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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

SARS-CoV-2 Induces a More Robust Innate Immune Response and Replicates Less Efficiently Than SARS-CoV in the Human Intestines: An Ex Vivo Study With Implications on Pathogenesis of COVID-19

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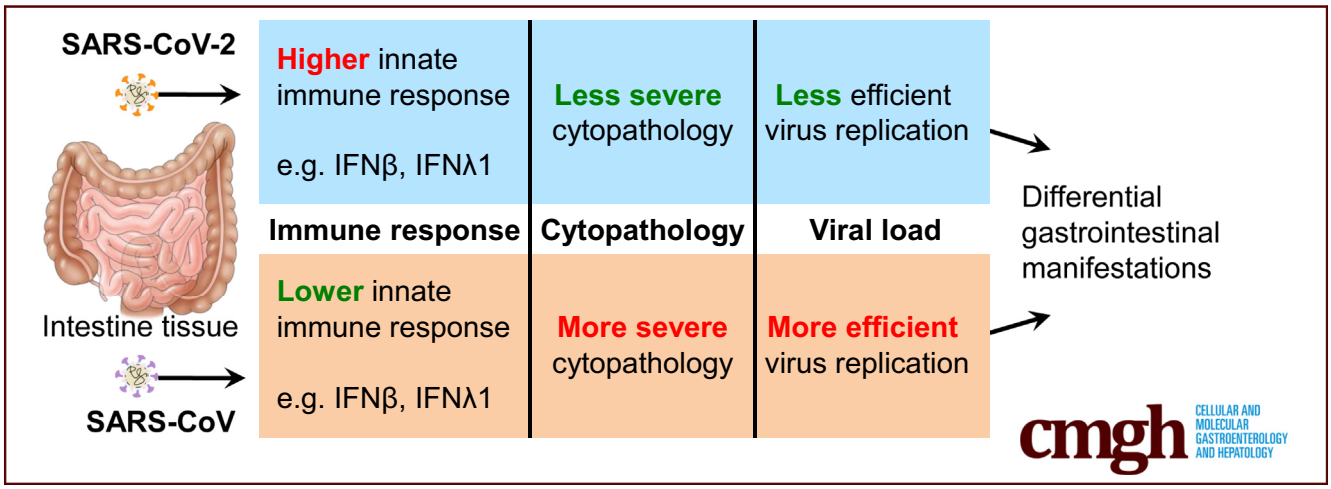

SUMMARY

SARS-CoV-2 induces a more robust innate immune response while replicates less efficiently and induces less cytopathology than SARS-CoV in human intestinal tissues. These findings potentially explained the differential gastrointestinal manifestations observed in COVID-19 and SARS.

BACKGROUND AND AIMS: Besides prominent respiratory involvement, gastrointestinal manifestations are commonly reported in Coronavirus Disease 2019 (COVID-19) patients. We compared infection of ex vivo human intestinal tissues by SARS-CoV-2 and SARS-CoV with respect to their replication kinetics and immune activation profile.

METHODS: Human intestinal tissues were obtained from patients while undergoing surgical operations at Queen Mary Hospital, Hong Kong. Upon surgical removal, the tissues were immediately processed and infected with SARS-CoV-2 or SARS-CoV. Replication kinetics were determined with immunohistochemistry, qRT-PCR, and plaque assays. Immune activation in the infected intestinal tissues was assessed by detecting the gene expression of interferons and representative pro-inflammatory cytokines and chemokines.

RESULTS: SARS-CoV-2 could infect and productively replicate in the ex vivo human intestinal tissues with release of infectious virus particles, but not in ex vivo human liver and kidney tissues. Importantly, SARS-CoV-2 replicated less efficiently than SARS-CoV, induced less cytopathology in the human intestinal epithelium, and induced a more robust innate immune response including the activation of both type I and type III interferons, than SARS-CoV in human intestinal tissues.

CONCLUSION: Using the ex vivo human intestinal tissues as a physiologically relevant model, our data indicated that SARS-CoV-2 could productively replicate in the human gut and suggested that the gastrointestinal tract might serve as an alternative route of virus dissemination. SARS-CoV-2 replicated less efficiently and induced less cytopathology than SARS-CoV in keeping with the clinical observations reported for COVID-19 and SARS, which might be the result of a more robust immune activation by SARS-CoV-2 than SARS-CoV in the human intestine. *(Cell Mol Gastroenterol Hepatol 2021;11:771–781; https://doi.org/10.1016/j.jcmgh.2020.09.017)*

Keywords: COVID-19; SARS-CoV-2; SARS-CoV; Replication; Immune Activation; Intestine.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread rapidly among the human population,[1,2] resulting in more than 20 million laboratory-confirmed cases and over 750,000 deaths within 8 months. In addition to systemic and respiratory manifestations, gastrointestinal symptoms including diarrhea, vomiting, and abdominal pain, were also commonly reported in patients with Coronavirus Disease 2019 (COVID-19).[3–9] Viral RNA was persistently detected in the stool specimens of 28.8%–70.3% of COVID-19 patients,[10] and viral protein expression or virus particles were also observed in intestinal tissues on biopsy.[11,12] These findings suggested that SARS-CoV-2 might be able to infect and replicate in the human gastrointestinal tract, which might serve as an alternative route of virus dissemination.

In addition to SARS-CoV-2, gastrointestinal involvement was also reported in SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) infection. Approximately 20%–70% of SARS[13–16] and 26% of MERS[17] patients had diarrhea during the course of illness. In comparison, we have recently estimated the pooled prevalence of diarrhea in COVID-19 to be 12.5% (95% confidence interval, 9.6%–16.0%),[10] which is considerably lower than those of SARS and MERS. In line with this observation, fecal shedding of viral RNA was detected in 28.8%–70.3% of COVID-19 patients, which was substantially lower than the 86%–100% reported in SARS patients.[10] These findings indicated a differential degree of gastrointestinal tract involvement in COVID-19 and SARS, which is intriguing because both of these lineage B betacoronaviruses use angiotensin-converting enzyme 2 (ACE2) as the entry receptor[18] and have similar tissue tropism.[19]

To physiologically simulate SARS-CoV-2 infection in the human intestinal tract, we investigated the infection of SARS-CoV-2 in ex vivo human intestinal tissue explants, which authentically represent the cell-type composition and protein expression profile of the human intestine as they were directly extracted and immediately infected upon resection from patient donors. We further performed side-by-side comparison of SARS-CoV-2 and SARS-CoV infection and investigated the replication kinetics and host innate immune activation of the ex vivo human intestinal tissue explants in response to the 2 coronaviruses. Our study revealed a number of intriguing findings. First, we demonstrated that SARS-CoV-2 could infect and productively replicate in the human intestinal tissues. Second, SARS-CoV-2 replicated less efficiently than SARS-CoV and induced less cytopathology in the human intestinal epithelium. Third, SARS-CoV-2 induced a more robust innate immune response, including the activation of both type I and type III interferons, than SARS-CoV in the human intestinal tissues. Overall, our study revealed that SARS-CoV-2 and SARS-CoV exhibited different replication efficiencies and induced differential immune activation profiles in human intestinal tissues, thus providing novel insights into our understanding on the relatively less severe and frequent gastrointestinal involvement reported in COVID-19 than in SARS.

Results

Patient Donors

A total of 14 patients with large intestine tumors who underwent surgery joined the study. There were 7 women and 7 men and their median age was 68.1 (range, 54–81) years. Additionally, 3 patients with renal tumors and 3 patients with hepatocellular carcinoma who underwent surgery donated their kidney and liver tissues, respectively.

SARS-CoV-2 Replicates Less Robustly and Induces Less Cytopathic Effects in the Human Intestinal Epithelium Than SARS-CoV. To perform side-by-side comparison of the virological and host response characteristics, we divided intestinal tissues from each individual donor into 2 parts for parallel challenge by SARS-CoV-2 and SARS-CoV. The intestinal tissues were infected with SARS-CoV-2 or SARS-CoV with an inoculum of 1×10^6 plaque-forming units (PFU)/mL. At 24 hours postinfection (hpi), the tissues were harvested for histology and immunohistochemistry. As demonstrated in Figure 1, intestinal epithelial cells (Figure 1*A–D*), including the ones on intestinal villi (Figure 1*E–H*), were readily infected by SARS-CoV-2, as indicated by the positive nucleocapsid protein (N) signal

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Abbreviations used in this paper: **ACE2, angiotensin-converting enzyme 2; COVID-19, Coronavirus Disease 2019; DMEM, Dulbecco's modified Eagle's medium; hpi, hours postinfection; IFN, interferon; MERS-CoV, Middle East respiratory syndrome coronavirus; N, nucleocapsid protein; PBS, phosphate-buffered saline; PFU, plaque-forming units; qPCR, quantitative polymerase chain reaction; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.**

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(arrows). In agreement with previous reports,[20] SARS-CoV was capable of infecting human intestinal epithelial cells (Figure 1*I–L*) (arrows). Importantly, SARS-CoV infection induced substantial cytopathic effects on the infected intestinal epithelium at 24 hpi (Figure 1*I–L*). In contrast, SARS-CoV-2-infected intestinal epithelium remained largely intact at the same time point (Figure 1*A–H*). As a control, viral N antigen or cytopathic effects were not detected in mock-infected intestinal tissues (Figure 1*M–P*). In addition to the human intestinal tissues, we also obtained human liver tissues from 3 different donors and human kidney tissues from another 3 different donors. The liver tissues and kidney tissues were similarly processed and challenged with SARS-CoV-2. Interestingly, immunohistochemistry staining did not identify SARS-CoV-2 antigen in the liver or kidney tissues from any of the evaluated donors, suggesting that SARS-CoV-2 did not efficiently infect the human liver (Figure 1*Q–S*) and kidney (Figure 1*T–V*). Collectively, our results suggested that although both SARS-CoV-2 and SARS-CoV could target the human intestinal epithelium, SARS-CoV-2 induced remarkably less cytopathic effects in the infected epithelial cells than SARS-CoV.

SARS-CoV-2 and SARS-CoV Exhibited Different Replication Kinetic Profiles in Ex Vivo Human Intestinal Tissues. Next, we compared the replication kinetics of SARS-CoV-2 and SARS-CoV in the ex vivo intestinal tissues. To this end, the intestinal tissues were infected with SARS-CoV-2 or SARS-CoV with an inoculum of 1×10^6 PFU/mL. The supernatant samples were harvested at 2 hpi, 12 hpi, and 24 hpi. SARS-CoV replicated more efficiently than SARS-CoV-2 in 5 of the 6 (83.3%) evaluated donors (Figure 2*A*). Using the area under the curve analysis, we quantified the total virus growth from baseline between 2 hpi and 24 hpi. Our results indicated that SARS-CoV-2 produced significantly less (40.9%; $P < .03$) virus genome copies than SARS-CoV over the 22-hour incubation period (Figure 2*B*). We further assessed the infectious virus titer from supernatants of the infected intestinal tissues harvested at 24 hpi with plaque assays. Our results demonstrated that the SARS-CoV-2 generated significantly less (76.2%; $P < .04$) infectious particles than that of SARS-CoV at 24 hpi (Figure 2*C*). Overall, our findings indicated that SARS-CoV-2 could infect and replicate in the human intestinal tissues. Together with the immunohistochemistry and virus replication assays, we demonstrated that SARS-CoV-2 replicated less robustly than that of SARS-CoV and induced less cytopathology than SARS-CoV in the human intestinal epithelium.

SARS-CoV-2 Induces a More Robust Interferon and Proinflammatory Response Than SARS-CoV in Ex Vivo Human Intestinal Tissues. We recently demonstrated that SARS-CoV-2 triggered an attenuated interferon response in ex vivo human lung tissues.[21] To investigate the innate immune response in the human intestinal tract in response to SARS-CoV-2 infection, we challenged ex vivo human intestinal tissues with SARS-CoV-2 with SARS-CoV included as a control, and harvested the infected samples at 2 hpi and 24 hpi for gene expression assessment. Intriguingly, SARS-CoV-2 significantly induced the expression of type I interferons (IFNs), including IFNα and IFNβ, and type III IFNs, including IFNλ1, IFNλ2, and IFNλ3, in the infected human intestinal tissues. In contrast, SARS-CoV only significantly upregulated the expression of IFNα but not that of IFNβ or type III IFNs (Figure 3). Next, we evaluated the expression of 13 representative proinflammatory cytokines and chemokines in the human intestinal tissues upon SARS-CoV-2 or SARS-CoV infection. Our results demonstrated that SARS-CoV-2 significantly upregulated the expression of 7 of the 13 (53.8%) evaluated proinflammatory mediators including IL12, IL8, MCP-1, MIP1α, CXCL2, CXCL5, and CXCL9 (Figure 4). In stark contrast, SARS-CoV only significantly triggered the expression of 1 of the 13 (7.7%) evaluated proinflammatory mediators, RANTES (Figure 4). Overall, our findings suggested that SARS-CoV-2 triggered a more robust innate immune response than SARS-CoV in the human intestinal tissues, which was directly opposite to the observation in ex vivo human lung tissues.

Discussion

In this study, we developed an ex vivo human intestinal tissue explant culture model and exploited it to systematically compare the virus replication characteristics and virus-induced host immune responses upon SARS-CoV-2 and SARS-CoV infection. Our study revealed that SARS-CoV-2 could infect and productively replicate in the ex vivo human intestine but not kidney or liver tissues. In addition, we identified important differences between SARS-CoV-2 and SARS-CoV infection in the human intestinal epithelium. First, although both SARS-CoV-2 and SARS-CoV could target the intestinal epithelium, SARS-CoV-2 induced substantially less cytopathic effects at the intestine epithelium in comparison to SARS-CoV. Second, SARS-CoV-2 replicated less efficiently than SARS-CoV in the human intestinal tissues, producing significantly less (76.2%; $P = .0396$) infectious virus particles than did SARS-CoV at 24 hpi. Third, SARS-CoV-2 induced a substantially more robust IFN and proinflammatory response than did SARS-CoV in the infected human intestinal tissues. Overall, our ex vivo human intestinal tissue culture model revealed key differences between SARS-CoV-2 and SARS-CoV infection of the human intestinal tract, which provided novel insights into the differential clinical manifestations observed in COVID-19 and SARS patients.

Gastrointestinal involvement is well documented in COVID-19.[3–9] In animal models for COVID-19, SARS-CoV-2 RNA was found in the intestine of infected cats[22] and minks.[23] Similarly, viral RNA and antigens were detected from the intestine of infected ferrets[24] and hamsters.[25] However, SARS-CoV-2 RNA was only detectable at 1 day postinfection in the intestine of infected hACE2 transgenic mice[26] and was detected in all respiratory tissues but not in the intestinal tissues of rhesus macaques.[27] In COVID-19 patients, SARS-CoV-2 RNA was frequently identified in patient fecal samples.[10] Live SARS-CoV-2 could occasionally be recovered from patient fecal samples by VeroE6 and intestinal organoids,[28,29] and SARS-CoV-2 antigens had been

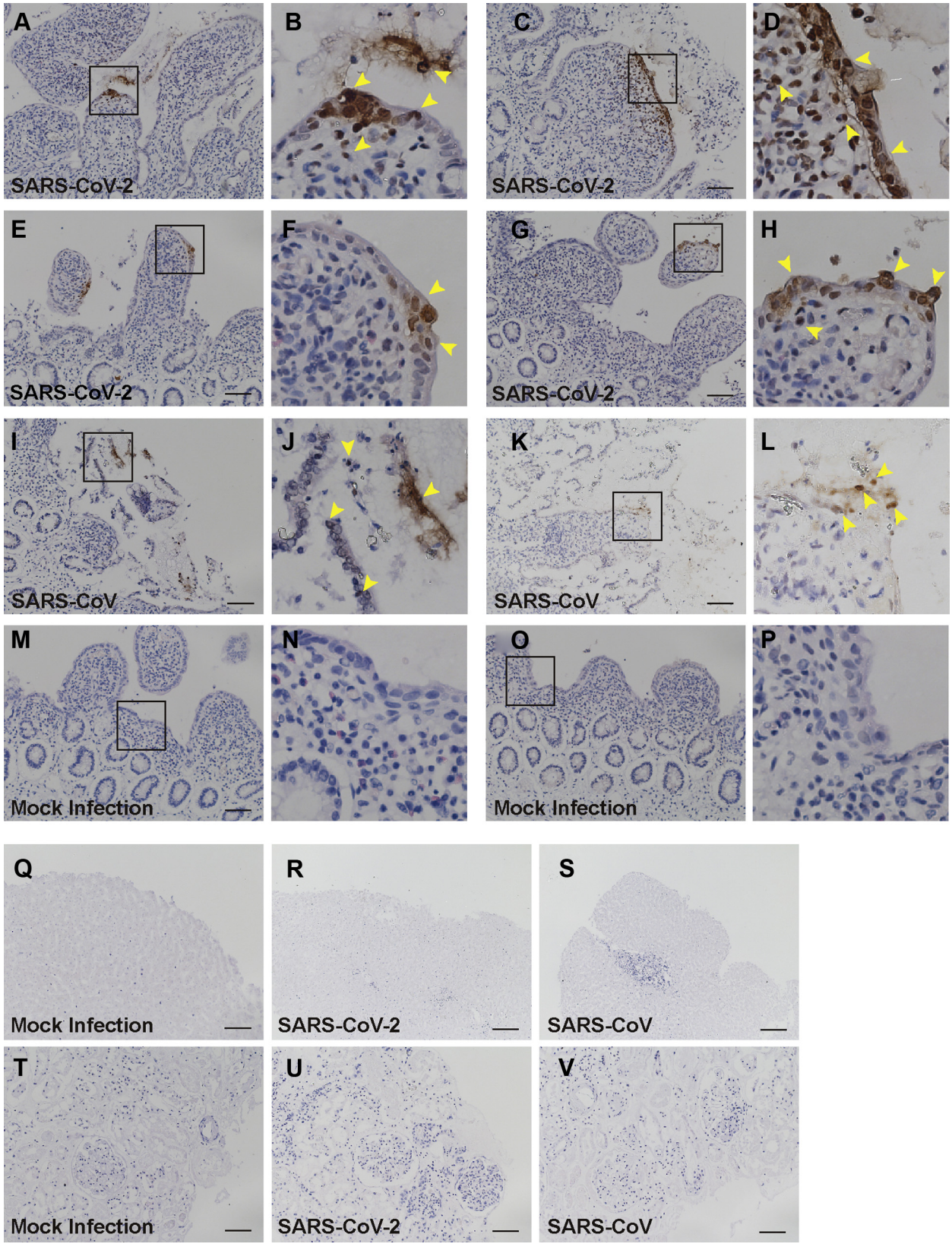

Figure 1. SARS-CoV-2 infects human intestinal epithelium. Ex vivo human intestinal, liver, and kidney tissues were challenged with (*A-H*, *R*, and *U*) SARS-CoV-2 or (*I-L*, *S*, and *V*) SARS-CoV at 1×10^6 PFU/mL, or (*M-P*, *Q*, and *T*) were mock-challenged. Tissues were harvested at 24 hpi and fixed in 4% paraformaldehyde overnight for virus inactivation. Immunohistochemistry staining was performed with an in-house mouse anti-SARS-CoV-2 serum against SARS-CoV-2-N or an in-house mouse anti-SARS-CoV serum against SARS-CoV-N. Yellow arrowheads indicate SARS-CoV-2-N and SARS-CoV-N signals. Scale bar = 50 um. Representative images from 2 independent experiments using human intestinal tissues from 2 donors are shown.

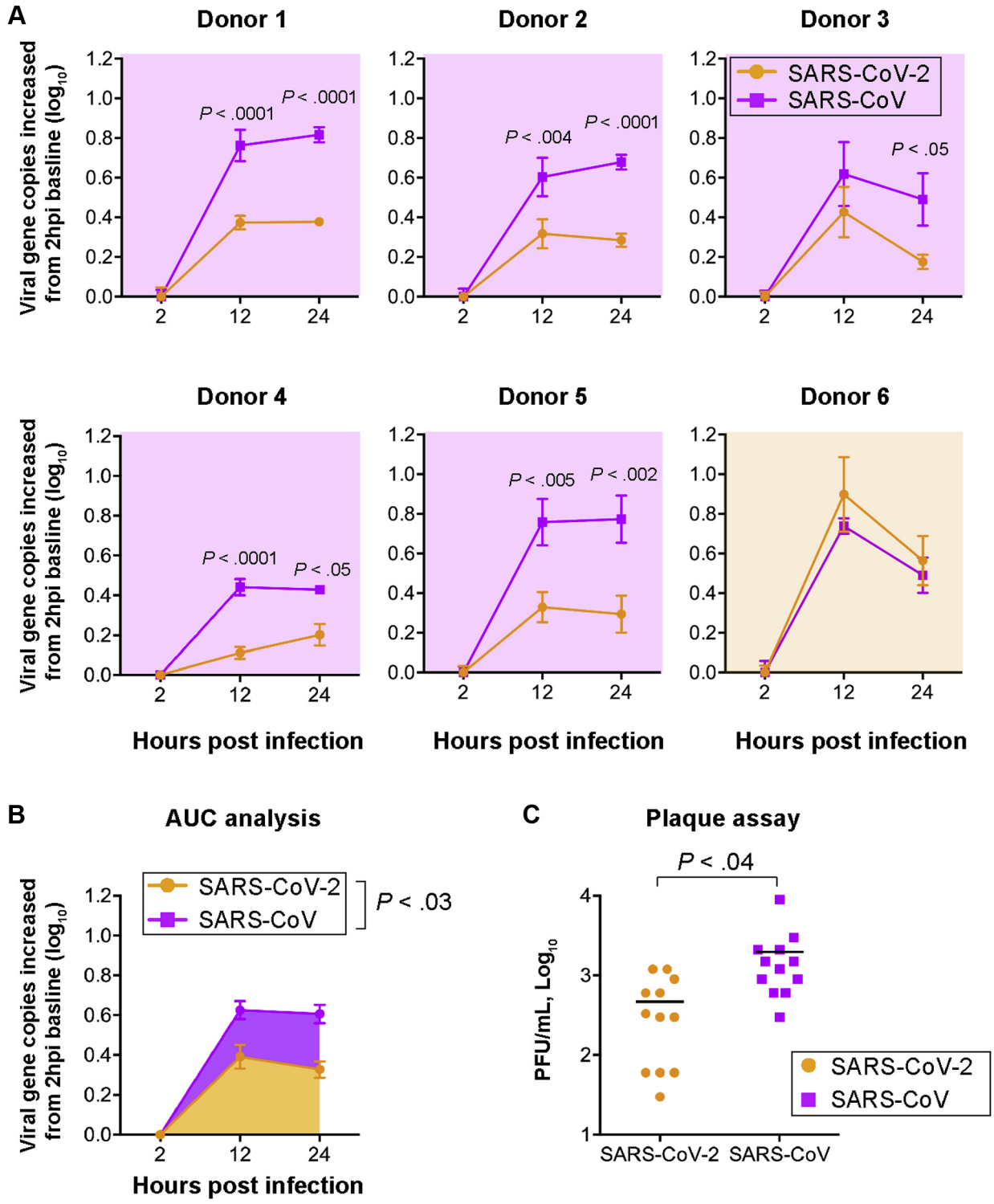

Figure 2. SARS-CoV-2 replicates less efficiently than SARS-CoV in ex vivo human intestinal tissues. Ex vivo human intestinal tissues were challenged with SARS-CoV-2 or SARS-CoV at 1×10^6 PFU/mL for 2 hours. (*A*) Viral supernatant samples were harvested at 2, 12, and 24 hpi. Virus replication was quantified by qRT-PCR. (B) Area under the curve (AUC) analysis of the human intestinal tissues. (*C*) Plaque assays were performed for the supernatant samples harvested at 24 hpi. Results represented values from 6 independent human intestinal tissue donors in 6 independent experiments. Statistical significance in panel *A* was determined by 2-way analysis of variance. Statistical significance in panels *B* and *C* was determined by Student's *t* test.

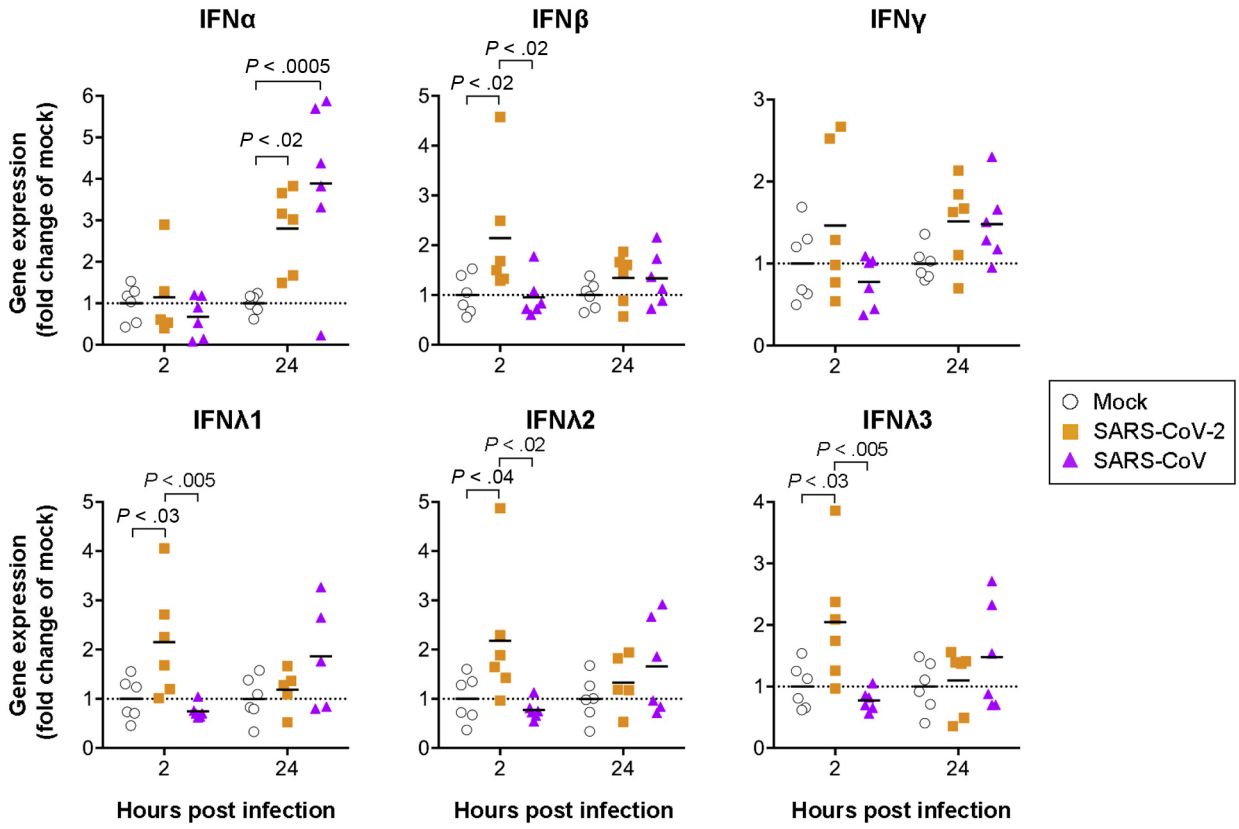

Figure 3. SARS-CoV-2 activates a significantly higher interferon response than SARS-CoV in ex vivo human intestinal tissues. Human intestinal tissues were challenged with SARS-CoV-2 or SARS-CoV. At 2 and 24 hpi, the infected intestinal tissues were harvested for qRT-PCR analysis of type I (IFNα and IFNβ), type II (IFNγ), and type III (IFNλ1, IFNλ2, and IFNλ3) interferons. Results represented values from 6 independent intestinal tissue donors in 6 independent experiments. Statistical significance was determined with 2-way analysis of variance.

identified in biopsies of COVID-19 patients.[11,12] Nevertheless, none of these findings conclusively answered the question of whether SARS-CoV-2 could productively replicate in the human intestinal tract. Here, with the ex vivo human intestinal tissue model, our results unambiguously demonstrated that SARS-CoV-2 could infect and replicate in the human intestine epithelium with the release of infectious virus particles. The ex vivo tissue models most authentically represent the cell-type composition and protein expression profile of the human organs because they are directly extracted from patient donors and are immediately processed and infected, usually within 1 hour of surgical removal. In this regard, the ex vivo tissue models are unique models that serve to complement findings obtained from cell lines, which may be defective in the expression of certain genes; from organoids, in which gene expression varies substantially based on differentiation methods and the composition of growth factors and chemicals used; and from animal models, which may be physiologically different from humans.

Gastrointestinal manifestations are reported in both COVID-19 and SARS patients.[10,30] However, the gastrointestinal symptoms are apparently milder in COVID-19 than in SARS. For example, diarrhea was reported in 2.6%–33.6% of COVID-19 patients—1 of 38 (2.6%),[5] 42 of 1099 (3.8%),[4] 3 of 62 (4.8%),[8] 14 of 138 (10.1%),[7] 12 of 116 (10.3%),[3] 3 of 18 (16.7%),[9] 13 of 59 (22.0%),[10] and 107 of 318 (33.6%)[6]—but was reported in 23.6%–73.3% of SARS patients—34 of 144 (23.6%,[13] 3 of 10 (30%),[16] 53 of 138 (38.4%),[20] 5 of 10 (50%),[15] and 55 of 75 (73.3%).[14] Here, with side-by-side comparison of SARS-CoV-2 and SARS-CoV infection in human intestinal tissues, we demonstrated that SARS-CoV-2 replicated less efficiently in the human intestinal tissues than SARS-CoV, as evidenced by the lower genome copy numbers and infectious titers. This result corroborated with our previous finding using the human colorectal epithelial cell line, Caco2.[19,31] Interestingly, the expression of known SARS-CoV-2 and SARS-CoV entry factors including ACE2 and TMPRSS2 (transmembrane protease, serine 2) were more robust in the human intestine than in the human lung.[32,33] In this regard, because SARS-CoV spike lacks the additional furin-like cleavage site at the S_1/S_2 junction compared with the SARS-CoV-2 spike,[34,35] the higher ACE2 and TMPRSS2 expression in the intestine may have a larger impact on facilitating SARS-CoV replication than on SARS-CoV-2 replication. In addition to virus replication, SARS-CoV-2 also triggered less severe cytopathology than did SARS-CoV in the infected intestine

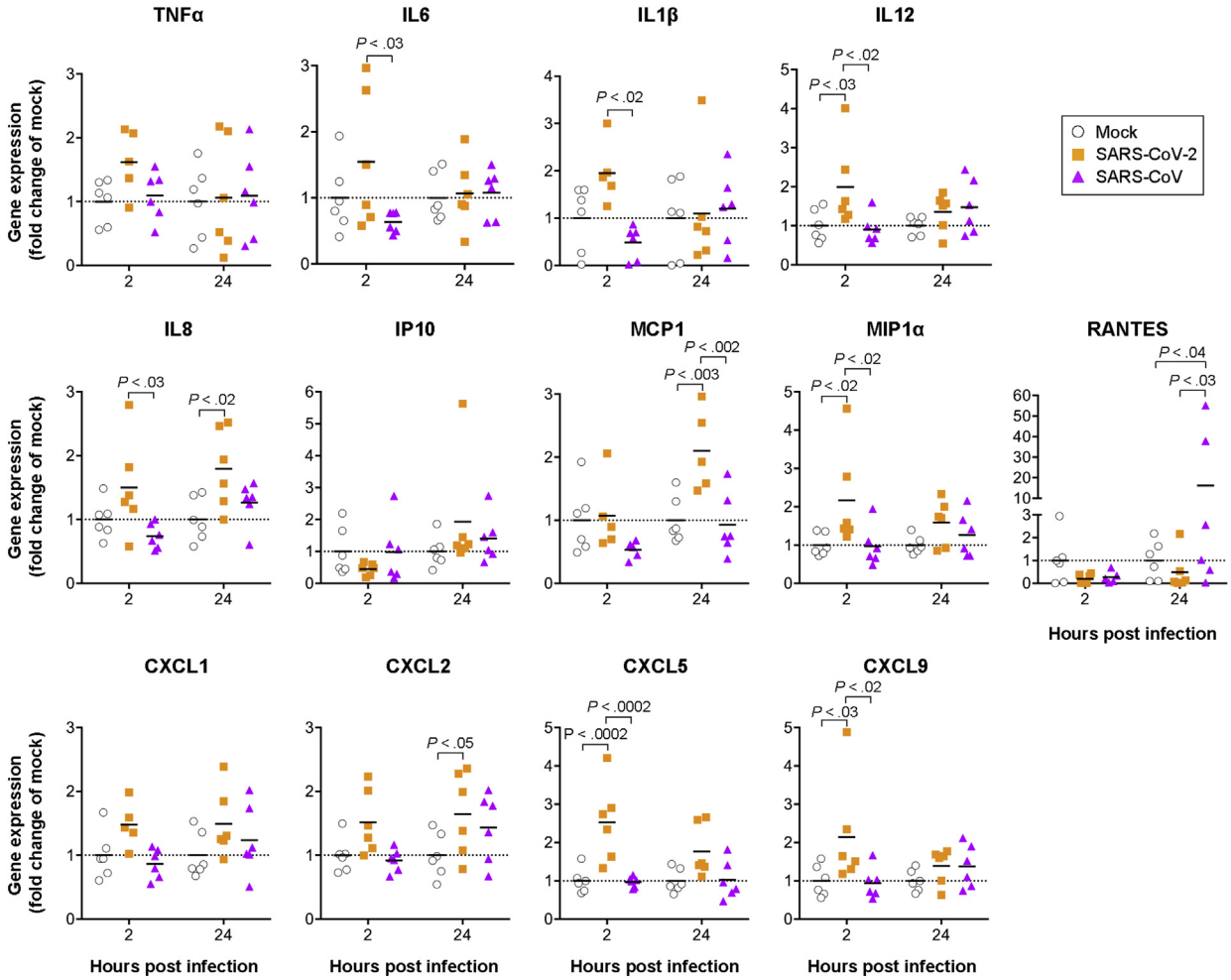

Figure 4. SARS-CoV-2 triggers higher expression of proinflammatory cytokines and chemokines than SARS-CoV in ex vivo human intestinal tissues. Human intestinal tissues were challenged with SARS-CoV-2 or SARS-CoV. At 2 and 24 hpi, the infected intestinal tissues were harvested for qRT-PCR analysis on the expression of representative proinflammatory cytokines and chemokines. The results represented values from 6 independent intestinal tissue donors in 6 independent experiments. Statistical significance was determined with 2-way analysis of variance.

epithelium. Together, the lower replication efficiency and less severe cytopathology upon SARS-CoV-2 infection in the human intestine epithelium potentially contributed to the differential gastrointestinal manifestations observed in COVID-19 and SARS.

SARS-CoV-2 launched an attenuated IFN response in human lung cell lines,[31,36] lung organoids,[37] lung tissues,[21] animal lungs,[36] and patient lungs,[36] which might result in its efficient replication in the human respiratory tract.[21,38] In this study, our results demonstrated that the IFN response was largely intact in SARS-CoV-2-infected human intestinal tissues, as evidenced by the significantly activated type I (IFNα and IFNβ)- and type III (IFNλ1, IFNλ2, and IFNλ3)-IFNs. In addition, both of the IFN and proinflammatory responses were more robustly initiated in human intestinal tissues infected by SARS-CoV-2 than by SARS-CoV. Taken together, the better immune response triggered in the human intestinal tissues upon SARS-CoV-2 infection might contribute to its less efficient virus replication and milder virus-induced cytopathology in comparison with SARS-CoV. The tissue-specific induction of IFN upon SARS-CoV-2 infection should be further investigated. Interestingly, while SARS-CoV-2 targets type I pneumocytes, type II pneumocytes, and alveolar macrophages in the human lung,[21] it predominantly infects enterocytes in the human intestine.[29,39] In this regard, the cell type–dependent expression of pathogen-recognition receptors and their role in sensing SARS-CoV-2 infection should be further explored.

Our study has a few limitations. First, the intestinal tissues could not be maintained for a long time in ex vivo settings. Thus, the infection of SARS-CoV-2 and SARS-CoV in the intestinal tissues at later time points was not evaluated. In this regard, virus replication and immune response described in this ex vivo study only represents the scenario during the early phase of infection. Second, the viral replication kinetics of SARS-CoV-2 in tissues originating from different anatomical sites along the human gastrointestinal

tract should be compared in future studies to determine the susceptibilities of the different organ tissues. Collectively, by utilizing the ex vivo human intestinal tissue model, we provided direct evidence that SARS-CoV-2 could productively replicate in the human intestine epithelium with release of infectious virus particles. We further demonstrated that SARS-CoV-2 replicated less efficiently than did SARS-CoV and induced less cytopathology than SARS-CoV in the infected intestinal tissues, which might be a result of the better immune response mounted by SARS-CoV-2 in the human intestinal tissues. Overall, our study performed the first side-by-side comparison of SARS-CoV-2 and SARS-CoV in the human intestines, which revealed novel information that contributed to our understanding on the gastrointestinal manifestations of COVID-19 and SARS.

Materials and Methods

Viruses and Biosafety

SARS-CoV-2 HKU-001a (GenBank accession number MT230904) was obtained from the nasopharyngeal aspirate of a laboratory-confirmed COVID-19 patient from Hong Kong as we described previously.[19] SARS-CoV GZ50 (GenBank accession number AY304495) was previously isolated and archived at the Department of Microbiology, The University of Hong Kong.[40] SARS-CoV-2 and SARS-CoV were expanded in VeroE6 cells. Virus titers were determined by plaque assays. All experiment protocols followed the standard operating procedures of the Biosafety Level 3 facility at the Department of Microbiology, the University of Hong Kong.

Ex Vivo Human Intestinal Tissues

Human large intestinal, liver, and kidney tissues were obtained from patients who underwent surgical operations at the Queen Mary Hospital, Hong Kong.[21] All donors gave written consent as approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. The freshly collected human intestinal tissues were prepared into small squares of approximately 1 mm^3 and submerged in advanced Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific, Waltham, MA), supplemented with 2 mM HEPES, 1× GlutaMAX, 100-U/mL penicillin, and 100-μg/mL streptomycin (Thermo Fisher Scientific). The human ex vivo intestinal tissue cubes were incubated with SARS-CoV-2 or SARS-CoV inoculum of 1×10^6 PFU/mL for 2 hours. After 2 hours, the viruses were removed and tissue cubes were washed 3 times with phosphate-buffered saline (PBS). The infected human intestinal tissues were incubated in 1.2-mL advanced DMEM/F12 medium supplemented with 2 mM HEPES, 1× GlutaMAX, 100-U/mL penicillin, 100-μg/mL streptomycin, 20-μg/mL vancomycin, 20-μg/mL ciprofloxacin, 50-μg/mL amikacin, and 50-μg/mL nystatin per well. Supernatants were harvested at 2, 12, and 24 hpi for real-time quantitative polymerase chain reaction (qPCR) analysis. Intestinal tissues were collected at 2 and 24 hpi in RL buffer (Qiagen, Hilden, Germany) with 50 mM DTT (Qiagen) for real-time qPCR analysis or were fixed overnight in 4% paraformaldehyde for immunohistochemistry staining.

Histology and Immunohistochemistry Staining

Histology and immunohistochemistry staining were performed as we previously described.[21,25,41] Briefly, fixed human intestinal tissues were dehydrated, embedded, and sectioned to prepare 4-μm tissue sections on glass slides. Sample slides were placed on a rack, and were dewaxed with xylene and successively decreased concentrations of ethanol. Slides were incubated with 0.3% hydrogen peroxide for 15 minutes to inactivate endogenous peroxidase. An in-house mouse anti-SARS-CoV-2-N serum and in-house mouse anti-SARS-CoV-N serum was applied for SARS-CoV-2 and SARS-CoV detection, respectively. Following incubating with mouse on mouse polymer (Abcam, Cambridge, United Kingdom), the signal was detected by the DAB (3,3'-diaminobenzidine) substrate kit (Vector Laboratories, Burlingame, CA). Nuclear counterstaining is performed by hematoxylin staining according to the manufacturer's instructions (Thermo Fisher Scientific). The slides were mounted with VectaMount permanent mounting medium (Vector Laboratories). Images were acquired with the Olympus BX53 light microscope (Olympus, Tokyo, Japan) using 20× and 40× objectives.

RNA Extraction and Quantitative Reverse-Transcription PCR

RNA extraction and quantitative reverse-transcription PCR (qRT-PCR) were performed as we previously described.[42,43] Infected intestinal tissues were collected and homogenized in RLT buffer (Qiagen) and extracted with the RNeasy Mini kit (Qiagen). To quantify the expression level of interferon and cytokine/chemokine markers, qRT-PCR was performed using the QuantiNova SYBR Green RT-PCR kit (Qiagen) with LightCycler 480 Real-Time PCR System (Roche, Basel, Switzerland). Each 20-μL reaction consists of 0.2μl of QuantiNova SYBR Green RT-Mix, 1.6-μL each of 10 μM gene-specific forward and reverse primer, 4 μL of extracted RNA as template, 10-μL of 2× QuantiNova SYBR Green RT-PCR Master Mix, and 2.6 μL of RNase-free water. Reactions were incubated at 45°C for 10 minutes for reverse transcription and at 95°C for 5 minutes for denaturation, followed by 45 cycles of 95°C for 5 seconds and 55°C for 30 seconds. The final cooling step of cycling profile is set at 40°C for 30 seconds. The primer sequences are available upon requests. SARS-CoV-2 and SARS-CoV gene copy was quantified using the QuantiNova Probe RT-PCR Kit (Qiagen) with the LightCycler 480 Real-Time PCR System (Roche). The primer and probe sequences were previously described.[19]

Plaque Assays

Plaque assays were performed as we previously described with slight modifications.[21] In brief, VeroE6 cells were seeded in 12-well plates 1 day before the experiment. The harvested supernatant samples were serially diluted and inoculated to the cells for 1 hour at 37°C. After

inoculation, the cells were washed with PBS 3 times, and covered with 3% agarose/PBS mixed with DMEM/1.5% fetal bovine serum at 1:2 ratio. The cells were fixed in 4% paraformaldehyde after incubating at 37°C for 96 hours. Fixed samples were stained with 1% crystal violet in 20% ethanol/distilled water for 15 minutes for plaque visualization.

Statistical Analysis

GraphPad Prism 6 (GraphPad Software, San Diego, CA) was used for statistical analyses. Area under the curve analysis for SARS-CoV-2 and SARS-CoV-replication in ex vivo human intestinal tissues was compared with Student's *t* test. The expression of interferon and cytokine or chemokine markers from SARS-CoV-2- or SARS-CoV-infected human intestinal tissues was compared with 2-way analysis of variance. The difference was considered statistically significant when $P < .05$.

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