SARS-CoV-2 pathogenesis in the gastrointestinal tract mediated by Spike-induced intestinal inflammation

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Dear Editor,

The pathogenesis of COVID-19 in the gastrointestinal (GI) tract is well-documented [1]. However, due to a lack of simple animal models, the pathogenic mechanisms of SARS-CoV-2 in the GI tract is not well established. Currently, common animal models for COVID-19 include mice, rats, hamsters, ferrets and non-human primates. Among them, SARS-CoV-2 infection in ferrets, cynomolgus monkeys, rhesus macaques and Cynomolgus macaques exhibits clinical symptoms and pathological changes similar to those in humans [2, 3]. However, development of animal models using ferrets and non-human primates is associated with complex procedures, high maintenance costs and strict environmental requirements, making them less feasible for widespread use. The primary objective of our study was to construct a mouse model replicating intestinal inflammation post-SARS-CoV-2 infection.

We present clinical data of RNA sequencing in colonocytes and undifferentiated colonic epithelial cells and found that angiotensin converting enzyme 2 (ACE2) receptor, the entry receptor for SARS-CoV-2 Spike protein, was highly expressed. Single-cell RNA sequencing data was obtained from a previous study which obtained biopsies of 17 subjects with colitis or inflammatory bowel disease [4]. We found that ACE2 receptor was specifically and highly expressed in undifferentiated colonic stem cells, absorptive progenitor cells, secretory progenitor cells and in colonocytes that are responsible for nutrient absorption and metabolism as well as extracellular redox homeostasis (Fig. 1A). Interestingly, ACE2 receptors were not highly expressed in all colonic epithelial subsets. The ACE2 receptor is required for maintaining amino acid homeostasis, antimicrobial peptide expression and the ecology of gut microbiome in the intestine [5] in addition to its known role as an entry receptor for SARS-CoV-2 [1].

Given that ACE2 receptor expression in colonic epithelial cells aids SARS-CoV-2 pathogenesis in the GI tract, we created an animal model to investigate the effects of the Spike-Fc protein (referred to as Spike protein) from SARS-CoV-2 on intestinal inflammation. We performed hematoxylin and eosin staining to evaluate (1) the effects of different concentrations of acetic acid enema to induce inflammation in the intestines prior to Spike protein administration, (2) the route of administration for Spike protein and (3) the correlation between the duration of Spike protein exposure and degree of inflammation in mice intestines. A previous model for SARS-CoV-2 lung infection utilized acetic acid-induced tissue injury to facilitate binding of the Spike protein to lung tissues [6]. Our previous study found that the intestinal mucosal barrier protected epithelial cells from Spike protein-induced inflammation [7]. Hence, we utilized the same model of acid-induced tissue injury to induce intestinal-specific inflammation and optimized parameters that resulted in histological changes most similar to SARS-CoV-2 pathology in the GI tract. All mice used were 7–8-week-old C57BL/6J mice (referred to as B6J mice). Histological comparisons of the duodenum, jejunoileum (J&I), colon and rectum in mice treated with phosphate buffered saline (PBS), 2% acetic acid and 5% acetic acid showed that mice without acetic acid treatment, regardless of whether they were the PBS + Spike group or the PBS + IgG protein injection group, did not exhibit significant inflammatory reactions in the intestinal tissues (Fig. 1D). Inflammatory histology was only found after Spike protein treatment as there wase no apparent inflammation observed in the mucosa of the intestines of IgG-treated mice. Mild to moderate inflammation was observed in the duodenal mucosa and the J&I of 5/6 mice with loose stool formation in mice treated with 2% acetic acid and Spike protein. No histological changes were observed in the colon and rectum (Fig. 1D). We found that 5% acetic acid enema treatment resulted in significant tissue damage with mucosal necrosis and dissolution with or without Spike protein-induced inflammation, suggesting that this is not a suitable model (Fig. 1D). Inflammatory pathology was only restricted to the GI tract as there was no significant inflammatory response in lung, testis and kidney tissues (Fig. 1E).

The route of Spike protein administration is crucial to the intestinal inflammation phenotype. Spike protein administration via oral gavage in B6J mice resulted in a higher degree of inflammation in the stomach and duodenum with no inflammatory phenotype in the colon and rectum. Intraperitoneal injection of Spike protein resulted in a relatively uniform exposure of intestinal tissue to Spike protein, with inflammation in the duodenum, stomach and the J&I and no inflammation in the colon (Fig. 1G). We also found that Spike protein induced intestinal inflammation rapidly after administration. Examination of intestinal histology
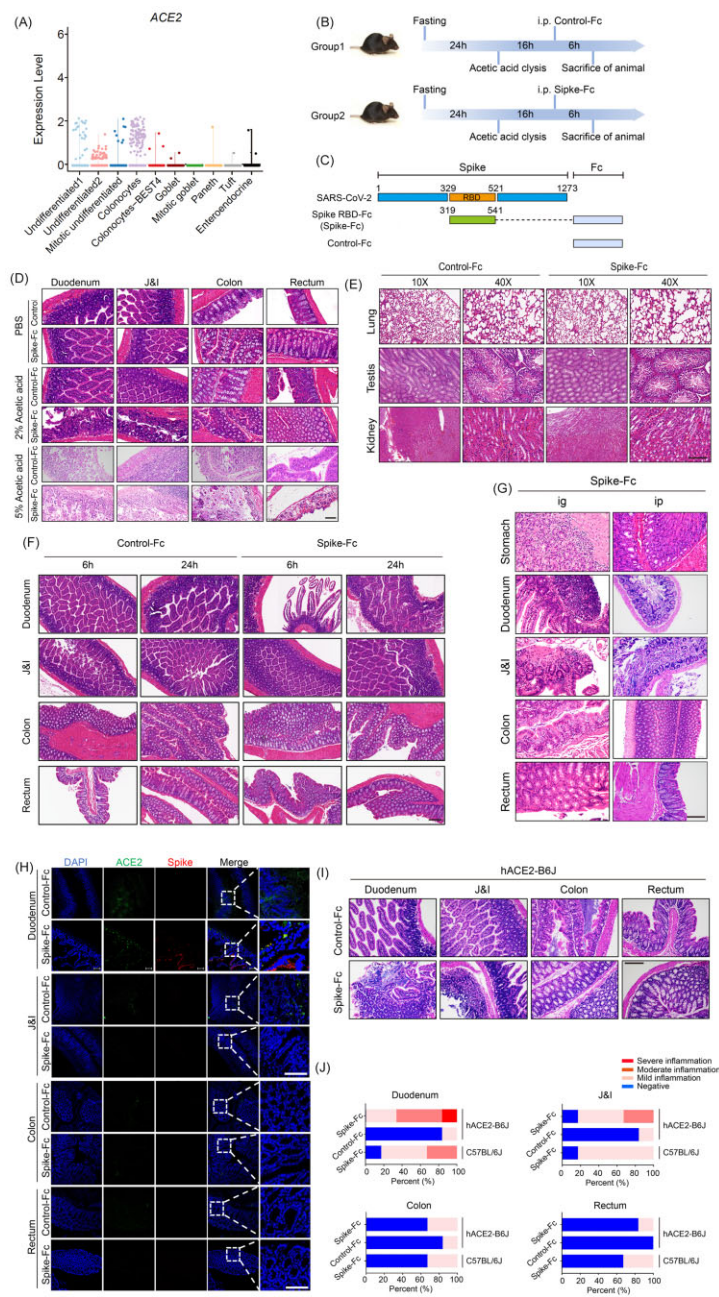
Figure 1. (**A**) Violin plots showing the normalized expression of ACE2 in the colonic epithelial subsets. Colonic epithelial cells were separated into 10 subsets according to the expression of canonical markers. Undifferentiated 1 = undifferentiated colonic stem cells, undifferentiated 2 = absorptive progenitor cells, mitotic undifferentiated = secretory progenitor cells. ACE2 was mainly expressed by colonocytes and undifferentiated stem and progenitor cells in the colon. BEST4^{+} colonocytes goblet cells, secretory goblet cells, Paneth cells and tuft cells did not highly express ACE2 receptors. The y-axis represents log-scaled normalized unique molecular identifiers (UMI) counts ranging from 0 to 6. (**B**) Schematic diagram illustrating the construction of the Spike protein-induced mouse intestinal inflammation model. (**C**) The receptor binding domain (RBD, Arg319-Phe541) and the Fc region at the C-terminus make up the recombinant SARS-CoV-2 Spike. (**D**) Eighteen B6J mice were divided into three groups. Enema of PBS, 2% acetic acid or 5% acetic (*n* = 6 each) were delivered via a catheter. Mice from each group received either 5.5 nmol/kg of Spike protein or 5.5 nmol/kg of IgG protein (*n* = 3 per treatment). (**E**) Inflammatory response in the lungs, testes and kidneys of mice in the 2% acetic acid + Spike group and the 2% acetic acid + IgG group. (**F**) Degree of intestinal inflammation in B6J mice after 2% acetic acid enema and 6 or 24 h of Spike protein exposure (*n* = 6 per group). (**G**) Spike protein-induced intestinal inflammation in B6J mice by oral gavage or intraperitoneal injection (*n* = 6 per group). Two pathologists independently evaluated the differences in inflammatory changes based on the consensus opinion on chronic gastritis in China [8] and the visual analog scale of the Sydney system [9]. (**H**) Expression and localization of ACE2 protein and Spike protein in the duodenum, jejunoileum (J&I), colon and rectum of mice treated with 2% acetic acid + Spike protein. (**I**) Intestinal inflammatory response in hACE2-B6J mice. (**J**) Quantitative analysis of the degree of intestinal inflammation in Spike protein-induced hACE2-B6J mice and B6J mice (*n* = 6 per treatment group). Scale bar: 100 μm.

of 2% acetic acid enema + Spike protein treatment showed that intestinal samples harvested 16 h after enema and 6 h after Spike protein treatment resulted in the same tissue morphology compared to 16 h after enema and 24 h after Spike protein treatment (Fig. 1F). To investigate whether the intestinal inflammation observed in our animal model is specifically induced by Spike protein, we performed immunofluorescence staining of Spike protein and ACE2 receptors on the intestinal tissues of mice treated with 2% acetic acid enema and Spike protein intraperitoneal (i.p.) injection. Figure 1H shows ACE2 and Spike protein colocalization in the glandular epithelium of the duodenum, but not in colon and rectum. The expression of both proteins was higher in the duodenum and lower in the J&I, similar to the observations in COVID-19 patients. These findings suggest that Spike protein infects intestinal tissue via the ACE2 receptor to cause inflammation in the duodenum.

Previous studies have suggested that Spike protein does not directly bind or has a weak binding ability to mouse ACE2 (11 out of 29 of the critical amino acids in the binding region are different from human ACE2) [10]. This raised concerns about whether the regular B6J mouse model adequately represents the intestinal changes observed in COVID-19 patients. Therefore, we compared the intestinal inflammatory response in our model using mice expressing the human ACE2 receptors (hACE2-B6J mice) and wild-type mice [7]. Comparison between hACE2-B6J mice and the regular B6J mice treated with 2% acetic acid enema + Spike protein i.p. injection showed similar degrees of intestinal inflammation in both mouse models, with mild to moderate inflammation observed in the duodenum and weaker inflammation in the J&I, colon and rectum (Fig. 1I). However, mice expressing hACE2-B6J had a higher proportion of inflammation in the duodenum and J&I (100% vs 83.7%, 83.7% vs 83.7%), with 16.7% severe inflammation and 33.3% moderate inflammation in hACE2-B6J mice duodenum and J&I, respectively (Fig. 1J). These results suggest that Spike protein can induce similar intestinal inflammation in both mouse models. The humanized hACE2-B6J mice have a higher success rate in developing intestinal inflammation, however, the B6J mice are also an effective model for studying Spike protein-induced intestinal inflammation.

Our results indicate that our model using wild-type B6J or hACE2-B6J mice accurately simulates intestinal inflammation induced by Spike protein and mimics the pathogenesis of SARS-CoV-2 intestinal infection. This model will serve as a valuable research tool for assessing potential therapeutic interventions such as drug screening to develop inhibitors and antagonists for downstream signaling pathway alterations brought on by the binding of Spike protein to ACE2 receptor. The model allows for the investigation of the direct effects of the Spike protein on the intestines, without involving secondary effects from viral infection in other organs. It is a feasible, simple and reproducible model that can be used to study the pathogenic mechanisms and potential treatments for Spike protein-induced intestinal inflammation caused by SARS-CoV-2.

Acknowledgements

Ethical statement

The study was approved by the Medical Ethics Committee of Guangzhou Women and Children's Medical Center (ID: 2 017 021 504) and clinical trial (ID: 2 017 052 401). The implementations were in concordance with the International Ethical Guidelines for Research Involving Human Subjects as stated in the Helsinki Declaration. Informed written consent was obtained from the legal guardians of all participants.

Conflict of interest

Authors have no conflict of interest to declare.

Author contributions

FZ: Conceptualization, funding acquisition, methodology, project administration, resources, validation, writing – review & editing; OM: Funding acquisition, visualization, writing original draft, writing – review & editing; YL: Funding acquisition, supervision, methodology, writing – review & editing; ZL, JH, YY, LT: Data curation, formal analysis, investigation, writing – review & editing.

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