

Life Sciences and Medicine

Special Topic: COVID-19: Virus, Immunity and Vaccines

Broadly neutralizing antibodies against SARS-CoV-2 variants

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Received 8 November 2021; Revised 19 February 2022; Accepted 4 March 2022; Published online 24 April 2022

Antibody response plays a critical role in protective immunity against SARS-CoV-2 infection and disease progression [1–3]. As the current COVID-19 pandemic continues to rage around the world, there is growing concern that new SARS-CoV-2 variants may emerge that are antigenically distinct from the prototype strain, rendering the current antibody and vaccine strategies ineffective [4–15]. Indeed, the spike (S) glycoprotein D614G mutation becomes dominant just a few months into the pandemic and is associated with greater infectivity and transmissibility, as well as moderately decreased susceptibility to antibody neutralization [16,17]. Starting from the end of 2020, multiple waves of steeply increased infections are associated with emergence of variants of concerns (VOCs) such as Alpha, Beta, Gamma, Delta, and Omicron that are able to escape from antibody and serum neutralization of convalescent and vaccinated individuals. As a result, efficacies of all vaccine modalities are severely compromised, particularly toward Omicron, followed by Beta, Delta, Gamma, and Alpha [9–13,18–20]. Similarly, many antibodies approved for emergency use authorization (EUA) also lose their neutralizing activities [7,8,11,14,15,19–22] (Figure 1). Genetic and phenotypic characterization of VOCs have nailed down to a few key mutations in the S glycoprotein that are responsible for neutralization escape and many of which are shared among VOCs. For instance, the N501Y mutation previously shown to enhance binding affinity to the angiotensin-converting enzyme 2 (ACE2) [23,24] is found in Alpha, Beta, Gamma, and Omicron. Beta, Gamma, and Omicron each have three mutation sites in common within the receptor-binding domain (RBD)—K417N/T, E484K/A, and N501Y—which markedly reduce or completely escape neutralizing activities of many mAbs including some already approved for EUA [3,8,14,15,20,25–27]. Delta and lambda variants have respective L452R and L452Q mutations within the RBD that also facilitate virus escape [18,19,21]. In the N-terminal domain (NTD), short deletions such as $\Delta 69/70$, $\Delta 142-144$, $\Delta Y144$, $\Delta 156-157$, $\Delta Y211$, and/or $\Delta 242-244$ together with point mutations were found in Alpha, Beta, Gamma, and Omicron [20,28,29]. While these findings indicate the dominance of RBD and NTD in immune recognition and selection, it is also a worrying sign that antibody responses induced by infection or current vaccination are likely subjected to the similar escape mechanisms, highlighting the importance of identifying broadly neutralizing antibodies and universal vaccines that are able to overcome these major escape mutations.

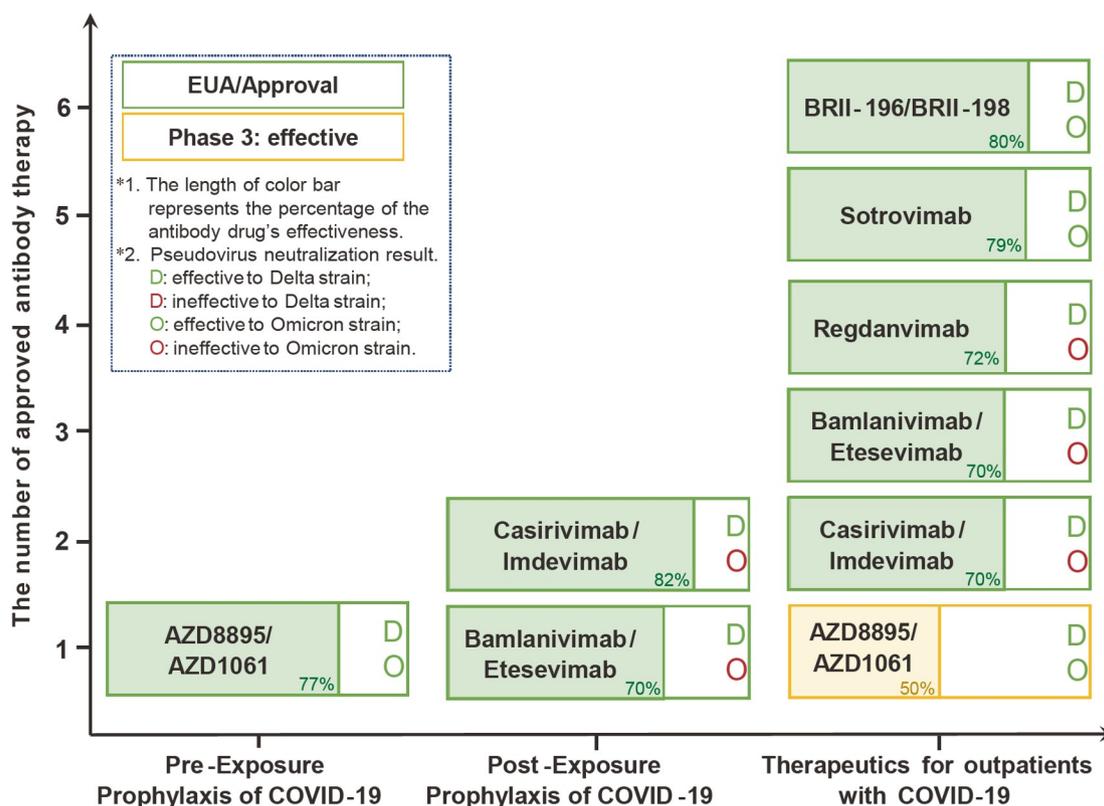


Figure 1 Summary of clinical efficacy for licensed antibodies or under EUA. Bamlanivimab/Etesevimab are combination of monoclonal antibody products developed by Lilly, Casirivimab/Imdevimab by Regeneron, Tixagevimab/Cilgavimab (AZD8895/AZD1061) by AstraZeneca, BRII-196/BRII-198 by Bii Biosciences, and CT-P59 (Regdanvimab) by Celltrion. The first three combinations have been approved by the U.S. Food and Drug Administration, the fourth one by the Chinese National Medical Products Administration, and the last one by South Korea regulatory agencies.

Whether the broadly neutralizing antibodies exist and, if so, how to identify them are largely dependent on our capacity to isolate and characterize the full-spectrum of S glycoprotein-specific antibodies derived from infected and vaccinated individuals. From the S glycoproteins perspective, we learned their critical and indispensable roles in binding to the host receptor ACE2 to initiate and mediate viral entry [30–33]. In doing so, the S glycoproteins undergo a series of spontaneous and dramatic structural and conformational changes leading to the ultimate membrane fusion and viral entry. On the surface of matured virion and infected cells, S glycoproteins exist as transmembrane homotrimers and each monomer is cleaved by a furin-like protease into two subunits: the receptor-binding S1 subunit and the fusion machinery S2 subunit. The majority of S1 and S2 subunits remain noncovalently bound in the prefusion conformation while some lose their S1 subunit and transformed into the postfusion conformation [33–37]. In the full-length or stabilized S ectodomain, S1 subunit folds into four domains, the NTD, the RBD, and two C-terminal domains (CTDs) that wrap around the threefold axis and protect the prefusion conformation of S2 [34–39]. Cryo-EM and crystal structural analysis reveal that RBD undergoes spontaneous structural fluctuations between an “up” and a “down” conformation. But only the “up” conformation enables the exposure of the receptor-binding motif (RBM) of RBD, allowing binding to or becoming accessible by the host receptor ACE2. The “up” conformation is believed to be less stable, which may explain why the dominant trimer state has only one of the three RBDs

standing up [34,36–39]. Receptor engagement induces further proteolytic cleavage at the S2's site by a transmembrane serine protease 2 (TMPRSS2) or the endosomal cysteine proteases cathepsins B and L (CatB/L) [40,41]. This cleavage is believed to activate membrane fusion process which involves irreversible refolding of the spring-loaded S2 subunit into a postfusion conformation and insertion of the fusion peptide into the target membrane. The concomitant folding back of heptad repeat 2 (HR2) onto the heptad repeat 1 (HR1) triggers the formation of long and three-stranded coiled coil, which brings the viral and cellular membrane into proximity and leads to ultimate membrane fusion and viral entry [32,33,37].

From the antibody perspective, we learned that they can exert their neutralizing activities by targeting various antigenic domains on the S glycoprotein, such as RBD, NTD, S2 domain, and quaternary epitopes only exist in the form of S trimer [8,15,42–48]. Some of these domains are exposed throughout the entry process while others are hidden and become exposed only when RBD in the “up” conformation or during the entry process. Antibodies are found to target these structurally diverse configurations, indicating their capacity in capturing the highly dynamic and even transiently exposed cryptic antigenic domains [8,15,42–48]. Quantitatively, more antibodies target to the exposed than to the cryptic domains, consistent with spatial requirement for antibody access and binding. The majority of neutralizing antibodies target to the RBD, followed by those to the NTD. S2-directed antibodies are generally poor in neutralizing activity although some broad and reasonably good neutralizing ones have recently been identified [49,50]. NTD-directed antibodies are in general not broadly neutralizing, largely due to the highly variable nature of NTD involving multiple deletion/insertion and point mutations surrounding the N3 and N5 loops [15,28]. S2-directed antibodies, although high in numbers in relative to those RBD- and NTD-directive ones, are rarely potent although some are indeed broad against many VOCs and variant of interests (VOIs) [49,50]. Such pattern of antibody recognition is highly consistent in naturally infected and vaccinated individuals [46,51], indicating the S glycoprotein expressed by the vaccines resembles fairly well with those on the infectious particles.

A recent comprehensive study has categorized 186 RBD-directed antibodies with potential therapeutic use into seven core “communities” (RBD-1 to RBD-7) based on their distinct footprints and broad competition profiles (Figure 2) [52]. Within each community, finer clusters are also defined by cross-competition and the ability to compete with ACE2 [52]. These antibody communities align well with previously defined antibodies classes based on germline or structural information [8,15,42]. Broadly speaking, these antibodies target the three major surfaces on RBD, namely the top RBM face, the outer face, and the inner face (Figure 2). Antibodies targeting to the RBM face are among the most potent neutralizing antibodies against SARS-CoV-2 and belong to the three major communities RBD-1, RBD-2, and RBD3 [52]. They compete with ACE2 and generally require the RBD in the “up” conformation for binding. The three communities overlap extensively in their binding sites on RBM surface although each demonstrates somewhat unique binding pose and specificity. Many antibodies in RBD-2 overlap with those previously categorized in Class 1, such as P2C-1F11 (7CD1), A23-38.1 (7LRS), and B1-182.1 (7MLZ) (Figure 2). The heavy chain germline IGHV3-53/3-66 were overly represented while the light chain genes were rather diverse [47,53]. They carry limited somatic hypermutation with relatively short HCDR3 loops (<15 residues). RBD-2a antibodies are heavily affected by the K417N mutation, while RBD-2b antibodies by the E484K mutation are found in Beta and Gamma variants [52]. RBD-3 antibodies, however, are more sensitive to mutations at positions N501 and E484. RBD-1 antibodies are rather variable as a community in their sensitivity to various mutations [52].

On the other hand, antibodies targeting to the outer face are among the most broad and potent neutralizing

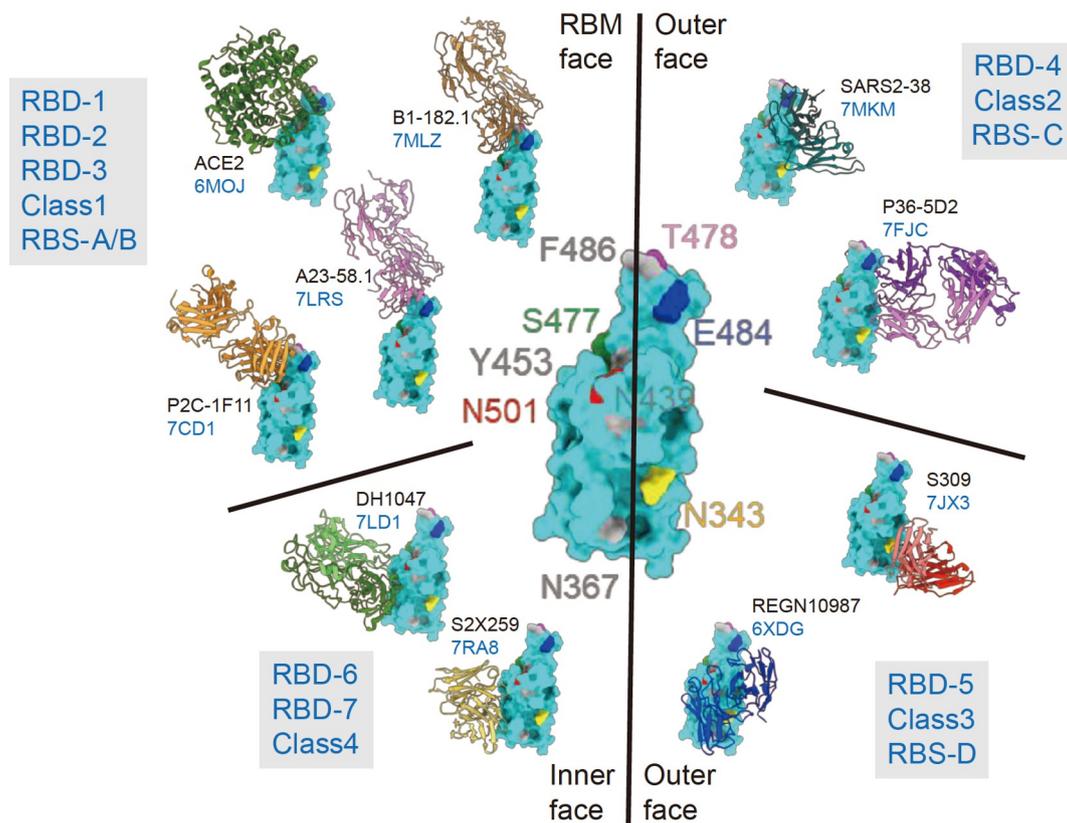


Figure 2 Classification of RBD-directed antibodies into seven major communities. The figure was a modification of that in Hastie *et al.* [52]. In the center, the entire RBD is colored in cyan together with mutations identified among the VOCs. The RBM face of RBD is depicted on the up-left corner, together with some of the representative antibodies with unknown crystal structure information. The inner and outer faces of RBD are separated by the black line in the middle. On the periphery, RBD-1 to RBD-7 communities are separated by the black lines. The relationship with previously used classification methods (Class 1–4 and RBS-A-D) are shown in grey boxes. The representative antibodies in each community are presented by their complex structures with RBD (cyan) together with their names and PDB accession numbers.

antibodies against SARS-CoV-2 variants and belong to recently classified RBD-4 and RBD-5 communities [52]. Their footprints on RBD are solvent-exposed, accessible in both “up” and “down” conformations, and largely overlapping with those previously categorized in Class 2 and Class 3. RBD-4 antibodies such as SARS2-38 (7MKM) are sensitive to E484K mutation found in the Beta and Gamma variants. L452R found in the Delta variant also has noticeable impact on RBD-4 antibodies such as P36-5D2 (7FJC) [52,54]. RBD-5 community, however, show broad resistance to almost all mutations analyzed so far and exemplified by S309 (7JX3). It appears to neutralize SARS-CoV-2 independent of blocking interaction between RBD and ACE2. Unfortunately, many of antibodies in this community including REGN10987 (6XDG) are inactivated by Omicron [20]. By contrast, antibodies targeting to the inner face are generally weaker in neutralizing potency compared to RBM face and outer face antibodies. They belong to recently classified RBD-6 and RBD-7 community antibodies and bind to cryptic epitopes on the opposite of outer surface accessible only when the RBD is in “up” conformation. Some members require at least two or three RBD in the “up” conformation for binding [45,52,55]. RBD-6 and RBD-7 antibodies have remarkable capacity to resist the escape mutations found surrounding the RBM and inner face of RBD and demonstrate substantial cross reactivity against

SARS-CoV-1 and other ACE2-using coronaviruses [52,55]. However, recently identified Omicron variant results in partial or full escape from some of this community antibodies such as DH1047 (7LD1) and S2X259 (7RA8) [20]. Many members in RBD-7 community also neutralize SARS-CoV-2 not through blocking interaction between RBD and ACE2 [52]. Lastly, several antibodies isolated from SARS-CoV-1 have demonstrated impressive cross-neutralizing activity to SARS-CoV-2, such as S309 of RBD-5, and DH1047 of RBD-6 communities [50,56].

In summary, by screening and characterizing hundreds and thousands of monoclonal antibodies from convalescent or vaccinated individuals, we and others have identified a small but compelling number of broad and potent neutralizing antibodies directed to RBD capable of neutralizing all variants identified thus far [8,14,15,50,55–59]. These antibodies were distributed in all seven antibody communities (Figure 2), although recent studies suggested they are disproportionately prevalent in RBD-6 and RBD-7 as their cognate epitopes are the most conserved [52]. Their broad and potent activity may contribute to the residual serum neutralizing activity in the infected or vaccinated individuals against SARS-CoV-2 VOCs. More importantly, these neutralizing antibodies can be substantially boosted by mRNA vaccines, particularly in individuals with pre-existing SARS-CoV or SARS-CoV-2 infection [51,60,61]. These results suggest the existence of broadly neutralizing antibodies against RBD of all VOCs identified so far and perhaps sarbecoviruses in general. While these antibodies are waiting for further evaluation and development, the footprint of these antibodies represent the precise and vulnerable target for the development of next generation vaccines capable of inducing such broad and potent immunity against SARS-CoV-2 variants.

Funding

This work was supported by the National Key Plan for Scientific Research and Development of China (2020YFC0849900 and 2021YFC0864500) and the National Natural Science Foundation of China (92169205).

Conflict of interest

The author declares patent has been filed for BR11-196 and BR11-198 antibodies in 2020.

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