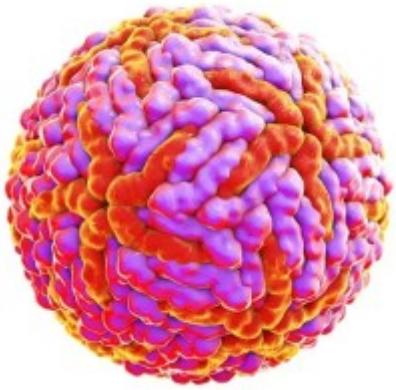
The authors from India used a combination of experimental and computational studies to investigate whether the anti-SARS-CoV-2 antibodies have any impact on Dengue virus (DENV)-2 infection. The results showed that anti-SARS-CoV-2 antibodies acquired through natural infections in humans or through experimental immunization in animals were cross-reactive with DENV-2 and had the potential to enhance DENV-2 infection in K562 and U937 cells. The results of *in silico* and *in vitro* studies showed a strong interaction between SARS-CoV-2 antibodies and DENV-2 envelope (E) protein, providing a molecular basis for these findings. This study is the first to show that anti-SARS-CoV-2 antibodies can cross-react with DENV-2 and enhance Dengue virus infection through antibody-dependent enhancement (ADE).

Dengue virus is a positive-sense single-stranded RNA virus that causes dengue disease, which is highly prevalent in tropical and subtropical regions worldwide. The virus circulates as four serotypes (DENV 1-4), each containing multiple distinct genotypes. Any serotype can lead to mild or severe forms of infection, including dengue hemorrhagic fever and dengue shock syndrome.

For DENV, ADE is a well-known phenomenon. Given the recent increase in cases of dengue disease, researchers aimed to examine the potential cross-reactivity between DENV and SARS-CoV-2 antibodies.



About the study

Researchers examined a total of 48 samples of human convalescent plasma collected during different waves of COVID-19: before the Delta wave (n=21), after the Delta wave (n=17) and during Omicron wave (n=10).

Human myeloid cell lines K562 and U937 were used *in vitro* to determine whether the SARS-CoV-2 antibodies enhance the infection with Dengue virus.

A panel of commercially available monoclonal and polyclonal antibodies raised against SARS-CoV-2, and animal sera raised against SARS-CoV-2 were used to examine their potential to enhance dengue virus infection through ADE. An *in-silico* approach was employed to test the cross-reactivity of SARS-CoV-2 antibodies with DENV E protein. The cross-reactivity was also assessed by immunofluorescence assay. The binding kinetics of SARS-CoV-2 antibodies with DENV envelope and SARS-CoV-2 spike (S) protein were assessed by a biolayer interferometry.

The results

The findings revealed that anti-SARS-CoV-2 antibodies from convalescent plasma samples of SARS-CoV-2 infected individuals (confirmed by qRT-PCR), from pre-delta, delta and post-delta waves significantly enhanced DENV-2 infection in cultured K562 and U937 cells.

The samples obtained from convalescent COVID-19 patients (n=48) significantly augmented DENV-2 infection in K562 cells. The percentage of K562 cells infected with DENV-2 was increased in 14 of 21 samples from the pre-delta wave, in 15 of 17 samples from the delta wave, and in 8 of 10 samples from the omicron wave, compared with the untreated viral control.

Similar findings were observed when samples from convalescent COVID-19 patients (n=15) were tested with U937 cells, and 9 of 15 serum samples enhanced DENV-2 infection. The quantification of the neutralizing antibody titers against the SARS-CoV-2 Wuhan strain in these samples by live-virus neutralization assay failed to demonstrate any correlation between the neutralizing antibody titer and ADE efficiency.

Since DENV is endemic in India, it is possible that pre-existing anti-DENV antibodies are responsible for ADE. To eliminate this possibility, researchers tested commercially available monoclonal and polyclonal antibodies and sera raised against the S protein and receptor-binding domain (RBD) of the SARS-CoV-2 to determine their potential to enhance the DENV-2 infection in an ADE assay using K562 cells.

In both the model systems, the commercially available anti-S protein and anti-RBD SARS-CoV-2 antibodies and S protein-immunized mice and hamster sera showed an increased DENV-2 infection. All of these antibodies enhanced the DENV-2 infection, confirming that antibodies to SARS-CoV-2 cross-react with DENV and cause ADE. The RBD-specific antibody showed a stronger enhancement compared to the antibody against S protein with dose-dependent increase in DENV-2 infection. Similarly, serum from hamsters immunized with purified SARS-CoV-2 S protein showed ADE of DENV-2 infection in K562 cells.

A monoclonal antibody against Rabies virus, which does not cross-react with DENV, did not show any ADE of dengue infection in K562 cells, indicating that anti-SARS-CoV-2 antibodies have specific effect.

To assess the cross-reactivity of SARS-CoV-2 antibodies with the DENV-2 E-protein dimer, the authors used an *in-silico* approach. The findings revealed a significant interaction between SARS-CoV-2 antibodies and DENV-2 dimeric E protein. The free energy of binding of three antibodies, namely CR3022 (6W7Y), S2E12 ab (7K45) and DMAb2196 (8D8R) was comparable to that of DENV-2 dimer specific antibody, C8 (4UTA). These top three binders shared their interacting interface with the C8 antibody.

The residues involved in the interaction of the C8 antibody with the DENV-2 E protein that were also involved in the interaction of at least one of three major binders with E protein were: R73, E84, R99, G102 and E311. The residues, most stabilizing the interaction of these top three SARS-CoV-2 antibodies with E protein were S72, R73, Q77, S81, N83, and R99. All the residues involved in the interaction of these three SARS-CoV-2 antibodies with E-protein were found to lie in the Domain-II and fusion-loop of E-protein.

The authors concluded that this study is the first to demonstrate that anti-SARS-CoV-2

antibodies can cross-react with DENV-2 and can enhance Dengue virus infection through ADE. The study also identified the cross-reactive epitopes and paratopes and listed some critical residues involved in the interaction of SARS-CoV-2 antibodies with DENV-2 E-protein.

The authors stated that their findings have implications for the development and use of SARS-CoV-2 vaccines in regions where Dengue disease is endemic. Further studies should investigate whether antibodies produced in response to various SARS-CoV-2 vaccines also have the potential to enhance DENV-infection. The researchers also suggested that seasonality of the DENV should be considered when using SARS-CoV-2 vaccines to prevent severe infections, especially in countries where DENV is endemic.

The results of the study have been published on a preprint server and are currently being peer-reviewed.

Journal Reference

Jakhar K, Sonar S, et al. SARS-CoV-2 antibodies cross-react an 1 d enhance dengue infection. bioRxiv preprint https://doi.org/10.1101/2023.10.09.557914