Molecular Therapy
Commentary

Tackling COVID19 by Exploiting Pre-existing Cross-Reacting Spike-Specific Immunity

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The novel coronavirus disease 2019 (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The lack of targeted molecular therapy for patients with SARS-CoV-2 infection has led to high mortality.[1] The study of pre-existing cross-reacting immunity to pre- and post-pandemic coronaviruses provides direction for the development of targeted molecular therapy for members of this class of virus.[2–6] Combining two or more anti-SARS-CoV-2 spike receptor binding domain (RBD) antibodies should strengthen SARS-CoV-2 neutralization and might restrict the generation of neutralization-evading mutants.

Coronaviruses are a group of single-stranded RNA-enveloped viruses that infect the respiratory tract, with some species causing severe acute respiratory syndrome (SARS). SARS-CoV-2 is the causative pathogen of the ongoing COVID-19 pandemic.[1] The membrane surface of SARS-CoV-2 is covered by spike proteins, which are comprised of 1273 amino acids (aa), including an N-terminal signal peptide (aa 1–13), an S1 subunit (aa 14–685; Figure 1), and an S2 subunit (aa 686–1273; Figure 1). The S1 subunit is composed of an N-terminal domain (aa 14–305) and an RBD motif (aa 319–541) that binds to the host cell receptor, angiotensin-converting enzyme 2 (ACE2), to mediate entry into epithelial cells of the lung.[1] Phylogenetic analysis of full-length genome sequences obtained from infected patients showed that SARS-CoV-2 is similar to the originally described SARS-CoV, which caused an outbreak of SARS in Asia in 2003, and uses the same cell entry receptor. The RBD region is a critical target for neutralizing antibodies, and SARS-CoV-2 and SARS-CoV RBDs are ~75% similar in sequence.[1,2]

Four known seasonal "common cold" human coronaviruses (HCoVs), namely HCoV-OC43, HCoV-HKU1, HCoV-NL63, and HCoV-229E, cause human upper respiratory tract infections annually.[2] Grifoni and colleagues[2] investigated serum from donors sampled in 2015 and 2018 and who had not been exposed to SARS-CoV-2. All of these donors were positive for HCoV-OC43 and HCoV-NL63, as assessed by an immunoglobulin G (IgG) ELISA test for reactivity to the corresponding spike RBDs. Approximately 40%–60% of these SARS-CoV-2-unexposed individuals exhibited SARS-CoV-2-reactive non-spike-specific $CD4^+$ T cells upon stimulation with SARS-CoV-2 non-spike peptide pools. These results suggest that there is pre-existing SARS-CoV-2 non-spike-specific immunity in people infected by seasonal "common cold" HCoV-OC43 and HCoV-NL63 viruses. This pre-existing SARS-CoV-2-specific immunity might reduce the severity of subsequent infection with the latter.[2]

Further studies showed that although antibody cross-reactivity appears relatively common, cross-neutralization reactions may be rare. Lv and colleagues[3] reported that plasma samples from 15 patients with COVID-19 showed significant cross-reactivity to the SARS-CoV spike non-RBD, and 5 of the 15 samples showed convincing cross-reactivity with SARS-CoV RBD. However, only 1 of the 15 samples could weakly cross-neutralize SARS-CoV. The authors also reported plasma samples from 7 patients with SARS that could significantly cross-react with both SARS-CoV-2 spike non-RBD and spike RBD, although none of these plasma samples were able to cross-neutralize SARS-CoV-2. These findings indicate that infection by one subtype of coronavirus induces antibodies that can bind to the non-RBD and RBD regions of the spike protein on other subtypes of coronavirus.[2,3]

Yuan and colleagues[4] recently described a neutralizing monoclonal antibody (mAb; CR3022) previously isolated from a convalescent SARS patient that could bind the RBD of the SARS-CoV-2 spike protein. Other recent studies have shown that this cross-reactive antibody has relatively strong binding affinity to SARS-CoV-2. CR3022 targets a highly conserved cryptic epitope in the RBD, which enables cross-reactive binding to both the SARS-CoV-2 and SARS-CoV RBDs. A key finding is that the antibody binding sites in the two coronaviruses are very similar, differing by only 4 amino acids. This high degree of similarity suggests that this site within the RBD contains an important viral function that could be lost if the site underwent significant mutations. The fact that the mAb binding site is highly conserved between SARS-CoV and SARS-CoV-2 also indicates that antibodies yet to be identified might effectively neutralize both viruses and prevent a pandemic in the future.[4]

Pinto and colleagues[5] reported another neutralizing mAb (S309) isolated from a convalescent SARS patient. This antibody also bound the previously mentioned conserved area of the RBD (17 out of 22 residues of the epitope) of SARS-CoV and SARS-CoV-2. Importantly, the data showed that S309 could neutralize potentially 11,839 SARS-CoV-2 isolates known, to date, to be circulating. However, S309 IgG-mediated neutralization reached 100%,

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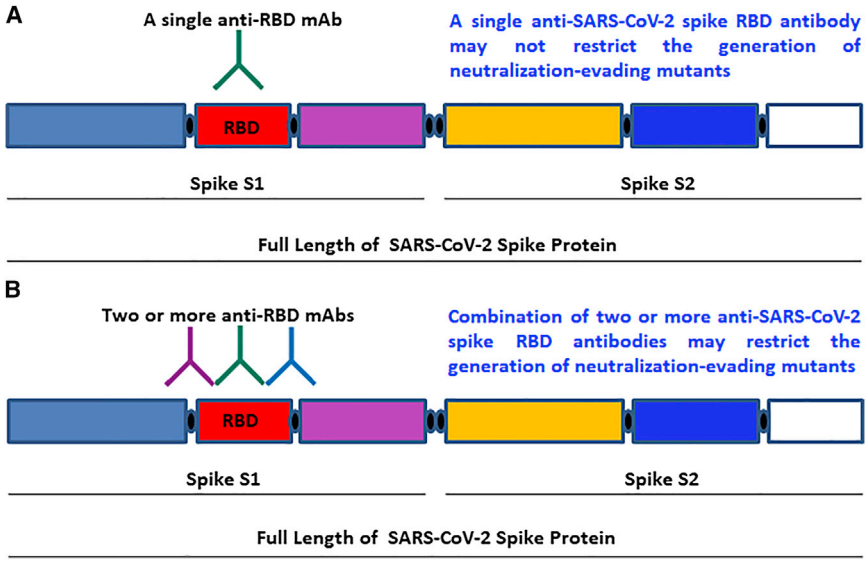

Figure 1. The Monoclonal Antibody (mAb) Recognition Sites of the SARS-CoV-2 Spike RBD and the Strategy for the Potential Future Therapeutic Treatment of COVID-19

(A) A single anti-SARS-CoV-2 spike RBD antibody may not restrict the generation of neutralization-evading mutants. (B) Combination of two or more anti-SARS-CoV-2 spike RBD antibodies or "antibody cocktail" may restrict the generation of neutralization-evading mutants. Antibodies of different isotypes activate different effector mechanisms in response to antigens. Therefore, combination of two or more isotypes of anti-SARS-CoV-2 spike RBD antibodies or "antibody cocktail" against the RBD may enhance this effect.

whereas neutralization plateaued at approximately 80% in the presence of S309 Fab. This result indicates that one or more IgG-specific bivalent mechanisms—such as S-glycoprotein trimer cross-linking, steric hindrance, aggregation of virions, or antibody-dependent cell cytotoxicity—may contribute to the ability of S309 to fully neutralize pseudovirions. Furthermore, S309 could recruit effector mechanisms, and showed increased neutralization of other weakly neutralizing mAbs in combination with such a neutralizing mAb, which may reduce the risk of viral escape.[5] Antibodies of different isotypes activate different effector mechanisms in response to antigens. Therefore, a combination of IgG1 and IgG2 isotypes against the same antigen may enhance this effect.

Baum and colleagues[6] reported the important finding that 2 antibodies binding distinct and non-overlapping regions of the RBD could concurrently bind and block RBD function (Figure 1). The authors demonstrated that growing a pseudovirus in the presence of any one of these two antibodies led to the development of spike mutants with resistance to the antibody. In contrast, escape mutants did not develop when the pseudovirus was cultured in the presence of pairs of antibodies that either do not compete or only partially compete for binding to the RBD. The discovery of spike protein cross-reactivity provides direction for future research. The combination of 2 or more anti-SARS-CoV-2 spike RBD antibodies should strengthen SARS-CoV-2 neutralization and may restrict the generation of neutralization-evading mutants.[5,6] Although no clinical trials have yet been reported, SARS-CoV-2-neutralizing mAb combination therapy might prove beneficial to curb pandemics in the future.

CONFLICTS OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. Vabret, N., Britton, G.J., Gruber, C., Hegde, S., Kim, J., Kuksin, M., Levantovsky, R., Malle, L., Moreira, A., Park, M.D., et al.; Sinai Immunology Review Project (2020). Immunology of COVID-19: current state of the science. Immunity *52*, 910–941.

2. Grifoni, A., Weiskopf, D., Ramirez, S.I., Mateus, J., Dan, J.M., Moderbacher, C.R., Rawlings, S.A., Sutherland, A., Premkumar, L., Jadi, R.S., et al. (2020). Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell *181*, 1489–1501.e15.

3. Lv, H., Wu, N.C., Tsang, O.T., Yuan, M., Perera, R.A.P.M., Leung, W.S., So, R.T.Y., Chan, J.M.C., Yip, G.K., Chik, T.S.H., et al. (2020). Cross-reactive antibody response between SARS-CoV-2 and SARS-CoV infections. Cell Rep. *31*, 107725.

4. Yuan, M., Wu, N.C., Zhu, X., Lee, C.D., So, R.T.Y., Lv, H., Mok, C.K.P., and Wilson, I.A. (2020). A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. Science *368*, 630–633.

5. Pinto, D., Park, Y.J., Beltramello, M., Walls, A.C., Tortorici, M.A., Bianchi, S., Jaconi, S., Culap, K., Zatta, F., De Marco, A., et al. (2020). Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. Nature *583*, 290–295.

6. Baum, A., Fulton, B.O., Wloga, E., Copin, R., Pascal, K.E., Russo, V., Giordano, S., Lanza, K., Negron, N., Ni, M., et al. (2020). Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. Science *369*, 1014–1018.