



## Review

## SARS-CoV-2 and immune-microbiome interactions: Lessons from respiratory viral infections



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## ABSTRACT

By the beginning of 2020, infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had rapidly evolved into an emergent worldwide pandemic, an outbreak whose unprecedented consequences highlighted many existing flaws within public healthcare systems across the world. While coronavirus disease 2019 (COVID-19) is bestowed with a broad spectrum of clinical manifestations, involving the vital organs, the respiratory system transpires as the main route of entry for SARS-CoV-2, with the lungs being its primary target. Of those infected, up to 20% require hospitalization on account of severity, while the majority of patients are either asymptomatic or exhibit mild symptoms. Exacerbation in the disease severity and complications of COVID-19 infection have been associated with multiple comorbidities, including hypertension, diabetes mellitus, cardiovascular disorders, cancer, and chronic lung disease. Interestingly, a recent body of evidence indicated the pulmonary and gut microbiomes as potential modulators for altering the course of COVID-19, potentially via the microbiome-immune system axis. While the relative concordance between microbes and immunity has yet to be fully elucidated with regards to COVID-19, we present an overview of our current understanding of COVID-19-microbiome-immune cross talk and discuss the potential contributions of microbiome-related immunity to SARS-CoV-2 pathogenesis and COVID-19 disease progression.

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## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped RNA beta-coronavirus that was reported to have emerged in Wuhan, China, in December 2019. This virus caused the coronavirus disease 2019 (COVID-19) pandemic. The rapid spread of SARS-CoV-2 exerted significant effect on the healthcare system and crippled the global economy. At present, around 93 million people have been infected and over 2 million people have died in more than 180 countries and territories (Dong et al., 2020). The SARS-CoV-2 virus is highly contagious and remains viable on surfaces and closed atmospheres for up to 3 days, thus promoting

the transfer of interpersonal aerosols and fomites (Van Doremalen et al., 2020). Owing to its highly infective nature, the rapidity of its spread, and the emergence of new variants, countries have employed international travel bans and implemented home quarantine to limit its burden (Conti et al., 2021).

The symptoms of COVID-19, which typically appear 2–14 days post-viral exposure, include fever, cough, shortness of breath, diarrhea, and pneumonia. Severe COVID-19 cases exhibit similar respiratory, gastrointestinal, hepatic, and neurological complications, requiring patient hospitalization (for oxygen supplementation and mechanical ventilation), and in some cases, resulting in death (Al-Tawfiq, 2020). Such complications and fatal outcomes are commonly observed in aged patients and those with comorbidities, including hypertension (HTN), cardiovascular disorders, cancer, diabetes mellitus (DM), chronic lung disease, and acute respiratory distress syndrome (ARDS). COVID-19 is characterized by bilateral pulmonary infiltrates, diffused alveolar damage (DAD), potential tracheobronchitis, vascular injury with

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inflammatory microthrombi, and other infections (Zhou et al., 2020a). Many of these progressing infections are thought to be related to the microbiome (Borczuk et al., 2020; Fan et al., 2020a), as impaired microbiota has been reported in COVID-19 infections (Gu et al., 2020). The microbiota of SARS-CoV-2 patients, for instance, was shown to be similar to that of patients with community-acquired pneumonia, with either pathogenic dominance or elevation in the levels of oral and upper respiratory commensal bacteria (Shen et al., 2020). Interestingly, substantial differences in COVID-19-associated mortality have been recorded between developed and developing countries (Kumar and Chander, 2020). These authors found that COVID-19-associated mortality was positively correlated with hygiene, water quality, and overall health care efficiency. Therefore, it was suggested that high microbial exposure due to a lack of sanitation could possibly induce interferons, which might have a protective effect against COVID-19 (Kumar and Chander, 2020). Individuals from low-income countries with reduced COVID-19-related mortality are thought to have more diverse microbiota as the microbiota regularly provides tonic immune signals, thereby influencing host susceptibility to infections (Pang and Iwasaki, 2012). Microbiome-induced immune-priming, for example, can activate type I interferon production, providing protection against COVID-19 (Monroe et al., 2010; Taefehshokr et al., 2020).

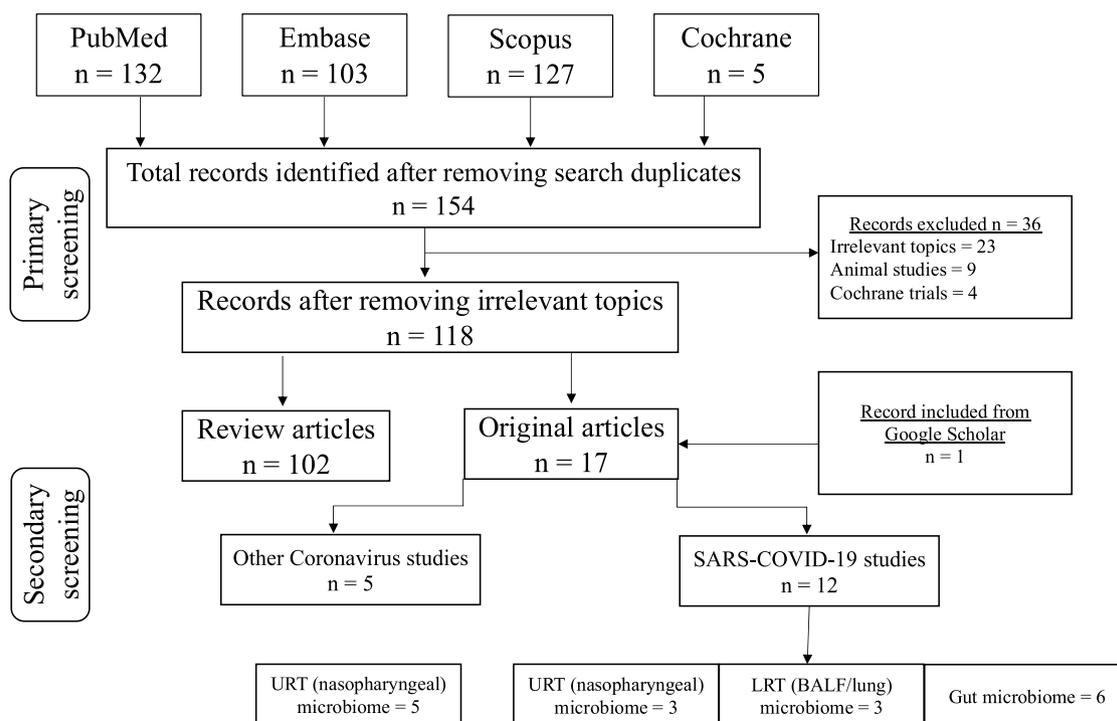
Antibiotics are widely used as a broad-spectrum therapeutic approach to prevent secondary infections. For instance, 58–71% of COVID-19 patients in China received antibiotics, while 2–36% of patients suffered gastric complications, including diarrhea (Guan et al., 2020). It is well known that antibiotics alter the flora of the normal gastrointestinal tract (GIT). In this context, Zuo et al. (2020b) observed that COVID-19 patients who did not receive antibiotics had a higher number of opportunistic pathogens known to cause bacteremia (Dakshinamoorthy et al., 2019; Elsayed and Zhang, 2004). However, the empirical antibiotic treatment of patients with COVID-19 demonstrated a further depletion of several bacterial species beneficial for host immunity, including

*Eubacterium rectale*, *Faecalibacterium prausnitzii*, *Ruminococcus obeum*, *Dorea formicigenerans* and the family Lachnospiraceae. This is further supported by reports that microbial dysbiosis; characterized by lower microbial diversity, richness, and evenness; has been associated with DM, obesity, and autoimmune disorders, the major risk factors of severe COVID-19 (Gou et al., 2020). Of the resident flora in different organs, several studies have focused particularly on characterizing the flora of the lungs and the gut in COVID-19 patients. Here, we shed more light on the interplay between microbiota, immunity, and COVID-19, with particular focus on how microbiome-associated immune crosstalk can shape outcome of COVID-19. Enhancing our understanding of such interplay could help us to develop targeted therapies for COVID-19 and associated secondary infection.

**Review methods**

We performed a comprehensive literature review of COVID-19 and the microbiome using MEDLINE (PubMed database), SCOPUS, Cochrane, and EMBASE, using the following search terms: “microbiome”, “microbiota”, “coronavirus”, “SARS-CoV”, “MERS-CoV”, “Middle East respiratory syndrome”, “SARS\_COV2”, and “COVID-19”. References and bibliographic lists in original and review papers were also scanned to identify relevant studies that may have been missed during database searches.

In total, 154 research articles were retrieved from these databases using the aforementioned key terms and appropriate filters for the title, abstract, and keywords. We then read the titles and abstracts and removed 36 articles because they were not relevant. The remaining 118 articles, which investigated or reviewed COVID-19 or coronavirus and the microbiome of the gut or respiratory tract, were included (Figure 1). In order to expand on the main objective of our review (to investigate the relationships between the severity of COVID-19 disease, changes in the microbiome, and immunity), we also explored relevant review articles and mechanistic studies.



**Figure 1.** Flow-chart of the inclusion/exclusion criteria and search strategy used in this study.

## The human microbiome with regards to age and comorbidity

It is well known that persistent exposure to bacterial toxins as we age will result in chronic low-grade inflammation, recurrent infections, excessive medication, and hospitalization (Nagpal et al., 2018). Moreover, owing to the parallel evolution between our microbiome and immunity, the diversity of the gut microbiome also tends to fluctuate with age (Nagpal et al., 2018), with a significant decline in the numbers of lactobacilli and bifidobacteria, and an increase in *Clostridium perfringens*, *Clostridium difficile*, and enterobacteria, as we grow older (Gavini et al., 2001; Odamaki et al., 2016). Other studies have shown a similar age-related shift in the composition and diversity of the gut microbiome and the respiratory tract microbiome, particularly in the excessive growth of *Rothia* and *Streptococcus* (de Steenhuijsen Pijters et al., 2016; Stearns et al., 2015). Although it remains unclear whether microbiome dysbiosis is a cause or effect of aging and senescence-related inflammatory disorders, it has been hypothesized that a healthy microbiome is essential for healthy aging and that restoring microbiome homeostasis can promote human longevity.

As with aging, comorbid conditions such as hypertension and cancer have also been associated with microbial dysbiosis in the gut and respiratory tract (Hosgood et al., 2019; Michalovich et al., 2019; Zhang et al., 2020a). An emerging body of evidence has highlighted the changes in the gut microbiome of obese and diabetic patients and addresses their role in the pathogenesis of disease. Hyperglycemia and hyperlipidemia are likely to create suitable environments that may attract some bacteria that could subsequently flourish (Sohail et al., 2017). Furthermore, hypoxic niches that impact on bacterial survival may also be generated due to the airway closure that often accompanies obesity (Salome et al., 2010). Although much has yet to be elucidated on these topics, it is very conceivable that microbial communities in the lungs are also altered by DM and obesity. In a previous study, Zhang et al. reported that patients with hypertension exhibit an upregulation in bacterial invasion-related genes and bacterial toxins in the respiratory tract, along with an overgrowth of several opportunistic pathogens, including *Lautropia*, *Streptococcus*, and *Ralstonia* (Zhang et al., 2020a). Similarly, the cancer-associated lung microbiome is also distinct, represented by low diversity and species richness but with an overgrowth of *Streptococcus* when compared to a control group (Liu et al., 2018). This suggests a direct connection between lung cancer and the microbiome, where microbial agents and/or products may promote carcinogenesis that eventually favors immunosuppression and provides an environment that is suitable for lung infections.

In order to gain a better understanding of the wide spectrum of clinical manifestations in COVID-19 patients ranging from minor upper respiratory tract infection (URTI) to sepsis, researchers have studied a plethora of data and identified several risk factors that are associated with adverse outcomes, including hypertension, DM, cancer, and ischemic heart diseases (Robilotti et al., 2020; Zhou et al., 2020a). Interestingly, microbiome dysbiosis was also classified as a significant risk factor that may contribute to these chronic diseases (Rook, 2010) as both microbial dysbiosis and pro-inflammation are known to initiate and accumulate epigenetic changes that culminate in chronic inflammatory responses in DM, obesity, and cancer, thus increasing morbidity due to sepsis (Serino, 2018). These observations are similar to the immune response observed in sepsis where activation of the innate immune system via pattern recognition receptors (PRR) and TLR-4 recognizing pathogen associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), leads to the overproduction of pro-inflammatory cytokines.

## The respiratory tract microbiome in respiratory tract infections and COVID-19 patients

### The microbiome of the upper respiratory tract

The upper respiratory tract (URT) is directly exposed to the outside atmosphere and is also closely correlated with the skin microbiome. Therefore, the URT provides a diverse landscape of micro-niches for microbial attachment. For instance, the anterior nares harbors commensal and opportunistic pathogens, including *Staphylococcus aureus*, *Anaerococcus*, *Dolosigranulum pigrum*, *Corynebacterium* spp., and *Moraxella* spp., which are distinct from the mucosal communities of the nasal cavity and the pharynx (Wos-Oxley et al., 2010). Examples of commensal microbiota that normally colonize the URT include *Staphylococcus*, *Streptococcus*, *Prevotella*, and *Veillonella*. These presumably prevent overgrowth and the dissemination of potential pathogens towards the lower respiratory tract (LRT), thereby acting as gatekeepers for the lungs (Kumpitsch et al., 2019). Physiological and microbial gradients along the URT indicate that the highest bacterial load is in the oropharynx while the lowest is in the nares. Interestingly, several studies have reported changes in the URT microbiome during a range of viral infections, including coronavirus influenza, rhinovirus, and respiratory syncytial virus infections, thus rendering the host more susceptible to infection. Acute viral infections, for example, are associated with significant changes in the nasopharyngeal core microbiome and are characterized by a reduction in alpha-diversity and an abundance of beneficial microbes, specifically anaerobes and *Prevotella* (Edouard et al., 2018). Moreover, respiratory pathogens, including *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Corynebacterium propinquum*, have been found to be enriched in patients with viral infections. Interestingly, the type of viral infection does not appear to influence the microbial profile (Bouquet et al., 2020; Edouard et al., 2018). Similarly, Yi et al. did not find any relationship between the oropharyngeal microbiota and the type of viral agent when studying different viral respiratory illnesses. Instead, microbiome clustering was found to be related to subject age, with over-representation of *Moraxella nonliquefaciens* in younger patients (Yi et al., 2014).

Previous studies have shown that viral infections enhance bacterial adherence to the epithelium and increase pathogenic bacterial colonization. The microbial composition of the URT, for example, is altered by viral infections, thus promoting the translocation of pathogens to the lungs and thereby predisposing infected hosts to secondary infections (Dube et al., 2016). Safaeyan et al. demonstrated that symptomatic patients infected with influenza A have higher colonization rates of *Staphylococcus aureus*, *S. pneumoniae* and *H. influenzae* (Safaeyan et al., 2015). In contrast, asymptomatic influenza A virus carriers exhibit a microbiome that is strictly comparable to healthy subjects, thus suggesting a critical role for the nasopharyngeal microbiome in the clinical presentation of viral infections (Yi et al., 2014). It is well known that the commensal microbiotas are essential for the development of the mucosal immune system (Neish, 2014). The mucosal microbiome has been shown to influence the immune system locally across multiple pathways by either enhancing or minimizing viral infection via bacterial ligands that occupy viral receptors (Bosch et al., 2013). In particular, the genus *Prevotella*, constituting 50% of the core microbiome of healthy subjects (de Steenhuijsen Pijters et al., 2016), has many member strains (*Prevotella melaninogenica*, *Prevotella nanceiensis*, and *Prevotella salivae*) that show no pro-inflammatory effects on human dendritic cells (DCs) and play a protective role in reducing the secretions of pro-inflammatory cytokines induced by pathogens, such as *H. influenzae* (Larsen et al., 2012). The prevalence of *Prevotella* has also been shown to

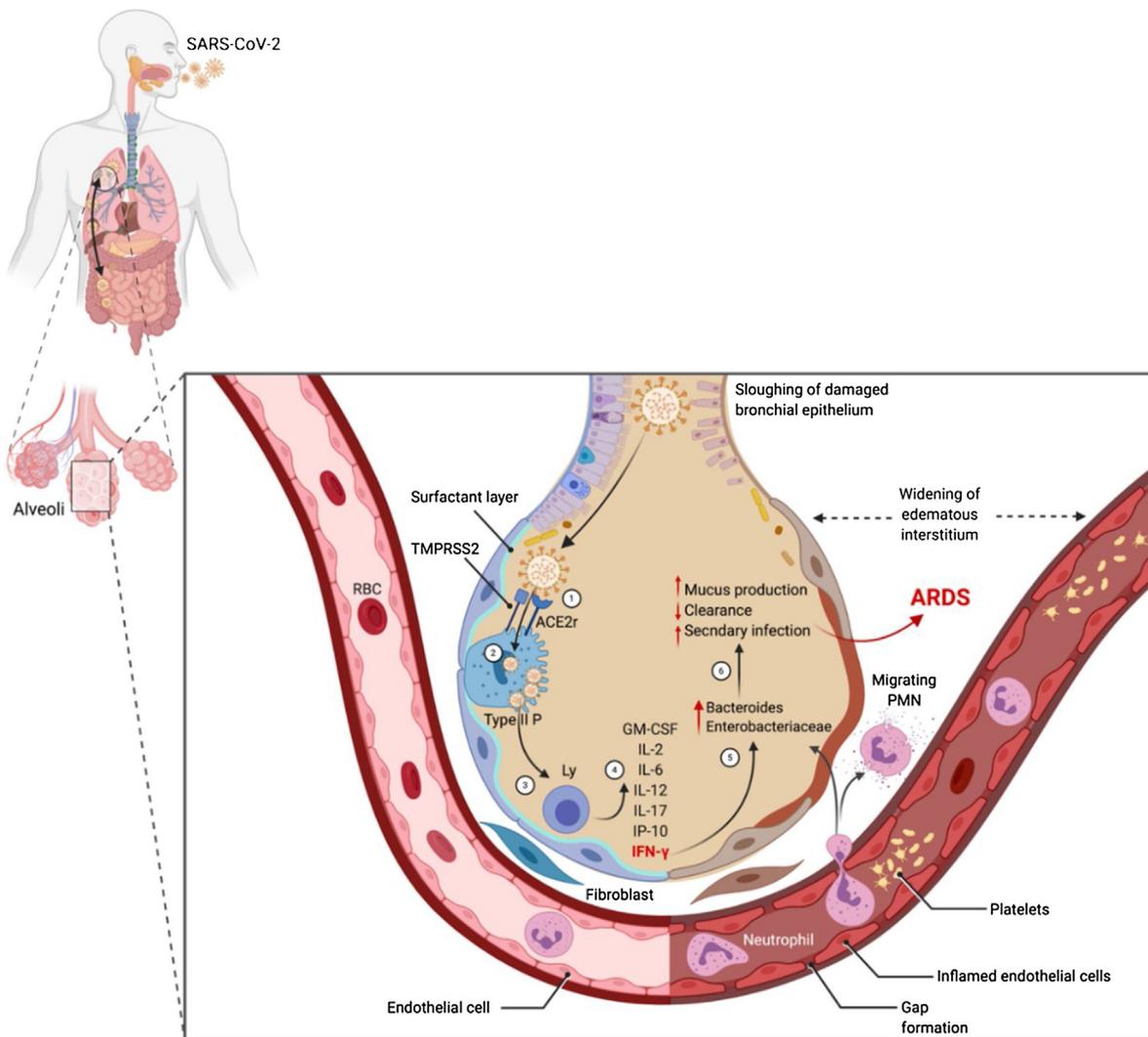
decrease significantly in influenza and sinusitis patients, while *Corynebacterium propinquum* has been associated with susceptibility to viral infections (Edouard et al., 2018; Leung et al., 2013). Therefore, preventive or therapeutic interventions may be guided to preserve or restore a healthy microbiome of the nasopharynx in order to regulate a healthy innate and adaptive immune responses against viral infections.

The lung microbiome

Since the dawn of germ theory, it was widely assumed that healthy lungs are germ-free, a thought that initially excluded studying the lungs in the National Institutes of Health Human Microbiome Project (Proctor, 2011). Recently, however, high throughput sequencing techniques have revealed that healthy lungs are indeed populated by a diverse range of bacterial communities albeit relatively fewer in number when compared to those in the GIT (Sohail et al., 2015). Thus, studying the lung microbiome under healthy and pathological conditions is

emerging as one approach with which to re-visit respiratory diseases, particularly those involving an infectious aspect, including pneumonia, cystic fibrosis, asthma, emphysema, and chronic obstructive pulmonary disease (COPD). Interestingly, similar to that of the intestine, the immunity of the lung mucosa has also been shown to be engaged in maintaining an equilibrium state of tolerance and immune activation against pathogens (Lloyd and Marsland, 2017).

Although only a few studies have been directed towards unravelling the human lung microbiome in a healthy status, most of the evidence gathered thus far indicates the existence of a complex and dynamic microbial community that is predominantly composed of the Firmicutes, Proteobacteria, and Bacteroidetes phyla (Dickson et al., 2015). At the genus level, *Prevotella*, *Veillonella*, *Streptococcus*, *Methylobacterium*, *Neisseria*, *Pseudomonas*, and *Fusobacteria*, predominate, and to a lesser extent potential pathogens, including *Leptotrichia* and *Haemophilus* (Dickson et al., 2015; Moffatt and Cookson, 2017). It is worth noting that lung sampling for microbiome analysis is considered invasive and could



**Figure 2.** Lung microbiome changes in COVID-19. (1) SARS-CoV-2 infects target cells in the lung by engaging ACE2r and TMPRSS2 followed by (2) intracellular viral replication and localized inflammation (3) accompanied by the activation of immune cells (4) further adding to the inflammatory microenvironment via cytokine release. (5) Localized IFN- $\gamma$  release contributes to microbiome dysbiosis characterized by increased populations of *Bacteroides* and *Enterobacteriaceae* which in turn (6) lead to increased mucus production and decreased mucociliary clearance. Collectively, these features increase the risk of secondary infections and the development of ARDS.

Abbreviations: ACE2r, angiotensin-converting enzyme 2 receptor; ARDS, acute respiratory distress syndrome; Ly, lymphocyte; PMN, polymorphonuclear neutrophils; RBC, red blood cell; TMPRSS2, transmembrane protease serine protease 2; Type-II P, type-II pneumocyte.

therefore be technically problematic. Instead, sputum collection is considered to be better for the diagnosis of lower respiratory tract (LRT) infections. Contamination is another challenging aspect here. The passage of the bronchoscope through the oral or nasal route is associated with the possibility of sample contamination. Moreover, oropharyngeal contamination renders a sputum specimen unfit for microbiome studies. As such, and in conjunction with the low biomass availability during sampling, the validity of lung microbiome reports have been challenged (O'Dwyer et al., 2016).

To date, only a few studies have investigated the lung microbiome of viral infections in the respiratory tract. Existing evidence has mainly arisen from *in vivo* or cross-sectional studies conducted on infections caused by influenza, rhinovirus, and human immunodeficiency virus (HIV). These studies suggested that microbial communities in the lung are altered in viral infections, thus improving host immunity and disease severity (Eskind et al., 2020; Hanada et al., 2018; Ichinohe et al., 2011; Molyneaux et al., 2013). For instance, the lung microbiome analysis of HIV-positive patients showed that T-helper cell response and mortality rate were correlated via a clustering pattern with a distinct microbiome (Shenoy et al., 2017). Moreover, the respiratory microbiome of HIV-patients with advanced disease were shown to exhibit reduced alpha diversity and increased beta diversity compared to healthy individuals (Twigg et al., 2016). Along the same lines, Eskind et al. reported that respiratory microbiome diversity is compromised by acute viral infections with a decline in alpha diversity accompanied by a higher prevalence of bacterial pathogens (Eskind et al., 2020). In contrast, Yildiz et al. observed no changes in the alpha and beta diversity of the lung microbiome in mice infected with influenza (Yildiz et al., 2018). Another study, investigating influenza infections, found that commensals are essential for generating specific CD4 and CD8 T cells and antibody responses. In particular, the existing microbiome provided signals for pro-IL-1 $\beta$  and pro-IL-18 cytokines and inflammasome activation, thus leading to the migration of DCs and the priming of T-cells (Ichinohe et al., 2011). Moreover, influenza infection was shown to alter the composition of the microbiome and promote the release of pro-inflammatory cytokines, C reactive protein, and increase mucus production in the respiratory tract. Similarly, rhinovirus exacerbation in COPD patients resulted in an increased bacterial pathogen load, especially *Haemophilus influenzae*; this was correlated with an increase in inflammatory markers (Molyneaux et al., 2013).

Acute respiratory distress syndrome (ARDS) is a serious complication that often occurs in severe influenza, MERS-CoV, and SARS-CoV infections. Irrespective of the source of infection, the lung microbiome of patients with ARDS was found to be enriched with gut bacteria and correlated with the intensity of alveolar and systemic inflammation (Dickson et al., 2016). Bacterial toxins, such as lipopolysaccharides and flagellin, are known to cause inflammation in patients with ARDS, thus resulting in pneumonia; this occurs mainly by engaging PRRs such as TLRs 4 and 5. The accumulation of inflammatory cells and cytokines, along with the excessive production of mucus during ARDS, leads to obstruction of the airway by impeding mucociliary clearance, thus providing an anaerobic environment for putative bacterial growth and secondary infections. The over-accumulation of gut pathogens, including *Enterobacteriaceae* and *Bacteroides*, in the lungs of critically ill ARDS patients may lead to sepsis and respiratory failure (Dickson et al., 2020, 2016). Dickson et al. observed that patients with severe cases of ARDS had a higher bacterial burden and richness but a lower bacterial diversity (Dickson et al., 2020). Furthermore, microbial dysbiosis has been shown to be significantly associated with TNF- $\alpha$ , a principal mediator of inflammation in ARDS (Dickson et al., 2016).

## The respiratory tract microbiome and COVID-19 patients

Recent studies have analyzed the respiratory tract microbiome of patients with COVID-19 (Figure 2). A meta-transcriptomic analysis of nasopharyngeal swabs and sputum samples from patients with pneumonia revealed a reduced alpha diversity in patients infected with SARS-CoV-2 when compared with patients with other forms of pneumonia. This finding could be attributed to other manifestations in these patients, including an increased susceptibility to respiratory viruses and the upregulation of several immune pathways related to cytokine signaling (Zhang et al., 2020c, d). Interestingly, COVID-19 patients were also found to develop concurrent infections with other viruses, bacteria, and fungi, more frequently than patients with non-COVID-19 pneumonia with cytokine signatures that were indicative of Gram-negative infections. Similarly, the meta-transcriptomic analysis of bronchoalveolar lavage (BAL) fluid from both COVID-19 and patients with non-COVID-19 showed a significant increase in bacterial load and other pathogens, thus emphasizing the occurrence of lung microbial dysbiosis in COVID-19 (Shen et al., 2020). Along these lines, Fan et al. reported that the microbiome of lung tissue in 20 deceased COVID-19 patients was dominated by *Acinetobacter*, a pathogen that is commonly associated with the lung infections that may cause pneumonia (Fan et al., 2020b). Furthermore, opportunistic pathogens, such as *Cryptococcus*, *Cladosporium*, *Issatchenkia*, *Alternaria*, *Aspergillus*, and *Candida*, were also dominant in the lung mycobionomes of deceased patients. Some of these pathogens can be lethal, particularly *Cryptococcus* infection, which is linked to a high morbidity and mortality rate (Fan et al., 2020b). On the other hand, De Maio et al. analyzed the nasopharyngeal microbiomes of patients suffering from acute respiratory illness who attended clinic due to a suspicion of COVID-19; these authors used 16S rRNA amplicon sequencing and observed no difference in the composition or diversity of the microbiomes when comparing between patients who were confirmed to have COVID-19 and those who were negative (De Maio et al., 2020). Similarly, Minich et al. also analyzed the nasopharyngeal microbiomes of COVID-19 patients but compared sampling techniques and swabs-types; these authors reported that the microbiome composition was not affected by swab-type, but rather by sampling technique (Minich et al., 2020). Similar to De Maio et al. (2020); Minich et al. (2020) also reported that Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria, were the predominant phyla in the nasopharynx of COVID-19 patients. These are also the most common phyla in nasopharyngeal samples, regardless of COVID-19 disease. Therefore, these data suggest that COVID-19 does not significantly alter the microbiome from a healthy state.

## The gut microbiome in respiratory tract infections and COVID-19 patients

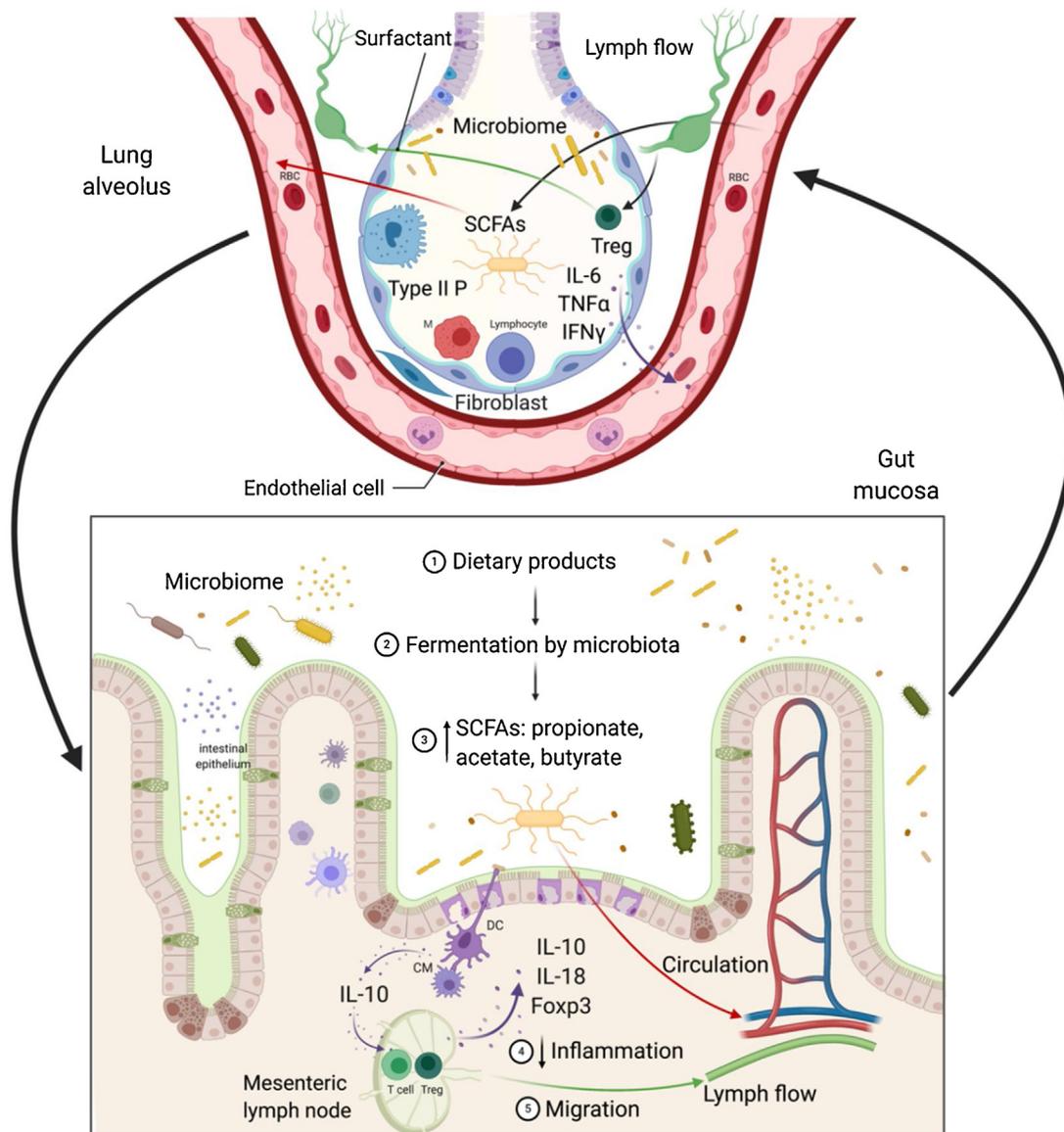
### The gut microbiome

The gut microbiome performs several important functions, including metabolizing nutrients, resisting pathogenic colonization, maintaining the bowel barrier, and educating the immune system (Al-Asmakh and Hedin, 2015; Althani et al., 2016; Sohail et al., 2019). In addition, it has been recently reported that changes in the composition of the gut microbiome has various systemic effects, particularly those exhibited via the gut-brain neuroendocrine axis and the gut-lung inflammatory axis (El Aidy et al., 2015). Germ-free mice were shown to have substantially underdeveloped gut-associated lymphoid tissues, reduced Peyer patches and mesenteric lymph nodes, as well as compromised production of antibodies against pathogens. Similarly, recent studies investigated the effects of the gut microbiome on respiratory tract immunity

(Anand and Mande, 2018; Deriu et al., 2016; Groves et al., 2018; Marsland et al., 2015; Zhang et al., 2020b) and reported the existence of bidirectional cross talk (Figure 3) between the gut microbiome composition and respiratory tract infections. For example, mice maintained on a fibrous diet showed an increased survival rate and resisted lung damage, a phenotype attributed to the increased production of short-chain fatty acids (SCFAs) during influenza infection (Trompette et al., 2018). Interestingly, SCFAs reduced inflammation and conferred protection against influenza by promoting Ly6c<sup>-</sup> patrolling monocytes, CD8<sup>+</sup> T cell effector functions, and by activating macrophages (Trompette et al., 2018). In addition, Smith et al. (2013) demonstrated the importance of SCFAs and microbial metabolites in promoting the immune suppressive regulatory T cell (Treg) population in the gut to maintain tolerance. Similarly, a higher population of butyrate-producing bacteria in the fecal microbiota is associated with an increased resistance to respiratory viral infection (Haak et al.,

2018). Moreover, a study by Sencio et al. (2020) showed that gut microbiome dysbiosis during influenza infection promotes respiratory pneumococcal superinfection via alterations in SCFAs production. These authors also showed that acetate supplementation decreased susceptibility to secondary bacterial infection by enhancing alveolar macrophage activity.

More recently, Gu et al. compared the gut microbiome of patients infected with influenza virus with healthy controls and COVID-19 patients (Gu et al., 2020) and observed that patients with influenza infection exhibited lower diversity and a different microbial composition than healthy controls and COVID-19 patients. In particular, the populations of butyrate-producing bacteria, including *Ruminococceae* and *Lachnospiraceae* families, were decreased significantly in influenza patients. In a study conducted in germ-free mice, Abt et al. revealed the importance of commensal bacteria in calibrating the threshold activation of innate antiviral immunity (Abt et al., 2012). Within this frame of



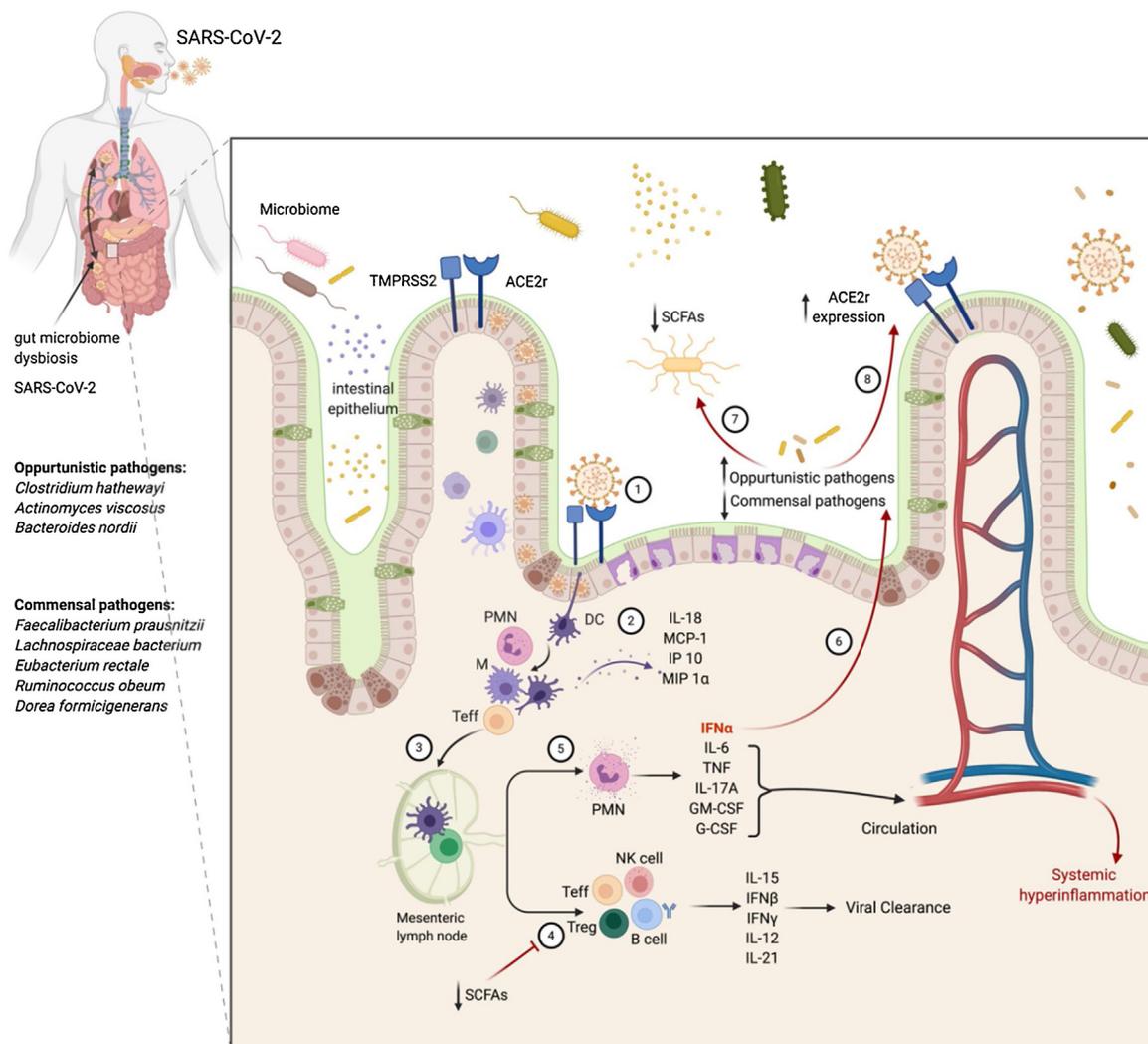
**Figure 3.** Gut-lung Cross-talk. (1) The ingestion of dietary fibers is followed by (2) fermentation by anaerobic intestinal commensals that leads to the increased (3) production of SCFAs that are absorbed and transported through the blood circulation to the lungs. (4) In the intestinal micro-environment SCFAs induce an anti-inflammatory cytokine response that promotes Treg formation in the local LN. (5) Immune suppressive lymphocytes migrate to the pulmonary lymphatics maintaining cross-talk between the gut and the lung.

Abbreviations: CM, colonic macrophage; DC, dendritic cell; Foxp3, forkhead box P3; LN, Lymph node; M, macrophage; SCFAs, short chain fatty acids; Treg, regulatory T cell.

reference, respiratory influenza infection was shown to induce intestinal inflammation, promote immune dysregulation, and alter the gut microbiome by increasing *Enterobacteriaceae* and decreasing *Lactococci* and *Lactobacilli* populations in murine models (Wang et al., 2014). Interestingly, lung-derived CD4<sup>+</sup> T cells in the gut were shown to be responsible for these changes by producing IFN- $\gamma$  that mediates microbiome-associated shifts and promotes immune-mediated injury in the intestine. Similarly, gut microbiome dysbiosis was shown to be associated with the severity of respiratory syncytial virus (RSV) disease in infants. Patients with severe RSV disease have lower alpha diversity in the gut microbiota, as characterized by the enrichment of *Actinomyces*, *Clostridiales*, *Lactobacillaceae*, and *Odoribacteraceae* (Cormier et al., 2020). These data collectively suggest the existence of cross-talk between respiratory viral infections and the intestinal microbiome, an interplay that questions whether these gut microbiome changes and the associated immune responses are the cause or the effect of respiratory infections.

The gut microbiome and COVID-19 patients

Coronavirus-based infections, such as SARS and MERS, show a range of gastrointestinal tract-like symptoms. As a consequence of COVID-19 infection, viral-specific antibodies and inflammatory cytokines are produced (Neurath, 2020; Sundararaman et al., 2020; Zuo et al., 2020b) (Figure 4); these are detectable in the stool samples of COVID-19 patients (Britton et al., 2020). Interestingly, even when GIT manifestations are absent or after respiratory recovery from the infection, the virus is thought to remain active in the GIT, potentially in a dormant stage (Zuo et al., 2020a). Gou et al. were among the first to report gut microbiome dysbiosis and immune-inflammatory phenotypes that may predispose patients to severe/fatal consequences of COVID-19 (Gou et al., 2020). These authors developed a proteomics-based risk score by machine learning and found that the gut microbiome has better predictive capacity when compared to various demographic and laboratory findings, including gender, age, BMI, blood pressure, and lipid



**Figure 4.** Gut microbiome changes in COVID-19. (1) SARS-CoV-2 infects gut epithelial cells via ACE2/TMPRSS2 receptors, (2) triggering local inflammatory response and stimulating cytokine release along with (3) antigen presentation and the activation of immune responses. (4) Anti-viral immunity leads to the effective clearance of the viral infection or (5) sustained infection leads to the over-activation of the immune system. The pro-inflammatory cytokine (6) IFN $\alpha$  acts locally to augment opportunistic pathogens and suppress commensals. (7) Microbial dysbiosis prompts a reduction in SCFAs and (8) an upregulation of ACE2 receptor expression. The magnitude of these responses may contribute to systemic inflammation.

Abbreviations: ACE2r, angiotensin-converting enzyme 2 receptor; DC, dendritic cell; M, macrophages; NK, natural killer; PMN, polymorphonuclear neutrophils; SCFAs, short chain fatty acids; Teff, effector T cells; TMPRSS2, transmembrane protease serine protease 2; Treg, regulatory T cell.

profiles (Gou et al., 2020). Their study revealed that inflammatory cytokines were positively correlated with *Blautia*, *Lactobacilli*, and *Ruminococci*, while *Bacteroides*, *Streptococcus*, and *Clostridiales* were, on the contrary, negatively correlated. Another study found that hospitalized COVID-19 patients had significant alterations in their fecal microbiomes and mycobiomes when compared with controls (Zuo et al., 2020a, b), and correlated disease severity with fecal shedding of the virus. The microbiome of COVID-19 patients was also enriched with opportunistic fungal (*Candida albicans*, *C. auris*, and *A. flavus*) and bacterial pathogens (*Clostridium hathewayi*, *C. ramosum*, and *Coprobacillus*) (Zuo et al., 2020a, b). Furthermore, beneficial commensals, such as *Faecalibacterium prausnitzii*, *Bacteroides massiliensis*, *B. dorei*, *B. thetaiotaomicron*, and *B. ovatus*, were also depleted in severe COVID-19 patients. Interestingly, these bacteria are known for their anti-inflammatory effects and have been linked to the downregulation of angiotensin-converting enzyme-2 (ACE2) receptors (Geva-Zatorsky et al., 2017; Miquel et al., 2013).

In another study, Gu et al. examined the gut microbiome data of COVID-19 patients in a plot receiver-operating characteristic (ROC) curves context and investigated whether microbiome-based biomarkers could be used to predict the course of COVID-19 (Gu et al., 2020). As anticipated, these authors identified five microbial species *Actinomyces*, *Fusicatenibacter*, *Erysipelatoclostridium*, *Intestinibacter*, and *Romboutsia*, as possible markers to distinguish COVID-19 patients from control groups, thus presenting a GIT microbiome trademark to distinguish between healthy individuals and COVID-19 patients. The outcome of such studies highlights the importance of the microbiome in identifying non-invasive biomarkers and unravelling potential therapeutic targets for the management and treatment of COVID-19. In contrast to these studies, however, Britton et al. did not find any changes in the composition of the microbiome related to the severity of COVID-19 or gut inflammatory markers, thus suggesting that only patients treated with antibiotics exhibit substantial microbiome changes with low diversity in microbiome composition (Britton et al., 2020).

### Host immune responses in COVID-19 infection

Accumulating evidence currently supports the rationale that the mortality rate of COVID-19 can be greatly reduced by gaining a better understanding of the behavior of the immune system within the course of SARS-CoV-2 infection. COVID-19 presents with a vast array of clinical outcomes ranging from asymptomatic to severe ARDS, including fever hyper-inflammation, coagulopathy, and multi-organ failure (Gibson et al., 2020).

Initially, the virus uses its spike protein, primed by TMPRSS2, to attach to the ACE2 receptor on target cells. The co-expression of ACE2 and TMPRSS2 is essential for the initiation of virus infection in the respiratory tract and gastrointestinal epithelial cells, including, nasal goblet cells, alveolar epithelial type II cells, esophageal keratinocytes, colonocytes, pancreatic  $\beta$ -cells, cholangiocytes, renal podocytes, and proximal tubule cells (Qi et al., 2020). The initial infection of the nasal mucosa triggers a limited innate immune response to the virus that starts propagating towards lower respiratory tract (LRT). There, the virus faces a more robust innate immune response characterized by pulmonary infiltration with eosinophils and the production of cytokines, interferons, and chemokines (Fung and Liu, 2019). The PRRs, including the TLR family, retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and NLRs, recognize the expression of PAMPs on infected epithelial and immune cells, such as alveolar macrophages (Fung and Liu, 2019). Molecular interactions between PRRs and PAMPs induce phagocytosis and trigger intracellular signaling pathways to promote the production of pro-inflammatory

cytokines and chemokines that inhibit viral propagation through multiple mechanisms. Furthermore, the anti-viral signaling cascade attracts other innate immune cells (DCs), natural killer (NK) cells, monocytes, and polymorphonuclear leukocytes, that further produce a range of chemokines, including MCP-1, MIG, and IP-10. These chemokines are capable of recruiting lymphocytes that, in turn, recognize the viral antigens presented by DCs (Alon et al., 2020). Studies on COVID-19 have revealed that macrophages, NK cells, and DCs, are the main players in innate immune responses as they have the ability to kill the virus through phagocytosis, apoptosis, and the production of cytokines, including interferon  $IFN-\alpha/\beta$  and  $IFN-\gamma$  (Cervantes-Barragan et al., 2007). Furthermore, macrophages, NK cells, and Dendritic cells, are responsible for the presentation of viral antigens to initiate adaptive immune responses. The loss of epithelial barrier function on account of SARS-CoV-2 infection and antiviral immune responses may lead to additional bacterial infections via other pathogens, including *Cryptococcus*, *Cladosporium*, *Issatchenkia*, *Alternaria*, *Aspergillus*, and *Candida*. The superimposed bacterial and fungal infections are likely to trigger a robust cytokine response. The virus itself can also infect alveolar macrophages and cause a “cytokine storm”; this could damage pulmonary tissues and result in multiple organ failure (Wang et al., 2020a). In the following section, we summarize the broad innate and adaptive immune responses against SARS-CoV-2.

### Innate immune responses in COVID-19

Recent research related to SARS-CoV-2 has shed light on different aspects of SARS-CoV-2 pathogenesis, including viral entry, shedding, clearance or persistence, reinfection, and the host immune responses. One of the first molecular executors of innate resistance a host mounts in response to viral infection is the release of interferons (IFNs). These IFNs are potent cytokines that perform multiple roles to block viral replication through innate immune cells and the priming of adaptive immune responses (Schoggins, 2019). Recent transcriptomic studies of SARS-CoV-2-infected bronchial epithelial cells have shown that type I and type III interferon responses were impaired. However, the levels of different chemokines and IL-6 were increased (Blanco-Melo et al., 2020). Similarly, another study observed an impairment of the IFN-I responses in the peripheral blood of patients with severe COVID-19; this was accompanied by high levels of tumor necrosis factor (TNF) and IL-6, with a reported increase of NF- $\kappa$ B-mediated inflammatory responses (Zhou et al., 2020b). PAMPs can also induce the activation of Toll-like receptors (TLRs) which has been proposed as a player in fatal cases of severe COVID-19, including the development of ARDS and respiratory failure. These cytokines are typically secreted following LPS-induced TLR-4 activation, which is a well-known endotoxin of Gram-negative pulmonary bacteria (e.g. *Prevotella*, *Veillonella*, *Neisseria*, *Fusobacteria*). Furthermore, several other studies have highlighted the important role of type I interferons in the development of severe COVID-19. For example, delayed IFN-I responses lead to the accumulation of inflammatory monocyte-macrophages, as well as increased levels of pro-inflammatory cytokines and chemokines, along with the impairment of virus-specific T cell responses. Indeed, the genetic ablation of the  $IFN-\alpha\beta$  receptor and the depletion of inflammatory monocyte-macrophages has been shown to protect mice from lethal infections, with no effect on viral load (Channappanavar et al., 2016; Lee et al., 2020). PAMPs are another activator of innate immune cells that can induce anti-viral responses. Studies have revealed that the membrane (M) protein of SARS-CoV can activate  $IFN-\beta$  and NF- $\kappa$ B signaling through a non-canonical TLR-related signaling pathway independent of TRAF3 (Wang and Liu, 2016).

Hence, several therapeutic strategies for targeting TLRs are in various phases of clinical trials; these aim to target the detrimental immune responses and inflammatory mediators against SARS-CoV-2 (Patra et al., 2020).

A number of studies have applied single-cell RNA sequencing (scRNA-seq) to profile peripheral blood mononuclear cells (PBMCs) from patients hospitalized for COVID-19, including mild and severe cases. Results revealed a depletion of various innate immune cell subsets, including  $\gamma\delta$  T cells, (pDCs), (DCs), CD16+ monocytes, and NK cells, with NK cell exhaustion. This depletion of multiple innate immune cell subsets in the periphery of COVID-19 patients was accompanied by other immune perturbations, including the expansion and elevated frequency of plasmablasts, abundant antibody heavy chain sequences with long, diverse CDR3 sequences, and higher levels of somatic hypermutation in severe cases of COVID-19 (Kuri-Cervantes et al., 2020; Wilk et al., 2020). Another study has shown that an increased neutrophil-to-lymphocyte ratio can be considered as a prognostic marker of COVID-19 respiratory failure and disease severity. Laing et al. observed that patients with severe COVID-19 had depleted pDCs, monocytes, and basophils, but increased counts of neutrophils (Laing et al., 2020). This increased neutrophil count can be attributed to additional bacterial infections in patients infected with COVID-19. However, the concentrations of interleukin (IL)-8, IL-6, IL-10, and interferon gamma-induced protein 10 (IP-10) concentrations, were higher in COVID-19 patients and associated with disease severity. Furthermore, IP-10 levels were associated with IFN- $\gamma$ , while IFN- $\alpha/\beta$  responses were reported to be low in COVID-19 patients (Laing et al., 2020; Lee et al., 2020). Similarly, Wilk et al. (2020) reported a reduction in DC, pDC, NK, and  $\gamma\delta$  T cells, in ARDS-associated COVID-19 patients. Alveolar macrophages were significantly reduced while monocytes-derived inflammatory macrophages (FCN1<sup>+</sup> and SPP1<sup>+</sup>) were dominant in patients with severe COVID-19. These inflammatory macrophages upregulated INF signaling genes, monocytes recruiting chemokines, and pro-fibrotic cytokines (Wilk et al., 2020). However, the existing literature does not always concur; indeed, there are reports of heterogeneous phenotypic patterns of neutrophils, CD14+ and CD16+ monocytes, as well as cytokines and IFN secretions, across several COVID-19 studies (Kuri-Cervantes et al., 2020; Laing et al., 2020; Lee et al., 2020; Merad and Martin, 2020; Wilk et al., 2020). Therefore, the presented COVID-19 innate immune traits decipher multiple immunopathology and immune protection settings, thus emphasizing the reinvestigation of immune cells and their secreted molecules in other COVID-19 cohorts; these were strongly associated with disease severity and clinical outcomes.

#### *Adaptive immune responses in COVID-19 patients*

To investigate the adaptive immune response in COVID-19 patients, several studies performed in-depth immune-phenotyping to identify relationships between cell-mediated responses, disease severity, and poor clinical outcomes, in COVID-19 cases (Mathew et al., 2020). Indeed, CD4+ and CD8+ T cells have been characterized as key players in SARS-CoV-2 clearance and found to be strongly correlated with IgG and IgA antibody titers in COVID-19 patients. Interestingly, SARS-CoV-2-reactive CD4+ T cells were detected in unexposed individuals, indicating a cross-reactivity between circulating coronaviruses and SARS-CoV-2 (Grifoni et al., 2020; Pia, 2020; Weiskopf et al., 2020). Wang et al. (2020a; Wang et al., 2020b) reported lower levels of CD4+ T cells, CD8+ T cells, B cells, and natural killer (NK) cells, in COVID-19 patients, especially in severe cases. In particular, CD8+ T cells and CD4+/CD8+ ratio showed a significant association with inflammatory status in COVID-19 patients (Wang et al., 2020a, b).

Another potential immunological marker is the levels of IFN- $\gamma$  released by CD4+ T cells; levels were found to be lower in severe cases in comparison to moderate cases (Chen et al., 2020). Microbial dysbiosis due to the loss of commensals, along with reduced SCFAs, can cause a negative impact on Treg populations that are crucial for suppressing an overactive immune response. Data available from SARS-CoV-2 serological studies indicate that IgM and IgG antibodies to SARS-CoV-2 are detected between 6–15 days post-COVID-19 onset (Liu et al., 2020). In a previous study, antibodies were detected in <40% of patients within the first week of disease onset, and promptly increased to 100% for total antibodies, 94.3% for IgM, and 79.8% for IgG, starting from day-15 following the onset of COVID-19 (Zhao et al., 2020). Serological studies that measure the levels of antibodies in patients and recovered individuals over an extended period of time further demonstrated a decline in the antibody concentration after 8 months and therefore question protective long term immunity against SARS-CoV-2; however, further studies are needed to investigate this further (Anichini et al., 2021; Zhou et al., 2021). Furthermore, preliminary data by the COVID-IP project identified several prominent immunological signatures in the peripheral blood of COVID-19 patients that are linked to disease progression, including the over-expression of CXCL10/IP10, the proliferation of T cells, and the depletion of basophils and plasmacytoid dendritic cells (Laing et al., 2020).

#### **Conclusion**

Although, there is a general consensus that an over-activated immune system leads to massive pulmonary damage in COVID-19 patients, the reasons underpinning the variable disease spectrum observed in these patients have yet to be delineated. Conventional research direction is focused on the role of genetic susceptibility, viral strain, and comorbidities, in determining the outcome of COVID-19 infection. In this review, we focused on the rather overlooked role of microbiome dysbiosis in two major organs that have been involved in COVID-19 disease severity, the GIT and the respiratory tract. In this context, some of the factors that have been identified include the effect of aging and comorbidities on the diversity of the microbiota, as well as the use of antibiotics. There is now compelling evidence that the milieu of gut flora can exert influence on pulmonary immune responses. Studies indicate that a unique cross-talk exists between the pulmonary and gut microbial compartments, and that influences the microbial profiles in both compartments. Interestingly, these microbial compartments are important for educating the innate and adaptive immune cells present at these mucosal interfaces. The gut microbiota has been shown to modulate several innate and adaptive immune-regulatory pathways (e.g., SCFAs released in the gut exhibit a protective role by limiting the over-activation of the immune system). These observations might explain why alpha diversity in the microbiome could potentiate severity in viral respiratory infections, in particular, SARS-CoV-2. Further studies that delineate the role of the microbiota and their products in the immune dysregulation observed in SARS-CoV-2 infections are urgently needed; such studies could identify the molecular mechanisms that underlie severe cases of ARDS. In addition, longitudinal studies could potentially shed light on the cause or effect relationships of the microbial dysbiosis known to be associated with COVID-19 infection as we begin to understand the complex role of the microbiome in health and disease.

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## Ethical approval

This study did not require ethical approval.

## Author contributions

Conceptualization, M.A.-A.; methodology, M.U.S.; writing—original draft preparation, F.C. and M.U.S.; writing—review and editing, M.A.-A. and L.K.; figures—content and design, I.A. and S.S.; referencing, M.U.S., I.A. and Z.A.; supervision, M.A.-A. and F.C.; funding acquisition, M.A.-A. and F.C. All authors have read and agreed to the published version of the manuscript.

## Conflicts of interest

The authors declare no conflict of interest

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ijid.2021.02.071>.

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