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Letter to the Editor

Modeling of COVID-19 disease disparity in gastric organoids reveals the spatiotemporal dynamics of SARS-CoV-2 infectivity

Dear Editor,

The promptness and continuous expansion of the coronavirus disease 2019 (COVID-19) pandemic, elicited by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its variants, has presented an unprecedented impact on human health (WHO Coronavirus (COVID-19) Dashboard, 2021). Although vaccination has attenuated the severe symptoms, there is no specific antiviral medication available for preventing the viral spread (Drayman et al., 2021). A well-documented COVID-19 case study exhibited the temporal dynamics of disease progression and related symptoms (Holshue et al., 2020), and gastrointestinal epithelial cells are susceptible to SARS-CoV-2 infection (Han et al., 2021).

In order to delineate how host cell polarity regulates SARS-CoV-2 infectivity and how host genetic factors contribute to COVID-19 susceptibility, we established 3D human gastric organoids for real-time imaging of cellular response to enteric infection (Yao and Smolka, 2019). In this 3D model system, enteric infection can be mimicked using intraluminal microinjection by which pathogens and their metabolites can enter the host cell via the apical membrane. Figure 1A illustrates our experimental scheme in which we compare the consequence of microinjection-based apical infection versus the typical basolateral infection with matrigel.

As shown in Figure 1B, 3D gastric organoids exhibit a well-defined cell polarity as the apical membranes of gastric parietal cells (PCs), chief cells (CCs), and enterochromaffin-like (ECL) cells line up the lumen of gastric organoids (Supplementary Figure S1A and B). The apical polarity was also confirmed by F-actin labelling (Figure 1B, top panel; enlarged montage *a*), while basolateral membrane was marked by b-catenin (Figure 1B, bottom panel; enlarged montage *a′*). As depicted in Supplementary Figure S1A, PCs secrete hydrochloric acid and CCs are responsible for protease pepsin secretion. ECL cells secrete inflammatory factors such as histamine and IL2β (Yao and Smolka, 2019). Thus, the gastric epithelial lineage is well preserved in 3D organoids grown *in vitro*.

To examine the subcellular localization of the host cell protein factors implicated in SARS-CoV-2 infection, we carried out immunofluorescence studies as shown in Supplementary Figure S1C, in which both ACE2 and TMPRSS2 localized with the basolateral membrane marker Na,K-ATPase (arrows). In contrast, F-actin labelling was readily apparent in the apical membrane of the gastric lumen (arrowheads). Importantly, the basolateral distribution of ACE2 and TMPRSS2 was also confirmed (Supplementary Figure S1D). We next sought to inject engineered SARS-CoV-2 into gastric lumen and compare its infection efficiency with that of basolateral viral entry (Figure 1C and D). As shown in Supplementary Figure S2A, microinjection of Alexa647-conjugated dextran resulted in an even distribution of the liquid into the lumen (lower right panel; blue color), suggesting the reliability of the technique. In order to evaluate the efficiency of infection, we carried out electron microscopic analyses of organoids in which the samples were fixed 1 h after the microinjection followed by immune-gold labelling of spike protein. As shown in Supplementary Figure S2B, microscopic analyses revealed an abundant deposition of 10-nm gold particles into the microvilli structure (arrows), indicating efficient entry into PCs 1 h after the injection, which is consistent with our hypothesis of primary viral infection via the apical membrane (Figure 1D). In contrast, no apparent gold particles were seen from basolateral infection (Supplementary Figure S2C; arrows). The results of immuno-electron microscopic analyses were consist with that of the measurement of SARS-CoV-2 spike protein (Supplementary Figure S2D). Thus, SARS-CoV-2 is an enteric virus that efficiently enters host epithelial cells via apical membranes.

To assess the cytokine secretion in response to SARS-CoV-2 infection, we carried out enzyme-linked immunosorbent assay of culture media in which organoids were infected or microinjected with SARS-CoV-2 at difference time points after the infection. As shown in Figure 1E, microinjected SARS-CoV-2 elicited a time-dependent stimulation of IL-1β secretion from gastric organoids ($P < 0.001$; $n = 5$). In contrast, basolateral infection of SARS-CoV-2 in the parallel experiments did not exhibit a significant stimulation ($P > 0.05$; $n = 5$), suggesting that enteric administration of SARS-CoV-2 is a much more efficient infection. Interestingly, the levels of IL-6 secretion did not change in response to either microinjection or infection of SARS-CoV-2, which is consistent with

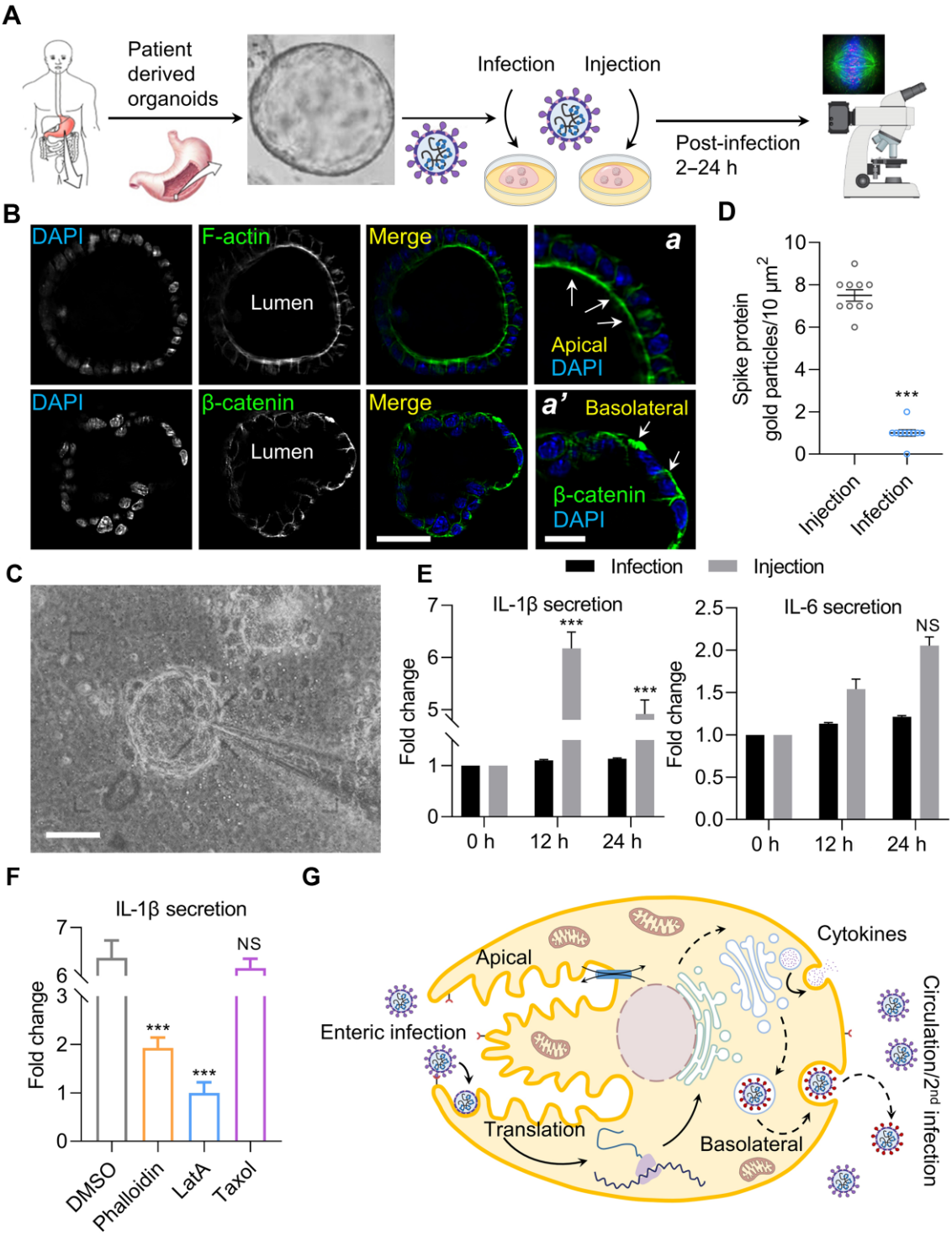

Figure 1 Modeling of SARS-CoV-2 infection in 3D gastric organoids reveals the apical entry of the virus. (**A**) Schematic diagram of the experimental design and flow. (**B**) Characterization of the apical and basolateral membranes of 3D gastric organoids. Note that F-actin labels the apical membrane, while β-cetenin marks the basolateral membrane. Scale bar, 100 μm. Magnified montages show the characteristics of the apical versus basolateral membranes of the 3D organoids. Scale bar, 30 μm. (**C**) A representative image of the microinjection of a 3D gastric organoid. Scale bar, 150 μm. (**D**) Statistical analyses of SARS-CoV-2 entry efficiency judged by immune-gold labelling (Supplementary Figure S2B and C). (**E**) Representative cytokine secretion profiles elicited by apically microinjected and basolaterally infected SARS-CoV-2. Note that IL-1β secretion is responsive to enteric but not basolateral infection in acute infection. Data represent mean ± SD; $n = 5$. Ordinary

the findings in the clinics (Stone et al., 2020). Thus, modeling enteric infection of SARS-CoV-2 validated that the virus primarily enters through the apical membrane of host cells. Consistent with this notion, measurement of cytokine levels of the organoids after a longer time of incubation revealed the time-dependent increase of M-CSF and IL-1β (Supplementary Figure S3A). Thus, SARS-CoV-2 preferentially enters via the apical membrane of host cells in gastric organoids.

Since apical membranes of PCs undergo dramatic remodeling in response to acid secretion and the actin-based cytoskeleton operates the reorganization (Yao and Smolka, 2019), we sought to test whether the actin cytoskeleton is involved in enteric SARS-CoV-2 infection. To this end, we incubated 3D gastric organoids with the actin stabilizer phalloidin and the microtubule stabilizer taxol for 30 min before microinjection of SARS-CoV-2. We measured the cytokine levels 12 and 24 h after the microinjection. Importantly, the perturbation of actin cytoskeleton dynamics by treatment of organoids with phalloidin and the actin disruptor latrunculin inhibited the elevation of cytokine releases (Figure 1F), while treatment with the microtubule stabilizer taxol did not alter cytokine secretion in response to SARS-CoV-2 infection, indicating that the actin-based cytoskeleton is critical for primary entry (Supplementary Figure S3B). Surprisingly, treatment with kinesin inhibitors, such as syntelin (Liu et al., 2020; Mullen et al., 2021), inhibited CCL18 secretion from SARS-CoV-2-infected (24 h) but not microinjected organoids, suggesting that apical infection represents the primary entry whereas basolateral infection provides a secondary phase of viral stimulation of different cytokine secretion (Supplementary Figure S3C). Future studies will be directed to delineating the host cell machineries underlying apical and basolateral infection of SARS-CoV-2.

In conclusion, this study has revealed the primary and secondary entries of SARS-CoV-2 infection in gastric organoids and their spatiotemporal profilings on cytokine secretion. We reason that SARS-CoV-2 infection resembles the enteric infection of *Helicobacter pylori*, which targets gastric PCs through the actin-based cytoskeleton network (Wang et al., 2008). Based on the results of the current study, we offer a working model accounting for a potential mechanism for primary infection of SARS-CoV-2 in the gastrointestinal tracts and the lung (Figure 1G). Thus, the enteric infection model system established here would provide a valuable platform to screen targeted therapeutics for virus variants based on infectivity and host susceptibility. Systemic assessment of the spatiotemporal profile of cytokines secreted from organoids of SARS-CoV-2-infected patients would help the rational design of targeted therapeutics for precision interrogation of disease disparity.

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Figure 1 *(Continued)* one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test was used to determine statistical significance. ***$P < 0.001$. NS, no significant difference. (**F**) Enteric infection of SARS-CoV-2 requires the apical actin-based cytoskeleton and its regulatory network. Data represent mean ± SD; $n = 3$. Ordinary one-way ANOVA followed by Tukey's *post hoc* test was used to determine statistical significance. ***$P < 0.001$. NS, no significant difference. (**G**) Working model to account for stage-distinct SARS-CoV-2 infection in gastrointestinal tracts. The primary infection is mediated by the apical membrane, while the secondary infection is facilitated at the basolateral membrane. Molecular delineation of primary and secondary infection will provide a novel niche for interrogation of virus–host interaction for disease disparity and severity.

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