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

Kuttuvan Valappil Sajna*,**, Siya Kamat*

Department of Biochemistry, Indian Institute of Science, Bangalore, India

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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
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 organotypic 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-1β, 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 chemotactrant 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 lymphocytosis, 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 1α (MIP1α), 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+CD16+ 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 hyaluronal 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.

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Comorbidity</th>
<th>Current therapy</th>
<th>Risks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diabetes (type 1 and 2)</td>
<td>ACE inhibitors and ARBs</td>
<td>Increased expression of ACE2, poor prognosis</td>
<td>[25]</td>
</tr>
<tr>
<td>2</td>
<td>Hypertension, cardiovascular diseases</td>
<td>ACE inhibitors, ARBs, ibuprofen and thiazolinediones</td>
<td>Increased expression of ACE2, poor prognosis</td>
<td>[26,27]</td>
</tr>
<tr>
<td>3</td>
<td>Inflammatory lung diseases</td>
<td>Macrolide or quinolone antibiotics and steroids</td>
<td>Excess inflammation</td>
<td>[28]</td>
</tr>
<tr>
<td>4</td>
<td>Cancer</td>
<td>Surgery or chemotherapy</td>
<td>Increased severity due to delays in treatment and low immunity</td>
<td>[29-30]</td>
</tr>
<tr>
<td>5</td>
<td>Liver injury</td>
<td>Macrolide or quinolone antibiotics and steroids</td>
<td>Excess inflammatory response</td>
<td>[31]</td>
</tr>
<tr>
<td>6</td>
<td>Autoimmune diseases: rheumatoid arthritis</td>
<td>Corticosteroids, NSAIDs, acetaminophen and TNF inhibitors</td>
<td>Increased risk of secondary infection due to immunosuppression</td>
<td>[32,33]</td>
</tr>
</tbody>
</table>

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 Biotec 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-2 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\textsubscript{resus} 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

**Figure 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. (Color version of figure is available online).

**Figure 2.** SARS-CoV-2-neutralizing mAb production using transgenic H2L2 mice [8]. Created with BioRender.com. ELISA, enzyme-linked immunosorbent assay. (Color version of figure is available online).
outbreak in 2003/2004. Zhu et al. [58] reported that humanized mAbs m396 and S230 successfully cross-react with the GDO3 strain, Urbani and Tor2 isolates (first SARS outbreak) and S23 and S216 (pseudo-typed 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 receptor activation 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 Fcy 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 Fcy 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 variants [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 against 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.

<table>
<thead>
<tr>
<th>mAbs</th>
<th>Source</th>
<th>Mechanism of neutralization</th>
<th>IC50</th>
<th>In vivo study</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>47D11</td>
<td>Transgenic H2L2 mice</td>
<td>Binding to conserved epitope of spike RBD</td>
<td>0.061 µg/mL (SARS-CoV-2 pseudotyped virus) 0.57 µg/mL (SARS-CoV-2 virus)</td>
<td>**</td>
<td>[8]</td>
</tr>
<tr>
<td>311mAb-31B5</td>
<td>RBD-specific single B-cell repertoire from recovered COVID-19 patient</td>
<td>Blocking binding of SARS-CoV-2 RBD to hACE2</td>
<td>311 mAb-31B5 = 0.0338 µg/mL 311 mAb-32D4 = 0.0098 µg/mL (SARS-CoV-2 pseudotyped virus)</td>
<td>NI</td>
<td>[60]</td>
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<td>311mAb-32D4</td>
<td></td>
<td></td>
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<td>hACE2 transgenic mice model</td>
<td>[53]</td>
</tr>
<tr>
<td>B38</td>
<td>RBD-specific single B-cell repertoire from COVID-19 patient</td>
<td>Blocking binding of SARS-CoV-2 RBD to hACE2</td>
<td>B38 = 0.177 µg/mL H4 = 0.896 µg/mL (SARS-CoV-2 virus)</td>
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<td>HA001</td>
<td>Phage display 311mAb-31B5 Memory B cells of person previously infected with SARS-CoV</td>
<td>Blocking binding of SARS-CoV-2 RBD to hACE2</td>
<td>311 mAb-31B5 = 0.0338 µg/mL 311 mAb-32D4 = 0.0698 µg/mL (SARS-CoV-2 pseudotyped virus)</td>
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<td>[59]</td>
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<tr>
<td>S309</td>
<td>Phage display 311mAb-31B5 Memory B cells of person previously infected with SARS-CoV</td>
<td>Blocking binding of SARS-CoV-2 RBD to hACE2</td>
<td>79 ng/mL (SARS-CoV-2 virus)</td>
<td>NI</td>
<td>[61]</td>
</tr>
<tr>
<td>BD-368-2</td>
<td>High-throughput scRNA/VDJ sequencing 311mAb-31B5 Memory B cells of person previously infected with SARS-CoV</td>
<td>Blocking binding of SARS-CoV-2 RBD to hACE2</td>
<td>1.2 ng/mL (SARS-CoV-2 pseudotyped virus) 15 ng/mL (SARS-CoV-2 virus)</td>
<td>hACE2 transgenic mice model</td>
<td>[62]</td>
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<tr>
<td>PC2-1F11</td>
<td>RBD-specific single B-cell repertoire from COVID-19 patient</td>
<td>Blocking binding of SARS-CoV-2 RBD to hACE2</td>
<td>PC2-1F11 = 0.03 µg/mL P2B-2F6 = 0.05 µg/mL P2C-1A3 = 0.62 µg/mL (SARS-CoV-2 pseudotyped virus)</td>
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<td>[63]</td>
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<tr>
<td>P2C-1A3</td>
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<tr>
<td>ADI-56046</td>
<td>RBD-specific single B-cell repertoire from SARS-CoV survivor</td>
<td>Binding to conserved epitopes in RBD and competing with hACE2 binding</td>
<td>&lt;0.05 µg/mL (SARS-CoV-2 pseudotyped virus) &lt;0.1 µg/mL (SARS-CoV-2 virus)</td>
<td>NI</td>
<td>[64]</td>
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<tr>
<td>COV2-2196, COV2-2130, COV2-2381</td>
<td>SARS-CoV-2 S2P, RBD or S2Preactive single B cells from convalescent COVID-19 patients</td>
<td>Blocking binding of SARS-CoV-2 RBD to hACE2</td>
<td>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)</td>
<td>Ad-hACE2 mouse model and rhesus macaque model</td>
<td>[65]</td>
</tr>
<tr>
<td>CC12.1</td>
<td>SARS-CoV-2 RBD and S antigen-specific single memory B cells from convalescent COVID-19 patients</td>
<td>Targeting RBD-A epitope by competing with hACE2 and blocking its binding to SARS-CoV-2 RBD</td>
<td>0.019 µg/mL (SARS-CoV-2 pseudotyped virus) 0.022 µg/mL (SARS-CoV-2 virus)</td>
<td>Syrian hamster model</td>
<td>[66]</td>
</tr>
<tr>
<td>REGN10933</td>
<td>Anti SARS-CoV-2 antibodies from immunized VI mouse and RBD-specific single B cells from convalescent COVID-19 patients</td>
<td>Blocking binding of SARS-CoV-2 RBD to hACE2</td>
<td>REGN10933-42 pM REGN10987- 40 pM (SARS-CoV-2 pseudotyped virus) REGN10933-37 pM REGN10987- 42 pM (SARS-CoV-2 virus)</td>
<td>Rhesus macaque model and golden hamster model</td>
<td>[67]</td>
</tr>
<tr>
<td>REGN10987</td>
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</table>
| 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 µg/mL (SARS-CoV-2 pseudotyped virus) ND<sub>50</sub> = 0.036 µg/mL (SARS-CoV-2 virus) | Rhesus macaque model | [68] | (continued on next page)
Table 2 (Continued)

<table>
<thead>
<tr>
<th>mAbs</th>
<th>Source</th>
<th>Mechanism of neutralization</th>
<th>IC50</th>
<th>References</th>
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<tbody>
<tr>
<td>H014</td>
<td>4A8 SARS-CoV-2 S-specific single B cells from convalescent COVID-19 patients</td>
<td>Neutralizing activity</td>
<td>10.6 nM (SARS-CoV-2 pseudotyped virus)</td>
<td>NI [69]</td>
</tr>
<tr>
<td>2-15</td>
<td>4A8 SARS-CoV-2 RBD-binding antibody from camelid</td>
<td>Neutralizing activity and blocking RBD from interacting with hACE2</td>
<td>0.009 μg/mL (SARS-CoV-2 pseudotyped virus)</td>
<td>[70]</td>
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<tr>
<td>414-1</td>
<td>4A8 SARS-CoV-2 S-specific single B cell from COVID-19 patients</td>
<td>Neutralizing activity and blocking RBD from interacting with hACE2</td>
<td>0.0013 μg/mL (SARS-CoV-2 pseudotyped virus)</td>
<td>[71]</td>
</tr>
<tr>
<td>CV30</td>
<td>2C11 SARS-CoV-2 S1 or RBD-specific single memory B cells from convalescent COVID-19 patients</td>
<td>Neutralizing activity and blocking RBD from interacting with hACE2</td>
<td>0.008 μg/mL (SARS-CoV-2 pseudotyped virus)</td>
<td>[72]</td>
</tr>
<tr>
<td>CV30</td>
<td>2C11 SARS-CoV-2 S1 or RBD-specific single memory B cells from convalescent COVID-19 patients</td>
<td>Neutralizing activity and blocking RBD from interacting with hACE2</td>
<td>0.008 μg/mL (SARS-CoV-2 pseudotyped virus)</td>
<td>[73]</td>
</tr>
<tr>
<td>CV30</td>
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<td>0.008 μg/mL (SARS-CoV-2 pseudotyped virus)</td>
<td>[74]</td>
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</table>

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 (~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 immuno-therapy 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 IVg 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 pandemic threat. Apart from the lack of a successful therapy, the high mortality rate associated with COVID-19 has been attributed 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 pandemic threat. Apart from the lack of a successful therapy, the high mortality rate associated with COVID-19 has been attributed 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 pandemic threat. Apart from the lack of a successful therapy, the high mortality rate associated with COVID-19 has been attributed 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 pandemic threat. Apart from the lack of a successful therapy, the high mortality rate associated with COVID-19 has been attributed 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 pandemic threat.

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Drafting or revising the manuscript: KVS and SK. Both authors have contributed in data: KVS and SK. Analysis and interpretation of data: KVS and SK.

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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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References


