

Antibodies to SARS-CoV-2 and their potential for therapeutic passive immunization

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Abstract

We review aspects of the antibody response to SARS-CoV-2, the causative agent of the COVID-19 pandemic. The topics we cover are relevant to immunotherapy with plasma from recovered patients and with monoclonal antibodies against the viral S-protein. The development of vaccines against SARS-CoV-2, an essential public health tool, will also be informed by an understanding of the antibody response in infected patients. Although virus-neutralizing antibodies are likely to protect, antibodies could potentially trigger immunopathogenic events in SARS-CoV-2-infected patients or enhance infection. An awareness of these possibilities may benefit clinicians and the developers of antibody-based therapies and vaccines.

Introduction

Passive immunization with plasma from patients who have seroconverted to and recovered from infection with a pathogen has a long and generally successful history. It was used on a small scale during the 1995 and 2014-2015 Ebola epidemics (1, 2). In recent years, highly specific and often broadly active neutralizing monoclonal antibodies (MAbs) have been developed against several viruses, as a more advanced substitute for patient plasma (3-8). These methods are now being considered for treating COVID-19, the disease caused by the SARS-CoV-2 coronavirus (9-12). The US Food and Drug Administration has recently approved plasma immunotherapy for this purpose (<https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-ind-or-device-exemption-ide-process-cber/recommendations-investigational-covid-19-convalescent-plasma>). Here, we review aspects of the antibody response to SARS-CoV-2, which may be relevant to immunotherapy with plasma or MAbs. A major goal of viral vaccine development is the induction of strong and broadly active neutralizing antibodies (NAbs), and that goal applies also to SARS-CoV-2 (9, 13, 14). The development of vaccines, an essential public health tool, will also be informed by an understanding of the antibody response during COVID-19.

Assays are now available for detecting IgA, IgM, and IgG specific for SARS-CoV-2 in patient serum, i.e., to demonstrate seroconversion and also for detecting NAbs (15-17). These techniques are rapidly evolving, and additional information on the antibody response to CoV-2 infection is emerging almost daily. Analyses of antibody kinetics and how long predictably protective titers are maintained have not yet been performed. They will be a priority once enough time has elapsed for long-term studies to be feasible.

The natural history of COVID-19 and some lessons from the previous SARS coronavirus (SARS-CoV-1) and the more distantly related MERS-CoV, including animal model studies, do raise some potential concerns about NAb-based therapies and vaccines, warranting careful surveillance by clinicians during human trials. Furthermore, certain approaches may minimize risks while preserving the benefits of passive immunization for curing COVID-19.

Antibody-mediated neutralization of SARS-CoV-2

The entry of SARS-CoV-2 into cells is initiated by the interaction of the receptor-binding domain (RBD) of the viral Spike (S) glycoprotein with the angiotensin converting enzyme-2 (ACE2), which acts as a receptor for the virus on the target cell surface (18, 19). The most potent NAbs are directed to the RBD and some may act by simply competing with the receptor for binding to the S-protein. Antibodies to SARS-CoV-1 and MERS-CoV generally do not cross-neutralize SARS-CoV-2; although cross-reactive antibodies are frequently detected in S-protein ELISA (11, 17, 19-22). Recently, however, the S-protein-specific NAb S309, isolated from memory B cells of a patient who had recovered from CoV-1 infection in 2003, was shown to neutralize both SARS-CoV-1 and -2 potently by ligating the RBD. Cryo-electron microscopy and binding assays demonstrated that the conserved S309 epitope comprises glycans and that in spite of its specificity for the RBD, S309 does not interfere with ACE2 binding (23).

The neutralizing potency of antibodies against the RBD may be determined not only by their own affinity for the S-protein but also by the affinity of the latter for ACE2, at least when they act by a competitive mechanism of action (11). In this context, it is notable that the SARS-CoV-2 S-protein has a 10-20-fold higher affinity for ACE2 than its counterpart from SARS-CoV-1 (22). Although most NAbs are directed to the RBD (23-30), some antibodies that recognize the SARS-CoV-1 S2 fragment can also neutralize (31). In addition, antibodies to the ectodomain of another surface SARS-CoV-1 protein, Orf3a, are also reported to have neutralizing activity, while

antibodies to the M and E proteins can potentiate neutralization (32, 33). Whether SARS-CoV-2 is similar to CoV-1 in these respects remains to be determined. Nonetheless, passive and active immunization approaches to COVID-19 are generally focused on NAb against the S-protein.

The kinetics of NAb and other antibody responses in SARS-CoV infection

Little information on the antibody responses elicited in COVID-19 patients is available, and none from prolonged studies. Data on SARS-CoV-1 infection may, however, be informative. Surprisingly, the NAb response in patients who later succumbed to the infection was faster than in those who recovered; in the patients who later died, the titers had peaked on day 15 after the onset of symptoms, whereas similar titers and extents of neutralization were reached only after day 20 in the patients who recovered. The NAb titers in the moribund patients declined or disappeared after the early rise, as their conditions deteriorated towards death (34). It is unknown whether this titer loss reflects an inability to produce antibodies due to lymphocyte losses or antibody elimination by immune complexing as the viral load rises. In plasma collected from 175 patients who had recovered from mild COVID-19, NAb and S-binding-antibody titers correlated positively with age and CRP (C-reactive protein) levels, but negatively with lymphocyte counts; and the NAb did not cross-neutralize SARS-CoV-1 (35). Since no severe cases were included and viral loads were not monitored, it is unclear what promoted the NAb responses within the patient cohort in which antibody titers, age, lymphopenia, and inflammation were associated. Other studies have shown higher binding-antibody titers to the nucleocapsid protein N among patients who recovered than those who did not (34, 36). Such antibodies to the intra-virion N-protein completely lack neutralizing capacity but their production might reflect the strength of T-helper cell responses (37).

NAb immunotherapy against SARS-CoV-1 and SARS-CoV-2

Will passive immunization with plasma from convalescent patients be beneficial for treating COVID-19? Anti-S antibodies are known to protect against lethal CoV challenge and clear the virus in mice and ferrets (38-41). In a small experiment, SARS-CoV-2 infection reportedly protected against a second challenge of macaques, which was attributed to the development of protective antibodies (42). The outcome of human clinical trials will, of course, outweigh animal-model experiments. No significant adverse reactions were noted when plasmas with high NAb titers were given to SARS-CoV-1 patients; clinical benefits such as lower viral loads and earlier release from hospital were noted in retrospective analyses (43, 44). Recently, five critically ill COVID-19 patients were transfused at 10-22 days post-admission with a pool of plasma derived from 5 convalescent patients; the RBD-binding antibody endpoint titers in ELISA were >1000, and the neutralization endpoint titers were >40 (45). All the patients (36-65 years; 3 male, 2 female) were receiving mechanical ventilation. After plasma transfusion, body temperatures normalized while organ-failure and respiratory-function scores improved to different extents. Nasopharyngeal viral load decreased and became undetectable within 12 days in all five patients, while SARS-CoV-2 ELISA and NAb titers increased, reflecting the antibody-content of the transfused plasma. Thus, in this preliminary and necessarily uncontrolled case series of five critically ill COVID-19 patients with acute respiratory distress syndrome (ARDS), the transfusion of NAb-containing convalescent plasma was associated with improved clinical status (45). A subsequent larger study yielded similar results: ten patients with severe COVID-19 received 200 mL of convalescent plasma obtained from recently recovered donors with NAb inhibitory-dilution factors > 640. Three days later, clinical, pulmonary-radiological, and laboratory parameters were improved, the latter including oxyhemoglobin saturation, lymphocyte counts, and C-reactive protein levels; viral loads

in serum became undetectable in seven patients (46). Overall, both studies showed plasma transfusion to be well tolerated. Although beneficial effects were reported, they could not be proven because neither study was controlled and both involved other antiviral interventions.

Can antibodies contribute to SARS pathogenesis?

Strategies for passive and active immunization to combat and prevent SARS-CoV-2 infection should take into account the pathogenesis of COVID-19, which can lead to death. The inflammatory response to SARS-CoV-2 is thought to drive or at least exacerbate the disease process, particularly during the second week after infection becomes symptomatic. Daily transcriptomic profiling of three COVID-19 cases showed a highly dynamic early immune response to SARS-CoV-2. The expression of many inflammatory genes peaked after respiratory function reached its nadir. Pro-inflammatory responses may be intertwined with T-cell activation that could exacerbate the disease; IL-1 secretion and related pro-inflammatory pathways may be prognostic and could serve as therapeutic targets in COVID-19 (47). How may these immune responses that modulate pathogenesis be affected by NABs?

The lethal coronaviruses cause fatal acute lung injury (ALI) by driving hypercytokinemia and aggressive inflammation through incompletely understood mechanisms. In macaque models of SARS-CoV-1 infection that involve both passive and active immunization, IgG specific for the S-protein was reported to exacerbate ALI by counteracting inflammation-resolving responses, abrogating wound-healing, promoting MCP1 and IL-8 production, and increasing proinflammatory monocyte and macrophage recruitment (48). Likewise, in human patients who died of SARS-CoV-1 infection, pulmonary proinflammatory macrophages accumulated in the lungs while wound-healing macrophages were absent (48). Moreover, two observations noted above raise questions about the causal relationship between antibodies and severity of infection: NAb responses were faster in the patients who later died than in those who recovered (34, 48), and older patients who had recovered from mild COVID-19, had significantly stronger NAb and S-protein-binding antibody responses than younger ones, while higher age is a major risk factor for lethal COVID-19 (17).

In vitro, sera from subsequently deceased patients enhanced SARS-CoV-1 induced MCP1 and IL-8 production by human monocyte-derived wound-healing macrophages, whereas blockade of the Fc γ R receptor reduced these effects (48). One must be prudent when extrapolating from a macaque model of SARS-CoV-1 infection to human COVID-19 patients, but the antibody response to these lethal coronaviruses might play a role in disease progression, perhaps by formation of immune complexes, and by promoting macrophage infiltration and sustained inflammation. We hypothesize that there may be a causal link between seroconversion and the rapid deterioration that can take place in the second week after the first symptoms, but this remains to be established.

There are some other reports that anti-S and other CoV-specific antibodies may have pathogenic effects in animal models. Thus, multiple CoV vaccines were associated with an increase in eosinophilic proinflammatory pulmonary responses upon challenge of the immunized animals (49-51). Previous SARS-CoV-1 infection limited virus replication in African green monkeys but not lung inflammation, when the animals were re-challenged with the same virus (52). It has not been determined which factors, such as viral dose and the extent of the innate and adaptive immune responses, yield these problematic effects. A particularly important knowledge gap is whether certain specificities and other properties of antibodies are responsible.

Preexisting serum antibodies against influenza antigens were consistently associated with severe illness in patients during the 2009 influenza A H1N1 pandemic (53, 54). Of note is that those antibodies did not neutralize influenza virus (53) and that immune complex formation was suggested to be a pathogenic trigger (54). Whether these observations are linked to the findings reported by Liu et al. remains to be seen (48).

Antibody-dependent enhancement of infection (ADE)

Antibodies can also exacerbate viral infection by different mechanisms that have long been described (55). In the vaccine context, infection by alpha- and flaviviruses (such as Dengue and Zika viruses) is enhanced when the antibody occupancy on the virion-surface epitopes falls below a critical threshold (56). This is the stoichiometric condition of an Fc-receptor-dependent form of ADE: the same antibodies that mediate ADE can be neutralizing and protective at higher occupancies on virions (56, 57). The *in vitro* observations of ADE seem to account for the unfortunate outcome of recent Dengue vaccine trials, where examples of worsened disease post-infection occurred (58). ADE has been reported in the CoV literature, although there is no strong evidence that it will be as problematic as for alpha- and flaviviruses (59-64). One recent report described an unusual mechanism of MERS-CoV infection enhancement *in vitro*, whereby the antibody binding to the S protein RBD promoted endocytic uptake by engaging with an Fc-receptor and triggered fusion by inducing a conformational change (62). It augurs well for vaccine development, however, that a SARS-CoV-2 RBD used as an immunogen elicited strong NAb responses in rats, without any ADE (21). These topics will, no doubt, be investigated thoroughly as various designs of the much-needed SARS-CoV-2 vaccine undergo pre-clinical and clinical testing.

Possible improvements to immunotherapy

How could therapeutic interventions be improved so as to preserve the capacity of the infused NABs to reduce virus replication while preventing the possible induction of fatal ALI through promotion of IL-8 and MCP-1 production and inflammatory macrophage accumulation? One precaution would be to administer NABs with Fc deletions. In principle, this could be accomplished by enzymatic treatment of polyclonal IgGs purified from plasma to generate bivalent F(ab')₂ fragments. But in practice this would probably be too onerous. More feasible is the genetic engineering of neutralizing MAbs to eliminate the ability of the Fc-domains to bind FcR. Although such mutations would also eliminate potentially beneficial Fc-mediated effects such as ADCC, there is no evidence that these effector functions play a role in reducing viral load. Here, virus neutralization may be necessary and sufficient, at least during the COVID-19 acute phase.

An alternative neutralizing intervention, which eliminates some risks associated with polyclonal and monoclonal antibodies, is the use of a soluble, recombinant form of the ACE2 receptor, which is potent (nM range) and effective (depending on target cells) at blocking SARS-CoV-1 infection *in vitro* (34). Since SARS-CoV-2 has a 10-20-fold higher affinity than CoV-1 for ACE2, it may be more sensitive to this particular intervention, at least under some infection conditions (22). The entry of both SARS-CoV-1 and SARS-CoV-2 is blocked by recombinant IgG Fc-fusion proteins of both the S-protein RBD (RBD-Fc), binding to the host cells, and soluble ACE2 (ACE2-Fc), binding to the virus; an even more potent variant of the latter has also been described (65). The advantages of these constructs are their potency and potential breadth of action against new viral variants. Furthermore, if Fc-receptor ligation is pathogenic (cf. 48), methods of increasing avidity other than fusing the soluble receptor to the Fc portion of IgG could be explored.

The effects on angiotensin activation and its pharmacological inhibition may also need to be evaluated (18, 67).

Conclusions

Plasma infusion as therapy for COVID-19 is a stop-gap measure that is now being used in a medical emergency. Within the next year, effective drugs are likely to emerge, and they may well include highly potent and specific MAbs to the SARS-CoV-2 S-protein. Animal experiments, particularly in macaques, will be valuable for comparing the capacity of different monoclonal and polyclonal antibodies, including combinations, or of recombinant receptor mimics, to clear CoV-2 infection. Ideally, the intervention should permit or even promote the emergence of favorable innate responses and the resolution of inflammation (66). However, given the urgency of the COVID-19 pandemic it may be impossible to perform such studies before human trials. In these circumstances, an awareness of what has occurred in other viral infections, particularly with SARS-CoV-1, and what is now being published on SARS-CoV-2 may guide both treatment strategies and the development of antibody-based vaccines (61, 68, 69). Prospective or retrospective analyses of how the binding-antibody and NAb titers of transfused plasmas are associated with clinical improvements should also guide both MAb-based therapies and vaccine evaluation. If apparently antibody-mediated adverse events do occur, they too should help to improve these important public health measures against the COVID-19 pandemic.

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