Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Recombinant Antibodies to Face SARS-CoV-2 Syndemic

Maykel González-Torres[1*], Gerardo Leyva-Gómez[2], María Luisa Del Prado-Audelo[3], Hernán Cortés[4], Jonathan J. Magaña[3,4]

[1] Conacyt & Laboratorio de Biotecnología, Instituto Nacional de Rehabilitación, 14389, México
[2] Facultad de Química, Universidad Nacional Autónoma de México, 04510, México
[3] Tecnológico de Monterrey, 14380, México
[4] Laboratorio de Medicina Genómica, Instituto Nacional de Rehabilitación, 14389, México

ARTICLE INFO

Mini Review

Article history:
Received: January 27, 2023
Accepted: June 06, 2023
Published: July 31, 2023

Keywords:

SARS-CoV-2; syndemic; coronavirus; perspective; antibody

ABSTRACT

In recent years, increasing interest has been paid to using antibody-based therapies for clinical applications. However, it is unclear whether recombinant antibodies can be combined with other scientific approaches to generate innovative solutions for mitigating severe acute respiratory syndrome coronavirus 2. In this context, the increase in this virus transmission, the number of infected people, and the interaction between social and biological processes have led to a syndemic, exacerbating the public health problem. Here, we argue about recent advances in recombinant antibody strategies and the perspective of using them to face this syndemic. Thus, the most promising methods in sample readiness, potency, and reduction of manufacturing time frame have been highlighted.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.7.4

Introduction

The adaptive resilience of humans has gone beyond the usual due to the appearance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The synergistic epidemic has more extensive ranges than a coronavirus disease. SARS-CoV-2 has crumbled the world's lifestyle from research exceptionalism to conspiracy theories. The airborne transmission of the virus and its ability to spread from asymptomatic individuals have been underestimated. While researchers and governments struggle to find more efficient treatments and suitable epidemiological surveillance systems, the virus has taken advantage of such disparity in criteria to unleash chaos. SARS-CoV-2 has already mutated thousands of times and seems to be here to stay. In retrospect, evidence for the failure of the classical epidemiological perspective as the only way to address this issue is overwhelming. Scientists realized that much remains unknown about SARS-CoV-2 (1). The road ahead is, therefore, challenging.

The ability of SARS-CoV-2 to adapt and re-establish infection in patients has caused periodic outbreaks. The virus has severely damaged the environment and political, social, economic, and cultural development (2). People's well-being deteriorates because of uncertainty, confinement, poverty, financial crisis, social or traditional media misinformation, stressors, and inadequate care for comorbidities (3). One solution to curb the spread of coronaviruses involves behavioral science, community engagement, and social discipline, coupled with an ethical and multidisciplinary scientific vision without borders. This approach allows the coronavirus to be socially contextualized (4).

Research teams have been working to create effective vaccines against the virus due to the severity of the pandemic, with a focus on the spike (S) protein of SARS-CoV-2, which is essential for virus-cell absorption. To meet the demands of this pandemic, new plant-based platforms have arisen in addition to the conventional technologies used for generating medications or vaccines as an effective approach for bulk production. (5–7). Tobacco, potato, and turnip are models of plants employed in producing vaccines (5). For example, the production of virus-like particles (VLPs) formed by the Hepatitis E virus in *Nicotiana benthamiana* was carried out, resulting in high levels of Hepatitis E-specific serum antibodies in VLPs immunized mice (8). Similarly, *N. benthamiana* produced hemagglutinin antigens from seasonal influenza viruses, which induced high serum HI antibody responses (9).

Various strategies for transient expression systems to produce VLPs by plants as an alternative to SARS-CoV-2 treatments have been reported. In this context, in 2022, Jung et al. studied the expression of the native version of the S protein of SARS-CoV-2 in Nicotiana benthamiana and evaluated the resulting VLPs (10). They reported significant levels of VLPs when the S protein is expressed in the absence of E and M proteins; interestingly, this protein S was recognized by antibodies in human sera from convalescent patients. These results suggested that the protein S expressed in plants presented biological

* Corresponding author. Email: maykel.gonzalez@conacyt.mx

characteristics like the parent virus. Similarly, other studies have been carried out using *N. benthamiana* to express the receptor binding domain of the S protein (11,12). Here, the perspective of recombinant antibodies is argued in light of facing the SARS-CoV-2 syndemic.

The immunologists' perspective

Albeit more research has yet to be conducted, it is known that antibodies can inhibit the S1 domain binding to angiotensin-converting enzyme 2 (ACE2)/ ACE enzyme complex, which leads to therapeutic alternatives. Also, envelope (E), membrane (M), and nucleocapsid (N) proteins are potential targets. Bamlanivimab illustrates the successful use of Immunoglobulin G (IgG) against the Spike (S) glycoprotein of SARS-CoV-2. Recombinant antibodies (rAb) arise from B cells and antibodies of convalescent patients. Intravenous Immunoglobulin (IVIG) used to address SARS-CoV-2 has shown the drawback of only partially protecting patients. However, IVIG can be beneficial in preventing and decreasing the viral burden (13). Therefore, the current epidemic situation requires original approaches to face uncertainty.

The role of antibodies

Unfortunately, the role of the antibodies in treating SARS-CoV-2 remains uncertain. The antibody response is affected by ethnicity, gender, age, virus variations, comorbidities, waning immunity, and infection severity. Notably, adult patients with serious COVID-19 (even those dying) exhibit high viral loads and high titers of antibodies to SARS-CoV-2 (14,15). Contrariwise, other patients with documented COVID-19 have low antibody production but mild or moderate symptoms or are asymptomatic (16–18). Likewise, children are generally less severely affected than adults and present lower antibody levels (19,20).

Similarly, the levels of specific neutralizing antibodies do not necessarily mirror protection against the severity of the infection. Therefore, these findings put doubts about the protective role of humoral immunity. On the other hand, other reports suggest that a robust T-cell response may benefit COVID-19 patients (21,22). Thus, these apparent inconsistencies in neutralizing antibody titers could be attributed to T cell responses to SARS-CoV-2 under diverse host and viral risk factors. Also, the antibodies produced for specific epitopes revealed antibody-dependent enhancement. Antibodies wane in long-term or chronic COVID-19 patients but are advantageous for immunodeficient individuals. This outcome underscored the significance of the T-cell assay for SARS-CoV-2 (23).

Antibiotics are vital in infection control, therapeutic design, or adjuvant therapy. In the case of monoclonal (mAbs) and polyclonal (pAbs) antibody therapy for COVID-19, the main benefit implies reducing viral load, hospitalizations, and deaths. Despite this therapy's low risks, adverse injection and infusion-related reactions are the most common side effects. A study in vitro revealed that a human mAb neutralizes SARS-CoV-2 and SARS-CoV (24). The ability to cross-neutralize the virus is related to the antibody (47D11) binding a conserved epitope on the spike receptor binding domain (RBD) by an independent receptor-binding inhibition mechanism. Interestingly, therapeutic antibodies failed to recognize new SARS-CoV-2 variants, so flow cytometry-based novel mAb binding assays have been developed to neutralize emerging threats (25).

Furthermore, antibodies show limitations such as size, stability, and possible immunogenicity. A promising alternative to avoid these disadvantages is using nanobodies, which are variable domains of camelid heavy chain-only antibodies (26). Nanobodies presented low immunogenicity and high specificity and were obtained easier and lower cost than conventional antibodies. Huo et al. reported using four nanobodies with affinity to the RBD domain of the spike protein of SARS-CoV-2 (27). Their preliminary results evaluated one of these engineered nanobodies in Golden Syrian hamsters inoculated with SARS-CoV-2, observing that those treated with 4 mg/kg of the nanobody presented a reduced pulmonary infection than those without treatment. In addition, the treatment induced a strong macrophage response.

Discussion

mAbs have helped treat a number of diseases, and the FDA permitted them to be used as a therapy for SARS-CoV-2 (23). Thus, the urgency of producing more therapeutic options on a massive scale has focused on repositioning indoor farms into biomanufacturing facilities (28–30), the utility platform: Nicotiana benthamiana. The agroinfiltration technique in Nicotiana benthamiana has allowed the production of two anti-SARS-CoV-2 mAbs on a pilot scale (CR3022 and nanobody 72), with a production capacity of grams in an estimated time of 6 weeks. CR3022 antibody is an ScFv-Fc IgG1 (VL-Linker-VH) with a size of 56.2 kDa and targets RBD (SARS-CoV and SARS-CoV-2), and nanobody 72 is a VHH-Fc IgG1 with a size of 43.3 kDa and also targets RBD (SARS-CoV and SARS-CoV-2) with neutralizing activity (31). The authors cloned and assembled the antibodies using the GoldenBraid assembly system (32) and improved cloning prediction using the Codon Optimization Tool (Integrated DNA Technologies). The Codon Optimization Tool rebalances codon usage for a sequence from one species to that of the organism chosen for expression based on a codon sampling strategy in which the reading frame is recorded based on the frequencies of each codon's usage in the new organism. The prediction is strengthened because the algorithm eliminates codons with less than 10% frequency and re-normalizes the remaining frequencies to 100%, removing the presence of repeats, hairpins, and extreme GC content (31). However, it appears that mAbs are not sufficiently successful regarding hospital admissions or mortality of patients with COVID-19 (33).

On the other hand, encouraging results have been recently revealed from rAb research. The use of universal libraries has allowed the generation of antibodies from only healthy human donors in an easy, quick, and efficient strategy (34)—these SARS-CoV-2 neutralizing human rAbs bind to the receptor-binding domain (RBD)-ACE2 interface. Also, recombinant hyperimmune globulins have been generated from various B-cell repertoires from the rAb molecular genomics strategy (35). Hyperimmune globulins offer immediate defense against the disease (but in the short term). These are usually obtained from newly vaccinated people or immunizing animals; however, this method exhibits some drawbacks, including impurities,

allergic reactions, and low potency. In the method described by Keating et al., B cells from mice or donors are processed in a microfluidic platform, and light and heavy chain immunoglobulin sequences are bonded to create antibody repertoires (35). As a result, these repertoires are mass-produced into constructs for full-length expression. The full-length antibody expression constructs are then site-directedly and stably inserted into Chinese hamster ovary (CHO) cells. The scientists claim that this method combines the benefits of plasma-derived antibodies with the advantages of rAbs (purity, consistency, and potency, and efficacy). Therefore, the research on B cells receptor sequencing combined with target–ligand blocking is critical for discovering more efficient neutralizing antibodies (36).

In hindsight, the rAb are detected more easily than mAbs and are produced using coding genes. It can be obtained from plants with rapid scale-up and reduced animal experiments. The rAb can be harnessed to create models of targeted and fast responses to future pathogens (SARS-CoV-2 variants). Furthermore, the upcoming rAb biotechnology is likely linked to mammalian cells, but it may also involve conjugating the antibodies with nanoparticles or biomatrixes to create therapeutic biomolecules. Therefore, it is tempting to speculate that using rAbs may prove crucial, feasible, and helpful in facing the SARS-CoV-2 syndemic over time. In this context, since the vaccine-induced antibodies appear to wane out, the concomitant use of rAb therapy and SARS-CoV-2 vaccine boosters could be efficacious in preventing severe cases of COVID-19 in this "new normal" syndemic stage.

Conclusions

In conclusion, from the routes reviewed, the most appropriate appears to consider any variant as a new viral infection contextualized in each social environment and perform large-scale antigen and viral screening, followed by a vaccine boost combined with rAb adjuvant therapy. In only three months, the hyperimmune globulins method engages high neutralizing activity against SARS-CoV-2. Therefore, it is the most promising rAb method considering potency, reduction of the manufacturing timeframe, and sample readiness.

Acknowledgments
The authors acknowledge the program "Investigadores por México" of CONACYT for the support in the publication of this work. We acknowledge Oswaldo González Mendoza for his comments.

Interest conflict
The authors declare no conflict of interest.

Consent for publications
The author read and proved the final manuscript for publication.

Availability of data and material
All data generated during this study are included in this published article.

Authors' Contribution
All authors had an equal role in study design and manuscript writing.

Funding
This research received no external funding.

Ethics approval and consent to participate
No humans or animals were used in the present research. Institutional Review Board Statement: Not applicable. Informed Consent Statement: Not applicable.

References

1. Leonard WR. Human Biologists confront the COVID-19 pandemic. Am J Hum Biol 2020; 32 (5): e23511 https://doi.org/10.1002/ajhb.23511
2. Bogin B, Varea C. COVID-19, crisis, and emotional stress: A biocultural perspective of their impact on growth and development for the next generation. Am J Hum Biol 2020; 32:e23474. https://doi.org/10.1002/ajhb.23474
3. Katzmarzyk PT, Salbaum JM, Heymsfield SB. Obesity, noncommunicable diseases, and COVID-19: A perfect storm. Am J Hum Biol 2020; 32:e23484. https://doi.org/10.1002/ajhb.23484
4. Gravlee CC. Systemic racism, chronic health inequities, and COVID-19: A syndemic in the making? Am J Hum Biol 2020; 32:e23482. https://doi.org/10.1002/ajhb.23482
5. Dhama K, Natesan S, Iqbal Y et al. Plant-based vaccines and antibodies to combat COVID-19: current status and prospects. Hum Vaccines Immunother 2020; 16(12):2913-2920. 10.1080/21645515.2020.1842034.
6. Peyret H, Steele JFC, Jung JW et al. Producing vaccines against enveloped viruses in plants: Making the impossible, difficult. Vaccines 2021; 9(7):780. doi: 10.3390/vaccines9070780.
7. Capell T, Twyman RM, Armario NV et al. Potential Applications of Plant Biotechnology against SARS-CoV-2. Trends Plant Sci 2020; 25(7):635-643. doi: 10.1016/j.tplants.2020.04.009.
8. Mardanova ES, Takova KH, Toneva VT et al. A plant-based transient expression system for the rapid production of highly immunogenic Hepatitis E virus-like particles. Biotechnol Lett 2020; 42(11):2441-2446. doi: 10.1007/s10529-020-02995-x.
9. Shoji Y, Farrance CE, Bautista J et al. A plant-based system for rapid production of influenza vaccine antigens. Influenza Other Respi Viruses 2012; 6: 204-210. https://doi.org/10.1111/j.1750-2659.2011.00295.x
10. Jung JW, Zahmanova G, Minkov et al. Plant-based expression and characterization of SARS-CoV-2 virus-like particles presenting a native spike protein. Plant Biotechnol J 2022; 20(7):1363-1372. doi: 10.1111/pbi.13813.
11. Makatsa MS, Tincho MB, Wendoh JM et al. SARS-CoV-2 Antigens Expressed in Plants Detect Antibody Responses in COVID-19 Patients. Front Plant Sci 2021; 12:589940. doi: 10.3389/fpls.2021.589940
12. Maharjan PM, Cheon J, Jung J et al. Plant-expressed receptor binding domain of the SARS-CoV-2 spike protein elicits humoral immunity in mice. Vaccines 2021; 9(9):978. doi: 10.3390/vaccines9090978.
13. De Sanctis JB, García AH, Moreno D, Hajduch M. Coronavirus infection: An immunologists' perspective. Scand J Immunol 2021; 93:e13043. https://doi.org/10.1111/sji.13043
14. Zhou Y, Liu Z, Li S et al. Enhancement versus neutralization by SARS-CoV-2 antibodies from a convalescent donor associates with distinct epitopes on the RBD. Cell Rep 2021; 34(5):108699. doi: 10.1016/j.celrep.2021.108699.
15. Negro F. Is antibody-dependent enhancement playing a role in COVID-19 pathogenesis? Swiss Med Wkly 2020; 150:w20249.

doi: 10.4414/smw.2020.20249.
16. Kowitdamrong E, Puthanakit T, Jantarabenjakul W et al. Antibody responses to SARS-CoV-2 in patients with differing severities of coronavirus disease 2019. PLoS One 2020; 15(10):e0240502. doi: 10.1371/journal.pone.0240502.
17. Marot S, Malet I, Leducq V et al. Rapid decline of neutralizing antibodies against SARS-CoV-2 among infected healthcare workers. Nat Commun 2021; 12(1):844. doi: 10.1038/s41467-021-21111-9.
18. Yang Y, Wang X, Du RH et al. Serological investigation of asymptomatic cases of SARS-CoV-2 infection reveals weak and declining antibody responses. Emerg Microbes Infect 2021; 10(1):905-912. doi: 10.1080/22221751.2021.1919032.
19. Bloise S, Marcellino A, Testa A et al. Serum IgG levels in children 6 months after SARS-CoV-2 infection and comparison with adults. Eur J Pediatr; 2021; 180(11):3335-3342. doi: 10.1007/s00431-021-04124-w.
20. Pierce CA, Preston HP, Dai Y et al. Immune responses to SARS-CoV-2 infection in hospitalized pediatric and adult patients. Sci Transl Med 2020; 12(564):eabd5487. doi: 10.1126/scitranslmed.abd5487.
21. Sekine T, Perez PA, Rivera BO et al. Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19. Cell 2020; 183(1):158-168.e14. doi: 10.1016/j.cell.2020.08.017.
22. Tan AT, Linster M, Tan CW et al. Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. Cell Rep 2021; 34(6):108728. doi: 10.1016/j.celrep.2021.108728.
23. Ameratunga R, Woon ST, Lea E et al. The (apparent) antibody paradox in COVID-19. Expert Rev Clin Immunol 2022; 18(4):335-345. doi: 10.1080/1744666X.2022.2044797
24. Wang C, Li W, Drabek D et al. A human monoclonal antibody blocking SARS-CoV-2 infection. Nat Commun 2020; 11(1):2251. doi: 10.1038/s41467-020-16256-y.
25. Schulz SR, Hoffmann M, Roth E et al. Augmented neutralization of SARS-CoV-2 Omicron variant by boost vaccination and monoclonal antibodies. Eur J Immunol 2022; 52(6):970-977. doi: 10.1002/eji.202249841.
26. Najmeddin A, Bahrololoumi M, Behdani M, Dorkoosh, F. Nanobodies as powerful pulmonary targeted biotherapeutics against SARS-CoV-2, pharmaceutical point of view. BBA Gen Subj 2021; 1865(11):129974. doi: 10.1016/j.bbagen.2021.129974.
27. Huo J, Mikolajek H, Le BA et al. A potent SARS-CoV-2 neutralising nanobody shows therapeutic efficacy in the Syrian golden hamster model of COVID-19. Nat Commun 2021; 12(1):5469. doi: 10.1038/s41467-021-25480-z.
28. Khan T, Khan MA, Karam K et al. Plant *in vitro* Culture Technologies; A Promise Into Factories of Secondary Metabolites Against COVID-19. Front Plant Sci 2021; 12:610194. doi: 10.3389/fpls.2021.610194.
29. Buyel JF. Plant molecular farming – Integration and exploitation of side streams to achieve sustainable biomanufacturing. Front Plant Sci 2019; 9:1893. doi: 10.3389/fpls.2018.01893.
30. Pandey A, Khan MK, Hamurcu M, Gezgin S. Natural Plant Products: A Less Focused Aspect for the COVID-19 Viral Outbreak. Front Plant Sci 2020; 11:568890. doi: 10.3389/fpls.2020.568890.
31. Borja D, González B, Vazquez VM et al. Pilot Production of SARS-CoV-2 Related Proteins in Plants: A Proof of Concept for Rapid Repurposing of Indoor Farms Into Biomanufacturing Facilities. Front Plant Sci 2020; 11:612781. doi: 10.3389/fpls.2020.612781.
32. Sarrion PA, Vazquez VM, Palacio J et al. Goldenbraid 2.0: A comprehensive DNA assembly framework for plant synthetic biology. Plant Physiol 2013; 162(3):1618-31. doi: 10.1104/pp.113.217661.
33. Kreuzberger N, Hirsch C, Chai KL et al. SARS-CoV-2-neutralising monoclonal antibodies for treatment of COVID-19. Cochrane Database Syst Rev 2021; 9(9): CD013825. doi: 10.1002/14651858.CD013825.pub2.
34. Bertoglio F, Meier D, Langreder et al. SARS-CoV-2 neutralizing human recombinant antibodies selected from pre-pandemic healthy donors binding at RBD-ACE2 interface. Nat Commun 2021; 12(1):1577. doi: 10.1038/s41467-021-21609-2.
35. Keating SM, Mizrahi RA, Adams MS et al. Generation of recombinant hyperimmune globulins from diverse B-cell repertoires. Nat Biotechnol 2021; 39(8):989-999. doi: 10.1038/s41587-021-00894-8.
36. Shiakolas AR, Kramer KJ, Johnso NVet al. Efficient discovery of SARS-CoV-2-neutralizing antibodies via B cell receptor sequencing and ligand blocking. Nat Biotechnol 2022; 40(8):1270-1275. doi: 10.1038/s41587-022-01232-2.