In this study, the authors from Spain and the United Kingdom investigated whether the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to and enters human erythrocytes, as well as the role of CD147 as an alternative receptor for SARS-CoV-2. They also examined whether the presence of the malaria parasite *Plasmodium falciparum* in human erythrocytes increases their susceptibility to SARS-CoV-2 infection and whether the presence of SARS-CoV-2 in blood culture affects the survival and growth of the malaria parasite.

Two host-cell factors are important for SARS-CoV-2 viral entry into many cell types: angiotensin-converting enzyme 2 (ACE2), which binds to spike (S) protein, and transmembrane serine protease 2 (TMPRSS2), which cleaves S protein, allowing this binding to take place. In addition to ACE2 and TMPRSS2, the S protein has been reported to engage other cell-surface factors proposed to serve as attachment factors promoting SARS-CoV-2 entry. CD147, expressed in many cell types, including human erythrocytes, has been proposed as an alternative route for SARS-CoV-2 entry. CD147 is a transmembrane glycoprotein that belongs to the immunoglobulin superfamily and is essential for red blood cell invasion by the malaria parasite *Plasmodium falciparum.* However, certain studies did not support the role of CD147 as a functional SARS-CoV-2 receptor.

The authors emphasize the co-occurrence and similarity between malaria and COVID-19. Several computational studies have found immunodominant epitopes shared by SARS-CoV-2 and *Plasmodium falciparum*. A study that employed a computational integrative approach for epitope prediction and identification found molecular similarity between antigenic sites in the SARS-CoV-2 receptor binding domain (RBD) and several antigenic determinants found in fifteen pathogenic bacteria, parasites, and viruses. Several predicted antigenic sites in SARS-CoV-2 RBD had molecular similarity with antigenic determinants from *Plasmodium falciparum,* including circumsporozoite protein, circumsporozoite protein-related antigen, malaria protein EXP-1, merozoite surface protein 1, ring-infected erythrocyte surface antigen, erythrocyte-binding antigen 175, liver stage antigen-1, and liver stage antigen-3.
https://discovermednews.com/molecular-similarities-between-sars-cov-2-rbd-and-pathogens/ Accordingly, it was hypothesized that previous exposure to *Plasmodium* parasites could indirectly protect against severe forms of infection with the SARS-CoV-2. This was attributed to cross-immunity due to the common immunodominant epitopes shared between the two pathogens.
https://discovermednews.com/immune-response-to-sars-cov-2-after-exposure-plasmodium-falciparum/

About the study

The authors initially investigated whether the transient overexpression of CD147 in HEK 293T cells could facilitate the internalization of SARS-CoV-2 pseudovirions. Due to its broad cell-type tropism, the vesicular stomatitis virus was used as a positive control for infection. At 48 hours after the infection of HEK 293T cells expressing the ACE2 or the CD147 receptors, the infection was quantified with luciferase activity assay. The magnitude of luciferase activity reflected the expression of integrated proviruses.

Researchers then tested whether the virus could bind to and enter human erythrocytes derived from the whole blood of healthy donors from the Andalusian Public Health System Biobank. The erythrocytes were analyzed by indirect immunofluorescence microscopy using an antibody against the SARS-CoV-2 nucleocapsid (N) protein.

As *Plasmodium falciparum* alters the erythrocyte membrane, the authors hypothesized that malaria-infected red blood cells may be more susceptible to the adhesion and entry of SARS-CoV2, potentially leading to coinfection. This hypothesis was tested by incubating a *Plasmodium falciparum* culture with SARS-CoV-2 for 1 hour. After incubation, the erythrocytes were analyzed by immunofluorescence and antibodies against the SARS-CoV-2 N protein, and by DAPI staining for parasite DNA.

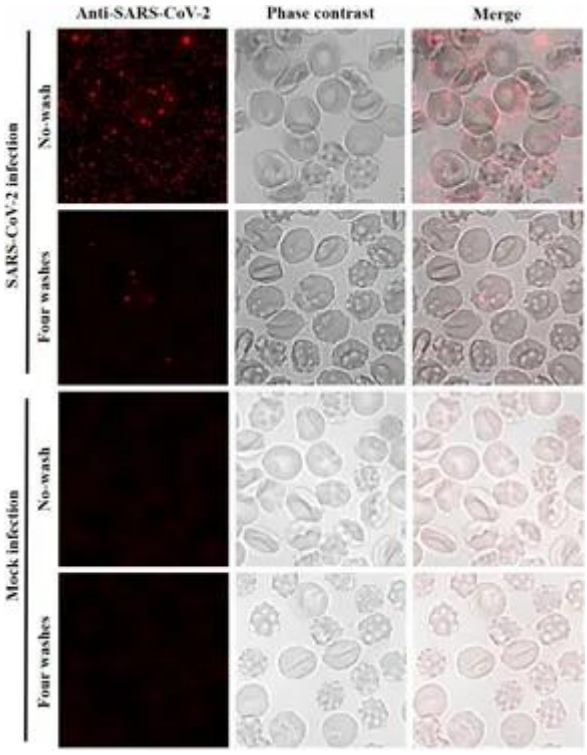
Finally, researchers determined whether SARS-CoV-2 may influence the growth and development of parasites in malaria-infected erythrocytes. After incubation of a *Plasmodium falciparum* culture with SARS-CoV-2, the development of the culture was monitored by

Giemsa-stained blood smears, which were quantified by microscopic examination.

Results

The overexpression of the CD147 receptors in HEK 293T cells did not mediate the infection with SARS-CoV-2 pseudovirions. Only cells that expressed the ACE2 receptor and TMPRSS2 protease were permissive for the entry of SARS-CoV-2 pseudovirions. Since some other studies suggest that CD147 receptors are alternative receptors for SARS-CoV-2 in cells with low ACE2 expression, the authors explained that the contradictory results regarding CD147-mediated entry could be due to the heterogeneous glycosylation of the expressed protein in the different cell lines or tissues studied.

The findings also revealed a low level of entry of SARS-CoV-2 into human erythrocytes *in vitro.* Immunofluorescence demonstrated that SARS-CoV-2 could adhere to and enter erythrocytes, but less efficiently than SARS-CoV-2 permissive cell lines such as VeroE6. Virions did not accumulate inside the erythrocytes or plasma membrane as observed in VeroE6 cells, and only 1-5 viral particles were observed in a low percentage of cells (10.9% of erythrocytes).



Original figure from the article of Lopez-Farfan et al. Low entry of SARS-CoV-2 to human erythrocytes. Immunofluorescence analysis using an anti-SARS-CoV-2 nucleocapsid antibody for detection of SARS-CoV-2 in infection assays with human erythrocytes. The percentage of erythrocytes with viral particles (red dots) attached after four washes was calculated by counting several fields with a total of 2,490 red blood cells

Incubation of a *Plasmodium falciparum* culture with SARS-CoV-2 showed similar results as incubation with uninfected erythrocytes, with only 9.1% of parasitized erythrocytes exhibiting few viral particles attached (1 to 5 particles per cell). This shows that the presence of *Plasmodium falciparum* inside erythrocytes did not facilitate or increase the entry of SARS-CoV-2 to these cells and that the interaction between the two pathogens is not frequent. The possibility of SARS-CoV-2 and *Plasmodium falciparum* coinfection in red blood cells was observed mainly during the ring stage of the parasite.

Also, SARS-CoV-2 did not affect the survival, growth, or development of *Plasmodium falciparum* in malaria-infected erythrocytes. The parasitemia of *P. falciparum* and cell stages did not differ between cultures incubated with SARS-CoV-2 and control cultures incubated with a medium without the virus.

Conclusion

According to these results, mature erythrocytes in the blood are not a critical niche for SARS-CoV-2 that could contribute to the spread or hiding of the virus, although they can be infected. The low coinfection rate suggests that *Plasmodium falciparum* does not facilitate the entry of SARS-CoV-2 into malaria-infected erythrocytes. The presence of SARS-CoV-2 in blood culture did not affect the survival or growth rate of the malaria parasite.

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