Antibody Response to Severe Acute Respiratory Syndrome- Corona Virus 2, Diagnostic and Therapeutic Implications

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The immune response against severe acute respiratory syndrome-corona virus 2 (SARS-CoV-2) is comprised of both cellular and humoral arms. While current diagnostic methods are mainly based on polymerase chain reaction, they suffer from insensitivity. Therefore, antibody-based serologic tests are being developed to achieve higher sensitivity and specificity. Current efforts in treating SARS-CoV-2 infection include blocking of viral entry into the host cells, prohibiting viral replication and survival in the host cells, and reducing the exaggerated host immune response. Administration of convalescent plasma containing antiviral antibodies was proposed to improve the outcome in severe cases. In this paper, we review some of the aspects associated with the development of antibodies against SARS-CoV-2 and their potential use for improved diagnosis and therapy. (*Hepatology Communications* 2020;4:1731-1743).

Severe acute respiratory syndrome-corona virus 2 (SARS-CoV-2) is an infectious RNA virus responsible for causing corona virus disease 2019 (COVID-19).[1] While current diagnostic methods for COVID-19 diagnosis are mainly based on polymerase chain reaction (PCR), they suffer from insensitivity. Widespread reports of both false-positive and false-negative tests have been reported. Therefore, serologic tests are being developed to identify patients suffering from COVID-19 and to assist in identifying subjects who have been diseased and may now be immune to reinfection or to severe disease.

The host immune response mounted toward the virus contributes to disease severity. The immune response toward SARS-CoV-2 is comprised of both the cellular and humoral arms. Current evidence points to the severe manifestation of COVID-19 disease as being driven by inappropriate hyperactivation of the immune system, associated cytokine storm, and end-organ damage.[2,3] Current efforts for the treatment of COVID-19 include blocking viral entry into the host cells, prohibiting viral replication and survival in the host cells, or reducing the exaggerated host immune response. However, these strategies have shown limited efficacy.[4] Administration of convalescent plasma was proposed to improve patient outcomes in severe cases. In this paper, we review some of the aspects associated with the development of antibodies against SARS-CoV-2, their biology, potential uses, expected advantage, and disadvantages.

SARS-CoV-2 Epitopes as Potential Targets for the Humoral Immune Response

SARS-CoV-2 is an enveloped single-stranded RNA virus. The viral genome encodes four structural

Abbreviations: ACE, angiotensin converting enzyme; COVID-19, corona virus disease 2019; ELISA, enzyme-linked immunosorbent assay; FcR, Fc receptor; h, human; Ig, immunoglobulin; mAb, monoclonal antibody; MERS, Middle East respiratory syndrome; N, nucleocapsid; NAb, neutralizing antibody; PCR, polymerase chain reaction; RBD, receptor binding domain; rN, recombinant nucleocapsid protein; rS, recombinant spike protein; S, spike; SARS-CoV-2, severe acute respiratory syndrome-corona virus 2; TLR, toll-like receptor.

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proteins, including the spike (S), envelope, membrane, and nucleocapsid (N), as well as other nonstructural proteins. The S protein of SARS-CoV-2 consists of two subunits, S1 and S2. Several distinctive elements of SARS-CoV-2 are compared with other coronaviruses in Fig. 1.

Acting as a homotrimer, the heavily glycosylated S protein binds its cellular receptor, angiotensin converting enzyme 2 (ACE2), present on the pneumocytes and enterocytes, by the C-terminal domain of the S1 subunit in the receptor binding domain (RBD) region.[5,6] Extending from the viral membrane, the S protein extends outward from the virion. While the S1 subunit extends furthest from the virus membrane, the inner S2 subunits consist of a mostly helical structure, leading toward the viral membrane. The interaction of the S1–ACE receptor leads to conformational changes in the helical S2 subunit. The next event in viral binding and entry includes cleavage of the S1/S2 protein subunits by cellular proteases. This proteolytic activity may be performed by furin protease, a feature not unique to SARS-CoV-2 among the coronaviruses but absent in SARS-CoV.[7] The cleaving protease, dictating the exact exposed viral amino acid sequence, also determines the pattern of viral–cell fusion.[8,9] The release of newly constructed virions and the later activities of these new virions are also dependent on specific protease activity.[6]

Among the sites enumerated in this description, several appear as attractive targets for biologically active antibodies. Of note, while new data are continuously and vigorously obtained, specifically regarding SARS-CoV-2, much of the functional data regarding coronavirus activity and mechanisms come from research on SARS-CoV and Middle East respiratory syndrome-corona virus (MERS-CoV). This appears particularly poignant where homologies in the structure and function between these viruses are sought. While sequence and biological similarities are common, major differences exist, influencing virus function and antibody biology. These range from matters such as cleavage by similar proteases (although SARS-CoV-2 shows unique furin sensitivity) to receptor binding where it shares the affinity toward ACE2 with SARS-CoV through highly conserved RBD residues.[10]

Development of Antibodies Against SARS-CoV-2

The final event of protective and effective antibody production is the differentiation of B cells into plasma cells, a change accompanied by robust antibody production. A fraction of these cells will differentiate into memory B cells, allowing for an early antibody response following reinfection; this differentiation has been demonstrated after SARS-CoV infection.[11] Presumably, the "first contact" of SARS-CoV-2 with the immune system occurs following introduction of viable viral particles into the airways. The first responding part of the immune system may be the epithelial cells, both acting as antigen-presenting cells[12] and internally expressing antiviral proteins, specifically type-I interferons.[13] Type-I interferon signaling is usually initiated by toll-like receptors (TLRs). Variance in the vulnerability to the virus, namely men

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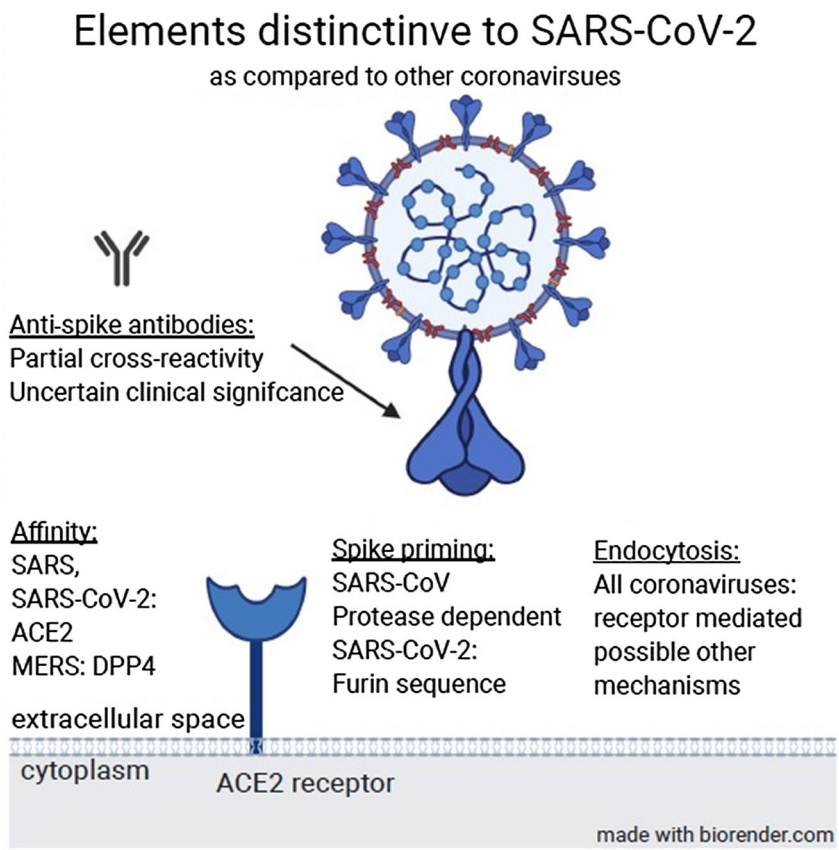

FIG. 1. Several distinctive elements of SARS-CoV-2 compared with other coronaviruses. Abbreviation: DPP4, dipeptidyl peptidase 4.

being more vulnerable, has been attributed partially to superior TLR7 signaling in women, possibly resulting in enhanced antibody production.[14] Notably, TLR7 functions in B cells as well and may contribute to enhanced function and differentiation of plasma cells.[15] Following initial contact with epithelium, innate immune cells come in contact with the virus and with infected cells. Superficial intraepithelial dendritic cells (DCs) in the lungs adjacent to the airways are required for antibody production.[16] After antigen encounter, they move to the regional lymph nodes and help trigger robust antibody production by activation of cluster of differentiation (CD)4 "follicular helper" T cells, supporting B-cell function.[17] Some DC functions, including type-I interferon secretion in response to viral stimulation, is also dependent on TLR signaling.[18]

While existing research is focused on the endogenic immune response to SARS-CoV-2 and its possible beneficial manipulations, isolation of neutralizing antibodies (NAbs) from infected persons or laboratory manufacturing of these antibodies is another subject of intense interest. Monoclonal antibodies (mAbs) with some neutralizing activities were demonstrated to occur in infected human sera.[19] NAbs may be defined in various ways and commonly as the antibody concentration required to prevent or decrease infectivity.[20] The most attractive antibodies are those targeting the S protein, whether in the RBD or other regions, including the S1/S2 proteolytic cleavage site.[21] It is plausible that antibodies targeting these sites will block essential viral functions, including viral antigen binding (expected from S1-RBD antibodies), and/or interfere with S protein-mediated viral fusion or cell entry.[21-23]

Multiple specific regions in SARS-CoV-2 show high homology to the SARS-CoV virus, suggesting potential B- and T-cell epitopes for SARS-CoV-2.[24] A set of B-cell and T-cell epitopes were derived from S and N proteins, which (excluding notable differences)

are generally conserved between SARS-CoV and SARS-CoV-2. The lack of mutations in these identified epitopes allows assessment of possible SARS-CoV-2 immune targets.[25] This study showed that no mutations occurred between SARS-CoV and SARS-CoV-2 in these sequences, confirming the possibility of antibody cross-reactivity and humoral immunity.

In spite of this high homology, cross-reactivity of SARS-CoV antibody is limited between two viral S proteins.[26,27] Murine polyclonal SARS-CoV antibodies directed against the S protein inhibited SARS-CoV-2 entry into cells, indicating that cross-NAbs targeting conserved S epitopes can be produced.[6] S1-targeting mAbs from immunized transgenic mice expressing human immunoglobulin (Ig) variable heavy and light chains can neutralize SARS-CoV-2 and SARS-CoV infections.[28,29] In a previously mentioned trial, 206 SARS-CoV-2 RBD-specific mAbs were generated, among which only two clones showed significant blocking of viral entry; this was associated with a high competitive capacity against ACE2 receptor binding.[19] Similar results were observed in studies using sera from patients recovered from SARS and COVID-19 where limited cross-neutralization occurred, suggesting that cross-NAbs are either incompletely reactive or insufficient for disease prevention.[28]

Before and concurrently with the isolation of specific antibodies, SARS-CoV S1-specific serum from patients convalescing from SARS or from animals was proposed to cross-neutralize the SARS-CoV-2 infection by reducing S protein-mediated SARS-CoV-2 entry.[8] Cross-reactivity of the antibodies from patients with SARS-CoV-2 against the S proteins but not against the RBD of SARS-CoV and MERS-CoV has been documented.

The roles played by the RBD in the invasion of SARS-CoV-2 into host cells make the RBD a potential target for NAbs. Blocking binding between the RBD and its respective receptor may restrict the conformational change of S or hamper S2-mediated membrane fusion, thereby inhibiting viral infection of host cells.[21] The human NAbs S230.15 and m396 were isolated from patients infected with SARS-CoV. They neutralize SARS-CoV infection by interacting with the RBD and by blocking binding between the viral RBD and ACE2 receptor.[30] The SARS-CoV RBD-specific human NAb, CR3022, binds the SARS-CoV-2 RBD with high affinity and recognizes an epitope on the RBD that does not overlap with the ACE2-binding site.[27] The S109.8 and S227.14 mAbs can neutralize the infectious clones of SARS-CoV and protect mice against four different homologous and heterologous SARS-CoV strains.[31,32] Of note, such mAbs produced in the chimeric mouse cells and originating from patients with SARS-CoV were shown to neutralize SARS-CoV-2 virus particles by an ACE2-independent mechanism; this probably has to do with S protein fusion or proteolysis and preventing viral fusion.[28]

While these studies hold both promise and interest, isolation and analysis of neutralizing antibodies remain a difficult task. A majority of 26 patients recovered from COVID-19 showed high titers of SARS-CoV-2 S1-specific IgG antibodies when tested by enzyme-linked immunosorbent assay (ELISA).[29] However, only 3 out of these 26 patients manifested an effective blockade of SARS-CoV-2 RBD binding to human (h)ACE2 when tested *in vitro*.[29] The transient and dynamic conformational states of the S protein have been suggested to provide a narrow window for exposure of the immunogenic epitopes of RBD to B lymphocytes.[33] Early and transient peak levels of anti-S antibody response were associated with a less favorable outcome for patients compared with a more delayed and sustained response.[34]

The phage display method, allowing rapid and wide display of proteins directly correlated to their associated genes, can detect NAbs against SARS-CoV from both naive and immune antibody libraries that are capable of blocking the binding of the S1 domain, thereby showing virus neutralization and prophylaxis capability either *in vitro* or in animal models.[30,32,35] Another method, possibly allowing the production and use of existing NAbs, may include the use of Epstein-Barr virus transformation of human B cells to improve the isolation of NAbs from the memory B cells harvested from patients infected with SARS-CoV.[11] Transgenic mice with human Ig genes that are effective for virus prophylaxis in animal models are being developed to produce NAbs against SARS-CoV by antigen immunization.[36,37]

Cloning of human mAbs using samples from patients recovered from COVID-19 whose sera showed hACE2 receptor binding inhibition has been reported.[38] Following antibody cloning, three pairs of IgG variable heavy-chain and light-chain inserted expression plasmids were expressed and named as

311mAb-31B5, 311mAb-32D4, and 311mAb-31B9. All three mAbs bind to the RBD protein. While mAb-31B5 and 311mAb-32D4 blocked SARS-CoV-2 RBD-hACE2 interaction and neutralized a SARS-CoV-2 S pseudotyped lentiviral particle,[28] 311mAb-31B5 and 311mAb-32D4 neutralized pseudovirus entry into host cells ectopically expressing hACE2.[29] Several NAbs, such as B1, 1F8, and 5E9, toward epitopes on SARS-CoV S2 manifested neutralization properties.[39,40]

N-specific antibodies have also been demonstrated in the sera of infected patients. Most studies assessing N antibodies have not differentiated these antibodies from other antibodies directed against SARS-CoV-2 in studies that seem to show similar kinetics to that of the general antibody response.[41] No studies have shown the occurrence of definitive NAbs directed at the N protein or the nature of the immune response triggered by such antibodies.

Antibody-Based Diagnosis of COVID-19

Serum IgG, IgM, and IgA antibodies against SARS-CoV appeared in patients after primary SARS infection.[42] Data on the production of IgG and IgM is important for improved diagnosis of COVID-19.[43] Several studies have described the dynamics of antibody production in these patients. While it is too early to definitively summarize the characteristics of antibody dynamics, certain conclusions seem consistent across these studies. Broadly, antibody titers increase and the prevalence of viral RNA decreases as time progresses from the onset of symptomatic disease.[44,45]

ELISA-based diagnostic kits often report a specificity of ~ 90%,[46] with some trials reporting a higher percentage.[44] While this is an impressive figure by itself, it may yield a relatively poor positive predictive value when employed on a large scale to a disease with relatively low prevalence. ELISA tests were argued to be efficient when trying to augment the sensitivity of testing of close contacts[45,47] or deciding to allow a person to leave from quarantine. This specificity may be further reduced when testing a person recently exposed to the milder coronaviruses circulating within humans and livestock. However, to our knowledge, this question has not been directly assessed.

IgG and IgM antibodies may appear simultaneously or sequentially, with cases of IgM antibodies appearing last being described in one study.[47] Conversion from seronegativity to seropositivity is likely to occur between 14 and 21 days after the onset of symptoms. Data from some of these studies show that patients with more severe illness were more likely to mount a high-titer and high-affinity antibody response, which was not necessarily associated with a reduction in the viral RNA assayed from their blood.[48] This is supported by reports of recurring PCR positivity after IgG seroconversion.[49] If these studies become the prevalent findings, they may stand in stark contrast to well-established viral disease behaviors where high IgG levels are thought to denote virtual immunity to the disease, allowing at most a mild manifestation following re-exposure. It seems that in COVID-19, as our current understanding stands, antibody titers should be thought of as disease markers and not as definitive markers of immunity or disease resolution in the actively ill.

In antibody detection, different ELISA kits show variable results based on recombinant SARS-CoV-2 N protein (rN) and recombinant S protein (rS). In a study of 214 patients with confirmed COVID-19, 68% were diagnosed with rN-based IgM, 70% with an IgG, 77% with rS-based IgM, and 74% with IgG tests. The positive rates for rN-based and rS-based ELISA detections were 80% for IgM and 82% for IgG. The sensitivity of the rS-based ELISA for IgM was higher than that of the rN-based test. Here also, antibody positivity increased as disease time progressed.[50]

Another stratum of results expected from antibodies is the identification of immune and recovered persons who may be able to work in critical locations during the times of pandemic. The ability to definitively identify specific NAbs in the serum of recovered patients could also allow identifying potential plasma donors for the development of passive immunization and may assist in evaluating the effectiveness of various treatments in addition to assisting in determining prognosis.[51]

Most convalescent plasmas obtained from individuals who recover from COVID-19 do not contain high levels of NAbs. A recent analysis of plasma from 149 individuals convalescing from COVID-19 that was collected an average of 39 days after the onset of symptoms showed variable half-maximal pseudovirus neutralizing titers below 1:50 in 33% and below

1:1,000 in 79%. Only 1% showed titers above 1:5,000. Expanded clones of RBD-specific memory B cells expressing closely related antibodies in different individuals were identified. The antibodies were directed against three distinct epitopes on the RBD. Rare but recurring RBD-specific antibodies with potent antiviral activity were identified in all recovered subjects.[52] The relevance of the titers for clinical effect has yet to be determined.

A recent review analyzed the diagnostic accuracy of antibody tests for SARS-CoV-2 infection, for assessing past infections, and for use in seroprevalence surveys.[53] A total of 57 publications reporting cohorts with 15,976 samples, of which 8,526 were from cases of SARS-CoV-2 infection, were evaluated. These showed substantial heterogeneity in sensitivities of IgA, IgM, and IgG Abs or combinations thereof for results aggregated across different time periods post-symptom onset. Pooled results for IgG, IgM, IgA, total antibodies, and IgG/IgM showed low sensitivity during the first week since onset of symptoms, rising in the second week, and reaching their highest values in the third week. The sensitivity of antibody tests was proposed to be too low in the first week since symptom onset to have a primary role for diagnosis but was suggested to have a role complementing other testing in individuals presenting later, when real-time PCR tests are negative. Antibody tests are useful for detecting previous SARS-CoV-2 infection if used 15 or more days after the onset of symptoms.[53]

Several currently available COVID-19 antibody tests that are used in diagnostics and epidemiology, with a focus on their strengths and weaknesses, are summarized in Table 1.

Using Convalescent Plasma as a Therapy for COVID-19

The lack of specific SARS-CoV-2-targeted treatments and vaccines poses great challenges for the management of patients with severe illness. IgG levels against SARS-CoV, drawn from affected patients, reach peak serum concentration during the convalescent phase and are reduced following recovery.[54] While the capacities of antibodies to neutralize the virus were highly variable in the required concentration, some of them indeed showed such capability and have been shown to provide protection against reinfection in a mouse model.[11] Use of convalescent plasma and development of NAbs are attractive methods for the treatment of viral infections.[55,56] Blocking mAbs with high antigen specificity have been proposed as potential candidates for neutralizing infections.[57-59]

Convalescent plasma has intermittently emerged during the last few decades as a treatment for various infectious diseases,[60-62] enjoying attention whenever diseases prove resistant to more conventional treatment methods. Plasma-derived NAbs can provide passive immune responses to viral infections and were effective in patients with severe illnesses caused by other viruses.[63,64] A meta-analysis showed that mortality was reduced after receiving various doses of convalescent plasma in patients with severe acute respiratory infections, with no adverse events or complications after treatment.[65] Antibodies from convalescent plasma were proposed to reduce viremia by enhancing viral clearance, blocking infection of new cells, and contributing in the clearance of infected cells.[56,66,67]

TABLE 1. SEVERAL COVID-19 ANTIBODY TESTS THAT ARE USED IN DIAGNOSTICS AND EPIDEMIOLOGY

	Technology Used	Sensitivity	Specificity	Strengths	Weaknesses
IgG	CGIA[96] CLIA[124] ELISA[125] LFA[126]	29.7%-88.2%	98.8%-99.5%	- High specificity - LFA available kits low cost	- Low early sensitivity - Scarce data after 35 days - Neutralizing effect N/A
IgM	CGIA[96] CLIA[124] ELISA[125] LFA[126]	23.2%-75.4%	97.3%-99.6%	- High specificity - LFA available kits low cost	- Low early sensitivity - Scarce data after 35 days - Neutralizing effect N/A
IgG/IgM	CGIA[44] CLIA[127] ELISA[128] LFA[96]	30.1%-91.4%	94.1%-99.4%	- High specificity - LFA available kits low cost	- Low early sensitivity - Scarce data after 35 days - Neutralizing effect N/A

Abbreviations: CGIA, colloidal gold immunoassay; CLIA, chemiluminescence immunoassay; LFA, lateral flow assay; N/A not applicable.

During the 2003 SARS epidemic, severely ill patients who deteriorated despite treatment with methylprednisolone were given convalescent plasma at around the fourteenth day of disease onset. Earlier plasma administration correlated with a better prognosis and higher rate of hospital discharge at day 22.[63] Convalescent plasma or Igs were effective in patients with SARS whose condition continued to deteriorate. Some studies suggested a shorter hospital stay and lower mortality rate following convalescent plasma administration.[56,63,68-72] A similar trend for treatment timing was described for 27 patients with Lassa fever in Nigeria treated with convalescent plasma.[73] The empirical use of convalescent plasma for Ebola virus disease showed some positive results.[64,74,75]

Experimental and clinical data on the use of convalescent plasma products and humanized monoclonal antibodies for H5N1 influenza infection have also shown positive outcomes, and this treatment was proposed as a means for overcoming antiviral drug resistance.[61,76,77] In a study involving 20 patients with severe pandemic influenza A virus subtype H1N1 2009 virus infection, administration of convalescent plasma reduced respiratory tract viral load, serum cytokine response, and mortality.[59] A prospective cohort study during the 2009 pandemic showed reduction in the relative risk of mortality in patients treated with convalescent plasma, demonstrating reduction of viral loads without any adverse effects.[59] A randomized trial of convalescent plasma failed to achieve its primary endpoint, a reduction of mortality; however, a subgroup multivariate analysis performed on 22 of the 35 patients enrolled in the trial demonstrated that human intravenous Ig treatment was the only factor independently associated with reduced mortality.[78]

Development of NAbs against SARS-CoV-2 was proposed as a method for developing therapeutic agents for COVID-19.[10,23,35,79] Several SARS-CoV-2 proteins (discussed above) prove attractive targets for NAbs. The SARS-CoV-2 S protein is a target for developing NAbs to block its binding and fusion.[35] Currently, no SARS-CoV-2-specific NAbs have been reported.[21] However, polyclonal antibodies from patients recovered from SARS-CoV-2 are being used to treat patients with severe infections. While many patients will develop an antibody response following their illness, specific characterization of these antibodies and their properties as NAbs have yet to be determined.[47,48] Early administration of convalescent plasma was advised in order to maximize its viral clearance effect.[80]

Plasma collection is done by apheresis. In order to qualify for donation, the donor must meet several conditions: diagnosis of prior COVID-19 infection confirmed by PCR, donation needs to take place 14-28 days after resolution of the symptoms followed by two consecutive negative PCR results, donors need to be tested for absence of transmissible pathogens, and donation should be done from male or nulliparous female donors, with no previous exposure to blood products in order to minimize the risk of transfusion-associated acute lung injury. Plasma (200-600 mL) is donated according to ABO compatibility. Pathogen inactivation measures need to be undertaken.[81] It is advised to administer up to 2 units of plasma, possibly from two different donors.[81]

Several studies that described the administration of convalescent plasma to patients critically ill with COVID-19 suggested posttransfusion viral elimination and clinical improvement. A study of critically ill patients (N = 5) reported clinical improvement in patients' status and laboratory indication of viral clearance for up to 12 days posttransfusion of two consecutive doses of convalescent plasma (total 400 mL).[82] Three of the patients were on mechanical ventilation and 2 on extracorporeal membrane oxygenation; treatment was provided between 10 and 20 days after hospitalization, following which improvements in fever, the ratio of arterial oxygen partial pressure to fractional inspired oxygen, and viral clearance were noted. Three patients were discharged from the hospital and 2 were in stable condition at the end of the follow-up period. Although a clinical effect was obtained, the delay of up to 3 weeks in administration and the concurrent use of other therapies make it difficult to assess the effect of plasma.[83]

Administration of convalescent plasma in 6 critically ill patients was followed by discontinued viral shedding 3 days after infusion, without reducing mortality.[84] A study of 6 patients with COVID-19 showed clinical, radiologic, and laboratory improvement following administration of ABO-compatible convalescent plasma, indicating that this therapy is effective and specific.[85] In a study of 10 patients with severe disease, administration with 200 mL of convalescent plasma showed improved clinical, laboratory, and radiologic status without severe adverse effects.[86] In this study, the antibody titers of donors' plasma were assessed and found to be elevated in the majority of donors, along with a concurrent increase in NAb titers in the patients' sera following transfusion. Treatment within 2 weeks of initial symptoms improved the response.[86] Differences

in outcomes between the studies may reflect temporal variations of administration, including the time lag between plasma donation and administration as well as the time from disease detection to treatment.

Safety evaluation of candidate antibodies must not be overlooked. Although antibodies are generally protective, the antibody-dependent enhancement (ADE) phenomenon of viral infections is documented for dengue virus and other viruses.[87] In SARS- CoV infection, ADE is mediated by the engagement of Fc receptors (FcRs) expressed on various immune cells, including monocytes, macrophages, and B cells.[88] Preexisting SARS-CoV-specific antibodies were proposed to promote viral entry into FcR-expressing cells. Internalization of virus–antibody immune complexes may induce inflammation and tissue injury by activating myeloid cells through FcRs.[88]

Several putative and proven NAb interactions in COVID-19, such as antibody targets and functions, including those associated with disruption and non-disruption binding mechanisms and those targeting the virus itself, are shown in Fig. 2. In addition, non-neutralizing antibodies, cross-reactive antibodies, and antibodies with low specificity or low titers, which are unable to act as Nabs, are also generated.

Several large trials using convalescent plasma are being conducted.[89] Identifying and cloning mAbs that target viral proteins to block entry into host cells is being explored for preventing and treating COVID-19.[29,35] Computational simulation of antibody–antigen complexes can improve the design of these therapies. Key residues between RBD and NAbs can be identified, and models are being used to assess the interaction between S protein and human ACE2 or antibodies.[27,35,90-93]

Methods for Improving Potential Use of Antibodies for COVID-19 Treatment

Several methods for improving the effectiveness of convalescent plasma or NAbs are being considered. The outcomes of passive convalescent plasma

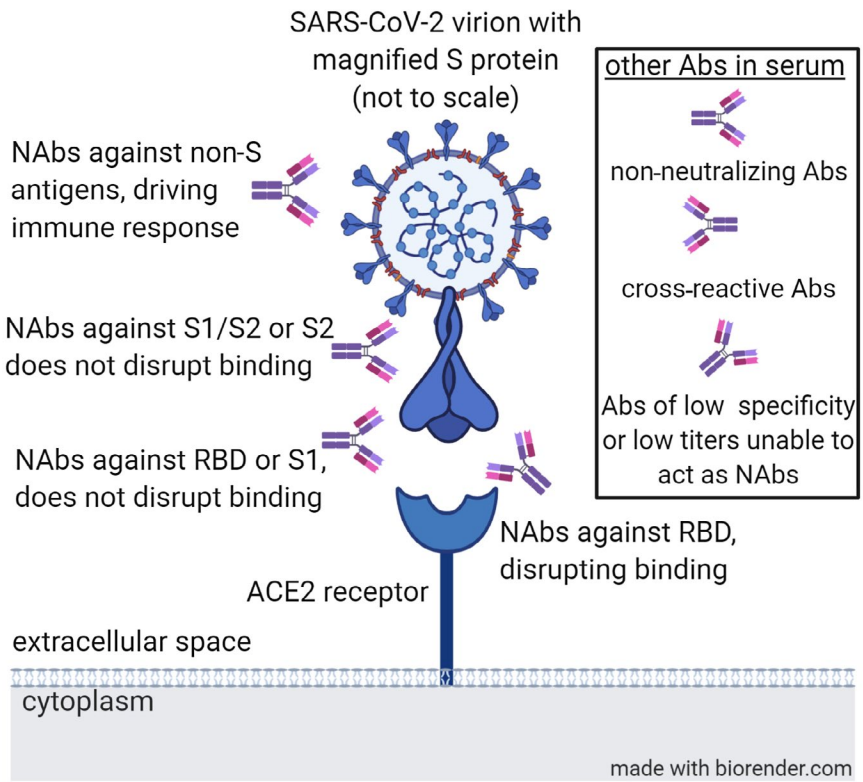

FIG. 2. Several putative and proven NAbs in COVID-19 antibody targets and functions. Abbeviation: Abs, antibodies.

therapy from recovered donors are unpredictable due to variability among donors in both the levels and types of antibodies.[94] Appropriate selection of donors is required for improving the quality of collected plasma. Assessment of antibody titers needs to be performed before harvesting due to marked variability in titers among donors. Titers correlate with disease severity, timing of donation, use of steroids during acute illness,[95,96] and quality of antibodies (i.e., whether they are NAbs or not). Timing of plasmapheresis is a major factor as lower levels of antibodies are detected within the first 2 weeks following recovery.[47] More data are required on the amount of virus neutralization by antibodies following exposure to convalescent plasma. *In vitro* testing for neutralizing and/or cross-neutralizing activity and *in vivo* evaluation in available COVID-19 animal models for protective efficacy along with preclinical studies and clinical trials testing safety and efficacy are needed for optimizing this therapeutic option.[21]

The sex of the donor also plays a role in mounting a significant response. The degree of activation of immune cells is higher in women than in men, which correlates with triggering of TLR7 and production of proinflammatory cytokines. TLR7 is expressed in innate immune cells, which recognize single-strand RNA virus by promoting production of viral antibodies and the generation of interleukin (IL)-6 and IL-1 inflammatory cytokines. TLR7 is higher in women than in men, and its expression may lead to better immune responses and increased resistance to viral infections.[14] Pairing human leukocyte antigen typing with COVID-19 was proposed to improve the assessment of disease severity and assist in preferred donor selection.[97]

The use of hyperimmune globulin rather than whole plasma was proposed for improving the efficacy and validity of the therapy. The main advantages are associated with an ability to provide patients with controlled quantities of antibodies in lower volumes.[82] Similar techniques for concentrating antibodies are being used for the treatment and prevention of other diseases.[98] This is similar to the concept of using hyperimmune globulin for various indications, including viral diseases in immunocompetent and immunocompromised hosts.[99-101] A "cocktail antibody approach" for SARS-CoV-2 was proposed based on studies suggesting that the combination of antibodies from diverse donors may exert a synergistic neutralization effect.[35] A mixture of two antibodies showed a synergistic neutralization effect due to recognition of different epitopes on the RBD.[102]

Use of immune adjuvants may also improve the response to the antibodies.[103] Sphingolipid-based adjuvants, when administered with antibodies, augmented the antiviral response[104] and improved the systemic anti-inflammatory effects of antibodies.[105] Use of hyperimmune bovine colostrum comprised of antibodies and sphingolipids was effective in reducing systemic inflammation.[106-108] Mode of antibody administration may also have an impact on the effect of antibody-based therapy. Oral administration of antibodies ameliorated viral-mediated chronic inflammation by promotion of regulatory T cells,[109] and oral administration of viral antigens augmented an antiviral immunity while reducing inflammation.[110,111]

Data on the possible harmful effects of antibody-mediated immune response in the development of pulmonary complications of SARS-CoV is controversial. Several patients who died of SARS manifested strong NAb responses and pulmonary inflammation, suggesting that the NAbs could be associated with deterioration of the lung disease.[35,112] Similar notions have been proposed for explaining the more severe phenotype of COVID-19 prevalent in China. This may be related to the higher degree of exposure to milder coronaviruses and a "priming" of the immune system by preexisting antibodies, leading to immune dysfunction and overfunction.[113] This notion is supported by mild disease manifestations in patients with agammaglobulinemia.[114] Previous exposure to coronaviruses may also explain a relatively high prevalence of S protein-reactive CD4 cells in healthy donors in a study.[115]

A major obstacle for implementing immune-based therapies for the viruses, including the administration of mAbs, is associated with development of viral resistance due to immune evasion mechanisms, which the virus generates in response to the immune pressure imposed on it by immunomodulatory agents.[116] Prolonged exposure to antiviral drugs is associated with drug resistance, leading to persistent viremia or severe disease. In cases where antiviral treatment is highly effective, leading to viral elimination, resistance is less likely to occur. However, immunotherapy, including administration of antibodies, is associated

with selective pressure that may result in rapid viral and host adaptations leading to resistance to the therapy.[117] Both host and viral factors are associated with development of resistance. Viral-related tools include mechanisms of viral replication, genomic inference, and high rates of viral mutations.[117-119]

An immune adaptation process toward antibody-induced pressure on the virus or on antiviral humoral and cellular responses may limit the efficacy and longevity of these therapies. A combination of several potent NAbs could improve the sensitivity to neutralization.[35] Methods for overcoming resistance by implementing host-tailored variability are being developed based on data generated from the use of these methods for improving the effects of other immunomodulatory drugs.[120-123] These include implementing artificial intelligence methods for overcoming host compensatory responses in sepsis and its sequel[120] and for improving the effects of adjuvants.[122] Algorithm-controlled treatment regimens are now being used in several clinical trials for overcoming drug resistance (NCT03843697; NCT03747705).

Concluding Remarks

The lack of accurate diagnostic and effective therapeutic methods for patients infected with SARS-CoV-2 led to the need to develop humoral-based approaches. While this approach holds promise, more data are needed for optimizing antibody-based diagnosis and for improving the implementation of convalescent plasma and other antibody-based therapies. The potential development of effective vaccines will benefit from the results achieved from these diagnostic and therapeutic attempts. Immunotherapeutic methods are expected to require targeting the cellular arm of the immune system either in addition to or as part of the design of antibody-based approaches, mainly for alleviating immune-mediated target organ damage in COVID-19.

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