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Reduced Replication Efficacy of Severe Acute Respiratory Syndrome Coronavirus 2 Omicron Variant in "Mini-gut" Organoids

See editorial on page 376.

Over 480 million infections and 6 million deaths have been recorded since the novel coronavirus disease 2019 pandemic began over 2 years ago. Coronavirus disease 2019 is principally a respiratory illness caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which infects the cells of the airway and can also replicate in the gastrointestinal tract. Notably, over half of those with SARS-CoV-2 infections shed SARS-CoV-2 genomes in their stool,[1] and infectious viruses can be isolated from their fecal samples,[2] suggesting that the virus also infects and replicates in the intestinal tract.

Intestinal organoids are versatile tools used to study viral infections ex vivo, and the clinical isolates of the SARS-CoV-2 outbreak strain can infect human and bat intestinal organoids.[3,4] Newly emerged variants, such as delta and omicron, have an increased capacity to infect the respiratory tract, and omicron preferentially infects the upper airway and spares the lower respiratory tract.[5] However, the replication preferences of these variants in the intestinal tract are currently unknown. Hence, we hypothesized that the omicron variant may have altered infective potential in the intestinal tract.

We previously reported on an induced pluripotent stem cell–derived apical-out intestinal organoid (mini-gut) containing different types of intestinal cells, including epithelial, goblet, Paneth, and enteroendocrine cells derived from 3 germ layers to form a 3-dimensional arrangement mimicking the intestinal tissue without introducing Matrigel or another extracellular matrix[6] (Figure 1*A* and *B*, Supplementary Figure 1). The epithelial cells were arranged on the surface of the mini-gut and stably expressed the SARS-CoV-2 entry receptor, angiotensin converting enzyme 2 (ACE2), and the facilitating enzyme, transmembrane serine protease 2 (TMPRSS2), on the cell surface (Figure 1*C*), suggesting susceptibility of the mini-gut to SARS-CoV-2 infection. We thus investigated if human mini-gut organoids could be infected with the prototype Wuhan strain and currently circulating delta and omicron (BA.1 and BA.2) SARS-CoV-2 strains to evaluate the proliferation efficiency of these virus strains in the gut model. By serial measurement of viral RNA and titer in the culture supernatants, viral replication peaked 4 days after infection, and the replication efficiency of the delta strain was approximately 4–6 times higher than that of the Wuhan strain. In contrast, the replication capacity of the omicron strains was extremely low in the mini-gut model (Figure 1*D* and *E*, Supplementary Figure 2*A*). Interestingly, this trend was seen in the intestinal epithelial Caco-2 cells, where viral shedding of the omicron strain was severely reduced compared with the Wuhan and delta strains. In contrast, the omicron strain showed replication capability almost equivalent to the Wuhan strain in lung epithelial Calu-3 cells and African green monkey kidney epithelial VeroE6 cells, irrespective of exogenous TMPRSS2 expression (Figure 1*F* and *G*, Supplementary Figure 2*B* and *C*). These results imply that the infection efficiency of omicron is selectively reduced in intestinal epithelial cells.

Levels of a cell death marker, lactate dehydrogenase (LDH), were significantly increased in the culture supernatants from the mini-guts infected with the Wuhan and delta strains, whereas the LDH levels in the supernatants of omicron-infected mini-guts were comparable with that of the uninfected control (Figure 1*H*). Similarly, the high mobility group box 1 (HMGB1), a representative of damage-associated molecular patterns that are secreted during cell destruction, was detectable in the mini-guts infected by both the Wuhan and delta strains but not in the omicron-infected mini-guts (Figure 1*I*). Simultaneously, higher levels of various cytokines were detected in delta-infected mini-guts than in the omicron-infected mini-guts (Supplementary Figure 2*D*). HMGB1 is secreted on viral infection and induces inflammatory responses by stimulating immune cells to produce pro-inflammatory cytokines. By failing to secrete HMGB1 and possibly other damage-associated molecular patterns, it can be presumed that omicron infection might not induce inflammatory responses in the intestines, whereas the delta strain could do so.

Immunostaining using SARS-CoV-2 nucleocapsid antibodies was performed to visualize the presence of the virus in the mini-gut. The Wuhan strain–infected mini-guts retained distinct cellular morphology, and the infected cells were sporadically distributed among the organoid tissue, whereas delta-infected mini-guts exhibited clustering of virus-infected cells (Figure 1*J* and *K*), presumably because of the enhanced fusogenicity (membrane fusion activity) of this strain (Supplementary Figure 2*E*). Virus-infected cells were extremely scarce in the omicron infection model (Figure 1*J*). Of note, viral antigens were restricted to the luminal side of the intestinal epithelium and were not observed in the basal lamina and submucosal tissues, suggesting that SARS-CoV-2 infection is unlikely to intrude into the lamina propria.

Abbreviation used in this paper: **ACE2, angiotensin converting enzyme 2; HMGB1, high mobility group box 1; LDH, lactate dehydrogenase; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane serine protease 2.**

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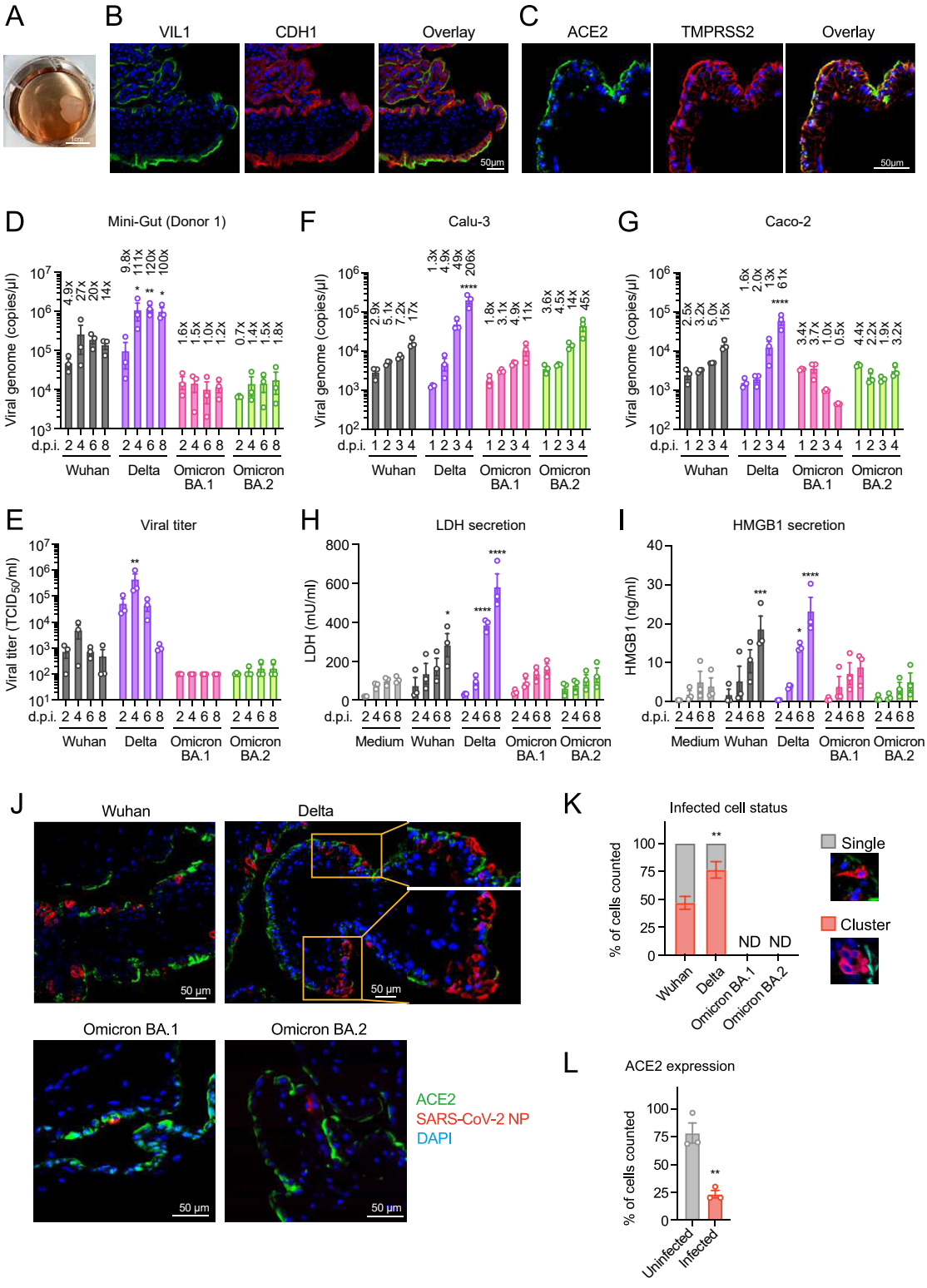
Interestingly, cell-surface expression of ACE2 was prominently downregulated in virus-infected cells (Figure 1*L*).

Intestinal organoids can also be used to test drug susceptibility of SARS-CoV-2,[7] but there are a few reports yet on these tests. Therefore, we evaluated the effect of a neutralizing antibody cocktail (imdevimab and casirivimab) to reduce viral cell-to-cell transmission. Immunofluorescence analysis showed that the antibody cocktail treatment reduced viral spread to adjacent cells, even 2 days after infection with the delta strain, thereby suppressing the formation of infected cell clusters (Supplementary Figure 2*F* and *G*). Although it may not be practical to administer antibody drugs to the intestinal tract, our observations indicate that neutralizing antibodies secreted into the intestinal mucosa can inhibit the lateral spreading of progeny viruses.



Studies have shown differences in the anatomic site preferences between omicron and other SARS-CoV-2 variants in the respiratory tract,[5] and we have revealed a similar phenomenon with respect to the intestinal tract. Currently, it is still elusive as to why the replication efficacy of the omicron variant is markedly reduced in gut organoids. The spike protein of the omicron variant is substantially mutated compared with the conventional strain (Supplementary Figure 2*H*), which may result in changes in its receptor binding, proteolytic cleavage, and stability in intestinal cells or tissues.[8] Further analysis is needed to confirm the precise molecular mechanisms involved. Moreover, the low replication ability and cytotoxicity properties of the omicron variant in certain tissues and contrastingly high replication ability in other tissues must be assessed further.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org and at https://doi.org/10.1053/j.gastro.2022.04.043.

KEI MIYAKAWA
Department of Microbiology
Yokohama City University School of Medicine
Yokohama, Japan

MASAKAZU MACHIDA
TOMOYUKI KAWASAKI
Center for Regenerative Medicine
National Center for Child Health and Development
Tokyo, Japan

MAYUKO NISHI
Department of Microbiology
Yokohama City University School of Medicine
Yokohama, Japan

HIDENORI AKUTSU[§]
Center for Regenerative Medicine
National Center for Child Health and Development
Tokyo, Japan

AKIHIDE RYO[§]
Department of Microbiology
Yokohama City University School of Medicine
Yokohama, Japan

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References

1. Xiao F, et al. Gastroenterology 2020;158:1831–1833.
2. Wang W, et al. JAMA 2020;323:1843–1844.
3. Zhou J, et al. Nat Med 2020;26:1077–1083.
4. Lamers MM, et al. Science 2020;369:50–54.
5. Hui KPY, et al. Nature 2022;603:715–720.
6. Uchida H, et al. JCI Insight 2017;2:e86492.
7. Kruger J, et al. Cell Mol Gastroenterol Hepatol 2021;11:935–948.
8. Hulswit RJ, et al. Adv Virus Res 2016;96:29–57.

[§]**Authors share co-corresponding authorship.**

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Correspondence
Address correspondence to: Hidenori Akutsu, MD, PhD, Center for Regenerative Medicine, National Center for Child Health and Development, 2-10-1 Okura, Setagaya, Tokyo 157-8535, Japan. e-mail: akutsu-h@ncchd.go.jp; or Akihide Ryo, MD, PhD, Department of Microbiology, Yokohama City University School of Medicine, 3-9 Fuku-ura, Kanazawa, Yokohama 236-0004, Japan. e-mail: aryo@yokohama-cu.ac.jp.

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CRediT Authorship Contributions
Kei Miyakawa, PhD (Investigation: Lead; Writing – original draft: Lead; Writing – review & editing: Lead).
Masakazu Machida, PhD (Investigation: Equal; Resources: Lead).
Tomoyuki Kawasaki, PhD (Investigation: Equal; Resources: Lead).
Mayuko Nishi, PhD (Investigation: Supporting).
Hidenori Akutsu, MD, PhD (Conceptualization: Equal; Supervision: Equal; Writing – review & editing: Equal).
Akihide Ryo, MD, PhD (Conceptualization: Lead; Supervision: Lead; Writing – original draft: Equal; Writing – review & editing: Equal).

Conflicts of Interest
The authors disclose no conflicts.

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Figure 1. Replication efficiency of SARS-CoV-2 variants in mini-gut organoids. (*A*) Macroscopic appearance of mini-guts cultured in 6-well plates. (*B* and *C*) Expression of VIL1, CDH1 (epithelial marker), ACE2, and TMPRSS2 in a mini-gut. Note that the mucosal epithelium is the outside of the mini-gut (apical-out). Nuclei were shown with 4',6-diamidino-2-phenylindole (DAPI) in *blue*. Scale bar: 50 μm. (*D*–*G*) SARS-CoV-2 propagation in (*D* and *E*) mini-gut, (*F*) Calu-3, and (*G*) Caco-2 cells. Cells were infected by SARS-CoV-2 strains at a multiplicity of infection of 1 (for mini-guts) or 0.5 (for Calu-3 and Caco-2 cells). Half of the culture supernatants were collected, and fresh medium was topped-up on designated days after infection (d.p.i.). (*D*, *F*, and *G*) Viral genomes and (*E*) titer from culture supernatants were quantified. Viral titer was determined by 50% tissue culture infectious dose (TCID50) assay in VeroE6/TMPRSS2 cells. The mean ± SEM of 3 independent determinations is plotted. Values indicate the ratio of the inoculum to the virus dose. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$; Šídák's multiple comparisons test was used to determine significant differences between groups. (*H* and *I*) Amount of (*H*) LDH and (*I*) HMGB1 in the supernatants of infected mini-guts. The mean ± SEM of 3 independent determinations is plotted. $^{*}P < .05$, $^{**}P < .01$; 2-tailed unpaired *t* test was used to determine significant differences between uninfected and infected groups. (*J*) Immunostaining images of the mini-gut 8 days after infection. ACE2 and SARS-CoV-2 nucleocapsid (NP) antigens were stained in *green* and *red*, respectively. Nuclei are shown with DAPI in *blue*. Scale bar: 50 μm. (*K*) Infected cell status is quantified as "cluster" or "single." A cluster is defined as 2 or more infected cells adjacent to each other. Of the randomly taken fields of view, >100 infected cells were examined. ND, not determined because omicron-infected cells were extremely scarce. (*L*) Percentage of ACE2-expressing epithelial cells in infected or uninfected mini-gut ($n > 50$ cells).