EDITORIAL

Antibodies for COVID-19 - which, when and how long?

T.C. Theoharides[1,2,3], D. Lauritano[4], G. Ronconi[5], V. Calvisi[6] and P. Conti[7]

[1]Laboratory of Molecular Immunopharmacology and Drug Discovery, Department of Immunology, Tufts University School of Medicine, Boston, USA; [2]School of Graduate Biomedical Sciences, Tufts University School of Medicine, Boston, MA, USA; [3]Department of Internal Medicine, Tufts University School of Medicine and Tufts Medical Center, Boston, MA,USA; [4]Medicine and Surgery Department, Centre of Neuroscience of Milan, University of Milan-Bicocca, Italy; [5]Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy; [6]Department of Orthopaedics, School of Medicine, University of L'Aquila, L'Aquila, Italy; [7]Postgraduate Medical School, University of Chieti, Chieti, Italy

Infection with SARS-CoV2 leads to COVID-19, the severity of which derives from the host's immune response, especially the release of a storm of pro-inflammatory cytokines. This coronavirus infects by first binding to the ectoenzyme Angiotensin Converting Enzyme 2 (ACE2), a serine protease acting as the receptor, while another serine protease is necessary for priming the viral spike "S" protein required for entering the cells. Repurposing existing drugs for potential anti-coronavirus activity have failed. As a result, there were intense efforts to rapidly produce ways of providing prophylactic active immunization (vaccines) or abortive passive (convalescent plasma or monoclonal antibodies) neutralizing antibodies. The availability of vaccines for COVID-19 have been largely successful, but many questions still remain unanswered. In spite of the original enthusiasm, clinical studies using convalescent serum or monoclonal antibodies have shown limited benefit. Moreover, the emergence of Long-COVID syndrome in most infected patients necessitates the development of treatment approaches that may prevent viral entry by blocking both serine proteases involved, as with a liposomal blend of the natural flavonoids luteolin and quercetin.

Infection with the recent Coronavirus [severe acute respiratory syndrome (SARS)-CoV2] leads to COVID-19, the severity of which derives from the host's immune response (1) especially the release of a storm of pro-inflammatory cytokines (2-9) mainly IL-6 (10-14), in what has been termed "cytokine storms" (15) or "cytokine release syndrome" (16). This coronavirus infects by first binding to the ectoenzyme Angiotensin Converting Enzyme 2 (ACE2), a serine protease acting as the receptor, while another serine protease is necessary for priming the viral corona spike "S" protein required for entering the cells (17, 18). The S protein is made up of two subunits, S1 containing a receptor-binding domain that binds to ACE2 on the surface of host cells, and the S2 containing a transmembrane anchor that mediates fusion of viral and host cell membranes.

Unfortunately, repurposing existing drugs for potential anti-coronavirus activity have not yielded positive results (19). As a result, there were intense efforts to rapidly produce ways of providing prophylactic active immunization (vaccines) or abortive passive (convalescent plasma or monoclonal antibodies) neutralizing antibodies (20, 21). Early studies used high-throughput methods to identify

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Corresponding Author
Theoharis C. Theoharides, MS, MPhil, PhD, MD
Department of Immunology,
Tufts University School of Medicine,
136 Harrison Avenue, Suite 304, Boston, MA 02111, USA
Tel.; +1 617 636 6866 - Fax: +1 617 636 2456
e-mail: theoharis.theoharides@tufts.edu

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antibodies from convalescent plasma using either B cells from patients (22) or a small animal model (23).

How antibodies may work

Neutralizing antibodies could block viral entry by acting in different ways (24): (a) preventing the S protein from binding to ACE2, (b) preventing the S protein conformational changes required for membrane fusion, (c) mimicking ACE2 and prematurely triggering S protein conformational changes before binding ACE2 and thus preventing actual binding, or (d) inhibiting the IL-6 receptor. Such antibodies would be produced after infection, could be present in convalescent serum, made after vaccination, or produced *ex vivo* and administered acutely (25, 26)

Vaccines

A number of vaccines for COVID-19 were developed rapidly using either mRNA or adenovirus vector technology aimed at production of the coronavirus S protein and subsequent innate synthesis of neutralizing antibodies, with apparently minor side-effects so far (27).

However, a number of questions remain unanswered such as the level of antibody titers produced, will they limit transmission, how long will they remain protective, will they prevent asymptomatic disease, whether coronavirus mutations will escape from any antibody neutralizing effect (28), whether the virus will continue to mutate becoming a "recurrent seasonal infection" (29) and requiring the production of new vaccines every year with the realization that such vaccines may have reduced efficacy as is the case for the vaccines against the influenza virus that had only 40% efficacy during 2020 in the US.

Convalescent plasma therapy

Passive immunization with convalescent plasma involves transfusing plasma from patients who have recovered from SARS-CoV2 infection to infected patients with severe disease, assuming that the donors have developed protective antibodies against the coronavirus (30). However, donors may have recovered without production of sufficient neutralizing antibodies. For instance, in one study of 149 convalescent patients whose plasma was collected at 39 days after the onset of symptoms, most did not contain high levels of neutralizing antibodies (only 1% had plasma titers >5000) (31). Moreover, harvesting of such antibodies may have occurred at a time when such antibodies were declining, and any such passive immunity is likely to be short-lived.

A number of studies have reported positive results. In one older study of SARS patients who were treated with high-dose methylprednisolone, the antiviral drug ribavirin and convalescent plasma had shorter duration of hospitalization (32). One uncontrolled study of patients with severe COVID-19, showed convalescent plasma resulted in rapid increase in serum neutralizing antibody titers, no detectable SARS-CoV2 viral RNA nor clinical improvement (33). In another uncontrolled study of five critically ill patients, administration of convalescent plasma led to clinical improvement (34). Three recent studies provided interesting positive results. In the first, administration of convalescent plasma containing high-titer anti-RBD immunoglobulin significantly reduced mortality if given within 44 hours of hospitalization (35) The second showed that convalescent plasma with high but not lower titers reduced risk of death in hospitalized COVID-19 patients not requiring mechanical ventilation (36). The third study reported that early administration of high-titer convalescent serum to mildly ill patients reduced the progression of the diseases (37). However, one randomized clinical trial using convalescent plasma given more than 20 days after viral shedding in patients with severe and life-threatening COVID-19 apparently increased viral clearance but had no effect on mortality (38). Another randomized, placebo-controlled, clinical trial using convalescent plasma in COVID-19 severe pneumonia found no significance in clinical status or overall mortality (39).

Convalescent plasma has several limitations including requirement for blood type matching and screening for blood-borne pathogens, as well as batch-to-batch variability.

Monoclonal antibodies

Passive immunity using human monoclonal antibodies was recognized as potentially serving

as an important abortive treatment for severe COVID-19 patients (40, 41). Such antibodies have been directed against epitopes of the coronavirus receptor-binding domain (RBD) (24, 42). In one instance, the antibody developed could bind potently with RBD, but did not overlap with the ACE2 binding domain (43). In another instance, using more than 1000 purified memory B cells, 11 potent neutralizing antibodies were selected, one of which had the additional ability to enhance the neutralizing ability of the other antibodies (44). There could be additional modifications in the structure of the Fc receptor binding region of the antibodies to increase affinity for Fc receptors.

A number of recent publications have reported on clinical trials using monoclonal antibodies for COVID-19. In one ongoing Phase 2 clinical rial, a single infusion of the neutralizing antibody LY-CoV555 to outpatients with mild to moderate COVID-19, one of the three doses used appeared to accelerate the natural decline of the viral load (45). In another randomized, placebo-controlled, trial administration of LY-CoV555 7000 mg by single infusion to hospitalized COVID-19 patients (who were also on rendemsivir and corticosteroids) did not demonstrate any efficacy (46).

Limitations of antibody use

Several critical questions remain unanswered. What critical epitopes in the S protein are targeted by neutralizing antibodies in convalescent plasma? How many neutralizing antibody epitopes can be targeted simultaneously on the S protein? How easily can the coronavirus develop mutations to allow it to escape neutralizing antibodies? What is the efficacy in different age, sex, or ethnic subpopulations? Are there any undesirable effects, such as antibody-dependent enhancement of infection of immune cells or worsening cytokine storms? Last but not least, antibodies must be given intravenously, even though inhaled delivery has been contemplated. 47

One way to overcome some of the issues raised, at least acutely (abortively) may be the use of a "cocktail" of monoclonal antibodies, rather than a single one, and may decrease the likelihood of rapid mutational escape from neutralization (48, 49). In one instance, the REGN-COV2 neutralizing antibody cocktail reduced viral load and the effect was more pronounced in patients with weak immune response or those with high viral load at baseline.

IL-6 receptor blocking antibodies

In spite of the fact that IL-6, 10-14 has been shown to be increased in the serum of most patients with severe COVID-19 (50), such increases have been mild and two recent clinical trials have also yielded inconsistent results. One study using both tocilizumab and sarilizumab showed better survival than controls, but this clinical study also included treatment with glucocorticoids (51). Another study using only tocilizumab reported no significant improvement in clinical status or death rates (52). Other pro-inflammatory molecules may prove to be better candidates for therapy.

Long-COVID syndrome

COVID-19 survivors experience fatigue and other neuropsychiatric symptoms, especially the presence of mental fatigue known as "brain fog" (53). Such patients have been called "long-haulers" and the illness has been termed "Long COVID syndrome". In addition to the well-known severe respiratory and inflammatory problems discussed above, infection with SARS-CoV-2 can also contribute to neurological (54-57) and mental (58-62) disorders. Increasing publications discuss Long-COVID syndrome (63, 64). A recent paper reported that post-COVID affects over 50% of COVID patients (65). Another showed persistent fatigue that was apparently independent of the severity of the initial symptoms (66). Symptoms experienced by Long-COVID syndrome patients are similar (67) to those present in patients with Mast Cell Activation Syndrome (MCAS) (68, 69).

CONCLUSION

The availability of vaccines for COVID-19 have been largely successful, but many questions still remain unanswered. Despite the original enthusiasm about convalescent serum and monoclonal antibodies, clinical studies have shown limited benefit. Moreover, the emergence of Long-COVID syndrome in most

infected patients necessitates the development of treatment approaches that may prevent viral entry by blocking both serine proteases involved, as with a liposomal blend of the natural flavonoids luteolin and quercetin (e.g., FibroProtek) (70, 71).

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