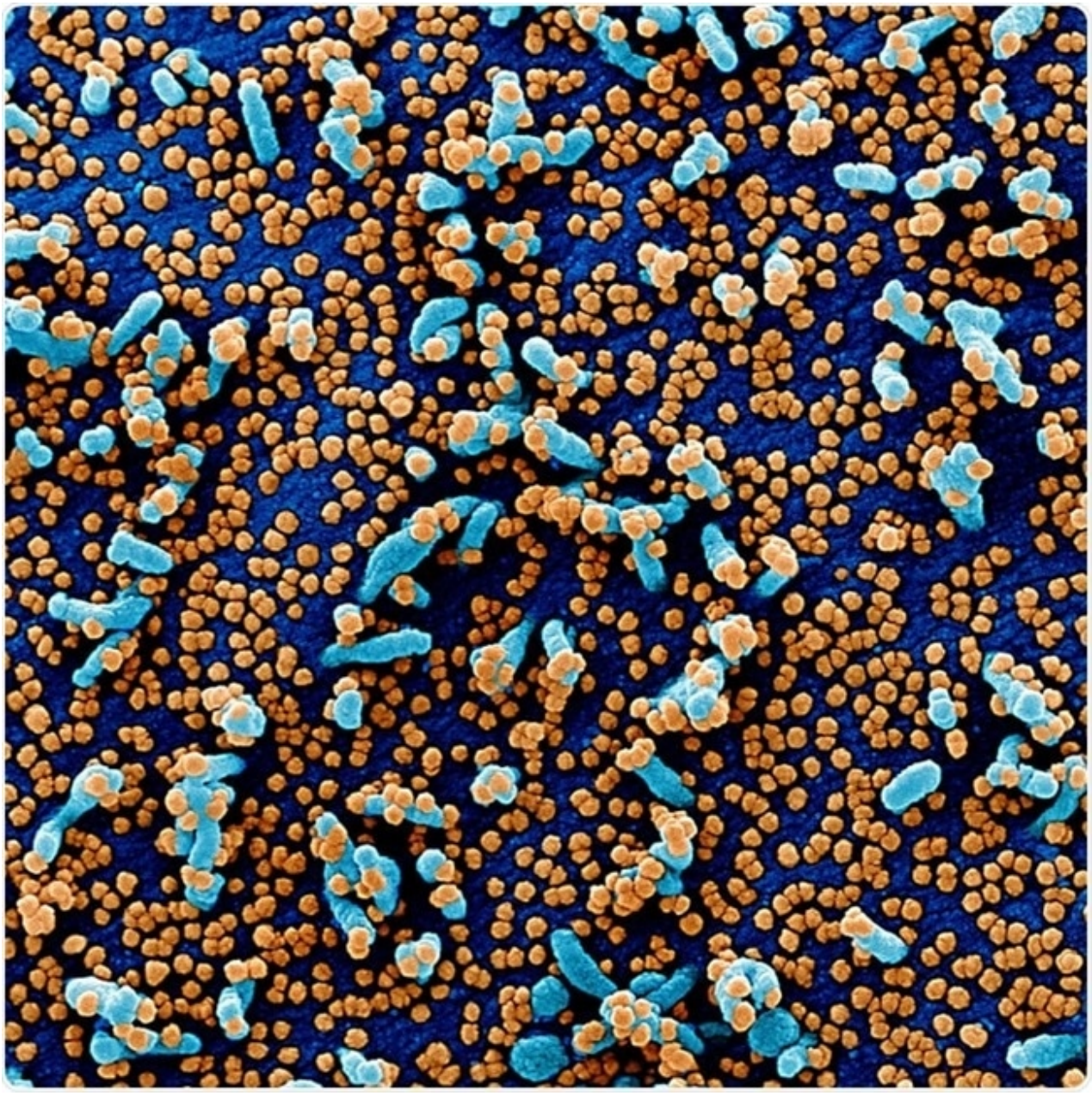
Recombinant protein produces neutralizing COVID-19 antibodies in primate model

By Dr. Liji Thomas, MD Apr 26 2020

A new study reports the induction of high levels of neutralizing antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus causing the current pandemic of COVID-19. This promising finding was reported on the preprint server *bioRxiv* on April 2020.



Novel Coronavirus SARS-CoV-2 Colorized scanning electron micrograph of a VERO E6 cell (blue) heavily infected with SARS-COV-2 virus particles

(orange), isolated from a patient sample. Image captured and color-enhanced at the NIAID Integrated Research Facility (IRF) in Fort Detrick, Maryland. Credit: NIAID

The novel coronavirus is causing hundreds of thousands of cases and deaths all over the world. Despite an early start with the release of the viral genome sequence, researchers are still searching for a cure or an effective vaccine.

This search includes the use of the viral RNA, DNA, vaccines introduced into the host by viral vectors, recombinant subunits that stimulate antibodies to the virus, and the inactivated virus. Some vaccines are entering or have just entered early clinical trials.

What was the focus of the current study?

The SARS-CoV-2 virus attaches to the host cell via a spike protein, which is the receptor-binding protein, mediating the attachment of the virus to the host cell.

The current study in non-human primates shows that the use of the recombinant spike fusion protein, S1-Fc, on the virus, prevents infection by inhibiting the fusion of the virus to the host cell membrane.

In other words, this protein antigen is a highly immunogenic molecule and stimulates the production of high levels of neutralizing antibodies.

Researchers in China tested the immune resistance of CHO-expressed recombinant SARS-CoV-2 S1-Fc fusion protein in mice, rabbits, and monkeys against SARS-COV-2 as a potential vaccine candidate.

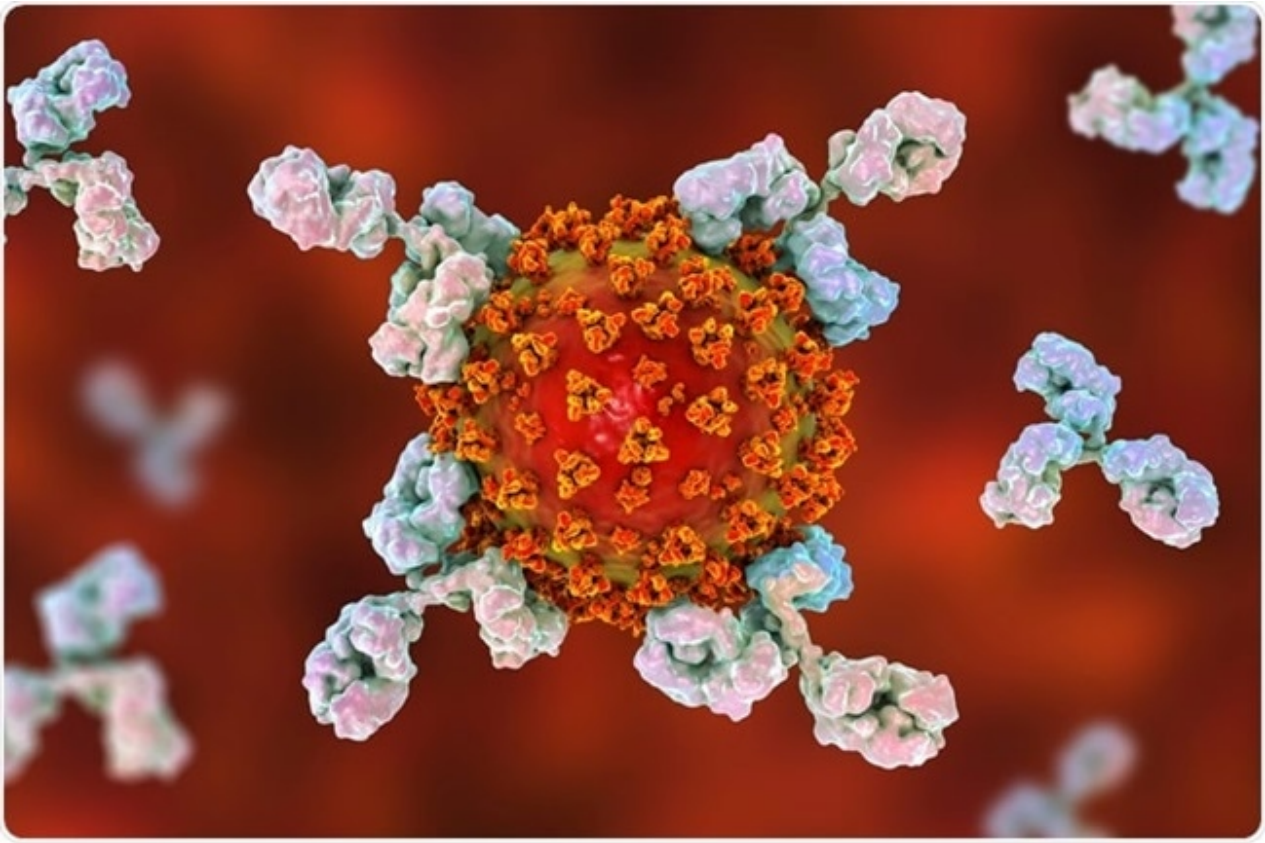
How was the vaccine synthesized?

Most vaccines against this virus target the receptor-binding spike protein, which helps the virus latch on to cells and infect them. SARS-Cov-2 S1-Fc fusion protein is the part of the virus that controls its ability to bind to and infect a cell, making it a logical target in vaccine development.

The spike protein has two major domains, the first being S1, which contains the receptor-binding domains (RBD), and the second the trimeric supporting

structure, including the fusion bundle, which is activated by virus-S1 binding and protrudes into the host cell membrane.

The researchers in the current study cloned the S1 protein into a cell line chosen for its proven performance in making human antibody preparations, and its ability to ensure the right glycosylation of the S1 antigen. They purified the resulting protein and added an adjuvant to increase its immunogenicity. This was then tested in mice, rabbits, and monkeys, and the antibodies formed were analyzed.



Antibodies attacking SARS-CoV-2 virus, the conceptual 3D illustration. Image Credit: Kateryna Kon / Shutterstock

What did the results show?

A virus neutralization assay detects antibodies that are capable of inhibiting virus replication (or, in other words, antibodies that can neutralize virus infection). A high concentration of such antibodies implies strong immune resistance to the virus.

The subjects displayed a high concentration of neutralizing antibodies by day 7. "On day 7, all of the immunized mice showed syndrome of fevers, much

earlier than usual, suggesting S1-Fc fusion protein is super immunogenic," the researchers said.

On day 14, rabbits displayed strong neutralizing activity against the virus when immunized with a pseudovirus neutralization assay.

In what researchers say is the most important finding, two monkeys developed higher concentrations of the antibody neutralizing the virus than those in the samples of a recovered COVID-19 patient in a live SARS-CoV-2 infection assay. This was accomplished in less than 20 days and three injections of the S1-Fc fusion protein.

While the female monkey showed anti-S1 IgG antibodies only in the female monkey on day 9, both monkeys developed strong titers of this isotype by day 16. Still, the IgM levels dropped below detectable concentrations.

An additional boost of the fusion protein on day 24 pushed up the IgG levels and IgM levels, the former to a much larger extent. This could throw light on the phenomenon in which both S1-IgM and IgG were found to be present in patients with COVID-19. This could be a sign of re-infection or that the dormant virus is re-emerging from the infected cells.

In the recovered COVID-19 primate patients, IgG levels were detected at four days from onset, to increase by day 12. On day 20 and 28, the levels dropped. IgM was not detectable at any time.

Why is this study important?

The investigators claim that their results show the S1-Fc protein is capable of effectively stimulating the host to induce humoral immunity (antibodies), and importantly, to produce neutralizing antibodies at high concentrations in a primate species other than humans. This, they say, is a strong indication that this recombinant fusion protein could be a strong candidate for COVID-19 vaccine development.

The most significant advantages of this approach are the reduced costs of production and the ease of approach.

The possible risks of this approach are dealt with by the authors. For

instance, multiple doses were required to produce a stable and robust antibody response. This is amenable to the same type of immunization schedule required by many other vaccines, including the vaccine against shingles.

Secondly, the paper claims that using a single 3,000 L CHO cell reactor (the CHO-K1 line) was used to generate the antibodies), 3 million doses of the recombinant S1-Fc fusion protein vaccine can be produced within two weeks. They say, "With so many antibody drug production plants in the world, it may be the ONLY feasible way to make enough COVID-19 vaccines for the entire world within one year."

Thirdly, they point out that many biologicals already in use contain Fc components derived from the host cell, without additional side effects. Indeed, the presence of the Fc part may help to mimic the 3D conformational structure of the S oligomer in vivo, by the formation of disulfide bonds between the Fc parts of two such molecules. Moreover, and most significantly, it is easy to purify the recombinant protein from the CHO-K1 cell when the S1 protein is fused to the human Fc domain, following the standardized antibody-drug protocols.

The study concludes, "Our data strongly suggests that the CHO-expressed SARS-CoV-2 S1-Fc recombinant protein could be a strong candidate for vaccine development against COVID-19."

Journal reference:

- Wenlin, R., Hunter S., Gao, G. F. et al. (2020). Recombinant SARS-CoV-2 spike S1-Fc fusion protein induced high levels of neutralizing responses in non-human primates. https://doi.org/10.1101/2020.04.21.052209 doi: bioRxiv preprint. https://www.biorxiv.org/content/10.1101/2020.04.21.052209v1

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