Adding Fuel to the Fire? A Role of Intraepithelial Lymphocytes in Enteric Immune Responses to SARS-CoV-2 Infection

Lehmann M, Allers K, Heldt C, et al. Human small intestinal infection by SARS-COV-2 is characterized by a mucosal infiltration with activated CD8^{+} T cells. Mucosal Immunol 2021;14:1381–1392.

Twenty to fifty percent of patients with coronavirus disease 2019 (COVID-19) with acute severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection report gastrointestinal (GI) symptoms, including abdominal pain, nausea, vomiting, anorexia, and diarrhea (J Med Virol 2021;93:2740–2768). Patients who experience "long COVID" symptoms often report ongoing GI symptoms as well as disorders, including persistent malnutrition weight loss (Clin Gastroenterol Hepatol 2021;19:2438–2440.e1). A deeper investigation into the impact and mechanisms of SARS-CoV-2 infection in the GI tract and how the virus interacts with the intestinal immune system in the context of acute and long-term GI diseases is needed.

Lehmann et al added new insights into the small body of human mechanistic investigations by studying the immunohistologic changes in the small intestines after SARS-CoV-2 infection (Mucosal Immunol 2021;14:1381–1392). To do this, the authors used methods of deep immune profiling on endoscopically obtained duodenal biopsies from 5 patients, namely imaging mass cytometry (IMC) and multiplex immunohistochemistry (IHC). They found that among these 5 patients' tissues, 2 were positive for both SARS-CoV-2 RNA and viral nucleocapsid staining. Four out of 5 biopsies showed blunted villi and increased numbers of intraepithelial lymphocytes (IELs). A single-cell level IMC analysis of COVID-19 and control GI samples revealed 41 cell clusters based on a panel of 25 antibodies. Specifically, COVID-19 patients had increased intraepithelial CD8^{+} T cells that express markers phenotypic for activated antigen-experienced effector cells (positive for CD45, CD3, CD45RO, and CD7; negative for CD27). These results were confirmed by multiplexed IHC, which offers a higher spatial resolution than IMC. Additionally, multiplex IHC revealed high levels of cleaved caspase-3 staining in the intestinal epithelial cells (IECs) of COVID-19 samples, suggesting either infection-induced or T-cell–mediated IEC apoptosis. The authors also found increases in the number of Ki67^{+} IECs, indicative of epithelial regeneration after injury, a finding that was consistent with a prior study that reported increased caspase-3 and Ki67 staining in SARS-CoV-2 infected enteroids (Science 2020;369:50–54). Overall, the authors concluded that SARS-CoV-2 infection of the gut epithelium leads to an accumulation of activated CD8^{+} IELs, increased IEC apoptosis, and a compensatory increase in regenerative epithelial proliferation.

Comment. To date, there remains a relative paucity of literature addressing the pathogenesis of SARS-CoV-2 GI infection and whether intestinal infection or the systemic immunological response precipitates the GI symptoms and pathology. Previous studies have shown that SARS-COV-2 can infect and replicate in human colonic cell lines, human small intestinal enteroids and colonoids, as well as ex vivo human intestinal explants (Emerging Microbes Infect 2020;9:2169–2179; Sci Immunol 2020;5:eabc3582; Cell Mol Gastroenterol Hepatol 2021;11:771–781). Two recent studies analyzed biopsies from endoscopically normal mucosa of patients with COVID-19 (Nature 2021;591:639–644; Gastroenterology 2021;160:2435–2450). Both studies found evidence of SARS-CoV-2 persistence in the GI tract long past shedding in the upper respiratory tract and neither study found overt acute intestinal inflammation. Gaebler et al found that in a small cohort (14 individuals), SARS-CoV-2 RNA was detectable in duodenal and ileal epithelial cells 3 to 5 months after a COVID-19 diagnosis. At this point in the disease, staining was patchy and no inflammatory infiltrate was noted. The persistence of intestinal viral antigens is consistent with the retained memory B-cell response and IgA levels, despite the time-dependent attenuation of IgG and IgM levels. Livanos et al also examined small bowel and colon biopsies and performed deep immune profiling by mass cytometry and RNA sequencing. COVID-19 samples taken from 10 to 106 days after diagnosis demonstrated an attenuation of inflammatory genes, including IFN-γ, CXCL8, CXCL2, and IL-1β, as well as a decrease in proinflammatory dendritic cells as compared with controls. This study further found that presentation with GI symptoms correlated with decreased COVID-19 severity and mortality across 2 populations. Because a decrease in inflammatory cytokines was also observed in the serum of patients with GI symptoms, the authors concluded that intestinal involvement may attenuate overall SARS-CoV-2 pathogenicity.

In another study, Chu et al infected surgically obtained human intestinal tissue with SARS-CoV-2 in an explant culture model and also identified an acute yet distinct inflammatory phenotype (Cell Mol Gastroenterol Hepatol 2021;11:771–781). They found that SARS-CoV-2 induced the expression of type I and III interferons, as well as proinflammatory mediators including IL-12, IL-8, CCL2, CCL3, CXCL2, CXCL5, and CXCL9, when examining tissues at very early time points (2 and 24 hours after infection). Although CXCL2 represents a shared cellular target in the Livanos and the Chu studies, it was down-regulated in the former and induced in the latter. Because CXCL2 is a known chemoattractant for neutrophils (Proc Natl Acad Sci U S A 1989;86:612–616), the role of neutrophil infiltration and intestinal injury during the course of SARS-CoV-2 infection is worth investigation in future studies. Unfortunately, intestinal neutrophils seemed to be overlooked in the Lehmann study, despite the application of the high dimensional mass cytometry imaging and higher resolution multiplex IHC immunophenotyping.

The study by Lehmann et al has other limitations, including the small cohort size in which evidence of SARS-CoV-2 intestinal infection was found in only 2 of the 5 patients. Moreover, sampling was restricted to the duodenum

and to only a single time point, which was early in the course of infection (8 days after symptom onset). Despite the small number of samples, the investigators missed the opportunity to correlate the patient histories with their actual samples, which may be relevant since natural and induced IELs vary with age (Front Immunol 2019;10). It would also be interesting to reanalyze the mass cytometry data for viral antigens in different cell types and assay the fecal samples for microbiota and viral shedding (quantities and sequence) information.

Despite these potential caveats, the current study provides an important framework for understanding the mechanisms and impact of GI COVID-19. Unraveling a more complete picture of GI COVID-19 will benefit from more tractable small animal models (Inflamm Bowel Dis 2022;28:318–321) that can integrate GI SARS-CoV-2 infection with controlled variables such as microbiota, sampling time, primary location of infection, pharmacologic intervention, and more. The importance of these future investigations is highlighted by the emerging data on the clinical impact of long COVID-19 and GI symptoms. A pathologically valid animal model will also help to address the relative contribution of intestinal SARS-CoV-2 infection and the induced immunopathology in COVID-19 GI diseases and sequelae.

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Positioning Ozanimod in Ulcerative Colitis: Restoring Leukocyte Traffic Under Control

Sandborn WJ, Feagan BG, D'Haens G. Ozanimod as induction and maintenance therapy for ulcerative colitis. N Engl J Med 2021;385:1280–1291.

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (Lancet 2017;389:1756–1770). Because UC is a chronic and disabling disease, the main goals of medical therapies are to maintain symptom-free clinical remission, to achieve and maintain mucosal healing, and to block the progression of the disease (Lancet 2017;389:1756–1770). In the recent years, UC management has significantly changed owing to the development of biological drugs such as anti-tumor necrosis factor (TNF), anti-integrins, inhibitors of IL-12/23, and small molecules such as anti-JAK (J Crohn's Colitis 2021:jjab178). However, a significant percentage of patients do not respond to therapy and require surgery. For this reason, there is a continuous effort to identify new therapeutic targets and to develop new molecules to offer further options to patients and guarantee them a better quality of life.

Ozanimod is a new oral drug included in the small molecule class (N Engl J Med 2021;385:1280–1291). It is a sphingosine-1-phosphate (S1P) receptor modulator (N Engl J Med 2021; 385:1280–1291). S1P is a pleiotropic lipid mediator that binds to 5 G protein-coupled receptors termed as SP1R 1-5 (Nat Immunol 2007;8:1295–1301). It is involved in the trafficking of immune cells by regulating the outflow of lymphocytes from the lymph nodes and their entry into the circulation (J Crohns Colitis 2018;12:S678–S686). Ozanimod selectively binds to S1P1 and S1P5 receptors, limiting the capacity of lymphocytes to egress from peripheral lymphoid organs and leading to a reduction of peripheral lymphocytes (N Engl J Med 2021;385:1280–12919). This mechanism of action is effective in treating multiple sclerosis and has been shown to be successful in UC as well.

Sandborn et al recently conducted a phase III randomized, double-blind, placebo-controlled clinical trial investigating the efficacy and safety of ozanimod in patients with moderate-to-severe UC (N Engl J Med 2021;385:1280–1291). The study consisted of a 10-week induction phase and a subsequent 42-week maintenance phase. In the first part of the study, a cohort of patients was randomized 2:1 to receive ozanimod 1 mg/d or placebo, and a second cohort of patients received open-label ozanimod 1 mg/d. Patients treated with ozanimod who achieved clinical response at 10 weeks (defined as a reduction of ≥3 points in total Mayo score, a decrease of ≥30% in Mayo score from baseline, or a decrease in bleeding subscore of ≥1 point), were rerandomized 1:1 to receive ozanimod 1 mg/d or placebo through week 52. The primary endpoint was the rate of clinical remission at week 10 and at week 52 (defined as rectal-bleeding subscore of 0, stool frequency subscore of ≤1, and endoscopy subscore of ≤1). Overall, 1012 patients were enrolled in the induction phase (645 in cohort 1 and 367 in cohort 2). After 10 weeks, ozanimod-treated patients had a significantly higher clinical remission rate compared with those treated with placebo (18.6% vs 6.0%; $P < .001$). Similarly, among the 457 patients who participated in the maintenance study, a higher rate of clinical remission at 52 weeks was detected in patients treated with ozanimod compared with placebo (37.0% vs 18.5%; $P < .001$). Several secondary end points were evaluated including clinical response, endoscopic improvement (endoscopic Mayo score of ≤1), mucosal healing (endoscopic Mayo score of ≤1 and