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Gut bacteria, bacteriophages, and probiotics: Tripartite mutualism to quench the SARS-CoV2 storm

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ABSTRACT
Patients with SARS-CoV-2 infection, exhibit various clinical manifestations and severity including respiratory and enteric involvements. One of the main reasons for death among covid-19 patients is excessive immune responses directed toward cytokine storm with a low chance of recovery. Since the balanced gut microbiota could prepare health benefits by protecting against pathogens and regulating immune homeostasis, dysbiosis or disruption of gut microbiota could promote severe complications including autoimmune disorders; we surveyed the association between the imbalanced gut bacteria and the development of cytokine storm among COVID-19 patients, also the impact of probiotics and bacteriophages on the gut bacteria community to alleviate cytokine storm in COVID-19 patients. In present review, we will scrutinize the mechanism of immunological signaling pathways which may trigger a cytokine storm in SARS-CoV2 infections. Moreover, we are explaining in detail the possible immunological signaling pathway-directing by the gut bacterial community. Consequently, the specific manipulation of gut bacteria by using probiotics and bacteriophages for alleviation of the cytokine storm will be investigated. The tripartite mutualistic cooperation of gut bacteria, probiotics, and phages as a candidate prophylactic or therapeutic approach in SARS-CoV-2 cytokine storm episodes will be discussed at last.

1. Introduction

The emergence of SARS-CoV-2 as a global pandemic brings up the Coronaviridae alongside the Orthomyxoviridae as two virus families with the ability to threaten the lives of human beings around the world. Although the estimated patient fatality rate due to COVID-19 has been lower than the Spanish influenza pandemic, SARS-CoV-2 has by far the highest socio-economic impact than other infectious agents that humans have ever encountered to date. There have been almost over than 555 million confirmed cases of SARS-CoV-2 infection, including more than 6.3 million deaths till July 11, 2022, as reported by WHO.

SARS-CoV-2 infects target cells via the ACE2 receptor, a transmembrane protein found in a variety of human tissues such as the small intestine, testis, heart, and lung [1]. In a SARS-CoV-2 infected person, the virus exacerbates mainly respiratory involvement in the form of viral pneumonia. The status of Immune response against viral components depends on various factors including age, sex, and underlying diseases [2,3]. The severity of COVID-19 disease is likely due to not only viral replication but also the status of the host immune responses. Meanwhile, one of the main causes of death in COVID-19 patients is due to aggressive inflammatory responses by hyper-production of an array of pro-inflammatory cytokines that are intensely associated with lung damage and multiorgan dysfunction with a low chance of recovery. The clinical condition is referred as to SARS-CoV-2 cytokine storm (SARS-CS) [4,5]. In the lack of sufficient therapeutic interventions, the SARS-CS comes to be a life-threatening situation with a series of serious clinical manifestations. The exact mechanisms of triggering cytokine...
storm scenarios among vulnerable high-risk groups are not yet well understood. Nevertheless, dysregulation of immune homeostasis plays an important role in the development of COVID-19 severity among high-risk groups [6,7]. The gut microbiota provides essential health benefits by regulating immune homeostasis. There is growing evidence, which has illustrated dysbiosis or disruption of gut microbiota could promote severe complications including asthma, cardiovascular disease, and autoimmune disorders [8,9]. Moreover, several layers of investigations have been conducted to assess the impact of gut microbiota dysbiosis (genetics, underlying disease, diet, antibiotics consumption, etc.), which results in cytokine storm in COVID-19 patients [10,11]. Furthermore, recent studies have demonstrated that COVID-19 patients have represented gut microbiota dysbiosis, which also could result in cytokine storm [12,13]. Unrevealing the cross-talk between gut bacteria and immune response could provide efficient strategies to intervene or prevent cytokine storm in SARS-CoV-2 patients. Although some aspects of this topic have been partly reviewed previously, a comprehensive view of SARS-CS to facilitate its treatment is still lacking with unmet clinical needs [14,15]. Herein, we provide an updated scenario of immunological signaling pathways of SARS-CoV-2 infection and SARS-CS. Initially, we are explaining the currently identified immunological signaling pathway features directed by the gut bacterial community, and also cross-talk between gut bacteria and SARS-CoV-2. Consequently, the feasibility of specific manipulation of imbalanced gut bacteria by using the probiotics and bacteriophages through their mechanism of action for alleviation of drastic inflammatory responses resulting in cytokine storm will be investigated. Overall, we aimed to suggest the tripartite mutualistic cooperation of gut bacteria, phages, and probiotics as a candidate prophylactic or therapeutic approach in SARS-CS episodes.

2. Immunopathology of SARS-CoV-2

2.1. Innate immune response

The first line of innate immune defense against viral infections includes a set of PRRs, including TLRs and RLRs that recognize the viral RNA genome and its replication intermediaries. Upon recognition, a variety of mediators and cell types can be induced by TLR pathways. This activates of inflammatory signaling pathways, results in a cytokine storm and widespread damage to the host inducing the stimulation of antiviral interferon response. TLR9/MyD88 leads to the production of type I interferons (IFN-α and IFN-β) and pro-inflammatory cytokines (IL-6, IL-8, IL-10, IL-17, and TNF-α), through SARS-CoV-2 infection [16,17]. TLR3, TLR7/8, and RIG-I/MDA-5 pathss through ACE2, detect PAMPs, and trigger downstream signaling by binding the adapter proteins MyD88 and MAVS, which cause the transcription factors IFR3/7 and NF-κB to be activated, resulting in the production of IFN-1 and pro-inflammatory cytokines. Also, the NF-κB pathway can be activated by TLR2, which detects Spike (S) protein and causes the production of inflammatory cytokines and chemokines [18–20]. Active viral replication leads to activation of neutrophils and monocytes/macrophages (MQ), which results in the overproduction of pro-inflammatory cytokines. Neutrophils are an early representative of SARS-CoV-2 infection, and the development of extracellular NETs is one of the processes by which neutrophils clear the infection. NETs may contribute to cytokine release and progression to respiratory failure. Activated neutrophils also release leukotrienes and ROS, thereby inducing cytotoxicity such as a cytokine storm [21,22]. In addition, activated neutrophils appropriately express properdin, factors B and C3, thereby promoting complement activation, a hallmark of severe COVID-19 disease [21]. Complement is a key player in the innate immune system’s antiviral response, but its excessive activation can lead to pro-inflammatory responses and tissue damage. A recent study on SARS-CoV-2 showed that activation of the complement component C3 exacerbates disease in SARS-CoV-2 associated with cytokine storm and ARDS [23].

COVID-19 manifests with MQ infiltration into lung tissue, with epithelial cell and lung cell apoptosis, NF-κB pathway induction, and cytokine production. This inflammatory response is required to initiate immune responses against SARS-CoV-2 and excessive inflammation in the form of a cytokine storm contributes to COVID-19-related mortality such as TGF-β that promotes fibroblast proliferation and contributes to pulmonary fibrosis in COVID-19 patients [24,25]. In addition, cytokines such as IL-12 secreted by MQs and DCs can promote NK cell proliferation, cytotoxicity and immunostimulatory cytokine secretion such as; IFN-γ. NK cell stimulation may play a role in limiting SARS-CoV-2 infection [26]. SARS-CoV-2 also enhances the production of pro-inflammatory cytokines by DCs in response to signals activated by bacterial LPS via TLR-4, which further contributes to the induction of a destructive inflammatory response. The upregulation of IL-6 is an established effect of TLR-4 signaling, which occurs through the NF-κB signaling pathways [27,28]. In addition, innate APCs, such as DCs and MQs, provide an important bridge connecting innate and adaptive immune responses and present viral antigens to virus-specific T cells at the site of infection. This triggers the host’s adaptive immunity, which is mediated by virus-specific B (humoral immunity) and T (cellular immunity) cells [29,30]. Although DC infection appears to play an important role in driving the cytokine storm and in regulating the T-cell response to SARS-CoV-2, the exact mechanisms used by the virus to change DC function demand more investigation [31,32].

2.2. Adaptive immune response

Once the innate immune system has been activated, the adaptive immune system runs in. The adaptive immune system comprises three main cell types: CD4 + T helper cells (TH), CD8 + Cytotoxic T cells (CTL), and B cells. Normally, during infection, activated Th1 effector T cells produce pro-inflammatory cytokines (IFN-γ, IL-2, and GM-CSF) and antiviral cytokines, such as granzyme B, TNF-α, TNF-β, and TNF-κ to
stimulate responses of T cells. In addition, GM-CSF activates the CD14 + inflammatory monocytes to produce large amounts of IL-6, TNF-α, and other cytokines. This is followed by an infiltration of macrophages and neutrophils for pulmonary tissues, resulting in a cytokine storm [33]. It has been reported that significant increases in typical Th2 cytokines, including IL-4, IL-5, IL-10, and IL-13. Both IL-4 and IL-13 are primarily involved in inflammatory fibroblast remodeling, while Th1 cells exert anti-fibroblast activity by secreting IFN-γ- and IL-2 [34].

Circulating CD8 + T cells specific for SARS-CoV-2 were observed less consistently than CD4+ T cells. CD8+ T cells are important in clearing many viral infections because of their ability to target the infected cells. During acute COVID-19, SARS-CoV2 specific CD8 + T cells express high levels of molecules involved in potent cytotoxic function, such as IFN-γ, granzyme B, and perforin, [35]. A decrease in peripheral blood T cells associated with disease severity and inflammation is now well documented in COVID-19. The number of T cells was inversely correlated with IL-6, IL-10, and TNF-α levels. High concentrations of TNF-α, IL-6, and IL-10 in cytokine storm induce negative regulatory effects on T cell survival and proliferation [36-38].

CD4+ T cells drives B lymphocytes to produce virus-specific antibodies such as IgG and IgM. In addition to producing virus-specific antibodies, activated B cells also secrete IL-1, IL-6, IL-8, TNF-α, GMCSF, and other cytokines, which can intensify the cytokine storm. As a result, viral replication will not be inhibited and viral infection will spread throughout the body, leading to further imbalance in the immune response and the release of many pro-inflammatory cytokines by non-specific immune cells [33,39]. In addition, a Th17 response has been detected and confirmed in patients with COVID-19. There is increasing evidence that Th17 cells that produce the inflammatory cytokine IL-17 recruit more monocytes/MQs and neutrophils to the site of infection and stimulate other cytokine cascades, such as IL-1β and IL-6, among others [40]. Elevated levels of IL-17 have been reported in COVID-19 patients as part of a cytokine storm, and are associated with viral load and disease severity [37]. In addition, the number of Treg cells, which regulate the induction and proliferation of effector T cells, was significantly lower in COVID-19 patients and this manifests as a lack of functional immunosuppression. Treg populations suppress innate and adaptive activation of immune cells by multiple mechanisms and secretion of immunosuppressive cytokines (IL-10, TGF-β, and IL-35) [41]. The loss of peripheral Tregs in COVID-19 patients may maintain a delicate balance between regulatory arms and immune system effects, leading to significant growth and activation of neutrophils, MQ, DCs, mast cells, and Th17 cells [42]. Locally and during SARS-CoV-2 infection, uncontrolled innate inflammatory responses and disturbances in Treg/Th17 balance can promote tissue damage [43]. Soluble substances, especially NO and TGF-β, decrease the proliferation and activation of Th1 and Th17 cell lines and decrease IFN-γ and IL-17 production. They also reduce direct damage to lung parenchyma by inhibiting the activation of cytotoxic CD8+ cells. TGF-β would be able to increase the release of IL-4 and IL-6, as well as IL-13 by alveolar MQ, and accelerate fibrosis. On the other hand, inhibition of TGF-β is thought to decrease the inflow of neutrophils, MQs, and lymphocytes to the site of injury. TGF-β promotes redox imbalance by increasing ROS levels and decreasing antioxidant enzymes on the one hand. ROS also promotes the fibrinogenic effects of TGF-β by inducing it [44,45]. Besides aforementioned immune modulators, some investigations have mentioned the key role of the balanced gut microbiota in providing immune homeostasis [14,46,47]. The Immunopathology pathway of SARS-CoV-2 with host cells has been illustrated in Fig. 1.
3. Gut bacteria in human health and SARS-CoV-2 cytokine storm

Commensal bacteria regulate innate and adaptive immune responses and affect the activation threshold for pathogenic stimulations, in massive part by producing small molecules that mediate host-microbial relations, such as SCFAs, AHR, PSA, and Polyamines. SCFAs are inhibitors of HDACs and act as signaling molecules that increase FOXP3 expression in an HDAC-dependent manner to raise tolerogenic anti-inflammatory cells by inactivating NF-κB, decreasing TNF-α production [48]. Gut bacteria induce a noticeable response of the gut immune system to the production of IgA by B cells, which has an important role in the regulation of gut bacterial populations in the small intestine [49]. SCFAs decrease the expression of T cell-activating molecules on antigen-presenting cells, such as DCs, and increase the number and function of colonic Treg cells, promoting the development in the lamina propria of tolerogenic DCs that stimulate the differentiation of T cells into Treg. B cells differentiate into plasma cells (PCs) secreting IgA that translocate through the epithelium and are released into the mucus layer where control bacteria adhesion to host tissues. MqS, stimulated by signals such as flagellin, release IL-23, which in turn promotes the production of IL-22 by ILC3. (Figure has been created in biorender).

**Fig. 2. Intestinal microbiota-immunity interplay in immune homeostasis.** Microbiome-derived TLR and NOD ligands and metabolites (e.g., SCFAs, AHR ligands) act directly and indirectly. The effects of SCFAs include enhanced epithelial barrier function and immune tolerance, which promote gut homeostasis through specific mechanisms: 1. enhanced production of mucus by intestinal goblet cells, 2. inhibition of NF-κB; production of IL-18, 3. increased secretion of secretory IgA by B cells, 4. reduced expression of T cell-activating molecules on antigen-presenting cells, such as DCs, 5. and increased number and function of colonic Treg cells, including their expression of FOXP3 and their production of anti-inflammatory cytokines (TGF-β and IL-10). PSA is taken up by lamina propria DCs through a TLR2-dependent mechanism and presented to naïve CD4+ T cells. In the simultaneous presence of activated TGF-β, these cells can differentiate to Treg. IL-10 produced by these cells promotes immune homeostasis. Contrarily, IL-23 licensed through the same cascade promotes the expansion of pro-inflammatory Th17 cells. IECs-released factors such as retinoic acid (RA) and TGF-β, promote the development in the lamina propria of tolerogenic DCs that stimulate the differentiation of T cells into Treg. B cells differentiate into plasma cells (PCs) secreting IgA that translocate through the epithelium and are released into the mucus layer where control bacteria adhesion to host tissues. MqS, stimulated by signals such as flagellin, release IL-23, which in turn promotes the production of IL-22 by ILC3. (Figure has been created in biorender).
flagellin, activate NF-κB, and control the expression of several inflammation-associated cytokines [52]. Gut dysbiosis has been reported to associate with a reduction in the production of gut bacteria-derived SCFAs such as butyrate resulting in enhanced gut permeability. This eases the translocation of microbiota-derived lipo-polysaccharides (LPS), particularly from gram-negative bacteria and inflammatory substances to general circulation resulting in immune activation and inflammatory responses [58]. TLR4, which has been known for its ability to bind with bacterial LPS, is induced by Fusobacterium and results in stimulation of inflammatory genes expression via NF-κB signaling, which also could cause cytokine storm [39,60]. Peptidoglycan and teichoic acid of Streptococcus attach to TLR-2 leading to the production of IL-1b, IL-6, IL-8, TNF-α, and inflammatory response [61]. As dysbiosis of the gut bacteria populations can result in many diseases, such as inflammatory bowel disease, obesity, and cancer [51] the analysis of fecal samples in 15 patients of SARS-CoV-2 has represented major changes in the fecal microbiome in comparison with controls [62]. Dysbiosis of gut bacteria disrupts the integrity of the gut barrier and induces the translocation of SARS-CoV-2 from the lung into the gut through the circulatory and lymphatic systems. It seems that the level of Enterobacteriaceae has raised during inflammatory conditions and exaggerated release of ROS and nitrogen intermediates by epithelial cells and transmigrating neutrophils in the gut lumen, which enhance inflammatory response [61]. Moreover, SARS-CoV-2 infection results in dysbiosis of gut flora and causes dominance of the pathogenic commensal bacterial. Higher levels of Klebsiella, Streptococcus, and Ruminococcus graucus in SARS-CoV-2 patients have been associated with some proinflammatory cytokines (IFN-γ, TNF-α), which causes cytokine storm and Th1 cell response activation [63]. Magalhães et al., reported in their study that people belonging to risk groups for COVID-19-related death showed hyperimmune activation in the intestine, increased Th17 cells and IL-17 production. These patients also showed an increase in the circulating levels of bacterial endotoxins such as LPS, as well as pro-inflammatory cytokines including IL-1β, IL-6, and TNF [64]. Also, studies have demonstrated that COVID-19 patients present with decreased levels of Lactobacillus and Bifidobacterium. The high levels of Lactobacillus spp. correlate with increased anti-inflammatory IL-10 cytokine [14]. The interaction between gut bacteria and host cells is showed in Fig. 2.

4. Probiotics and SARS-CoV2 cytokine storm

Balancing the immune responses and enhancing host immunity is paramount in COVID-19 pandemic scenario. Probiotics consumption upsurges immunological protection in the human host by maintaining the balance between the types of T cell responses (Th1/Th2, Th17, and Treg) and has a vital role in limiting various pathogens [65,67,68]. In vivo effects of probiotics evaluation has illustrated the increased peripherical immunoglobulin production and decreased pro-inflammatory cytokine production. As discussed before, SARS-CoV-2 infection leads to a cytokine storm that causes deterioration in patient’s lung condition and gastrointestinal tract infections. Therefore, these probiotic strains may facilitate the alleviation of cytokine storm by balancing cellular and humoral immune responses as seen in animal experiments [65,69,70]. There are not any available data verifying the effectiveness of probiotics on SARS-CoV-2 infection, but previously proven antiviral action of probiotics against different respiratory viruses could suggest probiotics as a harmless and accessible complementary medicine against COVID-19 disease. It has been illustrated that upon infection with influenza virus, many cytokines such as IL-12, IFN-γ, IL-4, IL-10, IL-1α, IL-1β, IL-6, TNF-α, IFN-α, and IFN-β are produced in the respiratory tract. Research on alleviating infection in mice and the symptoms attempt to correct the imbalance caused by the out-of-control cytokine storm after infection [66,71]. Kawashima’s group studied the antiviral effect of the YU strain of Lactobacillus plantarum (L. plantarum) in a mouse model of influenza virus (IFV). These probiotic strains activate the Th1 immune response, leading to increased levels of IFV-specific secretory IgA and neutralizing antibodies, resulting in the eradication of IFV from the lungs and other infection sites. These results confirm that the application of probiotic strains has a protective effect on IFV infection [72]. Bottari et al. reported that immune benefits of probiotics in SARS-CoV-2 infection could be obtained by developing mucosal immunity via the stimulation of IgA secretion, improvement of the biological functions of phagocytosis, and MQs, and adjustment of regulatory cells [73]. Additionally, there are scientific evidences that confirm the role of probiotics in enhancing immune functions [74].

Songa’s group conducted a study to analyze the antiviral efficacy of L. rhamnosus in IFV infection. They found higher survival rate after administration lives probiotic bacteria. Treatment with live bacteria increased the production of secretory IgA and decreased the expression levels of the pro-inflammatory cytokines TNF-α and IL-6 in the lungs of infected mice. IL-6 is the major component of the cytokines released by activated MQs, and an IL-6 storm may increase the mass release of various inflammatory cytokines in patients with severe COVID-19 [75]. Pham et al. showed that L. rhamnosus EH8 strain yielded butyric acid that inhibits the secretion of IL-6 in MQs. In addition, this bacterium leads to the activation of free fatty acid receptor 2 (Ffar2) on the gut axis to suppress IL-6 signaling, which could be a target to treat the cytokine storm in COVID-19 [76]. Interestingly, a retrospective, single-center study of 311 consecutive heavy patients with confirmed COVID-19 showed that probiotics cannot lower IL-6 levels, but control the ability to moderate immunity and reduce the incidence of secondary infection in COVID-19 patients [77]. Also, Hou et al. showed that the presence of excess IL-6 promotes Th17 cell formation; IL-6 and IL-17 synergistically promote viral replication and may be important targets for anti-coronaviral therapy [78]. However, Colonari et al. reported that anti-IL-6 therapy alone did not reduce ICU admissions and mortality in COVID-19 patients [79]. The findings represent a strategy that includes a more comprehensive approach to immune modulation rather than the suppression of host cytokines that could be more effective against the cytokine storm in virus-infected individuals. IL-17 and IL-6, stimulate viral persistence by immune cross-talk through autophagy [79]. Schett et al. reported that blocking IL-17 could decrease viral replication in COVID-19 patients [80]. The pathogenesis of the immune coronavirus responses is similar to Th17–Th1 driven autoimmune diseases and these interactions seem to perform a significant role in virus replication. Immune modulating effects of Bifidobacteria species, including an anti-IL-17 effect, could be important in both treatment and vaccine development [79]. Also, some clinical studies in humans have illustrated the favorable role of probiotic interventions in fighting against viral infections. Treatment with L. reuteri ATCC 55730 improves the mucosal expression level of IL-10 and decreases the inflammatory cytokine expression, including TNF-α, IL-1β, and IL-8 [81,82]. Bibiloni’s group stated the efficacy of VSL#3 in patients who suffer from clinical remission of ulcerative colitis. VSL#3 is a probiotic that is a mixture of eight strains, which modulate the secretion of the anti-inflammatory cytokine, IL-10, and inhibit the secretion of IL-6, IL-8, TNF-α, and IFN-γ. Thus, probiotic consumption strengthens gut barrier integrity and improves inflammatory responses by various signaling pathways [83]. In a clinical survey, the administration of probiotics resulted in IL-6 and C-reactive protein (CRP) decrease, while there were increased levels of IL-10 in multiple sclerosis patients’ sera [84]. Another study has shown that some Bifidobacterium species can promote TGF-β signaling and increase the number of peripheral Tregs. L. plantarum NCU169 induced the expression of Th17 and Treg immune responses. This strain enhances the immunity of the intestinal mucosa and modulates the Th17/Treg balance which is ascribed to the TLR pathway in DC. After the hyper inflammation, there is a rapid increase in anti-inflammatory mediators such as Treg cells and IL-10 in the lungs that reduce lung damage. Together, they suggest an immunomodulatory potential in reducing cytokine storms. Thus, the use of anti-inflammatory probiotics can keep the gut microbiology in balance and prevent secondary COVID-19
infection [85–87]. The probiotic strains Clostridium butyricum MIYAIRI 588, L. plantarum CAI6, L. rhamnosus GG, and VSL#3 can successfully control redox homeostasis in the host cell, resulting in an overall antioxidant capacity enhancement. This significant aspect of probiotic bacteria offers a unique opportunity to manage COVID-19 as redox homeostasis performs a vital role in slowing down the disease progression [65]. Regarding the effects of probiotics on respiratory disease, two randomized controlled trials found that critically ill, ventilated patients who received probiotics (L. rhamnosus GG, live Bacillus subtilis, and Enterococcus faecalis) had significantly less ventilator-associated pneumonia compared to placebo; therefore, it can be assumed that COVID-19 pneumonia can be alleviated in the same way [88, 89]. It has been shown that the use of certain Lactobacillus strains can improve the gut microbial population by increasing mucus secretion and also prevent the breakdown of tight junction proteins by reducing the number of LPS [84]. Some studies have reported that SARS-CoV-2 can directly interact with LPS via S protein. While, neither the S protein alone nor the LPS alone induce any activation of pro-inflammatory NF-κB, the combination of the S protein, even at low LPS levels, dose-dependently increases NF-κB activation followed by the cytokine response in monocytes in vitro. Furthermore, ACE2 has been reported to exert a protective effect against LPS-induced acute lung injury in mice; therefore, viral ACE2 suppression may lead to a stronger inflammatory response in the lungs [90, 91]. Probiotics likely interfere with the treatment of viral infections such as COVID-19. Therefore, it is important to better understand the immunomodulatory mechanisms of probiotic function to improve the targeting of probiotic interventions by selecting the optimal strain (s). Talking about probiotics without mentioning bacteriophages is impaired discussion, due to the crucial phage performance both in probiotic industry and also human microbiota. Cross talk between probiotics and host immune is represented in Fig. 3.

5. Bacteriophages and SARS-CoV2 cytokine storm

Dominated by bacteria, the gut microbiome is made up of different taxonomic groups including viruses, fungi, and archaea which are also important members of the community with potential effects on human health [92, 93]. Bacteriophages not only acted as particularly insensitive bacteria, but they can also have facilitative and inhibitory functions in the surrounding gut microbiota. Yet, phage interactions with bacterial hosts and the immune system in the human gut remains poorly described [94, 95]. The ubiquity of phages in the gut and their ability to modulate bacterial communities in other ecosystems suggest that they could be active players in human health and interact with the host immune system. Phages can enhance phagocytosis of bacteria by MQs, due to administered phages together with the host bacteria, which were able to incite bacterial phagocytosis. This was referred to opsonization of bacterial cells by phages, where the phage covers the bacteria and makes it more recognizable to the immune system [96, 97]. A survey showed that T4 phage in E. coli cells by binding to LPS could prevent LPS induced production of proinflammatory cytokines in mice [98]. The phage-mediated changes in gut bacterial communities could have downstream effects on immune signaling. Bacteriophages are very specific to their hosts, which minimize the risk of secondary infections, with no side effects during or after phage administration, while resistant bacteria, allergies, and secondary infections are common side effects of antibiotic treatment [99]. One of the critical problems of a viral infection is the super infection caused by pathogenic bacteria. To solve this problem, antibiotics come first in therapeutic methods. Antibiotics
Table 1

| GUT                           | Probiotic   | Function                                                      | References |
|-------------------------------|-------------|---------------------------------------------------------------|------------|
| Lactobacillus                 | L. plantarum| gastrointestinal barrier function, IgA ↑                      | [114]      |
|                               | L. plantarum| IgG2a, IFN-γ ↑                                               | [115]      |
|                               | NCMB8826    | Th2 ↓                                                       |            |
|                               | L. plantarum| TLR1/2, TLR2/6, TLR10, anti-apoptotic, tight junction, intestinal barrier ↑ | [116]      |
|                               | MB452       | nfκβ ↑                                                      |            |
|                               | L. reuteri  | Modulation of DC function, IL-10, Tregs ↑                     | [118]      |
|                               | L. reuteri ATCC| Modulation of TLR9, IL-5, and IL-13 ↓                       | [119]      |
|                               | L. reuteri DSM| Treg ↑                                                      | [120]      |
|                               | 17938       | Th1 ↓                                                       |            |
|                               | L. rhamnosus| Phagocytosis, tight junction, IgA ↑                           | [121,122] |
|                               |             | IL-6, TNF-α, TLR2 ↓                                         |            |
|                               | L. rhamnosus GG| Phagocytosis, Th1 (IL-12 and IFN-γ), Tregs, TGF-β, IgA ↑ | [123-125] |
|                               |             | Neutrophil, eosinophil, IL-6, TNF-α, TLR2, Th2 (IL-4, IL-5, and IL-13) ↓ | | |
|                               | L. casei    | Modulation of DC function, TLR2, Tregs, IgA, IFN-γ, TNF-α, IL-10 ↑ | [118,126] |
|                               | L. casei IBS041| Stimulation of DCs, TGF-β ↑                                  | [127]      |
|                               | L. paracasei| MQ2 ↑                                                       | [128]      |
|                               | L. fermentum| TLR1/2, IL-12/6, TLR10, anti-apoptotic, tight junction, intestinal barrier ↑ | [129,130] |
|                               | 1930BB      | nfκβ ↑                                                      |            |
|                               |             | IL-10, IFN-γ, TGF-β ↑                                       | [131,132] |
|                               | L. fermentum| TLR4, IL-18, IL-6, IL-8, MCP-1, TNF-α ↑                      |            |
|                               | L. paragasseri K7| TLR2/6, TLR10, anti-apoptotic, tight junction, intestinal barrier ↑ | [129]      |
|                               |             | nfκβ ↑                                                      |            |
|                               | L. amylovorus| Th17 ↓                                                       | [131]      |
|                               | L. bulgaricus| IL-10, IL-12, IFN-γ, IgA ↑                                   | [69,133]  |
|                               | L. acidophilus| Phagocytosis, improved the Treg/Th17 imbalance                | [131,134] |
|                               |             | IL-1, IL-6, IFN-γ, IgA ↑                                     | [135]      |
|                               | L. acidophilus AD031| restored the balance of inflammatory cytokines and Th17/Treg cells, Treg, TGF-β, IgA ↑ | [127]      |
| Bifidobacterium FoW176        | B. breve    | IgA ↑                                                       | [121,136] |
|                               |             | TNF-α ↓                                                      |            |
|                               |             | tight junction ↑                                             | [137]      |
|                               | B. longum   | IL-6, TNF-α, TLR2 ↓                                         | [123]      |
|                               | B. longum ATCC| Neutrophil, IL-6, TNF-α, TLR2 ↓                             | [123]      |
|                               | B. lactis Bb-12| Tregs, TGF-β ↑                                             | [138]      |
|                               | B. infantis | mucin, IgA ↑                                               | [139]      |
|                               | B. animalis IM386| TLR1/2, TLR2/6, TLR10, anti-apoptotic, tight junction, intestinal barrier ↑ | [129]      |
| Streptococcus                 | S. thermopiles| IgA ↑                                                       | [140]      |

Table 1 (continued)

| GUT                           | Probiotic     | Function                                                      | References |
|-------------------------------|---------------|---------------------------------------------------------------|------------|
| Enterococcus                  | S. thermopiles ST28| TNF-α ↓, TLR-9, IFN-γ, modulation of Th1/Th17 balance ↑ | [141]      |
|                               | E. faecium/MNM13/NM213| TLR2, IL-6, TNF-α ↑                                        | [142]      |
| Lactococcus                   | L. lactis K24| IL-10, Anti-inflammatory effects ↑                           | [145]      |

usually cause intestinal dysbiosis and lead to inflammation and cytokine storms. Hence, phage therapy can act as a targeted therapy only to kill specific pathogenic bacteria, help maintain balance in the gut microbiome, and may prevent cytokine storms. A study by Prazak et al. found that phage therapy could be a possible advanced option that could play an important role in eliminating the pathogen as well as preventing gut dysbiosis, which can lead to a cytokine storm. Several studies have confirmed that phages have not only antibacterial but also antiviral properties. Anti-immunoregulatory and anti-inflammatory activity has also been demonstrated by phage particles, and these features may be useful in restoring immune homeostasis [104,105]. Recently, it has been shown that some phages endocytosed by leukocytes activate TLR3-dependent pattern recognition receptors and inhibit TNF-α-driven IFN-I production [106,107]. There is also data suggesting that T4 phage via inhibiting activation of NF-kB and ROS production can reduce extreme inflammatory reactions in pathology and clinical course of SARS-CoV-2 [108]. Phages also work to activate NK cells, which could be a key feature in their therapeutic actions. A study of staphylococcal phages on the expression of genes that are involved in antimicrobial immunity suggested that there is an upregulated translation of IL-2, which improves the activity of NK cells and hence causes a progressive cellular immune response [109]. Another investigation has shown that a Pseudomonas phage upregulates IL-10 expression in human mononuclear cells, while a Staphylococcus phage does not [110,111]. It must be ensured that bacteriophages are selected correctly which target both optimal bacteria and should be most effective against the growth of the bacterial population. Bacteriophages should not interfere with the patient’s innate or adaptive immunity. It is also very important to exclude that the patient does not use antibodies to bacteriophages and does not produce antibodies to bacteriophages to get rid of the bacteriophage earlier than against SARS-CoV-2 [109,112]. Laboratory
studies, clinical trials, and randomized steps of one to three human trials can be conducted to prove their therapeutic utility [113]. Phage therapy may also show promise as a treatment for SARS-CoV-2. Due to the data have been mentioned in Table 1&2, similar modulatory function on the immune response of probiotics and bacteriophages casts light on co-administration of them to quench cytokine storm in patients with extreme immune response, or ameliorate immune system in patients with immune deficiency, which both lead into the death in some chronic or acute cases. To develop this stance, it could be suggested that co-administration of phages, such as T4 and Pf4 with probiotics such as L. rhamnosus, L. paracasei, and L. fermentum L930BB, may help to modulate the immune response. Phage direct and indirect responses have been illustrated in Fig. 4.

6. Concluding remarks: a glimpse into the future

The human gut microbiota is an exclusive community of microorganisms including bacteria, Mycobio, Virome, Archaeome, and Eukaryotic Parasites that has received much attention because of their impacts on human health and diseases. SARS-CoV-2 infection can lead to widespread tissue damage resulting in multi-organ failure and death. This is due to an aggressive inflammatory response caused by releasing many proinflammatory cytokines, known as a cytokine storm. The gut microbiota regulates multiple host functions, including immune homeostasis. By contrast, imbalances in their community composition are associated with several human diseases or conditions. In the present study, we tried to discuss how the imbalanced gut bacteria could contribute to the development of cytokine storms in COVID-19 patients.

We then suggested targeted interventions using probiotics and bacteriophages to mitigate this in COVID-19 patients by restoring balanced gut bacterial communities. In vitro and in vivo evidence supports the potential role of gut microbiota in regulating the immune system, suggesting a definitive role for probiotics and bacteriophages in viral

| bacteriophage | Anti-bacterial function | Anti-virus function | Reference |
|---------------|------------------------|---------------------|-----------|
| Lytic Lactococcus phage | Anti-inflammatory response ↑ | TLR3, IL-1α/β, IL-6, IL-8, IFN-γ ↑, TNF-α ↑ | [98] |
| P4 (Pseudomonas aeruginosa phages) | TLR3, IL-12, IFN-γ ↑ | [98,110] |
| T4 phage | TR2L, IL-10 ↑ | hBD2, defensin-2, TLR2 ↑ | [108,109] |
| Escherichia coli phages | IL-6, IL-8, IL-10, IFN-γ↑, TLR-α ↑ | TLR9, Th, CTL, IL-12, IFN-γ ↑ | [146,147] |
| Staphylococcus aureus phages | IL-1β, IFN-γ, IL-10 ↑ | [110] |
| Staphylococcus aureus phages VB-SauM-JS25 | NF-kB, pro-inflammatory cytokines ↓ | [148] |
| A5/80 | TLR10, TLR2, IL-2, NK cell, anti-inflammatory effect ↑ | IL-2, NK cell ↑ | [109,149] |
| M13 | TLR4 ↓ | [150,151] |
| MS2 phages | Apoptosis ↓ | [152] |
| Bacteroides phages | IFN-γ ↑ | TLR9, IFN-γ ↑ | [153] |
| Actinobacter. baumannii phage Bφ-R2096 | IL-6, TNF-α ↓ | [153] |
| qkm18p phage | IL-6, TNF-α ↓ | [154] |

Fig. 4. Interaction between phages and immune system. Phage infection led to the release of PAMPs which translocate the gut epithelium and trigger TLR pathways and induce a transcriptional response in monocytes, MQs, DCs, and particularly improved the transcription of IL-1, IL-6, and TNF-α. Besides, by inhibiting activation of NF-κB and ROS production, phages can reduce extreme inflammatory reactions. Phages induce antiviral immunity via activating NK cells, TLR3, and TLR-9, and inhibiting TNF-α by driving IFN-1 production. Phages can trigger a strong immune response by activation of Th1 (IFN-γ and TNF-α), Th2 (IL-4, IL-6, and IL-10) cells, and also B cells. The activation of Th1 generated a CTL response. (Figure has been created in biorender).
Infections including COVID-19. Since one of the main reasons for death among covid-19 patients is excessive immune responses directed toward cytokine storm we introduced the potential probiotics and bacteriophages that could alleviate the immune responses via induction of Treg, Th1, IFN-γ, and IgA responses and suppression of proinflammatory responses including IL-6 and Th2. This can be a safe and promising low-cost method to balance the gut microbiota by supplementation of specific probiotics and phages to reduce the severity of COVID-19 morbidity and mortality. Probiotics and phages can prevent the formation of cytokine storms by simultaneously boosting innate immunity and avoiding the exaggeration of adaptive immunity challenged to respond quickly to viral propagation. Suppression of inflammatory cytokine responses by administration of probiotics and phages can prevent both the severity and development of ARDS. Since there are no effective therapy methods in SARS-CoV2 CS scenarios, it seems the targeted manipulation of the gut microbiome especially in high-risk groups in the early days of infection could potentially decrease the risk of development of cytokine storm in these patients.

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Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability
Data will be made available on request.

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