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Potential causes and consequences of gastrointestinal disorders during a SARS-CoV-2 infection

François Trottein[1,5*] & Harry Sokol[2,3,4]

[1]Centre d'Infection et d'Immunité de Lille, Inserm U1019, CNRS UMR 9017, University of Lille, CHU Lille- Institut Pasteur de Lille, F-59000 Lille, France.

[2]Sorbonne Université, INSERM, Centre de Recherche Saint-Antoine, CRSA, AP-HP, Saint Antoine Hospital, Gastroenterology department, F-75012 Paris, France

[3]INRA, UMR1319 Micalis & AgroParisTech, F-78350 Jouy en Josas, France

[4]Paris Center for Microbiome Medicine, Fédération Hospitalo-Universitaire, F-75012 Paris, France

[5]Lead contact

* Correspondence: francois.trottein@pasteur-lille.fr

SUMMARY

Coronaviruses cause several human diseases, including severe acute respiratory syndrome. The global coronaviruses disease 2019 (COVID-19) pandemic has become a huge threat to humans. Intensive research on the pathogenic mechanisms used by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is urgently needed – notably in order to identify potential drug targets. Clinical studies of patients with COVID-19 have shown that gastrointestinal disorders appear to precede or follow the respiratory symptoms. Here, we review gastrointestinal disorders in patients with COVID-19, suggest hypothetical mechanisms leading to gut symptoms, and discuss the potential consequences of gastrointestinal disorders on the outcome of the disease. Lastly, we discuss the role of the gut microbiota during respiratory viral infections and suggest that targeting gut dysbiosis may help to control the pathogenesis of COVID-19.

KEY WORDS

SARS-CoV-2, COVID-19, gastrointestinal symptoms, gut microbiota, microbial dysbiosis, disease outcomes.

INTRODUCTION

Coronaviruses are enveloped RNA viruses containing a large (25 to 32 kb) single-stranded, positive-sense RNA genome. These viruses circulate continuously in human populations and generally cause mild respiratory diseases, including the common cold. In contrast, the zoonotic severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) cause severe respiratory diseases and are associated with a high mortality rate when they spread to humans (Ferh et al., 2017). No specific antiviral drugs or vaccines have approved for combating infections by SARS and MERS. In late 2019, a new infectious respiratory disease (now termed coronavirus disease 19 (COVID-19)) emerged in Wuhan, China (Wang et al., 2020a; Zhu et al., 2020). This disease spread rapidly across China and to other countries. The World Health Organization recently declared the COVID-19 outbreak to be a pandemic. SARS-CoV-2 is the etiologic agent of COVID-19 (Zhu et al., 2020). A phylogenetic analysis has shown that SARS-CoV-2 is most closely related to SARS-CoV (nucleotide similarity: 89.1%). COVID-19 is characterized by acute pathological outcomes, including pneumonia and acute respiratory distress syndrome (ARDS), and has a substantial mortality rate (Chen et al., 2020; Chen et al., 2020b). The ARDS during a SARS-CoV-2 infection is also associated with dysregulation of cytokine production, barrier leakage, and organ dysfunction. COVID-19 is more severe in individuals with comorbidities (diabetes mellitus, cardiovascular diseases, hypertension) and in the elderly (Guan et al., 2020; Wu et al. 2020).

SARS-CoV-2 can target the gastrointestinal tract in humans

Both SARS-CoV and SARS-CoV-2 can infect host cells when the viral spike protein binds to its cell surface receptor angiotensin-converting enzyme II (ACE2). It is noteworthy that with the exception of human coronavirus hCoV-NL63, the seven other human coronaviruses have

other host cell receptors (also exopeptidases). ACE2 is a homolog of angiotensin I-converting enzyme (ACE), the central enzyme in the renin-angiotensin-aldosterone system (RAAS) which is crucial for the physiology and pathology of all the organs (Gheblawi et al., 2020). ACE2 maintains the homeostasis of RAAS as a negative regulator. Virus entry also requires the cleavage of the spike protein ("priming") by a type II transmembrane serine protease called TMPRSS2. This priming step is essential for fusion of the viral and cell membranes (Matsuyama et al., 2010; Hoffman et al., 2020; Zhou et al., 2020a). Additional proteases (including furin) might be involved in SARS-Cov-2 priming (Coutard et al., 2020). It is noteworthy that SARS-CoV-2's binding affinity for human ACE2 is 10 to 20 times greater than that of SARS-CoV; this disparity might be due to differences in spike protein methylation and/or cleavage sites (Jin et al., 2020, Wang et al. 2020b). Hence, the expression of ACE2 is essential for SARS-CoV-2's entry into host cells. As discussed below, changes in ACE2's functional activity might have a critical impact on the disease outcome. In humans, ACE2 and TMPRSS2 are strongly expressed in lung tissue in general and by epithelial cells in particular. This strong expression explains why the lung appears to be the most vulnerable target organ during COVID-19. A recent single-cell analysis revealed that more than 80% of ACE2-expressing pulmonary cells were type II alveolar cells (Zhao 2020); hence, this cell type might have a crucial role in coronavirus invasion and replication. Bronchial transient secretory cells (with a phenotype between those of goblet cells and ciliated cells) also express high levels of ACE2 (Lukassen et al., 2020). Furthermore, epithelial cells in the upper respiratory tract (specifically nasal goblet/secretory cells and ciliated cells) express both ACE2 and TMPRSS2 (Sungnak et al., 2020). This combined expression in the upper airways may be correlated with enhanced transmission of the virus. ACE2 is also expressed in many extrapulmonary tissues, including the heart, liver, kidney, and intestine (Crakower et al., 2002; Hamming et al., 2004; Hashimoto et al., 2012). In the latter, high levels of ACE2 are

found on the luminal surface of differentiated epithelial cells in the small intestine, whereas levels are lower in the crypt cells and in the colon (Hashimoto et al., 2012; Liang et al., 2020). It is noteworthy that human proximal and distal enterocytes co-express dipeptidyl peptidase-4 (the receptor for MERS-CoV) and alanine aminopeptidase (the receptor for HCoV-229E) (Liang et al., 2020). In the intestine, ACE2 acts as a co-receptor for nutrient uptake and especially amino acid absorption from food (Hashimoto et al., 2012). On one hand, the gut might be a major entry site for SARS-CoV-2; firstly, this would suggest that consumption of contaminated food can propagate the virus in humans. On the other, the intestinal expression of ACE2 might have essential implications for fecal-oral transmission and thus the containment of viral spreading. As seen during previous coronavirus outbreaks, around half of all COVID-19 patients have detectable SARS-CoV-2 RNA in their stools - even when it is no longer found in the respiratory tract (Zhang et al., 2020; Xiao et al., 2020, Wang et al., 2020c). The virus has also been detected in gastrointestinal histological samples and by endoscopy (Xiao et al., 2020a; Lin et al., 2020). Importantly, infectious viruses were detected in fecal samples of COVID-19 patients suggesting that the digestive tract might be a site of viral replication and activity (Xiao et al., 2020b; Zhou et al. 2020c). In line, several recent studies using human small intestinal organoids have shown that SARS-CoV-2 replicates in enterocytes (Zhou et al., 2020c; Zang et al., 2020; Lamers et al., 2020). The presence of virus in the stools and its fecal persistence suggest that fecal-oral transmission is possible. In line, a recent study (mouse system) reported that intragastric inoculation of SARS-CoV-2 causes productive infection and leads to pulmonary pathological changes (Sun et al., 2020). Collectively, the lung is not the only site targeted by SARS-CoV-2 and enteric infection occurs. Here, we review gastrointestinal disorders in patients with COVID-19, suggest hypothetical mechanisms leading to gut symptoms, and discuss the potential consequences of gastrointestinal disorders on the outcome of the disease.

SARS-CoV-2 causes gastrointestinal disorders in humans

Various research groups have analyzed the epidemiological and clinical characteristics of gastrointestinal disorders in patients with COVID-19. The percentage of patients with gastrointestinal disorders (as evidenced by vomiting, nausea and/or diarrhea) varies depending on the study. In China, Jin and colleagues enrolled 651 patients on admission, i.e. before treatment with antiviral or antibiotic drugs which might have biased the analysis (Jin et al., 2020). Interestingly, 11.4% of COVID-19 patients had at least one gastrointestinal tract symptom - the most common being diarrhea (in 5 to 8% of patients). In Jin and colleagues' study, the intestinal disorders lasted for a median of 4 days and appeared to precede the respiratory symptoms. Gastrointestinal symptoms were more frequent in severe/critical cases of COVID-19 (23%) than in mild COVID-19 (8%). In other studies performed in China and Hong Kong, the proportion of COVID-19 patients (n total = 254, 59, 204, 58, 138 and 1099 respectively) having developed gastrointestinal disorders was 26%, 25.4%, 18.6%, 11%, 13.7% and 8.7%, respectively (Zhou et al., 2020b; Cheung et al., 2020; Pan et al., 2020; Lin et al., 2020; Wang et al., 2020c, Guan et al., 2020). Other studies (of 40 COVID-19 patients in Europe and 278 and 318 in the USA) reported that 55%, 35% and 61% of the patients displayed gastrointestinal signs and symptoms (Effenberger et al. 2020; Nobel et al., 2020; Redd et al. 2020). Importantly, meta-analyses of studies totaling 4.243, 6.686 and 10.890 patients found that the pooled prevalence of gastrointestinal symptoms was 17.6%, 15% and 10% (Cheung et al., 2020; Mao et al., 2020; Sultan et al., 2020). It is noteworthy that SARS-CoV-2 infection triggers an inflammatory response in the gut, as evidenced by elevated fecal levels of calprotectin (a marker protein expressed mainly by neutrophils) (Effenberger et al., 2020). Lin and colleagues also observed that while the presence of SARS-CoV-2 in the stool does not correlate with gastrointestinal symptoms, the presence of SARS-CoV-2 in intestinal

tissue is generally associated with severe gastrointestinal symptoms (Lin et al., 2020). The use of human small intestinal organoids will be instrumental to better analyze the modalities of enterocyte infection and its impact on the inflammatory response. Furthermore, it will be interesting in the future to determine the consequences of pre-existing digestive diseases (*i.e.* inflammatory bowel disease) on gastrointestinal disorders caused by SARS-CoV-2 and on the severity of COVID-19.

Potential mechanisms of gastrointestinal disorders in COVID-19

Gastrointestinal disorders in COVID-19 patients are likely to have several etiologic factors. The recruitment of inflammatory cells (*i.e.* from the bone marrow) and/or the systemic and local production of inflammatory cytokines are likely to have a role. In this setting, local inflammation might weaken the epithelial barrier. Cellular damage induced directly by viral replication and spreading might also actively contribute to injury and inflammation of the gut epithelium. Gastrointestinal symptoms in COVID-19 patients might also be due to dysfunction of ACE2. As mentioned above, ACE2 is a key regulatory enzyme in the RAAS, and the latter is known to influence immune functions and inflammation (Ranjbar et al. 2019). Indeed, ACE2 is an essential regulator of intestinal homeostasis, and a lack of this enzyme accentuates the intestine's susceptibility to inflammation (Hashimoto et al., 2012). The intestinal phenotype is due (at least in part) to ACE2's non-catalytic function. A lack of ACE2 alters the expression of the neutral amino acid transporter B^0AT1 on intestinal epithelial cells, which lowers the intake of tryptophan and thus reduces the production of nicotinamide and (potentially) other tryptophan-derived metabolites with a critical role in intestinal homeostasis (Hashimoto et al., 2012; Agus et al., 2018). Binding of the viral spike protein to ACE2 leads to reduced expression of the latter but does not change the expression of ACE (Kuba et al., 2005). During SARS-CoV infections, the expression of ACE2 is considerably reduced - at

least in the lungs (Kuba et al., 2005). Given the key role of ACE2 in intestinal homeostasis, reduced expression and impaired function of this protein during a SARS-CoV infection might substantially contribute to local disorders and pathogenesis. Of note, it still remains to be seen whether drugs able to modulate ACE2 expression, such as ACE inhibitors or angiotensin II receptor blockers (used in hypertension and diabetes), can modulate gastrointestinal disorders, including gut dysbiois, in the context of SARS-CoV-2 infection. Along with inflammation and dysfunction of ACE2, other mechanisms might be implicated in gastrointestinal symptoms in COVID-19 patients. As hypoxia is a major clinical symptom in COVID-19 patients (Vavezzi et al. 2020) and is known to be critical in intestinal homeostasis, including microbiota composition and function (Singhal & Shah., 2020), it is also likely that oxygen deprivation might be important in gastrointestinal disorders and disease severity. Recent evidence suggests that SARS-CoV-2 may affect the central nervous system (Paniz-Mondolfi et al. 2020; Helms et al. 2020). Regarding the importance of the gut-brain axis, one can speculate that this may play a role in gastrointestinal disorders during SARS-CoV-2 infection. It is possible that the enteric nervous system could be impacted by SARS-CoV-2, either by direct viral infection or through the elicited immune response (*e.g.* inflammatory cytokines), amplifying the diarrhea and possibly stimulating the vagus nerve to promote vomiting.

ACE2 dysfunction alters the composition of the gut microbiota

It is well established that the gut microbiota has a critical role in intestinal homeostasis and that changes in its composition and function activity are involved in local inflammation (Blander et al., 2017; Lavelle & Sokol. 2020). A lack of ACE2 leads to a substantial alteration in the composition of the gut microbiota in mice; this is due in part to reduced production of the antimicrobial peptides that control the gut's microbial community (Hashimoto 2012). Moreover, disruption of the ACE/ACE2 axis during pulmonary hypertension (loss of ACE2)

is associated with alteration of the gut microbiota in humans (Santisteban et al., 2016; Kim et al., 2020). One can speculate that the decrease in ACE2 availability during SARS-COV-2 infection is enough to alter the composition of the gut microbiota. Recent pilot studies suggest gut microbiota alterations during SARS-CoV-2 infection (Yu et al., MedRxiv 2020, Gu et al. 2020b, Zuo et al. 2020). In a cohort of 30 COVID-19 patients (fecal sample analysis using 16S rRNA gene sequencing), infection associated with a significant decrease of bacterial diversity and abundance (Gu et al., 2020b). Significant changes in gut microbial communities were noticed including lower relative abundance of beneficial (butyrate-producing) bacteria such as several genera from the *Ruminococcaceae* and *Lachnospiraceae* families. On the other hand, a significantly higher relative abundance of opportunistic pathogens including *Streptococcus*, *Rothia*, *Veillonella* and *Actinomyces* occurred. Of interest, the gut microbial signature of patients with COVID-19 was different from that of H1N1 patients (Gu et al. 2020b). Enrichment of opportunistic pathogens and depletion of beneficial commensals was also observed in another (longitudinal) study using deep shotgun metagenomics (15 COVID-19 patients) (Zuo et al. 2020). In particular, an enrichment of opportunistic pathogens known to cause bacteremia, including *Clostridium hathewayi*, *Actinomyces viscosus* and *Bacteroides nordii* was observed. A lower relative abundance of beneficial commensals including the anti-inflammatory bacterium *Faecalibacterium prausnitzii, Alistipes onderdonkii*, *Roseburia and Lachnospiraceae taxa* was also noticed. The authors found a correlation between the rise or the drop of these bacteria and disease severity. Of note, altered microbiota composition persisted after clearance of SARS-CoV-2 and resolution of respiratory symptoms indicating that resiliency is long-lasting (Zuo et al. 2020). Hence, SARS-CoV-2 infection modifies the composition of the gut microbiota in humans. It is possible that reduced levels of commensal bacteria with important physiological functions, such as butyrate producers, may promote the overgrowth of intestinal conditional pathogenic bacteria. Whether these changes increase

intestinal mucosal permeability and endotoxin concentrations in the blood, ultimately triggering inflammation and cytokine release exacerbation, remains to be investigated. Further analyses are urgently needed in larger human cohorts to confirm and to further detail gut microbiota alterations in COVID-19 patients. Studies should prospectively include asymptomatic COVID-19 confirmed subjects and patients at disease onset, during disease course, and long term after discovery to delineate the role of microbiome changes in SARS-CoV-2 infection and post-infection recovery. It will be critical to consider patients treated or not with antibiotics (and other medications) and the co-morbidity status of those patients. The consequences of SARS-CoV-2 infection on the gut microbiota should also be investigated in relevant animal models of COVID-19. Possible models include non-human primates, ferrets, human ACE2-expressing mice, and hamsters (Callaway 2020). The impact of SARS-CoV-2 on the gut microbiota' metabolic output also remains to be defined. This aspect is of particular importance because, in a mouse model, the altered gut microbiota associated with ACE2 deficiency (i) favors intestinal inflammation and (ii) confers susceptibility to colitis when transferred to wild-type animals (Hashimoto et al., 2012). In parallel, it remains to be determined whether alterations in the microbiota influence the extra-intestinal outcomes of COVID-19. Together, direct enterocyte infection, disruption of the enteric ACE2 axis (and interference with nutrient absorption), hypoxia, alterations of the enteric nervous system and local immune response, and changes in inflammatory cytokine levels might lead to adverse intestinal outcomes (including dysbiosis) in patients with COVID-19.

The potential role of the gut microbiota in COVID-19 outcomes

Recent evidence suggests that the gut microbiota can remotely boost the host's response to respiratory viral infections. Paradoxically, dysbiosis of the microbiota can also worsen the outcome of the disease. The gut microbiota's role in human coronavirus infections has yet to

be defined. In the setting of influenza infections, the results of experiments with antibiotic treatment (to deplete the residual microbiota) have demonstrated that the gut microbiota is critical for controlling viral replication (Ichinohe et al. 2011; Abt et al., 2012; Steed et al. 2018; Bradley et al. 2019). Bacterial cell wall components and bacterial metabolites (such as desaminotyrosine) favor the production of type I interferons and inflammasome-dependent cytokines by pulmonary cells. The gut microbiota also boosts $CD8^+$ T cell effector function, a process that also contributes to viral clearance (Ichinohe et al. 2011). Whereas altering the gut microbiota with antibiotics increases the severity of the infection, stimulating the microbiome with a high-fiber diet has the opposite effect (Trompette et al., 2018). The stimulatory mechanisms involve short-chain fatty acids (SCFAs, the end-products of dietary fiber fermentation by commensal bacteria) that are absorbed by and act on immune cells to reduce the inflammatory component of infection. SCFAs also enhance the effector activity of $CD8^+$ T cells by stimulating cellular metabolism (Trompette et al., 2018). Similar protective effects have been observed with the respiratory syncytial virus (Antunes et al., 2019). It remains to be seen whether the gut microbiota also participates in the early control (through innate immunity) and late control (through adaptive immunity) of coronavirus replication, and this topic warrants investigation. It would also be useful to investigate the effect of SCFA-promoting high-fiber diets and/or probiotics on the outcome of SARS-CoV-2 infection.

Patients with respiratory infections generally have gut-dysfunction-related complications that worsen the clinical course. For example, this type of gut-lung crosstalk has been described during influenza infections in both humans and animal models. Severe influenza A virus infection is associated with intestinal disorders and gut microbiota alterations (Wang et al., 2014; Qin et al., 2015; Deriu et al., 2016; Groves et al., 2018; Yildiz et al., 2018; Sencio et al., 2020). These changes were attributed in part to an infection-related reduction in food consumption (Sencio et al., 2020) and to interferon production (Wang et al., 2014, Deriu et

al., 2016). We recently looked at whether influenza A virus-conditioned microbiota enhanced susceptibility to bacterial superinfection - a major cause of mortality during epidemics and pandemics (McCullers 2014). In fecal transfer experiments, we showed that the dysbiotic microbiota remotely compromises the lung's defenses against pneumococcal infection. In mechanistic terms, a reduction in SCFA production is associated with a decrease in the alveolar macrophages' bactericidal activity (Sencio et al. 2020).

Whether gut disorders (including microbiota alterations) influence on outcomes in COVID-19 is still an open question. Recent evidence suggests that SARS-CoV-2 infection alters the gut-blood barrier and thus leads to systemic dissemination of bacteria, endotoxins, and microbial metabolites (Wang et al., 2020; Huang et al., 2020; Guan et al., 2020). This might affect the host's initial response to SARS-CoV-2 infection and might then contribute to multisystem dysfunction, septic shock, and the systematic inflammation storm seen in the second phase of SARS-CoV-2 infection, which typically result in death (Guan et al., 2020; Huang et al., 2020; Wang et al., 2020c). In fact, clinical studies have demonstrated that the presence of a gastrointestinal disorder in COVID-19 patients is associated with a more aggressive clinical course, including ARDS, liver injury, a higher body temperature, and shock (Jin et al. 2020). Moreover, the risk factors for severity and mortality in COVID-19 (such as diabetes (Singh et al. 2020)) are known to be associated with disturbances of the intestinal microbiota and, in particular, low production of SCFAs. This is particularly the case for patients with metabolic syndrome (obesity, high blood pressure and diabetes), who exhibit severity factors for viral infections (including respiratory infections) (Badawi et al. 2018; Honce et al. 2019). The role of intestinal dysbiosis in this setting needs to be investigated, e.g. by performing fecal transfer experiments in relevant animal models. We speculate that ACE2 disruption, changes in microbial profiles, and intestinal inflammation and leakiness during SARS-CoV infection strongly amplify systemic pathways in co-morbid individuals and exacerbate underlying

pathologies (e.g. diabetes and hypertension). SARS-CoV-2 infection may also deteriorate the course of human inflammatory bowel diseases (Effenberger et al. 2020). The mortality rate associated with SARS-CoV2 infection is higher in older adults, who also tend to display chronic low-grade inflammation with underlying microbiota alterations and gut leakiness. In the setting of COVID-19, can we exploit the gut microbiota for the patient's benefit? At present, there is no direct clinical evidence to suggest that modulation of the gut microbiota has therapeutic value in patients with COVID-19. However, it is tempting to speculate that targeting gut microbiota might be a valuable therapeutic option.

CONCLUDING REMARKS

The complex pathogenesis of SARS-CoV-2 infection is only starting to be deciphered. Along with the respiratory tract, the gastrointestinal tract is an entry and replication site for SARS-CoV-2. The gut constitutes one of the main extrapulmonary target organs with regard to symptoms and is a potential route for virus dissemination; its role therefore needs to be actively explored. In particular, several lines of evidence suggest that SARS-CoV-2 infection is associated with alteration of the gut microbiota. It remains to be determined whether the gut microbiota influences the gastrointestinal and pulmonary signs and symptoms of COVID-19 and overall mortality. Putative changes in the gut microbiota's composition and functional activity might be biomarkers of disease severity. Lastly, and if the gut microbiota does prove to have an impact on the disease's severity and mortality rate, targeting the microbiota's various components might be an attractive therapeutic strategy. In this setting, conventional approaches like antibiotic treatment targeting pathobionts (preferably with a narrow-spectrum drug), probiotics, and fecal transfer might be relevant. However, more specific interventions (e.g. live biotherapies or microbiota-derived metabolites) based on an in-depth understanding of the mechanisms involved are attractive.

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AUTHOR CONTRIBUTIONS

F.T. and H.S. wrote the manuscript.

DECLARATION OF INTERESTS

The authors have no conflicts of interest.

FIGURE TITLES AND LEGENDS

Figure 1. The gut microbiota's hypothetical role in SARS-CoV-2 infection.

A healthy gut microbiota boosts the lungs' antiviral response, including interferon production and the effector function of $CD8^+$ T cells (step 1). Soon after infection, SARS-CoV-2 replicates in the intestinal compartment and decreases the expression and activity of ACE2 (step 2). This leads to gut dysbiosis and gastrointestinal symptoms (step 3). Meanwhile, viral infection of the lungs triggers the systemic production of interferons and elicits weight loss - both of which triggering gut dysbiosis (step 3). Changes in the composition and functional activity of the gut microbiota and impairment of the gut's barrier function contribute to

disease outcomes, including ARDS, a systemic cytokine storm, and multi-organ dysfunction (step 4).

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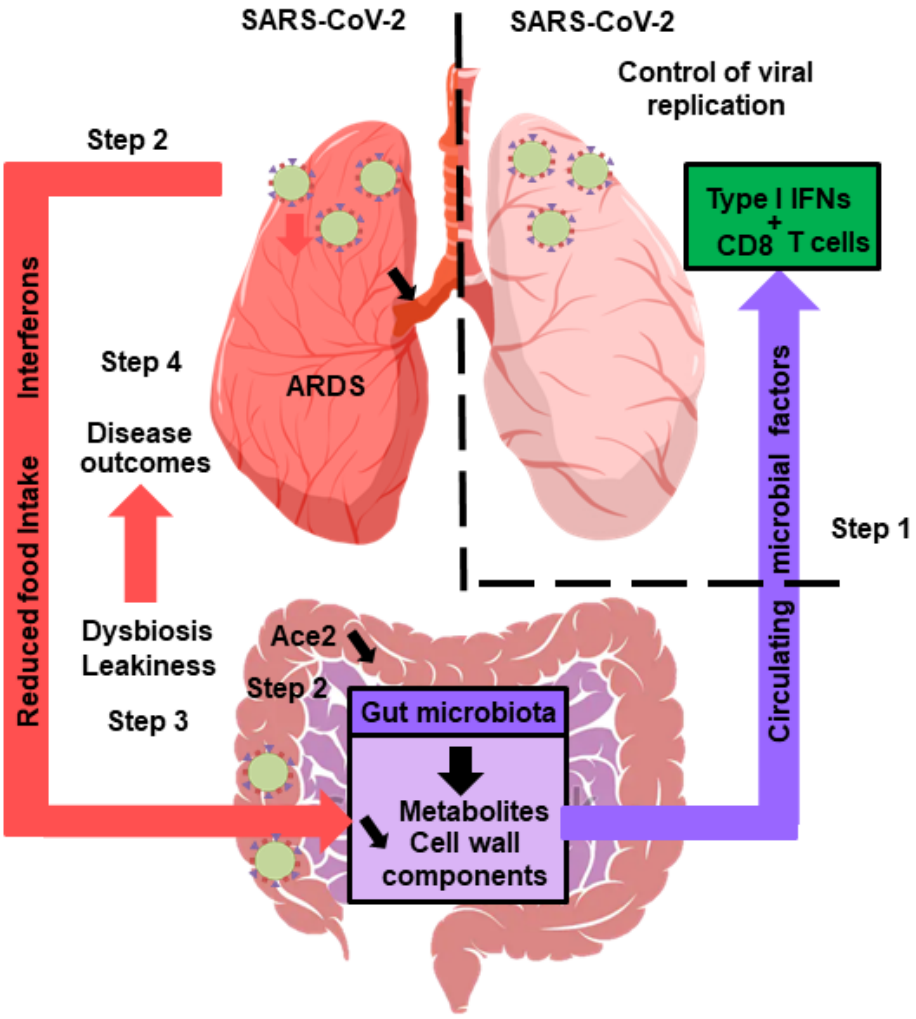
In Brief

In this review, Trottein & Sokol present hypothetical mechanisms leading to gut symptoms in patients with COVID-19 and discuss their potential consequences on disease severity. They also discuss the role of the gut microbiota in disease and the potential interest of targeting it to improve COVID-19 pathogenesis.


SARS-CoV-2
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Control of viral replication
Step 2
Type I IFNs + CD8 T cells
Interferons
Step 4
ARDS
Disease outcomes
Reduced food Intake
Step 1
Circulating microbial factors
Dysbiosis Leakiness
Step 3
Ace2
Step 2
Gut microbiota
Metabolites Cell wall components