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Review article

## Antibodies at work in the time of severe acute respiratory syndrome coronavirus 2

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### ABSTRACT

In view of devastating effects of COVID-19 on human life, there is an urgent need for the licensed vaccines or therapeutics for the SARS-CoV-2 infection. Age-old passive immunization with protective antibodies to neutralize the virus is one of the strategies for emergency prophylaxis and therapy for coronavirus disease 2019 (COVID-19). In this review, the authors discuss up-to-date advances in immune-based therapy for COVID-19. The use of convalescent plasma therapy as the first line of defense to treat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has been established, with encouraging results. Monoclonal antibodies (mAbs) that bind to the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein or block the interaction between SARS-CoV-2 RBD and the human angiotensin-converting enzyme 2 receptor have been found to be very promising as a countermeasure for tackling the SARS-CoV-2 infection, and clinical trials are underway. Considering the counterproductive antibody-dependent enhancement of the virus, mAbs therapy that is safe and efficacious, even in people with underlying conditions, will be a significant breakthrough. In addition, emerging immunotherapeutic interventions using nanobodies and cellular immunotherapy are promising avenues for tackling the COVID-19 pandemic. The authors also discuss the implication of mAbs as mediators of cytokine storm syndrome to modify the immune response of COVID-19 patients, thus reducing the fatality rate of COVID-19 infection.

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### Introduction

The latest 21st century pandemic, coronavirus disease 2019 (COVID-19), by the etiological agent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to a worldwide disruption of human activities. Globally, as of August 21, 2020, SARS-CoV-2 has infected 22,536,278 people and led to 789,197 deaths, as reported by the World Health Organization [1]. The unprecedented clinical challenges posed by human-to-human transmission of SARS-CoV-2 begin with a range of clinical manifestations, including fever, cough and dyspnea, with no or mild pneumonia. Severe cases present with dyspnea, hypoxia and >50% pulmonary damage, requiring intensive care for respiratory support, whereas critical cases are characterized by respiratory and multi-organ failure. SARS-CoV is also known to cause the common complication of acute respiratory distress syndrome, thus requiring mechanical ventilation [2].

The high morbidity and mortality rates worldwide demonstrate that there seem to be differential responses in COVID-19 patients. The global imperative of the current hour is to rapidly develop

immune therapy to prevent COVID-19 [3]. Hence, it is necessary to understand the immunological basis of this infection, the implications of which will offer better insight into the development of new therapies. Extracorporeal membrane oxygenation is one of the evolving strategies that can be utilized in treating patients with refractory hypoxemia and altered lung properties despite optimal conventional treatment, including mechanical ventilation [4]. Combining anti-viral and anti-inflammatory treatments is also being investigated. Repurposing drugs for COVID-19, with the challenge of appropriate dosage, remains an attractive treatment modality [5]. Many studies have also tried to investigate the function of glucocorticoids in modulating inflammation-mediated lung injury, thereby mitigating the progression of respiratory failure and mortality [6].

### SARS-CoV-2: the escape pathogen

The first event in the chronology of SARS-CoV-2 infection is the virus binding to a host cell. The cytopathic virus utilizes its spike glycoprotein (S) located on its surface to bind with the angiotensin-converting enzyme 2 (ACE2) receptor for cell entry. Various research groups have targeted the receptor-binding domain (RBD) of SARS-CoV, SARS-CoV-2 and Middle East respiratory syndrome CoV (MERS-CoV) with neutralizing antibodies to combat the infection [7]. The S protein has two functional subunits: the S1 subunit mediates cell attachment, and the S2

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subunit is involved in the fusion of viral and cellular membranes [8]. SARS-CoV-2 principally targets airway, alveolar and vascular epithelial cells and lung macrophages, all of which express the ACE2 entry receptor. SARS-CoV-2, in combination with ACE2, is endocytosed by cells. As a result of the inability of ACE2 to regulate the renin-angiotensin system, blood pressure and electrolyte imbalances occur [9]. Furthermore, loss of ACE2 promotes accumulation of angiotensin II, which eventually activates A disintegrin and metalloproteinase 17 activity, perpetuating membrane shedding of ACE2, renin-angiotensin system overactivation and inflammation [10,11].

Zhu *et al.* [12] investigated the morphogenetic process and cytopathic effect of SARS-CoV-2 infection in organotropic human airway epithelial cultures. It was observed that the virus infected both ciliated and secretory cells, because of which the authors suggest the possibility of the involvement of other receptors in addition to ACE2. This is because ACE2 is mainly expressed on ciliated epithelial cells of human lungs [12]. Viral infection and replication in upper respiratory tract epithelial cells induce pyroptosis. This inflammatory phenomenon of programmed cell death is commonly observed in SARS-CoV-infected cells too [13]. The epidemiology working group for novel COVID-19-infected pneumonia epidemic response reported the differential fatality rate in males and females, where the percentage of deaths was higher in males [14]. This correlates with the immunoregulatory functions of estrogen and testosterone [15]. A cellular serine protease, transmembrane protease serine 2, is observed to process the S protein and eventually contribute to host cell entry. Hence, transmembrane protease serine 2 is also a potential drug target that has attracted a lot of attention in the field of repurposed drugs for SARS-CoV-2 [14].

## The immunity battles

The destruction of pulmonary cells initiates a local immune response involving macrophages and monocytes, which release an array of cytokines. This action also primes adaptive immunity by T and B cells. Mild cases of COVID-19 are primarily resolved at this stage. However, severe lung destruction is associated with a dysfunctional immune response. Pyroptosis of airway epithelial cells releases IL-1 $\beta$ , a potential trigger for the eventual inflammatory response [16]. This wave of local inflammation is sensed by alveolar epithelial cells and alveolar macrophages and leads to the secretion of pro-inflammatory cytokines and chemokines like IL-6, interferon gamma, monocyte chemoattractant protein 1 and interferon gamma-induced protein 10. This starts the pulmonary recruitment of monocytes and T lymphocytes into the infected site. Thus, the infiltration of lymphocytes into the respiratory airway manifests as T-cell lymphopenia, which is observed in the majority of COVID-19 patients [13,17]. Like SARS-CoV, the escape pathogen SARS-CoV-2 can sabotage the innate response by antagonism of the interferon response and disrupt the host protein translation process. This could potentially support viral

replication and eventually manifest as pyroptosis-associated aberrant inflammation in the lungs [13,18].

In most mild cases, recruited immune cells clear the respiratory airway, after which the immune response recedes. But a dysfunctional immune response is observed in severe cases, which triggers a massive cytokine storm that mediates extensive lung inflammation. The cytokine storm involves elevated blood plasma levels of macrophage inflammatory protein 1 $\alpha$  (MIP1 $\alpha$ ), tumor necrosis factor (TNF), IL-2, IL-7, IL-10, interferon gamma-induced protein 10, monocyte chemoattractant protein 1 and granulocyte-macrophage colony-stimulating factor. A high population of CD14<sup>+</sup>CD16<sup>+</sup> inflammatory monocytes, which also contribute to the cytokine storm, is also observed in severe cases. In addition, lymphocyte counts in peripheral blood are observed to significantly decrease [19]. Other observed consequences of T-cell lymphopenia and cytokine storm are hyaluronan formation and pulmonary edema. This contributes to severe breathlessness and vulnerability to secondary infections. The ripple effect of the cytokine storm brings about myocardial damage and multi-organ failure [3]. Transcriptomic analysis of three COVID-19 patients has revealed a dynamic early immune response [20]. After reaching the lowest point in respiratory function, a peak in most inflammatory and cytokine signaling gene expression, except expression in the IL-1 pathway, which preceded the decrease in respiratory function, was observed.

B-cell immune response is concomitantly observed 1 week after the onset of symptoms. The antibody response is observed against nucleocapsid and S protein by week 3 of SARS-CoV infection symptoms. However, some COVID-19 patients do not develop long-lasting antibodies [13,21]. Although many studies have made significant inroads, a clear understanding of the important host immune factors involved in the development of COVID-19 inflammation remains incomplete.

## COVID-19 and comorbidities

The most distinctive comorbidities seen in fatalities or severe cases of COVID-19 are diabetes, cerebrovascular diseases, hypertension, coronary heart diseases and cancer. Since cells expressing ACE2 are normally found in the epithelial lining of the lungs, intestines, kidneys and blood vessels, patients with these comorbidities are more susceptible to SARS-CoV-2 [22]. Although efforts to develop targeted immune therapies and vaccines are underway, many health organizations are advocating the practice of yoga and Ayurveda and maintaining a healthy lifestyle to boost immunity [23,24]. Most therapies routinely prescribed for these comorbidities favor the gateway of SARS-CoV-2 (Table 1).

## Non-vaccine pharmacological swords against SARS-CoV-2

Although some laboratories are involved in repurposing old antiviral drugs for use with the novel virus, some are involved in

**Table 1**  
Current therapies recommended for COVID-19 comorbidities.

Sl. no.	Comorbidity	Current therapy	Risks	Reference
1.	Diabetes (type 1 and 2)	ACE inhibitors and ARBs	Increased expression of ACE2, poor prognosis	[25]
2	Hypertension, cardiovascular diseases	ACE inhibitors, ARBs, ibuprofen and thiazolidinediones	Increased expression of ACE2, poor prognosis	[26,27]
3	Inflammatory lung diseases	Macrolide or quinolone antibiotics and steroids	Excess inflammation	[26]
4	Cancer	Surgery or chemotherapy	Increased severity due to delays in treatment and low immunity	[28–30]
5	Liver injury	Macrolide or quinolone antibiotics and steroids	Excess inflammatory response	[31]
6	Autoimmune diseases: rheumatoid arthritis	Corticosteroids, NSAIDs, acetaminophen and TNF inhibitors	Increased risk of secondary infection due to immunosuppression	[32,33]

Sl. no., Serial number; ARBs, angiotensin receptor blockers; NSAIDs, non-steroidal anti-inflammatory drugs.

designing targeted immune therapy. Remdesivir and lopinavir have shown a potential anti-viral effect and are being clinically investigated for safety and efficacy [34]. The efficacy of corticosteroid treatment for lung inflammation is also being questioned because of complications and delayed clearance of the infection [33]. Clinical trials to validate the efficacy of the anti-malarial drug hydroxychloroquine, which is also used to treat certain autoimmune diseases (lupus and rheumatoid arthritis); anti-cytokine therapies like IL-6 inhibitors and mesenchymal stromal cell-based therapies in COVID-19 patients are underway [35]. In such a scenario, where the production of an effective vaccine and anti-viral medicines is underway, convalescent plasma (CP) therapy and nanobodies have become exciting potential non-vaccine pharmacological tools for prevention [36].

### CP therapy: a pandemic savior?

CP therapy is a typical adaptive immunotherapy that has been applied successfully to stem outbreaks of SARS, MERS, the H1N1 pandemic of 2009, poliomyelitis, measles and mumps [37,38,39]. However, the application of CP was not able to treat the Ebola virus. Since SARS, MERS and COVID-19 share some similarities in the immunopathogenesis of their etiological agent, CP is looked upon as a promising option for treating COVID-19 [39,40]. It has been reported by Walls *et al.* [41] that murine SARS-CoV S protein polyclonal antibodies could inhibit S protein-mediated SARS-CoV-2 entry, shedding light on the cross-neutralizing antibodies that target the S epitope.

A study by Duan *et al.* [42] on the feasibility of CP transfusion in COVID-19 patients reported that a cohort of severely ill adult patients tolerated the therapy, and it significantly increased the titer of neutralizing antibodies, leading to reduction of clinical symptoms within 3 days and disappearance of viremia in a week. In a trial by China National Biotech Group Co Ltd (<http://www.chictr.org.cn/showprojen.aspx?proj=49861>), it was reported that 10 severely ill COVID-19 patients receiving CP therapy demonstrated significant improvement in oxygenation and pulmonary injury. Mechanistically, the high-titer-specific neutralizing antibodies could bring about complement activation and phagocytosis [42].

The key factors associated with CP therapy are neutralizing antibody titer, treatment time point and safety. Earlier studies on CP therapy for MERS-CoV demonstrated that the antibody titer should exceed 1:80 for full clearance. Hence, finding donors who have high titers is a prerequisite. It has been observed in SARS-CoV- and MERS-CoV-infected individuals that IgG titer reaches undetectable levels 3–4 months after recovery. Hence, it can be concluded that neutralizing antibodies represent a transient humoral immune response, and plasma from newly recovered patients should be more effective. The neutralizing antibody titer of recently recovered COVID-19 patients is above 1:640, which is higher than that seen in MERS patients [42].

SARS patients who were given CP before 14 days post onset of illness demonstrated a better outcome. Similarly, in the study by Duan *et al.* [42], patients who received CP transfusion before 14 days post onset of illness exhibited a spike in lymphocyte count and reduction in C-reactive protein, which is a marker of inflammation and cytokine storm. Patients who received CP after 14 days post onset of illness showed relatively less improvement, emphasizing the importance of an optimal transfusion time point. Stony Brook Hospital, New York, has initiated trials to test CP therapy for COVID-19 patients (<https://clinicaltrials.gov/ct2/show/NCT04344535>). Recently, the US Food and Drug Administration approved access to CP for COVID-19 patients while emphasizing the use of regulatory measures and clinical trials before routinely administering this therapy [43]. It has been reported that worldwide use of this therapy has mushroomed very rapidly because of encouraging results in patients [44].

In a study by Joyner *et al.* [43], early implications of CP therapy in 500 individuals of diverse races and a median age of 62 years who had severe or life-threatening COVID-19 were studied. The

researchers observed <1% severe adverse events, including 0.3% mortality, within the first 4 h of transfusion. The 7-day mortality rate was 14.9%, which could also be due to underlying multi-organ failure, sepsis and other significant comorbidities. The researchers discussed the possibility of developing transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO), which are often difficult to identify, as associated pulmonary complications post therapy. TACO results in pulmonary edema and hypertension. TRALI is characterized by bilateral pulmonary edema with or without acute respiratory distress syndrome. In certain cases, it has been observed that an underlying lung injury or comorbidities in COVID-19 patients further complicate the diagnosis of TACO and TRALI, which could exacerbate transfusion-related risks in severe patients [43]. Transmission of pathogens is also a major risk associated with CP therapy [40].

To maintain the activity of antibodies and inactivate the virus in donated plasma, Duan *et al.* [42] applied Methylene Blue photochemistry, which is reported to be better than UV-C light. Earlier studies on CP therapy for Ebola have reported transfusion-related acute lung injury [42]. Antibody-dependent infection enhancement is an uncommon risk that can suppress innate anti-viral immunity and thus support intracellular growth of the virus. However, it may be assumed that because of the high titer of neutralizing antibodies, optimal time of infusion and transfusion volume, the risk of antibody-dependent infection can be avoided. Thus, the use of CP therapy may be beneficial for COVID-19 patients [45].

### Hyperimmune globulin treatment: a safe option?

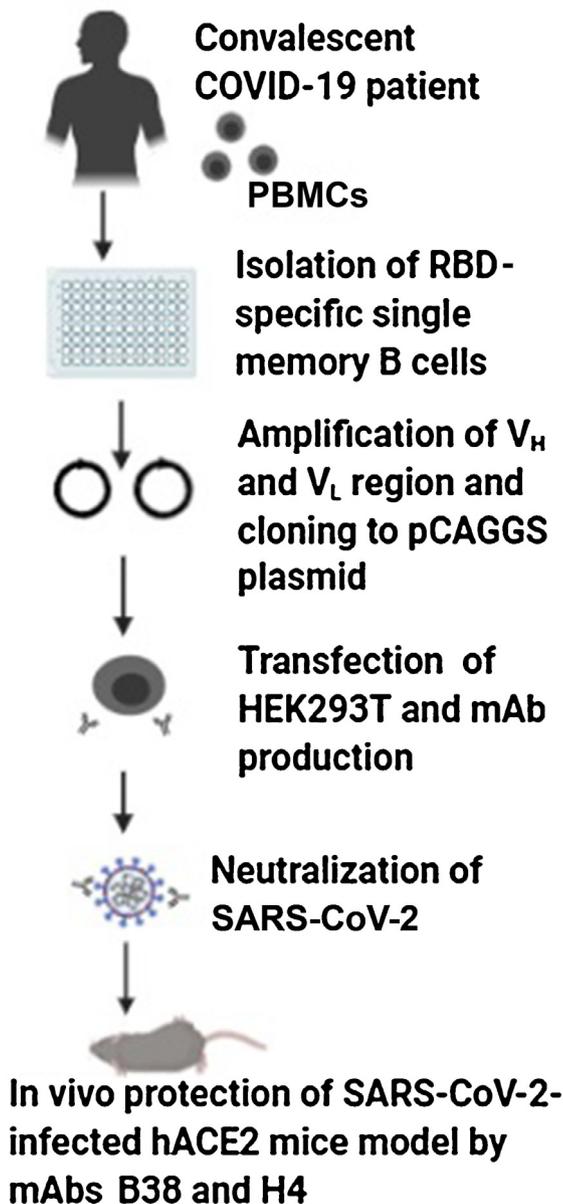
Nguyen *et al.* [46] define hyperimmune globulin as a product that is manufactured from the CP of thousands of donors with high antibody titers to a specific pathogen. It consists of an immune globulin fraction with well-defined properties. Plasmapheresis-derived plasma from recovered COVID-19 patients has been proposed to contain polyclonal hyperimmune globulin. This approach has already been explored for patients with SARS and severe influenza. It has been routinely used to treat patients infected with hepatitis B and rabies, proving its precedence and sound therapeutic plausibility. Díez *et al.* [47] tested the hypothesized cross-reactivity using currently available intravenous immunoglobulin (IVIg) products Gamunex-C and Flebogamma DIF with SARS-CoV-2, SARS-CoV and MERS-CoV. The researchers observed noteworthy cross-reactivity to antigenic components, including the S protein. It has been anticipated that vaccine administration may not be able to elicit an immune response of high magnitude in acutely ill COVID-19 patients. Hyperimmune globulin treatment could treat the viral infection and reduce the cytokine storm in severely ill COVID-19 patients. To date, a number of mechanisms behind the anti-inflammatory effects of IVIg therapy have been discussed, including anti-complement mechanisms, anti-idiotypic neutralization of pathogenic auto-antibodies, regulation via Fc receptors, enhanced production of regulatory T cells and inhibition of Th17 differentiation [47]. Hence, there have been suggestions for intravenous immunoglobulin therapy [48]. Although this needs to be confirmed through clinical trials, Takeda is advocating with regulatory agencies in the USA, Asia and Europe to expedite the use of anti-SARS-CoV-2 polyclonal hyperimmune globulin in high-risk patients with COVID-19 [49]. This could be a life-saving medicine for thousands of patients near death because of SARS-CoV-2.

### Therapeutic monoclonal antibodies against SARS-CoV-2: current scenario and challenges

Many companies and academic laboratories across the world are striving to develop therapeutic antibodies against SARS-CoV-2 for the emergency prophylaxis and treatment of COVID-19. Regeneron, AstraZeneca, GlaxoSmithKline, AbCellera, Eli Lilly and Company,

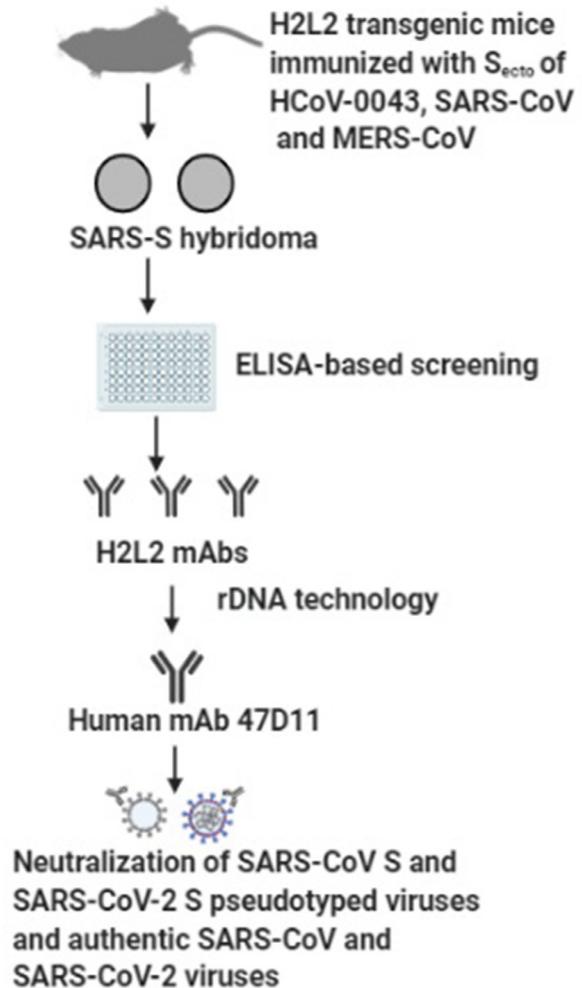
ImmunoPrecise, Mount Sinai Health System, Harbour BioMed and Vir Biotechnology, Inc, are some of the companies actively involved in developing functional therapeutic antibodies against SARS-CoV-2 [50]. Regeneron's double mAbs combination (REGN-COV-2) and a therapeutic antibody (LY-CoV555) developed by Eli Lilly in collaboration with Abcellera are in Phase 3 and Phase 2 clinical trials, respectively. [<https://clinicaltrials.gov/ct2/show/NCT04452318>; <https://clinicaltrials.gov/ct2/show/NCT04427501>].

Memory B cells of convalescent COVID-19 patient are the most used source for the identification of high-affinity neutralizing antibodies against SARS-CoV-2 (Figure 1). Another potential source is human trial testing for vaccines since an early immune response to the antigen is enough to release specific neutralizing antibodies with a high binding affinity [51]. S-RBD is an effective candidate for use in screening antibody-producing memory B cells for the development of therapeutics and diagnostics for SARS-CoV-2.



SARS-CoV-2 has a genome sequence that is 79.6% identical to that of SARS-CoV and 96% identical to the bat coronavirus [52]. The RBD sequences of SARS-CoV-2 share 73.8–74.9% amino acid identity with SARS-CoV [53]. Both SARS-CoV and SARS-CoV-2 possess a conserved epitope in RBD that makes them cross-reactive [54]. S glycoprotein of SARS-CoV-2 has 10 times higher affinity to ACE2 than that seen with SARS-CoV. Much of this affinity is attributed to RBD's structural features, such as a more compacted ACE2 binding ridge and well-stabilized hotspots [55,56]. CR3022, a neutralizing antibody obtained from a convalescent SARS-CoV-infected patient, cross-reacted with SARS-CoV-2 S but could not cross-neutralize SARS-CoV-2 *in vitro* [54]. A cross-reactive mAb that neutralizes both SARS-CoV and SARS-CoV-2 pseudoviruses and authentic viruses has been generated using transgenic H2L2 mice immunized with a combination of  $S_{\text{ecto}}$  from different coronaviruses, such as human CoV-OC43, SARS-CoV and MERS-CoV (Figure 2), though the identified mAb exhibited higher binding affinity toward SARS-CoV S [8].

Past research on the development of therapeutic antibodies for SARS-CoV serves as a backbone for the development of feasible therapeutic antibodies for SARS-CoV-2. In 2004, Sui *et al.* [57] reported high-affinity humanized mAbs based on single-chain variable region fragment antibody 80R against the S1 domain of the S protein of SARS-CoV. These could effectively neutralize SARS-CoV and block the S1 protein binding with ACE2. However, 80R was not effective against the GD03 strain of SARS CoV, which caused the second



**Fig. 1.** SARS-CoV-2-neutralizing mAb production using single B-cell repertoire from recovered COVID-19 patient [53]. Created with BioRender.com. PBMCs, peripheral blood mononuclear cells.

**Fig. 2.** SARS-CoV-2-neutralizing mAb production using transgenic H2L2 mice [8]. Created with BioRender.com. ELISA, enzyme-linked immunosorbent assay.

outbreak in 2003/2004. Zhu *et al.* [58] reported that humanized mAbs m396 and S230 successfully cross-react with the GD03 strain, Urbani and Tor2 isolates (first SARS outbreak) and SZ3 and SZ16 (pseudotyped viruses bearing spikes from palm civet), except bat-derived strains. Both antibodies block the SARS-CoV–ACE2 interaction by binding to the RBD of the SARS-CoV S protein. When 80R, m396 and S230 were tested against SARS-CoV-2 RBD, none of them showed significant binding regardless of the degree of structural homology between SARS-CoV S RBD and SARS-CoV-2 S RBD. Hence, the SARS-CoV-2 S protein would be the ideal target for designing therapeutic mAbs against SARS-CoV-2 [55]. Yi *et al.* [59] provided some interesting insight into the critical amino acid differences between the SARS-CoV and SARS-CoV-2 RBDs that result in their distinct immunogenicity and limit the cross-neutralizing activity of therapeutic antibodies.

Table 2 summarizes the neutralizing mAbs with therapeutic potential against SARS-CoV-2 reported thus far. Cao *et al.* [62] identified some potent SARS-CoV-2 mAbs based on the CDR3H structural similarity to SARS-CoV-neutralizing mAbs (m396 and 80R) from the large antigen-binding clonotype library produced by high-throughput single-cell 5' mRNA and VDJ sequencing. The mAbs derived from memory B cells of a SARS survivor were cross-reactive to SARS-CoV and SARS-CoV-2 but not to OC43 and MERS-CoV [61]. Similarly, sarbecovirus cross-neutralizing antibodies were identified from memory B cells of a SARS survivor [64]. However, SARS-CoV-2-generated mAbs showed no cross-reactivity to SARS-CoV and MERS-CoV RBDs [63]. The mechanism of action for SARS-CoV-2 neutralizing mAbs is either by blocking the interaction between RBD and human ACE2 (hACE2) or by binding to the conserved epitope of S-RBD without competing for receptor binding. Neutralizing human mAbs exhibiting intense competition with the hACE receptor for RBD binding are quite potent against SARS-CoV-2 [62,63]. Yi *et al.* [59] reported that the RBD binding affinity and neutralizing ability of SARS-CoV-2-targeted mAbs generated by phage display were much weaker for SARS-CoV, which strengthens the species-specific nature of anti-RBD antibodies. A cocktail of mAbs binding to the different epitopes on RBD will be more efficacious in neutralizing SARS-CoV-2 and preventing immune escape mutants than monotherapy with a candidate therapeutic mAb [53,62].

A major concern that should be addressed before implementation of antibody-based drug therapy or vaccination is antibody-dependent enhancement (ADE) of viral infection, in which virus-specific antibodies facilitate viral entry to immune cells by cross-linking the viral antibody or virus-activated complement complex to the cell surface Fc receptor or complement receptor, leading to viral replication and subsequently high viral load [75,76]. ADE has been reported for other coronaviruses [77–79]. Antibody-dependent infection with SARS-CoV-1 could allow the virus to widen the tropism by providing additional entry routes and contribute to pathogenicity. In ADE of SARS-CoV-1 infection, anti-S antibodies mediate viral entry to immune cells by cross-linking the virus-antibody complexes to the Fc $\gamma$  receptor II, followed by internalization through a pH- and cysteine protease-independent pathway, which is distinct from the hACE2-mediated endosomal/lysosomal pathway [78]. Wan *et al.* [79] demonstrated cell surface IgG Fc receptor-mediated viral entry of MERS-CoV into Fc receptor-expressing cells through canonical viral receptor-dependent pathways.

It has been suggested that low concentration of neutralizing antibodies could cause ADE of SARS-CoV-2, as reported in the case of SARS-CoV-1 [80–82]. Specificity, concentration, affinity and isotope of the antibody are factors that influence the infection enhancing tendency of an antibody. Designer antibodies with higher affinity to RBD are less likely to induce ADE [81]. Engineering the Fc region of mAbs to abolish its affinity for the Fc $\gamma$  receptor is a feasible solution to mitigate the risk of ADE [80]. To abrogate the risk of ADE of SARS-CoV-2, LALA mutations were introduced to the Fc region of CB6, a potent SARS-CoV-2-neutralizing antibody. CB6 (LALA) effectively reduced

viral load and infection-related lung damage associated with SARS-CoV-2 infection in rhesus macaques [68]. The possibility of ADE can also be minimized by maintaining a high concentration of circulating therapeutic antibodies, which is more easily achieved by improving the half-life of mAbs [83]. Lurie *et al.* [84] suggested that rigorous animal testing and pre-clinical trials should be conducted for therapeutic candidates against SARS-CoV-2, considering the adverse effects caused by ADE. The current uncertainty regarding the duration of functional protection by antibodies against SARS-CoV-2 RBD until the second wave of infection must be addressed to enhance the therapeutic efficacy of mAb products [85].

The ability of a virus to generate escape mutants because of immune pressure is a major challenge for successful vaccine and therapeutic antibody development. A pandemic scenario influenced by a highly diverse infected population also facilitates the generation of escape mutants [86]. RNA viruses such as SARS-CoV-2 exhibit a high mutation rate—up to a million times higher than the host—which drives viral evolution and adaptability [87,88]. High viral load of SARS-CoV-2 at the early stage of the disease might also contribute to anti-viral resistance [89]. Molecular modeling simulation studies of the antigen-antibody complex predict mutation of antigens that could disrupt the binding of antibodies and reduce the efficacy of mAb therapy [90]. It has been reported that escape mutants attenuate SARS-CoV infection. Formulation of more than one therapeutic antibody that binds to non-overlapping epitopes and/or parts of S other than RBD has been proposed to neutralize resistant variants [91,92]. A cocktail of mAbs S227.14 and S230.15 has effectively cross-reacted with a broad range of human and zoonotic SARS-CoV isolates, including escape mutants [93]. The mAb combination targeting the N-terminal domain as well as RBD of SARS-CoV-2 could be effective against viral escape [69]. Single antibody treatment against SARS-CoV-2 has induced escape mutants. Regeneron's mAbs cocktail, REGN10933 and REGN10987, each binds to the distinct epitopes of RBD prevented generation of escape mutants [94].

An effective passive immune therapy, along with stimulation of the endogenous humoral and cellular immune response, may provide durable protective immunity. Combining mAbs with immunostimulatory agents and altering antibody effector function through Fc-engineered mAbs are possible options to improve the long-lasting protective vaccine-like effects of mAbs [95]. Half-life and effector functions of S309, a mAb with neutralizing activity against SARS-CoV-2, were improved with FC engineering [61].

The prospect of highly potent super-antibodies that can neutralize multiple viruses of a single family looks quite promising in terms of developing broadly active therapeutic agents against Coronaviridae viruses. Large-scale screening for a donor with broadly neutralizing serum and high-throughput B-cell isolation are critical steps in the discovery of super-antibodies [96]. Defining the immunodominant regions of the S protein of SARS-CoV-2 is critical, as it can reveal potential targets for immune response. Development of mAbs targeting conserved epitopes of RBD and the highly similar S2 subdomain is likely to give cross-protection across betacoronaviruses [71,97].

### Immunomodulation of cytokine storm syndrome using mAb therapy: need of the hour

Targeted drug development is a time-consuming process. Repurposed drugs and therapeutic mAbs that modulate the immune response and improve the prognosis of COVID-19 patients should be considered at this critical time until a successful drug for viral clearance is on the market. An anti-IL6 mAb, tocilizumab, routinely used in the treatment of rheumatoid arthritis, has been found to mitigate cytokine storm syndrome and improve the outcome of COVID-19 patients. Apart from the anti-inflammatory action of tocilizumab, its role in halting coagulation activation greatly benefits COVID-19 patients with severe coagulopathy, which makes this therapy a more

**Table 2**  
Neutralizing antibodies with therapeutic potential against SARS-CoV-2.

mAbs	Source	Mechanism of neutralization	IC50	In vivo study	References
47D11	Transgenic H2L2 mice	Binding to conserved epitope of spike RBD	0.061 $\mu$ g/mL (SARS-CoV-2 pseudotyped virus) 0.57 $\mu$ g/mL (SARS-CoV-2 virus)		[8]
311mAb-31B5 311mAb-32D4	RBD-specific single B-cell repertoire from recovered COVID-19 patient	Blocking binding of SARS-CoV-2 RBD to hACE2	311 mAb-31B5 = 0.0338 $\mu$ g/mL 311 mAb-32D4 = 0.0698 $\mu$ g/mL (SARS-CoV-2 pseudotyped virus)	NI	[60]
B38 H4	RBD-specific single B-cell repertoire from COVID-19 patient	Blocking binding of SARS-CoV-2 RBD to hACE2	B38 = 0.177 $\mu$ g/mL H4 = 0.896 $\mu$ g/mL (SARS-CoV-2 virus)	hACE2 transgenic mice model	[53]
HA001 S309	Phage display Memory B cells of person previously infected with SARS-CoV	Blocking binding of SARS-CoV-2 RBD to hACE2 Binding to conserved epitope of spike RBD	0.016 $\mu$ g/mL (SARS-CoV-2 pseudotyped virus) 79 ng/mL (SARS-CoV-2 virus)	NI NI	[59] [61]
BD-368-2	High-throughput scrRNA/VDJ sequencing of COVID-19 convalescent patient's B cells	Blocking binding of SARS-CoV-2 RBD to hACE2	1.2 ng/mL (SARS-CoV-2 pseudotyped virus) 15 ng/mL (SARS-CoV-2 virus)	hACE2 transgenic mice model	[62]
PC2-1F11 P2B-2F6 P2C-1A3	RBD-specific single B-cell repertoire from COVID-19 patient	Blocking binding of SARS-CoV-2 RBD to hACE2	PC2-1F11 = 0.03 $\mu$ g/mL P2B-2F6 = 0.05 $\mu$ g/mL P2C-1A3 = 0.62 $\mu$ g/mL (SARS-CoV-2 pseudotyped virus) PC2-1F11 = 0.03 $\mu$ g/mL P2B-2F6 = 0.41 $\mu$ g/mL P2C-1A3 = 0.28 $\mu$ g/mL (SARS-CoV-2 virus)	NI	[63]
ADI-56046	RBD-specific single B-cell repertoire from SARS-CoV survivor	Binding to conserved epitopes in RBD and competing with hACE2 binding	$\approx$ 0.05 $\mu$ g/mL (SARS-CoV-2 pseudotyped virus) <0.1 $\mu$ g/mL (SARS-CoV-2 virus)	NI	[64]
COV2-2196, COV2-2130, COV2-2381	SARS-CoV-2 S2P <sub>ecto</sub> or RBD– mFc-reactive single B cells from convalescent COVID-19 patients	Blocking binding of SARS-CoV-2 RBD to hACE2	COV2-2196 = 0.7 ng/mL COV2-2130 = 1.6 ng/mL (SARS-CoV-2 pseudotyped virus) COV2-2196 = 15 ng/mL COV2-2130 = 107 ng/mL (SARS-CoV-2 virus)	Ad-hACE2 mouse model and rhesus macaque model	[65]
CC12.1	SARS-CoV-2 RBD and S antigen-specific single memory B cells from convalescent COVID-19 patients	Targeting RBD-A epitope by competing with hACE2 and blocking its binding to SARS-CoV-2 RBD	0.019 $\mu$ g/mL (SARS-CoV-2 pseudotyped virus) 0.022 $\mu$ g/mL (SARS-CoV-2 virus)	Syrian hamster model	[66]
REGN10933 REGN10987	Anti SARS-CoV-2 antibodies from immunized V1 mouse and RBD-specific single B cells from convalescent COVID-19 patients	Blocking binding of SARS-CoV-2 RBD to hACE2	REGN10933-42 pM REGN10987- 40 pM (SARS-CoV-2 pseudotyped virus) REGN10933-37 pM REGN10987- 42 pM (SARS-CoV-2 pseudotyped virus)	Rhesus macaque model and golden hamster model	[67]
CB6	RBD-specific memory B cells from convalescing COVID-19 patients	Blocking binding of SARS-CoV-2 RBD to hACE2	ND <sub>50</sub> – 0.036 $\mu$ g/mL (SARS-CoV-2 pseudotyped virus) ND <sub>50</sub> – 0.036 $\mu$ g/mL (SARS-CoV-2 virus)	Rhesus macaque model	[68]
4A8	SARS-CoV-2 S-specific single B cells from convalescent COVID-19 patients	Viral neutralization by targeting NTD of S1.	EC <sub>50</sub> – 49 $\mu$ g/mL (SARS-CoV-2 pseudotyped virus) EC <sub>50</sub> – 0.61 $\mu$ g/mL (SARS-CoV-2 virus)	NI	[69]
H014	SARS-CoV-2 RBD-binding antibody from phage display	Blocking binding of SARS-CoV-2 RBD to hACE2	3 nM (SARS-CoV-2 pseudotyped virus) 38 nM (SARS-CoV-2 virus)	hACE2 transgenic mice model	[70]
COVA1-18, COVA2-15	SARS-CoV-2 S-specific single B cells from convalescent COVID-19 patients	Competitively blocking hACE2 binding to RBD	COVA1-18, 0.008 $\mu$ g/mL (SARS-CoV-2 pseudotyped virus) COVA2-15, 0.008 $\mu$ g/mL (SARS-CoV-2 pseudotyped virus) COVA1-18, 0.007 $\mu$ g/mL COVA2-15, 0.009 $\mu$ g/mL (SARS-CoV-2 virus)	NI	[71]

(continued on next page)

Table 2 (Continued)

mAbs	Source	Mechanism of neutralization	IC50	In vivo study	References
2-15	SARS-CoV-2 S-specific single memory B cells from COVID-19 patients	Targeting RBD of SARS-CoV-2 and blocking its binding to hACE2	0.005 µg/mL (SARS-CoV-2 pseudotyped virus)	Golden Syrian hamster model	[72]
414-1	SARS-CoV-2 S1 or RBD-specific single memory B cells from convalescent COVID-19 patients	Binding to RBD-A epitope	0.0007 µg/mL (SARS-CoV-2 virus) 3.09 nM (SARS-CoV-2 pseudotyped virus) 1.75 nM (SARS-CoV-2 virus)	NI	[73]
CV30	SARS-CoV-2 S-specific single B cells from COVID-19 patients	Targeting RBD of SARS-CoV-2 and blocking its binding to hACE2	0.03 µg/mL (SARS-CoV-2 pseudotyped virus)	NI	[74]

NI, not investigated; NTD, N-terminal domain.

effective treatment than general anti-inflammatory interventions [98]. Tocilizumab has been recommended for critical COVID-19 patients in China and India who have significantly high levels of IL-6 [99,100]. A clinical trial is underway to investigate the use of mAbs targeting GM-CSF for the intervention of inflammatory cytokine storm caused by SARS-CoV-2 (<https://clinicaltrials.gov/ct2/show/NCT04341116>). Moreover, since TNF is a major player in the inflammatory response, targeting it with an anti-TNF antibody is a feasible strategy to reduce COVID-19-induced lung inflammation and related inflammatory markers [101]. In addition, administration of anti-C5a mAbs is presumed to have a role in the mitigation of COVID-19-associated lung injury [50].

### Nanobodies as a promising therapeutic agent against SARS-COV-2

Llama-based nanobodies have been implicated in designing anti-viral therapy against HIV, rotavirus and respiratory syncytial virus [102–104]. Nanobodies are fully functional single-domain antibodies obtained from the camelid family, comprising a single variable domain (VHH) instead of two variable domains, as in the case of human antibodies. Their small size ( $\approx 14$  kDa), good solubility and excellent stability, along with high antigen specificity, make them a superior candidate over conventional antibodies [105]. Nanobodies can be easily produced in yeast or a bacterial host by recombinant technology [106]. The potency of nanobodies can be enhanced by engineering monovalent VHH into multivalent VHH [102]. SARS-CoV S-directed VHH (SARS VHH-72) generated in immunized llama cross-react with the SARS-CoV-2 S protein. Though the binding of SARS VHH-72 to SARS-CoV-2 RBD-SD1 is weaker, an engineered bivalent VHH Fc construct results in successful neutralization of SARS-CoV-2 pseudoviruses *in vitro*. Upon binding of VHH, RBD undergoes a dynamic conformational change that prevents its binding to the host cell receptor. Antibody panning with SARS-CoV-2 S can yield VHH with more stable binding and affinity toward SARS-CoV-2 RBD [107]. Further investigation is warranted to determine the feasibility of nanobodies as anti-viral therapy. Two nanobodies (H11-D4 and H11-H4) that neutralize SARS-CoV-2 by blocking the RBD and hACE2 interaction were identified by Huo *et al.* [108]. Fully human single-domain antibodies against SARS-CoV-2 were constructed using the human single-domain antibody phage display library [109]. An engineered trivalent nanobody with remarkable potency and stability was made into an inhalable formulation that could give protection against SARS-CoV-2, though effectiveness is yet to be proven by clinical trials [110].

### Cellular immune therapy for SARS-CoV-2 infection: a field worth exploring?

T cells play an important role in the viral clearance of SARS-CoV. Reduction in viral titer and improvement in survival were observed in SARS-CoV-infected SCID and BALB/c mice on adoptive transfer of *in vitro*-generated SARS-CoV-specific effector CD4+ and CD8+ T cells [111]. Channappanavar *et al.* [112] suggested that boosting virus-specific memory T-cell responses of the host may be a good therapeutic strategy against SARS-CoV infection. A drastic reduction in total T cells was reported in COVID-19 patients [113]. Sekine *et al.* [114] demonstrated T-cell phenotypes associated with acute and convalescent COVID-19 patients [114]. Strong memory T-cell responses were elicited in mild and asymptomatic COVID-19 patients, implicating the potential role of T cells in immunity against SARS-CoV-2. A clinical trial is ongoing for the treatment of severe COVID-19 patients using clinical-grade SARS-CoV-2-specific T cells from the blood of convalescent donors (<https://www.clinicaltrials.gov/ct2/show/NCT04351659>). However, serious concerns, such as donor matching and induction of cytokine storm, should be addressed before the implication of adoptively transferred T cells for viral infection [115].

## Discussion

Unlike a newly emerged virion, the distance to successful immunotherapy against SARS-CoV-2 is not long, as there have been some advances in the therapeutic management of infections caused by previously emerged coronaviruses (SARS-CoV and MERS-CoV). CP therapy and the more refined IVIg approach are the currently available effective treatment options for SARS-CoV-2 infection. Human mAbs that prevent viral entry into the host by inhibiting viral attachment to the receptor show efficient SARS-CoV-2 neutralization *in vitro* and *in vivo*. The protective ability of a developed therapeutic antibody can be challenged by viral mutation under selection pressure. The use of combination mAbs has been a promising approach to overcoming the emergence of escape mutants. Treatment benefits of these promising candidates can only be assessed through randomized controlled trials. Further, if therapeutic candidates are proven safe and efficacious in the treatment of SARS-CoV-2 infection, policy makers should be well equipped for the mass production and distribution of anti-viral therapeutics in response to the pandemic threat. Apart from the lack of a successful therapy, the high mortality rate associated with COVID-19 has been attributed to the paucity of understanding regarding the immunopathology of SARS-CoV-2 infection and underlying comorbidities. Triggering immunomodulation using mAbs targeting inflammatory cytokines can be used to treat the life-threatening cytokine storm syndrome. Though developing therapeutic antibodies targeting SARS-CoV-2 is the primary need of the current scenario, designing super-antibodies that neutralize multiple coronaviruses can make the world ready to tackle a future coronavirus outbreak.

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The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

## Author Contributions

Conception and design of the study: KVS and SK. Acquisition of data: KVS and SK. Analysis and interpretation of data: KVS and SK. Drafting or revising the manuscript: KVS and SK. Both authors have approved the final article.

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