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Gastrointestinal mucosal immunity and COVID-19

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Abstract

As the gastrointestinal tract may also be a crucial entry or interaction site of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the role of the gut mucosal immune system as a first-line physical and immunological defense is critical. Furthermore, gastrointestinal involvement and symptoms in coronavirus disease 2019 (COVID-19) patients have been linked to worse clinical outcomes. This review discusses recent data on the interactions between the virus and the immune cells and molecules in the mucosa during the infection. By carrying out appropriate investigations, the mucosal immune system role in SARS-CoV-2 infection in therapy and prevention can be established. In line with this, COVID-19 vaccines that stimulate mucosal immunity against the virus may have more advantages than the others.

Key Words: Mucosa; Gut mucosa; Mucosa-associated lymphoid tissue; SARS-CoV-2; COVID-19; Secretory immunoglobulin A; Gut microbiota

Core Tip: The gastrointestinal tract is a frequent route of infection with severe acute

respiratory syndrome coronavirus 2. Given the complex interactions between the virus and the mucosal immune system after exposure, additional research is needed to elucidate the immune mechanisms and processes in the gut mucosa. The hallmark of all immune responses is the recruitment of various immune cells, such as neutrophils, dendritic cells, macrophages, and T cells in the gut mucosa. However, the mucosal inflammatory response could change intercellular space between enterocytes, leading to an increase in intestinal permeability that allows various bacterial antigens and toxins to enter the bloodstream, further complicating the disease state of coronavirus disease 2019 patients.

INTRODUCTION

It is well-established that nasopharynx-associated lymphoid tissue (NALT) and mucosa-associated lymphoid tissue (MALT) are first-line defenses. Therefore, airborne infections start by penetrating the upper airway mucosa, where a higher viral load is found, compared with the throat. NALT is involved in the induction of the immune response towards the microorganisms by promoting the differentiation and activation of immune cells such as Th1- and Th2 cells, dendritic cells, macrophages, resident microfold M cells, innate lymphoid cells, immunoglobulin (Ig)A-switched B cells, as well as immune mediators and molecules (*i.e.* beta-defensins, galectins, collectins, and cytokines)[1]. The same goes for the gut mucosal immune system.

It is thus not surprising that NALT exerts "gate control" on many infections that penetrate the mucosa, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[2]. SARS-CoV-2 acts cytopathically at the mucosal level by inducing injury and death of the infected cells. That can be accomplished by pyroptosis as a consequence of activation of host-cell released damage-associated (DAMPs) and viral pathogen-associated molecular patterns (PAMPs) and innate immunity along with the secretion of many cytokines [interleukin (IL)-6, interferon (IFN)-gamma, MCP1, and IP-10)][1]. On the other hand, pattern-recognition receptors and their soluble forms are mainly involved in SARS-CoV-2 infection[3,4].

Amongst the immune molecules, the collectins have a significant role in recognizing glycoside structures of the virus. For example, deficiency of mannose-binding lectin (MBL) has been associated with increased susceptibility to viral infections, including SARS-CoV[5]. Additionally, MBL can inhibit S protein by binding to it and inhibiting structural S rearrangements needed for optimal infection, thus leading to reduced virulence of SARS-CoV-2. Furthermore, as the expression of MBL declines with aging, older adults may be more prone to infection because of lack of an effective innate response[5].

Additionally, the presence of natural, pre-existing IgM and IgA antibodies produced in the absence of any antigen, provides the first-line defense[6]. Furthermore, some of the natural antibody subsets can recognize ABO blood-group antigens, which are expressed on many epithelial cells, including the lung[7]. As enveloped viruses like SARS-CoV-2 are highly glycosylated, it is thought that when virions reproduce in the alveolar epithelial cells in people with group A or B blood, those antigens may be expressed on their envelope. Thus, one may suggest that natural antibodies against A and B antigens may be protective[8]. Studies have so far revealed that anti-A antibodies can inhibit S protein binding to angiotensin-converting enzyme 2 (ACE2) receptors when the host cells express A antigen. Individuals with group O blood have a reduced risk of infection compared with those who have non-O blood groups, and those with group A blood are prone to severe coronavirus disease 2019 (COVID-19)[8].

EPIDEMIOLOGICAL SIGNIFICANCE OF MUCOSAL PENETRATION AND REPLICATION FOR THE SPREAD OF SARS-COV-2

COVID-19 is an infectious disease in which the primary mode of transmission of its causative agent, SARS-CoV-2, is by transfer of saliva microdroplets between people in close contact. The microdroplets are produced while coughing, sneezing, or talking. Infection by contact with contaminated surfaces followed by touching the face is less common. Most microdroplets fall to the ground or surfaces and are not effective over long distances. The first 3 d after the onset of symptoms is when the patient is most contagious. Transmission may occur before symptoms appear. Asymptomatic people may thus be contagious, too[9].

COVID-19 is an airborne viral disease. It was shown that SARS-CoV-2 penetration into the upper airways is the first step of the infection, as higher viral loads were found in nasal swabs than throat swabs[10,11]. The same distribution as in symptomatic patients was observed in asymptomatic patients, implicating the nasal epithelium as a portal for initial infection and transmission[12]. The nose is a critical component of mucosal immunity, providing protection in the upper airway. It is involved both in host protection and immune homeostasis between the commensal microbiota and invading pathogens. The mucosal immune system is the first line of physical and immunological defense against invading pathogens[13]. Current evidence indicates that SARS-CoV-2 enters the human body mainly through the ACE2 + transmembrane protease/serine subfamily member 2 (TMPRSS2) + nasal epithelial cells. The initial host response to this pathogen begins in the NALT system[1].

VIRAL FACTORS OF SARS-COV-2 AND IMPACT ON THE MUCOSA

Coronaviruses are enveloped, positive-sense single-stranded RNA viruses that are members of families *Coronaviridae*, order Nidovirales. There are four known genera, *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. SARS-CoV-2 belongs to the family *Coronaviridae* and genus *Betacoronavirus*[14,15]. Diseases caused by coronaviruses comprise symptoms that range from mild respiratory illness like the common cold to severe infections causing death. These viruses can infect humans, mammals, and avian species, including farm and companion animals (pets). Hence they represent not only a challenge for public health but also are of veterinary and economic concern. From the beginning of the 21st century, the SARS epidemic in 2002-2003, the Middle East respiratory syndrome (MERS) in 2012, and the emergence of the new SARS-CoV-2, are examples of human infections caused by coronaviruses[16,17].

Coronaviruses are spherical, enveloped RNA viruses containing an impressively large (25 kb to 32 kb), nonsegmented, single-stranded, positive-sense RNA genome, which is the same sense as the messenger RNA (mRNA) found in cells. The genome codes four main structural proteins, nonstructural proteins (NSPs), and accessory proteins. The structural proteins, which include the spike (S), nucleocapsid (N), membrane (M), and envelope (E) proteins, play essential roles in the arrangement of the virus particles and other aspects of the viral life cycle[18]. Among the structural proteins, the most important is S protein, which is required for viral entry as it binds to the target cell receptors and initiates fusion with the cell membrane[19].

SARS-CoV-2 spike (S) protein is a large glycosylated transmembrane, homotrimeric protein. Each monomer has a molecular mass of about 150-200 kDa[18]. Each subunit of the protein consists of two functionally distinct domains, S1 and S2. S1 forms the bulb portion of the spike protein on the virion surface. S2 attaches the S proteins to the viral membrane. The receptor-binding domain is located on S1; S2 is necessary for membrane fusion to allow the viral cell entry[20]. Transmembrane ACE2 is the functional host receptor for SARS-CoV-2[21]. ACE2 is widely expressed in the ciliated, goblet, and surfactant-producing type-2 alveolar cells of the lungs, intestinal, cardiac, and vascular endothelia, the kidney, and the liver[22].

Other receptors, such as DC-SIGN, L-SIGN, Neuropilin-1, furin, and cathepsin B and L can serve as portals of virus entry into the cell. Taken together, the findings provide a possible explanation for the occurrence of COVID-19 complications in organs expressing those receptors. The binding of S protein to the host ACE2 receptor alone is not enough for the viral fusion. The spike protein needs to be cleaved by cell surface serine proteases at specific sites (S1/S2 boundary and S2`), releasing the S1 domain, which subsequently activates the S2 domain, leading to fusion of the viral cellular membranes[23]. Host-cell surface serine proteases shown to cleave the S proteins include, but are not limited to, TMPRSS2, furin, and trypsin. Both ACE2 and

TMPRSS2 (also furin) are highly expressed in the gastrointestinal (GI) tract, particularly intestinal epithelial cells[24]. The primary entry site of SARS-CoV-2 is host lung cells. Nevertheless, the GI may also be a crucial entry or interaction site. SARS-CoV-2 viral particles are preferentially released apically and not at the basement of the airway cells. Thus, the released SARS virus may be removed by mucociliary clearance with access to the GI *via* luminal exposure. Moreover, the early appearance of gastrointestinal symptoms such as nausea, vomiting, abdominal pain, and diarrhea in almost 30% of COVID-19 patients supports this hypothesis.

Viral factors of SARS-CoV-2 and host innate immune response

After the fusion with the cell membrane, the viral genome is released into the host-cell cytoplasm, and the highly controlled process of viral RNA replication and transcription occurs[23]. The virus interacts with cellular compartments and proteins to make its RNAs and proteins. It has been shown that some viral proteins influence critical host-cell processes such as apoptosis, necrosis, innate immunity, and others. One of the structural proteins, nucleocapsid (N) protein, despite its central role in binding to the viral RNA genome, was shown to inhibit type I IFN production and signaling. Recent studies provide evidence that NSP1 (participates in host-cell mRNA degradation and translation inhibition[25]. Hence, the translation of vital cellular proteins, including type I IFN, is shut down, allowing viral RNA to be translated effectively[26]. That may be the reason why NSP1 is cleaved and activated immediately after the production of polypeptide pp1a.

PLpro and Mpro (3CLpro) viral proteases are necessary for the proteolytic cleavage of the polyproteins (pp1a and pp1ab)[20]. Furthermore, it has been shown that the proteases play a role in inhibition of type I IFN signaling. PLpro is responsible for only a few cleavage events in pp1a, but it also can act as a deubiquitinase and deISGylating (removal of IFN-stimulated gene 15 from proteins), which are enzyme activities that lead to evasion and the initial steps of the antiviral response[27,28].

During viral RNA replication and transcription, various PAMPs are produced in the form of double-stranded RNA intermediates. It has been suggested that some of the viral proteins (*i.e.* E, N, NSPs) are involved in the formation of convoluted membranes and double-membrane vesicles to create a protective microenvironment for genomic RNA replication and transcription of subgenomic mRNAs[23]. Furthermore, the PAMPs are recognized by endosomal Toll-like receptors (TLR 3, 7, 8) or cytoplasmic RNA PRRs, such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5). The proper function of TLRs, RIG-I, and MDA-5 is crucial for host-cell survival, as the receptors provide a first-line defense against infections[29-31]. The viral genome attachment to the receptors, especially the TLRs, gives rise to innate immune response signaling pathways. In leukocytes (*e.g.*, dendritic cells, macrophages, natural killer cells, T cells of the adaptive immune system, and B cells), interaction with viral RNA triggers innate immune responses and development of acquired antigen-specific immunity[31]. The innate immune system senses the foreign viral materials that are possibly pathogenic, which initiates downstream signaling to the nucleus, and in turn promotes the expression of types I and III IFNs and other proinflammatory cytokines. Once activated, the IFNs induce a cascade of cellular and molecular events that lead to the suppression of viral replication and reduction in the number of infected cells. Many viral proteins influence the IFN signaling pathway, thus providing a favorable environment for virus development. PLpro, NSP1, ORF3b (a viral accessory protein), and N inhibit two proteins (IRF3 and IRF7) required for INF transcription, thus inhibiting the first steps in the innate immune response against the virus[29-31]. ORF3a and ORF3b are viral proteins that induce caspase-independent necrotic cell death and initiate an inflammatory cascade through activation of the NLRP3 inflammasome[32].

It should be noted that necroptosis and pyroptosis are highly inflammatory mechanisms of cell death that lead to increased secretion of proinflammatory cytokines and chemokines, thus contributing to further tissue damage. Several studies have found that expression of N, E, M, ORF3a, ORF3b, ORF7a, ORF8a, or ORF9b proteins in various cell lines triggered apoptosis through cytochrome C release and caspase-dependent pathways. Apoptosis is a form of noninflammatory cell death that often serves as a host response during viral infection. At the moment, it is not yet clearly elucidated what the exact role of SARS-CoV-2 induced cell death is. It may be an exit strategy to increase viral spread, a form of immune evasion, or just an indirect effect of viral replication on the host cell cycle[23].

Proteome analysis of SARS-CoV-2 has shown that viral proteins interact with more than 300 host-cell proteins, leading to cellular mRNA degradation, inhibition of translation, inhibition of IFN production and signaling, induction of apoptosis and

necrosis, and other activities. The hallmark of all these functions is an evasion of host innate immune responses that could facilitate viral spreading to nearby cells. The changes in infected enterocytes could result in the recruitment of neutrophils, dendritic cells, macrophages, and T cells in the gut mucosa. The mucosal inflammatory response could change the intercellular space between enterocytes, leading to an increase in intestinal permeability that provides an opportunity for bacterial antigens and toxins to enter the bloodstream and further complicated the disease state of COVID-19 patients.

SARS-COV-2 AND MUCOSAL IMMUNITY OF THE GI

An effective and powerful adaptive immune response follows the early antiviral innate response in the mucosa. Expansion of CD4+ T-helper cells, CD8+ cytotoxic T cells, and plasma cells simultaneously with the ongoing innate immune response is critical for virus elimination[33]. However, many factors can alter the immune response and control of the viral replication cycle. External factors such as smoking, pollutants, temperature, humidity, and internal factors such as age and genetics negatively affect the effective response to SARS-CoV-2. Additionally, defects in the immune response towards the virus, such as reduced MBL levels and natural antibodies, anti-IFN autoantibodies, and impaired cytotoxic CD8+ cells, may lead to severe infection because of a lack of effective control of viral replication and the spread of propagation from the upper to the lower airway. Simultaneously, a vast release of proinflammatory cytokines and the recruitment of neutrophils, macrophages, and other cell types contribute to uncontrolled systemic inflammation and cytokine storm [33].

We have to mention the thick layer of mucus on the respiratory, gastrointestinal, and reproductive mucosal surfaces in most mammals that contributes to the first-line defense against various infections. Many studies have reported the essential role that mucins play in infectious diseases, including COVID-19[34,35]. In addition, glycans are complex molecules glycans that play a critical role in communication between cells, including adhesion. The extracellular N-terminal domain and intracellular C-terminal domain undergo biochemical changes during bacterial, viral, and parasitic infections by directly influencing both proinflammatory and anti-inflammatory responses[36]. Mucins sense ligands of pathogenic origin and pass the information downstream by activating immunomodulatory pathways. Currently, 22 genes for membrane-bound and secretory mucins have been documented in humans. Recent data suggest that they can be an entry and/or exit for SARS-CoV-2[36]. Furthermore, mucin levels in bronchoalveolar lavage fluid were shown to correlate with cytokine levels, predicting the magnitude of inflammation (*i.e.* cytokine storm), the hallmark of severe COVID-19 and acute respiratory disease syndrome (ARDS). Prognosis and the response to therapy were also influenced by mucin levels[34].

As the virus can enter through the mouth mucosa or the conjunctival surface of the eye, an excellent immune system response would begin with primed and activated immune cells and molecules, including secretory IgA, and SIgA and then spread through the entire mucosa. Moreover, bronchus-associated lymphoid tissue might contribute to the greater resistance to COVID-19 in children, adolescents, and young people compared with older adults[37]. Particular attention should be paid to SIgA, which plays an effective role in protection against various pathogens by neutralization, inhibition of adherence, and agglutination. As SIgA does not activate the classical complement cascade pathway, it is more anti- than proinflammatory[38]. Furthermore, IgA can inhibit IgM or IgG antibody-activated complement. It has been shown that the mucosal immune response with involvement of SIgA begins around 6-10 d after SARS-CoV-2 infection, with the expression of α4β7 integrin mucosal homing receptors and terminal differentiation of B cells into pIgA-secreting plasma cells in NALT[39]. Serum and salivary IgA antibodies against the spike protein of SARS-Cov-2 have also been reported[40]. Moreover, the salivary IgA has been shown to persist for at least 3 mon. Indeed, IgA antibodies against SARS-CoV-2 were found to be higher in the nasal mucosal fluids, tears, and saliva of infected subjects[41,42], IgA-switched plasmablasts that bear the mucosal chemokine receptor CCR10 were increased in the peripheral blood of SARS-CoV-2-infected subjects[42]. Thus, now we have more data on the IgA antibody production in response to SARS-CoV-2 infection.

In addition to MALT, mucosal-associated invariant T cells were also described. They comprise innate-like T cells (*e.g.*, invariant natural killer T, innate lymphoid cells, and γδ T cells) involved in pulmonary mucosal antiviral immunity and tissue protection

and repair after resolving the infection[43]. Pearson *et al*[44] focused on local mucosal responses during viral infection, particularly with SARS-CoV-2 in both lungs and gut. They found that IL-33 and IL-8 were increased in fecal samples of COVID-19 patients due to intestinal involvement[44,45]. Simultaneously, cytokines such as IL-1b, tumor necrosis factor alpha, and IL-6 were found to decrease. IL-7, a critical cytokine for T cell development and survival was also increased during gastrointestinal infection [46]. In addition to the other T cells in the gut mucosa during COVID-19 infection, and enhanced effector function of Th17 cells has also been seen[47]. By secreting many cytokines, they contribute largely to the acute lung injury observed in severe COVID-19 cases. However, their role in mucosal SARS-CoV-2 infection needs to be elucidated.

Considering the route of infection and the relative independence of mucosal and systemic immune responses, one can suggest that appropriate investigations can establish the role of mucosal immune system in SARS-CoV-2 infection for therapy and prevention. In line with this, intranasal COVID-19 vaccines are an additional hope to promote mucosal immunity against the virus, an apparent advantage of other nasal vaccines (*i.e.* influenza)[48]. Moreover, the advantages of such vaccines including generation of both mucosal (SIgA) and circulating (IgG and IgA) antibodies and SARS-specific effector and memory T cell responses have not be seen in conventional vaccines[49].

It is speculated that anti-SIgA antibodies can neutralize and eliminate SARS-CoV-2 in the mucosa without inflammatory consequences. Furthermore, testing for IgA antibodies in nasal and saliva samples might indicate the presence of mucosal immune responses against SARS-CoV-2. Additionally, serum IgA is distinct from locally secreted IgA$^+$ dimers in plasma cells in the lamina propria of mucosal tissues[50]. The interaction between the virus and the mucosal immune system is shown in Figure 1.

GASTROINTESTINAL INVOLVEMENT DURING SARS-COV-2 INFECTION

Along with its other functions, ACE2 participates in the uptake of amino acids in intestinal epithelial cells, expression of antimicrobial peptides, and gut microbiome ecology[21,51]. As stated above, ACE2 is expressed in almost all human organs, but in varying degrees. Active replication of the SARS-CoV-2 virus has been detected in small-intestine enterocytes isolated from fecal specimens[52]. Other studies showed that the SARS-CoV-2 spike glycoprotein had a 10- to 20-fold higher binding affinity to ACE2 compared with SARS-CoV[53]. ACE2 is highly expressed in the GI[11], and in addition to the small intestine, ACE2 is also highly expressed in the pancreas[54]. Recent studies of single-cell mRNA expression found enriched expression of ACE2 and TMPRSS2 in enterocytes and mucus-producing cells[11,55].

The physiological activities of ACE2 include the absorption of nutrients from digested food. It also maintains osmotic and electrolyte balance across the GI lining epithelium by regulating sodium-dependent amino acid and glucose transporters in the enterocyte brush border[51]. Infectious diarrhea and malabsorption disorders that result from SARS-CoV-2 infection can be explained from a pathophysiological standpoint by the dysregulation of intestinal ion transporters[56]. Studies also suggest dysregulation of these transporters leads to inflammation and GI symptoms[57]. A similar mechanism of enhanced ACE2 expression is known to occur in irritable bowel disease patients who present symptoms similar to those of patients with SARS-CoV-2 [58]. GI cells are potential sites for virus replication of SARS-CoV-2 because of the enriched expression of ACE2 receptors in the mucosal glands and enterocytes[53]. A study using a recombinant strain of SARS-CoV-2 confirmed in situ that the virus could potentially infect and replicate in human intestinal tissue[52]. Once the virus enters the GI cells, it can replicate there, and viral toxin-mediated cell injury can cause gastroen-teritis-like symptoms, including diarrhea, nausea, vomiting, and abdominal pain[59].

Infection caused by SARS-CoV-2 is often associated with typical respiratory response and prevalent gastrointestinal symptoms. ACE2 receptors in the GI play a vital role in the genesis of gastrointestinal symptoms. The mechanism underlying the gastrointestinal symptoms may involve damage to the intestinal mucosal barrier and promote the production of inflammatory factors[60]. Studies show that the incidence of gastrointestinal symptoms in SARS-CoV-2 and MERS-CoV infection is more than 20%[61]. Gastrointestinal symptoms may include vomiting, diarrhea, or abdominal pain in the disease's early phases[62]. The cause of the symptoms is an alteration of intestinal permeability and enterocyte dysfunction[63]. One of the first COVID-19 studies included 204 patients from Wuhan, China, infected with the virus with typical respiratory symptoms, many of whom also showed gastrointestinal symptoms, most
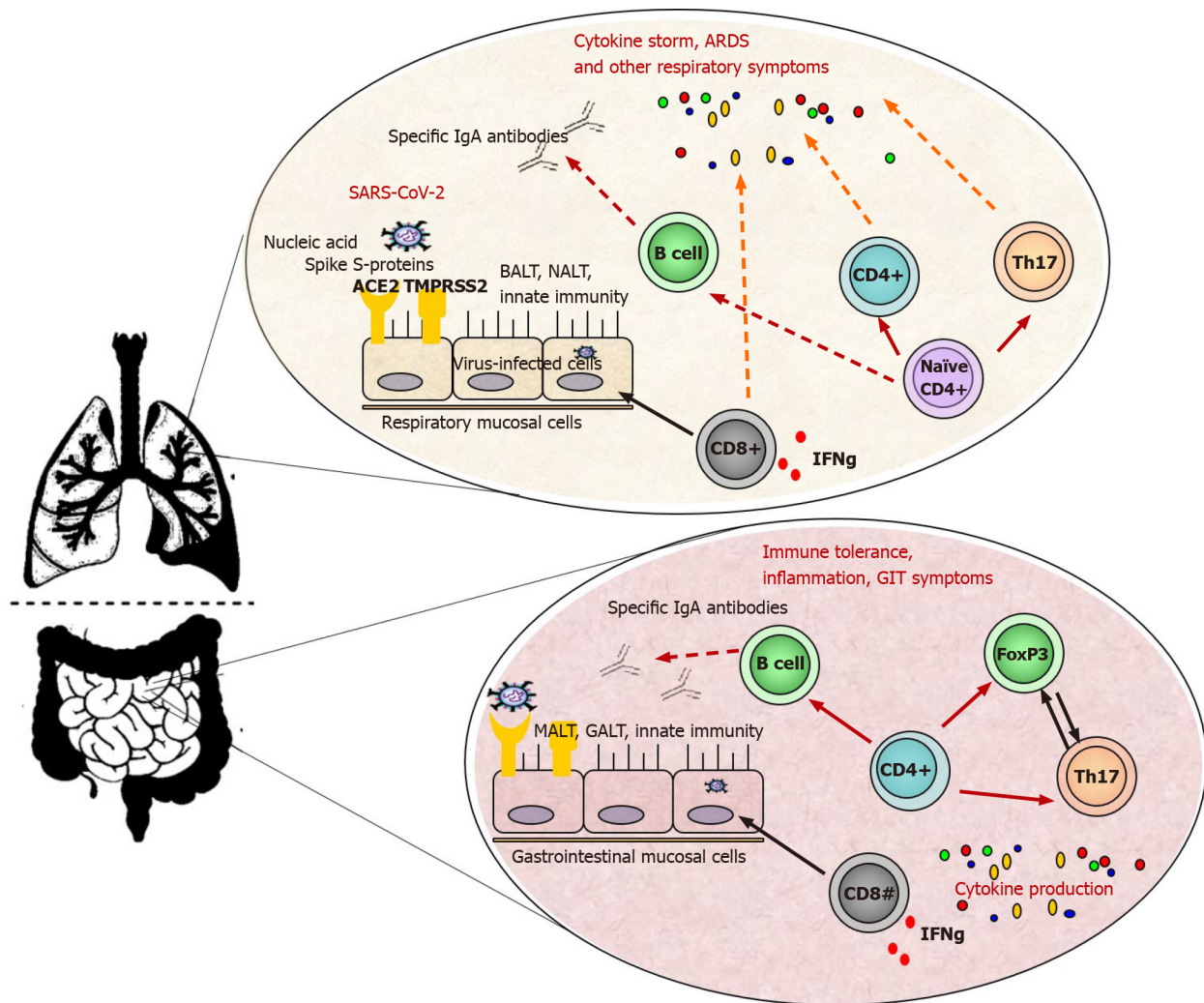
Figure 1 Nasal-, bronchial- and mucosa-associated lymphoid tissue are the first line of defense. Airborne infections usually penetrate the upper airway mucosa, where a higher viral load is found. Nasopharynx-associated lymphoid tissue is involved in the induction of the immune response against the microorganisms by promoting the differentiation and activation of immune cells such as Th1- and Th2 cells, dendritic cells, macrophages, resident microfold M cells, innate lymphoid cells, immunoglobulin (Ig)A-switched B cells, as well as immune mediators and molecules (*i.e.* beta-defensins, galectins, collectins, cytokines). Similar immune processes are also observed in the gut mucosa. However, the expansion of CD4+ T-helper cells, CD8+ cytotoxic T cells, and plasma cells simultaneously with the ongoing innate immune response is critical for virus elimination. Additionally, specific secretory IgA (SIgA) plays an effective role in protection against severe acute respiratory syndrome coronavirus 2 by neutralization, inhibition of adherence, and agglutination. Additionally, SIgA does not activate the classical complement cascade pathway, and thus greater anti- than proinflammatory activity. Innate immune system and some innate receptors (PAMPS, DAMPS) are not shown for simplification of the figure. ACE2: Angiotensin-converting enzyme 2; ARDS: Acute respiratory distress syndrome; BALT: Bronchial-associated lymphoid tissue; GALT: Gut-associated lymphoid tissues; GIT: Gastrointestinal tract. IFN: Interferon; Ig: Immunoglobulin; MALT: Mucosa-associated lymphoid tissue; NALT: Nasopharynx-associated lymphoid tissue; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; TMPRSS2: Transmembrane protease/serine subfamily member 2.

commonly diarrhea. Patients with digestive symptoms have a worse clinical outcome and a longer hospital stay than patients who do not suffer from these symptoms[64].

Although the underlying pathophysiology of gastrointestinal involvement of infection with SARS-CoV-2 is not fully understood, some loss of intestinal barrier integrity and gut microbes is observed. A disruption of intestinal barrier integrity activates innate and adaptive immune cells, which in turn release proinflammatory cytokines into the circulatory system, leading to systemic inflammation[65]. One piece of evidence that SARS-CoV-2 causes an inflammatory response in the gut is elevated levels of fecal calprotectin in patients infected with the virus[66]. Researchers suggest that measuring calprotectin concentrations may play a role in tracking patients infected with SARS-CoV-2. In patients with diarrhea as a symptom, the calprotectin concentrations are elevated, and higher serum IL-6 levels have been reported. It is possible that the disruption of the gut microbiota may be caused by the entry of inflammatory cells, including neutrophils and lymphocytes, into the intestinal mucosa [67]. Studies show that 34% of COVID-19 patients have digestive symptoms, with anorexia and diarrhea being the most common symptoms in adults, while vomiting is

more common in children[68]. Patients with severe COVID-19 have a higher incidence of gastrointestinal symptoms, such as diarrhea and abdominal pain, compared with patients with a mild form of the virus[69]. Nausea and/or vomiting, diarrhea, and loss of appetite are the digestive system's three most common symptoms. Their overall prevalence is around 15% in SARS-CoV-2 infections according to a recent systematic study and meta-analysis involving 6686 patients with GI manifestations. The same study also reported a loss of appetite, ranging from 1% to 79%[69]. The analysis showed that the most common symptom was anorexia (26.8%), but the mechanism remains unclear. The presumption is that widely spread taste and olfactory dysfunctions played a role[70]. Liver injury has also been reported in some patients, with an incidence of 39.6% to 43.4%. The most commonly found elevations are of alanine aminotransferase and aspartate aminotransferase as well as hypoalbuminemia [71].

GUT MICROBIOTA AND COVID-19

The gut microbiota consists of 10^{14} resident microorganisms, including bacteria, viruses, archaea, and fungi[72]. Principally, the gut bacteria in healthy people is dominated by four phyla Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes [73]. The gut microbiota has a validated health role through its protective, trophic, and metabolic actions[73]. Loss of healthy commensal bacteria and overgrowth of pathogenic microbes is described as dysbiosis and critical illness. Dysbiosis is related to increased susceptibility to sepsis, multiorgan failure, and nosocomial infections[74]. The development of gut microbiota alternations in COVID-19 depends on SARS-CoV-2 occurrence, the pharmacotherapy of COVID-19, and the disease-associated GI symptoms[75,76].

In a recent study, Xu *et al*[76] described a decrease of beneficial genera, such as *Lactobacillus* and *Bifidobacterium*, in some patients with COVID-19. In another study, Zuo *et al*[77] investigated gut microbiota in 15 SARS-CoV-2 patients by taking fecal samples 2-3 times during their hospital stay. They found reduced commensal bacteria (*Faecalibacterium prausnitzii*, *Eubacterium ventriosum*, *Roseburia*, and *Rachnospiraceae* taxa) and an increased amount of opportunistic pathogens (*Actinomyces viscosus*, *Clostridium hathewayi*, and *Bacteroides nordii*). Furthermore, the abundance of *Clostridium ramosum*, *Coprobacillus*, and *Clostridium hathewayi* was associated with COVID-19 severity[78]. Another exciting study noted that COVID-19 patients had a significantly reduced microbial diversity, a higher abundance of opportunistic bacteria (Streptococcus, Rothia, Veillonella, and Actinomyces), and an increased abundance of beneficial microbes. Additionally, it showed that the microbial signature in the patients was different from those with influenza A and in healthy controls[78]. In summary, the gut microbiota in SARS-CoV-2 infected patients is modified by the reduction of commensal microbes, loss of bacterial diversity, and increased opportunistic pathogens.

The pharmacological therapies used to treat COVID-19 contribute to gut microbiota alterations. A variety of drugs used to treat COVID-19. Among them are chloroquine phosphate, lopinavir, ritonavir, and remdesivir. In cases with pneumonia, broad-spectrum antibiotics are also administered[79]. Antibiotics are well-known modifiers of gut microbiota, and even if short-term use can reduce microbial diversity and cause dysbiosis[80]. Angelakis *et al*[81] demonstrated that gut microbiota alterations were associated with long-term doxycycline and hydroxychloroquine use, leading to significantly decreased amounts of Bacteroidetes, Firmicutes, and *Lactobacillus*. Such changes may also occur in COVID-19 patients, causing gut dysbiosis. Therefore, they may cause the development of gut dysbiosis-related diseases even after improvement of COVID-19 infection. Consequently, it is suggested to screen stool samples taken from recovered patients at least 35 d after the clearance of the virus from the respiratory tract. Before 35 d, SARS-CoV-2 may still be detected in feces[82]. It is also advised to screen the composition and the activity of gut microbiota to describe its balance.

CONCLUSION

As the virus can enter through mouth mucosa, the expectation is that the triggered immune response that occurs somewhere in the mucosa will spread throughout the entire mucosa. This is especially valid for the GI, which may also be a crucial entry or

interaction site of SARS-CoV-2 infection, leading to complex immune activation, digestive symptoms, altered microbiome, development of complications, and eventually to severe COVID-19 and fatal outcome. However, the role of the gut mucosal immune system as the first line of physical and immunological defense is critical. By carrying out appropriate investigations, the mucosal immune system's role in SARS-CoV-2 infection for therapy and prevention can be established. In line with that, COVID-19 vaccines that stimulate mucosal immunity against the virus may have more advantages than other types of vaccines.

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