Review

The Interplay Between Multisystem Inflammatory Syndrome in Children, Interleukin 6, Microbiome, and Gut Barrier Integrity

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Abstract: A severe consequence of SARS-CoV-2 infection that manifests as systemic inflammation and multi-organ involvement is called Multisystem Inflammatory Syndrome in Children (MIS-C). This review examines the possible relationship between gut barrier integrity, the microbiome, dysregulation of interleukin-6 (IL-6) signaling, and MIS-C. Clinical and biochemical features of MIS-C are similar to those of other hyperinflammatory syndromes, suggesting a dysregulated immune response. One possible explanation for the systemic inflammation seen in MIS-C patients is SARS-CoV-2-induced dysregulation of the IL-6 signaling pathway. In addition, new data suggest a reciprocal link between gut barrier integrity and IL-6. SARS-CoV-2 exhibits bacteriophage-like behavior, highlighting the role of bacteria as a reservoir for the virus and emphasizing the importance of understanding the bacteriophagic mechanism of the virus in fecal-oral transmission. Increased translocation of viral products and bacterial toxins may result from disrupting the intestinal barrier and cause systemic inflammation. On the other hand, systemic inflammation can weaken the integrity of the intestinal barrier, which feeds back into the loop of immunological dysregulation. In the context of MIS-C, understanding the interaction between SARS-CoV-2 infection, IL-6, and gut barrier integrity may shed light on the etiology of the disease and guide treatment options.

Keywords: MIS-C; IL-6; gut barrier dysfunction; zonulin levels; microbiome; SARS-CoV-2 bacteriophage behavior; Long COVID-19

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1. Introduction

COVID-19 is not as severe in children as it is in adults in most situations. Children are the target of around 18% of coronavirus infections overall and 1% of COVID-19 cases. The 5-15 age group has a higher incidence of COVID-19. Children in the United States have a seropositivity rate of approximately 75%, which is greater than that of adults. Fortunately, the majority of pediatric patients (>90%) with SARS-CoV-2 infection are asymptomatic or only show moderate signs such as weakness, dry cough, and low fever. In 2021, only 2% of all infected children in the United States required intensive care unit (ICU) care. 27% had moderate symptoms similar to influenza, 5% had substantial pneumonic symptoms, and 66% of all infected children exhibited no symptoms at all [1]. SARS-CoV-2 caused about 48 out of every 100,000 children under the age of 18 to be hospitalized. The prevalence is highest in children aged 0–4 years (66.8 per 100,000), next in children aged 12–17 years (59.9 per 100,000), and lowest in children aged 5–11 years (25 per 100,000 cases). As of February 2021, the number of child deaths from COVID-19 had dropped to 0.17 per 100,000 persons in seven countries—the US, the UK, Italy, Germany, Spain, and France. Among children under 18, COVID-19 was responsible for only 0.48% of the total predicted deaths from all causes in an average year [2]. Similar findings have been reported in other works [3-9].

Several investigations have shown that with the appearance of new less pathogenic variants, the percentage of children and adolescents with COVID-19 who needed to be admitted to the ICU fell sharply from the beginning of the pandemic across all age categories, even though community infection rates eventually increased [10,11]. Wang et al. [11] found that in children under five years old, Omicron infection was associated with a decreased probability of hospitalization, intensive care support, and hospitalizations than Delta infection in the United States. 1.7% of children and adolescents with COVID-19 were admitted to ICU by the Omicron wave in England. All children and adolescents who died within 28 days of being hospitalized for COVID-19 had medical comorbidities, as did the majority of those who needed to be admitted to the ICU [12,13].

Numerous theories have been presented to explain why COVID-19 is less severe in infants. Thymic involution is one plausible cause. A group of researchers has demonstrated that the frequency of COVID-19 hospital admissions in several nations regularly doubles every 16 years of age. They concluded that, in contrast to T-cell production, the probability of hospitalization for COVID-19 increased exponentially with age [14]. Moreover, men are more likely than women to be hospitalized with COVID-19 [15-17]. Men generate fewer T cells and have a higher rate of thymic involution than women. Consequently, thymic involution may be partially responsible for the hospitalization rate associated with COVID-19. However, the researchers found that a further 49-75% of the under-20s are protected from serious disease by other factors. Thymic involution is therefore one, but not the only, explanation for the increasing hospitalization rate with age [14].

To evaluate the immunological responses of children and adults, asymptomatic individuals, those with moderate disease, and individuals who experienced severe COVID-19, Banoun [18] conducted a review study. The majority of adults have antibodies (and T cells) that cross-react with SARS-CoV-2 and other human coronaviruses such as common colds. In adults, however, this response is not related to the severity of the disease; rather, there appears to be a qualitative rather than a quantitative difference (i.e., children have IgM and anti-S, whereas adults have IgG, IgA, and anti-N). Adults who are mildly affected share some of the child's cross-reactivity. The immaturity of the T cells involved is where the differences between children and adults lie at the cellular level [18]. Salivary and mucosal IgA levels are positively associated with the absence of SARS-CoV-2 infection and negatively correlated with age, and are distinct and more potent than serum IgA [19]. In children without symptoms, the innate cellular response in the upper and lower airways is more efficient; the interferon response occurs earlier and involves immune cells instead of epithelial cells, which are linked to hyper-inflammation [20,21]. Given that SARS-CoV-2 has the capacity to inhibit interferon 1 responses, this initial reaction is essential [21].

Regulatory T cells (Tregs), which play an important role in keeping the inflammatory response under control, are found in the respiratory tissues of children. Studies have shown that the lungs and other mucosal and non-lymphoid tissues in children have the highest levels of Tregs [22]. Adult lung

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tissue has a much lower frequency of Tregs among all CD4+ T cells, at about 5%, compared to pediatric lung tissue, which has a frequency of 15%. The increased concentration of Tregs in a child's respiratory tissues is probably caused by the respiratory system's persistent exposure to inhaled pathogens and antigens, which requires a careful balance between tissue maintenance and immunological responses [22].

When comparing mildly affected children and adults to those severely affected with COVID-19, there is a qualitative difference in the response of neutrophils and macrophages/monocytes, which are also accountable for hyper-inflammation: in severe cases, there is an increase in the number of classical monocytes and dysfunctional neutrophils [20,23].

Banoun [18] also reiterated the importance of the gut microbiome (GM) on the severity of COVID-19, as GM and respiratory infections are strongly linked and may influence the host's response to pneumonia. The synthesis of short-chain fatty acids (SCFAs), the control of systemic inflammation, the establishment of oral immunological tolerance via Tregs, and the management of extra-intestinal T cell populations are some of the potential pathways [24]. Therefore, this paper hypothesizes that gut dysbiosis may also influence the pathogenesis of the multisystem inflammatory response syndrome in children **(**MIS-C) and clinical outcomes in affected children, and presents evidence to support this proposal.

2. Long COVID-19 and (MIS-C) are Different

Long COVID-19 and MIS-C are two distinct disorders caused by the SARS-CoV-2 virus [25]. Long COVID-19, sometimes called Post-Acute Sequelae of SARS-CoV-2 (PASC), is a syndrome in which people diagnosed with COVID-19 have a variety of symptoms that affect their daily activities, including physical, mental, emotional, and behavioral problems that persist long after the initial infection [26]. These symptoms, which include cardiovascular symptoms (heart palpitations, for example), breathing difficulties, and extreme fatigue, can last for months or even years [26]. On the other hand, MIS-C is an uncommon illness that may occur in children infected with SARS-CoV-2. It involves inflammation in various organs, including the brain, skin, eyes, heart, lungs, kidneys, and the gastrointestinal tract. Although it can be life-threatening, the majority of children recover with medical attention [25,27,28].

3. The GM Supports Barrier Protection Functionality

The pathophysiology of numerous inflammatory and immunological disorders is intimately linked to the integrity of the intestinal barrier [29-31], a dynamic structure that interacts with and responds to a range of stimuli. It is composed of surface mucus, the epithelial layer, and immunological defenses [30]. The mucosal and epithelial components of the physical barrier are closely associated with several cellular junctions, such as adherens junctions (AJ), tight junctions (TJs), and desmosomes [32]. The intestinal epithelium serves its main function as a barrier limiting the interaction between luminal elements (e.g., gut bacteria), the core immune system, and other parts of the organism [33]. Additionally, the normal GM constitutes the majority of the biological barrier, which also controls the intestinal micro-ecological equilibrium [34]. Increased epithelial permeability and possibly microbial dysbiosis lead to a disruption of the intestinal barrier, resulting in a leaky gut. This can allow antigens, bacteria, and toxins from the lumen to enter the circulation and cause a variety of systemic side effects, such as oxidative stress, increased inflammation, and reduced insulin sensitivity (i.e. insulin resistance) [29,35,36].

The GM plays a critical role in maintaining the integrity of the intestinal barrier through several mechanisms, including the production of metabolites that directly regulate the barrier's permeability and the modulation of the immune system's response to the microbiome [37,38]. SCFAs, a type of microbial metabolite, have been shown to improve intestinal barrier function by stimulating the synthesis of TJ proteins, which are critical for preserving the integrity of the epithelial layer. In addition, SCFAs contain anti-inflammatory properties that can help reduce the permeability of the barrier and prevent the entry of hazardous chemicals and pathogens [37].

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4. The Link between Gut Microbiome Dysbiosis and MIS-C

Constant fever, vomiting, diarrhea, skin irritation, abdominal pain, and, in severe cases, hypotension and shock are the hallmarks of MIS-C [25-27] One of the most common MIS-C symptoms is gastrointestinal distress, raising the possibility that the GM may act as both a local and systemic inflammatory modulator [39,40].

The human gastrointestinal tract, particularly the colon, is home to trillions of microorganisms, that contain hundreds of times more genes in them than there are in the human genome [41,42]. In this sense, the human body can be thought of as a supra (or meta)-organism. The composition and activity of the GM are co-evolved with the host. It is influenced throughout life, from birth to old age, by a dynamic and complex interaction between the host genome and lifestyle variables, particularly nutrition, which is becoming recognized as the primary modulator of microbial activity [43,44].

This microbial population produces vitamins and ferments macromolecules like proteins, lipids, and carbohydrates. These metabolic effects are collectively known as co-metabolism [45]. Furthermore, the GM is primarily responsible for maintaining host homeostasis through the "training" of host immunity and structural/protective actions against commensal pathogens, specifically safeguarding the intestinal barrier [45]. Eubiosis is maintained by the GM when it is healthy. In pathological circumstances, the GM experiences an unbalanced state of dysbiosis wherein there is either a decrease in beneficial commensals, a proliferation of opportunistic pathogens, or both [46].

The intestinal barrier typically blocks substances and microbes from moving from the lumen to the circulation. However, intestinal dysbiosis, or dysregulation of the gut flora, may result in a disorder called "leaky gut syndrome", characterized by increased permeability that may trigger the innate immune system and promote low-grade inflammation. In recent times, GM dysbiosis has been linked to extra-intestinal as well as intestinal diseases. These include chronic diseases that are particularly prevalent in the elderly, such as diabetes and its severe vascular consequences [47-49]. In adults, it is increasingly recognized that the gut acts as a reservoir for SARS-CoV-2 [50], and that dysbiosis and GI barrier disruption induce inflammatory activation in severe COVID-19 [51,52].

Numerous investigations have revealed that GM is also affected in adult patients with long-term COVID. One study found that GM dysbiosis continued for up to 30 days after disease resolution, which may be related to persistent symptoms of PASC [53]. It is known that angiotensin-converting enzyme 2 (ACE2) regulates the synthesis of neutral amino acid transporters in the gut [54], which regulate the composition of the GM and, ultimately, the immune responses in the body [55,56]. In COVID-19 patients who already had age-related disorders, ACE2 imbalance has been associated with poor outcomes (including increased disease severity and mortality rate) through its impact on intestinal dysbiosis [57].

In a 6-month follow-up, Liu et al. [58] confirmed that long-term COVID patients had different GM species than controls and a consistently decreased diversity. Remarkably, the dysbiosis pattern was different among those who initially had COVID-19 but did not have long-term COVID-19. In the long-COVID-19 subgroup, tiredness, respiratory, and neuropsychiatric problems were strongly correlated with rising fecal relative abundance of opportunistic microorganisms [58]. Additional research revealed that long COVID-19 is associated with dysbiosis of the GM in patients who have recovered a year after being discharged, suggesting that the GM may be crucial in long COVID-19 [59].

Although the aforementioned findings explicitly demonstrated that alterations in the microbiome have a deleterious effect on the extended clinical course of COVID-19 in adults, there has been a lack of research on this crucial topic in MIS-C. The composition, diversity, and abundance of the gut microbiome were found to be different in MIS-C cases compared to COVID-19 cases and healthy controls. At the phylum level, the MIS-C group had an abundance of Bacteroidetes, whereas the healthy children had Firmicutes. When MIS-C was compared to the SARS-CoV-2 group and the healthy control group, the relative abundance of Bacteroidetes increased, and the Firmicutes: Bacteroides ratio significantly dropped [60]. Romani et al. [61] investigated the GM of children with COVID-19, including four cases of MIS-C. Veillonella, Ruminococcus, Clostridium, Dialister, and

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Streptococcus were more prevalent in the GM of MIS-C patients, while Bifidobacterium, Blautia, Granulicatella, and Prevotella were less prevalent.

Pro-inflammatory taxa were more prevalent and anti-inflammatory taxa were less prevalent in children with MIS-C. Children with MIS-C and COVID-19 [60] had lower concentrations of *Faecalibacterium prausnitzii*, which has been demonstrated to use butyrate to reduce intestinal mucosal inflammation and maintain gut physiology [62,63]. Therefore, it has been proposed that "alterations of the intestinal microbiome may contribute to the pathophysiology of MIS-C predisposing factors" [60].

4.1. SARS-CoV-2 Infection Impairs Gut Barrier Integrity by Inducing Zonulin Release

For successful maintenance of intestinal homeostasis, mucosal integrity and management of intestinal permeability are essential [64]. From a structural perspective, the gut barrier can be defined as a working structure composed of mechanical components like mucus and the layer of epithelial cells. Intestinal bacteria are secluded from the underlying lamina propria by the dynamic physical and biochemical barrier known as the intestinal mucosa, which also inhibits the infiltration of pathogenic and antigenic molecules [65]. Despite the presence of chemical and physical barriers in the intestines, some pathogens can cross through it and interact with intestinal epithelial cells (IECs). Several sensing pattern recognition receptors (PRRs) are expressed by IECs, which are essential for maintaining the intestinal mucosal immune response's homeostasis [66].

In response to bacterial signals, PRRs can initiate a physiological inflammatory response that promotes progenitor cell proliferation, epithelial cell survival, and pathogen elimination—all of which enhance intestinal integrity [67]. Crucially, intercellular junctional complexes consisting of desmosomes, TJs, AJs, and gap junctions (GJ) connect IECs. Together, these complexes establish intercellular communication, control paracellular permeability, and allow passive entry of water, ions, and nutrients. Maintaining intracellular adhesion helps to create a semi-permeable protective barrier that prevents pathogenic and commensal microbes and antigens from penetrating the lamina propria [68,69].

As was already mentioned, children with MIS-C frequently experience GI symptoms, which can lead to a severe hyper-inflammatory reaction and cardiac problems. A SARS-CoV-2 infection is known to cause MIS-C several weeks later, and it is known that the viral load in respiratory secretions decreases for 7–10 days following infection [70-72]. It seems doubtful that the initial respiratory tract infection in children with MIS-C is related to the disease, as the majority of them have negative nasopharyngeal viral swabs [70]. Furthermore, several lines of evidence demonstrated that SARS-CoV-2 can establish robust infection and replication in human IECs, which may contribute to GI dysfunction and potential fecal-oral transmission in some patients [73-75]. Patients with SARS-CoV-2 infection had elevated zonulin levels, which were linked to worse outcomes [40,76-79]. Furthermore, high serum zonulin levels disrupt the brain-blood barrier (BBB), allowing viruses to enter the brain and induce serious neurological symptoms [40,80]. Numerous autoimmune and hyper-inflammatory illnesses, including celiac disease [81], inflammatory bowel disease [82], and Kawasaki disease [83], have been linked to elevated circulating zonulin levels and increased intestinal permeability [82,84].

In 2021, Yonker et al. [40] examined bio specimens from 100 children: 19 had acute COVID-19, 26 had MIS-C, and 55 were controls. Reverse transcription PCR (RT-PCR) was used to detect SARS-CoV-2 in stool samples, and zonulin and other indicators of mucosal barrier integrity were tested in plasma. Zonulin is a member of a structurally and functionally similar family of proteins that modulate intercellular TJss to reversibly regulate intestinal permeability [85-87]. Large antigens, such as viral antigens generated from SARS-CoV-2 present in the GI tract, should not be able to pass through the gut lumen and into the bloodstream when the intestinal mucosal barrier is intact and functioning [50]. According to this seminal research, the prolonged presence of undigested SARS-CoV-2 viruses causes the gastrointestinal tract to release more zonulin, which increases intestinal permeability [40].

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Elevated zonulin levels suggest that intestinal epithelial TJss are failing, possibly allowing SARS-CoV-2 antigens to penetrate the blood. Several events, including gut dysbiosis [88], trigger the release of zonulin in a myeloid differentiation primary response 88 (MyD88)-dependent manner, which enables zonulin to bind to its target, the protease-activated receptor 2 (PAR2), and subsequently transactivate the epidermal growth factor receptor (EGFR) [89]. This initiates a chain of events that results in the phosphorylation of TJ proteins, such as myosin 1c and zonula occludens 1 (ZO1), which in turn leads to the disintegration of TJs and an increase in the permeability of paracellular membranes to macromolecules [90]. This, in turn, accelerates the trafficking of viral antigens into the bloodstream (Figure 1), resulting in hyper-inflammation [40].

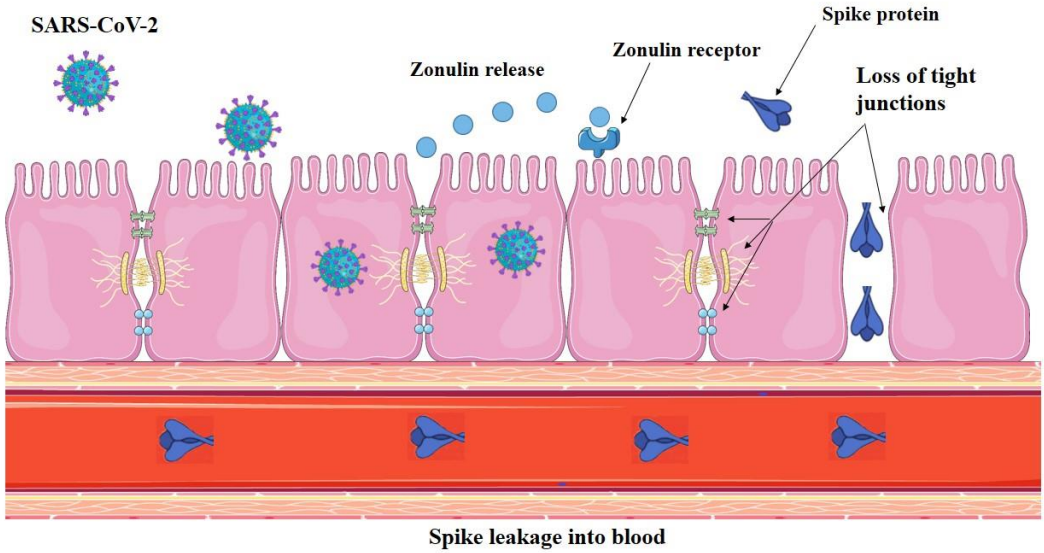

Figure 1. Zonulin is a molecular gatekeeper that regulates TJs between intestinal epithelial cells. Normally, these TJs serve as a selective barrier, preventing chemicals and pathogens from entering the bloodstream from the intestines. SARS-CoV-2 infection in the GI tract causes localized inflammation of the mucosa, which in turn triggers zonulin release. This, in turn, enhances gut permeability, allowing SARS-CoV-2 antigens, such as the spike protein's superantigen-like motif, to pass through mucosal barriers and enter the bloodstream. Parts of the figure were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

The development of zonulin-dependent loss of gut integrity in infants affected with MIS-C, but not COVID-19, suggests that a chronic form of SARS-CoV-2-induced dysbiosis in the gut leads to a progressive deterioration of mucosal barrier integrity. Viremia was not found in MIS-C, although antigenemia and viremia have been demonstrated to correlate with severe acute COVID-19 in adults [72,91].

Yonker et al. [40] found SARS-CoV-2 cleaved soluble component of spike (S1), and nucleocapsid antigens in the blood of children suffering from MIS-C, even though the children had been exposed to or had been infected with the virus weeks before. Compared to MIS-C patients and healthy controls ($p < 0.0001$), and children with acute COVID-19 ($p < 0.001$), SARS-CoV-2 spike protein levels were significantly higher. They also found that patients with MIS-C had significantly higher levels of SARS-CoV-2 S1 protein than healthy controls ($p = 0.004$) and children with acute COVID-19 ($p = 0.02$). Compared with healthy controls, the researchers found no discernible increase in blood levels of SARS-CoV-2 antigen in children with acute COVID-19 [40].

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4.2. Bacteriophage-Like Behavior of SARS-CoV-2 and MISC

In addition to SARS-CoV-2-mediated damage to the integrity of the intestinal barrier induced by zonulin release, other important research performed by Brogna and colleagues revealed that this virus is capable of infecting intestinal bacteria, acting like a bacteriophage [92-96]. According to a previous study by that group, the blood, feces, and urine of COVID-19 patients contained toxin-like peptides that were almost identical to the poisonous elements of animal venoms, including conotoxin, phospholipases A2, phosphodiesterases, zinc metal proteinases, and bradykinins [97]. Later research revealed that SARS-CoV-2 might replicate autonomously in bacterial cultures derived from patient feces for up to 30 days and longer [98]. Viral-like structures ranging in size from 25 to 100 nm were observed by electron microscopy interacting with and within bacterial cell walls (Figure 2). The presence of SARS-CoV-2 nucleocapsid protein both inside and outside of the bacteria was verified by immunolabeling; specifically two anti-inflammatory gut bacterial species normally present in a healthy human GM (Faecalibacterium prausnitzii and Dorea formicigenerans) [94]. These species were previously found to be considerably reduced in severe COVID-19 cases [53,99] and in children with MIS-C [60]. Importantly, both bacteria have been shown to use butyrate, thereby preserving gut physiology and reducing gut mucosal inflammation [62,63].

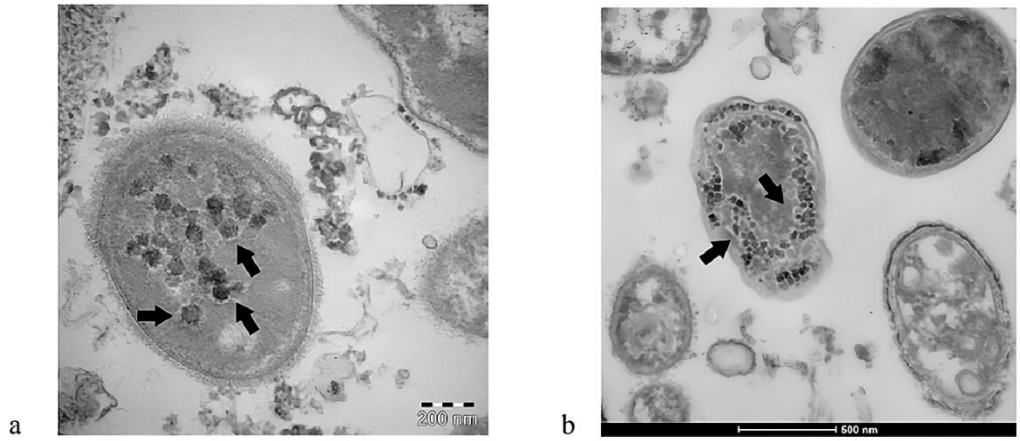

Figure 2. SARS-CoV-2 can infect intestinal bacteria, thus showing a bacteriophage-like behavior. Panels a and b: Images from a transmission electron microscope revealed the presence of SARS-CoV-2 inside two bacteria, marked by black arrows. Source: [93]. This figure is open access and is distributed under the Creative Commons Attribution Non-Commercial (CC BY-NC 4.0) license.

In addition, *de novo* synthesis of SARS-CoV-2 spike protein was detected in the bacterial cultures using 15N-labelled nitrogen as a source, accompanied by an increase in viral RNA load [94]. The discovery that SARS-CoV-2 can infect bacteria cells that are a component of the normal human GM and synthesize both nucleic acid and viral peptides has major consequences for human health, especially concerning the regulation and control of the virus spread. For example, the ability of SARS-CoV-2 to infect and replicate in human gut bacteria may lead to a scenario in which infected bacteria continue to harbor the virus for prolonged periods, possibly even after the systemic infection of the human host has resolved [94]. Epidemiological studies have shown that this may result in prolonged viral shedding through the intestinal tract, potentially increasing the likelihood of subsequent transmission. Furthermore, prolonged survival due to continuous replication of the virus in the intestinal lumen could allow recurrent revival of systemic SARS-CoV-2 infection in the person, leading to COVID-19 relapse without the need for external reinfection [94].

The combined deleterious effects of SARS-CoV-2 infection reflected as increased zonulin release [40] and the decrease in commensal bacterial populations due to the bacteriophage-like effect of SARS-CoV-2 [92-96] could further compromise the integrity of the intestinal barrier, allowing the passage of the spike protein and toxins released by infected bacteria.

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5. The Link between IL-6 Levels, Gut Barrier Integrity, and MIS-C

During the COVID-19 pandemic, several meta-analyses demonstrated that high levels of IL-6 have been linked to some adverse health effects, including acute respiratory distress syndrome, death, and ICU hospitalization. Serum IL-6 levels were almost three times greater in patients with such complex types of COVID-19 than in patients with less severe disease [100-104]. However, extensive research allows us to understand that, depending on the specific circumstances of the immune response, IL-6 is a cytokine with multiple functions, having both pro- and anti-inflammatory effects [105]. Initially discovered as B-cell stimulatory factor 2 (BSF-2), IL-6 stimulates activated B cells to produce immunoglobulin (Ig) [106]. At low, physiological concentrations, it regulates many important immune functions. Innate and adaptive immune systems are largely developed and activated in large part by IL-6. This cytokine induces monocytes to differentiate into macrophages rather than dendritic cells (DCs) in the innate immune system [107].

Moreover, the increase of anti-apoptotic proteins that support T cell survival is associated with IL-6 signaling [108-110]. Furthermore, IL-6 inhibits transforming growth factor beta (TGF-β)-mediated maturation of Tregs and promotes the development of untrained CD4+ T cells into effector T cell subgroups, such as pathogen-specific effector Th17 cells [111,112]. Therefore, an increase in IL-6 levels causes immune dysregulation, as in COVID-19′s cytokine storm, and also in other autoimmune disorders and cancer [111,113-118]. IL-6 is also essential for epithelial proliferation and wound repair [119] and for immune-epithelial-bacteria communication in both beneficial as well as harmful processes. For example, *Pediococcus acidilactici* K15, a lactic acid bacterial strain, promotes the secretion of IL-6 in BDCA1+ DCs (mDC1) via its double-stranded RNA and enhances the synthesis of protective IgA antibodies [120].

Conversely, high levels of IL-6 can cause inflammation, and many probiotics work to lower these levels to treat intestinal disease [121]. In the context of ulcerative colitis (UC), a study found that at physiological levels, IL-6 controls epithelial barrier function by modulating the expression of TJs-related proteins. In contrast, IL-6 levels in the plasma of UC patients were elevated and increased as the disease worsened. Overproduction of IL-6 has also been shown to damage the intestinal epithelial cell barrier and control barrier function by increasing zonulin release. Conversely, when an anti-IL-6 antibody was added, the amount of zonulin was lower than in the control group [122].

It was discovered that long-term SARS-CoV-2 infection in the GI tract triggered zonulin release in children with MIS-C, with subsequent trafficking of SARS-CoV-2 antigens into the circulation, resulting in hyper-inflammation and signs of a cytokine storm, including significantly higher levels of TNF-α, IL-6, IL-10, and IL-1β [40]. Scientists demonstrated that SARS-CoV-2 RNA is still present in the GI tract for weeks after initial infection and viral antigenemia is correlated with zonulin-induced increased permeability (leaky gut) of the mucosal barrier [40].

Thus, it is likely that the high zonulin levels found in children with MIS-C [40] are due to excessive concentrations of IL-6 released by SARS-CoV-2 infected cells at the GI. In addition, several works have demonstrated that in MIS-C, impairment of the innate and adaptive immune responses is responsible for the prolonged presence of the virus in the GI tract [123-128].

6. Preventive and Therapeutic Strategies for MIS-C

Yonker et al. [40] used larazotide, a zonulin antagonist, to treat children with MIS-C and evaluated the impact on antigenemia and the children's clinical outcomes. After receiving larazotide treatment for MIS-C, the patient's plasma SARS-CoV-2 spike antigen levels and inflammatory markers decreased concurrently, leading to a greater clinical improvement than what was currently possible with known treatments. In adults with COVID-19 and long COVID, Brogna et al. [129] discovered that patients who started early antibiotic treatment showed a statistically significant decrease in recovery time, and such treatment was critical for maintaining high blood oxygen saturation levels. Delayed antibiotic initiation within the first 3 days increased the risk of pneumonia in both vaccinated and unvaccinated patients.

Furthermore, it is noteworthy that a considerable proportion of patients who were administered antibiotics within the initial three days and throughout the full seven days of the acute phase did not

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experience long COVID. One of the main contributing factors to the development of the disease appears to be the bacteriophage behavior of SARS-CoV-2 during the acute and post-COVID-19 phases. Early antibiotic treatment appears to be essential for halting the progression of disease, controlling toxin release from infected bacteria, and preventing viral replication in the GM [129].

Another potential approach could involve employing anti-IL-6 antibodies such as tocilizumab or sarilumab. However, it has been shown that IL-6 suppression in COVID-19 patients affects the neutralizing capacity of anti-SARS CoV-2 antibodies [130]. Considering that the neutralizing activity of anti-SARS-CoV-2 antibodies determines protection against symptomatic infection [131], the study conducted by Della-Torre calls for a rigorous reevaluation of the risk of reinfection and severe illness in patients receiving anti-IL-6 antibodies [130].

Certain Gram-negative bacterial strains, such as *Escherichia coli*, Prevotella, Pseudomonas, and Salmonella spp., have been found to induce intestinal zonulin release, while other strains, primarily Gram-positive ones, like Bifidobacterium and Lactobacillus spp., have been found to decrease zonulin levels. These findings are consistent with previous research conducted on cell lines and animal models (reviewed in [132]).

Probiotics are live microorganisms that, when administered in the right quantities and for the right length of time, benefit the health of the host [133]. Through their surface molecules and metabolites, probiotics and intestinal symbionts can alter the host's intestinal barrier function [134]. Several studies have shown that probiotics reduce both intestinal permeability and epithelial barrier dysfunction in gastrointestinal disorders, thereby demonstrating the role of GM in improving intestinal barrier function and protection (Figure 3) against pathogens [135-137].

At present, there is no doubt about the link between GM and the regulation of zonulin release, as some probiotic strains have been shown to improve gut barrier function by affecting the expression of zonulin and TJ proteins [132,138-140]. Probiotics dramatically enhanced gut barrier functioning, according to a meta-analysis of data from a total of 26 randomized controlled trials (n = 1891). Specifically, the trans-epithelial resistance (TER) was significantly enhanced, while serum zonulin, endotoxin, and lipopolysaccharide levels were importantly reduced. Moreover, probiotic groups outperformed control groups in lowering inflammatory markers like IL-6, tumor necrosis factor-α (TNF-α), and C reactive protein. Additionally, probiotics can regulate the composition of the GM by increasing the enrichment of Lactobacillus and Bifidobacterium [137].

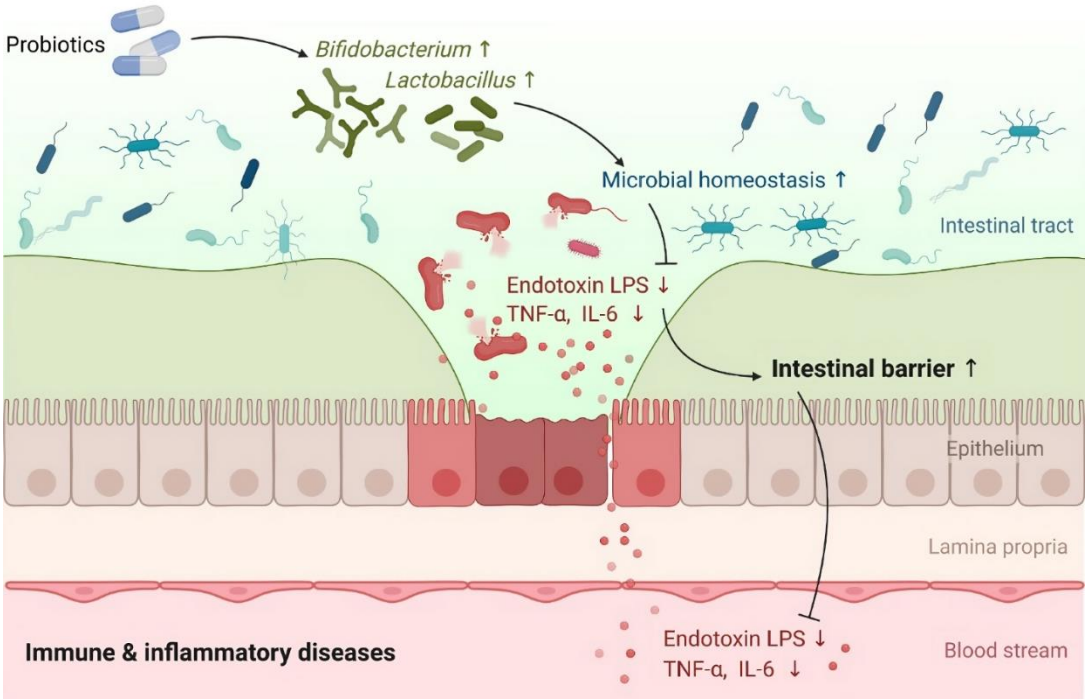

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Figure 3. A graphical representation shows the influence of probiotics on intestinal barrier function in immunological and inflammatory conditions. Source: [137]. This figure is open access and is distributed under the Creative Commons Attribution Non-Commercial (CC BY-NC 4.0) license.

Oral probiotic therapy has also been utilized in pediatric and adult populations to reduce the incidence and severity of respiratory infections. In this regard, it is interesting to mention that in 95% of MIS-C patients, hypovitaminosis D was found [141]. The severity of MIS-C and 25-hydroxyvitamin D [(25-(OH) D] levels were found to be significantly correlated; children with more severe MIS-C also had lower 25(OH) D levels. Echocardiograms showed worse left ventricular systolic and diastolic performance in MIS-C patients with decreased vitamin D levels [141]. The effects of vitamin D on the immune system and inflammatory response have been the subject of extensive investigation in recent years. Vitamin D affects immunological response as well as the proliferation and differentiation of immune cells through binding to vitamin D receptors [142-145]. Vitamin D additionally controls the proliferation and antibody synthesis in B lymphocytes [146]. It has been established that immunological illnesses and disorders are more likely to occur in people with low serum levels of 25(OH) D [147], and some scientists proposed that MIS-C may be the cause of hypovitaminosis D [148].

There is mounting evidence that vitamin D levels are related to the composition of the GM [149]. A systematic analysis identified 25 research papers that investigated the relationship between vitamin D and GM; it was found that supplementing with vitamin D caused a notable increase in the abundance of Firmicutes and Bifidobacterium, and Firmicutes were found to be correlated with serum Vitamin D levels [150]. Vitamin D insufficiency affects over 80% of people in certain geographic regions and has been connected to inflammation and gut dysbiosis [151-153]. In a study of 80 vitamin D-deficient women, its administration (after 8 weeks) markedly increased GM diversity. More precisely, the abundance of the probiotic taxa Bifidobacterium and Akkermansi increased in parallel with the Bacteroidetes to Firmicutes ratio [149].

By strengthening the gut-lung axis and controlling the host inflammatory response, probiotics can elicit antiviral effects [154]. In a pediatric experiment (n = 31 prebiotics, 31 probiotics, and 32 placebos), preterm infants were given a prebiotic combination of galacto-oligosaccharide and polydextrose or the probiotic *Lactobacillus rhamnosus* GG mixed with breast milk throughout the first 60 days of life. There were fewer cases of virus-associated respiratory tract infections in the probiotic and prebiotic groups ($p = 0.022$ and $p < 0.001$, respectively) [155]. Probiotics may lessen symptoms of upper respiratory tract infections and stabilize GM diversity, according to a study that supplied Lab4P probiotics (comprising lactobacilli and bifidobacteria) daily to 220 overweight and obese adults [154,156]. This may be especially important for COVID-19 infections, where obesity is linked to worse outcomes [157,158].

Research into the treatment of COVID-19 in children using oral microbial interventions is scarce, despite its potential [39,159]. Probiotics have only been tested in a small number of adult patients with COVID-19, but the results were encouraging, showing reductions in viral load, hospitalization duration, death, and diarrhea frequency [160-164]. Adults with moderate-to-severe COVID-19 were given a supplementary oral dose of the *Bifidobacterium animalis* sp. Lactis strain (n = 20 probiotic, 24 non-probiotic). The probiotic group experienced a five-day reduction in hospital stay ($p < 0.001$) and a corresponding decrease in IL-6 levels ($p < 0.001$) [162].

A single-center, quadruple-blinded, randomized trial was carried out by other researchers on adult outpatients with symptomatic Covid-19. For 30 days, subjects were randomly assigned to either a probiotic formula (including *Lactiplantibacillus plantarum* KABP022, KABP023, and KAPB033, as well as *Pediococcus acidilactici* KABP021) or a placebo [161]. Of the 147 patients, 78 (53.1%) in the probiotic group experienced complete remission, while 41 (28.1%) in the placebo group did. There were no hospitalizations or deaths during the research, and the nasopharyngeal viral load, lung infiltrates, and duration of both digestive and non-digestive symptoms were all reduced when compared to placebo. There were no significant differences in fecal microbiome composition between probiotic and placebo groups, although probiotic treatment boosted specific IgM and IgG antibodies against SARS-CoV2 when compared to placebo. Therefore, rather than altering the diversity of the

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colonic microbiome, it is assumed that probiotics predominantly enhance the host´s immune system [161]. They promote the synthesis of immunoglobulins, specifically IgA and type I interferons, and boost the induction of interleukins and the activation of macrophages, natural killer cells, and T-helper cells [165,166].

Probiotic supplementation may therefore help to restore gut health by enhancing immune responses, reducing inflammation, and reinforcing the epithelial barrier, thus possibly decreasing susceptibility to severe SARS-CoV-2 infection. It is suggested that probiotics should be used as a prophylactic rather than a treatment for severe cases of MIS-C, as once a cytokine storm is activated it is very difficult to control.

7. Conclusions

Research has shown that viral infections of the respiratory tract, such as H1N1 influenza A virus [167] and respiratory syncytial virus [168], can affect GM in children. Although the complex relationship between invasive viruses and host physiology is not yet fully understood, increasing data suggests that the microbiome may influence the course of viral diseases [169]. The GM diversity has been suggested as a predictive biomarker of COVID-19. This diversity is characterized by a lower ratio of Firmicutes to Bacteroidetes, a higher abundance of Proteobacteria, and a lower abundance of beneficial butyrate-producing bacteria [170], and it has been proposed that the microbiome of children may have a high level of protective resilience due to characteristics such as the comparatively high abundance of Bifidobacterium [39]. Research suggests that changes in the composition of the gut microbiome may play a role in the production of inflammatory cytokines induced by SARS-CoV-2, which could trigger the cytokine storm [171].

Lower levels of Bifidobacterium have been linked to the severity of SARS-CoV-2 infection [53,171-175]. Prebiotics are frequently used to enhance the effects of probiotics [176], which have been successfully tested and hypothesized to improve SARS-CoV-2 symptoms [160,161,177-179].

Through their metabolic byproducts and end products, Bifidobacterium spp. symbiotically feed other gut microorganisms, which in turn improve butyrate synthesis and decrease inflammation [180]. The anti-inflammatory effects of Bifidobacterium may be one of the methods by which it increases "natural immunity”," hence defending against the consequences of SARS-CoV-2 infection [181]. It has been demonstrated that Bifidobacterium binds TNF-α [182,183]. According to Cervantes and Hong [184], this binding could absorb TNF-α from the gut, which will then decrease it in the bloodstream and eventually adsorb it from the lungs and other affected tissues (the "gut-lung axis"). Moreover, Bifidobacterium species modulate the helper T cell response and raise levels of the anti-inflammatory cytokine interleukin IL-10. Furthermore, Bifidobacterium stimulates the Th1 immune response while suppressing the Th2 immunological response [185].

Regarding the pathogenic role of SARS-CoV-2, Brogna et al. [92,94,96] discovered that this virus, by exhibiting a bacteriophage-like behavior, infects and destroys two important anti-inflammatory bacteria (Faecalibacterium prausnitzii and Dorea formicigenerans) that are normally present in a healthy gut. Interestingly, both species are butyrate-producing bacteria. Butyrate is an important short-chain fatty acid that contributes to gut health by serving as an energy source for the body, stimulating immunological responses, having anti-inflammatory effects, and supporting colon cell function [62,186,187]. Previously, the prevalence of these species was found to be much lower in sever COVID-19 cases [53,99], in post-acute COVID-19 syndrome (PACS) [58,188], and in children with MIS-C [60]. Such a reduction in the number of these bacteria could be due to the destruction caused by the lytic phase of SARS-CoV-2 [92]. A relevant study demonstrated that fecal transplant from PACS patients into germ-free animals resulted in lung inflammation and worse outcomes following pulmonary infection with multidrug-resistant Klebsiella pneumoniae. Transplanted mice also performed poorly in cognitive tests. These findings showed that altered GM may significantly contribute to PACS [189].

In addition to the destruction of beneficial bacteria by SARS-COV-2 [92,94,96], this virus also induces an excessive IL-6-mediated zonulin release that increases intestinal permeability (leaky gut), so the spike protein and toxins released by the bacteria pass into the bloodstream causing MIS-C [40].

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Finally, there is evidence that if damage to the mucosal epithelium is prevented and treated early in the disease, MIS-C may not develop [40]. Given that gut dysbiosis may predispose children to MIS-C, it is important to strengthen their microbiome by consuming probiotics, particularly butyrate-producing Bifidobacterium, and diets high in plant fiber (prebiotics).

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