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Gut Microbiota Dysbiosis and COVID-19: Possible Links

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Microbiota and Gut Dysbiosis

The human body hosts thousands of microbial species with more than 20 million unique microbial genes that support the health and maintenance of their host (Proctor, 2011). Based on bacterial distribution in the human body, 29% of the sequenced bacterial species are associated with the gastrointestinal tract (The NIH HMP Working Group, 2009).

Although each individual has a unique gut microbiome composition just like a fingerprint, the most abundant microbial phyla found in a healthy human gut are *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*, with mostly the predominance of genera including *Bacteroides*, *Lachnospiraceae*, *Faecalibacterium*, and *Bifidobacterium* (Arumugam et al., 2011; Huttenhower et al., 2012; Rinninella et al., 2019). Gut microbiota harbors not only bacteria species but also fungal microbiome called gut mycobiome (Nash et al., 2017; Rinninella et al., 2019). Diversity in the fungal community is much lower relative to bacterial diversity. Gut mycobiome is mostly dominated by *Candida*, *Saccharomyces*, and *Malassezia* with *C. albicans*, *S. cerevisiae*, and *M. restricta* at high prevalence (Nash et al., 2017). Gut microbiome is also colonized by extensive populations of viruses (Rinninella et al., 2019; Shkoporov et al., 2019). The vast majority of the gut virome are bacteriophages shaping the gut microbial composition.

More than 99% of microbial organisms found in complex environments are not easy to culture in laboratory conditions; therefore, culture-independent technologies have been developed to allow us to study microbial communities directly in these complex environments (Qin et al., 2010; Gevers et al., 2012; Scholz et al., 2012; Morgan and Huttenhower, 2014). The challenge is to make sense of massive amounts of data accumulating day by day. The whole web of information can be unraveled if a link is found between healthy and non-healthy conditions, the link suggesting how microbes communicate in the gut. The Human Microbiome Project coordinates projects to identify microbial communities associated with certain diseases, allowing us to understand the relationships between gut microbiota and host (The NIH HMP Working Group, 2009; Proctor, 2011; Proctor et al., 2019). Researches on the gastrointestinal microbiota composition in animals and humans of varying different health conditions such as obesity, cancer, and diabetes suggest that gut microbiota is crucial in digestion, health and disease, impacting immunity, and pathogenesis of gut-related diseases (Sudo et al., 2004; Smith and Vale, 2006; Mayer et al., 2014; Shanahan and Quigley, 2014).

The composition of the gastrointestinal microbiota is influenced by various factors such as host genotype, age, physical activity, and diet, which may cause instability in the gut microbiota and change the relative bacterial predominance and diversity in the gut (Sommer and Bäckhed, 2013; Belizário and Faintuch, 2018). A persistent imbalance in the microbial composition is referred to as dysbiosis. Although gut microbiota is composed of both beneficial microorganisms and pathogens such as *Lactobacillus*, *Bifidobacterium*, *Clostridium* and *Bacteroides*, beneficial microorganisms are dominant in a healthy gut microbiome composition (Belizário and Faintuch, 2018). Change in the equilibrium of the abundance of beneficial microorganisms and pathogens with dysbiosis paves the way for opportunistic pathogens and leads to the altered intestinal barrier function and immune protection. There is a bidirectional interaction between the microbiota and the immune system, which is well-balanced (Gill et al., 2006; Proctor, 2011; Ostaff et al., 2013; Proctor et al., 2019). While the microbiota plays a fundamental role in the development of the immune system, the immune system shapes the microbiota composition and functions in return. Dysfunction in this balance can lead to inflammatory-related diseases such as inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis (Duboc et al., 2013). While the presence of *anti*-inflammation associated species such as *Faecalibacterium prausnitzii*, which is predominated in healthy individuals, decrease in IBD patients gut microbiota, proinflammatory species such as *Ruminococcus gnavus* and *Bacteroides* sp. increase (Nagalingam and Lynch, 2012; Duboc et al., 2013). Altered gut microbiota associate-bile acids due to dysbiosis have been shown in IBD patients, which impact the inflammatory loop in IBD (Duboc et al., 2013). The microbiome profile of IBD patients also demonstrates a characteristic shift in intestinal microbiota composition, resulting in an enrichment of *Proteobacteria* and *Actinobacteria* or depletion of *Firmicutes* and *Bacteroides* (Walters et al., 2014). Dysbiosis causing shifts in the composition of gut microbiota induces a reduction in short-chain fatty acids (SCFA), antimicrobial peptide secretion, and mucus production, which protects gut barrier integrity. Intestinal bacteria and bacterial products passing through the impaired gut barrier stimulate inflammatory cytokine (Tumor Necrosis Factor (TNF)-α, Interleukin (IL)-1, IL-6, IL-12, etc.) production in IBD patients (Antoni et al., 2014; Shamoon et al., 2019). Studies with IBD animal models exploring the impact of gut microbiota on IBD demonstrated the association between active IBD and dysbiosis and the protective roles of microbial metabolites including acetic acid, butyrate,

and propionic acids on IBD (Shamoon et al., 2019). Moreover, it has been shown that the exposure of infants to antibiotics increases their risk of developing IBD in the future since microbiota begin to influence immune development early in life (Shaw et al., 2010; Hviid et al., 2011).

Gut microbes also significantly influence metabolic activities in the host. Dysbiosis, recognized as a signal of an unhealthy microbiome, has been associated with various chronic conditions such as type 2 diabetes and obesity (Tai et al., 2015; Aleman and Valenzano, 2019). Reduction in the prevalence of *Firmicutes*, *Clostridia*, and *Betaproteobacteria* in the gut microbiota has been reported in type 2 diabetes with an increase in *Bacteroidetes/Firmicutes* ratio (Minemura and Shimizu, 2015). A low abundance of *Bacteroides* with a decreased *Bacteroidetes/Firmicutes* ratio in the gastrointestinal microbiota is linked to obesity (Verdam et al., 2013). Additionally, *Lachnospiraceae* has been found to be involved in the development of obesity and diabetes with an impact on glucose and lipid metabolism (Ravussin et al., 2012; Kameyama and Itoh, 2014). Turnbaugh et al. reported in a study of obese and lean mice that the microbiota of the obese mice had a higher energy harvesting capacity compared to the microbiota of the lean mice. Moreover, they demonstrated that when transplanting microbiota from an obese mouse into a germ-free mouse, the mouse gained more fat than when a germ-free mouse received microbiota from a lean mouse (Turnbaugh et al., 2006, 2008). It has been shown that the transplantation of fecal solution from lean mice can improve insulin sensitivity in mice with metabolic syndrome (Vrieze et al., 2012). In a study comparing the microbial composition of the fecal samples from the patients with gallstones with the fecal samples from the individuals with no gallstones, gut microbiota dysbiosis has been shown to be associated with the development of cholesterol gallstones (Wu et al., 2013). While *Proteobacteria* increased in gallstone patients, there was a reduction in the abundance of *Faecalibacterium*, *Lachnospira*, and *Roseburia*, known as inulin-oligofructose, inulin, and pectin degraders, respectively. Intestinal bacteria are capable of regulating bile acid metabolism via bile-specific enzymes and eventually impact gallstone pathogenesis. They can convert primary bile acids to secondary bile acids by 7α-dehydroxylase activity and deconjugate bile acids by bile salt hydrolases activity (Färkkilä and Miettinen, 1990; Thomas et al., 2000; Ridlon et al., 2006; Cai and Chen, 2014; Duparc et al., 2017).

Intestinal microbiota is closely interrelated with liver diseases as well. Individuals with nonalcoholic fatty liver disease, one of the most common diseases in the world, have been shown to have a low prevalence of *Bacteroides* and a high prevalence of *Prevotella* and *Porphyromonas* compared to healthy individuals (Boursier and Diehl, 2015; Minemura and Shimizu, 2015). Dysbiosis in the gut microbiota results in impaired gut barrier integrity and consequently causes metabolic toxicity (Tai et al., 2015; Aleman and Valenzano, 2019; Hufnagl et al., 2020). Increased intestinal epithelial permeability leads to intestinal bacteria and bacterial metabolites such as pathogenic bacteria-derived lipopolysaccharides (LPS) to translocate from the gut lumen to the circulatory system and influence the distal organs (Burcelin et al., 2012; Bajaj, 2019). Translocation of gut bacteria and/or their metabolites to the liver via the portal vein impact the liver. LPS reaching the liver with the increased intestinal permeability induces hepatic stellate cells and kupffer cells, which trigger proinflammatory cytokine production and cause liver damage (Minemura and Shimizu, 2015).

Bacterial population in the gut microbiota yield energy and SCFA such as acetate, butyrate, and propionic acids, by fermenting non-digestible complex carbohydrates in humans (Minemura and Shimizu, 2015). While acetate and propionic acid are energy sources for cells in peripheral tissues, butyrate is an energy source for epithelium cells in the large intestine and has been found to impact genes encoding β-defensin and leukocyte antimicrobial peptides (Vrieze et al., 2012; Zeng et al., 2013; Louis et al., 2014; Minemura and Shimizu, 2015). SCFA produced by intestinal microbiota regulate inflammation and cell proliferation and take a role in insulin metabolism (den Besten et al., 2013; Morgan and Huttenhower, 2014; Minemura and Shimizu, 2015). Hence, changes in the bacterial population in the gut alter the ratio of SCFA in the gut and lead to metabolic diseases.

It has been shown that gut microbiota can impact even the brain functions via modifying intermediaries resulting from the interaction between the gut immune system (Mayer et al., 2014; Shanahan and Quigley, 2014). Bidirectional interaction between the gut and the nervous system has been shown to be involved in irritable bowel syndrome (IBS) pathogenesis and elated functional gastrointestinal disorders with GI symptoms of abdominal pain, diarrhea, or/and constipation (Moayyedi et al., 2010; Jasper, 2015). Interestingly germ-free animals have been reported to overdraw the hypothalamic-pituitary-adrenal (HPA) axis activation, a central stress response system, in response to stress. This hyperresponsiveness was corrected by restructuring the microbiota with fecal suspension from animals kept in a pathogen-free environment or through *B. infantis* administration (Sudo et al., 2004; Smith and Vale, 2006). The intestinal microbiota and its metabolic products are capable of modifying intestinal functions by impacting the gut barrier integrity, immune function, enteric nervous system (ENS), and brain (Mayer et al., 2014). Human gut microbiota has been predicted to consist of genera capable of producing or consuming γ-aminobutyric acid (GABA, a major inhibitory neurotransmitter), such as *Escherichia*, *Bacteroides*, and *Parabacteroides* species. GABA level has been demonstrated to be modulated by altering the composition of gut microbiota, in particular *Bacteroides*, which is negatively correlated with depression-related brain signatures (Strandwitz et al., 2019). In a study on the impact of microbiota on inflammatory pain with germ-free and conventional mice, it has been shown that commensal microbiota is necessary for mice to generate inflammatory hypernociception (Amaral et al., 2008). Other studies also demonstrated that gut microbiota plays a role in adult neural plasticity such as microglia activation and neurogenesis and is required for normal brain development in adulthood (Hsiao et al., 2013; Ogbonnaya et al., 2015; Stilling et al., 2015). Antibiotic-induced microbiota damage influences anxiety and cognitive behaviors as well as neuromodulators in the gut-brain axis including monoamines, neuropeptides, and tryptophan (Desbonnet et al., 2015). Vice versa, the brain is able to alter the gut microbiota composition via ENS, autonomic nervous system, and hypothalamic-pituitary axis by affecting the epithelial integrity, mucosal immunity, luminal secretion, and neurotransmitters release (Rhee et al., 2009; Mayer, 2011).

Intestinal microbiota and gut epithelial barrier have been found to be disrupted in pulmonary diseases (Hanada et al., 2018; Rutten et al., 2014). Due to gut microbiota dysbiosis with the impaired intestinal barrier integrity, translocation of the intestinal microbes is followed by a secondary infection via circulatory systems (Aktas and Aslim, 2020). The bacterial translocation from the gut to the lungs

has been reported in acute respiratory distress syndrome (ARDS) and sepsis because of possible epithelial barrier damage (Dickson et al., 2017). It is well established that gut and lung have been associated with modulating immune responses, and dysbiosis in the gut microbiota is involved in disease pathogenesis in the respiratory tract (Fanos et al., 2020). Sencio et al. studied the impact of gut dysbiosis on bacterial superinfection during influenza in mice (Sencio et al., 2020). They demonstrated that influenza modified the SCFA composition by altering the gut microbiota and dysbiosis in the gut led to secondary bacterial infection in the respiratory system. Moreover, they showed that acetate supplementation reduced the susceptibility to bacterial superinfection.

Dysbiosis, disturbance of the healthy intestinal microbiota, has been involved in a wide range of disorders or diseases as mentioned above. Therefore, staying in balance is vital for health. The recipe for preventing or treating these non-healthy conditions could be located within the microbiota itself; hence, researchers ask if modification of microbiota could help to treat or prevent human diseases (Nagalingam and Lynch, 2012; Sommer and Bäckhed, 2013; Shanahan and Quigley, 2014; Kumar et al., 2015; Ohno, 2015).

Possible Link Between Gut Dysbiosis and COVID-19

The recent outbreak of the novel coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), COVID-19, in Chine affected millions of people around the world with a high mortality rate, much more than the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome-related coronavirus (MERS-CoV) combined (Mahase, 2020). In comparison, while the outbreak of SARS and MERS resulted in death of less than two thousand people, SARS-CoV-2 caused more than three million deaths around the world (Mahase, 2020; World Health Organization, 2021). While some of the SARS-CoV-2 infected individuals develop ARDS with respiratory failure requiring mechanical ventilation, shock, or other organ failure in intensive care unit (ICU) care, some remain asymptomatic or experience the disease with mild symptoms (Chen et al., 2020). Many of the patients in ICU develop multiple organ failure syndrome with damage to vital organs, which later may lead to death (Farooqui, 2021).

It is commonly known that SARS-CoV-2 mainly affects the lung or respiratory system; however, gastrointestinal symptoms have been described in COVID-19 patients as well. Furthermore, SARS-CoV-2 nucleic acid was detected in fecal samples collected from COVID-19 patients (Wu et al., 2020). Although the COVID-19 patients discharged from the hospital were respiratory negative for viral RNA, their fecal samples remained positive for about two weeks and more than 90 days for some patients (Zhang et al., 2020; Xu et al., 2020; Tao et al., 2021). The centers for research on intestinal microbiota and fecal microbiota transplantation (FMT), therefore, advised to screen FMT donors for SARS-CoV-2 and drew attention to the possible fecal-oral transmission of the virus (Holshue et al., 2020; Zhang et al., 2020; Chen et al., 2020; Zhang et al., 2020). This brings to mind the presence of extrapulmonary SARS-CoV-2 infection or involvement of the gastrointestinal system in the course of COVID-19. SARS-CoV-2 is a single-stranded RNA virus with a double-layer lipid envelope structure on the outer surface. The lipid envelop is embedded in various proteins such as spike (S) protein, envelope (E) protein, and membrane (M) protein (Xiao et al., 2020). The most crucial structure of the SARS-CoV-2 is the spike glycoprotein which is composed of two functional units, S1 and S2. Those units bind to the angiotensin-converting enzyme 2 (ACE2) receptor on the host cell. The role of ACE2 in viral invasion via SARS-CoV-2 S proteins has been well defined in the literature (Xiao et al., 2020; Fakhroo et al., 2021). SARS-CoV-2 uses ACE2 receptor to enter human cells same as SARS-CoV does but with a higher affinity. SARS- CoV-2 binding affinity has been found to be 10–20 times higher than that of SARS-CoV (Wrapp et al., 2020). A higher number of COVID-19 cases compared to the SARS cases could be explained by the binding affinity of the SARS-CoV-2 to the ACE2 receptors. Following the binding to ACE2 receptor, SARS-CoV-2 invades the host cells via type II transmembrane serine protease (TMPRSS2). For a successful SARS-CoV-2 infection, these two proteins are required to be co-expressed in the target cell (Zhang et al., 2020). Airways are one of the main sites of viral entry; however, it is now well recognized that ACE2 receptors are expressed in other sites of the body including heart, intestine, kidney, and liver (Dong et al., 2020). Even though both ACE2 receptor and TMPRSS2 enzyme are highly expressed in the gastrointestinal system, intestinal cells to be the SARS-CoV-2 infection site still remains debated because live viruses from intestinal tissues of patients with COVID-19 have not been titrated or isolated due to the technical limitations (Aktas and Aslim, 2020). In a study with human intestinal enteroid (3D multicellular *in vitro* intestinal tissues), whether SARS-CoV-2 is able to infect human intestinal epithelial cells were explored (Zang et al., 2020). They reported that these two proteins were not co-expressed on all enterocytes. TMPRSS2 was only expressed on ACE2 positive mature enterocytes. Other proteases including TMPRSS4 were involved in the cleavage of the SARS-CoV-2 spike proteins and enhanced membrane fusion. Taking together with the information of viral RNA detection in feces of COVID-19 patients, it could be considered that the infection by SARS-CoV-2 does not stay with the respiratory tract only and the gastrointestinal system may serve as an infection site for the SARS-CoV-2 as well. However, the data received until today is limited to ensure the fecal-oral transmission route for explaining the gastrointestinal symptoms in COVID-19 patients. ACE2 receptor is also part of the renin-angiotensin-aldosterone system (RAAS), which controls fluid balance and blood pressure. ACE2 receptor, therefore, has an important role in cardiovascular and renal diseases as well as pulmonary diseases including acute respiratory distress syndrome (Xiao et al., 2020; Fakhroo et al., 2021). Moreover, ACE2 is involved in regulating amino acid absorption in the intestine, which balances the intestinal bacteria and controls intestinal inflammation (Hashimoto et al., 2012). ACE2 receptors have been shown to maintain the neutral amino acid (tryptophan) transporter B^0AT1 in the epithelial cells of the small intestine and regulate the degree of antimicrobial peptides in the intestinal system, which is important for gut stability (Hashimoto et al., 2012). Decreased level of tryptophan due to impaired ACE2 expression fails to activate the antimicrobial peptide secretion in the gut, which then leads to pathogen survival and dysbiosis in the gut (Chhibber-Goel et al., 2021; Rajput et al., 2021). Changes in the level of antimicrobial peptides result in modulation in the intestinal microbial population. SARS infection was reported to induce downregulation of

ACE2 expression and lead to a reduction in antimicrobial peptides (Devaux et al., 2021). A decrease in the antimicrobial peptides such as α-defensin HD5 promotes the intestinal pathogens to be dominated in the gut and altered the gut microbial composition resulting in gut dysbiosis.

The most common clinical symptoms of COVID-19 patients were reported as fever, cough, dyspnea, sore throat, fatigue, and headache. However, as the pandemic data accumulating day by day around the world, gastrointestinal manifestations emerged as important clinical symptoms (Guo et al., 2021; Schettino et al., 2021; Schmulson et al., 2021). Most studies described diarrhea, vomiting, abdominal pain, and nausea as the common gastrointestinal symptoms (Guo et al., 2021). It was previously reported that patients with SARS represented common symptoms similar to COVID-19 with 16%–73% diarrhea (World Health Organization, 2003). The ratio ranged from 2% to 50% for diarrhea and 5% to 80% for all gastrointestinal symptoms in the patients with COVID-19 (Chen et al., 2020; Fortune and Sharaiha, 2020; Chen et al., 2020). It is worth mentioning that the ratio varies among reports. It could be due to different diagnosing criteria used for diarrhea in different hospitals or the gastrointestinal health status of the individual patients before infected. In some cases, gastrointestinal symptoms could appear even before the respiratory manifestations or fever (Lin et al., 2020; Song et al., 2020). Moreover, the COVID-19 patients with gastrointestinal symptoms, in particular diarrhea, experienced more severe respiratory symptoms requiring ventilation support in ICU admission (Jin et al., 2020; Pan et al., 2020; Wan et al., 2020). A study with 204 patients, exploring the clinical characteristics of COVID-19 reported digestive symptoms including abdominal pain, vomiting, and diarrhea in 50.5% of the patients (Pan et al., 2020). Some of the patients declared only the intestinal symptoms. They revealed that patients with gastrointestinal symptoms more often developed severe cases with a higher level of liver enzyme and lower monocyte count. In another retrospective study analyzing the enteric involvement in hospitalized patients, they showed that patients with diarrhea showed more severe pneumonia symptoms and the ratio in patients who required ventilation support was higher among patients with diarrhea (Wan et al., 2020). With these data documented about diarrhea in COVID-19 around the world, gastrointestinal signs along with respiratory symptoms should not be underestimated. Although the pathophysiology of gastrointestinal symptoms at the begging or during the clinical course of the illness is not well understood yet, it is certain that the gastrointestinal system contributes to the illness either at the beginning or during the treatment.

There are several hypotheses about how SARS-CoV-2 ends up in feces. Fecal-oral transmission of SARS-CoV-2 to infect the intestinal cells is one of them since the fecal RNA of SARS-CoV-2 has been detected in the feces of COVID-19 patients. For a successful intestinal infection via fecal-oral transmission, the structure of the virus must manage the barriers through the digestive tract after ingestion including acid in the stomach and bile salts in the intestine. Coronaviruses are lipid enveloped viruses with the lipid bilayer (Bertók, 2004). The digestive tract conditions may disrupt the lipid envelop of the virus and prevent infection, especially the bile salts, which have a detergent effect on lipid envelopes. Previously SARS-CoV was found to be inactivated under acidic conditions with a pH lower than 3 (Darnell et al., 2004). Similarly, Pratelli et al. reported complete inactivation of the canine coronavirus under pH 2.26 and pH 4.38 at 37 °C (Pratelli, 2008). Recently, a study investigated the impact of gastric and intestinal fluid on SARS-CoV-2 survival to explore the possible enterocyte infection by SARS-CoV-2 (Zang et al., 2020). The virus was shown to be able to enter the intestinal cells; however, they lost their infectivity under the simulated gastrointestinal conditions with low gastric pH, bile acids, and digestive enzymes. Moreover, infectious virus was not recovered from the fecal specimens of COVID-19 patients. These results suggest that the virus can be deactivated under gastrointestinal tract conditions and prevented from causing intestinal infection. Intestinal SARS-CoV-2 infection is possible, but it requires more research to ensure that intestinal SARS-CoV-2 infection occurs via the fecal-oral route. Recently Zuo et al. investigated the SARS-CoV-2 transcriptional activity in fecal samples from 15 hospitalized COVID-19 patients (Zuo et al., 2021). They quantified SARS-CoV-2 genome sequencing coverage using RNA shotgun metagenomics sequencing. They hypothesized that if SARS- CoV-2 virions end up in feces without infecting intestinal cells, metagenomics reads should cover the full-length SARS- CoV-2 RNA. Notwithstanding, if SARS- CoV-2 infects the intestinal cells, the 3′ end of the SARS-CoV-2 genome should be covered more highly than the 5′ end regions of the viral RNA by metagenomics reads. They reported that around 47% of the patients had considerably higher coverage of the 3′ end compared to the 5′ end of SARS- CoV-2 genome in the viral metagenome profile of their feces. Moreover, three patients continued to exhibit viral infection signs with higher 3′ end region coverage up to 6 days after negative throat swab results for SARS-CoV-2. However, they could not isolate live SARS- CoV-2 viruses from the feces samples of COVID-19 patients due to methodologic limitations. They also examined the fecal microbial composition of the patients. In fecal microbiota of the patients with viral infectivity sign, while abundance of opportunistic pathogens was higher, SCFA producing bacterial populations were lower. Similarly, Gu et al. demonstrated that hospitalized COVID-19 patients harbored more potential pathogens in the gut microbiota relative to the healthy controls (Gu et al., 2020). Several studies have revealed that COVID-19 infection causes significant alterations in the fecal microbiota (Ferreira et al., 2021). *Streptococcus*, *Faecalibacterium*, *Clostridium*, *Lactobacillus*, *Veillonella*, *Bacteroides*, and *Eubacterium* are the genera commonly altered in the fecal microbiota of COVID-19 patients (Gu et al., 2020; Tao et al., 2020; Zuo et al., 2020; Lv et al., 2021). Tao et al. showed that while *Streptococcus*, *Clostridium*, *Haemophilus*, and *Proteobacteria* increased in abundance, the population of *Prevotella*, *Akkermansia*, *Paraprevotella*, and *Lachnospira* decreased in the feces of patients with COVID-19 (Tao et al., 2021). In another study with COVID-19 patients, the abundance of *Ruminococcus gnavus*, *Ruminococcus torques*, and *Bacteroides dorei* was increased while *Bifidobacterium adolescentis*, *Faecalibacterium prausnitzii*, and *Eubacterium rectale* were depleted in the feces of the patients (Yeoh et al., 2021). Although the microbiota patterns vary among different studies examining the fecal microbiota composition in COVID-19 patients, which is probably due to different geographic locations with different lifestyle or treatment procedures, some consistent changes in microbial composition across different studies have been reported (Aktas and Aslim, 2021). In general, COVID-19 infection enriches the opportunistic pathogens and lessens the populations commonly known as beneficial. Depletion in some bacterial groups, such as *Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Collinsella aerofaciens* were even higher in the

COVID-19 patients using antibiotics during treatment, with a lower bacterial diversity (Zuo et al., 2020; Cao et al., 2021; Yeoh et al., 2021). Alteration in the gut microbiota have been reported to be associated with COVID-19 severity and some of the populations, such as *Faecalibacterium* were negatively correlated with the disease severity while some of them, such as *Coprobacillus* correlated positively (Zuo et al., 2020; Cao et al., 2021; Yeoh et al., 2021). The severity of COVID-19 patients has been determined with an elevated level of inflammatory cytokines in blood or feces including IL-18, IL-10, TNF-α, C- X- C motif ligand (CXCL)8 and CXCL10, blood markers such as C reactive protein (CRP), lactate dehydrogenase, and natural killer cells (Tang et al., 2020; Tao et al., 2020; Yeoh et al., 2021). A decrease in diversity of the gut microbial composition results in altered intestinal microbiota to an imbalanced state and causes immune dysfunction and generalized inflammation (Aleman and Valenzano, 2019). As mentioned above, there is a bidirectional interaction with a graceful balance between the immune system and the gut microbiota (Gill et al., 2006; Proctor, 2011; Ostaff et al., 2013; Proctor et al., 2019). Dysfunction in this equilibrium with dysbiosis can lead to inflammatory diseases.

Gut-Lung Axis

Gut microbiota serving as a separate organ can influence physiological and homeostatic functions in the host and interact with distal organs via immune system (Zhang and Frenette, 2019; Zhang et al., 2020). Altered gut microbial composition and disrupted intestinal barrier have been described in pulmonary diseases as well (Rutten et al., 2014; Hanada et al., 2018). Antibiotic alterations of the gut microbiota have been linked to viral infections with the capability of impacting on viral infection in distal organs. It has been shown that antibiotic administration increased the susceptibility to influenza infection in the lung by affecting the adaptive immune response in mice, indicating a relationship between gut and lung (Ichinohe et al., 2011). More than 60% of the patients in China received antibiotics during COVID-19 treatment and this massive amount of antibiotic use breaks the microbial balance in the gut (Guan et al., 2020; Chen et al., 2020).

COVID-19 generates unfavorable outcomes associated with the immune response. Increased proinflammatory cytokines and lymphocytopenia have been shown to be linked to severe SARS-CoV-2 infection (Huang et al., 2020; Zheng et al., 2020). Inflammatory cytokines including IL-6, IL-10, interferon (IFN)-γ, and TNF-α, are raised in severe/critical patients during COVID-19 infection (Costela-Ruiz et al., 2020). Boosted production of chemokine and cytokine tends to create a "cytokine storm" leading to a severe acute respiratory syndrome in the lung along with multi-organ failure (Huang et al., 2020; Kalantar-Zadeh et al., 2020). During the early stage of the outbreak, Ruan et al. conducted a retrospective multicenter study on the mortality of COVID-19 patients with a 45% death rate (Ruan et al., 2020). When they compared the blood parameters of the patients who died and were discharged, there were significant differences in the level of blood creatinine, cardiac troponin, myoglobin, IL-6, CRP, white blood cell counts, and lymphocyte counts. Furthermore, 16% of the patients who died displayed secondary infections. Their results support virus-induced hyper-inflammation, so-called cytokine storm, to be the reason for the COVID-19 mortality. Both adaptive immune system and innate immune system commit to SARS-CoV-2 infection. Lymphopenia with severely low numbers of T cells ($CD4^+$ and $CD8^+$), B cells, and monocytes was identified in severe COVID-19 patients (Cao, 2020; Diao et al., 2020). It has been known that gut microbiota, with its immunomodulatory effect, takes a fundamental role in the development of immune cells including regulatory T cells and innate lymphoid cells (He et al., 2020). Commensal bacteria in the gut microbiota can lead to inflammasome activation and stimulate dendritic cells migration for T-cell responses against influenza virus to initiate antiviral responses in macrophages (Ichinohe et al., 2011).

Intestinal microbiota and gut epithelial barrier are found disrupted in pulmonary diseases or respiratory infections (Rutten et al., 2014; Hanada et al., 2018). Murine studies show that depletion of beneficial bacteria within the gut microbiota due to antibiotic intake is associated with lung diseases and allergic inflammation (Trompette et al., 2014). There is a link between the gastrointestinal system and the respiratory tract to modulate immune responses and dysbiosis in the gut microbiota contribute to disease pathogenesis in respiratory tract (Fanos et al., 2020). It has been reported that patients with asthma and chronic lung problems develop gastrointestinal tract complications such as IBS and the intestinal mucosa and permeability in some of those patients are found to be impaired (Roussos et al., 2003; Rutten et al., 2014). Additionally, nearly half of the adult IBD patients display an impaired lung function with no history of a respiratory disease indicating an inflammatory cross-talk between gut and lung (Keely et al., 2012). Research on gastroenteritis-like symptoms such as diarrhea in influenza using a mouse model of respiratory infection demonstrated that the mice exposed to an intranasal virus developed intestinal immune damage following lung injury (Wang et al., 2014). Interestingly, they showed that the reason for intestinal injury was the change in the gut microbial composition due to influenza rather than direct intestinal infection. Gut microbiota in infected mice was altered by IFN-γ produced by lung-derived T cells accompanied with an increased level of T helper (Th)17 cell in the small intestine. Studies support the network between the immune system, gut microbiota, and lungs. This gut-lung interaction may influence the severity of COVID-19 in patients with extrapulmonary conditions. Viral SARS-CoV-2 infection induces intestinal inflammation in the COVID-19 patients. Fecal calprotectin has been commonly used as a protein marker for intestinal inflammation in patients with infectious colitis and IBD (Effenberger et al., 2020). A high fecal calprotectin level was detected in COVID-19 patients with diarrhea even after the diarrhea was discontinued. Moreover, the concentration of proinflammatory cytokine IL-6 in serum was correlated with the fecal calprotectin level in COVID-19 patients (Effenberger et al., 2020). Increased inflammation in intestine causes a decrease in the gut integrity, commonly named leaky gut, and leads to bacteria and bacterial antigens passing to the systemic circulation and inducing the septic state of the patients with COVID-19 (Mahase, 2020). It has been reported that many of the deaths in the influenza pandemic called Spanish Flu was due to bacterial infection leading to secondary pneumonia in the patients and most of the severe cases occurred as a result of bacterial complications (Hanada et al., 2018). Similarly, around 30% of the patients in the 2009

H1N1 pandemic developed bacterial co-infection. It has been shown that lung microbiota of patients with acute pulmonary diseases and acute respiratory distress syndrome were increased in the abundance of intestinal bacteria such as *Bacteroidetes* and *Enterobacteriaceae* (Fanos et al., 2020). These are the signature of an impaired gut permeability associated with the gut-lung axis. In like manner, severe COVID-19 patients developed a secondary bacterial infection (Cucchiari et al., 2020; Lehmann et al., 2020; Peddu et al., 2020). In a study, the death rate due to secondary infection was reported as 16% with an elevated level of inflammatory response (Ruan et al., 2020). Elevated systemic inflammation induces multiple organ failure. Critically ill patients in intensive care unit develop multi organ failure associated with impaired intestinal permeability (Doig et al., 1998). Dysfunction in intestinal barrier causes bacterial translocation from the intestine to the lungs in sepsis and acute respiratory distress syndrome (Dickson et al., 2017). Vice versa, infectious viruses could travel to gastrointestinal system from infected lung via systemic circulation. Lymphatic and circulatory system members including circulating white blood cells, lymphocytes, monocytes, lymph nodes, and lymphoid tissues in SARS-CoV infected patients were found to be positive for the viral sequences (Gu et al., 2005). Additionally, immunopathological modifications in the intestinal tissues were detected indicating that viral infection was carried with the infected immune cells via lymphatic or circulatory system. This could explain the fecal SARS-CoV-2 RNA detected in fecal samples. With this way viruses could avoid the digestive tract conditions that may inhibit its infectivity.

Gut Microbiota-Targeted Therapeutics

Dysbiosis in the intestinal microbiota is associated with aging and various chronic conditions such as asthma, arthritis, obesity, liver cirrhosis, and type 2 diabetes and results in increased gut permeability and consequently cause metabolic toxicity (Tai et al., 2015; Aleman and Valenzano, 2019; Hufnagl et al., 2020). Impaired gut permeability impacts the disease severity via bacterial translocation from the intestinal lumen to the mucosa that triggers systemic inflammation (Burcelin et al., 2012; Bajaj, 2019). COVID-19 severity and mortality have mostly been associated with elders and patients with underlying medical conditions. Given the research on coronaviruses to date and the interaction between the gut microbiota and the immune system with the association of the lung axis, targeted modifications could be considered to get the microbial composition back to normal or healthy microbiota status alleviate the disease severity by protecting intestinal integrity. Probiotics are one of the approaches to alter the microbial composition of the gut. Promising impacts of probiotics to improve the severity of viral respiratory tract infections and to reduce the infection risk have been well documented with experimental and clinical studies on respiratory tract viruses including influenza (Singh and Rao, 2021). The clinical trials and the studies were performed mostly using the strains from *Lactobacillus* and *Bifidobacterium* genera commonly used as probiotics. However, it is worth noting that the benefits of probiotics are dependent on the particular strain and dose administered. As a consequence, there is growing interest in altering the composition of intestinal microbiota via probiotics. Many cohort studies and clinical trials are ongoing all around the world to test their impact on modulation of gut microbiota and the effectiveness in COVID-19 patients, with only two studies published to date (ClinicalTrials.gov, 2021). In a trial by d'Ettorre et al. seventy patients who tested positive for SARS-CoV-2 RNA received either the required drug therapy or a multistrain probiotic formulation containing *Streptococcus thermophilus* DSM 32345, *Lactobacillus acidophilus* DSM 32241, *L. helveticus* DSM 32242, *L. paracasei* DSM 32243, *L. plantarum* DSM 32244, *L. brevis* DSM 27961, *Bifidobacterium lactis* DSM 32246, *B. lactis* DSM 32247 in addition to the same drug therapy while hospitalization (d'Ettorre et al., 2020). All patients included in the study had a fever, cough, headache, dyspnea, myalgia, asthenia, and diarrhea. They all were required non-invasive oxygen support. Drug therapy used during the disease treatment contained hydroxychloroquine, antibiotics, and tocilizumab; however, some of the patients received only one of them while some received two or more in combination. The probiotic formulation was administered three times a day at a dose of 2.4×10^{12} CFU/day for 14 days. Their results showed that probiotic supplementation reduced the severity of COVID-19 by improving and clearing diarrhea within about 72 h and other COVID-19 associated symptoms including fever, asthenia, headache, myalgia, and dyspnea. Additionally, the risk of developing respiratory failure and ratio of patients shifted to ICU were lower in the probiotic group compared to the non-probiotic group. In another study including 200 patients with severe COVID-19 pneumonia, the same research group evaluated the commercial probiotic formulation mentioned above in addition to the drug therapy (Ceccarelli et al., 2021). They reported that the mortality rate was lower in the probiotic treated group with 11% compared to the non-probiotic group with 30%. In addition, to live bacterial cells, bacterial products such as exopolysaccharides (EPS) or dietary supplements could be used as a gut microbiota-altering strategy. EPSs have been used as prebiotics altering the gut microbial composition and improve health with *anti*-tumor and *anti*-oxidant activities (Hong and Hee, 2014; Caggianiello et al., 2016). EPSs also have been shown to enhance the gut mucosal barrier by increasing IgA production and influence systemic immune response by increasing T cell population in mice (Matsuzaki et al., 2015). Moreover, prebiotics, which are complex carbohydrates inducing the growth of beneficial microorganisms and support their activity, such as galactooligosaccharides and inulin, are capable of altering gut microbiota to prevent dysbiosis and immunomodulation (Salazar et al., 2017). Studies in elderly and overweight individuals demonstrated that galactooligosaccharides enhance the bifidobacteria population in the gut with reduced proinflammatory cytokines including TNF-α, IL-6, and IL-1β and increased *anti*-inflammatory cytokine IL-10 (Vulevic et al., 2013, 2015).

Antibiotics, although used for certain types of diarrhea, can cause antibiotic-associated diarrhea (AAD), which is a very common side effect with an imbalance in gut microbiota. AAD can cause *Clostridium difficile* associated diarrhea (CDAD) in hospitalized patients and mostly required patient isolation since it is highly contagious. Since AAD and CDAD are associated with the disruption of the microbial composition in the gut, there is growing interest in microbiota-targeted therapy for AAD and CDAD patients (Hickson, 2011). One of the microbiota-targeted strategies to treat *C. difficile* infection is fecal microbiota transplantation (FMT) from

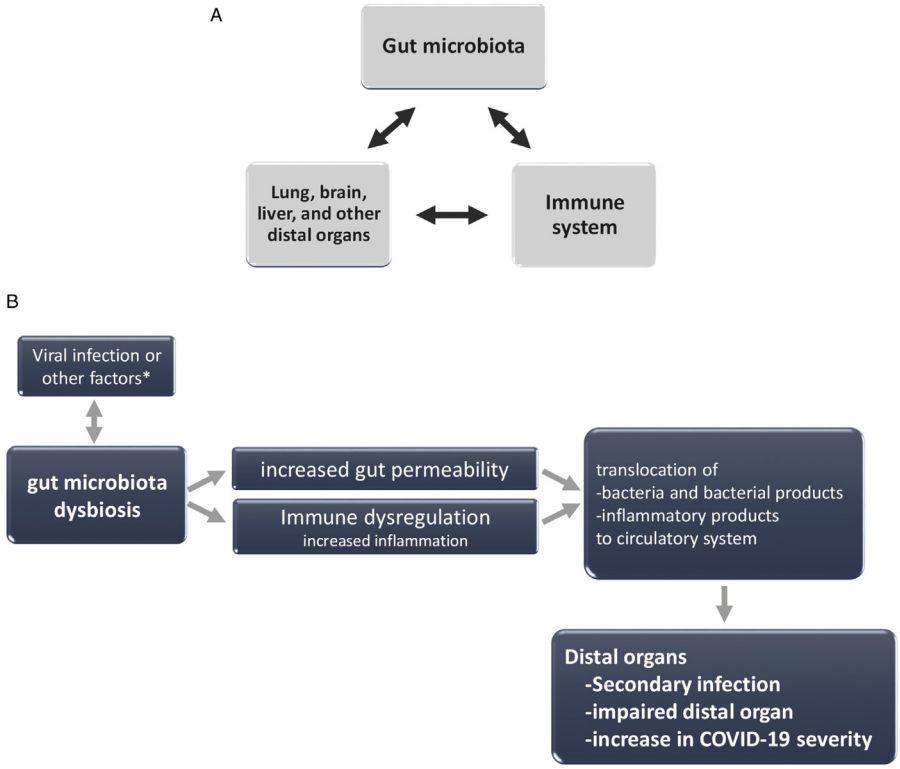

Fig. 1 Association between gut microbiota and COVID-19. (A) Cross-talk between gut microbiota and other organs with a balanced relationship in healthy individuals; (B) Possible role of gut dysbiosis in COVID-19. Adapted from (Aktas and Aslim, 2020). *Underlying medical conditions, dietary habits, age, medication, etc.

a healthy donor, which cures more than 90% of the patients (Bakken et al., 2011). The success of fecal transplantation as a treatment for patients with *C. difficile* infection can be shown as a good example of maintaining health through microbiota modification. Fecal microbiota transplantation is a powerful way of altering the disrupted gut microbiota and improving immunity and could be considered to apply to COVID-19 patients to reduce the disease severity. There is one study published on the FMT approach to SARS-CoV-2 infection; however, it has some limitations such as small sample size and non-randomized design and is performed with patients cured and discharged from the hospital rather than COVID-19 patients under treatment in hospital (Liu et al., 2021). They reported that FMT restored the gut microbiota with an increase in *Bifidobacterium* and *Faecalibacterium* and suggested that FMT could be a potential rehabilitative approach for the COVID-19 via improving gut dysbiosis. Additionally, one clinical trial is ongoing with patients hospitalized due to COVID 19 disease to investigate the impact of FMT on COVID-19 progression with increased inflammation (ClinicalTrials.gov, 2021).

Conclusion

The balanced relationship between the host immune system and the gut microbiota plays an important role in calibrating the host responses against infections. Disruption of this homeostatic relationship influences the immune response, inflammation and disease severity. Healthy gut microbiota ensures immune homeostasis. Studies show that there is an interaction between gastrointestinal microbiota and lung, protecting host homeostasis and disease development. Gut-lung-axis involvement in COVID-19 severity, therefore, is possible with the association of dysbiosis (**Fig. 1**). Targeted alterations in the gut microbiota could be considered to alleviate the disease severity.

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