EDITORIAL

Monoclonal antibody therapy in COVID-19 induced by SARS-CoV-2

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Acute severe respiratory syndrome coronavirus-2 (SARS-CoV-2) infection causes coronavirus disease-2019 (COVID-19) which is associated with inflammation, thrombosis edema, hemorrhage, intra-alveolar fibrin deposition, and vascular and pulmonary damage. In COVID-19, the coronavirus activates macrophages by inducing the generation of pro-inflammatory cytokines [interleukin (IL)-1, IL-6, IL-18 and TNF] that can damage endothelial cells, activate platelets and neutrophils to produce thromboxane A2 (TxA2), and mediate thrombus generation. In severe cases, all these phenomena can lead to patient death. The binding of SARS-CoV-2 to the Toll Like Receptor (TLR) results in the release of pro-IL-1β that is cleaved by caspase-1, followed by the production of active mature IL-1β which is the most important cytokine in causing fever and inflammation. Its activation in COVID-19 can cause a "cytokine storm" with serious biological and clinical consequences. Blockade of IL-1 with inhibitory and anti-inflammatory cytokines represents a new therapeutic strategy also for COVID-19. Recently, very rare allergic reactions to vaccines have been reported, with phenomena of pulmonary thrombosis. These side effects have raised substantial concern in the population. Highly allergic subjects should therefore be vaccinated under strict medical supervision. COVID-19 has accelerated vaccine therapy but also the use of drugs and monoclonal antibodies (mABs) which have been used in COVID-19 therapy. They are primarily adopted to treat high-risk mild-to-moderate non-hospitalized patients, and it has been noted that the administration of two mABs gave better results. mABs, other than polyclonal plasma antibodies from infected subjects with SARS-CoV-2, are produced in the laboratory and are intended to fight SARS-CoV-2. They bind specifically to the antigenic determinant of the spike protein, inhibiting the pathogenicity of the virus. The most suitable individuals for mAB therapy are people at particular risk, such as the elderly and those with serious chronic diseases including diabetics, hypertension and obesity, including subjects suffering from cardiovascular diseases. These antibodies have a well-predetermined target, they bind mainly to the protein S (formed by the S1A, B, C and D subtypes), located on the viral surface, and to the S2 protein that acts as a fuser between the virus and the cell membrane. Since mABs are derived from a single splenic immune cell, they are identical and form a cell clone which can neutralize SARS-CoV-2 by binding to the epitope of the virus. However, this COVID-19 therapy may cause several side effects such as mild pain, bleeding, bruising of the skin, soreness, swelling, thrombotic-type episodes, arterial hypertension, changes in heart activity, slowed bone marrow activity, impaired renal function,

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diarrhea, fatigue, nausea, vomiting, allergic reaction, fever, and possible subsequent infection may occur at the site of injection. In conclusion, the studies promoting mAB therapy in COVID-19 are very promising but the results are not yet definitive and more investigations are needed to certify both their good neutralizing effects of SARS-CoV-2, and to eliminate, or at least mitigate, the harmful side effects.

Key words: SARS-CoV-2, COVID-19, pro-inflammatory cytokines, anti-phospholipid antibodies, IL-1 family

The infectious SARS-CoV-2 pandemic emerged in 2019 and so far, has rapidly affected millions of people around the world. In coronavirus-19 disease (COVID-19), dyspnoea, progressive respiratory failure, hypoxemia, pulmonary edema, and other symptoms can occur (1). In the severe pathogenesis of COVID-19, anti-phospholipid antibodies are produced which, in conjunction with activated neutrophils and platelets, and pro-inflammatory cytokines, can damage endothelial cells and generate thrombotic events (2). In these cases, the spike protein of the virus by binding to the ACE2 receptor and to CD147 activates platelet PF4 which by binding to endothelial cells generates thrombi (3). Furthermore, SARS-CoV-2 activates granulocytes and platelets to produce thromboxane A2 (TxA2) (detected as TxB2) which also promotes thrombus generation (4). SARS-CoV-2 causes inflammation of tissues and organs, including lungs, with the production of pro-inflammatory cytokines and chemokines that can even cause death (5). Indeed, interleukin (IL)-1, one of the most important cytokines in the inflammatory process, is extensively studied in microbial infections, including viruses. Excessive production of IL-1 causes exaggerated inflammation that triggers pro-inflammatory cytokines that include members of the IL-1 family (such as IL-18), but also IL-6 and TNF, causing a "cytokine storm". Therefore, targeting IL-1 with anti-inflammatory cytokines may represent an interesting therapeutic strategy in inflammatory diseases, including COVID-19.

In a short time, safe and effective COVID-19 vaccines have been generated, representing a true scientific "illuminism" that will save millions of lives (6). However, allergic reactions to the vaccine or monoclonal antibody (mAB) in COVID-19 treatment have raised much concern due to the rare cases, albeit of uncertain origin, occurring globally. Therefore, the doctor must warn people who have a mast cell disorder or who have high allergic reactions before carrying out these therapies, even if serious events rarely occur.

Individuals highly allergic to mRNA or vector (adenovirus) vaccines can undergo allergic phenomena with the risk of an immediate reaction with the production of chemical mediators or delayed response with the generation of pro-inflammatory cytokines. Immediate systemic allergy can occur within the first 2 hours of treatment and can be fatal in some cases. The first allergic reaction can occur at the injection site, but also in tissues and organs, including lungs. After treatment with vaccines or mABs, reactions may be more frequent in highly allergic people and this must be taken into consideration (7). Therefore, before treatment, an anamnesis should be made to assess the pathological risk to which the subject is exposed. The most severe allergic reactions occurred in people who had had a previous life-threatening anaphylactic reaction. Some vaccines have shown life-threatening clotting disorders and have been paused at the present time, although the risk of these potential side effects are very small compared to the risks associated with coronavirus-19 infection. Furthermore, it is not yet certain whether these adverse effects result from vaccination and this should be clarified. In addition, it is recommended not to use these vaccines for people who have a high risk of clotting or a history of anaphylactic reaction. However, failure to use available vaccines could cost many lives.

COVID-19 has accelerated not only the production of vaccines but also the production of drugs and antibodies for the development of clinical therapy. Recently, the FDA approved (not as part of the usual standard review, but with a shorter, simpler, and more incomplete review process) the use of some mABs for COVID-19 therapy in order to reduce the severity and duration of the disease,

as well as hospitalization rates of high-risk patients, improving the chances of recovery (8).

In 1984, Köhler, Milstein and Jerne were awarded the Nobel Prize in Medicine for the discovery of mABs (9). These proteins have previously been generated for the prophylaxis and therapy of viral infections, such as Ebola, HIV, Zika, influenza and MERS-CoV. Officially, only a few mABs have been shown to be very effective in human studies, but they have paved the way for clinical progress, although, a number of mABs are still under study and require wider investigation.

Treatment of COVID-19 patients with mAB used in clinical trials may reduce hospitalization and emergency room visits. mABs were generated for the purpose of fighting the SARS-CoV-2 virus and it is hoped that this treatment represents a new and effective therapeutic strategy against the rapid expansion of COVID-19 which, as we know, can be globally lethal. In SARS-CoV-2 infection, mABs act by specifically binding to the antigenic determinant of the spike protein and inhibiting the pathogenicity of the virus. mABs are proteins produced in the laboratory with the aim of fighting pathogens including SARS-CoV-2. These antibodies have a well-predetermined target and are different from the plasma antibodies from previously infected patients with SARS-CoV-2.

Hence, mABs can be used to treat COVID-19 adult individuals who are not hospitalized and who are at high risk of developing severe symptoms. In particular, the treatment should be administered to patients with mild-to-moderate disease. mABs are recommended to avoid the risk of progression to severe disease and/or hospitalization. In SARS-CoV2 infection, the most suitable subjects for mAB therapy are people at particular risk, such as the elderly and those with serious chronic diseases including diabetes, hypertension and obesity, as well as subjects suffering from cardiovascular diseases. The therapy is directed against the antigenic determinant of the spike protein that the coronavirus uses to enter the host cell. The mABs bind on the receptor domain region of the viral spike protein and carry out its inhibiting action. The antibody binds mainly to the protein S formed by the S1A, B, C and D subtypes located on the viral surface, and to the S2 protein which acts as a fuser between the virus and the cell membrane (10).

The NIH researchers recommend administering 2 mABs in combination for COVID-19 therapy to outpatients with mild-to-moderate disease at high risk of clinical progression. Two different mAbs administered together against the virus can better reduce the effects and symptoms of the disease. In fact, in the therapy with a cocktail of mABs a better functioning was observed compared to the single administration and more effective reduction of the viral load (11). The treatment must be carried out strictly in clinic and lasts about 1 hour.

The mABs against SARS-CoV-2 can be produced by injecting the spike protein into a mouse, causing the formation of reactive splenic immune cells. Genes of the heavy and light chains of antibodies from virus-activated immune cells are isolated to produce mABs. Lymphocytes sensitized and activated by the spike protein are subsequently cloned to produce specific antibodies against this protein, preventing the virus from entering the cells. These antibodies are generated from patient-specific type B immune cells activated against SARS-CoV-2 or from genetically modified mice. Activated immune cells on recognition of the antigenic spike protein are fused with myeloma cells to form a hybridoma, whose reactive positive cells produce mAB against the antigenic spike protein. Since these antibodies are derived from a single splenic immune cell, they are identical and form a cell clone different from the polyclonal antibodies of convalescent patients, therefore, human mAB can neutralize SARS-CoV-2 by binding to an epitope of the virus, offering potential prevention and therapeutic treatment in COVID-19. Thus, mABs can potentially improve disease status by helping to protect the patient and eliminate the virus (12).

Side effects such as mild pain, bleeding, bruising of the skin, soreness, swelling, and possible subsequent infection may occur at the site of injection of the monoclonal antibody. In addition, the most common side effects of mAB therapy are: thrombotic-type episodes, arterial hypertension, changes in heart activity, constipation, slowed bone

marrow activity, impaired renal function, diarrhea, fatigue, nausea, vomiting, pain, allergic reaction and fever. Patients treated with mAB should continue to follow COVID-19 prevention rules such as staying at home, washing hands, disinfecting shared surfaces, and respecting 2 meters of physical distance. Following mAB therapy, individuals can test positive for SARS-CoV-2 for up to three months. COVID-19 vaccination should be performed approximately 90 days after mAB therapy. Therefore, neutralizing mABs are a potential treatment for COVID-19 and therapeutic administration is recommended in the mild or moderate COVID-19 form.

Outpatient treatment should be performed on adult patients at risk of disease progression. These patients include those with chronic diseases e.g., chronic kidney disease, diabetes, cardiovascular disease, hypertension, chronic obstructive pulmonary disease, and other conditions. Patients with a positive SARS-CoV-2 test should be treated with mAB as soon as possible and, if patients have received vaccination, the benefit of monoclonal therapy should be weighed. mAB treatment is a potential effective and novel therapeutic approach in preventing SARS-CoV-2 infection and they represent passive immunotherapy that plays a vital role as a possible treatment for COVID-19. mABs open up new therapeutic hopes that can reduce both the number of hospitalized individuals and mortality, however, this therapy has the disadvantage of being expensive and the effects can be short-lived.

In spite of the therapeutic benefits of mAB, some studies were suspended after finding in several cases that the therapy was ineffective and caused problems. Other studies using mAB were also discontinued because after treatment, hospitalized patients who received oxygen and were on mechanical ventilation had a high risk, greater than the therapeutic benefit.

Moreover, it has been observed that rare cases of immediate, non-fatal hypersensitivity may occur following therapy. In clinical studies, a certain number of excess antibodies that fail to neutralize SARS-CoV-2 can lead to the formation of immune complexes which are subsequently phagocytosed by macrophages within which viruses can replicate inside the cell and increase their virulence. Furthermore, viruses that bind to the gamma Fc fragment of macrophage cells stimulate pro-inflammatory cytokines, causing inflammation.

In conclusion, therapies with anti-SARS-CoV-2 mABs can have side effects aggravating the COVID-19 disease and consequent worsening of respiratory activity. This negative reaction adds to the inflammation due to the "cytokine storm" with deleterious effects that can even be deadly (13).

In light of the observations reported herein, we can say that the studies promoting mAB therapy are very promising but the results are not yet definitive as there are some concerns about their safety, efficacy and use; therefore, research in this field is still under investigation and, based on the potential side effects of this therapy, it should be used with caution also in COVID-19.

REFERENCES

1. Kazi DS, Martin LM, Litmanovich D, Pinto DS, Clerkin KJ, Zimetbaum PJ, Dudzinski DM. Case 18-2020: A 73-year-old man with hypoxemic respiratory failure and cardiac dysfunction. N Engl J Med 2020; 382(24):2354-64.
2. Linnemann B. Antiphospholipid syndrome - an update. Vasa 2018; 47(6):451-64.
3. Düsing R. Pharmacological interventions into the renin-angiotensin system with ACE inhibitors and angiotensin II receptor antagonists: effects beyond blood pressure lowering. Ther Adv Cardiovasc Dis 2016; 10(3):151-61.
4. Conti P, Caraffa A, Gallenga CE, Ross R, Kritas SK, Frydas I, Younes A, Di Emidio P, Ronconi G, Toniato E.IL-1 induces throboxane-A2 (TxA2) in COVID-19 causing inflammation and micro-thrombi: inhibitory effect of the IL-1 receptor antagonist (IL-1Ra). J Biol Regul Homeost Agents 2020; 34(5):1623-7.
5. CORIMUNO-19 Collaborative group. Effect of anakinra versus usual care in adults in hospital with COVID-19 and mild-to-moderate pneumonia (CORIMUNO-ANA-1): a randomised controlled trial. Lancet Respir Med 2021; 9(3):295-304.
6. Krause PR, Gruber MF. Emergency Use Authorization of Covid Vaccines - Safety and Efficacy Follow-up Considerations. N Engl J Med 2020 ; 383(19):e107.
7. Shanmugaraj B, Siriwattananon K, Wangkanont

K, Phoolcharoen W. Perspectives on monoclonal antibody therapy as potential therapeutic intervention for Coronavirus disease-19 (COVID-19). Asian Pac J Allergy Immunol. 2020; 38(1):10-18.

8. Drożdżal S, Rosik J, Lechowicz K, Machaj F, Kotfis K, Ghavami S, Łos MJ. FDA approved drugs with pharmacotherapeutic potential for SARS-CoV-2 (COVID-19) therapy. Drug Resist Updat 2020; 53:100719.
9. Köhler G. The Nobel Lectures in Immunology. The Nobel Prize for Physiology or Medicine, 1984. Derivation and diversification of monoclonal antibodies. Scand J Immunol 1993; 37(2):117-29.
10. He Y, Zhou Y, Liu S, Kou Z, Li W, Farzan M, Jiang S. Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implication for developing subunit vaccine. Biochem Biophys Res Commun 2004; 324(2):773-81.
11. Nirula A, Chen P, et al. Effect of Bamlanivimab as monotherapy or in combination with etesevimab on viral load in patients with mild to moderate COVID-19: A Randomized Clinical Trial. Gottlieb RL, JAMA 2021; 325(7):632-44.
12. Wang C, Li W, Drabek D, et al. A human monoclonal antibody blocking SARS-CoV-2 infection. Nat Commun 2020; 11(1):2251.
13. Lee WS, Wheatley AK, Kent SJ, DeKosky BJ. Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies. Nat Microbiol 2020; 5(10):1185-91.