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Journal Pre-proof

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

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Summary Title:

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

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Summarized Article

HUMAN SMALL INTESTINAL INFECTION BY SARS-COV-2 IS CHARACTERIZED BY A MUCOSAL INFILTRATION WITH ACTIVATED $CD8^+$ T CELLS.

Lehmann M, Allers K, Heldt C, Meinhardt J, Schmidt F, Rodriguez-Sillke Y, Kunkel D, Schumann M, Böttcher C, Stahl-Hennig C, Elezkurtaj S, Bojarski C, Radbruch H, Corman VM, Schneider T, Loddenkemper C, Moos V, Weidinger C, Kühl AA, **Siegmund B.** Mucosal Immunol. 2021 Nov;14(6):1381-1392. doi: 10.1038/s41385-021-00437-z. Epub 2021 Aug 21.PMID: 34420043

Summary (340 words)

Twenty to fifty percent of COVID-19 patients with acute SARS-CoV-2 infection report GI symptoms, including abdominal pain, nausea, vomiting, anorexia, and diarrhea (Journal of Medical Virology 2021;93:2740-2768). Patients who experience “long COVID” symptoms often report ongoing GI symptoms as well as disorders including persistent malnutrition weight loss (Clinical Gastroenterology and Hepatology 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 and colleagues added new insights into the small body of human mechanistic investigations by studying the immunohistologic changes in the small intestines following SARS-CoV-2 infection (Mucosal Immunology 2021). To do this, the authors employed methods of deep immune profiling on endoscopically obtained duodenal biopsies from five patients, namely imaging mass cytometry (IMC) and multiplex immunohistochemistry (IHC). They found that among these five patients' tissues, two were positive for both SARS-CoV-2 RNA and viral nucleocapsid staining. Four out of five 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.

Commentary (677 words)

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 & Infections 2020;9:2169-2179) (Science Immunology 2020;5:eabc3582) (Cellular and Molecular Gastroenterology and Hepatology 2021;11:771-781). Two recent studies analyzed biopsies from endoscopically normal mucosa of COVID-19 patients (Gaebler et al., 2021) (Livanos et al., 2021). 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 three to five months after 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 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 reduction in proinflammatory dendritic cells as compared to controls. This study further found that presentation with GI symptoms correlated with reduced COVID-19 severity and mortality across two populations. As a reduction 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 (Cellular and Molecular Gastroenterology and Hepatology 2021;11:771-781). They found that SARS-CoV-2 induced

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 post infection). Although CXCL2 represents a shared cellular target in the Livanos and the Chu studies, it was downregulated in the former and induced in the latter. Since CXCL2 is a known chemoattractant for neutrophils (Proceedings of the National Academy of Sciences 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 appeared to be overlooked in Lehmann study, despite the application of the high dimensional mass cytometry imaging and higher resolution multiplex IHC immunophenotyping.

The study by Lehmann has other limitations including the small cohort size in which evidence of SARS-CoV-2 intestinal infection was evident in only two of the five 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 (Frontiers in Immunology 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 (bioRxiv 2021;2021.07.23.453393) 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 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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