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Mechanisms of gastrointestinal barrier dysfunction in COVID-19 patients

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Abstract

Coronavirus disease 2019 (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a major global public health event, resulting in a significant social and economic burden. Although COVID-19 was initially characterized as an upper respiratory and pulmonary infection, recent evidence suggests that it is a complex disease including gastrointestinal symptoms, such as diarrhea, nausea, and vomiting. Moreover, it remains unclear whether the gastrointestinal symptoms are caused by direct infection of the gastrointestinal tract by SARS-CoV-2 or are the result of systemic immune activation and subsequent dysregulation of homeostatic mechanisms. This review provides a brief overview of the mechanisms by which SARS-CoV-2 disrupts the integrity of the gastrointestinal barrier including the mechanical barrier, chemical barrier, microbial barrier, and immune barrier.

Key Words: Gastrointestinal barrier dysfunction; SARS-CoV-2; COVID-19; Angiotensin-converting enzyme 2; Microbiome; Immune cells

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Core Tip: Coronavirus disease 2019 (COVID-19) has become a major global public health event, resulting in a significant social and economic burden. Although COVID-19 was initially characterized as an upper respiratory and pulmonary infection, recent evidence suggests that it is a complex disease including gastrointestinal symptoms. Moreover, it remains unclear whether the gastrointestinal symptoms are caused by direct infection of the gastrointestinal tract by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or are the result of systemic immune activation and subsequent dysregulation of homeostatic mechanisms. This review provides a brief

overview of the mechanisms by which SARS-CoV-2 disrupts gastrointestinal barrier integrity.

INTRODUCTION

Since the advent of coronavirus disease 2019 (COVID-19), the disease has spread globally and had a profound impact on the lives and health of people around the world[1]. The virus that causes COVID-19, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), replicates and matures once it enters cells through the angiotensin-converting enzyme 2 (ACE2) receptor. SARS-CoV-2 can trigger an inflammatory response that involves the activation of immune cells by a variety of cytokines[2,3]. ACE2 receptors are present in multiple cell types throughout the body, including the oral and nasal mucosa, lung, and gastrointestinal tract, indicating that SARS-CoV-2 can cause multi-organ damage[4,5].

The intestinal tract is the digestive organ of the body, but also has endocrine and immune functions, and is the first line of defense against non-specific infections. However, the intestine is also the largest reservoir of bacteria and endotoxins in the body and is, therefore, a hidden source of infection. While digesting and absorbing nutrients, the intestine contains bacteria and their metabolites, and the gastrointestinal barrier plays a very important role in preventing systemic absorption of harmful microbes and substances[6,7]. The gastrointestinal barrier is composed of the intestinal epithelial cell layer, mucus layer, normal intestinal flora, intestinal immune system, and intestine-hepatic axis, which together perform the functions of mechanical barrier, chemical barrier, microbial barrier, and immune barrier (Figure 1). This barrier plays an important role in homeostasis by preventing harmful substances and pathogens in the gastrointestinal tract from entering the internal environment of the body[8]. In this review, we describe the pathophysiological mechanisms of gastrointestinal barrier dysfunction in COVID-19 patients (Figure 2).

MECHANISMS OF THE GASTROINTESTINAL BARRIER

Mechanical barrier

The intestinal mechanical barrier consists of intestinal mucosal epithelial cells, tight junctions (TJs) between epithelial cells, and a layer of bacteria and mucous on the surface of epithelial cells. TJs are the most important component of the intestinal mechanical barrier[9] and are composed of four transmembrane proteins: Zonula occludens (ZO); occludin; claudins, and junctional adhesion molecules (JAMs). TJs form an important barrier against the translocation of bacteria and toxins in the intestine[10]. The TJs between cells close the gaps between adjacent intestinal epithelial cells, preventing bacteria and antigens in the intestinal lumen from entering the lamina propria and activating immune cells in the lamina propria. Therefore, TJs maintain the stability of the intestinal mucosal barrier and prevent abnormal immune reactions in the mucosa. Moreover, the TJs between intestinal epithelial cells play an important role in maintaining the morphological structure of epithelial cells, regulating the differentiation and repair of epithelial cells and intercellular material transport, and maintaining the barrier function of the intestinal mucosa and intestinal mucosal permeability[10-12].

Chemical barrier

The intestinal chemical barrier, also called the mucus layer, is a general term for the antimicrobial substances produced by the resident intestinal bacteria and host cells *e.g.*, Paneth cells and the chemicals that inhibit bacterial adhesion and colonization, such as gastric acid, digestive enzymes, lysozyme, and mucin[13,14]. The mucus layer, which is mainly composed of mucins and secretory mucin MUC2, limits the contact of gastrointestinal tissues with the microbiota[15,16]. Therefore, the mucus layer separates bacteria from intestinal epithelial cells in the intestinal lumen while allowing nutrient absorption[17]. It also lubricates the luminal contents to prevent degradation of the gastrointestinal tissues[18,19]. Gastric acid acts mainly at the beginning of the small intestine to inactivate bacteria and other pathogenic microorganisms. Lysozyme exerts bactericidal and antibacterial effects on the intestinal epithelial surface and in the

intestinal lumen[20,21]. Moreover, bile is an important chemical barrier to endotoxins, as bile salts bind to endotoxins in the intestine and prevent absorption from the intestine into the portal vein[22].

Microbial barrier

The normal intestine is inhabited by a large number of bacteria, with at least 500 different species, most of which are anaerobic[23]. Under normal conditions, the normal flora maintain a relatively stable proportional relationship with each other. They combine with the intestinal mucosa to form a micro-ecosystem that is both interdependent and interactive with the micro-spatial structure of the host and this micro-ecosystem forms the microbial barrier of the intestine[24]. Under normal microecological conditions, the dominant replication of non-pathogenic intestinal flora can hinder the survival of pathogenic bacteria. At the same time, non-pathogenic flora also secrete some antibacterial and antimicrobial substances, such as lactic acid and bacteriocins, which can interfere with and inhibit the vitality and function of pathogenic bacteria[25-27].

Immune barrier

The intestine is both a digestive organ and the largest immune organ in the body and is, therefore, an important part of the systemic immune system. The intestine is constantly exposed to antigenic substances, such as microbial antigens and food antigens, but the gastrointestinal barrier, including the immune barrier, can effectively prevent the penetration of these antigens. The intestinal immune defense system is mainly composed of gut-associated lymphoid tissue (GALT) in the intestinal wall and secreted IgA, IgM, and IgE. GALT is the site of induction and activation of the immune response, mainly in the Peyer's patches that are distributed throughout the small intestine[28,29]. Most of the IgA in intestinal secretions is secretory IgA (sIgA), which is the most secreted immunoglobulin in the body and the main immunoglobulin on the intestinal mucosal surface. sIgA plays a leading role in humoral immunity and is the first line of

defense against the adhesion and colonization of pathogens in the intestinal mucosa[30,31]. Furthermore, perivascular macrophages in the lamina propria constitute an anatomical barrier that functions to prevent bacterial translocation[32]. Also, neutrophils play an important role in capturing and killing pathogens in enterocolitis[33].

FACTORS CONTRIBUTING TO THE GASTROINTESTINAL BARRIER DYSFUNCTION CAUSED BY SARS-COV-2

SARS-CoV-2 and the mechanical barrier

Damage to the gastrointestinal mechanical barrier can be caused by direct infection of intestinal cells with SARS-CoV-2. The spike protein of SARS-CoV-2 has a high affinity for ACE2, which is widely expressed in intestinal epithelial cells. SARS-CoV-2 enters target cells mainly through the ACE2 receptor thus causing primary damage to the intestine by altering the expression and function of TJ-related proteins, leading to disruption of the paracellular barrier function[34-37]. A study by Sun *et al*[38] that compared differentially enriched proteins in the stools of COVID-19 patients and normal participants found evidence of intestinal infection and intestinal damage caused by SARS-CoV-2. For example, certain protein components of human immunoglobulins and hemoglobin were upregulated in COVID-19 patients, suggesting an enhanced immune response and potential bleeding in the intestine.

Studies have also shown that ZO-1 interacts with: (1) Occludin, claudins, and JAM; (2) molecular components of intracellular TJ plaques, such as ZO-2, ZO-3, and cingulin; and (3) the actin cytoskeleton[39]. Thus, ZO-1 plays a key role in the structural and functional integrity of the paracellular barrier[39]. Furthermore, the cytoskeletal and barrier biomarkers KRT19 and ZO1 were respectively enriched in stool and blood samples from COVID-19 patients, suggesting increased intestinal paracellular permeability and injury[38,40]. Another group showed that the microbial metabolite d-lactate and the TJ regulator zonulin were increased in the serum of patients with severe COVID-19 and in COVID-19 patients with secondary infections[41]. These data suggest

that biomarkers of intestinal permeability may also be early biomarkers for a fatal outcome. Indeed, treatment with larazotide, a zonulin inhibitor, significantly improved time-to-resolution of gastrointestinal symptoms and time-to-clearance of spike antigenemia in pediatric COVID-19 patients[42,43].

In addition, apoptosis of intestinal epithelial cells in COVID-19 patients is another important factor affecting the integrity of the gastrointestinal mechanical barrier. A study by Lehmann *et al*[44] demonstrated a significant increase in the number of apoptotic epithelial cells in COVID-19 patients by using immunohistochemistry to detect cleaved caspase-3. They also examined the histomorphological changes of epithelial cells and found apoptosis and subsequent regenerative proliferation of epithelial cells in the small intestine of COVID-19 patients[44].

In addition, infection of lung and small intestinal epithelial cells with SARS-CoV-2 stimulates secretion of large amounts of pro-inflammatory cytokines (chemokine-1, TGF-β1, TNF-α, IL-1, and IL-6) causing a cytokine storm and damage to the small intestine that disrupts the integrity of the gastrointestinal barrier[45]. Interestingly, this phenomenon was confirmed using human induced pluripotent stem (iPS)-derived intestinal epithelium, suggesting that iPSC could be a useful *in vitro* model for evaluating COVID-19 pathology in gastrointestinal barrier dysfunction[46].

SARS-CoV-2 and the chemical barrier

Many COVID-19 patients have a significantly reduced appetite, and severely affected patients, such as those dependent on mechanical ventilation, may not be able to eat, resulting in reduced secretion of gastric acid and bile. Under normal conditions, the intestinal epithelial surface is less susceptible to damage by harmful substances due to the mucus cover, which plays an important role in the gastrointestinal barrier. Studies have shown that in rats receiving total parenteral nutrition, glands atrophy, the intestinal mucus layer is damaged and thinned, intestinal permeability increases, and the gastrointestinal barrier is disrupted[47,48]. In standard total parenteral nutrition, no food passes through the intestine in the loading state and certain essential intestinal

nutrients such as glutamine are lacking. Numerous subsequent studies have confirmed that parenteral nutrition tends to damage the intestinal epithelial mucus layer, leading to disruption of the gastrointestinal barrier[49,50].

SARS-CoV-2 is known to be neuroinvasive, and when the body is infected with SARS-CoV-2, a cytokine storm is generated and the release of large amounts of pro-inflammatory cytokines leads to blood-brain barrier damage[51,52]. In addition, lipopolysaccharide (LPS) is recognized by Toll-like receptors, which transduce signals that stimulate release of inflammatory cytokines[53-55]. ACE2 is expressed in certain regions of the human brain as well as in neurons and SARS-CoV-2 can directly damage these target organs[56]. After SARS-CoV-2 infection, impairment of the gastrointestinal barrier causes leaky gut and increased translocation of bacterial metabolic components and toxins into the bloodstream, which activates the immune response and leads to systemic inflammation[57-59]. This can promote neuronal degeneration and the development of psychiatric and neurodegenerative disorders[60]. Furthermore, neurological damage can affect the gastrointestinal barrier *via* the neuroendocrine pathway; this occurs mainly through the hypothalamic–pituitary–adrenal (HPA) axis[61-63]. Stress activates the HPA axis, which ultimately leads to the release of glucocorticoids such as cortisol from the adrenal cortex. Cortisol can alter gastrointestinal dynamics, increase intestinal permeability, and affect the intestinal microbiota[61].

SARS-CoV-2 and the microbial barrier

When the microbial balance in the intestine is altered, pathogenic bacteria adhere to the intestinal epithelium and grow dominantly to replace the normal flora[64]. Pathogenic bacteria can directly damage the microvillous membrane proteins of the intestinal epithelium by producing bacterial proteases. Furthermore, pathogenic bacteria can alter biochemical reactions in the intestinal epithelium, leading to damage, blunting, fusion, or obliteration of the villi[65]. In addition, pathogenic bacteria can produce various toxins or other metabolites that inhibit protein synthesis in the intestinal epithelium, thereby damaging the intestinal mucosal barrier. Some opportunistic pathogens can also

produce proteases that degrade IgA, which can weaken or eliminate the immune function provided by sIgA and promote bacterial translocation, leading to bacteremia and endotoxemia, thereby further damaging gastrointestinal barrier integrity[66].

A study by Sun *et al*[38] using metagenomic and metaproteomic methods showed significant changes in the composition of the gut microbiome in COVID-19 patients, characterized by a decrease in commensal species and an increase in opportunistic pathogenic species. Moreover, other studies have shown that the microbiota of COVID-19 patients is enriched with opportunistic pathogens, compared with healthy individuals[64,67-69]. Furthermore, the plasma concentration of the gut permeability marker FABP2 and gut microbial antigens PGN and LPS were significantly elevated in COVID-19 patients in comparison to healthy controls[70].

Hashimoto *et al*[71] found that ACE2 acts as a binding partner of amino acid transporter B0AT1 (SLC6A19) in the intestine and plays an important role in amino acid transport. Tryptophan is mainly absorbed through the B0AT1/ACE2 transport pathway in enterocytes, which activates the mammalian target of the rapamycin (mTOR) pathway to regulate the expression of antimicrobial peptides. These antimicrobial peptides affect the composition of the intestinal microbiota[71]. Competitive binding of ACE2 receptors by the SARS-CoV-2 virus prevents the absorption of tryptophan through the B0AT1/ACE2 transport pathway in enterocytes, impairs the regulation of antimicrobial peptide expression, and causes an intestinal dysbiosis, leading to disruption of the gastrointestinal barrier.

In addition, when patients with COVID-19 are admitted to hospital for treatment, changes in gastrointestinal barrier dysfunction are often induced by bacterial translocation and gut microbiome dysbiosis as a result of early and heavy use of antimicrobial drugs, such as macrolides, fluoroquinolones, or cephalosporin antibiotics[72-74]. Wu *et al*[75] reported that washed microbiota transplantation can improve the intestinal mucosal barrier function, inflammatory response, and immunity. Therefore, this treatment is expected to be an efficacious and safe therapeutic option for the treatment of COVID-19 patients with gut microbiota dysbiosis[75].

SARS-CoV-2 and the immune barrier

After binding to ACE2 receptors expressed on intestinal epithelial cells, SARS-CoV-2 is transported to the nuclear endosome and releases its RNA[76,77]. Toll-like receptors recognize viral RNA and signal downstream mediators, which induce IFN-α and -β production and then activate the transcription factor NF-κB to produce pro-inflammatory cytokines[78]. During SARS-CoV-2 infection, small intestinal tissue and feces show increased pro-inflammatory markers, including neutrophil and monocyte accumulation, increased chemokine-1, TGF-β1, IL-1, IL-6, IL-8, and IFN-γ expression and decreased levels of the anti-inflammatory cytokine IL-10[45,79,80]. IFN-γ is produced by several types of immune cells, especially T helper type 1 (Th1) cells. IFN-γ acts as a major inducer of the cell-mediated response to infection by activating macrophages, enhancing antigen presentation and T cell differentiation[81], and interacting directly with epithelial cells, leading to chemokine expression and antimicrobial peptide secretion[82,83]. In addition, mucosal $CD4^+$ and $CD8^+$ T cells, Th17 cells, neutrophils, dendritic cells, and macrophages were activated and T regulatory cells were reduced after intestinal epithelial cells were infected by SARS-CoV-2. Therefore, SARS-CoV-2 infection promotes an over-activated immune response that further damages intestinal epithelium[84-86]. Interestingly, one study found that SARS-CoV-2 was not always measurable in the stools of COVID-19 patients with gastrointestinal symptoms. These data further suggest that the gastrointestinal barrier dysfunction may not be a direct result of viral infection of intestinal mucosal epithelial cells, but rather a result of the immune response[87].

CCL25 is expressed in enterocytes and the CCL25-CCR9 axis mediates recruitment of lung-derived effector $CD4^+$ T cells to the small intestine[88]. $CCR9^+CD4^+$ T cells disrupt homeostasis of the intestinal flora, thereby promoting the in-situ polarization of small intestinal Th17 cells and the production of IL-17A, leading to neutrophil aggregation and ultimately mediating intestinal immune-mediated injury[88,89]. Moreover, damage to the intestinal mucosa and bacterial imbalance can lead to harmful metabolites in the

intestine being transferred to the liver through the portal vein, affecting liver function[90]. With impaired liver function, endotoxin inactivation is reduced, resulting in endotoxin entering the systemic circulation[91-94]. This induces an inflammatory response, damages intestinal mucosa, and causes bacterial translocation and bacteremia, inducing a systemic inflammatory response and thus forming a vicious cycle[95].

Furthermore, antiviral drugs can also cause gastrointestinal barrier dysfunction. For example, severe and persistent diarrhea may be associated with the use of oseltamivir and arborol. Other drugs that can cause gastrointestinal barrier dysfunction include chloroquine phosphate, lopinavir, remdesivir, and some proprietary Chinese medicines, as well as immunosuppressors[96].

CONCLUSION

Gastrointestinal barrier dysfunction in COVID-19 patients is not an independent symptom, but rather a trigger of other diseases. In critically ill patients, gastrointestinal barrier dysfunction, if not treated early, may aggravate primary disease or even cause multi-organ dysfunction syndrome, thus endangering patients' lives. To assess gastrointestinal barrier dysfunction in COVID-19 patients, clinicians should not only consider the effect of the primary disease, but also the pathophysiological situation within the intestine. The pathological mechanisms that cause gastrointestinal barrier damage and dysfunction are complex and various mechanisms are often intertwined, interacting with each other. Therefore, the clinical diagnosis and treatment of gastrointestinal barrier dysfunction should be comprehensive and targeted.

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