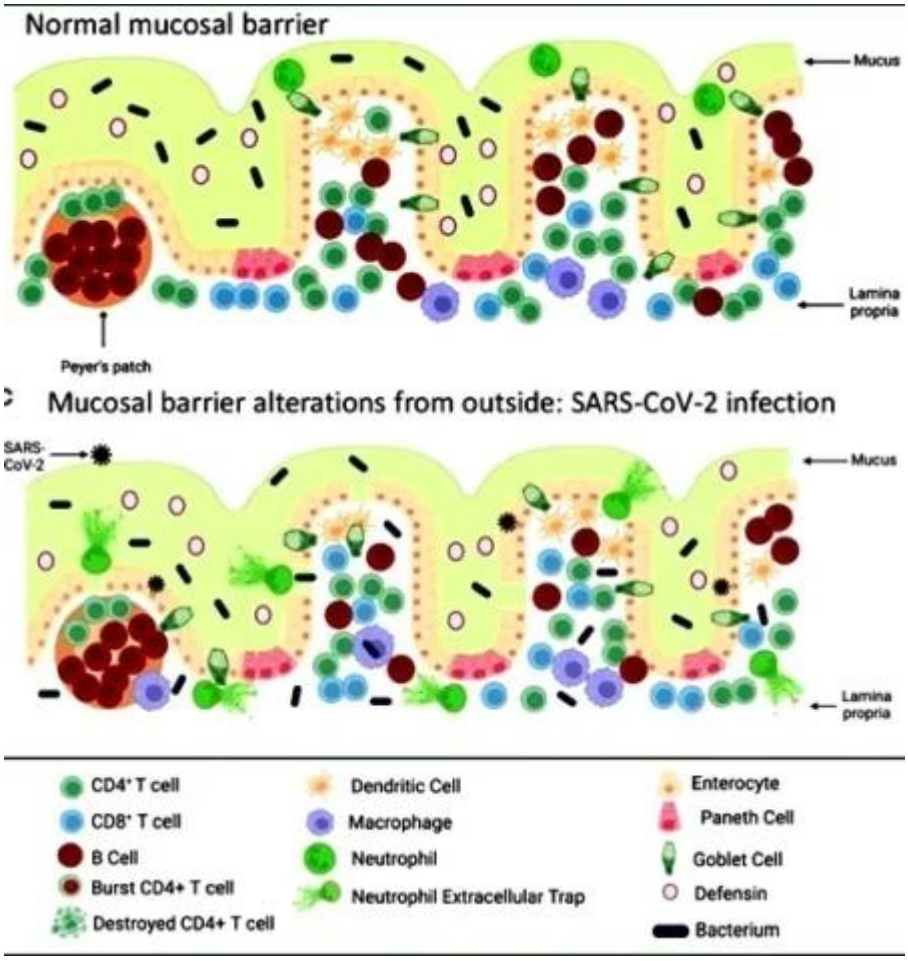
Coronavirus disease 2019 (COVID-19) is a systemic multiple-organ disease characterized by a broad spectrum of clinical manifestations and caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The gastrointestinal (GI) system is also affected, and the most frequent symptoms are abdominal pain, nausea, vomiting, and diarrhea. In this study, the authors from France investigated whether SARS-CoV-2 uses the intestinal epithelium as an alternative route of infection and which is the preferential side (the apical or basal side) for intestinal cell infection.

The intestinal epithelium is a monolayer of self-renewing epithelial cells linked together. The structural tissue integrity is preserved through intercellular junctions involving tight junctions, adherent junctions, desmosomes, and gap junctions. The epithelial cell layer has an apical domain that faces the lumen of the intestine and a basolateral domain, which is divided into a basal domain facing the basal membrane and a lateral domain facing the neighboring cells. This polarity of the epithelial cells allows a balanced communication between the tissues and the intestinal lumen and protects against GI pathogens.

According to some previous studies, the GI tract is considered a potential viral reservoir for SARS-CoV-2. Numerous studies reported the persistence of viral RNA and/or antigen(s) in specimens taken from the GI tract in individuals with long COVID syndrome. It was reported that SARS-CoV-2-specific antigens, spike (S), and nucleocapsid (N) proteins were detected in the appendix sample of a long COVID patient 426 days after the infection. https://discovermednews.com/sars-cov-2-rna-and-antigens-appendix-skin-breast-patients-long-covid/

Two host-cell factors are important for SARS-CoV-2 viral entry into many cell types: angiotensin-converting enzyme 2 (ACE2), which is bound by the S protein, and transmembrane serine protease 2 (TMPRSS2), which cleaves the S protein, allowing this binding to take place.

Previous studies suggested that SARS-CoV-2 initiates GI pathology from the luminal side. The SARS-CoV-2 enters enterocytes by binding to ACE2 and TMPRSS2. The infection of the enterocytes rapidly induces T-cell infiltration in the intestinal epithelium and lamina propria. Destruction of the epithelium leads to the transfer of the intestinal microbiota to the lamina propria and then systemically. (Frontiers in Immunology 2022; 13: 899559) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9411647



Original illustration from the article: Frontiers in Immunology 2022; 13: 899559.

About the study

To investigate the possibility that the intestinal epithelium serves as an alternative route for infection, the researchers employed a model of polarized intestinal cell monolayers inoculated with SARS-CoV-2. They used the human colon adenocarcinoma Caco-2 cell line, susceptible to SARS-CoV-2 infection and intense viral replication.

After 21 days of culture, Caco-2 cells adopted an adherent polarized cell architecture and formed an epithelial monolayer resembling an epithelial permeability barrier. However, the authors stated that Caco-2 cell monolayer systems are oversimplified and fail to replicate numerous cellular components and complex intestinal structures. Particularly, they lack the mucin microenvironment, a typical characteristic of the intestinal barrier that plays a major

role in the dynamics of viral infections, including SARS-CoV-2. Therefore, the authors used the HT29 human tumor epithelial cell line of intestinal origin, which produces mucin, to mimic intestinal goblet cells. The mucin produced by the HT29 cells is expected to protect the Caco-2 cells, similar to the protection of enterocytes in the intestinal mucosa.

The Caco-2 and HT29 cells were infected with SARS-CoV-2 either on the apical (upper chamber) or basolateral (lower chamber) sides. The integrity of the epithelial permeability barrier was assessed by measuring the transepithelial electronic resistance (TEER). The TEER was measured 4, 8, 16, 24, and 48 hours after the infection. A stable TEER of ~500–700 Ω/cm2 was considered a strong indicator of the integrity of the epithelial permeability barrier. A reverse transcription polymerase chain reaction (rt-PCR) was used to detect SARS-CoV-2 RNA. Tissue culture infectious dose 50 (TCID50) was used to evaluate viral release.

Results

24 hours after SARS-CoV-2 inoculation, TEER values decreased. The mean TEER value was 443 Ω/cm2 after apical inoculation and 556 Ω/cm2 after basolateral inoculation.

Confocal immunofluorescence, used to examine the expression of intercellular adhesion proteins in Caco-2 cell monolayers, revealed that E-cadherin (E-cad) expression decreased significantly 24 hours after apical inoculation of SARS-CoV-2. In contrast, after basolateral inoculation of SARS-CoV-2, the expression of E-cad protein was almost similar to that observed in virus-free Caco-2 cell monolayers, suggesting that the preserved integrity of the intercellular junctions. Decreased E-cad expression after SARS-CoV-2 inoculation *via* the apical side correlated with reduced TEER. These results were undetectable when SARS-CoV-2 was inoculated at the basolateral side.

After apical infection of the monolayer, the Ct values (results of qRT-PCR expressed as Ct) demonstrated the presence of SARS-CoV-2 only in samples from the upper chamber. The Ct values from the lower chamber were negative during the first 16 hours after the infection. 24 and 48 hours after infection at the apical side, viral particles were detected in the upper and lower chambers, indicating damage to intercellular junctions. Higher TCID50 titer in the samples collected from the apical side after apical inoculation indicated *de novo* synthesis of virions.

After basolateral inoculation, the Ct values showed the presence of the virus in the lower chamber, but not in the upper chamber. This suggests that the virus was kept in the lower

chamber due to the maintained integrity of the epithelial barrier.

Conclusion

This study showed that infection of the intestinal cells with SARS-CoV-2 from their apical side caused severe damage to the integrity of the intestinal epithelial barrier, indicating a disruption of tight junctions and paracellular trafficking of the virus. After damage to the intestinal epithelial barrier, the virus can enter the blood vessels and spread to various organs.

This study has been published on a preprint server and is currently being peer-reviewed.

Journal Reference

Garrec G, Arrindell J, Andrieu J et al. Preferential apical infection of intestinal cell monolayers by SARS-CoV-2 is associated with damage to cellular barrier integrity: Implications for the physiopathology of COVID-19. bioRxiv preprint. https://doi.org/10.1101/2024.01.08.574642