**Name of Journal:** *World Journal of Gastroenterology*

**Manuscript NO:** 65117

**Manuscript Type:** MINIREVIEWS

**Gastrointestinal mucosal immunity and COVID-19**

Velikova T *et al*. COVID-19 mucosal immunity

Tsvetelina Velikova, Violeta Snegarova, Alexander Kukov, Hristiana Batselova, Antoaneta Mihova, Radislav Nakov

**Tsvetelina Velikova, Alexander Kukov, Antoaneta Mihova,** Department of Clinical Immunology, University Hospital Lozenetz, Medical Faculty, Sofia University, St. Kliment Ohridski, Sofia 1407, Bulgaria

**Violeta Snegarova,** Clinic of Internal Diseases, Naval Hospital – Varna, Military Medical Academy, Medical Faculty, Medical University, Varna 9000, Bulgaria

**Hristiana Batselova,** Department of Epidemiology and Disaster Medicine, Medical University, Plovdiv, University Hospital "St George", Plovdiv 6000, Bulgaria

**Radislav Nakov,** Clinic of Gastroenterology, Tsaritsa Joanna University Hospital, Medical University of Sofia, Sofia 1527, Bulgaria

**Author contributions:** All the authors wrote sections in the paper; All authors revised and approved the final version of the manuscript.

**Corresponding author: Tsvetelina Velikova, MD, PhD, Assistant Professor,** Department of Clinical Immunology, University Hospital Lozenetz, Medical Faculty, Sofia University, St. Kliment Ohridski, Kozyak 1 str, Sofia 1407, Bulgaria. tsvelikova@medfac.mu-sofia.bg

**Received:** March 3, 2021

**Revised:** May 1, 2021

**Accepted:** July 12, 2021

**Published online:** August 14, 2021

**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

**©The** **Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Citation:** Velikova T, Snegarova V, Kukov A, Batselova H, Mihova A, Nakov R. Gastrointestinal mucosal immunity and COVID-19. *World J Gastroenterol* 2021; 27(30): 5047-5059

**URL:** https://www.wjgnet.com/1007-9327/full/v27/i30/5047.htm

**DOI:** https://dx.doi.org/10.3748/wjg.v27.i30.5047

**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 gastroenteritis-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 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 1014 resident microorganisms, including bacteria, viruses, archaea, and fungi[72]. Principally, the gut bacteria in healthy people is dominated by four phyla Actinobacteria*,* Proteobacteria*,* Firmicutes*,* andBacteroidetes[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.

**REFERENCES**

1 **Gallo O**, Locatello LG, Mazzoni A, Novelli L, Annunziato F. The central role of the nasal microenvironment in the transmission, modulation, and clinical progression of SARS-CoV-2 infection. *Mucosal Immunol* 2021; **14**: 305-316 [PMID: 33244161 DOI: 10.1038/s41385-020-00359-2]

2 **Lehtinen MJ**, Hibberd AA, Männikkö S, Yeung N, Kauko T, Forssten S, Lehtoranta L, Lahtinen SJ, Stahl B, Lyra A, Turner RB. Nasal microbiota clusters associate with inflammatory response, viral load, and symptom severity in experimental rhinovirus challenge. *Sci Rep* 2018; **8**: 11411 [PMID: 30061588 DOI: 10.1038/s41598-018-29793-w]

3 **Xu Z**, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang FS. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; **8**: 420-422 [PMID: 32085846 DOI: 10.1016/S2213-2600(20)30076-X]

4 **Matricardi PM**, Dal Negro RW, Nisini R. The first, holistic immunological model of COVID-19: Implications for prevention, diagnosis, and public health measures. *Pediatr Allergy Immunol* 2020; **31**: 454-470 [PMID: 32359201 DOI: 10.1111/pai.13271]

5 **Zhou Y**, Lu K, Pfefferle S, Bertram S, Glowacka I, Drosten C, Pöhlmann S, Simmons G. A single asparagine-linked glycosylation site of the severe acute respiratory syndrome coronavirus spike glycoprotein facilitates inhibition by mannose-binding lectin through multiple mechanisms. *J Virol* 2010; **84**: 8753-8764 [PMID: 20573835 DOI: 10.1128/JVI.00554-10]

6 **Holodick NE**, Rodríguez-Zhurbenko N, Hernández AM. Defining Natural Antibodies. *Front Immunol* 2017; **8**: 872 [PMID: 28798747 DOI: 10.3389/fimmu.2017.00872]

7 **Guillon P**, Clément M, Sébille V, Rivain JG, Chou CF, Ruvoën-Clouet N, Le Pendu J. Inhibition of the interaction between the SARS-CoV spike protein and its cellular receptor by anti-histo-blood group antibodies. *Glycobiology* 2008; **18**: 1085-1093 [PMID: 18818423 DOI: 10.1093/glycob/cwn093]

8 **Cheng Y**, Cheng G, Chui CH, Lau FY, Chan PK, Ng MH, Sung JJ, Wong RS. ABO blood group and susceptibility to severe acute respiratory syndrome. *JAMA* 2005; **293**: 1450-1451 [PMID: 15784866 DOI: 10.1001/jama.293.12.1450-c]

9 **Centers for Disease Control and Prevention**. Scientific Brief: SARS-CoV-2 Transmission. [cited 27 March 2021]. In: Centers for Disease Control and Prevention [Internet]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/more/scientific-brief-sars-cov-2.html

10 **Zou L**, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J, Guo Q, Song T, He J, Yen HL, Peiris M, Wu J. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *N Engl J Med* 2020; **382**: 1177-1179 [PMID: 32074444 DOI: 10.1056/NEJMc2001737]

11 **Sungnak W**, Huang N, Bécavin C, Berg M, Queen R, Litvinukova M, Talavera-López C, Maatz H, Reichart D, Sampaziotis F, Worlock KB, Yoshida M, Barnes JL; HCA Lung Biological Network. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med* 2020; **26**: 681-687 [PMID: 32327758 DOI: 10.1038/s41591-020-0868-6]

12 **Zhou P**, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. Addendum: A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; **588**: E6 [PMID: 33199918 DOI: 10.1038/s41586-020-2951-z]

13 **Kiyono H**, Fukuyama S. NALT- *vs* Peyer's-patch-mediated mucosal immunity. *Nat Rev Immunol* 2004; **4**: 699-710 [PMID: 15343369 DOI: 10.1038/nri1439]

14 **Wong SH**, Lui RN, Sung JJ. Covid-19 and the digestive system. *J Gastroenterol Hepatol* 2020; **35**: 744-748 [PMID: 32215956 DOI: 10.1111/jgh.15047]

15 **Coronaviridae Study Group of the International Committee on Taxonomy of Viruses.**. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol* 2020; **5**: 536-544 [PMID: 32123347 DOI: 10.1038/s41564-020-0695-z]

16 **Anindita PD**, Sasaki M, Setiyono A, Handharyani E, Orba Y, Kobayashi S, Rahmadani I, Taha S, Adiani S, Subangkit M, Nakamura I, Sawa H, Kimura T. Detection of coronavirus genomes in Moluccan naked-backed fruit bats in Indonesia. *Arch Virol* 2015; **160**: 1113-1118 [PMID: 25643817 DOI: 10.1007/s00705-015-2342-1]

17 **Guarner J**. Three Emerging Coronaviruses in Two Decades. *Am J Clin Pathol* 2020; **153**: 420-421 [PMID: 32053148 DOI: 10.1093/ajcp/aqaa029]

18 **Artika IM**, Dewantari AK, Wiyatno A. Molecular biology of coronaviruses: current knowledge. *Heliyon* 2020; **6**: e04743 [PMID: 32835122 DOI: 10.1016/j.heliyon.2020.e04743]

19 **Li F**. Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annu Rev Virol* 2016; **3**: 237-261 [PMID: 27578435 DOI: 10.1146/annurev-virology-110615-042301]

20 **V'kovski P**, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. *Nat Rev Microbiol* 2021; **19**: 155-170 [PMID: 33116300 DOI: 10.1038/s41579-020-00468-6]

21 **Li W**, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 2003; **426**: 450-454 [PMID: 14647384 DOI: 10.1038/nature02145]

22 **Dahiya DS**, Kichloo A, Albosta M, Pagad S, Wani F. Gastrointestinal implications in COVID-19. *J Investig Med* 2020; **68**: 1397-1401 [PMID: 32928903 DOI: 10.1136/jim-2020-001559]

23 **Hartenian E**, Nandakumar D, Lari A, Ly M, Tucker JM, Glaunsinger BA. The molecular virology of coronaviruses. *J Biol Chem* 2020; **295**: 12910-12934 [PMID: 32661197 DOI: 10.1074/jbc.REV120.013930]

24 **Zang R**, Gomez Castro MF, McCune BT, Zeng Q, Rothlauf PW, Sonnek NM, Liu Z, Brulois KF, Wang X, Greenberg HB, Diamond MS, Ciorba MA, Whelan SPJ, Ding S. TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Sci Immunol* 2020; **5** [PMID: 32404436 DOI: 10.1126/sciimmunol.abc3582]

25 **Kamitani W**, Narayanan K, Huang C, Lokugamage K, Ikegami T, Ito N, Kubo H, Makino S. Severe acute respiratory syndrome coronavirus nsp1 protein suppresses host gene expression by promoting host mRNA degradation. *Proc Natl Acad Sci U S A* 2006; **103**: 12885-12890 [PMID: 16912115 DOI: 10.1073/pnas.0603144103]

26 **Narayanan K**, Huang C, Lokugamage K, Kamitani W, Ikegami T, Tseng CT, Makino S. Severe acute respiratory syndrome coronavirus nsp1 suppresses host gene expression, including that of type I interferon, in infected cells. *J Virol* 2008; **82**: 4471-4479 [PMID: 18305050 DOI: 10.1128/JVI.02472-07]

27 **Clementz MA**, Chen Z, Banach BS, Wang Y, Sun L, Ratia K, Baez-Santos YM, Wang J, Takayama J, Ghosh AK, Li K, Mesecar AD, Baker SC. Deubiquitinating and interferon antagonism activities of coronavirus papain-like proteases. *J Virol* 2010; **84**: 4619-4629 [PMID: 20181693 DOI: 10.1128/JVI.02406-09]

28 **Mielech AM**, Kilianski A, Baez-Santos YM, Mesecar AD, Baker SC. MERS-CoV papain-like protease has deISGylating and deubiquitinating activities. *Virology* 2014; **450-451**: 64-70 [PMID: 24503068 DOI: 10.1016/j.virol.2013.11.040]

29 **Kikkert M**. Innate Immune Evasion by Human Respiratory RNA Viruses. *J Innate Immun* 2020; **12**: 4-20 [PMID: 31610541 DOI: 10.1159/000503030]

30 **Li G**, Fan Y, Lai Y, Han T, Li Z, Zhou P, Pan P, Wang W, Hu D, Liu X, Zhang Q, Wu J. Coronavirus infections and immune responses. *J Med Virol* 2020; **92**: 424-432 [PMID: 31981224 DOI: 10.1002/jmv.25685]

31 **Angelopoulou A**, Alexandris N, Konstantinou E, Mesiakaris K, Zanidis C, Farsalinos K, Poulas K. Imiquimod - A toll like receptor 7 agonist - Is an ideal option for management of COVID 19. *Environ Res* 2020; **188**: 109858 [PMID: 32846644 DOI: 10.1016/j.envres.2020.109858]

32 **Yue Y**, Nabar NR, Shi CS, Kamenyeva O, Xiao X, Hwang IY, Wang M, Kehrl JH. SARS-Coronavirus Open Reading Frame-3a drives multimodal necrotic cell death. *Cell Death Dis* 2018; **9**: 904 [PMID: 30185776 DOI: 10.1038/s41419-018-0917-y]

33 **Velikova TV**, Miteva L, Stanilov N, Spassova Z, Stanilova SA. Interleukin-6 compared to the other Th17/Treg related cytokines in inflammatory bowel disease and colorectal cancer. *World J Gastroenterol* 2020; **26**: 1912-1925 [PMID: 32390702 DOI: 10.3748/wjg.v26.i16.1912]

34 **Bose M**, Mitra B, Mukherjee P. Mucin signature as a potential tool to predict susceptibility to COVID-19. *Physiol Rep* 2021; **9**: e14701 [PMID: 33373502 DOI: 10.14814/phy2.14701]

35 **Zhao Y**, Sato Y, Isaji T, Fukuda T, Matsumoto A, Miyoshi E, Gu J, Taniguchi N. Branched N-glycans regulate the biological functions of integrins and cadherins. *FEBS J* 2008; **275**: 1939-1948 [PMID: 18384383 DOI: 10.1111/j.1742-4658.2008.06346.x]

36 **Bose M**, Mukherjee P. Potential of Anti-MUC1 Antibodies as a Targeted Therapy for Gastrointestinal Cancers. *Vaccines (Basel)* 2020; **8** [PMID: 33167508 DOI: 10.3390/vaccines8040659]

37 **Russell MW**, Moldoveanu Z, Ogra PL, Mestecky J. Mucosal Immunity in COVID-19: A Neglected but Critical Aspect of SARS-CoV-2 Infection. *Front Immunol* 2020; **11**: 611337 [PMID: 33329607 DOI: 10.3389/fimmu.2020.611337]

38 **Russell MW**, Kilian M, Mantis NJ, Corthésy B. Biological activities of mucosal immunoglobulins. In: Mestecky J, Strober W, Russell MW, Kelsall BL, Cheroutre H, Lambrecht BN. Mucosal Immunology. 4th ed. Amsterdam: Academic Press/Elsevier, 2015: 429-454

39 **Quiding-Järbrink M**, Nordström I, Granström G, Kilander A, Jertborn M, Butcher EC, Lazarovits AI, Holmgren J, Czerkinsky C. Differential expression of tissue-specific adhesion molecules on human circulating antibody-forming cells after systemic, enteric, and nasal immunizations. A molecular basis for the compartmentalization of effector B cell responses. *J Clin Invest* 1997; **99**: 1281-1286 [PMID: 9077537 DOI: 10.1172/JCI119286]

40 **Isho B**, Abe KT, Zuo M, Jamal AJ, Rathod B, Wang JH, Li Z, Chao G, Rojas OL, Bang YM, Pu A, Christie-Holmes N, Gervais C, Ceccarelli D, Samavarchi-Tehrani P, Guvenc F, Budylowski P, Li A, Paterson A, Yue FY, Marin LM, Caldwell L, Wrana JL, Colwill K, Sicheri F, Mubareka S, Gray-Owen SD, Drews SJ, Siqueira WL, Barrios-Rodiles M, Ostrowski M, Rini JM, Durocher Y, McGeer AJ, Gommerman JL, Gingras AC. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci Immunol* 2020; **5** [PMID: 33033173]

41 **Cervia C**, Nilsson J, Zurbuchen Y, Valaperti A, Schreiner J, Wolfensberger A, Raeber ME, Adamo S, Weigang S, Emmenegger M, Hasler S, Bosshard PP, De Cecco E, Bächli E, Rudiger A, Stüssi-Helbling M, Huber LC, Zinkernagel AS, Schaer DJ, Aguzzi A, Kochs G, Held U, Probst-Müller E, Rampini SK, Boyman O. Systemic and mucosal antibody responses specific to SARS-CoV-2 during mild versus severe COVID-19. *J Allergy Clin Immunol* 2021; **147**: 545-557.e9 [PMID: 33221383 DOI: 10.1016/j.jaci.2020.10.040]

42 **Sterlin D**, Mathian A, Miyara M, Mohr A, Anna F, Claër L, Quentric P, Fadlallah J, Devilliers H, Ghillani P, Gunn C, Hockett R, Mudumba S, Guihot A, Luyt CE, Mayaux J, Beurton A, Fourati S, Bruel T, Schwartz O, Lacorte JM, Yssel H, Parizot C, Dorgham K, Charneau P, Amoura Z, Gorochov G. IgA dominates the early neutralizing antibody response to SARS-CoV-2. *Sci Transl Med* 2021; **13** [PMID: 33288662 DOI: 10.1126/scitranslmed.abd2223]

43 **Flament H**, Rouland M, Beaudoin L, Toubal A, Bertrand L, Lebourgeois S, Rousseau C, Soulard P, Gouda Z, Cagninacci L, Monteiro AC, Hurtado-Nedelec M, Luce S, Bailly K, Andrieu M, Saintpierre B, Letourneur F, Jouan Y, Si-Tahar M, Baranek T, Paget C, Boitard C, Vallet-Pichard A, Gautier JF, Ajzenberg N, Terrier B, Pène F, Ghosn J, Lescure X, Yazdanpanah Y, Visseaux B, Descamps D, Timsit JF, Monteiro RC, Lehuen A. Outcome of SARS-CoV-2 infection is linked to MAIT cell activation and cytotoxicity. *Nat Immunol* 2021; **22**: 322-335 [PMID: 33531712 DOI: 10.1038/s41590-021-00870-z]

44 **Pearson CF**, Jeffery R; Oxford-Cardiff COVID-19 Literature Consortium, Thornton EE. Mucosal immune responses in COVID19 - a living review. *Oxf Open Immunol* 2021; **2**: iqab002 [PMID: 33585820 DOI: 10.1093/oxfimm/iqab002]

45 **Britton GJ**, Chen-Liaw A, Cossarini F, Livanos AE, Spindler MP, Plitt T, Eggers J, Mogno I, Gonzalez-Reiche AS, Siu S, Tankelevich M, Grinspan LT, Dixon RE, Jha D, Martinez-Delgado G, Amanat F, Hoagland DA, Oever BR, Dubinsky MC, Merad M, van Bakel H, Krammer F, Bongers G, Mehandru S, Faith JJ. SARS-CoV-2- specific IgA and limited inflammatory cytokines are present in the stool of select patients with acute COVID-19. 2020 Preprint. Available from: medRxiv:2020.09.03.20183947 [DOI: 10.1101/2020.09.03.20183947]

46 **Barata JT**, Durum SK, Seddon B. Flip the coin: IL-7 and IL-7R in health and disease. *Nat Immunol* 2019; **20**: 1584-1593 [PMID: 31745336 DOI: 10.1038/s41590-019-0479-x]

47 **Velikova TV**, Kotsev SV, Georgiev DS, Batselova HM. The role of Th17 cells in SARS-CoV-2 infection: implementation for the therapy of severe COVID-19 cases. *CellR4* 2021; **9**: e3058 [DOI: 10.32113/cellr4\_20212\_3058]

48 **Russell MW**, Mestecky J. Mucosal vaccines: Overview. In: Mestecky J, Strober W, Russell MW, Kelsall BL, Cheroutre H, Lambrecht BN, editors. Mucosal Immunology. 4th ed. Amsterdam: Academic Press/Elsevier, 2015: 1039-1046

49 **Velikova T**, Georgiev T. SARS-CoV-2 vaccines and autoimmune diseases amidst the COVID-19 crisis. *Rheumatol Int* 2021; **41**: 509-518 [PMID: 33515320 DOI: 10.1007/s00296-021-04792-9]

50 **Bidgood SR**, Tam JC, McEwan WA, Mallery DL, James LC. Translocalized IgA mediates neutralization and stimulates innate immunity inside infected cells. *Proc Natl Acad Sci U S A* 2014; **111**: 13463-13468 [PMID: 25169018 DOI: 10.1073/pnas.1410980111]

51 **Hashimoto T**, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, Wild B, Camargo SM, Singer D, Richter A, Kuba K, Fukamizu A, Schreiber S, Clevers H, Verrey F, Rosenstiel P, Penninger JM. ACE2 Links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* 2012; **487**: 477-481 [PMID: 22837003 DOI: 10.1038/nature11228]

52 **Lamers MM**, Beumer J, van der Vaart J, Knoops K, Puschhof J, Breugem TI, Ravelli RBG, Paul van Schayck J, Mykytyn AZ, Duimel HQ, van Donselaar E, Riesebosch S, Kuijpers HJH, Schipper D, van de Wetering WJ, de Graaf M, Koopmans M, Cuppen E, Peters PJ, Haagmans BL, Clevers H. SARS-CoV-2 productively infects human gut enterocytes. *Science* 2020; **369**: 50-54 [PMID: 32358202 DOI: 10.1126/science.abc1669]

53 **Wrapp D**, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation. *bioRxiv* 2020 [PMID: 32511295 DOI: 10.1101/2020.02.11.944462]

54 **Pal R**, Banerjee M. COVID-19 and the endocrine system: exploring the unexplored. *J Endocrinol Invest* 2020; **43**: 1027-1031 [PMID: 32361826 DOI: 10.1007/s40618-020-01276-8]

55 **Muus C**, Luecken MD, Eraslan G, Waghray A, Heimberg G, Sikkemaal L, Kobayashi Y, Vaishnav ED, Subramanian A, Smilie C, Jagadeesh K, Duong ET, Fiskin E, Triglia ET, Ansari M, Cai P, Lin B, Buchanan J, Chen S, Shu J, Haber AL, Chung H, Montoro DT, Adams T, Aliee H, Samuel J, Andrusivova AZ, Angelidis I, Ashenberg O, Bassler K, Bécavin C, Benhar I, Bergenstråhle J, Bergenstråhle L, Bolt L, Braun E, Bui LT, Chaffin M, Chichelnitskiy E, Chiou J, Conlon TM, Cuoco MS, Deprez M, Fischer FS, Gillich A, Gould J, Guo M, Gutierrez AJ, Habermann AC, Harvey T, He P, Hou X, Hu L, Jaiswal A, Jiang P, Kapellos T, Kuo CS, Larsson L, Leney-Greene MA, Lim K, Litviňuková M, Lu J, Ludwig LS, Luo W, Maatz H, Madissoon E, Mamanova L, Manakongtreecheep K, Marquette CH, Mbano I, McAdams AM, Metzger RJ, Nabhan AN, Nyquist SK, Penland L, Poirion OB, Poli S, Qi CC, Queen R, Reichart D, Rosas I, Schupp J, Sinha R, Sit RV, Slowikowski K, Slyper M, Smith N, Sountoulidis A, Strunz M, Sun D, Talavera-López C, Tan P, Tantivit J, Travaglini KJ, Tucker NR, Vernon K, Wadsworth MH, Waldman J, Wang X, Yan W, Zhao W, Ziegler CGK, The NHLBI LungMAP Consortium, The Human Cell Atlas Lung Biological Network. Integrated analyses of single-cell atlases reveal age, gender, and smoking status associations with cell type-specific expression of mediators of SARS-CoV-2 viral entry and highlights inflammatory programs in putative target cells. 2020 Preprint. Available from: bioRxiv:2020.04.19.04925 [DOI: 10.1101/2020.04.19.049254]

56 **Das S**, Jayaratne R, Barrett KE. The Role of Ion Transporters in the Pathophysiology of Infectious Diarrhea. *Cell Mol Gastroenterol Hepatol* 2018; **6**: 33-45 [PMID: 29928670 DOI: 10.1016/j.jcmgh.2018.02.009]

57 **Sueyoshi R**, Ignatoski KM, Daignault S, Okawada M, Teitelbaum DH. Angiotensin converting enzyme-inhibitor reduces colitis severity in an IL-10 knockout model. *Dig Dis Sci* 2013; **58**: 3165-3177 [PMID: 23949641 DOI: 10.1007/s10620-013-2825-4]

58 **Young BE**, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, Ng OT, Marimuthu K, Ang LW, Mak TM, Lau SK, Anderson DE, Chan KS, Tan TY, Ng TY, Cui L, Said Z, Kurupatham L, Chen MI, Chan M, Vasoo S, Wang LF, Tan BH, Lin RTP, Lee VJM, Leo YS, Lye DC; Singapore 2019 Novel Coronavirus Outbreak Research Team. Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. *JAMA* 2020; **323**: 1488-1494 [PMID: 32125362 DOI: 10.1001/jama.2020.3204]

59 **Haga S**, Yamamoto N, Nakai-Murakami C, Osawa Y, Tokunaga K, Sata T, Yamamoto N, Sasazuki T, Ishizaka Y. Modulation of TNF-alpha-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-alpha production and facilitates viral entry. *Proc Natl Acad Sci U S A* 2008; **105**: 7809-7814 [PMID: 18490652 DOI: 10.1073/pnas.0711241105]

60 **Ye Q**, Wang B, Zhang T, Xu J, Shang S. The mechanism and treatment of gastrointestinal symptoms in patients with COVID-19. *Am J Physiol Gastrointest Liver Physiol* 2020; **319**: G245-G252 [PMID: 32639848 DOI: 10.1152/ajpgi.00148.2020]

61 **Guan WJ**, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**: 1708-1720 [PMID: 32109013 DOI: 10.1056/NEJMoa2002032]

62 **Villapol S**. Gastrointestinal symptoms associated with COVID-19: impact on the gut microbiome. *Transl Res* 2020; **226**: 57-69 [PMID: 32827705 DOI: 10.1016/j.trsl.2020.08.004]

63 **Jin X**, Lian JS, Hu JH, Gao J, Zheng L, Zhang YM, Hao SR, Jia HY, Cai H, Zhang XL, Yu GD, Xu KJ, Wang XY, Gu JQ, Zhang SY, Ye CY, Jin CL, Lu YF, Yu X, Yu XP, Huang JR, Xu KL, Ni Q, Yu CB, Zhu B, Li YT, Liu J, Zhao H, Zhang X, Yu L, Guo YZ, Su JW, Tao JJ, Lang GJ, Wu XX, Wu WR, Qv TT, Xiang DR, Yi P, Shi D, Chen Y, Ren Y, Qiu YQ, Li LJ, Sheng J, Yang Y. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut* 2020; **69**: 1002-1009 [PMID: 32213556 DOI: 10.1136/gutjnl-2020-320926]

64 **Pan L**, Mu M, Yang P, Sun Y, Wang R, Yan J, Li P, Hu B, Wang J, Hu C, Jin Y, Niu X, Ping R, Du Y, Li T, Xu G, Hu Q, Tu L. Clinical Characteristics of COVID-19 Patients With Digestive Symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. *Am J Gastroenterol* 2020; **115**: 766-773 [PMID: 32287140 DOI: 10.14309/ajg.0000000000000620]

65 **Golonka RM**, Saha P, Yeoh BS, Chattopadhyay S, Gewirtz AT, Joe B, Vijay-Kumar M. Harnessing innate immunity to eliminate SARS-CoV-2 and ameliorate COVID-19 disease. *Physiol Genomics* 2020; **52**: 217-221 [PMID: 32275178 DOI: 10.1152/physiolgenomics.00033.2020]

66 **Mazza S**, Sorce A, Peyvandi F, Vecchi M, Caprioli F. A fatal case of COVID-19 pneumonia occurring in a patient with severe acute ulcerative colitis. *Gut* 2020; **69**: 1148-1149 [PMID: 32245909 DOI: 10.1136/gutjnl-2020-321183]

67 **Xiao F**, Sun J, Xu Y, Li F, Huang X, Li H, Zhao J, Huang J, Zhao J. Infectious SARS-CoV-2 in Feces of Patient with Severe COVID-19. *Emerg Infect Dis* 2020; **26**: 1920-1922 [PMID: 32421494 DOI: 10.3201/eid2608.200681]

68 **Cheung KS**, Hung IFN, Chan PPY, Lung KC, Tso E, Liu R, Ng YY, Chu MY, Chung TWH, Tam AR, Yip CCY, Leung KH, Fung AY, Zhang RR, Lin Y, Cheng HM, Zhang AJX, To KKW, Chan KH, Yuen KY, Leung WK. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples From a Hong Kong Cohort: Systematic Review and Meta-analysis. *Gastroenterology* 2020; **159**: 81-95 [PMID: 32251668 DOI: 10.1053/j.gastro.2020.03.065]

69 **Mao R**, Qiu Y, He JS, Tan JY, Li XH, Liang J, Shen J, Zhu LR, Chen Y, Iacucci M, Ng SC, Ghosh S, Chen MH. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020; **5**: 667-678 [PMID: 32405603 DOI: 10.1016/S2468-1253(20)30126-6]

70 **Lechien JR**, Chiesa-Estomba CM, De Siati DR, Horoi M, Le Bon SD, Rodriguez A, Dequanter D, Blecic S, El Afia F, Distinguin L, Chekkoury-Idrissi Y, Hans S, Delgado IL, Calvo-Henriquez C, Lavigne P, Falanga C, Barillari MR, Cammaroto G, Khalife M, Leich P, Souchay C, Rossi C, Journe F, Hsieh J, Edjlali M, Carlier R, Ris L, Lovato A, De Filippis C, Coppee F, Fakhry N, Ayad T, Saussez S. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur Arch Otorhinolaryngol* 2020; **277**: 2251-2261 [PMID: 32253535 DOI: 10.1007/s00405-020-05965-1]

71 **Chen N**, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020; **395**: 507-513 [PMID: 32007143 DOI: 10.1016/S0140-6736(20)30211-7]

72 **Gill SR**, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. Metagenomic analysis of the human distal gut microbiome. *Science* 2006; **312**: 1355-1359 [PMID: 16741115 DOI: 10.1126/science.1124234]

73 **Villanueva-Millán MJ**, Pérez-Matute P, Oteo JA. Gut microbiota: a key player in health and disease. A review focused on obesity. *J Physiol Biochem* 2015; **71**: 509-525 [PMID: 25749935 DOI: 10.1007/s13105-015-0390-3]

74 **Nakov R**, Segal JP, Settanni CR, Bibbò S, Gasbarrini A, Cammarota G, Ianiro G. Microbiome: what intensivists should know. *Minerva Anestesiol* 2020; **86**: 777-785 [PMID: 32368882 DOI: 10.23736/S0375-9393.20.14278-0]

75 **Kaźmierczak-Siedlecka K**, Vitale E, Makarewicz W. COVID-19 - gastrointestinal and gut microbiota-related aspects. *Eur Rev Med Pharmacol Sci* 2020; **24**: 10853-10859 [PMID: 33155247 DOI: 10.26355/eurrev\_202010\_23448]

76 **Xu K**, Cai H, Shen Y, Ni Q, Chen Y, Hu S, Li J, Wang H, Yu L, Huang H, Qiu Y, Wei G, Fang Q, Zhou J, Sheng J, Liang T, Li L. [Management of corona virus disease-19 (COVID-19): the Zhejiang experience]. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2020; **49**: 147-157 [PMID: 32096367 DOI: 10.3785/j.issn.1008-9292.2020.02.02]

77 **Zuo T**, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, Wan Y, Chung ACK, Cheung CP, Chen N, Lai CKC, Chen Z, Tso EYK, Fung KSC, Chan V, Ling L, Joynt G, Hui DSC, Chan FKL, Chan PKS, Ng SC. Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. *Gastroenterology* 2020; **159**: 944-955.e8 [PMID: 32442562 DOI: 10.1053/j.gastro.2020.05.048]

78 **Gu S**, Chen Y, Wu Z, Chen Y, Gao H, Lv L, Guo F, Zhang X, Luo R, Huang C, Lu H, Zheng B, Zhang J, Yan R, Zhang H, Jiang H, Xu Q, Guo J, Gong Y, Tang L, Li L. Alterations of the Gut Microbiota in Patients With Coronavirus Disease 2019 or H1N1 Influenza. *Clin Infect Dis* 2020; **71**: 2669-2678 [PMID: 32497191 DOI: 10.1093/cid/ciaa709]

79 **Kupferschmidt K**, Cohen J. Race to find COVID-19 treatments accelerates. *Science* 2020; **367**: 1412-1413 [PMID: 32217705 DOI: 10.1126/science.367.6485.1412]

80 **Mu C**, Zhu W. Antibiotic effects on gut microbiota, metabolism, and beyond. *Appl Microbiol Biotechnol* 2019; **103**: 9277-9285 [PMID: 31701196 DOI: 10.1007/s00253-019-10165-x]

81 **Angelakis E**, Million M, Kankoe S, Lagier JC, Armougom F, Giorgi R, Raoult D. Abnormal weight gain and gut microbiota modifications are side effects of long-term doxycycline and hydroxychloroquine treatment. *Antimicrob Agents Chemother* 2014; **58**: 3342-3347 [PMID: 24687497 DOI: 10.1128/AAC.02437-14]

82 **Ianiro G**, Mullish BH, Kelly CR, Kassam Z, Kuijper EJ, Ng SC, Iqbal TH, Allegretti JR, Bibbò S, Sokol H, Zhang F, Fischer M, Costello SP, Keller JJ, Masucci L, van Prehn J, Quaranta G, Quraishi MN, Segal J, Kao D, Satokari R, Sanguinetti M, Tilg H, Gasbarrini A, Cammarota G. Reorganisation of faecal microbiota transplant services during the COVID-19 pandemic. *Gut* 2020; **69**: 1555-1563 [PMID: 32620549 DOI: 10.1136/gutjnl-2020-321829]

**Footnotes**

**Conflict-of-interest statement:** The authors declare that they have no competing interests.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Peer-review started:** March 3, 2021

**First decision:** April 17, 2021

**Article in press:** July 12, 2021

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Bulgaria

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): B

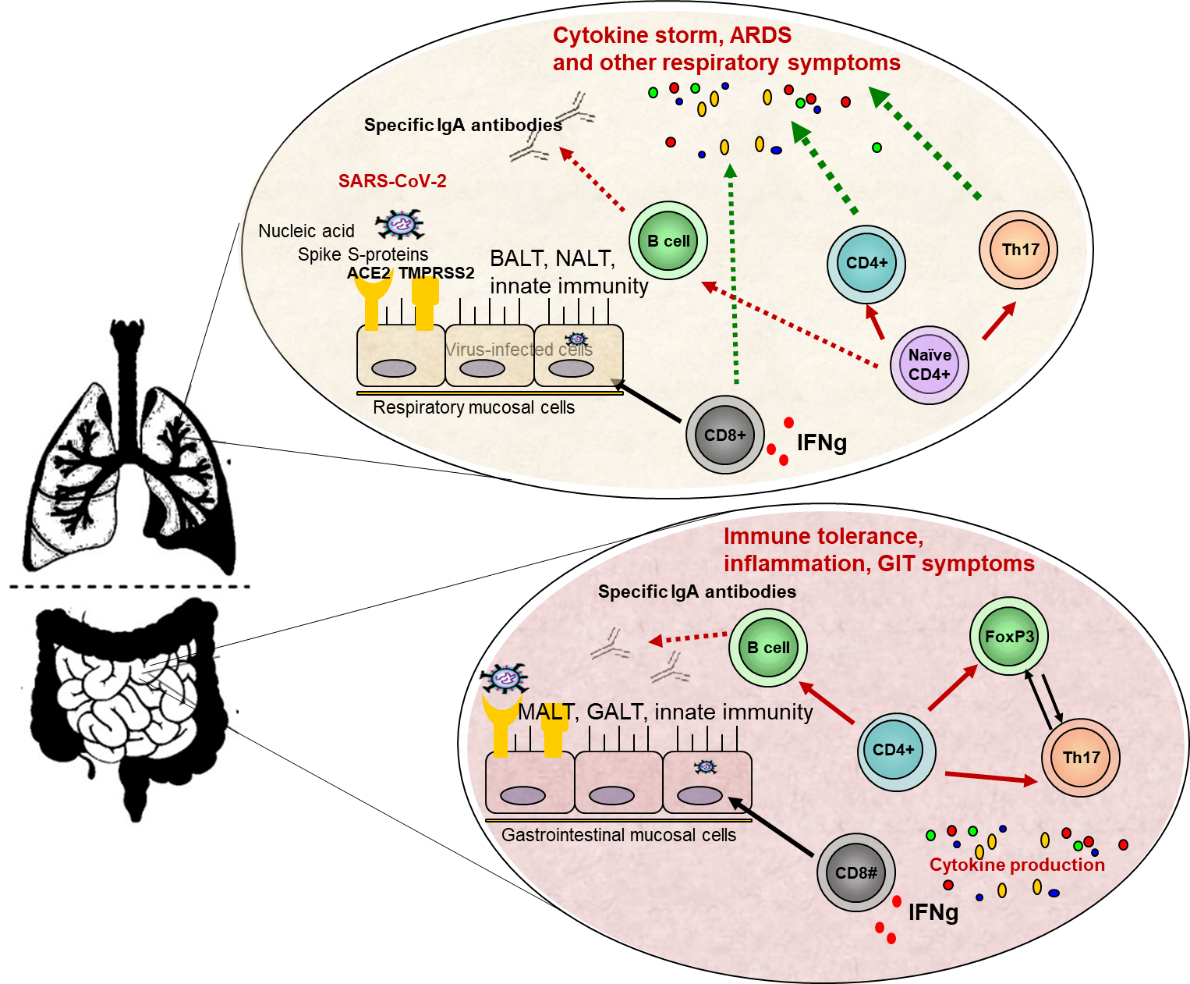
Grade C (Good): 0

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Lakshin G **S-Editor:** Gao CC **L-Editor:** Filipodia **P-Editor:** Liu JH

**Figure Legends**



**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.



Published by **Baishideng Publishing Group Inc**

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

**Telephone:** +1-925-3991568

**E-mail:** bpgoffice@wjgnet.com

**Help Desk:** https://www.f6publishing.com/helpdesk

https://www.wjgnet.com



**© 2021 Baishideng Publishing Group Inc. All rights reserved.**