in the prefusion conformation (S1 still associated with S2), when epitopes capable of blocking binding to host cell factors on S1 are exposed. Most

of the spike on SARS-CoV-2 virions is present in this metastable prefusion conformation, although some adopt an extended rod-like post-fusion conformation due to premature dissociation of S1 from S2 (Ke et al., 2020). The presence of neutralizing antibodies to prefusion spike is a strong correlate of protection for SARS-CoV-2 infection (Cromer et al., 2022; Dispinseri et al., 2021; Feng et al., 2021; Garcia-Beltran et al., 2021); development of anti-SARS-CoV-2 countermeasures has thus mainly focused on designing vaccines that induce neutralizing antibodies to prefusion spike for prophylaxis, or identification and large-scale production of monoclonal antibodies known to target neutralizing epitopes on spike.

2.3 Polyclonal antibody responses to SARS-CoV-2 spike protein

A polyclonal antibody response against spike results either from infection with SARS-CoV-2 or vaccination with platforms that introduce spike to a recipient immune system. To date, the most effective anti-SARS-CoV-2 tools developed have been vaccines based on spike, several of which are approved for use in humans, that utilize newer technologies, such as adenoviral vectors (Sadoff et al., 2021; Voysey et al., 2021), as well as more traditional approaches based on whole-virion inactivation (Xia et al., 2021). The two most widely used SARS-CoV-2 vaccines in the United States are Comirnaty (research name BNT162b2) and Spikevax (research name mRNA-1273), produced by BioNTech/Pfizer and Moderna, respectively (Baden et al., 2021; Polack et al., 2020). These mRNA vaccines use lipid nanoparticles to introduce encapsulated mRNA encoding a full-length SARS-CoV-2 spike protein into recipient cells, which then produce and secrete spike, leading to antibody production (Jackson et al., 2020; Mulligan et al., 2020; Pardi, Hogan, Porter, & Weissman, 2018). Although infection and vaccination broadly lead to functionally similar outcomes (i.e., induction of protective anti-spike antibodies), polyclonal antibody responses to each also differ in several key aspects.

Seroconversion to SARS-CoV-2 spike by infection is prevalent, with nearly all patients generating detectable anti-spike antibodies within 3 weeks of symptom onset (Long et al., 2020; Ma et al., 2020; Seow et al., 2020). Although there is significant variability in the reported kinetics of antibody responses in patients infected with SARS-CoV-2, in general, anti-spike IgM, IgA, and IgG develop simultaneously (Graham et al., 2020; Isho et al., 2020; Iyer et al., 2020; Seow et al., 2020), with some studies reporting a slight delay in IgG production compared to IgM and IgA (Ma et al., 2020).

Notably, delayed onset of IgG production correlates with moderate disease, and failure to produce functional IgG is associated with mortality in severe disease (Zohar et al., 2020). In the following months, IgM and IgA spike-binding titers undergo an acute drop followed by a more gradual decline, while IgG declines more steadily overall, but more steeply for anti-RBD antibodies compared to anti-spike (Isho et al., 2020; Wheatley et al., 2021). These findings initially led to concerns over the possibility of secondary SARS-CoV-2 reinfection (Ibarrondo et al., 2020; Seow et al., 2020); subsequent studies over extended time periods have shown persistence of neutralizing antibodies after the initial decline alongside hallmarks of durable humoral immunity, such as the generation of spike-specific memory B-cells and bone-marrow resident long-lived plasma cells (Dan et al., 2021; Turner et al., 2021). Notably, anti-spike IgA is implicated in effective neutralizing responses to SARS-CoV-2 (Gallo, Locatello, Mazzoni, Novelli, & Annunziato, 2021; Kaplonek et al., 2022; Russell, Moldoveanu, Ogra, & Mestecky, 2020; Sterlin et al., 2021; Wang, Lorenzi, et al., 2021), and intranasal vaccines candidates induce greater protection and control of viral replication in the upper airway when compared to intramuscular vaccination (Hassan et al., 2020, 2021; Lapuente et al., 2021; Park et al., 2021; van Doremalen et al., 2021), suggesting that robust mucosal IgA responses may be an important component of vaccination, particularly for controlling viral transmission in addition to systemic protection.

The quality of polyclonal antibody responses derived from SARS-CoV-2 infection are highly variable. Many convalescent patients, typically those with milder infection, fail to produce a neutralizing antibody response at all (Chen et al., 2020; Garcia-Beltran et al., 2021; Robbiani et al., 2020; Takeshita et al., 2021), while those with a greater duration and/or severity of COVID-19 symptoms who survive infection tend to mount higher quality neutralizing antibody responses (Gaebler et al., 2021; Long et al., 2020; Robbiani et al., 2020). In contrast, antibody responses elicited through vaccination are far more consistent, with 95% of vaccinees showing protection from SARS-CoV-2 infection after two doses (Baden et al., 2021; Polack et al., 2020). Interestingly, similar to patients with extended COVID-19 infection, vaccination with the Pfizer mRNA vaccine induces long-lasting germinal center responses (Turner et al., 2021), suggesting that extended germinal center responses may be an important factor in the development of high quality neutralizing responses (Laidlaw & Ellebedy, 2022; Lederer et al., 2020). Spike encoded by the mRNA vaccines also contains two

mutations, K986P and V987P, that stabilize the prefusion conformation (Hsieh et al., 2020; Pallesen et al., 2017), which has been shown to increase polyclonal serum neutralizing titers in mice (Bos et al., 2020) as well as human vaccinees (Bowen et al., 2021) and was previously shown to effectively generate higher neutralizing titers against other β -coronaviruses, including MERS (McLellan et al., 2013; Pallesen et al., 2017).

The majority of neutralizing activity in bulk polyclonal responses to spike for both convalesced patients and vaccinees localizes to epitopes on the RBD and NTD (Amanat et al., 2021; Piccoli et al., 2020). The most potently neutralizing antibodies target the RBM, and act by blocking the interaction of ACE2 with the RBD (Dejnirattisai, Zhou, Ginn, et al., 2021; Greaney et al., 2021; Piccoli et al., 2020; Steffen et al., 2020). Antibodies to NTD are the next most prevalent, and typically display lower neutralizing potency than RBD mAbs (Amanat et al., 2021; C. Graham et al., 2021; McCallum, De Marco, et al., 2021). The exact mechanism by which antibodies to the NTD neutralize SARS-CoV-2 is not well understood, although a recent study showed NTD antibodies to be capable of blocking proteolytic cleavage of the S2' site (Qing et al., 2022). Polyclonal responses directed against S2 are less common and tend to show very limited neutralization but high cross-reactivity to other coronaviruses, in line with the high degree of sequence conservation in S2 (Ladner et al., 2021; Ng et al., 2020; Poh et al., 2020; Shrock et al., 2020). In addition to neutralization, antibodies to SARS-CoV-2 spike can elicit Fc effector functions such as antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), which form an important component of protective responses (Chakraborty et al., 2022; Gorman et al., 2021; Kaplonek et al., 2022; McMahan et al., 2021; Zohar et al., 2020). These mechanisms must be approached with caution, however, as Fc-dependent effects have been implicated in increased lung damage and pathology (Liu et al., 2019) and increased viral infection of target cells via antibody-dependent enhancement of SARS-CoV infection (ADE) (Jaume et al., 2011); so far, however, there is little to no evidence of these effects manifesting clinically in humans.

Cross-reactivity of polyclonal antibodies between SARS-CoV-2 and SARS-CoV is prevalent, and in some cases can also extend to other coronaviruses (Bates et al., 2021; Hicks et al., 2021; Ou et al., 2020; Song et al., 2021). SARS-CoV-2S displays 76% amino acid identity to SARS-CoV S overall (Ou et al., 2020); the RBD has approximately 75% amino acid identity, while the RBM motif is notably divergent, with only

50% amino acid identity to SARS-CoV, despite identical function in ACE2 binding (Jaimes, André, Chappie, Millet, & Whittaker, 2020; Lan et al., 2020). The NTD is also highly variable, showing only 51% identity to SARS-CoV (Jaimes et al., 2020). In contrast, S2 displays approximately 90% sequence identity to SARS-CoV, consistent with functional requirements that constrain the mutational landscape (Jaimes et al., 2020). S2 is also highly glycosylated, and thus may not experience similar immunological pressures from antibodies as less glycosylated regions of spike (Casalino et al., 2020; Grant, Montgomery, Ito, & Woods, 2020; Watanabe, Allen, Wrapp, McLellan, & Crispin, 2020) (Fig. 1). Early investigations showed that although SARS-CoV convalescent serum is capable of binding to SARS-CoV-2 spike (and vice-versa), it loses almost all neutralizing activity (Lv, Wu, et al., 2020; Robbiani et al., 2020; Song et al., 2021; Zhu, Yu, et al., 2020). Unsurprisingly, cross-reactivity to MERS is even more limited, with almost no cross-neutralization apparent (Ju et al., 2020; Scheid et al., 2021).

2.4 Evasion of antibody-mediated immunity by SARS-CoV-2 variants

The most significant source of resistance to antibody-mediated immunity by SARS-CoV-2 has arisen from the accumulation of mutations in spike residues driven by antigenic drift, giving rise to SARS-CoV-2 variant viruses with higher transmissibility and varying degrees of resistance to neutralization by antibodies. A number of variants have emerged since the pandemic started, typically during periods of high transmission, and show variable numbers of mutations in spike, generally trending upwards over time. The first widely studied variant emerged early in the pandemic, and encoded a single amino acid substitution in spike: D614G, structurally located in SD2 near the hinge region of the RBD (Gobeil et al., 2021; Yurkovetskiy et al., 2020; Zhang, Cai, et al., 2021). This new variant had increased viral fitness and transmission, and quickly overtook the original wild-type virus in new infections, despite most studies reporting no greater resistance to antibody-mediated immunity (Hou et al., 2020; Korber et al., 2020; Volz et al., 2021). Since then, other instances of viruses with single substitutions have occurred alongside more heavily mutated variants that have earned their own classifications. In this review, we will refer to variants by either their official name derived from the Pango nomenclature system (Rambaut et al., 2020) or their WHO designation (alpha, beta, etc.) and limit our discussion to variants that caused large scale global infection.

Following D614G, several major variants with multiple spike mutations emerged, also containing D614G. The B.1.1.7 variant, called the alpha variant in WHO nomenclature, was first detected in September 2020 in the United Kingdom (Galloway, 2021), and by early 2021 had become the dominant variant causing new infections in the UK, before spreading worldwide (Davies et al., 2021; Washington et al., 2021). B.1.1.7 displays significantly enhanced transmissibility, mediated by several mutations in spike that enhance viral fitness; D614G, which enhances infectivity as previously described; N501Y, which enhances binding affinity between the RBD and ACE2 (Starr et al., 2020; Zhu et al., 2021); and a deletion in the NTD (del69-70) which is associated with increased viral infectivity in-vitro and causes certain PCR-based diagnostic tests to fail (Meng et al., 2021; Volz et al., 2021). Unlike the initial D614G variant, B.1.1.7 also displayed slightly increased resistance to neutralization by polyclonal sera from convalescent patients and vaccinees (Muik et al., 2021; Shen et al., 2021; Supasa et al., 2021), although this resistance was not great enough to cause significant breakthrough infection in vaccinated individuals (Abdool Karim & de Oliveira, 2021).

Alongside B.1.1.7, two other variants, termed B.1.351 (beta) and P.1 (gamma), emerged in South Africa and Brazil, respectively (Abdool Karim & de Oliveira, 2021; Faria et al., 2021; Tegally et al., 2021). These variants contain many of the mutations seen in B.1.1.7, including N501Y and D614G, and display similarly increased transmissibility (Abdool Karim & de Oliveira, 2021; Campbell et al., 2021). In contrast to B.1.1.7, B.1.351 and P.1 also carry additional mutations in the RBD (K417N/T and E484K) which confer much greater resistance to neutralization by sera from convalesced patients and vaccinees than N501Y alone (Abu-Raddad, Chemaitelly, & Butt, 2021; Chen et al., 2021; Hoffmann et al., 2021; Planas et al., 2021; Wang, Nair, et al., 2021) (Fig. 2). Indeed, a wave of infection by SARS-CoV-2 occurred in Manaus, Brazil despite high pre-existing seropositivity among the population (Sabino et al., 2021), with breakthrough infections observed in both vaccinated and convalescent individuals (Abu-Raddad et al., 2021; Kustin et al., 2021; Vignier et al., 2021).

The second major wave of global infections began with the emergence of B.1.617.2 (Delta variant in WHO nomenclature) in India in late 2020 (Cherian et al., 2021), which by June 2021 had become the dominant global strain, succeeding earlier waves caused by B.1.1.7, B.1.351, and P.1. Variant B.1.617.2 contains some substitutions seen in B.1.351 and P.1, such as

K417N and D614G, alongside less frequently observed changes such as L452R and T478K, and a number of changes at the NTD (Liu, Ginn, et al., 2021). B.1.617.1 and B.1.617.3, related subtypes, also contain a substitution at E484Q, which was shown to disrupt antibody-mediated neutralization (Cherian et al., 2021; Liu, Ginn, et al., 2021; Planas et al., 2021). These changes, in particular L452R for B.1.617.2, enhanced transmissibility of delta and also conferred resistance to neutralization from both vaccinated and convalescent serum (Deng et al., 2021), resulting in widespread infections and the second major wave of COVID-19 infections in the United States (Lopez Bernal et al., 2021; Mlcochova et al., 2021; Planas, Veyer, et al., 2021).

The most recent variant, B.1.1.529 (Omicron, also referred to as BA.1), emerged in Botswana and South Africa in Late 2021 before spreading internationally. In contrast to previous variants (B.1.1.7, 10; B.1.351, 12; P.1, 12; B.1.617.2, 8), BA.1 contains a staggering 32 mutations in Spike, with 15 localized to the RBD. Omicron displays robust evasion of antibody mediated immunity from both vaccinated (Andrews et al., 2022; Collie, Champion, Moultrie, Bekker, & Gray, 2022; Garcia-Beltran et al., 2022; Schmidt et al., 2022) and convalescent serum samples (Hoffmann et al., 2022; Liu, Iketani, et al., 2022; Planas et al., 2022). In combination with an apparent significant increase in transmissibility, B.1.1.529 fomented the third (and largest) wave of infections worldwide (Viana et al., 2022), despite record vaccination rates.

Even after Omicron's global sweep, B.1.1.529 lineage sub-variants, such as BA.2, have continued to evolve with additional mutations that confer resistance to antibody mediated neutralization (Case et al., 2022; Iketani et al., 2022). Although polyclonal responses have seen decreasing efficacy over time as new variants have emerged, generally they have retained enough neutralizing potency to provide protection from severe disease and mortality in vaccinated and convalescent individuals (Garcia-Beltran et al., 2022). In contrast, monoclonal antibodies, including those approved for clinical use in humans, have been more sensitive to these changes by virtue of their clonality.



3. Monoclonal antibodies targeting the receptor-binding domain of spike

Numerous panels of monoclonal antibodies (mAbs) have been generated over the course of the pandemic. These mAbs have utility not only as

reagents and therapeutics for clinical uses, but also as powerful tools to dissect antibody-mediated immunity to SARS-CoV-2 at the molecular level. Indeed, many of the trends observed for polyclonal responses can be understood by the properties of individual epitopes on SARS-CoV-2 spike antigens and the antibodies that target them. The majority of neutralizing mAbs characterized to date target the RBD; these antibodies can be roughly categorized into groups which cluster by the structural epitopes they target and their functional properties.

3.1 Receptor-binding domain structure and function

The receptor binding domain (RBD) is approximately 194 residues long and connects to the rest of the spike by a hinge formed at the N and C-terminal portions of the RBD, with several disulfide bonds stabilizing the fold (Fig. 1C). Structurally, the RBD contains 5 α -helices and 7 β -sheets, which form a core 5-strand antiparallel beta-sheet atop which two short antiparallel beta strands connect extended loops to form the receptor binding motif (RBM, residues 438–506) (Fig. 1C) (Lan et al., 2020). Because of the critical role of this motif in the viral life cycle and its exposure on the viral surface, antibodies targeting epitopes in this region frequently display exceptional neutralizing potency. Counterintuitively, despite this critical function, the RBM is highly permissive to mutation. SARS-CoV-2 variants frequently display numerous substitutions in the RBM, with the most recent sub-variants of the B.1.1.529 lineage encoding 7 mutations in ACE2 contact residues out of a total 15 on the RBD (Saxena et al., 2022) (Fig. 2). Indeed, the RBM motif in SARS-CoV-2 displays only \sim 50% amino acid identity to that of SARS-CoV, despite approximately 75% conservation in the RBD overall (Lan et al., 2020; Walls et al., 2020; Yi et al., 2020). In other non-sarbecovirus coronaviruses, the RBM displays significant structural and sequential diversity, enabling alternative binding modes of ACE2 in the case of the α -coronavirus hCoV-NL63 (Tortorici & Velesler, 2019), or utilization of different receptors, such as DPPN4 for the β -coronavirus MERS (Li, 2016; Wang et al., 2013). As such, epitopes localized to the RBM are generally moving targets.

3.2 RBM epitopes and antibodies

Antibodies targeting the RBM, commonly referred to as class 1 and class 2 antibodies (Barnes, Jette, et al., 2020), antigenic sites Ia and Ib (Piccoli et al., 2020), RBS-A, -B, -C epitopes (Yuan, Liu, Wu, & Wilson, 2021), or

RBD-1, -2, -3 antibodies (Hastie et al., 2021), display strong neutralizing potency against SARS-CoV-2 and primarily act by blocking ACE2 receptor binding. These RBM antibodies can be distinguished from one another by their binding orientation on the RBM motif (Figs. 1C and 2B); Class 1 RBM antibodies target residues that are sterically obscured in “down” conformation RBDs, and are thus observed binding to “up” conformation RBDs exclusively (Barnes, Jette, et al., 2020). Rotated over the RBM to the other side of the RBD, class 2 RBM antibodies target residues exposed in both “up” and “down” RBD conformations, enabling a full stoichiometry of binding regardless of RBD conformation. Antibodies to these class 1 and class 2 epitopes, jointly referred to as the RBM epitope in this review, share many properties and generally display potent neutralization.

Numerous independently identified RBM-epitope antibodies that bind the “up” RBD conformation utilize the IgHV3–53 heavy chain gene, including CB6 (Ly-CoV016, etesevimab), B38, CC12.1, CC12.3, C105, COVA2–04, and CV30, among others (Barnes, Jette, et al., 2020; Hurlburt et al., 2020; Wu, Yuan, et al., 2020; Wu, Wang, Shen, et al., 2020; Yuan et al., 2020). These antibodies target a nearly identical epitope centered on alpha helices $\alpha 4$ and $\alpha 5$ encoded by residues 400–410 and 415–420, respectively, a beta-strand $\beta 5$ encoded by residues 450–460, and a loop leading into the $\beta 6$ strand encoded by residues 473–477 and 486–505 that strongly overlaps with ACE2 binding residues in the RBM (Fig. 3A and B), and display relatively little somatic hypermutation and

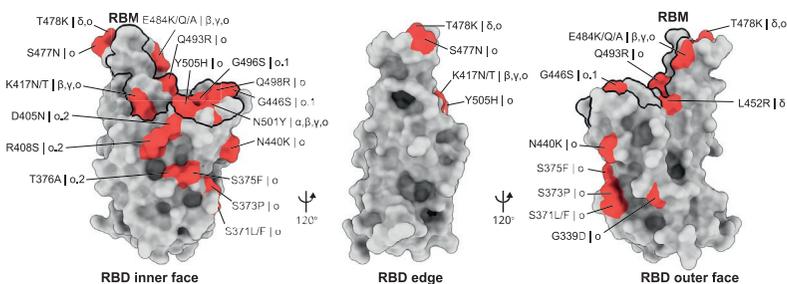


Fig. 2 RBD substitutions in SARS-CoV-2 variants. SARS-CoV-2 receptor binding domain (RBD) with substitutions from SARS-CoV-2 variants shaded red. Black outline indicates human ACE2 residues contact that define the RBM (Lan et al., 2020). Labels indicate amino acid changes and variants encoding each change using WHO nomenclature (α , B.1.1.7; β , B.1.351; γ , P.1; δ , B.1.617.2; $\alpha, 1$, B.1.1.529.1/BA.1; $\alpha, 2$, B.1.1.529.2/BA.2; α , Both BA.1 and BA.2).

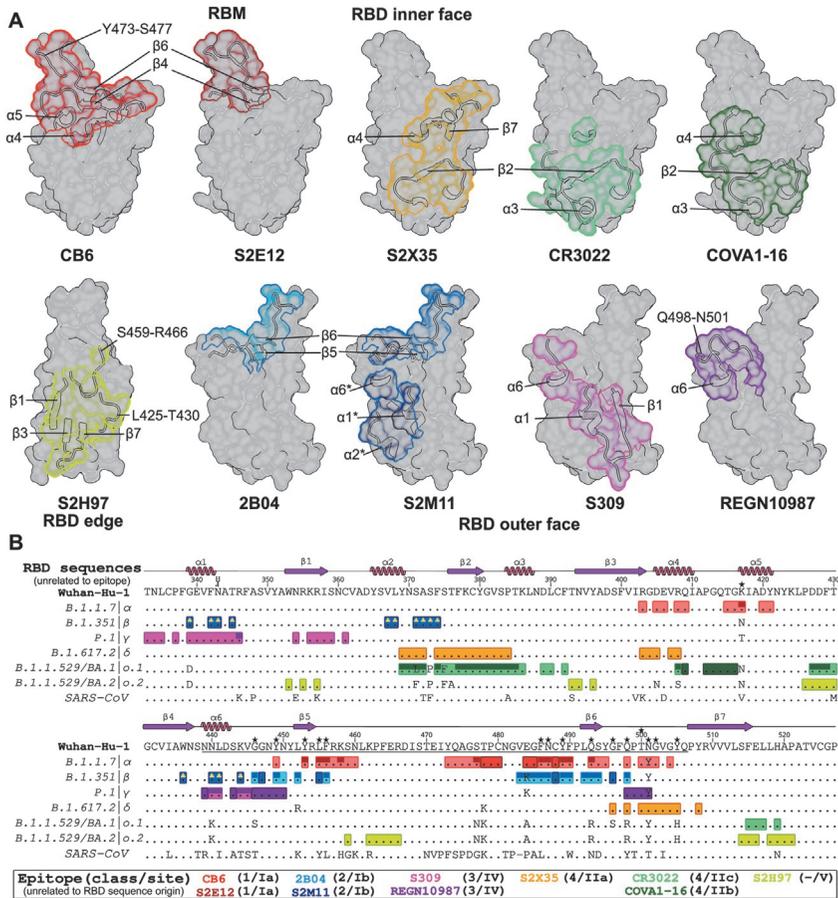


Fig. 3 Epitopes on the RBD. (A) RBD with epitopes from prototypical antibodies representing each class or antigenic site depicted as shaded region on RBD surface. Labels indicate specific strands and secondary structure elements involved in each epitope, depicted as cartoon ribbons underneath transparent RBD surface. (B) Multiple sequence alignment of SARS-CoV-2 RBD residues 333–527 for wild-type Wuhan-Hu-1 and variant strains as well as SARS-CoV; dots indicate fully conserved residues relative to Wuhan-Hu-1 reference strain. SARS-CoV-2 RBD Secondary structure is diagrammed above Wuhan-Hu-1 sequence as described (Lan et al., 2020). Representative antibodies with epitope class (Barnes et al., 2020) and antigenic site (Piccoli et al., 2020) indicated in parentheses have their RBD contacts highlighted over the variant RBD sequences and are unrelated to the variant sequence they are overlaid on. Half-shaded residues indicate binding by antibodies from both colors. Yellow triangles for S2M11 indicate quaternary contacts on an adjacent RBD. RBM motif is underlined; hACE2 contacts are identified with stars (Lan et al., 2020). Branch icon above N343 indicates N-linked glycan. RBD depictions use PDB: 6MOJ. Epitope contacts were identified by buried surface area measurement of atomic models (CB6, PDB: 7C01; S2E12, PDB: 7R6X; 2B04, PDB: 7K9I; S2M11, PDB: 7K43; S309, PDB: 7R6W; REGN10987, PDB: 6XDG; S2X35, PDB: 7R6W; CR3022, PDB: 6W41; COVA1-16, PDB: 7JMW; S2H97, PDB: 7M7W) using UCSF ChimeraX with a probe radius of 1.4 Å and the default cutoff of 1.0 Å² (Goddard et al., 2018).

shorter average CDR3 lengths (Tan et al., 2021). IgHV3–53 antibodies can also utilize a slightly different epitope by incorporating an extended CDR3 sequence. For example, COVA2–39 rotates slightly further up the RBD and avoids contact residues located in the 400–420 range whilst retaining heavy chain contacts on the bulk of the RBM motif (near residues 450–460, 473–477, and 483–498) (Wu, Yuan, Liu, et al., 2020). Subsequent work has shown that these IgHV3–53 antibodies encode conserved NY and SGGs motifs in their CDRH1 and CDRH2 loops, respectively, that facilitate RBD binding (Yuan, Liu, et al., 2020). Indeed, alternative IgH genes that contain these motifs and a very high degree of conservation to IgHV3–53, such as IgHV3–66, also frequently appear in human anti-SARS-CoV-2 RBM antibody repertoires (Barnes, Jette, et al., 2020; Robbani et al., 2020). Single mutations in the germline sequence of IgHV3–53 or IgHV3–66 containing antibodies, such as Y58F, are able to increase binding affinity for RBD up to 1000-fold, thus explaining how these antibodies are able to potently neutralize SARS-CoV-2 despite relatively little somatic hypermutation (Tan et al., 2021). As well, germline-reverted versions of IgHV3–53 and IgHV3–66 showed intact RBD binding and viral neutralization, suggesting these heavy chain genes are preconfigured for RBM binding (Clark et al., 2021; Shi et al., 2020; Yuan, Liu, et al., 2020). This germline affinity and short mutational pathway to strongly affinity-enhancing mutations may confer early proliferative advantages for B-cells bearing these heavy chain genes in nascent germinal centers, explaining their relatively high abundance among both convalescent patients and vaccinees.

The second class of RBM-targeting antibodies (Class 2 (Barnes, Jette, et al., 2020); antigenic site Ib (Piccoli et al., 2020); RBD-4 (Hastie et al., 2021)) binds on the opposite side of the RBD near the “outer face” (Figs. 2 and 3A). These antibodies also display potent neutralization and typically act primarily by blocking ACE2 receptor binding. Examples of human class 2 RBM antibodies include C144, BD23, 2–4, P2B-2F6, and mouse antibody 2B04 (Barnes, Jette, et al., 2020; Cao et al., 2020; Errico et al., 2021; Ju et al., 2020; Liu, Wang, et al., 2020). These antibodies display significantly greater variability in their epitope footprints, manifesting as variable rotation about the core RBM ridge motif. Members targeting epitopes closer to class I antibodies, such as BD23, 2B04, and 2–4, target a few residues in the 445–455 range; the bulk of their contacts, located in the 480–500 range, significantly overlap with class I epitope contacts as well as the RBM motif (Barnes, Jette, et al., 2020; Errico et al., 2021) (Fig. 3A). Other RBM antibodies rotated further away from the class I

epitope, such as P2B-2F6 and CV07-270, display sparser coverage of the core RBM residues in the 480–500 range, instead targeting alternative residues such as R346 and T470 (Ju et al., 2020; Kreye et al., 2020). Because of the diversity of epitope footprints among class 2 antibodies, alternative classification schemes split them into two further subgroups, termed “RBS-B” and “RBS-C” (Yuan et al., 2021). Uniting these antibodies, however, is a near universal lack of contacts in residues 404–421 and 473–477, leading to a shift in binding orientation that facilitates binding to “down” conformation RBDs, thus distinguishing both their target epitopes and functional capacity from class 1 antibodies. In general, class 2 RBM antibodies also display greater variability in heavy chain gene usage (Yuan et al., 2021), with notable exceptions including certain IgHV3-53 antibodies with longer CDR3 sequences and a class of potentially neutralizing antibodies derived from IgHV1-2 (Barnes, Jette, et al., 2020; Rapp et al., 2021).

RBM targeting antibodies generally neutralize SARS-CoV-2 by blocking the interaction of RBD with ACE2 on target cells. The most potently neutralizing RBM antibodies achieve single-digit ng/mL IC_{50} values (Alsoussi et al., 2020; Liu, Wang, et al., 2020; Tortorici et al., 2020; VanBlargan et al., 2021), with the majority falling between 10 and 100 ng/mL IC_{50} and upwards (Hansen et al., 2020; Ju et al., 2020; Robbiani et al., 2020; Rogers et al., 2020; Shi et al., 2020; Zost, Gilchuk, Case, et al., 2020). For many RBM antibodies, simply blocking ACE2 binding to RBD through direct binding competition is their primary mechanism of viral neutralization. Although binding affinity of RBM antibodies typically correlates with neutralizing potency, in cases of strong, low-nanomolar affinities, the trend becomes less clear (Asarnow et al., 2021; Wu, Wang, Shen, et al., 2020). For example, although BD-368 binds the RBD with a K_D of 1.2 nM and blocks ACE2-RBD interaction more potently than BD-368-2 (K_D 0.82 nM), it neutralizes SARS-CoV-2 much less potently (~ 1600 ng/mL vs. 15 ng/mL IC_{50} , respectively) despite targeting a similar epitope (Cao et al., 2020). Though unsurprising for antibodies whose binding affinities meet or exceed that of hACE2 for RBD ($K_D \sim 15$ nM), a model based only on direct competition with ACE2 fails to fully explain how some antibodies that do not exceed ACE2 binding affinity can neutralize as efficiently as antibodies that do, such as for CC12.1 and CC12.2 (Rogers et al., 2020). Although intra- and inter-spike avidity plays an important role in neutralizing potency (Barnes, Jette, et al., 2020; Huo et al., 2020; Koenig et al., 2021; Yan et al., 2021), alternative mechanisms of neutralization by RBM antibodies likely also contribute.

Cryo-EM studies of the class I RBM antibodies C105 and S2E12, for example, show that these antibodies promote conformational skewing of RBDs to the unstable “up” conformation, which could hypothetically induce S1 shedding, thus producing “inactivate” S2-only spike molecules incapable of ACE2 binding (Barnes, West, et al., 2020; Tortorici et al., 2020); later studies experimentally confirmed this mechanism for multiple ACE2 blocking antibodies (Ge et al., 2021; Hurlburt et al., 2020; Wec et al., 2020), with some, such as S2K146, also promoting fusogenic rearrangement of S2 in addition to S1 dissociation (Park et al., 2022). Indeed, this mechanism has been described for the anti-SARS-CoV RBD antibody S230 (Walls et al., 2019), and provides additional functionality by which RBM antibodies may enhance their neutralizing potency through noncompetitive inhibition of ACE2 binding by spike “deactivation.” As an alternative to S1 shedding, Cryo-EM studies have identified several class 2 RBM antibodies that lock spike RBDs in the “down” conformation, such as C144, S2M11, and the synthetic nanobody Nb6 (Barnes, Jette, et al., 2020; Liu, Wang, et al., 2020; Schoof et al., 2020; Tortorici et al., 2020). These antibodies overlap with other RBM-targeting antibodies but utilize extended CDR3 sequences to form bridging contacts with adjacent RBD molecules in the same spike trimer, and thus form a unique subclass of RBM antibodies that target a quaternary epitope (Rapp et al., 2021) (Fig. 3). By locking the RBDs “down,” hACE2 is unable to bind to the RBM due to steric hindrance, conferring exquisite neutralizing potency to these antibodies (2.35, 1.2, and 2.3 ng/mL IC_{50} , respectively). It is important to note that both S1-shedding class 1 and RBM-locking class 2 antibodies also inhibit interaction with ACE2, prohibiting disentanglement of the precise contributions of each mechanism to their sum neutralizing power.

Certain RBM antibodies can also mediate therapeutic protection from SARS-CoV-2 by engaging Fc receptors on effector immune cells, triggering protective mechanisms such as antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) (Schäfer et al., 2020; Winkler et al., 2021). S2M11 and S2E12 (both hIgG1 isotype) bound to spike on cell surfaces activated signaling through FcγRIIIa and FcγRIIa, respectively, with S2M11 showing significant activation of ADCC and ADCP of SARS-CoV-2 spike-expressing target cells in-vitro (Tortorici et al., 2020). Another RBM antibody, COV2-2050 (Zost, Gilchuk, Case, et al., 2020), displayed reduced protection in a mouse model of SARS-CoV-2 infection by introduction of the LALA-PG (L234A,

L235A, P329G) Fc mutations which eliminate FcγR binding (Lo et al., 2017; Winkler et al., 2021). The importance of these functions for ultra potent neutralizing RBM antibodies is unclear, however, as C144 and other ACE2-blocking antibodies can prophylactically and therapeutically protect mice from SARS-CoV-2 challenge by neutralization (Noy-Porat et al., 2021; Schäfer et al., 2020). In general, it seems that Fc effector functions may be more important for protection by less-potently neutralizing antibodies or with delayed onset of therapy (Chan et al., 2021; Winkler et al., 2021).

The polyclonal antibody response from SARS-CoV-2 survivors and vaccinees typically neutralizes heterologous coronaviruses poorly, if at all, and cross-neutralization by RBM targeting antibodies in particular is uncommon, likely because the RBM motif displays striking sequential and structural diversity among coronaviruses. At the monoclonal level, however, RBM targeting antibodies have been identified or engineered with varying degrees of cross-reactivity to other coronaviruses. S2K146, a class I RBM antibody, neutralizes SARS-CoV-2 and SARS-CoV with IC₅₀ values of 16 and 108 ng/mL, respectively (Y.-J. Park et al., 2022). S2K146 achieves this breadth of neutralization by mimicking key electrostatic interactions between ACE2 and RBD, sharing 18 of its 24 contact residues with ACE2. Due to functional constraints on the RBD for ACE2 binding, 9 contact residues for S2K146 are identical between SARS-CoV and SARS-CoV-2 RBD, with a further 4 conservatively substituted (Y.-J. Park et al., 2022). Indeed, although RBD escape mutations to S2K146 could be generated *in-vitro*, only one (Y489H) retained sufficient ACE2 binding affinity and consistently emerged in SARS-CoV-2 spike expressing VSV chimeric viruses grown under selective pressure from S2K146 (Y.-J. Park et al., 2022). Although SARS-CoV cross-reactive RBM (ACE2-blocking) antibodies exist in polyclonal settings (Walls et al., 2021; Wec et al., 2020), examples of true class 1 or 2 broad sarbecovirus neutralizing mAbs like S2K146 are rare; S2K146 is itself unlike other class I RBM antibodies in that it displays a higher number of somatic hypermutations than is typical for class I RBM mAbs which fine tune its paratope to target conserved residues (Y.-J. Park et al., 2022). More frequently observed pan-sarbecovirus neutralizing, ACE2-blocking mAbs, such as DH1047 (Martinez et al., 2022), S2X35 (Piccoli et al., 2020; Starr, Czudnochowski, et al., 2021), and the engineered ADG-2 (Rappazzo et al., 2021) only partially overlap the RBM while targeting a significant number of contacts breaching into the RBM-adjacent

(class 3, 4 (Barnes, Jette, et al., 2020); antigenic site IIa (Piccoli et al., 2020)) epitope space, which tends to be more highly conserved among coronaviruses (Fig. 3).

Given the mutational plasticity of the RBD, it is unsurprising that SARS-CoV-2 readily escapes class 1 and 2 RBM antibodies via antigenic drift. For class 1 nAbs in particular, the rapid production of high affinity public RBM antibodies with minimal somatic hypermutation would create strong, widespread selective pressure in infected individuals, which may have contributed to the emergence of RBM antibody-resistant variants as early as May 2020 (Deng et al., 2021; McCallum, Bassi, et al., 2021), well before the approval and use of vaccines or therapeutic monoclonal antibodies. The first major variant to evade neutralization by select RBD antibodies was B.1.1.7 via an N501Y mutation located on the periphery of the RBM motif (Fig. 2). N501Y is generally well tolerated by class 1 and class 2 antibodies (Chen, Zhang, et al., 2021; Collier et al., 2021; Shen et al., 2021; Supasa et al., 2021; Wang, Nair, et al., 2021), reflected by reports of relatively modest decreases in serum neutralizing potency from convalescent and vaccinated individuals (Muik et al., 2021; Planas, Bruel, et al., 2021; Wu et al., 2021), where RBM antibodies form the bulk of the neutralizing response (Greaney et al., 2021; Premkumar et al., 2020; Wang, Li, et al., 2020). Notable exceptions include certain IgHV3–53 antibodies, such as mAbs CB6 (Ly-CoV016), B38, and COVOX-269 (Cheng et al., 2021; Shen et al., 2021; Supasa et al., 2021; Wang, Nair, et al., 2021). For COVOX-269, N501Y slightly displaces light chain CDR1, causing collateral movement in the adjacent CDRL3 loop, resulting in disruption of an interaction between CDRL3 residue Y96 and R304/E406 on the RBD (Supasa et al., 2021). Notably, another IgHV3–53 antibody (mAb 222) paired with an alternative light chain that is not predicted to clash with N501Y displayed no loss in potency against B.1.1.7, suggesting that light chain pairing may be an important determinant of neutralizing potency for class 1 IgHV3–53 mAbs against N501Y-containing variants (Supasa et al., 2021).

Variants containing E484K and K417N/T mutations, the first being B.1.351 and P.1, show much greater resistance to RBM antibodies, reflected by larger decreases in neutralization for polyclonal serum samples (Chen, Zhang, et al., 2021; Edara et al., 2021; Planas, Bruel, et al., 2021; Wang, Casner, et al., 2021; Wang, Nair, et al., 2021; Wang, Zhang, et al., 2021; Wibmer et al., 2021; Zhou et al., 2021). Class 1 nAbs are vulnerable to K417 (Greaney et al., 2021; Harvey et al., 2021; Wang, Zhang, et al., 2021); some, such as CB6 (LY-CoV016, etesevimab) and 910–30, see a near

complete loss of potency against variants with K417N mutations while others, such as CV30, only suffer a partial loss (Stamatatos et al., 2021; Wang, Nair, et al., 2021). Interestingly, certain IgHV3–53 antibodies that lost neutralizing potency to B.1.351 and P.1 could be rescued by pairing them with the N501Y-resistant light chain from mAb 222, which also neutralizes B.1.351 and P.1 despite being an IgHV3–53 antibody (Dejnirattisai, Zhou, Supasa, et al., 2021). On the other side the RBM, E484K, which was identified even prior to the spread of B.1.351 and P.1 (Andreano et al., 2021; Greaney, Loes, Crawford, et al., 2021; Liu, VanBlargan, et al., 2021; Weisblum et al., 2020), disrupts neutralization by both class 1 and class 2 RBM antibodies (Barnes, Jette, et al., 2020; Greaney, Starr, Barnes, et al., 2021; Harvey et al., 2021; Planas, Bruel, et al., 2021; Wang, Nair, et al., 2021; Wang, Zhang, et al., 2021). Some of the most potently neutralizing RBM antibodies targeting these epitopes, such as C121, C144, S2M11, and 2B04 completely lost neutralization to viruses containing E484K (Errico et al., 2021; Tortorici et al., 2020; Wang, Nair, et al., 2021; Wang, Schmidt, et al., 2021; Weisblum et al., 2020). REGN-10933 (casirivimab), one of the components of the REGN-COV2 cocktail, targets a class 1 RBM epitope and displays significantly reduced neutralization against E484K-bearing variants (Chen et al., 2021; Dejnirattisai, Zhou, Supasa, et al., 2021; Wang, Casner, et al., 2021), although its use in combination with REGN-10987 (imdevimab), a class 3 epitope antibody, was sufficient to maintain protective efficacy. CT-P59 (regdanvimab), a therapeutic class 2 RBM antibody (Kim et al., 2021), also saw reduced neutralization against B.1.351 and P.1 due in part due to the E484K mutation, but retained protective efficacy in mouse models of SARS-CoV-2 challenge (Ryu et al., 2021; Ryu et al., 2021). As well, Ly-CoV555 (bamlanivimab) suffered complete loss of neutralization by B.1.351 and P.1 (Dejnirattisai, Zhou, Supasa, et al., 2021; Starr, Greaney, Dingens, & Bloom, 2021; Wang, Nair, et al., 2021; Wibmer et al., 2021). For many RBM antibodies, the combination of two or all three K417N, E484K, and N501Y mutants results in greater additive losses in neutralizing potency than any single mutation (Ryu, Kang, et al., 2021).

The Delta variant (B.1.617.2), which emerged separately from B.1.351 and P.1, contains alternative RBM substitutions L452R and T478K instead of K417N/T and E484K, in addition to N501Y. While L452R and T478K localize to the class 2 and 1 RBM epitopes, respectively, they appear less effective than E484K at escaping neutralization by RBM antibodies (Greaney, Starr, Barnes, et al., 2021; Starr, Greaney, et al., 2021).

Indeed, B.1.617.2 showed lesser resistance to neutralization by convalescent and vaccinee serum than B.1.351 or P.1 (Liu, Ginn, et al., 2021; Wang, Li, et al., 2020). Nonetheless, some RBM nAbs, such as P2B-2F6 (class 2) and LY-CoV555 (Bamlanivimab, class 2) lose neutralizing activity against B.1.617.2 as a result of L452R (Li, Wu, et al., 2020; Starr, Greaney, et al., 2021).

Omicron (B.1.1.529), with 8 mutations in the ACE2 binding footprint (K417N, G446S, S477N, Q493R, G496S, Q498R, N501Y, and Y505H), as well as mutations E484A and T478K, shows the most significant escape from neutralizing RBM antibodies and convalescent/vaccinee sera of all variants to date (Hoffmann et al., 2022; Liu, Wang, et al., 2020; Liu, Iketani, et al., 2022; Planas et al., 2022; VanBlargan et al., 2022). Some RBM antibodies, such as ADG-2 and DH1047, that had previously avoided escape mutations in B.1.1.7, B.1.351, P.1, and B.1.617.2 by targeting epitopes on the periphery of the RBM or by making contacts with more conserved RBM-adjacent regions of the RBD (i.e., antigenic site IIa) (Martinez et al., 2022; Piccoli et al., 2020) saw significantly reduced neutralization by B.1.1.529 due to new mutations such as G446S and S371L (Liu, Iketani, et al., 2022). Others, such as COV2-2196 (AZD-8895, tixagevimab) and S2E12, which suffered partial losses of neutralizing potency to B.1.1.529, form a subgroup of class I RBM antibodies targeting the IgHV1-58 “supersite,” that avoid contact with the bulk of the RBM ridge, where many escape mutations occur (Dong et al., 2021; Li, Chen, et al., 2022; VanBlargan et al., 2022; Wang, Zhou, et al., 2021; Zhou, Wang, et al., 2022) (Fig. 3). Besides S2K146, which retains full neutralizing potency against B.1.1.529 by targeting residues in the RBM with strong selection pressure for ACE2 binding (Park et al., 2022), the majority of neutralizing antibodies that retain full potency against B.1.1.529 target epitopes outside the RBM, where sequence conservation is higher.

3.3 Non-RBM RBD epitopes and antibodies

A smaller subset of antibodies target sites outside the RBM. These antibodies separate into several additional classes based on epitope, generally neutralize less potently than RBM antibodies, and display greater cross-reactivity to other coronaviruses and resistance to escape mutations in SARS-CoV-2 variants. Some nAbs in this class can neutralize SARS-CoV-2 by directly blocking ACE2 if their epitope extends far enough into the RBM or by indirectly blocking ACE2 via steric hindrance, while others neutralize independent of

ACE2 blockade. Epitopes in this class either border the RBM directly or do not have any direct connecting residues to the RBM, which we will refer to as broadly as RBM-adjacent and RBM-distal, respectively. As RBM mAb resistant variants have become widespread, increasing attention to these non-RBM epitopes will hopefully identify antibodies which retain potent neutralization against SARS-CoV-2 while leveraging conserved residues to increase neutralizing breadth and resistance to escape.

RBM-adjacent antibodies (Class 3 (Barnes, Jette, et al., 2020); site IV (Piccoli et al., 2020); RBD-5 (Hastie et al., 2021)) include members such as S309 (parent antibody of sotrovimab), REGN10987 (imdevimab), C135, and the murine antibodies SARS2-38 and 2H04 (Fig. 3A). These mAbs can bind both “up” and “down” conformation RBDs at an exposed epitope on the RBD “outer face” consisting of a loop-helix motif (residues 333–346), an RBM-adjacent loop connecting β -strands 4 and 5 (residues 439–450), and for some antibodies, a limited number of residues directly adjacent to the RBM near N501 (Barnes, Jette, et al., 2020; Errico et al., 2021; Pinto et al., 2020) (Fig. 3). SARS2-38 and REGN10987 in particular make multiple contacts near N501 while avoiding significant contact in the RBM-distal 333–346 loop-helix, positioning them upwards on the RBD in a binding orientation that enables steric ACE2 blockade despite little to no overlap with the RBM (Barnes, Jette, et al., 2020; Hansen et al., 2020; VanBlargan et al., 2021). C135 also targets P499, but in contrast to REGN10987 and SARS-38, forms more extensive contacts with residues V341-R346, positioning it further away from the RBM, where it is not predicted to block ACE2 binding (Barnes, Jette, et al., 2020; Robbiani et al., 2020). 2H04 and S309 form the bulk of their contacts with the distal 333–346 loop-helix and 439–450 loop, with S309 forming additional contacts on the β 1 strand encoded by residues K346-C361, and 2H04 making contact with the RBM-proximal P499 residue (Barnes, Jette, et al., 2020; Errico et al., 2021; Pinto et al., 2020).

Neutralizing potency for these antibodies varies, with REGN10987, SARS2-38, and C135 showing potent neutralization ($IC_{50} \sim 6.3, 9,$ and 2.98 ng/mL, respectively) and 2H04 and S309 slightly less so (154 and 79 ng/mL, respectively) (Errico et al., 2021; Hansen et al., 2020; Pinto et al., 2020; Robbiani et al., 2020). The high potency of REGN10987 and SARS2-38 is consistent with their ability to block ACE2. C135, although not predicted to block ACE2, was able to partially block SARS-CoV-2 spike binding to 293 T cells transfected with ACE2, suggesting at least partial blockade of ACE2 binding (Weisblum et al., 2020). In contrast, S309 and 2H04 are

both incapable of direct ACE2 blockade, implying their use of alternative mechanisms of neutralization. Although exactly how these antibodies neutralize is not yet fully understood, several studies have identified possible leads. Cleavage of 2H04 IgG to Fab, which lacks avidity, resulted in a nearly 3-log reduction in neutralizing potency for 2H04 (Errico et al., 2021). In contrast, S309 Fabs retained neutralizing potency but failed to fully neutralize SARS-CoV-2 compared to S309 IgG (Pinto et al., 2020), showing that both 2H04 and S309 require avidity as part of their mechanism of neutralization. Additionally, 2H04 neutralizes SARS-CoV-2 more effectively prior to viral attachment to host cells, and S309, 2H04, and C135 all make contact with the N343 glycan on the RBD. S309 was also shown to neutralize SARS-CoV-2 more potently on target cells over-expressing C-type lectins such as DC-SIGN (and less potently on ACE2-over-expressing cells) (Jackson et al., 2022; Lempp et al., 2021), suggesting that antibodies to these epitopes may neutralize SARS-CoV-2 in part by preventing viral recruitment to host cells via lectin attachment factors.

Alongside neutralization, class 3 antibodies are capable of eliciting Fc-receptor dependent effector functions to stimulate ADCC and ADCP (Hansen et al., 2020; Pinto et al., 2020). S309 in particular shows significant activation of ADCC of spike-expressing CHO cells through binding and activation of the high affinity variant of FcγRIIIa (V158) on primary natural killer cells and ADCP of target cells through activation of FcγRIIa and FcγRIIIa on PBMC-derived monocytes (Pinto et al., 2020), surpassing both REGN10987 and RBM antibodies S2E12 and S2M11 at NK-cell activation (Rappazzo et al., 2021; Tortorici et al., 2020). These effector functions appear particularly important for S309 protection in-vivo, as they mediate continued protection from B.1.1.529 sub variants that escape neutralization by S309, while effector-function deficient forms of S309 fail to do so (Case et al., 2022). Versions of S309 containing an Fc GAALIE mutation (G236A, A330L, I332E) that enhances binding to activating FcγRIIa and FcγRIIIa, reduces binding to inhibitory FcγRIIb, and promotes protective CD8⁺ T cell responses during viral respiratory infections (Bournazos, Corti, Virgin, & Ravetch, 2020) are currently under investigation (VIR-7382) (Cathcart et al., 2021).

In addition to the RBM-adjacent class 3 epitopes, which are exposed for binding in both the “up” and “down” RBD conformations, several epitopes localize to non-RBM cryptic regions of the RBD (the “inner” face) which are only exposed on “up” conformation RBDs (Class 4 (Barnes, West, et al., 2020); antigenic sites IIb, IIc, and V (Piccoli et al., 2020;

Starr, Czudnochowski, et al., 2021); RBD-6, -7 (Hastie et al., 2021)) (Fig. 3A). Antibodies to these epitopes typically are less neutralizing than RBM or RBM-adjacent mAbs and do not directly block ACE2 binding, although a subset are capable of indirect ACE2 blockade via steric hindrance. Similar to class 3 nAbs, they also display broader cross-reactivity to heterologous coronaviruses and greater resistance to escape via antigenic drift.

One of the earliest antibodies to this site to be widely studied was CR3022, which binds to SARS-CoV-2 RBD with high affinity (K_D estimates range from ~ 6.3 to ~ 115 nM (Tian et al., 2020; Yuan et al., 2020)) but does not block ACE2 (Tian et al., 2020). The binding site for this antibody lies on the “inner face” of the RBD, which is only exposed for binding in the RBD “up” conformation, and centers on residues Y369-F392, forming the $\beta 2$ strand and $\beta 2$ - $\beta 3$ loop, D427-T430, which make up the $\alpha 4$ - $\beta 4$ loop, and F515-H519 which lie at the end of the final $\beta 7$ strand just prior to the C-terminus of the RBD (Barnes, West, et al., 2020; Yuan, Wu, et al., 2020) (Fig. 3). CR3022 is thought only to be capable of binding when two RBDs are in the “up” conformation, and even then clashes with an N-terminal domain and S2 (Yuan, Wu, et al., 2020). Although CR3022 is neutralizing for SARS-CoV and synergies with other SARS-CoV neutralizing antibodies (ter Meulen et al., 2006), the majority of studies indicate it lacks neutralization against SARS-CoV-2 (Bates et al., 2021; Rattanapisit et al., 2020; Wrobel et al., 2020; Yi et al., 2020; Yuan, Wu, et al., 2020; Zost, Gilchuk, Case, et al., 2020). Initially, the absence of a CR3022 contact glycan present on SARS-CoV-2 at N370 (present on SARS-CoV structural equivalent N357) was speculated to contribute to the loss of neutralization (Yuan, Wu, et al., 2020); it was later revealed, however, that reversion of a single amino acid change between SARS-CoV-2 and SARS-CoV, P384A, was sufficient to restore neutralizing potency (IC_{50} 3.2 $\mu\text{g}/\text{mL}$) and binding affinity of CR3022 for SARS-CoV-2 (Wu, Yuan, Bangaru, et al., 2020). Mechanistically, CR3022 appears to slightly decrease the affinity of ACE2 for SARS-CoV-2 RBD (Huo et al., 2020), and cause disintegration of the SARS-CoV-2 spike trimer into monomers (Huo, Zhao, et al., 2020; Wrobel et al., 2020). Structural studies of the mAbs EY6A and S304, which strongly overlap with CR3022, reveal that full binding of spike by these mAbs forces the RBDs to rotate up and outwards, contorting the trimer into an unfavorable configuration, thus leading to a similar destruction of spike for EY6A (Zhou, Duyvesteyn, et al., 2020) and dissociation of intact S1 from S2 for S304 (Piccoli et al., 2020; Pinto et al., 2020). EY6A and S304 also decrease the ability of SARS-CoV-2 RBD to bind ACE2, which

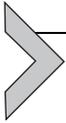
is speculated to result from steric clashes with the N322 glycan on ACE2, as well as potential clashes with the N-terminus of ACE2 on an adjacent RBD by S304 (Mehdipour & Hummer, 2021; Piccoli et al., 2020; Zhao et al., 2020; Zhou, Duyvesteyn, et al., 2020). Although these antibodies show poor neutralizing potency (despite their apparent ability to disassemble spike), S304 can synergistically enhance the neutralizing potency of S309 (Pinto et al., 2020), and CR3022 (as well as S304, to a lesser extent) is capable of eliciting robust Fc γ R-mediated effector functions (Atyeo et al., 2021; Pinto et al., 2020; Shiakolas et al., 2021).

A subset of class 4 antibodies display slightly greater neutralizing potency despite targeting similar epitopes as their modestly neutralizing counterparts. Examples of antibodies in this subset include COVA1-16, S2A4, H014, C022, C118, and the single-domain camelid antibody VHH-72 (Jette et al., 2021; Li, Wu, et al., 2020; Lv, Wu, et al., 2020; Piccoli et al., 2020). COVA1-16 was shown to target an epitope that overlaps with CR3022 but neutralizes SARS-CoV-2 with an IC₅₀ of \sim 745 ng/mL (Brouwer et al., 2020) (Fig. 3). Structural analysis reveals that COVA1-16 is able to indirectly block ACE2 binding to RBD through clashes with its light chain, resulting from its upright binding orientation (Liu, Wu, et al., 2020). S2A4 and H014 display lower neutralizing potency (IC₅₀ 3.5 and 5.7 μ g/mL, respectively), and S2A4 is also capable of inducing S1 shedding, similar to S304 (Lv, Wu, et al., 2020; Piccoli et al., 2020). C118 was also shown to promote shedding of the S1 subunit, while C022 shares a similarly long CDRH3 sequence as COVA1-16, although it showed broader neutralization against heterologous coronaviruses (Jette et al., 2021). Besides ACE2 blockade, these mAbs are distinguished from their non-ACE2 blocking counterparts by recognition of residues closer to the RBM (G404-K417, encoding the α 4-helix and subsequent loop) (Jette et al., 2021; Yuan et al., 2021) (Fig. 3), and for some, a significantly increased reliance on avidity for neutralization (Hastie et al., 2021; Jette et al., 2021; Liu, Wu, et al., 2020; Wrapp et al., 2020) not observed for CR3022 (N. C. Wu, Yuan, Bangaru, et al., 2020). Accordingly, class 4 epitopes are divided into two antigenic subsites made up by antibodies such as CR3022 and S304 that bind lower on the RBD and do not block ACE2 (antigenic site IIc (Piccoli et al., 2020)) and those that bind closer to the RBM and may indirectly compete for ACE2 blockade, such as S2A4 and COVA1-16 (antigenic site IIb) (Piccoli et al., 2020), with finer classifications also proposed (RBD-6, -7a, -7b, and -7c) (Hastie et al., 2021) (Fig. 3).

RBM-adjacent and RBM-distal epitopes show significantly greater sequence conservation than the RBM (Jette et al., 2021), with many

targeting antibodies showing varying degrees of broad neutralization against heterologous sarbecoviruses. Indeed, the prototypical class 3 and class 4 antibodies S309 and CR3022 were originally isolated from SARS-CoV convalescent patients and more potently neutralize SARS-CoV than SARS-CoV-2 (Pinto et al., 2020; ter Meulen et al., 2006). Notable exceptions include S2A4, which is blocked by a SARS-CoV specific glycan at N357 (Piccoli et al., 2020; Tortorici et al., 2021), and some class 3 antibodies which bind closer to the RBM, such as SARS2-38 (VanBlargan et al., 2021). An additional, seemingly infrequent class of antibodies to the RBD, prototyped by the pan-sarbecovirus neutralizing mAb S2H97, display exceptional breadth of binding and neutralization among sarbecoviruses (IC₅₀ 352 ng/mL for SARS-CoV-2). S2H97 targets a novel epitope on the edge of the core RBD motif, in-between the “inner” and “outer” faces of the RBD, designated antigenic site V (Piccoli et al., 2020; Starr, Czudnochowski, et al., 2021) (Fig. 3), and like its class 4 neighbors, only binds RBD in the up conformation and does not block ACE2, instead destabilizing spike, and thus leading to S1 dissociation and premature fusogenic rearrangement of S2 (Starr, Czudnochowski, et al., 2021). Although rare, other antibodies to this epitope with similar properties, albeit less neutralizing, have been described (Li, Xue, et al., 2021). Given lower selective pressure from the relatively weaker neutralizing epitopes encoded by highly conserved core RBD residues, antibodies to these epitopes have also remained relatively effective against SARS-CoV-2 variants (Hastie et al., 2021; Jette et al., 2021; Tortorici et al., 2021). Of particular note, S309 (sotrovimab) had until recently retained neutralizing potency against all major variants (Chen, Zhang, et al., 2021; VanBlargan et al., 2022), succumbing only to the Omicron subvariant BA.2 due to multiple substitutions in its epitope (Case et al., 2022; Iketani et al., 2022) (Fig. 2).

Overall, SARS-CoV-2 RBD antibodies display fluctuating, interlinked properties that broadly separate according to the structural epitope they target (Hastie et al., 2021; Piccoli et al., 2020; Starr, Czudnochowski, et al., 2021). In general, the closer to the RBM an epitope is, the more potently neutralizing an antibody will be, at the cost of decreased cross-reactivity and increased susceptibility to escape by antigenic drift. Antibodies to epitopes further from the RBM follow the inverse trend, characterized by decreasing neutralization and increasing cross-reactivity and resistance to escape mutations in SARS-CoV-2 variants. Going forward, identifying and engineering antibodies that blend the best features of these epitopes, while ensuring staying power against antigenic drift, is likely to yield the most ideal candidates for the next generation of monoclonal antibodies against SARS-CoV-2.



4. Monoclonal antibodies to the N-terminal domain of spike

While the majority of neutralizing SARS-CoV-2 monoclonal antibodies (mAbs) identified to date target the RBD, it is thought that the polyclonal response in infection is directed primarily against epitopes outside of the RBD (Voss et al., 2021), and neutralizing mAbs targeting other domains of spike have been described. Most of these mAbs appear to target the spike N-terminal domain (NTD). In contrast to RBD-targeting mAbs, anti-NTD mAbs tend to demonstrate less potent neutralization, and the means by which these mAbs inhibit the virus are less well-defined. Nevertheless, clear evidence has accumulated regarding critical NTD epitopes as well as some mechanistic features of NTD mAbs. Humoral targeting of the NTD contributes meaningfully to the neutralizing activity of polyclonal sera (Amanat et al., 2021; Andreano et al., 2021; Schmidt et al., 2021), and NTD-specific mAbs synergize with those targeting the RBD, enabling more potent neutralization and impairing viral escape (Dussupt et al., 2021; Haslwanter et al., 2021; Sun et al., 2021; Suryadevara et al., 2021; Zhang, Cao, et al., 2021). Thus, further investigation of anti-NTD mAbs is warranted.

4.1 NTD structure and function

The NTD (also called S^A, residues Q14–S305) of SARS-CoV-2S1 rests on the lateral exterior of the trimeric spike and comprises its three vertices. The NTD consists primarily of a galectin-like (beta-sandwich) core and a ceiling-like structure, shielded by disordered loops sometimes denoted N1 (residues 14–26), N2 (residues 67–79), N3 (residues 141–156), N4 (residues 177–186), and N5 (residues 246–260) (Chi et al., 2020) (Fig. 1D and 4).

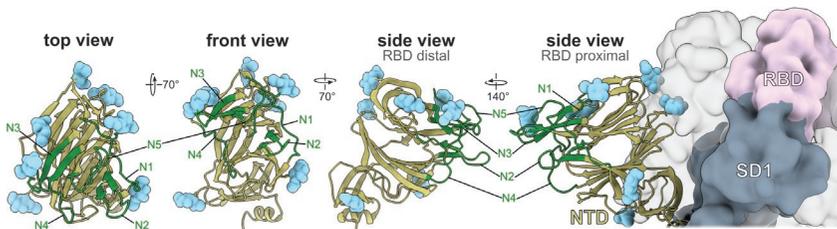


Fig. 4 Structure of the NTD. SARS-CoV-2N-terminal domain (NTD) with loops N1 through N5 shaded green and glycans shown as light blue blobs. The NTD is otherwise colored yellow. On the right, the RBD is shown in pink, with the remainder of the spike colored gray.

While the function of the SARS-CoV-2 NTD has yet to be determined, the susceptibility of the virus to neutralization at this site suggests that the NTD is functionally relevant to viral entry. Other β -coronaviruses are thought to interact with a variety of host factors via their NTDs. The NTDs of bovine coronavirus, HCoV-OC43, and HCoV-HKU1, for example, have retained a functional pocket in the core galectin-like fold, mediating viral attachment to 9-O-acetylated sialic acids on the cell surface (Huang et al., 2015; Hulswit et al., 2019; Künkel & Herrler, 1993; Peng et al., 2012; Schultze, Gross, Brossmer, & Herrler, 1991; Tortorici et al., 2019). The NTD of MERS-CoV likewise interacts with a variety of sialosides (Park et al., 2019). Alternatively, the ceiling of the NTD may serve as the principal binding motif for a proteinaceous receptor, as is the case for mouse hepatitis coronavirus and its receptor CEACAM1 (Peng et al., 2011). Various host proteins have been proposed to interact with the NTD of SARS-CoV-2, including the tyrosine-protein kinase receptor UFO (AXL), kringle containing transmembrane protein 1 (KREMEN1), asialoglycoprotein receptor 1 (ASGR1), and HDL-scavenger receptor B type 1 (Gu et al., 2022; Wang, Qui, et al., 2021; Wei et al., 2020), as have salic acids (Baker et al., 2020). Neither the significance nor specificity of these interactions have been extensively validated, however. It has alternatively been proposed that the NTD modulates entry through inter-domain interactions and allosteric transmission within the spike rather than through direct contact with specific host factors (Qing et al., 2021, 2022). In favor of this theory, it is widely accepted that 69–70del within the NTD somehow enhances S1/S2 cleavage distal to the site of the deletion (Meng et al., 2021). The mechanics and generalizability of this effect, however, are unclear.

As evidenced by the divergent functional properties of various NTDs, this domain displays considerable sequence diversity across related viruses. While the SARS-CoV-2 NTD shares high identity with its nearest neighbors (e.g., 99% identity BatCoV-RaTG13), the NTDs of many otherwise closely related sarbecoviruses share as little as 30–40% identity with that of SARS-CoV-2 (Jaimes et al., 2020; Temmam et al., 2022). Notably, SARS-CoV-2 and SARS-CoV share only 51% identity for the NTD despite sharing 74% identity for the RBD and 76% identity for spike overall (Jaimes et al., 2020). The NTD is highly mutable also among SARS-CoV-2 variants (Fig. 5A). In addition to featuring point mutations elsewhere in the NTD, B.1.1.7 (Alpha), B.1.351 (Beta), and B.1.617.2 (Delta) harbor respective deletions in loops N2, N5, and N3 (McCallum et al., 2021; Wang, Nair, et al., 2021); variant P.1 (Gamma) contains five NTD point mutations, with new glycosylation sites near loops N1 and N4 (Wang, Casner, et al., 2021);

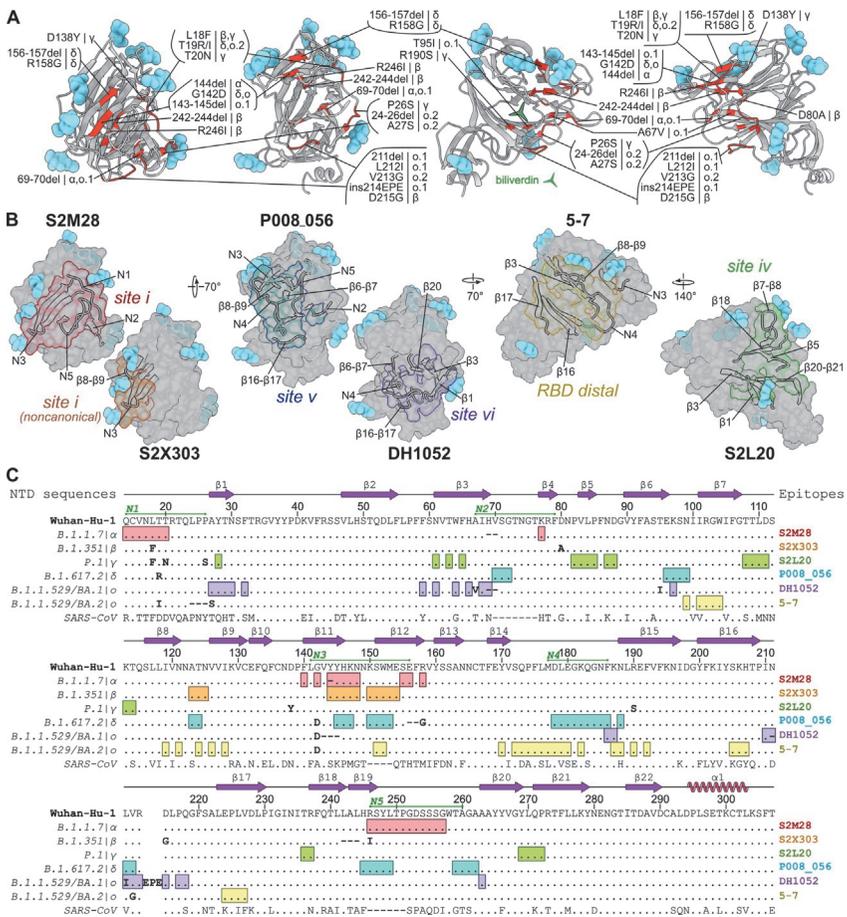


Fig. 5 Epitopes of the NTD. (A) NTD with mutations from SARS-CoV-2 variants shaded red. Labels indicate amino acid changes and variants encoding each change using WHO nomenclature (α , B.1.1.7; β , B.1.351; γ , P.1; δ , B.1.617.2; o.1, B.1.1.529.1/BA.1; o.2, B.1.1.529.2/BA.2; o, Both BA.1 and BA.2). A green tripod designates the tetrapyrrole-binding site. (B) NTD with epitopes from antibodies representing each antigenic site depicted as shaded region on NTD surface. Labels indicate secondary structures or specific loops, designated by name (N1 through N5) or flanking beta-strands, depicted as cartoon ribbons underneath transparent NTD surface. (C) Multiple sequence alignment of SARS-CoV-2 NTD residues 14–307 for wild-type Wuhan-Hu-1 and variant strains as well as SARS-CoV. Dots indicate fully conserved residues relative to Wuhan-Hu-1 reference strain; dashes indicate deletions, and gaps represent insertions. SARS-CoV-2 NTD Secondary structure is diagrammed above Wuhan-Hu-1 sequence determined via DSSP annotation of PDB: 7B62 in ChimeraX (Goddard et al., 2018), and loops N1 through N5 are delineated in green. NTD contacts for representative antibodies targeting distinct antigenic sites are highlighted over NTD sequences and are unrelated to the underlying variant sequence. NTD depictions use PDB: 7B62. Epitope contacts were identified by buried surface area measurement of atomic models (S2M28, PDB: 7LY3; S2X303, PDB: 7SOF; S2L20, PDB: 7N8I; P008_056, PDB: 7NTC; DH1052, PDB: 7LAB; 5–7, PDB: 7RW2) using ChimeraX with a probe radius of 1.4 Å and the default cutoff of 1.0 Å² (Goddard et al., 2018).

variant B.1.427/B.1.429 (Epsilon) contains a novel disulfide bond resulting in loop N3 rearrangement (McCallum, Bassi, et al., 2021); and some recent variants of the B.1.1.529 lineage (including BA.1 (Omicron)) harbor within the NTD four substitutions, deletions in loops N2 and N3, and neighboring indels in another exposed loop (McCallum et al., 2022). It has also been shown *in vitro* that the NTD tolerates large insertions within loop N5 (Andreano et al., 2021). The potential for dramatic remodeling of the NTD surface suggests that its potential function, if any, is either dispensable for the sake of antibody evasion or robust to such changes, perhaps relying primarily on the integrity of the galectin-like core.

4.2 NTD epitopes and antibodies

In June 2020, Chi et al. provided the first characterization of a neutralizing anti-NTD mAb, 4A8. Isolated from a convalescent patient, mAb 4A8 binds spike with high affinity ($K_D \sim 1.0$ nM) and moderately inhibits SARS-CoV-2 infection (EC_{50} of 610 ng/mL). Structural analysis via cryo-electron microscopy revealed that 4A8 targeted NTD loops N3 and N5 (Chi et al., 2020). In subsequent months, additional NTD-directed mAbs were described, frequently within larger panels of mAbs targeting various epitopes of spike. While select, highly potent anti-NTD mAbs have been identified (e.g., S2X333, IC_{50} of 2–6 ng/mL (McCallum et al., 2021)), mAbs targeting the NTD tend to be less potently neutralizing than those targeting the RBD, with typical IC_{50} values ranging from 20 to 700 ng/mL, or are non-neutralizing altogether (Brouwer et al., 2020; Liu, Wang, et al., 2020; McCallum et al., 2021; Suryadevara et al., 2021; Zost, Gilchuk, Chen, et al., 2020).

In April–May of 2021, McCallum et al. and Cerutti et al. independently concluded via extensive structural studies that, like mAb 4A8, the majority of neutralizing NTD-specific mAbs target a single antigenic supersite comprised of NTD loops N1, N3 (the “supersite β -hairpin”), and N5 (the “supersite loop”) (Cerutti, Guo, Zhou, et al., 2021; McCallum et al., 2021). The basis for this restriction is still unclear. Structurally delineated mAbs targeting the supersite include 4A8, COVA1–22, S2L28, S2M28 (Fig. 5B and C), S2X28, S2X333, COV2–2676, COV2–2489, 1–68, 1–87, 2–17, 2–51, 4–8, 4–18, 5–24, FC05, CM25, DH1049, DH1050.1, DH1050.2, DH1051, DH1048, 159, C12C9, C12C11, and WRAIR–2025, among others (Brouwer et al., 2020; Cerutti, Guo, Zhou, et al., 2021; Chi et al., 2020; Dejnirattisai, Zhou, Ginn, et al., 2021; Dussupt et al., 2021; Li, Edwards, et al., 2021; McCallum et al., 2021;

Suryadevara et al., 2021; Tong et al., 2021; Voss et al., 2021; Zhang, Cao, et al., 2021). Supersite-directed mAbs utilize a wide repertoire of heavy chain V segments, suggesting that the exceptional flexibility of the supersite enables stable interaction with a variety of paratopes; nevertheless, over-represented genes have been noted, particularly IgHV1–24 (also IgHV1–69, IgHV3–30, IgHV1–8, IgHV4–39, IgHV3–21, and IgHV3–33) (Cerutti, Guo, Zhou, et al., 2021; McCallum et al., 2021; Voss et al., 2021; Wang, Muecksch, et al., 2022). As previously described for anti-RBD mAbs, many NTD supersite-specific mAbs harbor few somatic hypermutations, suggesting that certain clones display high basal germline affinity for the NTD. Given the overall electropositive potential of the supersite, paratope charge complementarity is thought to predispose electronegative VH genes (e.g., IgHV1–24) for supersite recognition, with key germline residues forming recurrent salt bridges (e.g., IgHV1–24 residue E53 with NTD residue K150) (Cerutti, Guo, Zhou, et al., 2021).

Supersite-directed mAbs canonically contact some combination of loops N1, N3, N5, and sometimes N2, and generally approach the NTD from above, with the antigen-binding site oriented toward the viral envelope (Cerutti, Guo, Zhou, et al., 2021; McCallum et al., 2021). It has been observed also that some clonotypes target the NTD with a preferred orientation. Those utilizing IgHV1–24 (e.g., mAbs 1–68, 1–87, 2–51, and 4A8), for instance, approach the NTD ~ 45 degrees above the horizontal plane, skewing slightly counterclockwise with respect to the C3 symmetrical axis of spike when viewed from above (Cerutti, Guo, Zhou, et al., 2021; Li, Edwards, et al., 2021). Noncanonical supersite binding modes have also been observed, although infrequently. The pose of mAb S2X303, for example, is nearly orthogonal to the typical upward orientation of most supersite antibodies. S2X303 instead protrudes just above the horizontal plane, its epitope shifted toward the RBD distal side of the NTD to form novel contacts with residues 123–125 (McCallum, Walls, et al., 2021) (Fig. 5B and C). The implications of this unusual supersite binding mode for recognition of SARS-CoV-2 variants will be discussed below.

Relatively little is known about antibodies targeting the NTD outside the supersite. In August 2020, Barnes et al. structurally defined the first set of such antibodies, identifying a collection of Fabs (plasma sample COV57) via negative-stain electron microscopy polyclonal epitope mapping that recognized the underside (viral membrane proximal face) of the NTD; the activity of this antibody family was as that time unclear (Barnes et al., 2020). The antigenic anatomy of the NTD was further

outlined in April 2021 by McCallum et al., who identified six competition groups among NTD-specific mAbs and structurally mapped four of these sites. The supersite was designated site i; site iv (defined by mAb S2L20) resides on the RBD proximal side of the NTD and is flanked by glycans on residues N17, N61, and N234; site v (defined by mAb S2X316) comprises the outward face below the supersite; and site vi (defined by mAb S2M24) sits on the underside of the NTD where COV57 Fabs were previously observed (McCallum et al., 2021) (Fig. 5B and C). Sites ii and iii were not structurally defined, but it is probable that one or both sites are found on the RBD distal side of the NTD. Notably, of the 41 anti-NTD mAbs characterized by McCallum et al., only 14 neutralized SARS-CoV-2, all of which recognized the supersite.

Few neutralizing antibodies targeting the NTD outside the supersite have been identified. The reasons for this may be twofold. First, focused screenings suggest that such antibodies are fewer in number (Wang, Muecksch, et al., 2022). Second, the method by which NTD-specific mAbs are typically isolated (screening first for reactivity to trimeric spike, then recombinant NTD (Brouwer et al., 2020; Cerutti, Guo, Zhou, et al., 2021; McCallum et al., 2021)) may lack sufficient sensitivity to detect these less common antibodies. Nevertheless, it has become clear that the supersite is not the sole point of vulnerability on the NTD. For instance, mAb P008_056 (IC_{50} of 30 ng/mL) targets an epitope near site v, wedging between loops N3 and N4 just below the supersite (Rosa et al., 2021) (Fig. 5B and C). Neutralizing mAbs C1520 and 5–7 (respective IC_{50} values of 4 and 33 ng/mL) alternatively target the RBD distal side of the NTD, inserting extended HCDR3 loops into the hydrophobic cavity gated by loop N4 (Cerutti, Guo, Wang, et al., 2021; Liu, Wang, et al., 2020; Wang, Muecksch, et al., 2022) (Fig. 5B and C); the less potent mAb PVI.V6–14 ($IC_{50} > 1 \mu\text{g/mL}$) binds similarly (Altomare et al., 2022; Amanat et al., 2021). A mildly neutralizing antibody targeting the RBD proximal side (site iv) also has been identified, namely C1791 (IC_{50} of 824 ng/mL), which sits between the N61 and N234 linked glycans, similar to non-neutralizing mAb S2L20 (McCallum et al., 2021; Wang, Muecksch, et al., 2022) (Fig. 5B and C). What delineates neutralizing versus non-neutralizing binding at these sites has yet to be determined. A novel epitope proximal to the NTD/RBD interface (the medial surface of the NTD) also has recently been identified. The mildly neutralizing mAb COV2–3434 (IC_{50} of 5.5 $\mu\text{g/mL}$) contacts the NTD near residues 43, 175–176, and 226, sterically clashing with the RBDs of neighboring

protomers in a manner that causes separation of the spike trimer. Serum competition experiments suggest that antibodies targeting this site are common in some vaccinated individuals (Suryadevara et al., 2022).

Interestingly, some antibodies targeting the underside of the NTD (site vi) are thought to enhance infection *in vitro* in an FcγR-independent manner (Li, Edwards, et al., 2021; Liu, Soh, et al., 2021). Antibodies with this functional property include DH1052, DH1053, DH1054, DH1055, DH1056, COV2-2490, COV2-2210, COV2-2582, COV2-2369, COV2-2660, and 8D2 (Li, Edwards, et al., 2021; Liu, Soh, et al., 2021). These mAbs generally contact a narrow region near residues 27–32, 59–68, 185–187, and 211–218, with some (e.g., DH1052) minimally contacting SD2 within the hinge region of S1 (Li, Edwards, et al., 2021) (Fig. 5B and C). Enhancement at this site is mediated only by bivalent Ig or F(ab')₂ and involves augmentation of ACE2 engagement. Thus, it is speculated that the bridging of neighboring spikes by these mAbs may somehow stabilize the RBD in an up position (Liu, Soh, et al., 2021). The relevance of this effect *in vivo* is still unclear. High “enhancing” titers appear to correlate with COVID-19 severity in humans (Liu, Soh, et al., 2021), but “enhancing” mAbs (e.g., DH1052) have been reported to confer protection in animal models (Li, Edwards, et al., 2021). In addition, some antibodies *in vitro* have been found instead to neutralize infection at this site, such as mAb C1717 (IC₅₀ of 287 ng/mL). C1717 spans NTD site vi and the S^D domain, flanked by N282- and N603-linked glycans, binding near the S2 fusion peptide. Occluding the S2' cleavage site may account for its inhibitory activity, but this has not been experimentally demonstrated (Wang, Muecksch, et al., 2022).

While the mechanism of SARS-CoV-2 neutralization by NTD-specific mAbs has not yet been clearly defined, recurrent functional properties have been observed for those targeting the supersite. Neither affinity nor exact positioning of epitopes on the NTD supersite correlates with neutralization potency, but interestingly, the magnitude of induced NTD conformational change required for binding is inversely correlated with potency (Cerutti, Guo, Zhou, et al., 2021). Monovalent Fabs typically lose some or all neutralizing activity (McCallum et al., 2021; Suryadevara et al., 2021). While this could suggest that some anti-NTD antibodies act in part through steric hindrance of cellular attachment, the inhibitory activity of most anti-NTD mAbs is not attributed to competition with ACE2, steric or otherwise (Chi et al., 2020; Dejnirattisai, Zhou, Ginn, et al., 2021; Hastie et al., 2021; Li, Edwards, et al., 2021; Liu, Zhou, et al., 2022; Suryadevara et al., 2021;

Zost, Gilchuk, Case, et al., 2020). Moreover, anti-NTD mAbs generally do not prevent attachment to host cells but rather block viral entry post-attachment (McCallum et al., 2021; Suryadevara et al., 2021). Thus, the principal mechanism of these mAbs is neither steric occlusion of the RBD nor abrogation of NTD binding to alleged host factors (Gu et al., 2022; Wang, Qui, et al., 2021; Wei et al., 2020). It has further been demonstrated that NTD-directed mAbs can prevent spike-mediated cell-cell fusion (McCallum et al., 2021). It may be then that some NTD-specific antibodies stabilize the SARS-CoV-2 spike, pre- or post-attachment, in a fusion-incompetent conformation, consistent with previous descriptions of mAbs targeting the MERS-CoV NTD (Zhou et al., 2019). Although this proposed mechanism currently lacks experimental support in SARS-CoV-2, one NTD antibody (mAb 4-18) has been shown to induce conformational changes within S2 (Cerutti, Guo, Zhou, et al., 2021), and the NTD antibodies 4A8 and TRES328 have recently been demonstrated to impair S2' cleavage in a cell-free system (Qing et al., 2022).

A yet unexplained functional property of many NTD-directed mAbs is the inability to completely inhibit SARS-CoV-2 in conventional neutralization assays. Often a resistant viral fraction persists at high mAb concentrations, with total inhibition plateauing at 40–90% (Hastie et al., 2021; Liu, Wang, et al., 2020; McCallum et al., 2021; Tanaka et al., 2022). The proportion of resistant virus is protocol-, cell-type-, and VOC-dependent (Hastie et al., 2021; McCallum et al., 2021; Tanaka et al., 2022). It may be that host and viral factors modulate the stoichiometric requirements for neutralization by NTD-directed mAbs such that some virions are effectively resistant despite maximal epitope occupancy. Alternatively, virions may stochastically acquire resistance through an unknown mechanism influenced by host or viral factors. The ability of NTD-directed mAbs to recognize persistent virus retrieved after neutralization has not been investigated, nor has the relevance of this fraction to protection *in vivo*.

In addition to neutralization, NTD antibodies may combat SARS-CoV-2 via Fc-effector functions. It has been demonstrated *in vitro* that antibodies targeting the NTD bind infected cells with marked avidity, achieving higher density than RBD-directed mAbs (Suryadevara et al., 2021; Wec et al., 2020), and are particularly adept at mediating complement deposition and cell opsonization (Dussupt et al., 2021). The relevance of these findings also has been corroborated *in vivo*. Although potently neutralizing NTD-specific mAbs may confer Fc-independent protection (Noy-Porat et al., 2021), most NTD-specific mAbs require intact Fc-effector functions

for optimal performance in animal models (Dussupt et al., 2021; Suryadevara et al., 2021). Non-neutralizing NTD mAbs may also synergize with neutralizing mAbs in combination therapy (Beaudoin-Bussi eres et al., 2022), highlighting the potential clinical utility of Fc-engineered mAbs targeting more conserved, non-neutralizing NTD epitopes.

Due to high variability of the NTD between sarbecoviruses (described above in “NTD structure and function”), the humoral response against the NTD is poorly cross-reactive. Screening of COVID-19 convalescent sera has found that only 4.9% of SARS-CoV-2 NTD-reactive samples also cross-react with the SARS-CoV NTD, versus 77.5% for RBD cross-reactivity (Lv et al., 2021). Serum depletion experiments likewise suggest that NTD cross-reactivity between SARS-CoV-2 and other human coronaviruses, including OC43, HKU-1, NL63, and 229E, is minimal (Dowell et al., 2022). While most supersite-specific mAbs unsurprisingly recognize RaTG13 (which shares 99% NTD identity with SARS-CoV-2) and may weakly bind very closely related pangolin CoVs, they largely do not cross-react with more distant sarbecoviruses which lack the supersite loops, including SARS-CoV (McCallum et al., 2021). Anti-NTD mAbs that do recognize both SARS-CoV-2 and SARS-CoV (e.g., mAbs FC07 and 46472–6) are uncommon and likely target more conserved epitopes outside the supersite (Shiakolas et al., 2021; Zhang, Cao, et al., 2021; Zost, Gilchuk, Chen, et al., 2020).

The restriction of potent neutralization to a single site on the SARS-CoV-2 NTD is reflected unsurprisingly in the high mutability of loops N1, N3, and N5, with all major variants featuring altered NTD supersites. Although some recurrent mutations have independently emerged, mutations within the NTD are far less consistent than those in the RBD, perhaps because a relative lack of functional constraints permits greater plasticity for the NTD. In addition to relieving immune pressure, it is thought that some NTD mutations confer intrinsic advantages for viral fitness. The first designated VOC, B.1.1.7 (Alpha), harbors deletions of residues H69–V70 and Y144 in loops N2 and N3, respectively (Fig. 5A and C). The deletion of 69–70, though physically proximal to the supersite, minimally effects antibody binding and is instead thought to enhance infectivity, potentially offsetting the fitness cost of immune escape mutations (McCallum et al., 2021; Meng et al., 2021; Tong et al., 2021; Wang, Nair, et al., 2021). (This is also reflected by the independent recurrence of 69–70del in later variants, including B.1.1.529/BA.1 (Omicron).) Variant B.1.1.7 is refractory to neutralization by the majority of NTD supersite-targeting mAbs, however, owing primarily to the distortion of the supersite beta-hairpin N3 by

Y144del (equivalent to Y145del) (Cai et al., 2021; Tong et al., 2021; Voss et al., 2021; Wang, Nair, et al., 2021). Nevertheless, neutralizing antibodies targeting other sites of the NTD, such as site v (e.g., mAb N-612-014) or the RBD distal side of the NTD (e.g., mAbs 5–7 and C1520) may retain partial activity against B.1.1.7 or Y144del single mutants (Cerutti, Guo, Wang, et al., 2021; Hastie et al., 2021; Tanaka et al., 2022; Wang, Nair, et al., 2021; Wang, Muecksch, et al., 2022). Select supersite-directed mAbs (e.g., C12C9) also can neutralize B.1.1.7 (Tong et al., 2021), illustrating the utility of targeting this site redundantly in a polyclonal response.

Variant B.1.351 (Beta) emerged independently of B.1.1.7 and features more dramatic remodeling of the NTD supersite (Fig. 5A and C). A characteristic deletion of nonpolar residues L242-A243-L244 results in the retraction of N5 and the inward dislocation of polar residues H245-R246-S247, with a compensatory shift in neighboring loop N3 (Cai et al., 2021). This deletion alone confers resistance to nearly all supersite antibodies (Cai et al., 2021; Cao et al., 2021; Wang, Nair, et al., 2021). Antibody recognition of the supersite is also impacted by substitutions L18F and D80A, which reconfigure loop N1, and, in a rare subset of B.1.351 isolates, R246I within loop N5 (Cai et al., 2021; Wang, Nair, et al., 2021). A final substitution, D215G, minimally contributes to antibody escape (Wang, Nair, et al., 2021). B.1.351 consequently escapes most neutralizing, NTD-specific mAbs (Suryadevara et al., 2021; Tong et al., 2021; Voss et al., 2021). Like B.1.1.7, however, B.1.351 remains susceptible to neutralization outside the supersite, with mAbs 5–7 and C1520 retaining partial activity against B.1.351 (Cerutti, Guo, Wang, et al., 2021; Wang, Lorenzi, et al., 2021). Non-canonical supersite mAb S2X303 likewise recognizes B.1.351 spike, as its unusual pose entirely avoids contact with loop N5, minimizing the impact of 242-244del (McCallum, Walls, et al., 2021) (Fig. 5B and C). Nevertheless, the severe antigenic remodeling of the NTD, in combination with key RBD mutations, renders B.1.351 broadly resistant to vaccinated and convalescent sera (Hoffmann et al., 2021; Planas, Bruel, et al., 2021; Wang, Nair, et al., 2021; Zhou et al., 2021). Despite this advantage, the dominance of B.1.351 was short-lived, likely due to intrinsic disadvantages in infectivity and replication (Ulrich et al., 2022). Since that time, a B.1.351 NTD-specific mAb, Beta-43 (IC₅₀ of 48 ng/mL), has been isolated from a B.1.351 convalesced donor; it binds the supersite and is completely specific for B.1.351 (Liu, Zhou, et al., 2022).

In contrast to those of B.1.1.7 and B.1.351, the NTD of variant P.1 (Gamma) features no deletions or conspicuous conformational changes

within the supersite but instead contains five substitutions: L18F (previously seen in B.1.351), T20N, P26S, D138Y, and R190S (Fig. 5A and C). While the impacts of these changes have not been thoroughly characterized, it is predicted that T20N and R190S generate new glycosylation sites near the supersite and the RBD distal side of the NTD, respectively, conferring resistance to certain classes of antibodies. As a result, P.1 enjoys partial escape from supersite-directed mAbs. A study of five such mAbs found that three (4–18, 2–17, or 4–19) lost activity against P.1 due to various combinations of L18F, T20N, D138Y, and R190S, whereas two (5–24 and 4–8) retained potent activity against P.1 (Wang, Casner, et al., 2021). Unlike B.1.1.7 and B.1.351, however, P.1 escapes mAb 5–7 (Wang, Casner, et al., 2021). This antibody recognizes the RBD distal side of the NTD (Cerutti, Guo, Wang, et al., 2021) and thus may be impacted by R190S, but this substitution alone does not confer resistance (Wang, Casner, et al., 2021).

Coincident with B.1.1.7, B.1.351, and P.1, variant B.1.427/B.1.429 (Epsilon) emerged in California in July 2020 and flourished regionally in the following months, eventually remitting without global spread. Though briefly ascribed VOC status in the spring of 2021, B.1.427/B.1.429 lost this designation in July 2021 (Carroll et al., 2022; Deng et al., 2021). This variant presents an interesting case study of NTD remodeling, because its two substitutions (S13I within the signal peptide, and W152C within supersite beta-hairpin N3) have profound effects on antibody recognition. In a study of ten previously identified supersite mAbs, including 4A8, S2L26, S2L50, S2M28, S2X28, S2X158, S2X107, S2X333, S2X124, and non-canonical supersite mAb S2X303, these two deceptively simple point mutations were shown to mediate near complete escape from all antibodies tested (McCallum, Bassi, et al., 2021). Mass spectrometry analysis revealed that S13I shifts the signal peptide cleavage site from S13-Q14 to C15-V16, eliminating the disulfide bond typically found between C15 and C136; C136 is then free to form a novel disulfide with W152C (McCallum, Bassi, et al., 2021). This unusual pinning of loop N3 likely accounts for the broad resistance of B.1.427/B.1.429 to anti-NTD mAbs.

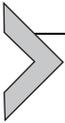
Variant B.1.617.2 (Delta) emerged separately from these previous VOC and harbors unique substitutions T19R, G142D, and R158G, as well as a deletion of E156–F157 (Fig. 5A and C). Mutation G142D, also shared by the related variant B.1.617.1 (Kappa), had previously been shown to abrogate the binding of supersite-directed mAbs S2L28, S2X28, and S2X333,

but not S2M28 or 4A8 (McCallum et al., 2021). It was later demonstrated that mutation of residues 156–158 in supersite beta-hairpin N3 resulted in helical remodeling of N3, enabling B.1.617.2 to evade 10 of 11 neutralizing antibodies tested (McCallum, Walls, et al., 2021). Only the non-canonical supersite mAb S2X303 retained the ability to bind B.1.617.2 spike on ELISA, but it could no longer neutralize the virus, perhaps reflecting impairments in binding affinity or kinetics not revealed by ELISA (McCallum, Walls, et al., 2021). Other studies likewise have found that B.1.617.2 escapes most but not all neutralizing anti-NTD mAbs, though the epitopes targeted in these studies are unclear (Changrob et al., 2021; Planas et al., 2021). Like previous variants, B.1.617.2 remains susceptible to some neutralizing mAbs targeting more conserved epitopes outside of the supersite, including C1520, which targets the RBD distal side of the NTD (Wang, Muecksch, et al., 2022).

The most recent variants of the B.1.1.529 lineage (e.g., BA.1 (Omicron) and BA.2) feature familiar mutations as well as more novel changes in and outside of the NTD supersite. For example, BA.1 harbors 69–70del and G142D, which are seen also in other variants, as well as novel mutations A67V, T95I, 143–145del, 211del, L212I, and ins214EPE (McCallum et al., 2022) (Fig. 5A and C). As previously described, G142D is known to confer resistance to several potently neutralizing mAbs (e.g. S2X333) (McCallum, De Marco, et al., 2021). The deletion of residues 143–145 is reminiscent of Y144del, which previously rendered B.1.1.7 broadly resistant to supersite-directed mAbs (Cai et al., 2021; Tong et al., 2021; Voss et al., 2021; Wang, Nair, et al., 2021). Indeed, BA.1 has demonstrated staunch resistance against several supersite-directed mAbs, including S2L50, S2X28, S2X333, and 4–18 (Cameroni et al., 2022; Liu, Iketani, et al., 2022). Broadly-neutralizing antibodies targeting more conserved epitopes, including S2X303 and 5–7, also have lost neutralization potency against BA.1 (Cameroni et al., 2022; L. Liu, Iketani, et al., 2022). The cluster of mutations around residues 211–214 is outside the supersite but could feasibly impair recognition of mAbs targeting site v or vi, such as P008_056 or C1717 (Rosa et al., 2021; Wang, Paulson, et al., 2022; Wang, Muecksch, et al., 2022), though this has not been demonstrated experimentally. The BA.2 sublineage diverges considerably from BA.1 and, in addition to G142D, is characterized by novel mutations T19I, 24–26del, A27S, and V213G. While antibody evasion of BA.2 remains to be thoroughly evaluated, it has been shown that 25–27del renders BA.2 resistant to mAbs 4–18 and 5–7, which respectively target the supersite and the RBD distal side of

the NTD (Iketani et al., 2022). Altogether, BA.1 and BA.2 appear to escape the majority of neutralizing, NTD-directed mAbs, highlighting the need for continued mAb discovery and development.

In addition to effects of immune escape mutations, it has been shown that the antigenic properties of the SARS-CoV-2 NTD may be modulated through the recruitment of host metabolites. Namely, heme products such as biliverdin and bilirubin may impair the activity of certain mAbs via occupation of the hydrophobic pocket on the RBD distal side of the NTD (Fig. 5A). This cleft is typically gated by loop N4, and antibodies such as P008_056 dislocate N4 into the cleft upon binding; accordingly, biliverdin confers resistance against P008_056 (Rosa et al., 2021). Antibodies that extend HCDR3 loops into this cavity are variably affected by biliverdin. While biliverdin completely abrogates the activity of PVI.V6–14, mAb 5–7 is only marginally impacted (Altomare et al., 2022; Cerutti, Guo, Wang, et al., 2021). Surprisingly, occupation of this site by biliverdin also attenuates the neutralization of some supersite-directed mAbs (e.g., COVA1–22, 5–24, 4–8) likely through an undefined allosteric mechanism (Cerutti, Guo, Wang, et al., 2021; Rosa et al., 2021). The relevance of biliverdin binding *in vivo* is unclear, but neutrophil-derived, heme-containing proteins are enriched in nasopharyngeal swabs of COVID-19 patients (Akgun et al., 2020). Thus, it has been speculated that parts of the NTD may be antigenically remodeled by heme byproducts within the inflammatory milieu.



5. Monoclonal antibodies targeting S2

5.1 S2 structure and function

S2 is composed of residues spanning S686 to the C-terminus of Spike, starting immediately downstream of a furin cleavage site (S1/S2) that distinguishes SARS-CoV-2 from SARS-CoV, and which is proteolytically processed during virion biogenesis, severing covalent coupling between S1 and S2 (Hoffmann, Kleine-Weber, & Pöhlmann, 2020; Hoffmann, Kleine-Weber, Schroeder, et al., 2020; Walls et al., 2020) (Fig. 1A and 6A). Further downstream, an additional dibasic cleavage site (S2', K814-R815) is cleaved by the host cell protease TMPRSS2 (or cathepsin L if the virion is internalized) (Hoffmann, Kleine-Weber, Schroeder, et al., 2020). Immediately downstream of S2' is the hydrophobic fusion peptide (FP) composed of two sequences (Lai & Freed, 2021), FP1 (~S816 to ~I835) which is inserted into host cell membranes during fusogenic rearrangement, and the fusion-peptide

proximal region (FPPR or “switch region,” \sim K836 to \sim F855) that plays a role in stabilizing RBD “down” conformations (Xiong et al., 2020; Zhang et al., 2021; Zhou, Tsybovsky, et al., 2020), and as such “senses” conformational switching of S1 RBD to the “up” position. This is speculated to result in S2' site exposure for cleavage by TMPRSS2 after ACE2 binding and promote S1 dissociation from S2, thus clearing the way for fusogenic rearrangements (Benton et al., 2020; Cai et al., 2020; Gobeil et al., 2021; C. B. Jackson et al., 2022).

Following the FPPR is the machinery responsible for fusogenic rearrangement, consisting of a helical sequence containing heptad repeats (HR1, G910 to D985) organized around a coiled-coil central helix (CH K986 to G1035), followed by a beta-hairpin motif (S2- β , G1046-A1076) (Henderson et al., 2020) and connector domain (CD, T1077 to T1137) (Wrapp, Wang, et al., 2020) which link HR1 and CH to a second set of coiled heptad repeats (HR2, P1162-L1211) below the bulk of the spike, before it anchors into the viral envelope via a transmembrane sequence (TM, W1212-L1234) (Cai et al., 2020). Upon S1 dissociation, fusogenic rearrangement of the metastable S2, driven by free energy loss, begin with a dramatic upward extension of HR1 and FP to form an elongated

Fig. 6 Epitopes on S2. (A) Pre- (left) and post-fusion (right) structures of SARS-CoV-2 spike S2 colored by domain. Approximate orientations of viral and host membranes at each stage of fusion depicted by curved dashed lines. (B) Ribbon diagram showing the fusion peptide epitope (residues 809–833). Purple side chains indicate important contact residues for COV44–62 and COV44–79. (C) Ribbon diagram showing the stem-helix epitope. Contact residues for CV3–25, S2P6, CC40.8, and B6 are colored blue and labeled. Exposed indicates side of stem-helix that is solvent accessible on the prefusion conformation; cryptic label indicates solvent inaccessible side of stem-helix in the prefusion conformation. Red star indicates H1159 clash predicted to prevent B6 neutralization of sarbecoviruses. (D) Multiple sequence alignment of the fusion peptide (left) and stem-helix (right) epitope for β -coronaviruses SARS-CoV-2, SARS-CoV, MERS-CoV, hCoV-OC43, hCoV-HKU1, and bCoV-HKU4; α -coronaviruses NL63 and 229E; and δ -coronavirus porcine deltacoronavirus (PDCoV). Dots indicate conserved residues relative to SARS-CoV-2 Wuhan-Hu-1 reference strain (top). Horizontal highlights mark contact residues for antibodies listed below alignment, either previously reported (COV44–62 & COV44–79 (Dacon et al., 2022)) or identified by buried surface area analysis in UCSF ChimeraX (S2P6, PDB: 7RNJ; B6, PDB: 7M55; CC40.8, PDB: 7SJS; CV3–25, PDB: 7NAB) with a probe radius of 1.4 Å and default cutoff of 1.0 Å² (Goddard et al., 2018). Horizontal highlights are not related to the originating viral sequence they cover. Vertical green outlines indicate totally conserved residues between all listed coronaviruses (FP) or only β -coronaviruses (SH). Stars above alignment indicate contact residues important for binding by each antibody if data is available. Branched icon above N1158 indicates N-linked glycan site on SARS-CoV-2.

three-helix coiled coil that forces insertion of the fusion peptide at its tip into a juxtaposed membrane (Tortorici & Veesler, 2019; Xia et al., 2020). S2 then folds back on itself, enabling hydrophobic residues in HR2, which sit in close proximity to the viral membrane, to tightly bind HR1 located near the host cell membrane, forming a highly stable six-helix bundle, and thus forcing the viral envelope and host cell membranes into contact, initiating membrane fusion (Cai et al., 2020; Harrison, 2008; Koppiseti, Fulcher, & Van Doren, 2021) (Fig. 6A).

5.2 S2 epitopes and antibodies

The S2 region of spike is highly conserved among β -coronaviruses, making it a possible target for broadly neutralizing antibodies (Ng et al., 2020; Shah, Canziani, Carter, & Chaiken, 2021). SARS-CoV-2 S2, like S2 in other coronaviruses, is covered in N-linked glycans, shielding much of S2 from binding by host antibodies (Grant et al., 2020; Walls, Tortorici, Frenz, et al., 2016; Watanabe et al., 2020). Despite this, antibodies to S2 can be observed at the polyclonal level in both convalescent patients and vaccinees (Amanat et al., 2021; Brewer et al., 2022; Lv, Deng, et al., 2020), and several groups have isolated and characterized S2-reactive monoclonal antibodies. Two regions of SARS-CoV-2 S2, FP and the region immediately upstream of HR2, termed stem-helix (SH), appear to account for nearly all SARS-CoV-2 S2-specific antibodies from natural infection or vaccination (Heffron et al., 2021; Hsieh et al., 2021; Ladner et al., 2021; Li, Lai, et al., 2020; Li, Ma, et al., 2021; Poh et al., 2020), although antibodies to HR1 and CD have been described for SARS-CoV and MERS-CoV, respectively (Elshabrawy, Coughlin, Baker, & Prabhakar, 2012; Pallesen et al., 2017).

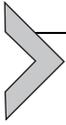
The majority of currently known neutralizing monoclonal antibodies to S2 target SH (Pinto et al., 2021; Sauer et al., 2021) (Fig. 6C). The mAb B6 was identified using an S2-specific selection strategy in CD-1 mice immunized twice with MERS-CoV spike ectodomain followed by SARS-CoV ectodomain. B6 recognizes MERS S2, hCoV OC43 S, and SARS-CoV/SARS-CoV-2S in order of decreasing avidity, and displayed no neutralizing potency for sarbecoviruses (Sauer et al., 2021). S2P6, identified in convalescent SARS-CoV-2 patients, targets SH and neutralizes SARS-CoV-2 virus (IC_{50} 1.67 μ g/mL) as well as SARS-CoV, hCoV OC43, MERS-CoV, and Pangolin Guandong 2019 CoV (PANG/GD) VSV pseudotypes (Pinto et al., 2021). Structural investigations revealed that S2P6 targets residues

D1146 through H1159 of SH by intercalation of hydrophobic residues F1148, L1152, Y1155, and F1156 into a groove in between S2P6 heavy and light chains, with specificity mediated through hydrogen bonding to the peptide backbone (Pinto et al., 2021) (Fig. 6C and D). Additional antibodies of this class include CC40.8 and 28D9, which both bind the same region of stem helix, and neutralize sarbecoviruses with low potency (IC_{50} 12.6 $\mu\text{g}/\text{mL}$ for CC40.8, 45.3 $\mu\text{g}/\text{mL}$ against pseudovirus for 28D9), although both (28D9 to a lesser extent) were capable of protecting mice from SARS-CoV-2 challenge (Song et al., 2021; Wang, van Haperen, et al., 2021; Zhou, Yuan, et al., 2022). CV3–25, which was mapped to residues in SH, neutralizes SARS-CoV-2 with an unusually potent IC_{50} of 340 ng/mL, and was also shown to protect mice from SARS-CoV-2 lethality (Jennewein et al., 2021; Li, Chen, et al., 2022). Unlike B6 and S2P6, CV3–25 targets residues further down the helical N-terminus of SH and into a C-terminal loop beyond, and binds at a different orientation on the helix, targeting charged, surface-exposed residues rather than the cryptic hydrophobic core (Hurlburt et al., 2022; Li, Chen, et al., 2022) (Fig. 6C and D). Recently, a series of fusion-peptide reactive monoclonal antibodies were identified, two of which, COV44–62 and COV44–79, showed broad coronavirus neutralization (Dacon et al., 2022). Despite low neutralizing potency ($NT_{50} \sim 21 \mu\text{g}/\text{mL}$ and 27.5 $\mu\text{g}/\text{mL}$ against SARS-CoV-2 pseudotyped lentivirus, respectively), both displayed variable prophylactic protection from SARS-CoV-2 infection in a Syrian hamster model (Dacon et al., 2022). Crystallographic analysis revealed both mAbs targeted the fusion peptide on S2, including R815 of the S1/S2 cleavage motif, and approach the fusion peptide from different binding orientations (Fig. 6B and D). Nonetheless, mutagenesis revealed that both antibodies rely on the same set of core residues (E819, D820, L822, and F823) for binding (Fig. 6B and D), and binding affinity was higher against S2 compared to full-length S (Dacon et al., 2022).

SH and FP-specific mAbs appear to neutralize by inhibiting the membrane fusion process (Dacon et al., 2022; Li, Chen, et al., 2022; Pinto et al., 2021; Sauer et al., 2021; Zhou, Yuan, et al., 2022). B6, CC40.8, and S2P6 all target the hydrophobic side of the SH helix which is cryptic in both the pre- and post-fusion states, suggesting these antibodies may be able to neutralize S by disrupting the quaternary helical assembly of SH in the pre-fusion state in order to attain binding (Pinto et al., 2021; Wang, van Haperen, et al., 2021; Zhou, Yuan, et al., 2022) (Fig. 6C). In contrast, CV3–25 targets non-cryptic residues on the exposed side of the SH helix (Fig. 6C), which may facilitate its greater neutralizing potency

(Li, Chen, et al., 2022). In any case, after binding to SH, it is thought these mAbs then sterically “jam” S2 from folding back on itself via interactions between HR1 and SH/HR2, preventing 6-HB formation, and thus membrane fusion (Hurlburt et al., 2022; Li, Chao, et al., 2022; Li, Chen, et al., 2022; Pinto et al., 2021; Zhou, Yuan, et al., 2022). Indeed, S2P6 potently inhibited cell-cell syncytium formation in SARS-CoV-2 spike-expressing Vero-E6 cells exposed to S2E12, which triggers fusogenic rearrangements by mimicking ACE2 binding (Pinto et al., 2021). In addition to neutralization, S2P6 and CV-325 showed robust in-vitro activation of ADCC and ADCP via FcγR activation. Further, S2P6, CV-325, and COV44-79 all show enhanced in-vivo protection mediated by effector functions (Dacon et al., 2022; Pinto et al., 2021; Ullah et al., 2021), suggesting alternative modes of antibody-mediated immunity may be especially important for S2 mAbs given their generally poor neutralizing potency.

In keeping with the strong conservation of SH and FP between coronaviruses, S2 mAbs display exceptionally broad cross-reactivity (Fig. 6D). SH-specific mAbs typically show cross-reactivity between β -coronaviruses (Fig. 6D); S2P6 showed the broadest cross-reactivity, able to bind hCoV-HKU1 spike and cross-neutralize representative viruses from all *sarbecovirus* clades, including SARS-CoV and SARS-CoV-2, as well as MERS-CoV (*merbecovirus*), and hCoV-OC43 (*embecovirus*) (Pinto et al., 2021). CV3-25 and CC40.8 were both able to neutralize SARS-CoV and SARS-CoV-2 and bind to hCoV-OC43 and hCoV-HKU1 spike, but displayed no cross-reactivity to MERS (Song et al., 2021). B6, originally raised against MERS spike, was able to bind and neutralize both MERS and the embecoviruses OC43 and HKU4, but was unable to neutralize SARS-CoV and SARS-CoV-2, likely due to clashes between B6 CDRH2 and H1159 on SH (Pinto et al., 2021; Sauer et al., 2021) (Fig. 6C). 28D9 and IgG22 were the least cross-reactive, only able to neutralize MERS-CoV despite binding SARS-CoV, SARS-CoV-2, and OC43 (28D9) or HKU1 (IgG22) spikes (Hsieh et al., 2021; Wang, van Haperen, et al., 2021). Antibodies to the even more highly conserved FP (Fig. 6D) showed the broadest cross-neutralizing activity of all, with COV44-62 able to neutralize SARS-CoV, SARS-CoV-2, MERS-CoV, hCoV-OC43 as well as the α -coronaviruses hCoV-NL63, hCoV-229E, and binding to δ -coronavirus Porcine Deltacoronavirus 0081-4 spike (Dacon et al., 2022). Not surprisingly, given the high degree of conservation of these epitopes and relatively low immunological pressure, none of the major SARS-CoV-2 variants appear to escape neutralization by these antibodies.



6. Conclusions

Trends seen in polyclonal responses, particularly neutralizing potency and cross-reactivity of antibodies as a function of spike domain binding target, can be understood through study of individual monoclonal antibodies directed against the epitopes within each domain. On the RBD, RBM-targeting antibodies show exceptional neutralizing potency and varied mechanisms of SARS-CoV-2 neutralization, but suffer from the malleable nature of their epitopes, displaying low cross-reactivity to other coronaviruses and a low threshold for SARS-CoV-2 variant escape. Nonetheless, certain RBM antibodies continue to display robust neutralization against resistant variants, and high resolution structural information has revealed unique binding modes for these mAbs that enable continued neutralization. In general, the further away from the RBM an epitope is, the less neutralizing and more cross-reactive an antibody becomes, as is seen for RBM-adjacent and RBM-distal antibodies. SARS-CoV-2 bears an additional site of vulnerability in the NTD, particularly within a well-defined antigenic supersite. This site unfortunately is prone to a variety of substantial structural changes that confer broad resistance to potentially neutralizing NTD-specific mAbs. Neutralizing mAbs targeting other portions of the NTD may circumvent these changes and thus are generally more cross-reactive, but such mAbs are rare. Antibodies to S2, although also rare, can be identified and display mild neutralizing potency but exceptional cross-reactivity, particularly for mAbs to the fusion peptide. It is unclear, however, if these mAbs can be leveraged as therapeutics. SARS-CoV-2 will likely continue to mutate and evade antibody responses, and the search for effective antibodies is likely to continue. These efforts will hopefully lead to the identification or engineering of an antibody from which SARS-CoV-2 cannot escape. In the meantime, continuous, thorough investigation of the variant-contextualized virology and immunology of SARS-CoV-2 will be critical for assessing and adjusting antiviral strategy so that we may safely navigate an end to this pandemic.

Acknowledgments

This work was supported by National Institute of Allergy and Infectious Diseases contracts 75N93019C00062 and HHSN272201700060C (to D.H.F.). We gratefully acknowledge Christopher A. Nelson for critical review of the manuscript, and other members of the Fremont and Diamond laboratories for valuable discussions.

Disclosures

The authors declare no competing interests.

References

- Abdool Karim, S. S., & de Oliveira, T. (2021). New SARS-CoV-2 variants—Clinical, public health, and vaccine implications. *New England Journal of Medicine*, *384*(19), 1866–1868. <https://doi.org/10.1056/NEJMc2100362>.
- Abu-Raddad, L. J., Chemaitelly, H., & Butt, A. A. (2021). Effectiveness of the BNT162b2 Covid-19 Vaccine against the B.1.1.7 and B.1.351 Variants. *New England Journal of Medicine*, *385*(2), 187–189. <https://doi.org/10.1056/NEJMc2104974>.
- Akgun, E., Tuzuner, M. B., Sahin, B., Kilercik, M., Kulah, C., Cakiroglu, H. N., et al. (2020). Proteins associated with neutrophil degranulation are upregulated in nasopharyngeal swabs from SARS-CoV-2 patients. *PLoS One*, *15*(10), e0240012. <https://doi.org/10.1371/journal.pone.0240012>.
- Alsoussi, W. B., Turner, J. S., Case, J. B., Zhao, H., Schmitz, A. J., Zhou, J. Q., et al. (2020). A potently neutralizing antibody protects mice against SARS-CoV-2 infection. *Journal of Immunology (Baltimore, Md.: 1950)*, *205*(4), 915–922. <https://doi.org/10.4049/jimmunol.2000583>.
- Altomare, C. G., Adelsberg, D. C., Carreno, J. M., Sapse, I. A., Amanat, F., Ellebedy, A. H., et al. (2022). Structure of a vaccine-induced, germline-encoded human antibody defines a neutralizing epitope on the SARS-CoV-2 Spike N-terminal domain. *mBio*, *13*(3), e0358021. <https://doi.org/10.1128/mbio.03580-21>.
- Amanat, F., Thapa, M., Lei, T., Ahmed, S. M. S., Adelsberg, D. C., Carreño, J. M., et al. (2021). SARS-CoV-2 mRNA vaccination induces functionally diverse antibodies to NTD, RBD, and S2. *Cell*, *184*(15), 3936–3948.e10. <https://doi.org/10.1016/j.cell.2021.06.005>.
- Amraei, R., Yin, W., Napoleon, M. A., Suder, E. L., Berrigan, J., Zhao, Q., et al. (2021). CD209L/L-SIGN and CD209/DC-SIGN act as receptors for SARS-CoV-2. *ACS Central Science*, *7*(7), 1156–1165. <https://doi.org/10.1021/acscentsci.0c01537>.
- Andreano, E., Piccini, G., Licastro, D., Casalino, L., Johnson, N. V., Paciello, I., et al. (2021). SARS-CoV-2 escape from a highly neutralizing COVID-19 convalescent plasma. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(36). <https://doi.org/10.1073/pnas.2103154118>.
- Andrews, N., Stowe, J., Kirsebom, F., Toffa, S., Rickeard, T., Gallagher, E., et al., & Lopez Bernal, J. (2022). Covid-19 vaccine effectiveness against the omicron (B.1.1.529) variant. *New England Journal of Medicine*. <https://doi.org/10.1056/NEJMoa2119451>.
- Asarnow, D., Wang, B., Lee, W.-H., Hu, Y., Huang, C.-W., Faust, B., et al. (2021). Structural insight into SARS-CoV-2 neutralizing antibodies and modulation of syncytia. *Cell*, *184*(12), 3192–3204.e16. <https://doi.org/10.1016/j.cell.2021.04.033>.
- Atyeo, C., Slein, M. D., Fischinger, S., Burke, J., Schäfer, A., Leist, S. R., et al. (2021). Dissecting strategies to tune the therapeutic potential of SARS-CoV-2-specific monoclonal antibody CR3022. *JCI Insight*, *6*(1), 143129. <https://doi.org/10.1172/jci.insight.143129>.
- Baden, L. R., El Sahly, H. M., Essink, B., Kotloff, K., Frey, S., Novak, R., et al., & Zaks, T. (2021). Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *New England Journal of Medicine*, *384*(5), 403–416. <https://doi.org/10.1056/NEJMoa2035389>.
- Bai, Y., Yao, L., Wei, T., Tian, F., Jin, D.-Y., Chen, L., et al. (2020). Presumed asymptomatic carrier transmission of COVID-19. *JAMA*, *323*(14), 1406–1407. <https://doi.org/10.1001/jama.2020.2565>.

- Baker, A. N., Richards, S. J., Guy, C. S., Congdon, T. R., Hasan, M., Zwetsloot, A. J., et al. (2020). The SARS-CoV-2 spike protein binds sialic acids and enables rapid detection in a lateral flow point of care diagnostic device. *ACS Central Science*, 6(11), 2046–2052. <https://doi.org/10.1021/acscentsci.0c00855>.
- Barnes, C. O., Jette, C. A., Abernathy, M. E., Dam, K.-M. A., Esswein, S. R., Gristick, H. B., et al. (2020). SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. *Nature*, 588(7839), 682–687. <https://doi.org/10.1038/s41586-020-2852-1>.
- Barnes, C. O., West, A. P., Huey-Tubman, K. E., Hoffmann, M. A. G., Sharaf, N. G., Hoffmann, P. R., et al. (2020). Structures of human antibodies bound to SARS-CoV-2 spike reveal common epitopes and recurrent features of antibodies. *Cell*, 182(4), 828–842.e16. <https://doi.org/10.1016/j.cell.2020.06.025>.
- Bates, T. A., Weinstein, J. B., Farley, S., Leier, H. C., Messer, W. B., & Tafesse, F. G. (2021). Cross-reactivity of SARS-CoV structural protein antibodies against SARS-CoV-2. *Cell Reports*, 34(7), 108737. <https://doi.org/10.1016/j.celrep.2021.108737>.
- Beaudoin-Bussi eres, G., Chen, Y., Ullah, I., Pr evost, J., Tolbert, W. D., Symmes, K., et al. (2022). A Fc-enhanced NTD-binding non-neutralizing antibody delays virus spread and synergizes with a nAb to protect mice from lethal SARS-CoV-2 infection. *Cell Reports*, 38(7), 110368. <https://doi.org/10.1016/j.celrep.2022.110368>.
- Benton, D. J., Wrobel, A. G., Xu, P., Roustan, C., Martin, S. R., Rosenthal, P. B., et al. (2020). Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion. *Nature*, 588(7837), 327–330. <https://doi.org/10.1038/s41586-020-2772-0>.
- Bos, R., Rutten, L., van der Lubbe, J. E. M., Bakkers, M. J. G., Hardenberg, G., Wegmann, F., et al. (2020). Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 Spike immunogen induces potent humoral and cellular immune responses. *Npj Vaccines*, 5(1), 1–11. <https://doi.org/10.1038/s41541-020-00243-x>.
- Bournazos, S., Corti, D., Virgin, H. W., & Ravetch, J. V. (2020). Fc-optimized antibodies elicit CD8 immunity to viral respiratory infection. *Nature*, 588(7838), 485–490. <https://doi.org/10.1038/s41586-020-2838-z>.
- Bowen, J. E., Walls, A. C., Joshi, A., Sprouse, K. R., Stewart, C., Tortorici, M. A., et al. (2021). SARS-CoV-2 spike conformation determines plasma neutralizing activity [Preprint]. *Immunology*. <https://doi.org/10.1101/2021.12.19.473391>.
- Brewer, R. C., Ramadoss, N. S., Lahey, L. J., Jahanbani, S., Robinson, W. H., & Lanz, T. V. (2022). BNT162b2 vaccine induces divergent B cell responses to SARS-CoV-2 S1 and S2. *Nature Immunology*, 23(1), 33–39. <https://doi.org/10.1038/s41590-021-01088-9>.
- Brouwer, P. J. M., Daniels, T. G., van der Straten, K., Snitselaar, J. L., Aldon, Y., Bangaru, S., et al. (2020). Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. *Science (New York, N.Y.)*, 369(6504), 643–650. <https://doi.org/10.1126/science.abc5902>.
- Cai, Y., Zhang, J., Xiao, T., Lavine, C. L., Rawson, S., Peng, H., et al. (2021). Structural basis for enhanced infectivity and immune evasion of SARS-CoV-2 variants. *Science (New York, N.Y.)*, 373(6555), 642–648. <https://doi.org/10.1126/science.abi9745>.
- Cai, Y., Zhang, J., Xiao, T., Peng, H., Sterling, S. M., Walsh, R. M., et al. (2020). Distinct conformational states of SARS-CoV-2 spike protein. *Science*, 369(6511), 1586–1592. <https://doi.org/10.1126/science.abd4251>.
- Cameron, E., Bowen, J. E., Rosen, L. E., Saliba, C., Zepeda, S. K., Culap, K., et al. (2022). Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. *Nature*, 602(7898), 664–670. <https://doi.org/10.1038/s41586-021-04386-2>.
- Campbell, F., Archer, B., Laurenson-Schafer, H., Jinnai, Y., Konings, F., Batra, N., et al. (2021). Increased transmissibility and global spread of SARS-CoV-2 variants of concern as at June 2021. *Eurosurveillance*, 26(24), 2100509. <https://doi.org/10.2807/1560-7917.ES.2021.26.24.2100509>.

- Cao, Y., Su, B., Guo, X., Sun, W., Deng, Y., Bao, L., et al. (2020). Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. *Cell*, 182(1), 73–84.e16. <https://doi.org/10.1016/j.cell.2020.05.025>.
- Cao, Y., Yisimayi, A., Bai, Y., Huang, W., Li, X., Zhang, Z., et al. (2021). Humoral immune response to circulating SARS-CoV-2 variants elicited by inactivated and RBD-subunit vaccines. *Cell Research*, 31(7), 732–741. <https://doi.org/10.1038/s41422-021-00514-9>.
- Carroll, T., Fox, D., van Doremalen, N., Ball, E., Morris, M. K., Sotomayor-Gonzalez, A., et al. (2022). The B.1.427/1.429 (epsilon) SARS-CoV-2 variants are more virulent than ancestral B.1 (614G) in Syrian hamsters. *PLOS Pathogens*, 18(2), e1009914. <https://doi.org/10.1371/journal.ppat.1009914>.
- Casalino, L., Gaieb, Z., Goldsmith, J. A., Hjorth, C. K., Dommer, A. C., Harbison, A. M., et al. (2020). Beyond shielding: The roles of glycans in the SARS-CoV-2 Spike protein. *ACS Central Science*, 6(10), 1722–1734. <https://doi.org/10.1021/acscentsci.0c01056>.
- Case, J. B., Mackin, S., Errico, J., Chong, Z., Madden, E. A., Guarino, B., et al. (2022). Resilience of S309 and AZD7442 monoclonal antibody treatments against infection by SARS-CoV-2 Omicron lineage strains [Preprint]. *Microbiology*. <https://doi.org/10.1101/2022.03.17.484787>.
- Cathcart, A. L., Havenar-Daughton, C., Lempp, F. A., Ma, D., Schmid, M. A., Agostini, M. L., et al., & Hebner, C. M. (2021). The dual function monoclonal antibodies VIR-7831 and VIR-7832 demonstrate potent in vitro and in vivo activity against SARS-CoV-2 [Preprint]. *Immunology*. <https://doi.org/10.1101/2021.03.09.434607>.
- Cerutti, G., Guo, Y., Wang, P., Nair, M. S., Wang, M., Huang, Y., et al. (2021). Neutralizing antibody 5-7 defines a distinct site of vulnerability in SARS-CoV-2 spike N-terminal domain. *Cell Reports*, 37(5), 109928. <https://doi.org/10.1016/j.celrep.2021.109928>.
- Cerutti, G., Guo, Y., Zhou, T., Gorman, J., Lee, M., Rapp, M., et al. (2021). Potent SARS-CoV-2 neutralizing antibodies directed against spike N-terminal domain target a single supersite. *Cell Host Microbe*, 29(5), 819–833.e7. <https://doi.org/10.1016/j.chom.2021.03.005>.
- Chakraborty, S., Gonzalez, J. C., Sievers, B. L., Mallajosyula, V., Chakraborty, S., Dubey, M., et al. (2022). Early non-neutralizing, afucosylated antibody responses are associated with COVID-19 severity. *Science Translational Medicine*, 14(635), eabm7853. <https://doi.org/10.1126/scitranslmed.abm7853>.
- Chan, C. E. Z., Seah, S. G. K., Chye, D. H., Massey, S., Torres, M., Lim, A. P. C., et al. (2021). The Fc-mediated effector functions of a potent SARS-CoV-2 neutralizing antibody, SC31, isolated from an early convalescent COVID-19 patient, are essential for the optimal therapeutic efficacy of the antibody. *PLoS One*, 16(6), e0253487. <https://doi.org/10.1371/journal.pone.0253487>.
- Chen, X., Pan, Z., Yue, S., Yu, F., Zhang, J., Yang, Y., et al. (2020). Disease severity dictates SARS-CoV-2-specific neutralizing antibody responses in COVID-19. *Signal Transduction and Targeted Therapy*, 5, 180. <https://doi.org/10.1038/s41392-020-00301-9>.
- Changrob, S., Fu, Y., Guthmiller, J. J., Halfmann, P. J., Li, L., Stamper, C. T., et al. (2021). Cross-neutralization of emerging SARS-CoV-2 variants of concern by antibodies targeting distinct epitopes on spike. *mBio*, 12(6), e0297521. <https://doi.org/10.1128/mBio.02975-21>.
- Chen, R. E., Winkler, E. S., Case, J. B., Aziati, I. D., Bricker, T. L., Joshi, A., et al. (2021). In vivo monoclonal antibody efficacy against SARS-CoV-2 variant strains. *Nature*, 596(7870), 103–108. <https://doi.org/10.1038/s41586-021-03720-y>.
- Chen, R. E., Zhang, X., Case, J. B., Winkler, E. S., Liu, Y., VanBlargan, L. A., et al. (2021). Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. *Nature Medicine*, 27(4), 717–726. <https://doi.org/10.1038/s41591-021-01294-w>.

- Cheng, L., Song, S., Zhou, B., Ge, X., Yu, J., Zhang, M., et al. (2021). Impact of the N501Y substitution of SARS-CoV-2 Spike on neutralizing monoclonal antibodies targeting diverse epitopes. *Virology Journal*, 18(1), 87. <https://doi.org/10.1186/s12985-021-01554-8>.
- Cherian, S., Potdar, V., Jadhav, S., Yadav, P., Gupta, N., Das, M., et al. (2021). SARS-CoV-2 Spike mutations, L452R, T478K, E484Q and P681R, in the second wave of COVID-19 in Maharashtra, India. *Microorganisms*, 9(7), 1542. <https://doi.org/10.3390/microorganisms9071542>.
- Chi, X., Yan, R., Zhang, J., Zhang, G., Zhang, Y., Hao, M., et al. (2020). A neutralizing human antibody binds to the N-terminal domain of the Spike protein of SARS-CoV-2. *Science*, 369(6504), 650–655. <https://doi.org/10.1126/science.abc6952>.
- Clark, S. A., Clark, L. E., Pan, J., Coscia, A., McKay, L. G. A., Shankar, S., et al. (2021). SARS-CoV-2 evolution in an immunocompromised host reveals shared neutralization escape mechanisms. *Cell*, 184(10), 2605–2617.e18. <https://doi.org/10.1016/j.cell.2021.03.027>.
- Clausen, T. M., Sandoval, D. R., Spliid, C. B., Pihl, J., Perrett, H. R., Painter, C. D., et al. (2020). SARS-CoV-2 infection depends on cellular heparan sulfate and ACE2. *Cell*, 183(4), 1043–1057.e15. <https://doi.org/10.1016/j.cell.2020.09.033>.
- Collie, S., Champion, J., Moultrie, H., Bekker, L.-G., & Gray, G. (2022). Effectiveness of BNT162b2 vaccine against Omicron variant in South Africa. *New England Journal of Medicine*, 386(5), 494–496. <https://doi.org/10.1056/NEJMc2119270>.
- Collier, D. A., De Marco, A., Ferreira, I. A. T. M., Meng, B., Dattir, R. P., Walls, A. C., et al. (2021). Sensitivity of SARS-CoV-2 B.1.1.7 to mRNA vaccine-elicited antibodies. *Nature*, 593(7857), 136–141. <https://doi.org/10.1038/s41586-021-03412-7>.
- Cromer, D., Steain, M., Reynaldi, A., Schlub, T. E., Wheatley, A. K., Juno, J. A., et al. (2022). Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: A meta-analysis. *The Lancet Microbe*, 3(1), e52–e61. [https://doi.org/10.1016/s2666-5247\(21\)00267-6](https://doi.org/10.1016/s2666-5247(21)00267-6).
- Dacon, C., Tucker, C., Peng, L., Lee, C.-C. D., Lin, T.-H., Yuan, M., et al. (2022). Broadly neutralizing antibodies target the coronavirus fusion peptide (p. 2022.04.11.487879). *bioRxiv*. <https://doi.org/10.1101/2022.04.11.487879>.
- Dan, J. M., Mateus, J., Kato, Y., Hastie, K. M., Yu, E. D., Faliti, C. E., et al. (2021). Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science (New York, N.Y.)*, eabf4063. <https://doi.org/10.1126/science.abf4063>.
- Davies, N. G., Abbott, S., Barnard, R. C., Jarvis, C. I., Kucharski, A. J., Munday, J. D., et al. (2021). Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. *Science*, 372(6538), eabg3055. <https://doi.org/10.1126/science.abg3055>.
- Dejnirattisai, W., Zhou, D., Ginn, H. M., Duyvesteyn, H. M. E., Supasa, P., Case, J. B., et al. (2021). The antigenic anatomy of SARS-CoV-2 receptor binding domain. *Cell*, 184(8), 2183–2200.e22. <https://doi.org/10.1016/j.cell.2021.02.032>.
- Dejnirattisai, W., Zhou, D., Supasa, P., Liu, C., Mentzer, A. J., Ginn, H. M., et al. (2021). Antibody evasion by the P.1 strain of SARS-CoV-2. *Cell*, 184(11), 2939–2954.e9. <https://doi.org/10.1016/j.cell.2021.03.055>.
- Deng, X., Garcia-Knight, M. A., Khalid, M. M., Servellita, V., Wang, C., Morris, M. K., et al. (2021). Transmission, infectivity, and neutralization of a spike L452R SARS-CoV-2 variant. *Cell*, 184(13), 3426–3437.e8. <https://doi.org/10.1016/j.cell.2021.04.025>.
- Dispinseri, S., Secchi, M., Pirillo, M. F., Tolazzi, M., Borghi, M., Brigatti, C., et al. (2021). Neutralizing antibody responses to SARS-CoV-2 in symptomatic COVID-19 is persistent and critical for survival. *Nature Communications*, 12(1), 2670. <https://doi.org/10.1038/s41467-021-22958-8>.

- Dong, E., Du, H., & Gardner, L. (2020). An interactive web-based dashboard to track COVID-19 in real time. *The Lancet Infectious Diseases*, 20(5), 533–534. [https://doi.org/10.1016/S1473-3099\(20\)30120-1](https://doi.org/10.1016/S1473-3099(20)30120-1).
- Dong, J., Zost, S. J., Greaney, A. J., Starr, T. N., Dingens, A. S., Chen, E. C., et al. (2021). Genetic and structural basis for SARS-CoV-2 variant neutralization by a two-antibody cocktail. *Nature Microbiology*, 6(10), 1233–1244. <https://doi.org/10.1038/s41564-021-00972-2>.
- Dowell, A. C., Butler, M. S., Jinks, E., Tut, G., Lancaster, T., Sylla, P., et al. (2022). Children develop robust and sustained cross-reactive spike-specific immune responses to SARS-CoV-2 infection. *Nature Immunology*, 23(1), 40–49. <https://doi.org/10.1038/s41590-021-01089-8>.
- Dussupt, V., Sankhala, R. S., Mendez-Rivera, L., Townsley, S. M., Schmidt, F., Wiczorek, L., et al. (2021). Low-dose in vivo protection and neutralization across SARS-CoV-2 variants by monoclonal antibody combinations. *Nature Immunology*, 22(12), 1503–1514. <https://doi.org/10.1038/s41590-021-01068-z>.
- Edara, V. V., Norwood, C., Floyd, K., Lai, L., Davis-Gardner, M. E., Hudson, W. H., et al. (2021). Infection- and vaccine-induced antibody binding and neutralization of the B.1.351 SARS-CoV-2 variant. *Cell Host & Microbe*, 29(4), 516–521.e3. <https://doi.org/10.1016/j.chom.2021.03.009>.
- Elshabrawy, H. A., Coughlin, M. M., Baker, S. C., & Prabhakar, B. S. (2012). Human monoclonal antibodies against highly conserved HR1 and HR2 domains of the SARS-CoV Spike protein are more broadly neutralizing. *PLoS One*, 7(11), e50366. <https://doi.org/10.1371/journal.pone.0050366>.
- Errico, J. M., Zhao, H., Chen, R. E., Liu, Z., Case, J. B., Ma, M., et al. (2021). Structural mechanism of SARS-CoV-2 neutralization by two murine antibodies targeting the RBD. *Cell Reports*, 37(4), 109881. <https://doi.org/10.1016/j.celrep.2021.109881>.
- Faria, N. R., Mellan, T. A., Whittaker, C., Claro, I. M., da Candido, D. S., Mishra, S., et al. (2021). Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science*, 372(6544), 815–821. <https://doi.org/10.1126/science.abh2644>.
- Feng, S., Phillips, D. J., White, T., Sayal, H., Aley, P. K., Bibi, S., et al. (2021). Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nature Medicine*, 27(11), 2032–2040. <https://doi.org/10.1038/s41591-021-01540-1>.
- Gaebler, C., Wang, Z., Lorenzi, J. C. C., Muecksch, F., Finkin, S., Tokuyama, M., et al. (2021). Evolution of antibody immunity to SARS-CoV-2. *Nature*, 591(7851), 639–644. <https://doi.org/10.1038/s41586-021-03207-w>.
- Gallo, O., Locatello, L. G., Mazzoni, A., Novelli, L., & Annunziato, F. (2021). The central role of the nasal microenvironment in the transmission, modulation, and clinical progression of SARS-CoV-2 infection. *Mucosal Immunology*, 14(2), 305–316. <https://doi.org/10.1038/s41385-020-00359-2>.
- Galloway, S. E. (2021). Emergence of SARS-CoV-2 B.1.1.7 lineage—United States, December 29, 2020–January 12, 2021. *MMWR. Morbidity and Mortality Weekly Report*, 70. <https://doi.org/10.15585/mmwr.mm7003e2>.
- Gandhi, M., Yokoe, D. S., & Havlir, D. V. (2020). Asymptomatic transmission, the Achilles' heel of current strategies to control Covid-19. *New England Journal of Medicine*, 382(22), 2158–2160. <https://doi.org/10.1056/nejme2009758>.
- Garcia-Beltran, W. F., Lam, E. C., Astudillo, M. G., Yang, D., Miller, T. E., Feldman, J., et al. (2021). COVID-19-neutralizing antibodies predict disease severity and survival. *Cell*, 184(2), 476–488.e11. <https://doi.org/10.1016/j.cell.2020.12.015>.
- Garcia-Beltran, W. F., St. Denis, K. J., Hoelzemer, A., Lam, E. C., Nitido, A. D., Sheehan, M. L., et al. (2022). MRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. *Cell*, 185(3), 457–466.e4. <https://doi.org/10.1016/j.cell.2021.12.033>.

- Ge, J., Wang, R., Ju, B., Zhang, Q., Sun, J., Chen, P., et al. (2021). Antibody neutralization of SARS-CoV-2 through ACE2 receptor mimicry. *Nature Communications*, 12(1), 250. <https://doi.org/10.1038/s41467-020-20501-9>.
- Gobeil, S. M.-C., Janowska, K., McDowell, S., Mansouri, K., Parks, R., Manne, K., et al. (2021). D614G mutation alters SARS-CoV-2 Spike conformation and enhances protease cleavage at the S1/S2 junction. *Cell Reports*, 34(2), 108630. <https://doi.org/10.1016/j.celrep.2020.108630>.
- Goddard, T. D., Huang, C. C., Meng, E. C., Pettersen, E. F., Couch, G. S., Morris, J. H., et al. (2018). UCSF ChimeraX: Meeting modern challenges in visualization and analysis: UCSF ChimeraX visualization system. *Protein Science*, 27(1), 14–25. <https://doi.org/10.1002/pro.3235>.
- Gorman, M. J., Patel, N., Guebre-Xabier, M., Zhu, A. L., Atyeo, C., Pullen, K. M., et al. (2021). Fab and Fc contribute to maximal protection against SARS-CoV-2 following NVX-CoV2373 subunit vaccine with Matrix-M vaccination. *Cell Reports Medicine*, 2(9), 100405. <https://doi.org/10.1016/j.xcrm.2021.100405>.
- Graham, C., Seow, J., Huettner, I., Khan, H., Kouphou, N., Acors, S., et al. (2021). Neutralization potency of monoclonal antibodies recognizing dominant and subdominant epitopes on SARS-CoV-2 Spike is impacted by the B.1.1.7 variant. *Immunity*, 54(6), 1276–1289.e6. <https://doi.org/10.1016/j.immuni.2021.03.023>.
- Graham, N. R., Whitaker, A. N., Strother, C. A., Miles, A. K., Grier, D., McElvany, B. D., et al. (2020). Kinetics and isotype assessment of antibodies targeting the spike protein receptor-binding domain of severe acute respiratory syndrome-coronavirus-2 in COVID-19 patients as a function of age, biological sex and disease severity. *Clinical & Translational Immunology*, 9(10), e1189. <https://doi.org/10.1002/cti2.1189>.
- Grant, O. C., Montgomery, D., Ito, K., & Woods, R. J. (2020). Analysis of the SARS-CoV-2 spike protein glycan shield reveals implications for immune recognition. *Scientific Reports*, 10(1), 14991. <https://doi.org/10.1038/s41598-020-71748-7>.
- Greaney, A. J., Loes, A. N., Crawford, K. H. D., Starr, T. N., Malone, K. D., Chu, H. Y., et al. (2021). Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. *Cell Host & Microbe*, 29(3), 463–476.e6. <https://doi.org/10.1016/j.chom.2021.02.003>.
- Greaney, A. J., Starr, T. N., Barnes, C. O., Weisblum, Y., Schmidt, F., Caskey, M., et al. (2021). Mapping mutations to the SARS-CoV-2 RBD that escape binding by different classes of antibodies. *Nature Communications*, 12(1), 4196. <https://doi.org/10.1038/s41467-021-24435-8>.
- Greaney, A. J., Starr, T. N., Gilchuk, P., Zost, S. J., Binshtein, E., Loes, A. N., et al. (2021). Complete mapping of mutations to the SARS-CoV-2 spike receptor-binding domain that escape antibody recognition. *Cell Host & Microbe*, 29(1), 44–57.e9. <https://doi.org/10.1016/j.chom.2020.11.007>.
- Gu, Y., Cao, J., Zhang, X., Gao, H., Wang, Y., Wang, J., et al. (2022). Receptome profiling identifies KREMEN1 and ASGR1 as alternative functional receptors of SARS-CoV-2. *Cell Research*, 32(1), 24–37. <https://doi.org/10.1038/s41422-021-00595-6>.
- Gui, M., Song, W., Zhou, H., Xu, J., Chen, S., Xiang, Y., et al. (2017). Cryo-electron microscopy structures of the SARS-CoV spike glycoprotein reveal a prerequisite conformational state for receptor binding. *Cell Research*, 27(1), 119–129. <https://doi.org/10.1038/cr.2016.152>.
- Hansen, J., Baum, A., Pascal, K. E., Russo, V., Giordano, S., Wloga, E., et al. (2020). Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science (New York, N.Y.)*, 369(6506), 1010–1014. <https://doi.org/10.1126/science.abd0827>.
- Harrison, S. C. (2008). Viral membrane fusion. *Nature Structural & Molecular Biology*, 15(7), 690–698. <https://doi.org/10.1038/nsmb.1456>.

- Harvey, W. T., Carabelli, A. M., Jackson, B., Gupta, R. K., Thomson, E. C., Harrison, E. M., et al. (2021). SARS-CoV-2 variants, spike mutations and immune escape. *Nature Reviews Microbiology*, 19(7), 409–424. <https://doi.org/10.1038/s41579-021-00573-0>.
- Haslwanter, D., Dieterle, M. E., Wec, A. Z., O'Brien, C. M., Sakharkar, M., Florez, C., et al. (2021). A combination of receptor-binding domain and N-terminal domain neutralizing antibodies limits the generation of SARS-CoV-2 spike neutralization-escape mutants. *mBio*, 12(5), e0247321. <https://doi.org/10.1128/mBio.02473-21>.
- Hassan, A. O., Kafai, N. M., Dmitriev, I. P., Fox, J. M., Smith, B. K., Harvey, I. B., et al. (2020). A single-dose intranasal ChAd vaccine protects upper and lower respiratory tracts against SARS-CoV-2. *Cell*, 183(1), 169–184.e13. <https://doi.org/10.1016/j.cell.2020.08.026>.
- Hassan, A. O., Shrihari, S., Gorman, M. J., Ying, B., Yaun, D., Raju, S., et al. (2021). An intranasal vaccine durably protects against SARS-CoV-2 variants in mice. *Cell Reports*, 36(4), 109452. <https://doi.org/10.1016/j.celrep.2021.109452>.
- Hastie, K. M., Li, H., Bedinger, D., Schendel, S. L., Dennison, S. M., Li, K., et al. (2021). Defining variant-resistant epitopes targeted by SARS-CoV-2 antibodies: A global consortium study. *Science (New York, N.Y.)*, 374(6566), 472–478. <https://doi.org/10.1126/science.abh2315>.
- Heffron, A. S., McIlwain, S. J., Amjadi, M. F., Baker, D. A., Khullar, S., Armbrust, T., et al. (2021). The landscape of antibody binding in SARS-CoV-2 infection. *PLoS Biology*, 19(6), e3001265. <https://doi.org/10.1371/journal.pbio.3001265>.
- Henderson, R., Edwards, R. J., Mansouri, K., Janowska, K., Stalls, V., Gobeil, S. M. C., et al. (2020). Controlling the SARS-CoV-2 spike glycoprotein conformation. *Nature Structural & Molecular Biology*, 27(10), 925–933. <https://doi.org/10.1038/s41594-020-0479-4>.
- Hicks, J., Klumpp-Thomas, C., Kalish, H., Shunmugavel, A., Mehalko, J., Denson, J.-P., et al. (2021). Serologic cross-reactivity of SARS-CoV-2 with endemic and seasonal betacoronaviruses. *Journal of Clinical Immunology*, 41(5), 906–913. <https://doi.org/10.1007/s10875-021-00997-6>.
- Hoffmann, M., Arora, P., Groß, R., Seidel, A., Hörnich, B. F., Hahn, A. S., et al. (2021). SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies. *Cell*, 184(9), 2384–2393.e12. <https://doi.org/10.1016/j.cell.2021.03.036>.
- Hoffmann, M., Kleine-Weber, H., & Pöhlmann, S. (2020). A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Molecular Cell*, 78(4), 779–784.e5. <https://doi.org/10.1016/j.molcel.2020.04.022>.
- Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*, 181(2), 271–280.e8. <https://doi.org/10.1016/j.cell.2020.02.052>.
- Hoffmann, M., Krüger, N., Schulz, S., Cossmann, A., Rocha, C., Kempf, A., et al. (2022). The Omicron variant is highly resistant against antibody-mediated neutralization: Implications for control of the COVID-19 pandemic. *Cell*, 185(3), 447–456.e11. <https://doi.org/10.1016/j.cell.2021.12.032>.
- Holshue, M. L., DeBolt, C., Lindquist, S., Lofy, K. H., Wiesman, J., Bruce, H., et al. (2020). First case of 2019 novel coronavirus in the United States. *New England Journal of Medicine*, 382(10), 929–936. <https://doi.org/10.1056/nejmoa2001191>.
- Hou, Y. J., Chiba, S., Halfmann, P., Ehre, C., Kuroda, M., Dinnon, K. H., et al. (2020). SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo. *Science*, 370(6523), 1464–1468. <https://doi.org/10.1126/science.abe8499>.
- Hsieh, C.-L., Goldsmith, J. A., Schaub, J. M., DiVenere, A. M., Kuo, H.-C., Javanmardi, K., et al. (2020). Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. *Science*, 369(6510), 1501–1505. <https://doi.org/10.1126/science.abd0826>.

- Hsieh, C.-L., Werner, A. P., Leist, S. R., Stevens, L. J., Falconer, E., Goldsmith, J. A., et al. (2021). Stabilized coronavirus spike stem elicits a broadly protective antibody. *Cell Reports*, 37(5), 109929. <https://doi.org/10.1016/j.celrep.2021.109929>.
- Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*, 395(10223), 497–506. [https://doi.org/10.1016/s0140-6736\(20\)30183-5](https://doi.org/10.1016/s0140-6736(20)30183-5).
- Huang, X., Dong, W., Milewska, A., Golda, A., Qi, Y., Zhu, Q. K., et al. (2015). Human coronavirus HKU1 spike protein uses O-acetylated sialic acid as an attachment receptor determinant and employs hemagglutinin-esterase protein as a receptor-destroying enzyme. *Journal of Virology*, 89(14), 7202–7213. <https://doi.org/10.1128/JVI.00854-15>.
- Huang, Y., Yang, C., Xu, X., Xu, W., & Liu, S. (2020). Structural and functional properties of SARS-CoV-2 spike protein: Potential antiviral drug development for COVID-19. *Acta Pharmacologica Sinica*, 41(9), 1141–1149. <https://doi.org/10.1038/s41401-020-0485-4>.
- Hulswit, R. J. G., Lang, Y., Bakkers, M. J. G., Li, W., Li, Z., Schouten, A., et al. (2019). Human coronaviruses OC43 and HKU1 bind to 9-O-acetylated sialic acids via a conserved receptor-binding site in spike protein domain A. *Proceedings of the National Academy of Sciences of the United States of America*, 116(7), 2681–2690. <https://doi.org/10.1073/pnas.1809667116>.
- Huo, J., Le Bas, A., Ruza, R. R., Duyvesteyn, H. M. E., Mikolajek, H., Malinauskas, T., et al. (2020). Neutralizing nanobodies bind SARS-CoV-2 spike RBD and block interaction with ACE2. *Nature Structural & Molecular Biology*, 27(9), 846–854. <https://doi.org/10.1038/s41594-020-0469-6>.
- Huo, J., Zhao, Y., Ren, J., Zhou, D., Duyvesteyn, H. M. E., Ginn, H. M., et al. (2020). Neutralization of SARS-CoV-2 by destruction of the prefusion spike. *Cell Host & Microbe*, 28(3), 445–454.e6. <https://doi.org/10.1016/j.chom.2020.06.010>.
- Hurlburt, N. K., Homad, L. J., Sinha, I., Jennewein, M. F., MacCamy, A. J., Wan, Y.-H., et al. (2022). Structural definition of a pan-sarbecovirus neutralizing epitope on the spike S2 subunit. *Communications Biology*, 5(1), 1–13. <https://doi.org/10.1038/s42003-022-03262-7>.
- Hurlburt, N. K., Seydoux, E., Wan, Y.-H., Edara, V. V., Stuart, A. B., Feng, J., et al. (2020). Structural basis for potent neutralization of SARS-CoV-2 and role of antibody affinity maturation. *Nature Communications*, 11(1), 5413. <https://doi.org/10.1038/s41467-020-19231-9>.
- Ibarrondo, F. J., Fulcher, J. A., Goodman-Meza, D., Elliott, J., Hofmann, C., Hausner, M. A., et al. (2020). Rapid decay of anti-SARS-CoV-2 antibodies in persons with mild Covid-19. *The New England Journal of Medicine*, NEJMc2025179. <https://doi.org/10.1056/NEJMc2025179>.
- Iketani, S., Liu, L., Guo, Y., Liu, L., Chan, J. F.-W., Huang, Y., et al. (2022). Antibody evasion properties of SARS-CoV-2 Omicron sublineages. *Nature*, 604(7906), 553–556. <https://doi.org/10.1038/s41586-022-04594-4>.
- Isho, B., Abe, K. T., Zuo, M., Jamal, A. J., Rathod, B., Wang, J. H., et al. (2020). Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Science Immunology*, 5(52), eabe5511. <https://doi.org/10.1126/sciimmunol.abe5511>.
- Iyer, A. S., Jones, F. K., Nodoushani, A., Kelly, M., Becker, M., Slater, D., et al. (2020). Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. *Science Immunology*, 5(52), eabe0367. <https://doi.org/10.1126/sciimmunol.abe0367>.
- Jackson, L. A., Anderson, E. J., Roupheal, N. G., Roberts, P. C., Makhene, M., Coler, R. N., et al. (2020). An mRNA vaccine against SARS-CoV-2—Preliminary report. *New England Journal of Medicine*, 383(20), 1920–1931. <https://doi.org/10.1056/nejmoa2022483>.

- Jackson, C. B., Farzan, M., Chen, B., & Choe, H. (2022). Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews Molecular Cell Biology*, 23(1), 3–20. <https://doi.org/10.1038/s41580-021-00418-x>.
- Jaimes, J. A., André, N. M., Chappie, J. S., Millet, J. K., & Whittaker, G. R. (2020). Phylogenetic analysis and structural modeling of SARS-CoV-2 spike protein reveals an evolutionary distinct and proteolytically sensitive activation loop. *Journal of Molecular Biology*, 432(10), 3309–3325. <https://doi.org/10.1016/j.jmb.2020.04.009>.
- Jaume, M., Yip, M. S., Cheung, C. Y., Leung, H. L., Li, P. H., Kien, F., et al. (2011). Anti-severe acute respiratory syndrome coronavirus spike antibodies trigger infection of human immune cells via a pH- and cysteine protease-independent FcγR pathway. *Journal of Virology*, 85(20), 10582–10597. <https://doi.org/10.1128/jvi.00671-11>.
- Jennewein, M. F., MacCamy, A. J., Akins, N. R., Feng, J., Homad, L. J., Hurlburt, N. K., et al. (2021). Isolation and characterization of cross-neutralizing coronavirus antibodies from COVID-19+ subjects. *Cell Reports*, 36(2), 109353. <https://doi.org/10.1016/j.celrep.2021.109353>.
- Jette, C. A., Cohen, A. A., Gnanapragasam, P. N. P., Muecksch, F., Lee, Y. E., Huey-Tubman, K. E., et al. (2021). Broad cross-reactivity across sarbecoviruses exhibited by a subset of COVID-19 donor-derived neutralizing antibodies. *Cell Reports*, 36(13), 109760. <https://doi.org/10.1016/j.celrep.2021.109760>.
- Ju, B., Zhang, Q., Ge, J., Wang, R., Sun, J., Ge, X., et al. (2020). Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature*, 584(7819), 115–119. <https://doi.org/10.1038/s41586-020-2380-z>.
- Kaploněk, P., Cizmeci, D., Fischinger, S., Collier, A.-R., Suscovich, T., Linde, C., et al. (2022). MRNA-1273 and BNT162b2 COVID-19 vaccines elicit antibodies with differences in Fc-mediated effector functions. *Science Translational Medicine*, eabm2311. <https://doi.org/10.1126/scitranslmed.abm2311>.
- Kaploněk, P., Fischinger, S., Cizmeci, D., Bartsch, Y. C., Kang, J., Burke, J. S., et al. (2022). MRNA-1273 vaccine-induced antibodies maintain Fc effector functions across SARS-CoV-2 variants of concern. *Immunity*, 55(2), 355–365.e4. <https://doi.org/10.1016/j.immuni.2022.01.001>.
- Ke, Z., Oton, J., Qu, K., Cortese, M., Zila, V., McKeane, L., et al. (2020). Structures and distributions of SARS-CoV-2 spike proteins on intact virions. *Nature*, 588(7838), 498–502. <https://doi.org/10.1038/s41586-020-2665-2>.
- Kim, C., Ryu, D.-K., Lee, J., Kim, Y.-I., Seo, J.-M., Kim, Y.-G., et al. (2021). A therapeutic neutralizing antibody targeting receptor binding domain of SARS-CoV-2 spike protein. *Nature Communications*, 12(1), 288. <https://doi.org/10.1038/s41467-020-20602-5>.
- Kirchdoerfer, R. N., Wang, N., Pallesen, J., Wrapp, D., Turner, H. L., Cottrell, C. A., et al. (2018). Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis. *Scientific Reports*, 8(1), 15701. <https://doi.org/10.1038/s41598-018-34171-7>.
- Koenig, P.-A., Das, H., Liu, H., Kümmerer, B. M., Gohr, F. N., Jenster, L.-M., et al. (2021). Structure-guided multivalent nanobodies block SARS-CoV-2 infection and suppress mutational escape. *Science (New York, N.Y.)*, 371(6530). <https://doi.org/10.1126/science.abe6230>.
- Koppiseti, R. K., Fulcher, Y. G., & Van Doren, S. R. (2021). Fusion peptide of SARS-CoV-2 spike rearranges into a wedge inserted in bilayered micelles. *Journal of the American Chemical Society*, 143(33), 13205–13211. <https://doi.org/10.1021/jacs.1c05435>.
- Korber, B., Fischer, W. M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., et al. (2020). Tracking changes in SARS-CoV-2 spike: Evidence that D614G increases infectivity of the COVID-19 virus. *Cell*, 182(4), 812–827.e19. <https://doi.org/10.1016/j.cell.2020.06.043>.

- Kreye, J., Reincke, S. M., Kornau, H.-C., Sánchez-Sendin, E., Corman, V. M., Liu, H., et al. (2020). A therapeutic non-self-reactive SARS-CoV-2 antibody protects from lung pathology in a COVID-19 Hamster Model. *Cell*, 183(4), 1058–1069.e19. <https://doi.org/10.1016/j.cell.2020.09.049>.
- Küinkel, F., & Herrler, G. (1993). Structural and functional analysis of the surface protein of human coronavirus OC43. *Virology*, 195(1), 195–202. <https://doi.org/10.1006/viro.1993.1360>.
- Kustin, T., Harel, N., Finkel, U., Perchik, S., Harari, S., Tahor, M., et al. (2021). Evidence for increased breakthrough rates of SARS-CoV-2 variants of concern in BNT162b2-mRNA-vaccinated individuals. *Nature Medicine*, 27(8), 1379–1384. <https://doi.org/10.1038/s41591-021-01413-7>.
- Ladner, J. T., Henson, S. N., Boyle, A. S., Engelbrekton, A. L., Fink, Z. W., Rahee, F., et al. (2021). Epitope-resolved profiling of the SARS-CoV-2 antibody response identifies cross-reactivity with endemic human coronaviruses. *Cell Reports Medicine*, 2(1), 100189. <https://doi.org/10.1016/j.xcrm.2020.100189>.
- Lai, A. L., & Freed, J. H. (2021). SARS-CoV-2 fusion peptide has a greater membrane perturbing effect than SARS-CoV with highly specific dependence on Ca²⁺. *Journal of Molecular Biology*, 433(10), 166946. <https://doi.org/10.1016/j.jmb.2021.166946>.
- Laidlaw, B. J., & Ellebedy, A. H. (2022). The germinal centre B cell response to SARS-CoV-2. *Nature Reviews Immunology*, 22(1), 7–18. <https://doi.org/10.1038/s41577-021-00657-1>.
- Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., et al. (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, 581(7807), 215–220. <https://doi.org/10.1038/s41586-020-2180-5>.
- Lapiente, D., Fuchs, J., Willar, J., Vieira Antão, A., Eberlein, V., Uhlig, N., et al. (2021). Protective mucosal immunity against SARS-CoV-2 after heterologous systemic prime-mucosal boost immunization. *Nature Communications*, 12(1), 6871. <https://doi.org/10.1038/s41467-021-27063-4>.
- Lederer, K., Castaño, D., Gómez Atria, D., Oguin, T. H., Wang, S., Manzoni, T. B., et al. (2020). SARS-CoV-2 mRNA vaccines foster potent antigen-specific germinal center responses associated with neutralizing antibody generation. *Immunity*, 53(6), 1281–1295.e5. <https://doi.org/10.1016/j.immuni.2020.11.009>.
- Lempp, F. A., Soriaga, L. B., Montiel-Ruiz, M., Benigni, F., Noack, J., Park, Y.-J., et al. (2021). Lectins enhance SARS-CoV-2 infection and influence neutralizing antibodies. *Nature*, 598(7880), 342–347. <https://doi.org/10.1038/s41586-021-03925-1>.
- Li, D., Edwards, R. J., Manne, K., Martinez, D. R., Schäfer, A., Alam, S. M., et al. (2021). In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and neutralizing antibodies. *Cell*, 184(16), 4203–4219.e32. <https://doi.org/10.1016/j.cell.2021.06.021>.
- Li, F. (2016). Structure, function, and evolution of coronavirus spike proteins. *Annual Review of Virology*, 3(1), 237–261. <https://doi.org/10.1146/annurev-virology-110615-042301>.
- Li, C.-J., Chao, T.-L., Chang, T.-Y., Hsiao, C.-C., Lu, D.-C., Chiang, Y.-W., et al. (2022). Neutralizing monoclonal antibodies inhibit SARS-CoV-2 infection through blocking membrane fusion. *Microbiology Spectrum*, 10(2), e0181421. <https://doi.org/10.1128/spectrum.01814-21>.
- Li, W., Chen, Y., Prévost, J., Ullah, I., Lu, M., Gong, S. Y., et al. (2022). Structural basis and mode of action for two broadly neutralizing antibodies against SARS-CoV-2 emerging variants of concern. *Cell Reports*, 38(2), 110210. <https://doi.org/10.1016/j.celrep.2021.110210>.
- Li, Y., Lai, D.-Y., Zhang, H.-N., Jiang, H.-W., Tian, X., Ma, M.-L., et al. (2020). Linear epitopes of SARS-CoV-2 spike protein elicit neutralizing antibodies in COVID-19 patients. *Cellular & Molecular Immunology*, 17(10), 1095–1097. <https://doi.org/10.1038/s41423-020-00523-5>.

- Li, Y., Ma, M., Lei, Q., Wang, F., Hong, W., Lai, D., et al. (2021). Linear epitope landscape of the SARS-CoV-2 Spike protein constructed from 1,051 COVID-19 patients. *Cell Reports*, 34(13), 108915. <https://doi.org/10.1016/j.celrep.2021.108915>.
- Li, Y., Qian, H., Hang, J., Chen, X., Cheng, P., Ling, H., et al. (2021). Probable airborne transmission of SARS-CoV-2 in a poorly ventilated restaurant. *Building and Environment*, 196, 107788. <https://doi.org/10.1016/j.buildenv.2021.107788>.
- Li, Q., Wu, J., Nie, J., Zhang, L., Hao, H., Liu, S., et al. (2020). The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity. *Cell*, 182(5), 1284–1294. e9. <https://doi.org/10.1016/j.cell.2020.07.012>.
- Li, T., Xue, W., Zheng, Q., Song, S., Yang, C., Xiong, H., et al. (2021). Cross-neutralizing antibodies bind a SARS-CoV-2 cryptic site and resist circulating variants. *Nature Communications*, 12(1), 5652. <https://doi.org/10.1038/s41467-021-25997-3>.
- Liu, C., Ginn, H. M., Dejnirattisai, W., Supasa, P., Wang, B., Tuekprakhon, A., et al. (2021). Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. *Cell*, 184(16), 4220–4236.e13. <https://doi.org/10.1016/j.cell.2021.06.020>.
- Liu, L., Iketani, S., Guo, Y., Chan, J. F.-W., Wang, M., Liu, L., et al. (2022). Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. *Nature*, 602(7898), 676–681. <https://doi.org/10.1038/s41586-021-04388-0>.
- Liu, Y., Ning, Z., Chen, Y., Guo, M., Liu, Y., Gali, N. K., et al. (2020). Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature*, 582(7813), 557–560 (<https://doi.org/10/ggtgng>).
- Liu, Y., Soh, W. T., Kishikawa, J.-I., Hirose, M., Nakayama, E. E., Li, S., et al. (2021). An infectivity-enhancing site on the SARS-CoV-2 spike protein targeted by antibodies. *Cell*, 184(13), 3452–3466.e18. <https://doi.org/10.1016/j.cell.2021.05.032>.
- Liu, Z., VanBlargan, L. A., Bloyet, L.-M., Rothlauf, P. W., Chen, R. E., Stumpf, S., et al. (2021). Identification of SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization. *Cell Host & Microbe*, 29(3), 477–488.e4. <https://doi.org/10.1016/j.chom.2021.01.014>.
- Liu, L., Wang, P., Nair, M. S., Yu, J., Rapp, M., Wang, Q., et al. (2020). Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature*, 584(7821), 450–456. <https://doi.org/10.1038/s41586-020-2571-7>.
- Liu, L., Wei, Q., Lin, Q., Fang, J., Wang, H., Kwok, H., et al. (2019). Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. *JCI Insight*, 4(4), 123158. <https://doi.org/10.1172/jci.insight.123158>.
- Liu, H., Wu, N. C., Yuan, M., Bangaru, S., Torres, J. L., Caniels, T. G., et al. (2020). Cross-neutralization of a SARS-CoV-2 antibody to a functionally conserved site is mediated by avidity. *Immunity*, 53(6), 1272–1280.e5. <https://doi.org/10.1016/j.immuni.2020.10.023>.
- Liu, C., Zhou, D., Nutalai, R., Duyvesteyn, H. M. E., Tuekprakhon, A., Ginn, H. M., et al. (2022). The antibody response to SARS-CoV-2 Beta underscores the antigenic distance to other variants. *Cell Host & Microbe*, 30(1), 53–68.e12. <https://doi.org/10.1016/j.chom.2021.11.013>.
- Lo, M., Kim, H. S., Tong, R. K., Bainbridge, T. W., Vernes, J.-M., Zhang, Y., et al. (2017). Effector-attenuating substitutions that maintain antibody stability and reduce toxicity in mice. *The Journal of Biological Chemistry*, 292(9), 3900–3908. <https://doi.org/10.1074/jbc.M116.767749>.
- Long, Q.-X., Liu, B.-Z., Deng, H.-J., Wu, G.-C., Deng, K., Chen, Y.-K., et al. (2020). Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nature Medicine*, 26(6), 845–848. <https://doi.org/10.1038/s41591-020-0897-1>.
- Lopez Bernal, J., Andrews, N., Gower, C., Gallagher, E., Simmons, R., Thelwall, S., et al. (2021). Effectiveness of Covid-19 vaccines against the B.1.617.2 (delta) variant. *New England Journal of Medicine*, 385(7), 585–594. <https://doi.org/10.1056/NEJMoa2108891>.

- Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., et al. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. *The Lancet*, 395(10224), 565–574. [https://doi.org/10.1016/s0140-6736\(20\)30251-8](https://doi.org/10.1016/s0140-6736(20)30251-8).
- Lv, Z., Deng, Y.-Q., Ye, Q., Cao, L., Sun, C.-Y., Fan, C., et al. (2020). Structural basis for neutralization of SARS-CoV-2 and SARS-CoV by a potent therapeutic antibody. *Science*, 369(6510), 1505–1509. <https://doi.org/10.1126/science.abc5881>.
- Lv, H., Tsang, O. T., So, R. T. Y., Wang, Y., Yuan, M., Liu, H., et al. (2021). Homologous and heterologous serological response to the N-terminal domain of SARS-CoV-2 in humans and mice. *European Journal of Immunology*, 51(9), 2296–2305. <https://doi.org/10.1002/eji.202149234>.
- Lv, H., Wu, N. C., Tsang, O. T.-Y., Yuan, M., Perera, R. A. P. M., Leung, W. S., et al. (2020). Cross-reactive antibody response between SARS-CoV-2 and SARS-CoV infections. *Cell Reports*, 31(9), 107725. <https://doi.org/10.1016/j.celrep.2020.107725>.
- Ma, H., Zeng, W., He, H., Zhao, D., Jiang, D., Zhou, P., et al. (2020). Serum IgA, IgM, and IgG responses in COVID-19. *Cellular & Molecular Immunology*, 17(7), 773–775. <https://doi.org/10.1038/s41423-020-0474-z>.
- Martinez, D. R., Schäfer, A., Gobeil, S., Li, D., De la Cruz, G., Parks, R., et al. (2022). A broadly cross-reactive antibody neutralizes and protects against sarbecovirus challenge in mice. *Science Translational Medicine*, 14(629), eabj7125. <https://doi.org/10.1126/scitranslmed.abj7125>.
- McCallum, M., Bassi, J., De Marco, A., Chen, A., Walls, A. C., Di Iulio, J., et al. (2021). SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern. *Science (New York, N.Y.)*, 373(6555), 648–654. <https://doi.org/10.1126/science.abi7994>.
- McCallum, M., Czudnochowski, N., Rosen, L. E., Zepeda, S. K., Bowen, J. E., Walls, A. C., et al. (2022). Structural basis of SARS-CoV-2 Omicron immune evasion and receptor engagement. *Science (New York, N.Y.)*, 375(6583), 864–868. <https://doi.org/10.1126/science.abn8652>.
- McCallum, M., De Marco, A., Lempp, F. A., Tortorici, M. A., Pinto, D., Walls, A. C., et al. (2021). N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *Cell*, 184(9), 2332–2347.e16. <https://doi.org/10.1016/j.cell.2021.03.028>.
- McCallum, M., Walls, A. C., Sprouse, K. R., Bowen, J. E., Rosen, L. E., Dang, H. V., et al. (2021). Molecular basis of immune evasion by the Delta and Kappa SARS-CoV-2 variants. *Science (New York, N.Y.)*, 374(6575), 1621–1626. <https://doi.org/10.1126/science.abl8506>.
- McLellan, J. S., Chen, M., Joyce, M. G., Sastry, M., Stewart-Jones, G. B. E., Yang, Y., et al. (2013). Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus. *Science*, 342(6158), 592–598. <https://doi.org/10.1126/science.1243283>.
- McMahan, K., Yu, J., Mercado, N. B., Loos, C., Tostanoski, L. H., Chandrashekar, A., et al. (2021). Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature*, 590(7847), 630–634. <https://doi.org/10.1038/s41586-020-03041-6>.
- Mehdipour, A. R., & Hummer, G. (2021). Dual nature of human ACE2 glycosylation in binding to SARS-CoV-2 spike. *Proceedings of the National Academy of Sciences*, 118(19), e2100425118. <https://doi.org/10.1073/pnas.2100425118>.
- Meng, B., Kemp, S. A., Papa, G., Datir, R., Ferreira, I. A. T. M., Marelli, S., et al. (2021). Recurrent emergence of SARS-CoV-2 spike deletion H69/V70 and its role in the Alpha variant B.1.1.7. *Cell Reports*, 35(13), 109292. <https://doi.org/10.1016/j.celrep.2021.109292>.
- Mlcochova, P., Kemp, S. A., Dhar, M. S., Papa, G., Meng, B., Ferreira, I. A. T. M., et al. (2021). SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. *Nature*, 599(7883), 114–119. <https://doi.org/10.1038/s41586-021-03944-y>.

- Muik, A., Wallisch, A.-K., Sanger, B., Swanson, K. A., Muhl, J., Chen, W., et al. (2021). Neutralization of SARS-CoV-2 lineage B.1.1.7 pseudovirus by BNT162b2 vaccine-elicited human sera. *Science*, 371(6534), 1152–1153. <https://doi.org/10.1126/science.abg6105>.
- Mulligan, M. J., Lyke, K. E., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., et al. (2020). Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature*, 586(7830), 589–593. <https://doi.org/10.1038/s41586-020-2639-4>.
- Naqvi, A. A. T., Fatima, K., Mohammad, T., Fatima, U., Singh, I. K., Singh, A., et al. (2020). Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. *Biochimica et Biophysica Acta, Molecular Basis of Disease*, 1866(10), 165878. <https://doi.org/10.1016/j.bbadis.2020.165878>.
- Ng, K. W., Faulkner, N., Cornish, G. H., Rosa, A., Harvey, R., Hussain, S., et al. (2020). Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science (New York, N.Y.)*, 370(6522), 1339–1343. <https://doi.org/10.1126/science.abe1107>.
- Noy-Porat, T., Edri, A., Alcalay, R., Makdasi, E., Gur, D., Aftalion, M., et al. (2021). Fc-independent protection from SARS-CoV-2 infection by recombinant human monoclonal antibodies. *Antibodies*, 10(4), 45. <https://doi.org/10.3390/antib10040045>.
- Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., et al. (2020). Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nature Communications*, 11(1), 1620. <https://doi.org/10.1038/s41467-020-15562-9>.
- Pallesen, J., Wang, N., Corbett, K. S., Wrapp, D., Kirchdoerfer, R. N., Turner, H. L., et al. (2017). Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. *Proceedings of the National Academy of Sciences of the United States of America*, 114(35), E7348–E7357. <https://doi.org/10.1073/pnas.1707304114>.
- Pardi, N., Hogan, M. J., Porter, F. W., & Weissman, D. (2018). mRNA vaccines—A new era in vaccinology. *Nature Reviews Drug Discovery*, 17(4), 261–279. <https://doi.org/10.1038/nrd.2017.243>.
- Park, Y.-J., De Marco, A., Starr, T. N., Liu, Z., Pinto, D., Walls, A. C., et al. (2022). Antibody-mediated broad sarbecovirus neutralization through ACE2 molecular mimicry. *Science (New York, N.Y.)*, 375(6579), 449–454. <https://doi.org/10.1126/science.abm8143>.
- Park, J.-G., Oladunni, F. S., Rohaim, M. A., Whittingham-Dowd, J., Tollitt, J., Hodges, M. D. J., et al. (2021). Immunogenicity and protective efficacy of an intranasal live-attenuated vaccine against SARS-CoV-2. *iScience*, 24(9), 102941. <https://doi.org/10.1016/j.isci.2021.102941>.
- Park, Y.-J., Walls, A. C., Wang, Z., Sauer, M. M., Li, W., Tortorici, M. A., et al. (2019). Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nature Structural & Molecular Biology*, 26(12), 1151–1157. <https://doi.org/10.1038/s41594-019-0334-7>.
- Peng, G., Sun, D., Rajashankar, K. R., Qian, Z., Holmes, K. V., & Li, F. (2011). Crystal structure of mouse coronavirus receptor-binding domain complexed with its murine receptor. *Proceedings of the National Academy of Sciences of the United States of America*, 108(26), 10696–10701. <https://doi.org/10.1073/pnas.1104306108>.
- Peng, G., Xu, L., Lin, Y. L., Chen, L., Pasquarella, J. R., Holmes, K. V., et al. (2012). Crystal structure of bovine coronavirus spike protein lectin domain. *Journal of Biological Chemistry*, 287(50), 41931–41938. <https://doi.org/10.1074/jbc.M112.418210>.
- Piccoli, L., Park, Y.-J., Tortorici, M. A., Czudnochowski, N., Walls, A. C., Beltramello, M., et al. (2020). Mapping neutralizing and immunodominant sites on the SARS-CoV-2 spike receptor-binding domain by structure-guided high-resolution serology. *Cell*, 183(4), 1024–1042.e21. <https://doi.org/10.1016/j.cell.2020.09.037>.

- Pinto, D., Park, Y.-J., Beltramello, M., Walls, A. C., Tortorici, M. A., Bianchi, S., et al. (2020). Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature*, 583(7815), 290–295. <https://doi.org/10.1038/s41586-020-2349-y>.
- Pinto, D., Sauer, M. M., Czudnochowski, N., Low, J. S., Tortorici, M. A., Housley, M. P., et al. (2021). Broad betacoronavirus neutralization by a stem helix-specific human antibody. *Science (New York, N.Y.)*, 373(6559), 1109–1116. <https://doi.org/10.1126/science.abj3321>.
- Planas, D., Bruel, T., Grzelak, L., Guivel-Benhassine, F., Staropoli, I., Porrot, F., et al. (2021). Sensitivity of infectious SARS-CoV-2 B.1.1.7 and B.1.351 variants to neutralizing antibodies. *Nature Medicine*, 27(5), 917–924. <https://doi.org/10.1038/s41591-021-01318-5>.
- Planas, D., Saunders, N., Maes, P., Guivel-Benhassine, F., Planchais, C., Buchrieser, J., et al. (2022). Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. *Nature*, 602(7898), 671–675. <https://doi.org/10.1038/s41586-021-04389-z>.
- Planas, D., Veyer, D., Baidaliuk, A., Staropoli, I., Guivel-Benhassine, F., Rajah, M. M., et al. (2021). Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. *Nature*, 596(7871), 276–280. <https://doi.org/10.1038/s41586-021-03777-9>.
- Poh, C. M., Carissimo, G., Wang, B., Amrun, S. N., Lee, C. Y.-P., Chee, R. S.-L., et al. (2020). Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising antibodies in COVID-19 patients. *Nature Communications*, 11(1), 2806. <https://doi.org/10.1038/s41467-020-16638-2>.
- Polack, F. P., Thomas, S. J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., et al. (2020). Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *The New England Journal of Medicine*, 383(27), 2603–2615. <https://doi.org/10.1056/NEJMoa2034577>.
- Premkumar, L., Segovia-Chumbez, B., Jadi, R., Martinez, D. R., Raut, R., Markmann, A., et al. (2020). The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. *Science Immunology*, 5(48), eabc8413. <https://doi.org/10.1126/sciimmunol.abc8413>.
- Qing, E., Kicmal, T., Kumar, B., Hawkins, G. M., Timm, E., Perlman, S., et al. (2021). Dynamics of SARS-CoV-2 spike proteins in cell entry: Control elements in the amino-terminal domains. *mBio*, 12(4), e0159021.
- Qing, E., Li, P., Cooper, L., Schulz, S., Jäck, H.-M., Rong, L., et al. (2022). Inter-domain communication in SARS-CoV-2 spike proteins controls protease-triggered cell entry. *Cell Reports*, 39(5), 110786. <https://doi.org/10.1016/j.celrep.2022.110786>.
- Rambaut, A., Holmes, E. C., O’Toole, Á., Hill, V., McCrone, J. T., Ruis, C., et al. (2020). A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nature Microbiology*, 5(11), 1403–1407. <https://doi.org/10.1038/s41564-020-0770-5>.
- Rapp, M., Guo, Y., Reddem, E. R., Yu, J., Liu, L., Wang, P., et al. (2021). Modular basis for potent SARS-CoV-2 neutralization by a prevalent VH1-2-derived antibody class. *Cell Reports*, 35(1), 108950. <https://doi.org/10.1016/j.celrep.2021.108950>.
- Rappazzo, C. G., Tse, L. V., Kaku, C. I., Wrapp, D., Sakharkar, M., Huang, D., et al. (2021). Broad and potent activity against SARS-like viruses by an engineered human monoclonal antibody. *Science (New York, N.Y.)*, 371(6531), 823–829. <https://doi.org/10.1126/science.abf4830>.
- Rattanapit, K., Shanmugaraj, B., Manopwisedjaroen, S., Purwono, P. B., Siri wattananon, K., Khorattanakulchai, N., et al. (2020). Rapid production of SARS-CoV-2 receptor binding domain (RBD) and spike specific monoclonal antibody CR3022 in *Nicotiana benthamiana*. *Scientific Reports*, 10(1), 17698. <https://doi.org/10.1038/s41598-020-74904-1>.
- Robbiani, D. F., Gaebler, C., Muecksch, F., Lorenzi, J. C. C., Wang, Z., Cho, A., et al. (2020). Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature*, 584(7821), 437–442. <https://doi.org/10.1038/s41586-020-2456-9>.

- Rogers, T. F., Zhao, F., Huang, D., Beutler, N., Burns, A., He, W.-T., et al. (2020). Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. *Science (New York, N.Y.)*, 369(6506), 956–963. <https://doi.org/10.1126/science.abc7520>.
- Rosa, A., Pye, V. E., Graham, C., Muir, L., Seow, J., Ng, K. W., et al. (2021). SARS-CoV-2 can recruit a heme metabolite to evade antibody immunity. *Science Advances*, 7(22). <https://doi.org/10.1126/sciadv.abg7607>.
- Russell, M. W., Moldoveanu, Z., Ogra, P. L., & Mestecky, J. (2020). Mucosal immunity in COVID-19: A neglected but critical aspect of SARS-CoV-2 infection. *Frontiers in Immunology*, 11. <https://doi.org/10.3389/fimmu.2020.611337>.
- Ryu, D.-K., Kang, B., Noh, H., Woo, S.-J., Lee, M.-H., Nuijten, P. M., et al. (2021). The in vitro and in vivo efficacy of CT-P59 against gamma, delta and its associated variants of SARS-CoV-2. *Biochemical and Biophysical Research Communications*, 578, 91–96. <https://doi.org/10.1016/j.bbrc.2021.09.023>.
- Ryu, D.-K., Song, R., Kim, M., Kim, Y.-I., Kim, C., Kim, J.-I., et al. (2021). Therapeutic effect of CT-P59 against SARS-CoV-2 South African variant. *Biochemical and Biophysical Research Communications*, 566, 135–140. <https://doi.org/10.1016/j.bbrc.2021.06.016>.
- Sabino, E. C., Buss, L. F., Carvalho, M. P. S., Prete, C. A., Crispim, M. A. E., Fraiji, N. A., et al. (2021). Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. *The Lancet*, 397(10273), 452–455. [https://doi.org/10.1016/S0140-6736\(21\)00183-5](https://doi.org/10.1016/S0140-6736(21)00183-5).
- Sadoff, J., Gray, G., Vandebosch, A., Cárdenas, V., Shukarev, G., Grinsztejn, B., et al. (2021). Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19. *New England Journal of Medicine*, 384(23), 2187–2201. <https://doi.org/10.1056/NEJMoa2101544>.
- Sauer, M. M., Tortorici, M. A., Park, Y.-J., Walls, A. C., Homad, L., Acton, O. J., et al. (2021). Structural basis for broad coronavirus neutralization. *Nature Structural & Molecular Biology*, 28(6), 478–486. <https://doi.org/10.1038/s41594-021-00596-4>.
- Saxena, S. K., Kumar, S., Ansari, S., Paweska, J. T., Maurya, V. K., Tripathi, A. K., et al. (2022). Characterization of the novel SARS-CoV-2 Omicron (B.1.1.529) variant of concern and its global perspective. *Journal of Medical Virology*, 94(4), 1738–1744. <https://doi.org/10.1002/jmv.27524>.
- Schäfer, A., Muecksch, F., Lorenzi, J. C. C., Leist, S. R., Cipolla, M., Bournazos, S., et al. (2020). Antibody potency, effector function, and combinations in protection and therapy for SARS-CoV-2 infection in vivo In vivo efficacy of anti-SARS-CoV-2 antibodies. *Journal of Experimental Medicine*, 218(3), e20201993. <https://doi.org/10.1084/jem.20201993>.
- Scheid, J. F., Barnes, C. O., Eraslan, B., Hudak, A., Keeffe, J. R., Cosimi, L. A., et al. (2021). B cell genomics behind cross-neutralization of SARS-CoV-2 variants and SARS-CoV. *Cell*, 184(12), 3205–3221.e24. <https://doi.org/10.1016/j.cell.2021.04.032>.
- Schmidt, F., Muecksch, F., Weisblum, Y., Da Silva, J., Bednarski, E., Cho, A., et al. (2022). Plasma neutralization of the SARS-CoV-2 Omicron variant. *New England Journal of Medicine*, 386(6), 599–601. <https://doi.org/10.1056/NEJMc2119641>.
- Schmidt, F., Weisblum, Y., Rutkowska, M., Poston, D., DaSilva, J., Zhang, F., et al. (2021). High genetic barrier to SARS-CoV-2 polyclonal neutralizing antibody escape. *Nature*, 600(7889), 512–516. <https://doi.org/10.1038/s41586-021-04005-0>.
- Schoof, M., Faust, B., Saunders, R. A., Sangwan, S., Rezelj, V., Hoppe, N., et al. (2020). An ultrapotent synthetic nanobody neutralizes SARS-CoV-2 by stabilizing inactive Spike. *Science (New York, N.Y.)*, 370(6523), 1473–1479. <https://doi.org/10.1126/science.abe3255>.
- Schultze, B., Gross, H. J., Brossmer, R., & Herrler, G. (1991). The S protein of bovine coronavirus is a hemagglutinin recognizing 9-O-acetylated sialic acid as a receptor determinant. *Journal of Virology*, 65(11), 6232–6237. <https://doi.org/10.1128/JVI.65.11.6232-6237.1991>.

- Seow, J., Graham, C., Merrick, B., Acors, S., Pickering, S., Steel, K. J. A., et al. (2020). Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nature Microbiology*, 5(12), 1598–1607. <https://doi.org/10.1038/s41564-020-00813-8>.
- Shah, P., Canziani, G. A., Carter, E. P., & Chaiken, I. (2021). The case for S2: The potential benefits of the S2 subunit of the SARS-CoV-2 spike protein as an immunogen in fighting the COVID-19 pandemic. *Frontiers in Immunology*, 12. <https://doi.org/10.3389/fimmu.2021.637651>.
- Shen, X., Tang, H., McDanal, C., Wagh, K., Fischer, W., Theiler, J., et al. (2021). SARS-CoV-2 variant B.1.1.7 is susceptible to neutralizing antibodies elicited by ancestral spike vaccines. *Cell Host & Microbe*, 29(4), 529–539.e3. <https://doi.org/10.1016/j.chom.2021.03.002>.
- Shi, R., Shan, C., Duan, X., Chen, Z., Liu, P., Song, J., et al. (2020). A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. *Nature*, 584(7819), 120–124. <https://doi.org/10.1038/s41586-020-2381-y>.
- Shiakolas, A. R., Kramer, K. J., Wrapp, D., Richardson, S. I., Schäfer, A., Wall, S., et al. (2021). Cross-reactive coronavirus antibodies with diverse epitope specificities and Fc effector functions. *Cell Reports Medicine*, 2(6), 100313. <https://doi.org/10.1016/j.xcrm.2021.100313>.
- Shrock, E., Fujimura, E., Kula, T., Timms, R. T., Lee, I.-H., Leng, Y., et al. (2020). Viral epitope profiling of COVID-19 patients reveals cross-reactivity and correlates of severity. *Science*, 370(6520), eabd4250. <https://doi.org/10.1126/science.abd4250>.
- Sia, S. F., Yan, L.-M., Chin, A. W. H., Fung, K., Choy, K.-T., Wong, A. Y. L., et al. (2020). Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature*, 583(7818), 834–838. <https://doi.org/10.1038/s41586-020-2342-5>.
- Song, G., He, W., Callaghan, S., Anzanello, F., Huang, D., Ricketts, J., et al. (2021). Cross-reactive serum and memory B-cell responses to spike protein in SARS-CoV-2 and endemic coronavirus infection. *Nature Communications*, 12(1), 2938. <https://doi.org/10.1038/s41467-021-23074-3>.
- Stamatatos, L., Czartoski, J., Wan, Y.-H., Homad, L. J., Rubin, V., Glantz, H., et al. (2021). mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. *Science*, 372(6549), 1413–1418. <https://doi.org/10.1126/science.abg9175>.
- Starr, T. N., Czudnochowski, N., Liu, Z., Zatta, F., Park, Y.-J., Addetia, A., et al. (2021). SARS-CoV-2 RBD antibodies that maximize breadth and resistance to escape. *Nature*, 597(7874), 97–102. <https://doi.org/10.1038/s41586-021-03807-6>.
- Starr, T. N., Greaney, A. J., Dingens, A. S., & Bloom, J. D. (2021). Complete map of SARS-CoV-2 RBD mutations that escape the monoclonal antibody LY-CoV555 and its cocktail with LY-CoV016. *Cell Reports Medicine*, 2(4), 100255. <https://doi.org/10.1016/j.xcrm.2021.100255>.
- Starr, T. N., Greaney, A. J., Hilton, S. K., Ellis, D., Crawford, K. H. D., Dingens, A. S., et al. (2020). Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE2 binding. *Cell*, 182(5), 1295–1310.e20. <https://doi.org/10.1016/j.cell.2020.08.012>.
- Steffen, T. L., Stone, E. T., Hassert, M., Geerling, E., Grimberg, B. T., Espino, A. M., et al. (2020). The receptor binding domain of SARS-CoV-2 spike is the key target of neutralizing antibody in human polyclonal sera (p. 2020.08.21.261727). *bioRxiv*. <https://doi.org/10.1101/2020.08.21.261727>.
- Sterlin, D., Mathian, A., Miyara, M., Mohr, A., Anna, F., Claër, L., et al. (2021). IgA dominates the early neutralizing antibody response to SARS-CoV-2. *Science Translational Medicine*, 13(577), eabd2223. <https://doi.org/10.1126/scitranslmed.abd2223>.

- Sun, Y., Wang, L., Feng, R., Wang, N., Wang, Y., Zhu, D., et al. (2021). Structure-based development of three- and four-antibody cocktails against SARS-CoV-2 via multiple mechanisms. *Cell Research*, 31(5), 597–600. <https://doi.org/10.1038/s41422-021-00497-7>.
- Supasa, P., Zhou, D., Dejnirattisai, W., Liu, C., Mentzer, A. J., Ginn, H. M., et al. (2021). Reduced neutralization of SARS-CoV-2 B.1.1.7 variant by convalescent and vaccine sera. *Cell*, 184(8), 2201–2211.e7. <https://doi.org/10.1016/j.cell.2021.02.033>.
- Suryadevara, N., Shiakolas, A. R., VanBlargan, L. A., Binshtein, E., Chen, R. E., Case, J. B., et al. (2022). An antibody targeting the N-terminal domain of SARS-CoV-2 disrupts the spike trimer. *The Journal of Clinical Investigation*, 132(11), e159062. <https://doi.org/10.1172/JCI159062>.
- Suryadevara, N., Shrihari, S., Gilchuk, P., VanBlargan, L. A., Binshtein, E., Zost, S. J., et al. (2021). Neutralizing and protective human monoclonal antibodies recognizing the N-terminal domain of the SARS-CoV-2 spike protein. *Cell*, 184(9), 2316–2331.e15. <https://doi.org/10.1016/j.cell.2021.03.029>.
- Takeshita, M., Nishina, N., Moriyama, S., Takahashi, Y., Uwamino, Y., Nagata, M., et al. (2021). Incomplete humoral response including neutralizing antibodies in asymptomatic to mild COVID-19 patients in Japan. *Virology*, 555, 35–43. <https://doi.org/10.1016/j.virol.2020.12.020>.
- Tan, T. J. C., Yuan, M., Kuzelka, K., Padron, G. C., Beal, J. R., Chen, X., et al. (2021). Sequence signatures of two public antibody clonotypes that bind SARS-CoV-2 receptor binding domain. *Nature Communications*, 12(1), 3815. <https://doi.org/10.1038/s41467-021-24123-7>.
- Tanaka, S., Olson, C. A., Barnes, C. O., Higashide, W., Gonzalez, M., Taft, J., et al. (2022). Rapid identification of neutralizing antibodies against SARS-CoV-2 variants by mRNA display. *Cell Reports*, 38(6), 110348. <https://doi.org/10.1016/j.celrep.2022.110348>.
- Tegally, H., Wilkinson, E., Giovanetti, M., Iranzadeh, A., Fonseca, V., Giandhari, J., et al. (2021). Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature*, 592(7854), 438–443. <https://doi.org/10.1038/s41586-021-03402-9>.
- Temmam, S., Vongphayloth, K., Baquero, E., Munier, S., Bonomi, M., Regnault, B., et al. (2022). Bat coronaviruses related to SARS-CoV-2 and infectious for human cells. *Nature*, 604(7905), 330–336. <https://doi.org/10.1038/s41586-022-04532-4>.
- ter Meulen, J., van den Brink, E. N., Poon, L. L. M., Marissen, W. E., Leung, C. S. W., Cox, F., et al. (2006). Human monoclonal antibody combination against SARS coronavirus: Synergy and coverage of escape mutants. *PLoS Medicine*, 3(7), e237. <https://doi.org/10.1371/journal.pmed.0030237>.
- Tian, X., Li, C., Huang, A., Xia, S., Lu, S., Shi, Z., et al. (2020). Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. *Emerging Microbes & Infections*, 9(1), 382–385. <https://doi.org/10.1080/22221751.2020.1729069>.
- Tong, P., Gautam, A., Windsor, I. W., Travers, M., Chen, Y., Garcia, N., et al. (2021). Memory B cell repertoire for recognition of evolving SARS-CoV-2 spike. *Cell*, 184(19), 4969–4980.e15. <https://doi.org/10.1016/j.cell.2021.07.025>.
- Tortorici, M. A., Beltramello, M., Lempp, F. A., Pinto, D., Dang, H. V., Rosen, L. E., et al. (2020). Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science (New York, N.Y.)*, 370(6519), 950–957. <https://doi.org/10.1126/science.abe3354>.
- Tortorici, M. A., Czudnochowski, N., Starr, T. N., Marzi, R., Walls, A. C., Zatta, F., et al. (2021). Broad sarbecovirus neutralization by a human monoclonal antibody. *Nature*, 597(7874), 103–108. <https://doi.org/10.1038/s41586-021-03817-4>.
- Tortorici, M. A., & Veesler, D. (2019). Chapter Four—Structural insights into coronavirus entry. In F. A. Rey (Ed.), *Vol. 105. Advances in virus research* (pp. 93–116). Academic Press. <https://doi.org/10.1016/bs.aivir.2019.08.002>.

- Tortorici, M. A., Walls, A. C., Lang, Y., Wang, C., Li, Z., Koerhuis, D., et al. (2019). Structural basis for human coronavirus attachment to sialic acid receptors. *Nature Structural & Molecular Biology*, 26(6), 481–489. <https://doi.org/10.1038/s41594-019-0233-y>.
- Turner, J. S., Kim, W., Kalaidina, E., Goss, C. W., Rauseo, A. M., Schmitz, A. J., et al. (2021). SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans. *Nature*, 595(7867), 421–425. <https://doi.org/10.1038/s41586-021-03647-4>.
- Turner, J. S., O'Halloran, J. A., Kalaidina, E., Kim, W., Schmitz, A. J., Zhou, J. Q., et al. (2021). SARS-CoV-2 mRNA vaccines induce persistent human germinal centre responses. *Nature*, 596(7870), 109–113. <https://doi.org/10.1038/s41586-021-03738-2>.
- Ullah, I., Prévost, J., Ladinsky, M. S., Stone, H., Lu, M., Anand, S. P., et al. (2021). Live imaging of SARS-CoV-2 infection in mice reveals that neutralizing antibodies require Fc function for optimal efficacy. *Immunity*, 54(9), 2143–2158.e15. <https://doi.org/10.1016/j.immuni.2021.08.015>.
- Ulrich, L., Halwe, N. J., Taddeo, A., Ebert, N., Schön, J., Devisme, C., et al. (2022). Enhanced fitness of SARS-CoV-2 variant of concern Alpha but not Beta. *Nature*, 602(7896), 307–313. <https://doi.org/10.1038/s41586-021-04342-0>.
- van Doremalen, N., Bushmaker, T., Morris, D. H., Holbrook, M. G., Gamble, A., Williamson, B. N., et al. (2020). Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *New England Journal of Medicine*, 382(16), 1564–1567. <https://doi.org/10.1056/nejmc2004973>.
- van Doremalen, N., Purushotham, J. N., Schulz, J. E., Holbrook, M. G., Bushmaker, T., Carmody, A., et al. (2021). Intranasal ChAdOx1 nCoV-19/AZD1222 vaccination reduces viral shedding after SARS-CoV-2 D614G challenge in preclinical models. *Science Translational Medicine*, 13(607), eabh0755. <https://doi.org/10.1126/scitranslmed.abh0755>.
- VanBlargan, L. A., Adams, L. J., Liu, Z., Chen, R. E., Gilchuk, P., Raju, S., et al. (2021). A potentially neutralizing SARS-CoV-2 antibody inhibits variants of concern by utilizing unique binding residues in a highly conserved epitope. *Immunity*, 54(10), 2399–2416.e6. <https://doi.org/10.1016/j.immuni.2021.08.016>.
- VanBlargan, L. A., Errico, J. M., Halfmann, P. J., Zost, S. J., Crowe, J. E. J., Purcell, L. A., et al. (2022). An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. *Nature Medicine*, 1–6. <https://doi.org/10.1038/s41591-021-01678-y>.
- Viana, R., Moyo, S., Amoako, D. G., Tegally, H., Scheepers, C., Althaus, C. L., et al. (2022). Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. *Nature*, 603(7902), 679–686. <https://doi.org/10.1038/s41586-022-04411-y>.
- Vignier, N., Bérot, V., Bonnave, N., Peugny, S., Ballet, M., Jacoud, E., et al. (2021). Breakthrough Infections of SARS-CoV-2 gamma variant in fully vaccinated gold miners, French Guiana, 2021. *Emerging Infectious Diseases*, 27(10), 2673–2676. <https://doi.org/10.3201/eid2710.211427>.
- Volz, E., Hill, V., McCrone, J. T., Price, A., Jorgensen, D., O'Toole, Á., et al. (2021). Evaluating the effects of SARS-CoV-2 spike mutation D614G on transmissibility and pathogenicity. *Cell*, 184(1), 64–75.e11. <https://doi.org/10.1016/j.cell.2020.11.020>.
- Volz, E., Mishra, S., Chand, M., Barrett, J. C., Johnson, R., Geidelberg, L., et al. (2021). Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England. *Nature*, 593(7858), 266–269. <https://doi.org/10.1038/s41586-021-03470-x>.
- Voss, W. N., Hou, Y. J., Johnson, N. V., Delidakis, G., Kim, J. E., Javanmardi, K., et al. (2021). Prevalent, protective, and convergent IgG recognition of SARS-CoV-2 non-RBD spike epitopes. *Science (New York, N.Y.)*, 372(6546), 1108–1112. <https://doi.org/10.1126/science.abg5268>.
- Voysey, M., Clemens, S. A. C., Madhi, S. A., Weckx, L. Y., Folegatti, P. M., Aley, P. K., et al. (2021). Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: An interim analysis of four randomised controlled trials in Brazil, South

- Africa, and the UK. *The Lancet*, 397(10269), 99–111. [https://doi.org/10.1016/S0140-6736\(20\)32661-1](https://doi.org/10.1016/S0140-6736(20)32661-1).
- Walls, A. C., Miranda, M. C., Schäfer, A., Pham, M. N., Greaney, A., Arunachalam, P. S., et al. (2021). Elicitation of broadly protective sarbecovirus immunity by receptor-binding domain nanoparticle vaccines. *Cell*, 184(21), 5432–5447.e16. <https://doi.org/10.1016/j.cell.2021.09.015>.
- Walls, A. C., Park, Y.-J., Tortorici, M. A., Wall, A., McGuire, A. T., & Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell*, 181(2), 281–292.e6. <https://doi.org/10.1016/j.cell.2020.02.058>.
- Walls, A. C., Tortorici, M. A., Bosch, B.-J., Frenz, B., Rottier, P. J. M., DiMaio, F., et al. (2016). Cryo-electron microscopy structure of a coronavirus spike glycoprotein trimer. *Nature*, 531(7592), 114–117. <https://doi.org/10.1038/nature16988>.
- Walls, A. C., Tortorici, M. A., Frenz, B., Snijder, J., Li, W., Rey, F. A., et al. (2016). Glycan shield and epitope masking of a coronavirus spike protein observed by cryo-electron microscopy. *Nature Structural & Molecular Biology*, 23(10), 899–905. <https://doi.org/10.1038/nsmb.3293>.
- Walls, A. C., Xiong, X., Park, Y.-J., Tortorici, M. A., Snijder, J., Quispe, J., et al. (2019). Unexpected receptor functional mimicry elucidates activation of Coronavirus fusion. *Cell*, 176(5), 1026–1039.e15. <https://doi.org/10.1016/j.cell.2018.12.028>.
- Wang, P., Casner, R. G., Nair, M. S., Wang, M., Yu, J., Cerutti, G., et al. (2021). Increased resistance of SARS-CoV-2 variant P.1 to antibody neutralization. *Cell Host & Microbe*, 29(5), 747–751.e4. <https://doi.org/10.1016/j.chom.2021.04.007>.
- Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., et al. (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*, 323(11), 1061–1069. <https://doi.org/10.1001/jama.2020.1585>.
- Wang, C., Li, W., Drabek, D., Okba, N. M. A., van Haperen, R., Osterhaus, A. D. M. E., et al. (2020). A human monoclonal antibody blocking SARS-CoV-2 infection. *Nature Communications*, 11(1), 2251. <https://doi.org/10.1038/s41467-020-16256-y>.
- Wang, S., Qiu, Z., Hou, Y., Deng, X., Xu, W., Zheng, T., et al. (2021). AXL is a candidate receptor for SARS-CoV-2 that promotes infection of pulmonary and bronchial epithelial cells. *Cell Research*, 31(2), 126–140. <https://doi.org/10.1038/s41422-020-00460-y>.
- Wang, Z., Lorenzi, J. C. C., Muecksch, F., Finkin, S., Viant, C., Gaebler, C., et al. (2021). Enhanced SARS-CoV-2 neutralization by dimeric IgA. *Science Translational Medicine*, 13(577), eabf1555. <https://doi.org/10.1126/scitranslmed.abf1555>.
- Wang, Z., Muecksch, F., Cho, A., Gaebler, C., Hoffmann, H.-H., Ramos, V., et al. (2022). Analysis of memory B cells identifies conserved neutralizing epitopes on the N-terminal domain of variant SARS-Cov-2 spike proteins. *Immunity*. <https://doi.org/10.1016/j.immuni.2022.04.003>.
- Wang, P., Nair, M. S., Liu, L., Iketani, S., Luo, Y., Guo, Y., et al. (2021). Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature*, 593(7857), 130–135. <https://doi.org/10.1038/s41586-021-03398-2>.
- Wang, H., Paulson, K. R., Pease, S. A., Watson, S., Comfort, H., Zheng, P., et al. (2022). Estimating excess mortality due to the COVID-19 pandemic: A systematic analysis of COVID-19-related mortality, 2020–21. *The Lancet*, 399(10334), 1513–1536. [https://doi.org/10.1016/s0140-6736\(21\)02796-3](https://doi.org/10.1016/s0140-6736(21)02796-3).
- Wang, Z., Schmidt, F., Weisblum, Y., Muecksch, F., Barnes, C. O., Finkin, S., et al. (2021). MRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. *Nature*, 592(7855), 616–622. <https://doi.org/10.1038/s41586-021-03324-6>.
- Wang, N., Shi, X., Jiang, L., Zhang, S., Wang, D., Tong, P., et al. (2013). Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. *Cell Research*, 23(8), 986–993. <https://doi.org/10.1038/cr.2013.92>.

- Wang, C., van Haperen, R., Gutiérrez-Álvarez, J., Li, W., Okba, N. M. A., Albulescu, I., et al. (2021). A conserved immunogenic and vulnerable site on the coronavirus spike protein delineated by cross-reactive monoclonal antibodies. *Nature Communications*, 12(1), 1715. <https://doi.org/10.1038/s41467-021-21968-w>.
- Wang, R., Zhang, Q., Ge, J., Ren, W., Zhang, R., Lan, J., et al. (2021). Analysis of SARS-CoV-2 variant mutations reveals neutralization escape mechanisms and the ability to use ACE2 receptors from additional species. *Immunity*, 54(7), 1611–1621.e5. <https://doi.org/10.1016/j.immuni.2021.06.003>.
- Wang, L., Zhou, T., Zhang, Y., Yang, E. S., Schramm, C. A., Shi, W., et al. (2021). Ultrapotent antibodies against diverse and highly transmissible SARS-CoV-2 variants. *Science (New York, N.Y.)*, 373(6556), eabh1766. <https://doi.org/10.1126/science.abh1766>.
- Washington, N. L., Gangavarapu, K., Zeller, M., Bolze, A., Cirulli, E. T., Schiabor Barrett, K. M., et al. (2021). Emergence and rapid transmission of SARS-CoV-2 B.1.1.7 in the United States. *Cell*, 184(10), 2587–2594.e7. <https://doi.org/10.1016/j.cell.2021.03.052>.
- Watanabe, Y., Allen, J. D., Wrapp, D., McLellan, J. S., & Crispin, M. (2020). Site-specific glycan analysis of the SARS-CoV-2 spike. *Science*, 369(6501), 330–333. <https://doi.org/10.1126/science.abb9983>.
- Wec, A. Z., Wrapp, D., Herbert, A. S., Maurer, D. P., Haslwanter, D., Sakharkar, M., et al. (2020). Broad neutralization of SARS-related viruses by human monoclonal antibodies. *Science (New York, N.Y.)*, 369(6504), 731–736. <https://doi.org/10.1126/science.abc7424>.
- Wei, C., Wan, L., Yan, Q., Wang, X., Zhang, J., Yang, X., et al. (2020). HDL-scavenger receptor B type 1 facilitates SARS-CoV-2 entry. *Nature Metabolism*, 2(12), 1391–1400. <https://doi.org/10.1038/s42255-020-00324-0>.
- Weisblum, Y., Schmidt, F., Zhang, F., DaSilva, J., Poston, D., Lorenzi, J. C., et al. (2020). Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. *eLife*, 9, e61312. <https://doi.org/10.7554/eLife.61312>.
- Wheatley, A. K., Juno, J. A., Wang, J. J., Selva, K. J., Reynaldi, A., Tan, H.-X., et al. (2021). Evolution of immune responses to SARS-CoV-2 in mild-moderate COVID-19. *Nature Communications*, 12(1), 1162. <https://doi.org/10.1038/s41467-021-21444-5>.
- Wibmer, C. K., Ayres, F., Hermanus, T., Madzivhandila, M., Kgagudi, P., Oosthuysen, B., et al. (2021). SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. *Nature Medicine*, 27(4), 622–625. <https://doi.org/10.1038/s41591-021-01285-x>.
- Winkler, E. S., Gilchuk, P., Yu, J., Bailey, A. L., Chen, R. E., Chong, Z., et al. (2021). Human neutralizing antibodies against SARS-CoV-2 require intact Fc effector functions for optimal therapeutic protection. *Cell*, 184(7), 1804–1820.e16. <https://doi.org/10.1016/j.cell.2021.02.026>.
- Wrapp, D., De Vlioger, D., Corbett, K. S., Torres, G. M., Wang, N., Van Breedam, W., et al. (2020). Structural basis for potent neutralization of betacoronaviruses by single-domain camelid antibodies. *Cell*, 181(5), 1004–1015.e15. <https://doi.org/10.1016/j.cell.2020.04.031>.
- Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C.-L., Abiona, O., et al. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science (New York, N.Y.)*, 367(6483), 1260–1263. <https://doi.org/10.1126/science.abb2507>.
- Wrobel, A. G., Benton, D. J., Hussain, S., Harvey, R., Martin, S. R., Roustan, C., et al. (2020). Antibody-mediated disruption of the SARS-CoV-2 spike glycoprotein. *Nature Communications*, 11(1), 5337. <https://doi.org/10.1038/s41467-020-19146-5>.
- Wu, A., Peng, Y., Huang, B., Ding, X., Wang, X., Niu, P., et al. (2020). Genome composition and divergence of the novel Coronavirus (2019-nCoV) originating in China. *Cell Host & Microbe*, 27(3), 325–328. <https://doi.org/10.1016/j.chom.2020.02.001>.

- Wu, Y., Wang, F., Shen, C., Peng, W., Li, D., Zhao, C., et al. (2020). A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. *Science*, 368(6496), 1274–1278. <https://doi.org/10.1126/science.abc2241>.
- Wu, K., Werner, A. P., Koch, M., Choi, A., Narayanan, E., Stewart-Jones, G. B. E., et al. (2021). Serum neutralizing activity elicited by mRNA-1273 Vaccine. *The New England Journal of Medicine*, 384(15), 1468–1470. <https://doi.org/10.1056/NEJMc2102179>.
- Wu, N. C., Yuan, M., Bangaru, S., Huang, D., Zhu, X., Lee, C.-C. D., et al. (2020). A natural mutation between SARS-CoV-2 and SARS-CoV determines neutralization by a cross-reactive antibody. *PLoS Pathogens*, 16(12), e1009089. <https://doi.org/10.1371/journal.ppat.1009089>.
- Wu, N. C., Yuan, M., Liu, H., Lee, C.-C. D., Zhu, X., Bangaru, S., et al. (2020). An alternative binding mode of IGHV3-53 antibodies to the SARS-CoV-2 receptor binding domain. *Cell Reports*, 33(3), 108274. <https://doi.org/10.1016/j.celrep.2020.108274>.
- Wu, F., Zhao, S., Yu, B., Chen, Y.-M., Wang, W., Song, Z.-G., et al. (2020). A new coronavirus associated with human respiratory disease in China. *Nature*, 579(7798), 265–269 (<https://doi.org/10/dk2w>).
- Xia, S., Zhang, Y., Wang, H., Yang, Y., Gao, G. F., et al. (2021). Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: A randomised, double-blind, placebo-controlled, phase 1/2 trial. *The Lancet Infectious Diseases*, 21(1), 39–51. [https://doi.org/10.1016/S1473-3099\(20\)30831-8](https://doi.org/10.1016/S1473-3099(20)30831-8).
- Xia, S., Zhu, Y., Liu, M., Lan, Q., Xu, W., Wu, Y., et al. (2020). Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. *Cellular & Molecular Immunology*, 17(7), 765–767. <https://doi.org/10.1038/s41423-020-0374-2>.
- Xiong, X., Qu, K., Ciazynska, K. A., Hosmillo, M., Carter, A. P., Ebrahimi, S., et al. (2020). A thermostable, closed SARS-CoV-2 spike protein trimer. *Nature Structural & Molecular Biology*, 27(10), 934–941. <https://doi.org/10.1038/s41594-020-0478-5>.
- Yan, R., Wang, R., Ju, B., Yu, J., Zhang, Y., Liu, N., et al. (2021). Structural basis for bivalent binding and inhibition of SARS-CoV-2 infection by human potent neutralizing antibodies. *Cell Research*, 31(5), 517–525. <https://doi.org/10.1038/s41422-021-00487-9>.
- Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., & Zhou, Q. (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*, 367(6485), 1444–1448. <https://doi.org/10.1126/science.abb2762>.
- Yao, H., Song, Y., Chen, Y., Wu, N., Xu, J., Sun, C., et al. (2020). Molecular architecture of the SARS-CoV-2 virus. *Cell*, 183(3), 730–738.e13. <https://doi.org/10.1016/j.cell.2020.09.018>.
- Yi, C., Sun, X., Ye, J., Ding, L., Liu, M., Yang, Z., et al. (2020). Key residues of the receptor binding motif in the spike protein of SARS-CoV-2 that interact with ACE2 and neutralizing antibodies. *Cellular & Molecular Immunology*, 17(6), 621–630. <https://doi.org/10.1038/s41423-020-0458-z>.
- Yu, S., Zheng, X., Zhou, B., Li, J., Chen, M., Deng, R., et al. (2022). SARS-CoV-2 spike engagement of ACE2 primes S2' site cleavage and fusion initiation. *Proceedings of the National Academy of Sciences*, 119(1), e2111199119. <https://doi.org/10.1073/pnas.2111199119>.
- Yuan, M., Liu, H., Wu, N. C., Lee, C.-C. D., Zhu, X., Zhao, F., et al. (2020). Structural basis of a shared antibody response to SARS-CoV-2. *Science (New York, N.Y.)*, 369(6507), 1119–1123. <https://doi.org/10.1126/science.abd2321>.
- Yuan, M., Liu, H., Wu, N. C., & Wilson, I. A. (2021). Recognition of the SARS-CoV-2 receptor binding domain by neutralizing antibodies. *Biochemical and Biophysical Research Communications*, 538, 192–203. <https://doi.org/10.1016/j.bbrc.2020.10.012>.
- Yuan, M., Wu, N. C., Zhu, X., Lee, C.-C. D., So, R. T. Y., Lv, H., et al. (2020). A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. *Science (New York, N.Y.)*, 368(6491), 630–633. <https://doi.org/10.1126/science.abb7269>.

- Yurkovetskiy, L., Wang, X., Pascal, K. E., Tomkins-Tinch, C., Nyalile, T. P., Wang, Y., et al. (2020). Structural and functional analysis of the D614G SARS-CoV-2 spike protein variant. *Cell*, 183(3), 739–751.e8. <https://doi.org/10.1016/j.cell.2020.09.032>.
- Zhang, J., Cai, Y., Xiao, T., Lu, J., Peng, H., Sterling, S. M., et al. (2021). Structural impact on SARS-CoV-2 spike protein by D614G substitution. *Science (New York, N.Y.)*, 372(6541), 525–530. <https://doi.org/10.1126/science.abf2303>.
- Zhang, J., Xiao, T., Cai, Y., & Chen, B. (2021). Structure of SARS-CoV-2 spike protein. *Current Opinion in Virology*, 50, 173–182. <https://doi.org/10.1016/j.coviro.2021.08.010>.
- Zhang, L., Cao, L., Gao, X. S., Zheng, B. Y., Deng, Y. Q., Li, J. X., et al. (2021). A proof of concept for neutralizing antibody-guided vaccine design against SARS-CoV-2. *National Science Review*, 8(8), nwab053.
- Zhao, P., Praissman, J. L., Grant, O. C., Cai, Y., Xiao, T., Rosenbalm, K. E., et al. (2020). Virus-receptor interactions of glycosylated SARS-CoV-2 spike and human ACE2 receptor. *Cell Host & Microbe*, 28(4), 586–601.e6. <https://doi.org/10.1016/j.chom.2020.08.004>.
- Zhou, D., Dejnirattisai, W., Supasa, P., Liu, C., Mentzer, A. J., Ginn, H. M., et al. (2021). Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. *Cell*, 184(9), 2348–2361.e6. <https://doi.org/10.1016/j.cell.2021.02.037>.
- Zhou, D., Duyvesteyn, H. M. E., Chen, C.-P., Huang, C.-G., Chen, T.-H., Shih, S.-R., et al. (2020). Structural basis for the neutralization of SARS-CoV-2 by an antibody from a convalescent patient. *Nature Structural & Molecular Biology*, 27(10), 950–958. <https://doi.org/10.1038/s41594-020-0480-y>.
- Zhou, T., Tsybovsky, Y., Gorman, J., Rapp, M., Cerutti, G., Chuang, G.-Y., et al. (2020). Cryo-EM structures of SARS-CoV-2 spike without and with ACE2 reveal a pH-dependent switch to mediate endosomal positioning of receptor-binding domains. *Cell Host & Microbe*, 28(6), 867–879.e5. <https://doi.org/10.1016/j.chom.2020.11.004>.
- Zhou, T., Wang, L., Misasi, J., Pegu, A., Zhang, Y., Harris, D. R., et al. (2022). Structural basis for potent antibody neutralization of SARS-CoV-2 variants including B.1.1.529. *Science (New York, N.Y.)*, 376(6591), eabn8897. <https://doi.org/10.1126/science.abn8897>.
- Zhou, H., Chen, Y., Zhang, S., Niu, P., Qin, K., Jia, W., et al. (2019). Structural definition of a neutralization epitope on the N-terminal domain of MERS-CoV spike glycoprotein. *Nature Communications*, 10(1), 3068. <https://doi.org/10.1038/s41467-019-10897-4>.
- Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, 579(7798), 270–273. <https://doi.org/10.1038/s41586-020-2012-7>.
- Zhou, P., Yuan, M., Song, G., Beutler, N., Shaabani, N., Huang, D., et al. (2022). A human antibody reveals a conserved site on beta-coronavirus spike proteins and confers protection against SARS-CoV-2 infection. *Science Translational Medicine*, 14(637), eabi9215. <https://doi.org/10.1126/scitranslmed.abi9215>.
- Zhu, X., Mannar, D., Srivastava, S. S., Berezuk, A. M., Demers, J.-P., Saville, J. W., et al. (2021). Cryo-electron microscopy structures of the N501Y SARS-CoV-2 spike protein in complex with ACE2 and 2 potent neutralizing antibodies. *PLoS Biology*, 19(4), e3001237. <https://doi.org/10.1371/journal.pbio.3001237>.
- Zhu, Y., Yu, D., Han, Y., Yan, H., Chong, H., Ren, L., et al. (2020). Cross-reactive neutralization of SARS-CoV-2 by serum antibodies from recovered SARS patients and immunized animals. *Science Advances*, 6(45). <https://doi.org/10.1126/sciadv.abc9999>.
- Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., et al. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine*, 382(8), 727–733. <https://doi.org/10.1056/nejmoa2001017>.
- Zohar, T., Loos, C., Fischinger, S., Atyeo, C., Wang, C., Slein, M. D., et al. (2020). Compromised humoral functional evolution tracks with SARS-CoV-2 mortality. *Cell*, 183(6), 1508–1519.e12. <https://doi.org/10.1016/j.cell.2020.10.052>.

- Zost, S. J., Gilchuk, P., Case, J. B., Binshtein, E., Chen, R. E., Nkolola, J. P., et al. (2020). Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature*, *584*(7821), 443–449. <https://doi.org/10.1038/s41586-020-2548-6>.
- Zost, S. J., Gilchuk, P., Chen, R. E., Case, J. B., Reidy, J. X., Trivette, A., et al. (2020). Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nature Medicine*, *26*(9), 1422–1427. <https://doi.org/10.1038/s41591-020-0998-x>.