COVID-19: are neutralizing antibodies neutralizing enough?

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Since its inception, coronavirus disease 2019 (COVID-19) has caused significant morbidity and mortality globally. For that reason, treatment and prophylaxis are two quintessential ways to reduce harm as much as possible. The use of convalescent-phase plasma (CP) in severely ill patients with COVID-19 has been attempted on an individual basis[1] and it is being used in the context of ongoing clinical trials; thus, it is pivotal to understand the potential risks and caveats of using CP particularly taking immunopathologic phenomena into account. In a recent study, Shen and colleagues[1] assessed the clinical outcomes of five critically ill patients with COVID-19 treated with CP. The study had several limitations mainly including lack of a control group; however, interesting results were derived from the study that are worth discussing. They used recombinant receptor-binding domain (RBD) of the SARS-CoV-2 spike protein in their IgG enzyme-linked immunosorbent assay (ELISA). The first important result was the apparent lack of correlation between IgG ELISA titers with those of the neutralization assay. Neutralization titers as low as 240 had different IgG ELISA titers of 5400 and 16,200. These results highly suggest that there must exist more epitopes on RBD that do not engage in receptor binding on the cultured cells used in neutralization assay that can still bind anti-RBD IgGs present in the sera, not to mention that the ELISA design, expression, and purification of RBD, and more importantly coating of RBD on ELISA plates, may create or unmask neoepitopes leading to eventual lack of correlation with the neutralizing antibody titer.

The second main speculation is the potential interference by the original antigenic sin (OAS) phenomenon. OAS, first proposed over 60 years ago, has been shown in the context of infection with a variety of viruses including influenza, Dengue, Zika, and coronaviruses (CoVs).[2-4] According to OAS, prior exposure to an antigen influences subsequent immune responses to the antigenically related agents because existing antibodies reduce the epitope burden; thereby this favors using memory instead of naïve B cells. This leads to a brisk and strong immune response that may not be "adequately neutralizing" while viral load remains high and immunopathologic mechanisms proceed such as in COVID-19. This may delay the generation of bona fide high-titer and high-avidity neutralizing antibody repertoire. In this context, previous exposure to common coronaviruses would lead to an early and high-titer immune response to SARS-CoV-2. A similar phenomenon was frequently observed in serologic testing for the Zika virus and Dengue virus.[3] Furthermore, in the above study, despite diluting sera 1:200, they still obtained extraordinarily high ELISA titers as high as 48,600 (mean titer, 25,200) and 145,800 (mean titer, 75,600) for IgM and IgG, respectively, in critically ill patients 2 to 3 weeks after onset of symptoms whereas serum IgM and IgG ELISA titers in asymptomatic convalescent donors 2 to 3 weeks after onset of symptoms only ranged 1800 to 16,200 (mean, 9000). The authors did not perform neutralization assays in parallel to assess cross-reactivity with common CoVs: 229E, OC43, NL63, and HKU1.

The last and perhaps another important observation is while patients had neutralizing antibody geometric mean titer (GMT) of 80 before transfusion, their GMT only increased to 151 1 day after transfusion of 400 mL of plasma. This negligible increase in titer is barely one dilution difference, which could very well be due to the known ±1 dilution subjectivity associated with all neutralization assays. The donors GMT of neutralizing antibody was only 192 as early as 10 days after the resolution of their symptoms. This begs the question whether the so-called neutralizing antibodies were indeed "neutralizing" or not.

In a more recent publication by Duan and coworkers,[4] 10 patients with severe COVID-19 transfused with CP collected from COVID-19–resolved asymptomatic donors. The donors had neutralizing antibody titers of more than 640 at the time of donation while severely ill patients had relatively similar titers before transfusion as high as 640 (range, 160-640; GMT, 367). It should be highlighted

ABBREVIATIONS: CP = convalescent-phase plasma; GMT = geometric mean titer; OAS = original antigenic sin; RBD = receptor-binding domain.

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that these titers were measured in patients 11 to 20 days (median, 16.5 days) after onset of symptoms. This study was also not controlled and, in addition to intensive supportive care, patients were on a range of agents including arbidol, ribavirin, remdesivir, interferon-α, oseltamivir, peramivir, and methylprednisolone; therefore, the observed slight clinical outcomes could not be reliably attributed to the infused plasma.

Historically, it was established that cats immunized with feline CoV recombinant spike protein experienced worse outcomes when they were subsequently exposed to wild-type feline CoV.[5] Liu and colleagues[6] had also shown that macaques that were immunized with SARS-CoV (close relative of SARS-CoV-2) spike protein mounted anti-spike IgG response that triggered acute lung injury. The anti-spike IgG also skewed alveolar macrophages toward an inflammatory phenotype to launch hypercytokinemia (producing high levels of interleukin [IL]-1, IL-6, tumor necrosis factor-α, among others). In their study, they showed that these newly polarized macrophages expressed high levels of CD32a (FcγRIIA). Antibody-dependent enhancement (ADE) has been shown to work through non- or subneutralizing levels of "neutralizing antibodies" and CD32a. ADE has also been demonstrated in other infectious diseases such as Zika, Dengue, Ebola, and human immunodeficiency virus.[5] ADE can lead to more viral propagation and/or generation of cytokine storm. More interestingly, patients who deceased due to SARS had significantly higher titers of neutralizing antibodies.[7] The US Food and Drug Administration currently recommends using donated plasma with neutralizing antibody titers of 160 or at a minimum a titer of 80 in severely ill patients.[8] Although these cutoffs seem arbitrarily chosen, based on the above discussions, there remains a theoretical possibility that plasma recipients experience adverse outcomes due to iatrogenic ADE. The potential issue of ADE interference, albeit in the context of vaccine development, was also raised by Coalition for Epidemic Preparedness Innovations published recently in the *New England Journal of Medicine*.[9] It needs emphasizing that the risk of ADE, despite being clear in the context of certain infectious diseases such as Dengue, has not been shown for coronaviruses in humans but this certainly remains a hypothetical risk. It is no-brainer that prime vaccine candidates would elicit neutralizing antibodies against SARV-CoV-2. All in all, whether CP therapy or prophylaxis would yield significant clinical benefits or not can only be answered through large-scale multicenter randomized clinical trials.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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