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MINIREVIEWS

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Abstract

The disease coronavirus disease 2019 (COVID-19) is a severe respiratory illness that has emerged as a devastating health problem worldwide. The disease outcome is heterogeneous, and severity is likely dependent on the immunity of infected individuals and comorbidities. Although symptoms of the disease are primarily associated with respiratory problems, additional infection or failure of other vital organs are being reported. Emerging reports suggest a quite common co-existence of gastrointestinal (GI) tract symptoms in addition to respiratory symptoms in many COVID-19 patients, and some patients show just the GI symptoms. The possible cause of the GI symptoms could be due to direct infection of the epithelial cells of the gut, which is supported by the fact that (1) The intestinal epithelium expresses a high level of angiotensin-converting enzyme-2 and transmembrane protease serine 2 protein that are required for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) entry into the cells; (2) About half of the severe COVID-19 patients show viral RNA in their feces and various parts of the GI tract; and (3) SARS-CoV-2 can directly infect gut epithelial cells *in vitro* (gut epithelial cells and organoids) and *in vivo* (rhesus monkey). The GI tract seems to be a site of active innate and adaptive immune responses to SARS-CoV-2 as clinically, stool samples of COVID-19 patients possess proinflammatory cytokines (interleukin 8), calprotectin (neutrophils activity), and immunoglobulin A antibodies. In addition to direct immune activation by the virus,

impairment of GI epithelium integrity can evoke immune response under the influence of systemic cytokines, hypoxia, and changes in gut microbiota (dysbiosis) due to infection of the respiratory system, which is confirmed by the observation that not all of the GI symptomatic patients are viral RNA positive. This review comprehensively summarizes the possible GI immunomodulation by SARS-CoV-2 that could lead to GI symptoms, their association with disease severity, and potential therapeutic interventions.

Key Words: COVID-19; Gastrointestinal symptoms; Pathogenesis; Innate immune response; Adaptive immune response; Gut microbiota; Dysbiosis; Therapeutics; Probiotic; Pre-existing diseases

Core Tip: Coronavirus disease 2019 (COVID-19) is a global pandemic. Many COVID-19 patients either present gastrointestinal (GI) symptoms in addition to respiratory symptoms or just GI symptoms. Syndrome coronavirus 2 (SARS-CoV-2) directly infects GI epithelial cells as they express significant levels of angiotensin-converting enzyme-2 and transmembrane protease serine 2 protein, required for SARS-CoV-2 entry. This article reviews gut infection and GI immunomodulation by SARS-CoV-2, leading to spectrum of GI symptoms and pathogenesis in COVID-19-patients. Special emphases are given on the innate and acquired immune responses in the GI tract due to intestinal and non-intestinal SARS-CoV-2 infection, COVID-19 severity in people with pre-existing intestinal diseases, role of gut microbiota, and possible therapeutic interventions are discussed.

INTRODUCTION

In December 2019, pneumonia cases with unrecognized etiology were reported in the Wuhan city of China, causing fever and acute respiratory distress. The causative agent is a novel coronavirus named syndrome coronavirus 2 (SARS-CoV-2), and the disease is referred to as coronavirus disease 2019 (COVID-19)[1,2]. SARS-CoV-2 belongs to the order "Nidovirales"; family of "Coronaviridae", and subfamily "Orthocoronavirinae" [3]. Coronaviruses are single-stranded RNA viruses, usually zoonotic but have regularly affected humans to cause a major health crisis[4,5]. COVID-19 rapidly spread like an epidemic in China, followed by worldwide transmission of the infection and, therefore, was declared a pandemic and global crisis by the World Health Organization (WHO) in March 2020. As of May 23, 2021, the WHO COVID-19 dashboard reported 166352007 confirmed cases of COVID-19, and 3449189 deaths worldwide, making this one of the worst pandemics in the 21st century. The high rate of human-to-human transmission, asymptomatic carriers, and the absence of therapeutic intervention led to the global pandemic.

The evolution of new variants of the virus has made the situation even worse. New SARS-CoV-2 strains are emerging like B.1.351 was detected in South Africa, B.1.207 in Nigeria, while strain B.1.1.7 was identified in the United Kingdom in December 2020 and is highly infectious. The new strains like B.1.1.7 strain harbor several mutations, especially in the S protein, including the N501Y (asparagine to tyrosine substitution), 69/70 deletion. P681H and enhances the virus-angiotensin-converting enzyme-2 (ACE-2) binding efficacy, thereby making the variants highly contagious. B.1.617.1, B.1.617.2, and B.1.617.3 are the three subtypes of the Indian variant reported in October 2020 is highly infectious and causing fresh waves of infection in many countries around the world. Three important mutations in the sequence coding for the viral spike protein co-occur in variant B.1.617.1: L452R, E484Q, and P681R. B.1.617.2 is also linked to the L452R, T478K, and P681R mutations[6]. Indications suggest that

these variants can trigger severe disease conditions or higher fatality rates. The complete impact of these mutations is not yet understood and is still being researched, but comprehensive genomic strain surveillance is needed to better understand the strain-specific infection, pathogenesis, epidemiological and therapeutic aspects. Several potential therapeutic and prophylactic interventions are under investigation or have undergone randomized controlled trials. Great strides have been made in vaccine development, and COVID-19 vaccines are now approved for mass use in several countries. Raising hopes for curbing the COVID-19 crisis and WHO's guidelines on wearing a mask, social distancing, and sanitization needs to be strictly followed to bend down the infection curve.

Though COVID-19 mainly causes respiratory illness, many patients experience gastrointestinal (GI) symptoms, including nausea, vomiting, belly pain, appetite loss, and diarrhea. GI symptoms are often associated with the presence of CoV2 RNA in many patients' stool (feces) samples[7]. Though the mechanism of lung infection is widely studied, there is a dearth of information regarding the enteric phase of SARS-CoV-2, especially the immune contexture and response. The gut microbiome is considered to play a key role in regulating the impact of SARS-CoV-2, and significant alterations in the microbiota profiles are reported in COVID-19 patients. The role of the gut-lung axis and the severe respiratory distress associated with gut imbalance is also very relevant[8]. COVID researchers have reported a disturbance of the gut microbiota and its association with lung and gut infections, which can cause hindrance in the gut-lung axis. Recent data suggest that GI symptoms might be a warning sign of a more serious condition with poor prognosis. Because of the GI infection and COVID severity, the present paper deals with a complete review of the COVID-19-associated gut-infection, pathogenesis, innate and acquired immune responses, gut microbiota, and possible therapeutic intervention. Figure 1, is a schematic showing SARS-CoV-2 infection and activation of cell death-associated release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), associated inflammation and host intracellular immune response.

COVID-19 PATHOGENESIS AND INFECTION PROCESS

Human-to-human transmission of COVID-19 can occur when the infectious respiratory droplets of patients are transmitted as droplets or aerosol that finally gets deposited into the nasal, oral, and conjunctival mucosa of an uninfected human being. SARS-CoV-2 prominently attacks the lungs and infects other organs such as the gut, heart, blood vessels, kidney, cortex, and central nervous system[9]. SARS-CoV-2 infects host cells when the viral spike (S) protein binds to the cell surface receptor ACE2. Thereby, ACE2 is the crucial cellular receptor for the entry of SARS-CoV-2[10]. Two functional domains are found in the S protein: A receptor-binding domain and a second domain with S1/S2 cleavage site containing multiple arginine residues that must be cleaved by cell proteases for cellular entry. The furin-mediated pre-cleavage of the S1/S2 site leads to further activation of viral fusion to the cells by trans-membrane protease serine 2 protein (TMPRSS2)[11,12]. ACE2 receptors and TMPRSS2 are expressed in various human cells susceptible to viral infection, including epithelial cells in the lungs, small intestine, and colon, tubular cells of the kidney, neuronal and glial cells in the brain, enterocytes, vascular endothelial cells, smooth muscle cells and cardiomyocytes[13]. Viruses are shed in the feces long after the resolution of the pulmonary symptoms, making the fecal-oral route of SARS-CoV-2 transmission a possibility. Single-cell transcriptomics data suggest that the GI epithelium, especially the enterocytes lining of the ileum and colon, shows a higher frequency of coexpression of both the ACE2 and TMPRSS2 and therefore, are conducive for SARS-CoV-2 interaction and infection, which may explain the GI pathogenesis[14,15]. The viral entry is associated with the release of proinflammatory cytokines, immune cell infiltration, and overall immune activation leading to inflammation. The infection-associated GI-specific symptoms include anorexia, watery diarrhea, nausea and vomiting, and associated abdominal pain[14] (Figures 1 and 2).

COVID-19 AND GI SYMPTOMS

Similar to lung infection, the GI-infection by SARS-CoV-2 triggers an antiviral immune response characterized by the release of interferon (IFN), cytokines, and chemokines in the infected cells. Figure 2 presents a brief overview of the GI infection routes and
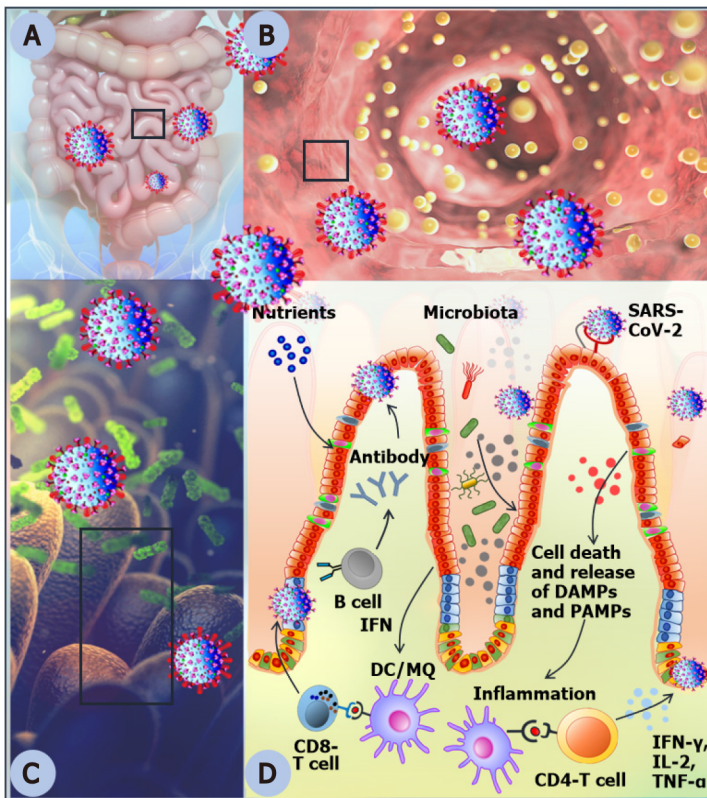
Figure 1 COVID-19 and gut Immunomodulation. A: Model is showing gut infection; B: Zoomed in area of the gut; C: Zoomed in representation of an area showing the intestinal crypts; D: Zoomed in C, showing a histological representation of intestinal crypts. The intestinal epithelium is folded and organized into crypts and villus. Villus is the finger-like projections gutting out towards the lumen of the intestine (red cells). The crypts base (shown in yellow and green cells) houses the intestinal stem cells, while the blue cells comprise the transit-amplifying cells. SARS-CoV-2 activates angiotensin-converting enzyme 2 receptors, and epithelial cell death-associated release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). DAMPs and PAMPs are considered a danger signal by immune cells, especially the dendritic cells, macrophages, and innate immune cells. This damage recognition is associated with proinflammatory cytokine production (like Interferon, tumor necrosis factor-α), followed by immune infiltration and virus-specific B and T cell response. CD8+ T cells undergo clonal expansion and kill the infected cells and launch an antiviral attack. B cells differentiation to plasma cells can lead to antiviral antibody production and subsequent neutralization of SARS-CoV-2[10,14]. Some images (Free Stock Media) are downloaded from Canva.com using subscription. IFN: Interferon; TNF-α: Tumor necrosis factor-α; DAMP: Damage-associated molecular patterns; PAMP: Pathogen-associated molecular patterns; DC: Dendritic cells; MQ: Macrophages.

symptoms. These inflammatory mediators promote infiltration of neutrophils, macrophages, and T cells to the site of infection, resulting in enteric inflammation that may lead to diarrhea and other GI symptoms[10]. Studies have shown that the elevated fecal levels of calprotectin (a marker protein expressed mainly by neut-rophils) in patients with COVID-19 adds to the growing evidence that SARS-CoV-2 infection triggers an inflammatory response in the intestine. Calprotectin concentrations were found to be significantly higher in COVID-19 patients who had suffered from diarrhea along with elevated serum interleukin (IL)-6 levels[16]. An alternate mechanism implicated in GI symptoms in COVID-19 patients is oxygen deprivation [17]. Hypoxia is one of the major clinical symptoms in COVID-19 patients known to influence intestinal homeostasis, including microbiota composition and immune function. It is shown that oxygen deprivation (exacerbated hypoxia) can contribute to GI disorders and inflammatory disease severity[18].

The tissues that are targeted by SARS-CoV-2 go through an early phase of infection where a high viral load induces intestinal symptoms such as vomiting and diarrhea associated with COVID-19 during the initial phase in some patients. Thus, diarrhea should also generate awareness of a possible SARS-CoV-2 infection and should be investigated to reach an early diagnosis of COVID-19 to slow down its transmission instead of waiting for the respiratory symptoms to develop.

The first results linking GI symptoms with COVID-19 were obtained from a study conducted in COVID-19 confirmed patients in Wuhan, China[19]. In this study, 204 patients with COVID-19 who presented at three hospitals were analyzed. Although most patients presented with respiratory symptoms, many patients also presented with GI -specific symptoms. It is possible that GI symptoms associated with COVID-19 could be underreported due to the focus on fatal respiratory symptoms. However, a
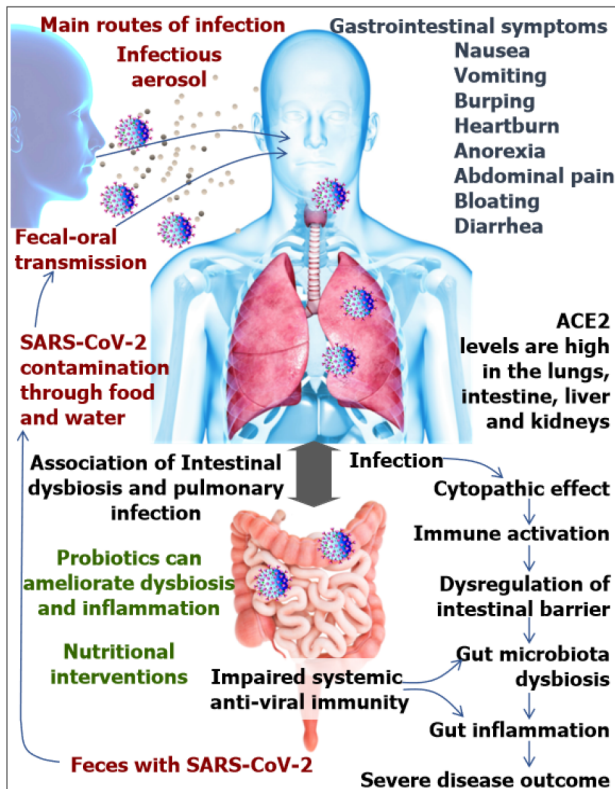
Figure 2 Illustrative model showing gastrointestinal infection routes and symptoms. The figure shows the main routes of infection. Infectious respiratory droplets or aerosols deposited on the nasal, oral, or conjunctiva may lead to person-to-person spread. SARS-CoV-2 is detected in feces of infected patients may infect by fecal-oral transmission. The right upper section of the figure also discusses the significant gastrointestinal symptoms associated with COVID-19 infection. The receptors (angiotensin-converting enzyme 2 and TMPRSS2) of SARS-CoV-2 are detected on various organs, especially the lungs, intestine, liver and kidneys. The right lower section describes the infection process leading to intestinal symptoms. After SARS-CoV-2 infection, cytopathic effect occurs due to infection and associated immune activation leading to compromised intestinal barrier function, microbial dysbiosis, and severe symptoms. Many studies have established the link between healthy intestinal flora and the gut-lung axis. COVID-19 severely induces the intestinal microbiota dysbiosis and affects the gut-lung axis, especially the immune response. Probiotics and appropriate nutritional supplements can help protect from SARS-CoV-2 associated symptoms[10,14]. Some images (Free Stock Media) are downloaded from Canva.com using subscription. ACE2: Angiotensin-converting enzyme 2.

study by Pan *et al*[20] reported that patients without GI symptoms were more likely to recover and be discharged than those with GI symptoms (60% *vs* 34%). This data indicates that GI symptoms like diarrhea may be associated with a worse outcome requiring respiratory assistance and intensive care admission. It was also found that patients with COVID-19, especially those with digestive symptoms, remained for a long time from the onset of symptoms to hospital admission with an average time of 9 d compared to patients with only respiratory symptoms who had an average admission time of 7.3 d[19]. This may indicate that those with digestive symptoms waited longer to be diagnosed in the hospital, as they were unsuspected of being SARS-CoV-2 positive in the absence of respiratory symptoms[21]. Besides, prolonged hospital stay could also be due to treatment time needed to resolve multiple symptoms in patients with GI and respiratory infections.

Wang *et al*[22] analyzed the biodistribution of SARS-CoV-2 in different tissues of patients with confirmed COVID-19[22]. In this study, SARS-CoV-2 was detected in multiple tissue specimens collected from 205 COVID-19 patients. Bronchoalveolar lavage fluid specimens showed the highest positive rates (14 of 15; 93%). However, the virus was also detected in feces suggesting that the infectious virions are secreted from the virus-infected GI cells. The virus has also been detected in GI histological samples and by endoscopy[23]. Therefore, the fecal-oral transmission could be a possible route for the viral spread. To further investigate the presence of SARS-CoV-2 in feces, Xiao *et al*[24] examined the viral RNA in feces from 73 patients with SARS-CoV-2 during their hospitalizations. Out of the 73 hospitalized patients infected with SARS-CoV-2, 39 (53.42%) tested positive for SARS-CoV-2 RNA in their stool. The study also found that 17 (23.29%) patients continued to have positive stool results after showing negative outcomes in the respiratory samples. Overall, these data suggest viral GI infection and a potential fecal-oral transmission that can last even after viral clearance in the respiratory tract, and also advocates implementing testing of the virus in feces by real-

time reverse transcription polymerase chain reaction for disease monitoring and surveillance.

IMMUNOMODULATION IN GI TRACT DUE TO INTESTINAL AND NON-INTESTINAL INFECTIONS

Clinical data suggest that co-infection of GI tract along with respiratory tract are quite prevalent[25]. Xiao *et al*[24] has reported the presence of replicating viruses in the epithelium of the GI tract[24], and the *in vitro* models of cell and organoid culture of human intestinal epithelial cells (hIECs) support efficient SARS-CoV-2 infection, replication and production of infectious de novo virus particles[25]. Intestinal viral load seems to show a stronger association with the severity of respiratory and GI symptoms in COVID-19 patients[26]. Recently, in a non-human primate (rhesus monkey) model of SARS-CoV-2 infection, *in vivo* infection of GI tract triggered reduced proliferation and increased apoptosis of intestinal epithelial and goblet cells along with intestinal inflammation by macrophages has been reported by performing immunohistochemistry for proliferation (Ki67), apoptosis (cleaved caspase 3), and recruited macrophages (CD68+), and multiplex cytokine assay of GI tract tissues[27]. These reports support immune modulation in the GI tract due to direct infection of GI tract cells by the virus or due to changes in the GI tract integrity and microbiota under the influence of systemic cytokines and hypoxic conditions or a combination of all. GI tract is a site of active immune reaction to generate tolerant immunity against various commensal pathogens and an effective immunity to fight the pathogenic infectious agents, such as bacteria, viruses, parasites, *etc.* Direct or indirect modulation in the GI tract's immune activation during SARS-CoV-2 infection seems a reason for observed GI symptoms in COVID-19 patients.

Innate immune response to SARS-CoV-2

The initial protection against pathogens is established by innate immunity. Although more studies are needed, it is reasonably convincing that intestinal epithelium gets infected and is associated with some sort of GI symptoms. Virally infected cells can recognize the virus and virus-associated molecular patterns to elicit initial innate immune pathways to release cytokines and chemokines to recruit body's innate-immune cells such as neutrophils, macrophages, *etc.* to the infected area of the gut, which further augments the inflammation in order to restrict the viral replication. This inflammatory response also promotes antigen processing and presentation to establish the adaptive immune response. However, some of the inflammatory cytokines are known to increase permeability of the intestinal lumen to the commensal microbes and may contribute to the onset of the GI-symptoms. The possible host immune responses during COVID-19 infection is discussed in this review.

Innate immune response mechanism to SARS-CoV-2: As explained in previous sections, it is evident that SARS-CoV-2 can infect various tissues of GI-tract followed by intestinal cell death, macrophage recruitment and release of various pro inflammatory cytokines to compromise the intestinal barrier[27]. Therefore, it is likely that SARS-CoV-2 infection of intestinal epithelial cells (IEcs) would trigger a coordinated innate immune response due to the recognition of SARS-CoV-2 associated molecular patterns (PAMPs), similar to that reported in the lung's epithelial cells[25,28]. The initial cytokine released by the infected cells can further recruit immune cells (neutrophils, macrophages, lymphocytes *etc.*), in the gut microenvironment to amplify the inflammatory response by recognition of PAMS and cytokines by their specialized receptors to restrict the virus propagation[29,30]. Notably, this early innate immune response is essential to facilitate the emergence of a more specific adaptive immune response by lymphocytes. The nature, timing and strength of innate and adaptive immune responses have been reported to be determining factors for the COVID-19 patient's symptoms[31]. Several components of inflammation exist but we have limited knowledge on the nature of inflammatory pathways triggered in the GI-tract by SARS-CoV-2.

One of the important components of inflammation is IFN response that includes large number of genes exerting antiviral effect. Recent report in a monkey model has shown many proinflammatory cytokines in the GI tract but they show no clear evidence on the IFN-I response genes and thus further omics studies may shed some lights in this regard[27]. It is apparent that asymptomatic and mild/moderate symptomatic patients develop a compelling early innate immune response to

successful viral clearance. While, patients with severe symptoms (especially the elderly and those with pre-existing health conditions) exhibit a dysfunctional early innate immune response against SARS-CoV-2 to allow the dissemination of infection leading to life-threatening complications[32,33]. In general, inadequate early innate immune response and failure to generate enough antiviral IFNs allow immune evasion, viral propagation, the spread of infection and subsequently cell death, and the release of PAMPs and DAMPs to cause cytokine storm. However, there is no strong correlation between viral load and severity of the disease highlighting the role of genetic or physiological state of the individual in developing the severe symptoms. Currently, we have little knowledge about the contribution of GI tract infection and inflammation towards cytokine storm and organ damage, which needs further exploration in the clinical and experimental setup. However, in the rhesus monkey model, it is evident that infection of GI-tract can contribute to systemic inflammation and inflammation to lungs[27].

IEcs and goblet cells undergo apoptosis[27]; however, other form of inflammatory cell death could be operational, which needs to be investigated in a preclinical and clinical setup as various types of cell death can occur due to the activation of innate immune recognition of PAMPs and DAMPs. The inflammatory cell death includes Pyroptosis, Apoptosis and Necroptosis, also termed as Panoptosis[30]. Pyroptosis is an inflammasome or Gasdermin mediated phenomena that involve caspase1, 4, and 5 activations (in humans) and gasdermin mediated pore formation and release of Il1b and IL-18. Recent data suggested a role of SARS-CoV-2 infection induced pyroptosis in peripheral blood mononuclear cells through NLRP3 (NLR family pyrin domain-containing 3) inflammasome activation, cleavage of caspase-1, and secretion of IL-1β and IL-18[34]. Necroptosis is a mixed-lineage kinase domain-like pseudokinase (MLKL)-mediated inflammatory cell death, during which oligomerized MLKL is translocated to form channels in the plasma membrane, which has been documented in SARS-CoV-2 infection[35]. Karki *et al*[35] have shown that a combination of just tumor necrosis factor (TNF)-α and IFN-γ can exert significant cell death in bone marrow-derived macrophages and their blockage can abrogate the cell death and severe symptoms in COVID-19 situation. We guess, possibly similar kind of cell death also operates in GI-tract as TNF-α and IFN-γ are induced in SARS-CoV-2 infected GI-tract[35]. Here, as part of an innate immune response, we elaborate on the evidence of IFN (IFN-I and IFN-III) and proinflammatory cytokines production in the context of human GI tract cells that may have consequences towards GI symptoms.

Induction of IFN and cytokines in the cells of the GI tract upon SARS-CoV-2 infection: To successfully combat and generate immune memory against SARS-CoV-2, the host' cells must generate an early innate immune response that includes the production of antiviral IFN and proinflammatory cytokines soon upon viral detection [36]. The severity of COVID-19 disease has been correlated with a defective or lower level of systemic IFN production but an elevated level of proinflammatory cytokines [37-39]. Since, infection of GI-tract contribute to the systemic cytokine pool[27], a detailed transcriptomic profile of GI-tract in non-human primate model can reveal some clues in future. In the *in vitro* models, similar to lung epithelial cells, the IEcs and intestinal organoids induce both type-I (IFN-I) and type-III IFN (IFN-III)[25,26,40]. Interestingly, SARS-CoV-2 induces stronger IFN-stimulated genes than SARS-CoV in the intestinal organoids, which seems similar to that observed in lung epithelial cells [26]. Analysis of feces of COVID-19 patients has revealed a significant association of elevated proinflammatory cytokine (IL-8) and lower level of anti-inflammatory cytokine (IL-10) in the COVID-19 patients as compared to healthy people, which indicate an inflammation/immune response in the intestine[26]. Post infection, expression of cytokines is evident in the time course analysis of 23 cytokines in the GI-tract of SARS-CoV-2 infected rhesus monkey[27]. Current studies analyzing the IEcs response upon SARS-CoV-2 infection have little data on IFN and ISG at later time points (24 h or longer). Also, analysis of cytokines in gut biopsy samples from various disease category may be useful. Future studies can be carried out to investigate other proinflammatory cytokines profiles that are usually observed in other viruses or bacterial infections. A comparative study would be necessary to dissect the molecular differences in response between IEcs and lung's epithelial cells. Whether intestinal inflammation contributes to the systemic cytokine pool (which seems convincing in rhesus monkey model), caused various types of cell death in intestinal epithelium and resident immune cells would be important aspects to explore.

Adaptive immune response to SARS-CoV-2 in the GI tract
The adaptive immune response mediated by B and T lymphocytes is usually

pathogen-specific and develops slowly relative to the innate immune response. B cells and T cells in the intestine are continually interacting with a vast amount of antigen-derived from diet and commensal microbes and maintain immune homeostasis. The interaction of gut-associated antigen and lymphocytes primarily happens in the gut-associated lymphoid tissues, including the Peyer's patches, isolated lymphoid follicles, and gut-draining mesenteric lymph nodes, leading to maturation and differentiation of lymphocytes[41]. We will discuss the potential adaptive immune response to SARS-CoV-2 infection.

Lymphopenia in COVID-19

Non-severe COVID patients show a near-normal number of circulating lymphocytes, while severe patients show a reduced number of circulating lymphocytes, a condition known as lymphopenia[42,43]. Whereas, a detailed analysis of lymphocyte subsets shows a significant reduction in T cells and NK cells, but without any alteration in B cell number in severe patients[22,42,43]. COVID-19 patients with pre-existing metabolic disease, like diabetes, show a higher proportion of severe infection[44], and lymphopenia has been linked to the severity of Crohn's disease[45]. Lymphopenia in severe COVID-19 patients may result from the synergistic effect of inflammation and metabolic disorder. The cellular mechanism of lymphopenia could be due to the following reasons and beyond. First, inhibition of lymphocyte proliferation in severe disease. Pre-existing metabolic disease, such as diabetes, enhances the propensity of the severity of COVID-19[46], and metabolic molecules, such as elevated blood lactic acid levels, may inhibit lymphocyte proliferation[47]. Second, higher lymphocyte death in severe disease. The potential mechanism of lymphocyte death could be due to metabolic disorder, inflammation, damage of lymphatic organs, and direct infection of lymphocytes[47]. Third, reduced lymphocyte production by skewed hematopoietic lineage cell fate decision. Metabolic disease (ulcerative colitis) and inflammation can skew hematopoietic fate decision towards the myelopoiesis with a concomitant decrease in lymphopoiesis[22,47,48]. Forth, infiltration of lymphocytes at the site of infection.

B cell and antibody-mediated immunity in COVID-19

In general, the intestine offers a model example of the diversity of antibody-secreting cells (ASCs) and comprises at least three subpopulations in humans[49]. Binding of antigen to antigen-specific B cells generates activated B cells that differentiates into ASCs with the help of T helper cells. It has been shown that 70% of non-severe COVID-19 patients have high and persistent SARS-CoV-2 neutralizing immuno-globulin (Ig)G in the sera after their recovery[50]. Antibody isotype analysis shows SARS-CoV-2 specific serum IgA and IgM in non-severe patients[51,52]. A longitudinal study in recovered patients showed that IgG antibodies are relatively stable up to 105 d post symptom onset while IgA and IgM antibodies rapidly decay[52]. Interestingly, an anti-SARS-CoV-2 antibody found in the mucosal fluid (saliva, nasal fluid, and tear fluid) and COVID-19 recovered patients have anti-SARS-CoV-2 specific IgG and IgA in saliva, indicating that antibody in the GI tract could be as crucial as antibodies in the serum for protective immunity[52-54]. ASCs in the intestine is a significant source of IgA producing cells in human. This indicates that the GI tract plays an essential role in generating anti-SARS-CoV-2 antibodies and protective immunity against SARS-CoV-2. The antibody can protect SARS-CoV-2 infection possibly by the following; antibody-mediated neutralization of the pathogen, phagocytosis of infected cells, and antibody-dependent cellular cytotoxicity. Patient-generated SARS-CoV-2-specific antibody can neutralize the virus SARS-CoV-2 in *ex-vivo* condition[53,55]. Furthermore, a mounting adaptive response in GI is supported by the prevalent presence of IgA in the stool of severe SARS-CoV-2 patients[26]. However, the importance and potency of antibody-mediated neutralization of SARS-CoV-2 *in vivo* require future studies in the model systems.

Longitudinal studies have shown that IgG and IgA levels to SARS-CoV-2 were significantly elevated as the disease progresses[52,56,57]. Anti-SARS-CoV-2 IgG was detected within the first week after onset of the symptoms in approximately 40% of patients. Within 15 d (late stage of infection), antibody levels increased by almost 100% of patients[57]. In general, severe patients showed a significantly higher IgG and IgA level compared to non-severe patients at late stages of infection. Surprisingly, the antibody level increases as the disease worsen in the severe group; on the contrary, the disease cured (patients recovered) in the non-severe group[51,56,57]. A few studies have shown that the severity of the disease positively correlated with an increased amount of IgG against S-protein and N-protein, especially in elderly patients[58].

Similarly, a very high level of anti-SARS-CoV-2 IgA correlated with severe acute respiratory distress syndrome[51]. Recent studies have shown that antibody levels (especially those of IgG and IgA) and B cell repertoire are highly dependent on the nature of microbiota in the gut[59,60]. Similarly, B cells and antibodies in the gut could affect the composition of the microbiota. B cell knock-out mice (a proxy for antibody deficiency) and AID deficiency mice (don't have secretory IgA in the gut) have reduced microbial diversity and alter the composition of gut microbiota[61]. Therefore, antibodies and microbiota have a feedback loop to maintain a healthy immune response. The current understanding is that people with dysbiosis (imbalance microbiota) have a prevalence of COVID-19[62]. Alternatively, it could be possible that high IgA levels in the severe patients who recovered have altered the composition of the microbiota and may have a long-term health effect. Figure 3 schematically represents the development of COVID-19 progress and its relationship to changes in the gut flora and disease progression.

T cell and cellular immunity in COVID-19

Cellular immunity is mediated by T cells, and microbiota profoundly affects T cell activation and differentiation, as observed in B cells. Dysbiosis can prompt multiple immune disorders mediated by T cells[63]. T cells have numerous subsets (CD4+ T cells, CD8+ T cells, and Treg) and distinct biological functions. CD4+ T cells primarily regulate the function of other immune cells, CD8+ T (cytotoxic T cells) cells can produce granzyme, and perforin results in the elimination of virus-infected cell, and regulatory T cell (Treg), which can restrain other activated T cells' function[64,65]. COVID-19 recovered patients have SARS-CoV-2 reactive IFN + T cells and granzyme B producing CD8+ T cells[66,67]. The correlation of IFN + T cells and granzyme B producing CD8+ T cells in recovered patients may indicate activated T cells mediated elimination of the virus-infected cells[54]. Interestingly, it has been observed that asymptomatic or mild SARS-CoV-2 infected patients have SARS-CoV-2 specific CD4+ T cells, which could be due to cross-reactive CD4+ T cell recognition between the common cold and SARS-CoV-2 coronaviruses[66,67].

The T cell functions are dysregulated in many severe patients[67,68]. The source of dysregulated T cell functions in severe disease could be due to the following reasons. First, it has been shown that both severe and non-severe patients have a comparable proportion of activated T cells suggesting functionality of activated T cells may be restrained by other immune cells, such as Treg, in non-severe patients[32,42]. In line with this, Qin *et al*[42] showed that severe patients have fewer Treg (specifically, induced Treg). Several studies have shown that GI tract dysbiosis can alter Treg/CD4+ T cell axis and may have a pathogenic outcome[69]. The generation of fewer Treg in severe patients can be a synergistic effect of inflammation and mucosal microbiota imbalance. Second, T cells are exhausted in severe patients than non-severe COVID-19 patients[67,68]. Dysbiosis can promote T cells exhaustion[70]. So, it could be possible that T cell exhaustion in severe patients is a combined effect of hyper inflammation and imbalanced GI microbiota. However, we can't exclude other possibilities (such as bystander T cells) of dysregulated T cells in severe disease.

COVID-19 IN PEOPLE WITH PRE-EXISTING INTESTINAL DISEASES

Patients with chronic GI conditions may be at an increased risk of severe COVID-related illness, therefore management of these patients becomes important. Although the primary source of transmission for SARS-CoV-2 is respiratory droplets, there is increasing evidence supporting the possibility of a fecal-oral route of transmission. Patients with active ulcerative colitis and Crohn's disease have a greater tissue concentration of ACE2, increasing the possibility of an infection[71]. Additionally, the level of serine protease TMPRSS2, is about ten times higher in patients with inflammatory bowel disease (IBD) than in healthy subjects, suggesting an increased risk of infection in these patients[72]. Brenner *et al*[73] created the Surveillance Epidemiology of Coronavirus Under Research Exclusion for IBD (SECURE-IBD) database to identify potential IBD-associated COVID-19 risk factors. Out of 525 patients with IBD and COVID-19, severe infection (defined as intensive care unit admission, ventilator use, or death) was reported in seven percent of patients. Potential risk factors in these patients include increasing age, ≥ 2 comorbidities like diabetes mellitus, chronic inflammatory disease, and systemic glucocorticoids use, but not with anti-TNF therapy[73]. Using anti-TNF antibodies has been shown to reduce inflammatory cell death during experimental COVID-19 situation[35].
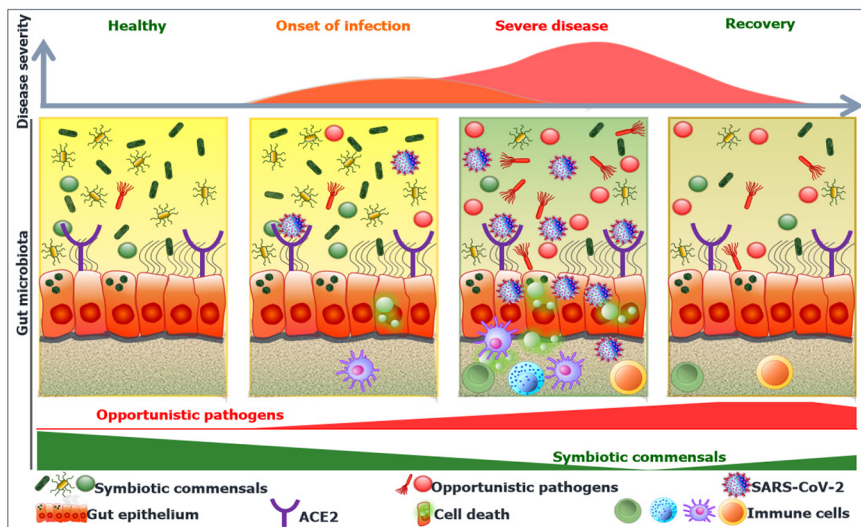
Figure 3 Schematic diagram showing COVID-19 disease progression and correlation with alterations with gut microbiota. The progression of gut microbiome alteration and its association with clinical symptoms and gut dysbiosis is evident. Cartoon inspired by[10,62,85]. ACE2: Angiotensin-converting enzyme 2.

The clinical presentation of several GI diseases (*e.g.*, Crohn's disease, ulcerative colitis) can mimic COVID-19 infection. Examples include diseases that manifest with diarrhea, nausea, vomiting, and/or anorexia. In a study by Mao *et al*[74], preliminary data have suggested that the prevalence of COVID-19 is not higher in IBD patients as compared to the general population[74]. Other studies have suggested that patients with IBD in remission are not at higher risk for SARS-CoV-2 virus infection and that such patients should continue maintenance therapy to sustain remission[74-76]. Digestive complications related to IBD relapse could be confused with symptoms of COVID infection and may skew the data for COVID symptoms in IBD patients. Discontinuing maintenance therapy for IBD has been associated with disease relapse and may lead to an increase of adverse outcomes such as hospitalizations, surgeries, and/or glucocorticoid therapy like prednisone that may increase the risk for severe COVID-19[76].

Patients with a flare of Crohn's disease/ulcerative colitis or active IBD, in the absence of COVID-19, may benefit from anti-inflammatory or biologic therapy to induce remission. Mild IBD therapeutic options include oral budesonide, aminosalicylates, and topical (rectal) therapy. While, the usual options for treating moderately to severely active IBD include biologic therapies (*e.g.*, anti-TNF agents, anti-integrin agents, and anti-interleukin agents)[76] are still viable. However, if systemic glucocorticoids are deemed necessary, the lowest dose of glucocorticoid with an appropriate clinical response is used for a short duration before transitioning to another therapy that is glucocorticoid-sparing[76]. Management of a patient hospitalized with severe ulcerative colitis in the absence of COVID-19 may include treatment with a glucocorticoid (like methylprednisolone) and in unresponsive cases medical therapy may be escalated to infliximab[76]. Surgery is an alternative option for patients who do not improve with medical therapy. Additionally, in the COVID-19 era, the initial use of infliximab at a dose of 5 mg/kg rather than glucocorticoid therapy is a reasonable approach.

IBD patients with known or suspected COVID-19 should have individualized medication regimen adjustments in order to balance the risk of disease flare[77]. The goal is to reduce immunosuppression during active viral infection to lower the risk of COVID-19-related complications (*e.g.*, pneumonia). Patients with suspected or confirmed COVID-19 infection can be treated with Budesonide, Aminosalicylates, including sulfasalazine, topical rectal therapy (*e.g.*, topical glucocorticoid), and antibiotics. However, Glucocorticoids require dose adjustment based on the severity of COVID-19 infection and Immunomodulators like thiotropines, methotrexate; Tofacitinib (Janus kinase inhibitor); biologic agents like anti-TNF agents, ustekinumab, or vedolizumab are held or delayed in patients with active symptoms of COVID-19 until symptoms resolve[78,79].

However, the association of comorbidities, and their effect on the prognosis of COVID-19 needs to be further evaluated. In a recent study, 18 (1%) of 1590 COVID-19

cases had a history of cancer of which three had a history of colorectal cancer, one each of colonic tubular adenocarcinoma, rectal carcinoma, and colorectal carcinoma. It was also noted that patients with a history of cancer and positive SARS-CoV-2 virus were observed to have a higher risk of severe events[80]. Several strategies have been proposed, such as delaying of adjuvant chemotherapy or elective surgery on a patient-by-patient basis, stronger personal protection provisions, and more intensive surveillance or treatment[80].

In a cross-sectional survey of 86602 individuals, 53130 reported prior abdominal pain, acid reflux, heartburn, and regurgitation with 6.4 percent COVID positivity. Proton pump inhibitors (PPI) users were shown to be considerably more likely than non-users to report a positive COVID-19 test result, with a dose-dependent increase in the likelihood of a positive test result, and further studies are required to ascertain the link. PPI increase the risk of enteric infections due to PPI-induced hypochlorhydria. The usage of Histamine-2 receptor antagonist was not associated with an increase in risk[81].

GUT MICROBE AND COVID-19

The gut microbiota, which includes approximately 10^{14} resident bacteria, archaea, virus, and fungi, regulates not only the metabolism and host immunity but also the overall health. The gut and the lung microbiota seem to bi-directionally modulate each other and maintain a healthy gut-lung axis and is reported to be altered in COVID-19 patients and other diseases[82]. Lung infections can also significantly change the composition of gut microbiota, a process collectively termed as "gut microbial dysbiosis." Viral respiratory infections are also known to induce inappetence and significantly impact the gut microbiota[7]. Severe pulmonary SARS-CoV-2 infection is associated with a hyperactive immune reaction and "cytokine storm". Inflammatory mediators cause significant lung cytopathy and hyper-permeability, leading to a viral transfer to the gut *via* circulation or some other unknown mechanisms. The inflammatory mediators also damage the intestinal barrier leading to the leakage of intestinal microorganisms and associated metabolites into the main bloodstream that may further the inflammation and GI symptoms.

Moreover, microorganism-associated molecular patterns and PAMPs are recognized by host immune mediators and evoke a strong detrimental immunological reaction in organs, including the lungs and intestine. This vicious cycle of chronic immune activation leads to tissue inflammation and damage. Giron *et al*[83], 2020 study the role of COVID-19-associated lung injury, systemic inflammation, and disruption of the gut's barrier functions, resulting in the enhanced vulnerability of microbial products [83]. Thus, COVID-19 affects the gut lung axis and induces microbial dysbiosis.

There is a dearth of information regarding the direct *vs* the indirect effect of SARS-CoV-2 on gut microbiota. Zuo *et al*[84], 2020 analyzed fecal microbiome from COVID-19 patients using shotgun metagenomic sequencing technology and detected higher opportunistic pathogens (including, *Clostridium hathewayi*, *Actinomyces viscosus*, and *Bacteriodes nordii*) and a concomitant decrease in beneficial commensals (including, *Faecalibacterium prausnitzii*, *Lachnospiraceae bacterium 5_1_63FAA*, *Eubacterium rectale*, *Ruminococcus obeum*, and *Dorea formicigenerans*[84]. Interestingly all patients in this study cohort did not present GI symptoms. Data regarding the use of probiotics and nutritional intervention can further confirm the hypothesis that SARS-CoV-2 associated disease severity may be dictated by the patient's microbiota status. Probiotics are live microbes, when consumed, can provide gut health. Several studies have shown that the administration of probiotics in COVID patients can ameliorate gut dysbiosis and improve host immune response[85,86]. In the absence of specific data, further investigation regarding the particular role of probiotics and supplements, microbial type or nutritional component needs investigation in larger SARS-CoV-2 infected patient cohorts[87].

Elderly people (> 60 years) are associated with severe symptoms and higher mortality rates. The link between aging and progressive alteration of detrimental gut microbiota is well worked out[88]. An increasing number of reports suggest that a strong relationship exists between the gut microbiome and SARS-CoV-2 infection severity. Therefore, the heightened risk of aged patients may be associated with microbial dysbiosis, leaky gut, inflammation, and a dysfunctional gut-lung axis in addition to pre-existing conditions. A major question that has not been addressed is why certain developed countries have significantly higher mortality rates as compared to some underdeveloped or developing nations. Amongst many possibilities, the role

of lung and gut microbiome and resulting interference with systemic immunity may also help explain the global disparities in COVID-19 associated disease severity and death[89]. To have a comprehensive idea investigation with a larger data set is warranted.

THERAPEUTICS OPTIONS FOR COVID-19

Though infection prevention, control strategies, and preventive treatment are the mainstay of the current management of COVID-19. Some glimmer of hope has arrived in the form of COVID-19 vaccines' approval for emergency use by many nations, but currently, no safe and effective treatment exists. The possible list of emerging therapeutics for the treatment of COVID-19 is steadily expanding and evolving, and that too in a short period of time. The United States Food and Drug Administration (FDA) has approved emergency use authorizations (EUAs) for a few medications and therapies and several others are under clinical trials[90]. This review will concentrate on the strategies especially aimed at prophylactic and therapeutic modulating the host immune system. Pre-infection immunoprophylaxis depends on the immune activation of the host immune system before infection and disease initiation. At the same time, therapeutic intervention strategies are focused to repair the immune systems during the duration of the illness, post-infection. Several therapeutic targets including IFN-I, TNF, JAK/STAT, IL-1, IL-6, GMCSF, convalescent plasma, and complements, are under investigation. A comprehensive list of all prophylactic and therapeutic molecules undergoing clinical trials is available online (https://www.who.int/ictrp/en/; clinicaltrials.gov).

Approved therapeutics for COVID-19

Several antiviral molecules are undergoing clinical trials, and Remdesevir has been approved by United States FDA for therapeutic management of COVID-19 patients [91]. Remdesevir (Veklury), being a nucleoside analog, prevents viral replication by inhibiting the viral RNA-dependent RNA polymerase (RdRp) activity, shortened recovery time, and reduced mortality rates. Eli Lilly and Company has also received EUA for a combinatorial use of Baricitinib (Olumiant; an inhibitor of JAK kinase) with Remdesevir in patients requiring supplemental oxygen.

The antibody cocktail of Casirivimab and Imdevimab by Regeneron Pharmaceuticals, Inc. has also obtained the EUA by the United States FDA for the treatment of mild to moderate COVID-19. Casirivimab and Imdevimab are monoclonal antibodies against spike protein of SARS-CoV-2, are supposed to neutralize the viral entry. Eli Lilly COVID-19 neutralizing antibody bamlanivimab (LY-CoV555) is also directed against the spike protein and has received EUA for non-hospitalized adults. Convalescent plasma (CP) is a passive immune therapy approach, where COVID-19 recovered patient can donate plasma rich in SARS-CoV-2-specific neutralizing antibodies to persons at high risk of contracting COVID-19[92]. United States FDA has provided a EUA for the use of CP as a treatment option for COVID-19 patients.

The potential vaccine can change the course of the COVID-19 pandemic. Researchers have used several technological vaccine development platforms, including nucleic acid-based (DNA and RNA), virus mimicking particle subunit vaccine, peptide vaccines, attenuated virus-based vaccines. mRNA vaccines developed by Pfizer-BioNTech and Moderna have received EUA from the United States FDA, bringing a big sigh of relief. mRNA vaccines prompt the cell to express the viral spike protein, which elicits a strong immune reaction against the infecting SARS-CoV-2 virus. The Oxford-AstraZeneca's COVID-19 vaccine has also received authorization in many countries. The Oxford-AstraZeneca vaccine uses the gene for the coronavirus S protein (double-stranded DNA) packed in an adenovirus. Other vaccine producers like Johnson & Johnson/Janssen Pharmaceuticals and Gam-COVID-Vac (Sputnik V) developed by the Gamaleya Research Institute of Epidemiology and Microbiology are also effective and are being used for mass vaccination in many countries. Bharat Biotech and the Indian Council of Medical Research collaborated to create Covaxin (codenamed BBV152), an inactivated virus-based COVID-19 vaccine. CoronaVac (inactivated vaccine) is produced by Sinovac is also used to vaccinate to fight against COVID-19. The new evolving strains with mutations in the S protein create a possibility of decreased susceptibility to monoclonal antibodies, vaccines and therapeutic agents. Scientists are investigating these mutations to help explain how quickly they can be spread and if vaccines will be effective.

Under trial therapeutics for COVID-19

Several strategies to target the uncontrolled host immune system have been attempted and are currently in clinical trials (clinicaltrials.gov). TNF-α, a proinflammatory cytokine, exhibits a positive correlation with advances in disease stages. Preliminary clinical data suggest the effectiveness of anti-TNF-α in reducing the cytokine storm as well as tissue inflammation. TNF-α-blockers, both small molecule and antibodies (Adalimumab and Otilimab), are currently under trial[93]. Patients with IBD with COVID respond better to anti-TNF-α blockers than alternative agents[94]. Dysregulated early IFN-I response may eventually lead to COVID complications, and an early IFN-Iα/β treatment with broad antiviral response can ameliorate disease progression [95]. IL-6 is associated with 'cytokine storm', and inhibition of IL-6 using a monoclonal antibody (Tocilizumab, Sarilumab) is under clinical trial and can be a potential treatment option. Initial clinical studies in China and a case study in France suggested a rapid favorable outcome on the therapeutic value of the anti-IL-6 receptor antibody [96]. More investigation is warranted as IL-6 is also reported to prevent enterocyte cell death after injury and help proliferation and repair[97]. Sanofi's KEVZARA Phase III trial investigating the efficacy of an anti-IL-6 receptor antibody in severe and critically ill patients did not yield promising results. Hence, further investigation is warranted to understand better the therapeutic advantage of inhibiting IL-6 signaling in COVID patients. Other than current therapeutics, patients suffering from systemic inflammation and IBD and associated diarrhea may benefit from the potential use of pro and pre-biotics[91,98].

CONCLUSION

SARS-CoV-2 has spread exponentially as a pandemic throughout the world. Scientists and researchers all over the world are working tirelessly to develop potential coronavirus treatment options. The United States FDA has recently granted EAU for several therapeutic modalities for targeting COVID-19. Pfizer and Moderna are producing United States FDA approved vaccines in millions of doses for the prophylactic use in COVID-19 patients. In this review, we have attempted to describe the link between COVID-19 associated GI infection, immune responses, and disease outcomes. SARS-CoV-2 primarily causes severe respiratory symptoms but also affects the GI system in many patients. The role of SARS-CoV-2 in gut infection, route of infection, relation with disease severity, localized *vs* systemic immune reaction, altered microbiota, dysbiosis, and the mechanism underlying pre-existing conditions and therapy. Many queries remain unexplored, especially in the context of GI infection, and need further investigation. The bidirectional gut-lung axis has been implicated in the homeostasis of the immune system. GI inflammation and dysbiosis may contribute to systemic inflammation and affect lung and other organs' health, and may be associated with severe COVID consequences. The vice versa may also be confirmed and the underlying mechanism that pathologically upsets the gut-lung communications during COVID-19 infection is not clearly understood. The role of probiotics in enhancing the immune system and the attenuation of dysbiosis may be a promising approach for reducing the GI-symptoms and preventing the COVID-19 severity. The emergence of new strains like B.1.207, B.1.351, B.1.1.7, B.1.617.1, B.1.617.2, and B.1.617.3 can impact GI significantly and therefore strain surveillance is important and its role in gut infection also needs to be studied. Hence the use of bioinformatics, mutational analysis, structural modeling to better understand the spike-ACE2 interaction, and the use of organoid and non-human primate models to study the viral infection process and therapeutic screening are key in the fight against COVID-19.

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