

Intestinal Dysbiosis and Probiotics in COVID-19

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ABSTRACT

The COVID-19 pandemic has been an emerging threat to global public health. Several lines of evidence suggest that the causative virus SARS-CoV-2 infects the human intestinal epithelial cells as well as airway epithelial cells, suggesting that the enteric infection of SARS-CoV-2 has destructive effects on the intestinal microbiota and subsequently airway physiology and immunity through the gut-lung axis. Despite the important roles of the gut-lung axis in the antiviral immunity, only limited information is currently available concerning COVID-19-specific changes in the gut microbiome. This review summarizes recent knowledge of intestinal dysbiosis associated with COVID-19 patients and its potential contribution to the respiratory symptoms through the gut-lung axis. We also discuss the possibility of prophylactic and therapeutic use of probiotics in COVID-19, including our ongoing trial using *Lactobacillus plantarum*, which is known to have a wide variety of immunomodulatory activity against respiratory viral infections.

Keywords: COVID-19; Dysbiosis; Gut-lung axis; Microbiome; Microbiota; Probiotics

INTRODUCTION

The recent pandemic of COVID-19 has been an emerging threat to global public health. COVID-19 patients present with a spectrum of disease severity, ranging from asymptomatic or mild flu-like to critically ill cases with severe pneumonia potentially accompanied by acute respiratory distress syndrome (ARDS) and multiple organ failure [1,2]. The causative coronavirus, SARS-CoV-2, primarily infects a subpopulation of airway epithelial cells co-expressing angiotensin-converting enzyme 2 (ACE2), a cell surface viral receptor, and transmembrane serine protease 2 (TMPRSS2), a transmembrane protease that facilitates viral entry into host cells [3-5].

Notably, several lines of evidence suggest that the human intestinal tract is an additional target of SARS-CoV-2 infection. Many clinical studies have revealed that a considerable percentage of COVID-19 patients suffer from concurrent gastrointestinal manifestations, most commonly diarrhea and abdominal pain [6-8]. SARS-CoV-2 viral RNA has been detected in stool specimens and anal/rectal swabs of patients even after the clearance of the virus in the nasopharynx [9-11], and furthermore, infectious viruses have been isolated from feces of a

COVID-19 patient with diarrhea [12]. ACE2 and TMPRSS2 are co-expressed in the lower gastrointestinal tract, particularly enterocytes and progenitor cells of the ileum and colon [4,5,13-15]. In fact, SARS-CoV-2 can infect human intestinal organoids and replicate actively [12,15-17]. These findings raise the possibility that the enteric infection of SARS-CoV-2 has negative effects on intestinal commensal microbes and subsequently airway physiology and immunity through the gut-lung axis.

In this review, we summarize recent knowledge of intestinal dysbiosis in COVID-19 patients and discuss its potential contribution to the respiratory symptoms through the gut-lung axis and the possibility of anti-COVID-19 therapy using probiotic intestinal bacteria.

INTESTINAL DYSBIOSIS ASSOCIATED WITH COVID-19

Accumulating evidence has highlighted a cross-talk between the intestinal microbiota and the respiratory tract, termed the gut-lung axis, in which gut microbe-derived components and metabolites modulate airway physiology and immunity [18-21]. Dysbiosis of the gut microbiota leads to aberrant immune tone

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in the respiratory epithelium and mucosa, which can trigger impaired immune responses to respiratory viral infections. Despite the important roles of the gut-lung axis in the antiviral immunity, only limited information is currently available concerning COVID-19-specific changes in the gut microbiome.

INTESTINAL MICROBIOTA AND HOST'S INFLAMMATORY TONE

Shen et al. previously conducted serum proteomic profiling of COVID-19 patients and established a blood proteomic risk score (PRS) for the prediction of the patients' clinical outcomes [22]. In a recent preprint paper, the same research group has discovered a core set of gut bacterial taxa that are closely associated with the blood PRS in uninfected individuals [23]. The core taxa include genus *Bacteroides*, genus *Streptococcus*, genus *Lactobacillus*, family Ruminococcaceae, family Lachnospiraceae and order Clostridiales. Furthermore, the authors suggest a link between compositions of specific gut bacteria and susceptibility to severe COVID-19. Interestingly, a subset of the core taxa is correlated with the serum levels of proinflammatory cytokines, including TNF- α , IFN- γ , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13. In general, *Bacteroides*, *Streptococcus*, and Clostridiales are negatively correlated with the serum levels of tested cytokines, whereas *Ruminococcus*, *Blautia*, and *Lactobacillus* are positively correlated. Fecal metabolome and pathway analyses have identified 45 metabolites associated with the core taxa and revealed that the metabolites are involved in the amino acid-related metabolic pathways. The core bacterial taxa and the relevant metabolites may mediate between the gut microbiota and host inflammatory tone.

In the above-mentioned cytokine test, *Ruminococcus gnavus* has a strong positive correlation with most of the inflammatory cytokines examined. *R.gnavus* is commonly present in the gut microbiome of more than 90% of individuals [24]. Several microbiome studies have linked *R. gnavus* to inflammatory bowel disease, two conditions [Crohn's disease (CD) and ulcerative colitis (UC)] characterized by chronic mucosal inflammation of the gastrointestinal tract [25-31], suggesting the possible role of *R. gnavus* in inducing aberrant inflammatory responses in the intestinal mucosa. Notably, *R. gnavus* produces a proinflammatory glucorhamnan polysaccharide that potently induces the Toll-like receptor 4-dependent secretion of TNF- α by dendritic cells [32]. In addition, *R. gnavus* can degrade mucin to utilize as a carbon source [29,33-35], which leads to the breakdown of the mucus layer and its barrier function. These metabolic properties may increase the exposure of the intestinal immune system to the glucorhamnan polysaccharide to enhance the host's inflammatory tone. *R. gnavus* and/or other mucolytic bacteria may contribute to systemic hyperinflammation characteristic for COVID-19 patients.

COVID-19-SPECIFIC ALTERATIONS IN INTESTINAL MICROBIOME

What are the gut microbiota changes that are specific for COVID-19 patients and how do the changes contribute to the COVID-19 symptoms? In a recent paper in Gastroenterology, Zuo and colleagues have clearly showed the COVID-19-specific

alterations in gut bacterial community [36]. They performed shotgun metagenomic sequencing to analyze fecal samples from 15 hospitalized mild to critical patients and 15 uninfected individuals and found patient-specific dysbiosis of the gut microbiome. The observed dysbiosis is characterized by an increase in opportunistic pathogens and a decrease in beneficial symbionts. Interestingly, COVID-19 severity is positively correlated with the relative abundance of genus *Coprobacillus*, *Clostridium ramosum*, and *Clostridium hathewayi* and inversely with *Faecalibacterium prausnitzii*. *F. prausnitzii* is known to have an immunomodulatory property by producing an anti-inflammatory protein and butyrate, a short-chain fatty acid that are essential for regulated intestinal immunity and epithelial barrier function [37]. Microbiome studies have associated the decreased relative abundance of *F. prausnitzii* with chronic inflammatory diseases such as CD [38,39], asthma [40,41], and obesity [42].

Zuo et al. have also presented interesting data that SARS-CoV-2 virus load in patients' fecal samples is inversely correlated with *Bacteroides dorei*, *B. thetaiotaomicron*, *B. massiliensis*, and *B. ovatus*. In this context, it is worthy of note that these *Bacteroides* species can downregulate intestinal *Ace2* expression when monoclonized in the colon of germ-free mice [43]. In particular, *B. thetaiotaomicron*, a dominant commensal anaerobe of the human intestine, is known to have an anti-inflammatory property and improve mucosal barrier function in rodents with chemically induced colitis, experimental models of CD [44-46]. Collectively, a decrease in *B. thetaiotaomicron* may lead to elevated *Ace2* expression and declined anti-inflammatory activity, which may enhance SARS-CoV-2 infectivity and intestinal/systemic inflammatory tone.

There is another piece of evidence that gut microbiota can modulate colonic *Ace2* expression and systemic inflammation in its host. Yang et al. have demonstrated that reconstitution of the gut microbiota in germ-free rats significantly decreased the colonic *Ace2* expression [47]. Serum metabolome analysis has revealed the higher levels of tryptophan metabolites, kynurenic acid and hydroxykynurenine, suggesting that these metabolites are implicated in the *Ace2* regulation. The conventionalization also resulted in elevated systemic inflammation as evidenced by the increased serum level of lipocalin-2, also known as neutrophil gelatinase-associated lipocalin that is involved in innate immunity, and increased proportion of CD11b+ granulocytes. This study suggests that gut microbiota can modulate intestinal *Ace2* expression and cause the immune dysregulation characteristic of COVID-19.

The two animal studies by Geva-Zatorsky et al. [43] and Yang et al. [47] raise further intriguing questions, and further studies are required to identify the bacterial species that release the metabolites with regulatory activity for *Ace2* expression and elucidate the mechanism of the *Ace2* downregulation by the bacterial metabolites. More importantly, we should clarify whether the intestinal *ACE2* expression is regulated by the similar microbiota-mediated mechanisms in human and whether the gut microbiota can also downregulate airway *Ace2* expression.

Identification of patient-specific changes in the gut microbiota may enable us to make a microbiome-based diagnosis of COVID-19. Gu et al. have identified differences in the gut microbial signature between 30 moderate to severe COVID-19 patients and 30 healthy controls using 16S ribosomal RNA gene amplicon sequencing [48]. The differences include reduced bacterial diversity, higher relative abundance of opportunistic pathogens such as genera *Streptococcus*, *Rothia*, *Veillonella* and *Actinomyces*, and lower relative abundance of beneficial commensals in patients. In addition, a combination of five genera, *Fusicatenibacter*, *Romboutsia*, *Intestinibacter*, *Actinomyces*, and *Erysipelato clostridium*, was identified as a biomarker that can distinguish patients from healthy individuals. This finding suggests the potential diagnostic and therapeutic value of the specific gut microbiota for COVID-19 even though further validation studies are required.

PROPHYLACTIC AND THERAPEUTIC USE OF PROBIOTICS IN COVID-19

Components and metabolites derived from gut commensal bacteria play a key role in gut-lung axis-mediated regulation of the airway physiology and immunity [18-21]. Hence, there is the possibility that normalization of intestinal dysbiosis using probiotics is effective for the control of COVID-19. Previous animal and clinical studies have shown that oral intake of probiotic strains has protective and therapeutic efficacy in respiratory tract infections caused by RNA viruses such as influenza virus and rhinovirus [49-53]. However, there have been no reports of clinical trials focusing on the efficacy of probiotics in COVID-19 to date, and the rationale for using probiotics against COVID-19 remains unclear. Life-threatening symptoms and complications of COVID-19 are caused by hyperinflammation due to complex immune dysregulation involving neutrophilia, lymphocytopenia, declined T-cell immunity, and excessive production of inflammatory mediators [54-56]. It is therefore particularly notable that specific probiotic strains have the superior strain-specific immunomodulatory ability against respiratory viral infections.

There is a large body of evidence that *Lactobacillus plantarum* has a wide variety of immunomodulatory activity particularly upon the infection of seasonal and highly pathogenic influenza viruses. Oral administration of *L. plantarum* in mice alleviates inflammation upon viral infection [57], enhances NK cell activity [58], activates Th1-mediated immune responses and Th1 cytokine production (IL-12 and IFN- γ) [57-59], and notably, promotes IgA-mediated mucosal immunity in the small intestine and lung [59,60]. Similarly, randomized controlled trials have demonstrated that oral intake of *L. plantarum* reduces the risk of acquiring upper/lower respiratory tract infections and alleviates the respiratory symptoms [61-63]. These effects are shown to be associated with the immunomodulatory ability of *L. plantarum*, including activation of CD8⁺ T cells, enhancement of the phagocytic activity of granulocytes, reduction of plasma proinflammatory cytokines IFN- γ and TNF- α , and elevation of anti-inflammatory cytokines IL-4 and IL-10 [62,64]. These findings suggest that *L. plantarum* strengthens many aspects of the host defense mechanism against viral infection. On the basis of these findings, we have employed *L. plantarum* as anti-

COVID-19 probiotics in our recent clinical trial for the immunological efficacy of probiotic lactic acid bacteria against COVID-19. The ongoing trial focusing on uninfected individuals is registered on University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR, <https://www.umin.ac.jp/ctr/index.htm>) under the trial number UMIN000040479.

FUTURE PERSPECTIVES

The composition of the intestinal microbiota is highly variable among individuals, which may lead to high interpersonal disparity in the susceptibility to SARS-CoV-2 infection and the severity of COVID-19-associated symptoms. To test this possibility, we need to conduct comparative studies of gut microbiota profiles among COVID-19 patients with different severity and identify the compositional changes in specific intestinal bacteria that determine the disparity. Similarly, it would be interesting to investigate the differences in the composition of the gut microbiome among COVID-19 patients with or without extra-respiratory manifestations such as thrombosis, myocardial injury, renal damage, or neurological symptoms [65-67].

To develop probiotics for the management of COVID-19, more effort is required to isolate and/or identify more probiotic strains with the ability to reduce the viral load and suppress hyperinflammation via different mechanisms of action. Bacterial species outside of the genera *Lactobacillus* and *Bifidobacterium* should be explored. Randomized controlled trials with large cohorts aimed at evaluating the prophylactic and therapeutic use of probiotics are also essential. As described above, the respiratory tract is inhabited by commensal bacterial communities, and the respiratory microbiota is known to be closely associated with susceptibility to and severity of respiratory infections. In fact, metatranscriptomic studies have revealed that COVID-19 patients have more disrupted airway microbiome. Interestingly, bacteria within gut-specific genus *Bacteroides* are commonly and abundantly found in bronchoalveolar lavage fluid from ARDS patients. Indirect manipulation of the respiratory microbiome with oral probiotics may be a promising adjunctive therapy to alleviate life-threatening hyperinflammation in COVID-19.

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