Review

Exploring the Role of Gut-Lung Interactions in COPD Pathogenesis: A Comprehensive Review on Microbiota Characteristics and Inflammation Modulation

Zi-Xuan Cheng[1] Jing Zhang[2]

[1] Shanghai Medical College of Fudan University, Shanghai, China

[2]Department of Pulmonary and Critical Care Medicine, Zhongshan Hospital, Shanghai, China

Address correspondence to:
Jing Zhang
Department of Pulmonary and Critical Care Medicine
Zhongshan Hospital, Shanghai Medical College, Fudan University
180 Fenglin Road
Shanghai, China
Phone: +86 134 7278 2754
Email: zhang.jing@zs-hospital.sh.cn

Running Head: **Gut-Lung Interactions in COPD Pathogenesis**

Keywords: gut-lung axis; gut microbiota; lung microbiota; COPD; inflammation

Abbreviations: COPD, chronic obstructive pulmonary disease; AECOPD, acute exacerbation of chronic obstructive pulmonary disease; FEV_1 forced expiratory volume in one second; FVC, forced vital capacity; IgE, immunoglobulin E; IL, interleukin; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; IFN-γ, interferon gamma; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor. PCT, procalcitonin; CRP, C-reactive protein; LPS, lipopolysaccharide; TLRs, Toll-like receptors; SCFAs, short-chain fatty acids; FFARs, free fatty acid receptors; HDAC, histone-deacetylase complex; NF-KB, nuclear factor-kappa B; NLRs, NOD-like receptors; HRV, rhinovirus; WGCNA, weighted gene co-expression networks; ICS, inhaled corticosteroids; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

Funding Support: This work is supported by the Shanghai Science and Technology Committee (Project number 19DZ1920104).

Date of Acceptance: March 21, 2024 | ***Published Online Date:*** April 1, 2024

Citation: Cheng Z, Zhang J. Exploring the role of gut-lung interactions in COPD pathogenesis: a comprehensive review on microbiota characteristics and inflammation modulation. *Chronic Obstr Pulm Dis*. 2024; Published online April 1, 2024.
https://doi.org/10.15326/JCOPDF.2023.0442

Abstract

Chronic obstructive pulmonary disease (COPD) is a paramount contributor to global morbidity and mortality. Over the past decade, the concept of the "gut-lung axis" has emerged, offering a lens through which to examine the intricate interplay between the host, microbiome, and respiratory diseases, including COPD. An expanding body of evidence underscores that the composition of both the gastrointestinal and respiratory microbiome deviates in COPD patients compared to healthy individuals, leading to distinct host immune responses and clinical manifestations. The objective of this review is to provide a concise overview of the role of both gut and respiratory microbiome plays in the development of COPD. This will be accomplished by compiling current literature on the microbiome profile in stable and exacerbated cases of COPD, as well as exploring the biological mechanisms through a discussion of relevant experiments conducted on murine models. Hallmark characteristics of the microbial profile in COPD encompass reduced *Prevotella spp*. in the respiratory microbiome, culminating in a loss of anti-inflammatory protection, and diminished Bacteroidetes in the gut microbiome, leading to a decrease in protective short-chain fatty acids (SCFAs). The proliferation of Proteobacteria, particularly *Haemophilus spp.*, *Moraxella spp.* and *Pseudomonas spp.* contributes to COPD pathologies via recognition of proinflammatory lipopolysaccharide (LPS) via Toll-like receptors (TLRs). As a consequence, deteriorated pulmonary function, enhanced severity, increased onset of exacerbations and elevated mortality were observed.

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide and is characterized by persistent airflow obstruction and respiratory symptoms. [1] Despite the heterogeneous pathogenesis of COPD, diagnostic and therapeutic approaches remain rather circumscribed[2], driving the need for further comprehending the underlying mechanisms of COPD etiology.

Over the past decade, the term "gut-lung axis" has gained significant attention as a way to elucidate the complex interplay between the host, microbiome, and respiratory diseases. Recently, there has been increasing interest in the potential role of the gut-lung axis in the pathogenesis of COPD. Growing evidence suggests that bidirectional communication between the two organs plays a crucial role in COPD progression and exacerbation.[3,4] In fact, dysbiosis, or an imbalance in the microbiome, contributes to COPD development[5,6], and disruption in intestinal barrier integrity and function was found in COPD patients but not in non-COPD control subjects.[7,8] An expanding corpus of research indicates that the constitution of both the gut and respiratory microbiome is distinct in individuals with COPD compared to healthy individuals. This underscores the plausible notion that alterations in the composition of the microbiome may lead to distinct host immune reactions and clinical presentations via intricate interplay with the host.

The rapid advancement in technologies has played a crucial role in comprehending the microorganisms residing in our body, their functionalities and their effects on human health and disease.[9] Sequencing of the 16S rRNA gene is a widely used method to classify microbial taxa due to its ability to provide a fast and cost-effective analysis of microbial composition.[10] The commonly used indices to evaluate overall patterns of variation in the microbiome are α-diversity

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

and β-diversity, which represent the diversity of the microbial community within individual samples and the dissimilarity in microbial composition within pairs of samples, respectively.[10,11] Investigating microorganisms that exhibit differential abundance between the groups of interest is another commonly employed analytical approach. Exploring the correlation between modified taxa and disease phenotypes can aid in identifying microbial indicators between groups and directing treatment.[11] Furthermore, the swift advancement of novel technologies based on exact sequence variants, integrating metagenomics and metabolomics, enables precise identification of particular species and analysis of microbial metabolism and functions.[11]

The objective of this review is to provide a concise overview of the role of both gut and respiratory microbiome plays in the development of COPD. This will be accomplished by compiling current literature on the microbiome profile in stable and exacerbated cases of COPD, as well as exploring the biological mechanisms through a discussion of relevant experiments conducted on murine models.

1. Dysbiosis of the gut microbiome in COPD

1.1 Gut microbiome in health

The microbial inhabitants in the gastrointestinal tract constitute the most abundant microbial group within the human body, so the gastrointestinal system represents the most extensively investigated microbiome in scientific inquiry. The structural composition of the gastrointestinal microbiome encompasses the microbiota inhabiting the oral cavity, esophagus, stomach, small intestine, and colon. In both rodents and humans, the regions of the cecum and proximal colon harbor the greatest microbial biomass, while the small intestine contributes to a

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

lesser but still significant extent. [12,13] Distinct microbial compositional profiles are discernible along the length of the gastrointestinal tract. Major phyla found in the oral cavity and esophagus include Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Fusobacteria. Because of the high acidic environment in the stomach, only a limited number of microorganisms can survive in the stomach and duodenum. Some of the common genera include *Bacillales incertae sedis*, *Streptococcaceae*, and *Enterobacteriaceae*. The intestine is dominated by Proteobacteria and Lactobacillales. Last, the featured phyla in the terminal ileum and colon are Bacteroidetes, Firmicutes, Verrucomicrobia, Proteobacteria, and Actinobacteria. [13]

1.2 Relationship between the gut microbiome and stable COPD

Studies have revealed that the composition of the gut microbiome in COPD patients varies significantly from that of healthy individuals. When assessed by β-diversity, significant differences in the composition of the gut microbiome were found in stable COPD subjects compared to healthy individuals, although the α-diversity between the two groups showed no significant difference.[6,14] When compared within COPD patients, both the bacterial richness and diversity failed to gauge the severity of stable COPD[6,15]. However, a decline in lung function within the same patient may be related to altered α-diversity. A 1-year follow-up study of COPD patients uncovered a compelling correlation: an increase in microbial diversity was concomitant with a marked reduction in forced expiratory volume in one second (FEV_1) exceeding 40 ml.[16] (It has been observed that the annual reduction in FEV_1 for nonsmokers is approximately 30 ml. In contrast, for individuals who partake in smoking, this annual diminution fluctuates between 40 and 60 ml.)[17,18]

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

Regarding the differential abundance of specific taxa, the most mentioned alteration in the gut microbiome of COPD is a reduction of the phylum Bacteroidetes[6,15,16] and its genus *Bacteroides*[14], which is related to decreased FEV_1 and forced vital capacity (FVC).[15,16] (Table 1) Other genera observed to be diminished in COPD include *Roseburia* and *Lachnospira*[14]. The phylum Firmicutes proliferated in COPD patients and was related to decreased lung function.[6,16] Dominating bacterial genera observed in fecal samples of COPD patients but not in healthy subjects are *Prevotella*[6], *Streptococcus*, *Rothia*, *Romboutsia* and *Intestinibacter*[14]. Several members from the genera *Prevotella* and *Streptococcus* were found to be negatively associated with FEV_1.[14,16] When compared within COPD patients, the genera *Fusobacterium* and *Aerococcus* had greater abundance in stage 3-4 COPD when compared to stage 1-2 COPD[15].

In addition to microbial profiling, a significant difference was also found in the metabolome of gut microbiota between healthy subjects and stable COPD patients.[14] It is interesting to note that the severity of COPD was found to be linked with the amount of short-chain fatty acids (SCFAs), which are gut microbial metabolites that demonstrate protective effects on respiratory diseases[6,19]. SCFAs are byproducts of the fermentation of dietary fibers that are released into the lumen and peripheral circulation.[20] In a mouse model of emphysema, SCFAs demonstrated significant prophylactic potential against the advance and intensity of emphysema, concurrently attenuating inflammatory responses.[21,22] A comprehensive study involving a cohort of 35,339 Swedish women revealed that long-term consumption of dietary fibers was associated with a 30% lower risk of COPD.[23] SCFAs have also exhibited therapeutic potential in asthma by mitigating airway inflammation and reducing the production of immunoglobulin E (IgE), interleukin (IL)-4 and IL-8, along with immune cell counts.[24] It is conceivable that individuals with more severe conditions lost the protective effects of specific metabolites generated by commensal intestinal microbiota.

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

1.3 Relationship between the gut microbiome and COPD exacerbations

The dysbiosis of the gut microbiome in acute exacerbation of COPD (AECOPD) patients is rarely investigated. In terms of microbial diversity and richness, contradictions exist. A study reported that decreased richness and diversity of gut microbiota were found in AECOPD patients but not in healthy subjects and stable COPD patients[25], while another study found no alterations in microbial diversity with varied abundances of bacteria. [26] Phylogenetically, increased abundances of Bacteroidetes and Proteobacteria, as well as decreased abundances of Firmicutes and Actinobacteria, were observed in AECOPD patients but not in stable COPD and healthy subjects. Moreover, weighted gene co-expression networks (WGCNA) revealed a significant correlation between members of the Firmicutes and Actinobacteria and lower lung function as well as higher levels of inflammatory markers, suggesting that these phyla may contribute to the progression of COPD by exacerbating inflammation.[25] (Table 2)

1.4 Relationship between the gut microbiome and host immunity in COPD

Scientific evidence underscores a correlation between alterations in the abundance of specific gut microbiota in COPD patients and systemic inflammation markers, alongside inflammatory cell counts. (Table 1) (Table 2) Therefore, it appears plausible that gut microbiota may contribute to COPD pathogenesis through the modulation of systemic inflammation. (Figure 1) Members from the phyla Firmicutes and Actinobacteria were reported to have a highly significant relationship with blood inflammatory indices, including IL-6, IL-8, tumor necrosis factor alpha (TNF-α), procalcitonin (PCT) and C-reactive protein (CRP), in COPD, suggesting

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

that these bacterial taxa may be involved in the development and progression of COPD by exacerbating systemic inflammation.[25] Another study revealed that a higher abundance of a member from *Bacteroides* was linked to better lung function and lower blood eosinophil counts. This is notable because high blood eosinophil counts are known to be associated with an increased risk of COPD exacerbations, mortality and reduced FEV_1.[15] The above changes are likely to arise from the epigenetic regulation of immune cells by SCFAs, which can bind to free fatty acid receptors (FFARs) on immune cells such as neutrophils, monocytes and macrophages, causing an inhibition of the expression of proinflammatory factors, including IL-6, IL-1β and TNF-α.[27] In addition to attaching to FFARs, SCFAs exert anti-inflammatory effects by inhibiting histone-deacetylase complexes (HDACs) and suppressing nuclear factor-kappa B (NF-κB) signaling.[28] (Figure 2) Notably, an elevated abundance of Bacteroidetes and a decreased abundance of Firmicutes were associated with higher levels of SCFAs[29], which is consistent with the aforementioned negative correlation between SCFA levels and COPD severity.[6] In other words, the reduction in Bacteroidetes and Firmicutes results in decreased levels of blood SCFAs, which deprives COPD patients of the protective effect of SCFAs against systemic inflammation. This ultimately leads to an increase in lung inflammation and may be a key mechanism by which alterations in the gut microbiome contribute to COPD. Another proposition that needs further inquiry is whether there exists specific gut microbiota that do not confer protection against COPD through the secretion of SCFAs.

1.5 Relationship between antibiotic therapy and the gut microbiome in COPD

Antibiotics are frequently used in the management of COPD, especially for addressing pneumonia during acute exacerbations. Nevertheless, their direct influence on microorganisms

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

poses a significant challenge in analyzing gut microbial sequencing. The effect of antibiotic use on gut microecology in COPD patients remains uncertain, primarily because most studies have excluded individuals who recently used antibiotics.[14–16,25] This lack of information also extends to severe AECOPD, despite the pressing need for further research and medical support for this specific population. Li et al. found that antibiotics led to a significant decrease in gut bacterial abundance in mice, resulting in dysbiosis and reduced levels of SCFAs, which played a role in COPD progression.[6] However, in clinical practice, the complexities of different pathogenic bacteria and bacterial loads in the lungs of AECOPD patients make it challenging to simply characterize the pros and cons of antibiotics. Further investigation is needed to better understand the impact of antibiotics on gut microecology in COPD. Longitudinal study designs could prove beneficial in providing more comprehensive insights into the long-term effects.

2. Dysbiosis of the respiratory microbiome in COPD

2.1 Respiratory microbiome in health

Lungs were previously thought to be completely sterile until researchers detected bacterial DNA in healthy lung samples.[30] In contrast to the gut, the respiratory tract is a low-biomass environment with microbial density diminishing in a gradient from the upper to the lower respiratory tract.[31] The most prevalent phyla of bacteria found in the respiratory tract are Firmicutes and Bacteroidetes, while Prevotella, *Streptococcus* and *Veillonella* are the most common genera. [32] The pulmonary microorganism community may have originated from the upper respiratory tract through microaspiration and been further shaped by host defense mechanisms; thus, a healthy respiratory microbiome is more likely to be a result of intermittent aspiration of taxa rather than reproduction of the core resident community.[4,20,32] Several sampling

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

methods are available to profile the respiratory microbiome, including nasal brushing, induced or expectorate sputum, bronchoalveolar lavage, epithelial brushing and bronchial biopsies, while sputum has been a preferred sampling method in terms of accessibility and difficulties with bronchoscopy in COPD patients. [31] The application of sputum sampling also eases the implementation of longitudinal studies, for example, comparing samples before and after exacerbations. [33–50] However, contamination from microorganism-rich oral cavities must be considered, especially the propensity for contamination increases in environments with a low biomass, such as the lower respiratory tract.[20,51] Therefore, sputum profiling cannot entirely encapsulate the composition of the pulmonary microbiome. Previous studies have suggested that oral antiseptic rinsing before collecting sputum samples may reduce contamination in sputum cultures.[52,53] However, the role of oral antiseptic rinsing in sputum microecological sequencing remains unexplored. Additional research is required to standardize protocols for spontaneous and induced sputum sampling.

2.2 Relationship between the respiratory microbiome and stable COPD

Studies have shown significant deviation of the respiratory microbiome in relatively severe COPD patients, whereas relatively milder patients had a respiratory microbiome more comparable to that of healthy individuals. When compared within stable COPD, the bacterial biodiversity can reflect the severity of COPD, as sputum samples from severe patients were found to have a decreased α-diversity compared to those from milder patients.[54–56] When compared with healthy individuals, several studies highlighted a decrease in α-diversity in stable COPD compared to healthy individuals[41,57–60], while others reported no significant changes or even a higher α-diversity.[61–63] A meta-analysis focusing on the alteration of α-diversity in COPD

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

found that there was no significant but a slight trend toward decreased microbial diversity measured by the Chao1 index, which is an index that measures microbial richness but not evenness.[64] The conflicting findings in α-diversity may be attributed to variations in the severity of the enrolled patients, as those with more severe symptoms are likely to have an altered respiratory microbiome with a declined microbial diversity. In terms of β-diversity, principal coordinate analysis was able to separate lung microbiota samples from healthy controls and COPD patients.[61,65,66] Taken together, changes in microbial richness and evenness indicate diminished commensal microbiota and proliferated potentially pathogenic microbiota, resulting in a deviated and restricted pattern in the respiratory microbiome of severe COPD patients.

In regard to the distribution of particular taxa, the most frequently mentioned alterations in the respiratory microbiome of COPD include an increase in the abundance of *Proteobacteria* (including *Haemophilus*[41,60,62], *Moraxella*[41,59,62] and *Pseudomonas*[58,60,67–69]) and a decrease in the abundance of *Prevotella*[58–60,70]. (Table 1) An elevated abundance of Proteobacteria was observed in COPD patients compared to healthy controls[62], and dominance of the phylum was correlated with more severe COPD.[56,71] This is likely related to the fact that COPD patients have a respiratory microbiome colonized by the pathogenic bacteria *Haemophilus influenzae* and *Moraxella catarrhalis*[36,72], as proliferation of representative pathogens (including *H. influenzae*, *M. catarrhalis*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*) was detected in patients with more severe symptoms.[42] However, it is worth mentioning that more studies have demonstrated an increase in multiple species from the same genera, resulting in an overall increase in the whole genera (*Haemophilus*[41,60,62] and *Morexella*[41,59,62]), which indicates a potential synergistic pathogenic effect among congeners. Correspondingly, *Haemophilus*[44] and *Pseudomonas*[67] were found to be related to more severe airflow limitation. The intricate interactions among taxa have resulted in greater heterogeneity of the respiratory microbiome in

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

COPD, which can be classified into subgroups characterized by distinct microbial dominance. For example, a study found that the abundance of *H. influenzae* was positively associated with *S. pneumoniae* but negatively associated with *P. aeruginosa*.[48]

A common pattern of intraspecies interaction is that the proliferation of potential bacteria is accompanied by a reduction of commensal bacteria. For instance, *Moraxella*[37] and *Pseudomonas*[63] were found to be negatively related to the α-diversity in sputum samples. The commensal bacteria reported to be reduced in COPD are *Prevotella*[58–60,68,70] and *Veillonella*[68,72], of which *Prevotella* was found to be associated with better lung function and alleviated symptoms. [59,73,74] Other genera that were reduced in COPD include *Treponema*[60,67], *Actinomyces*[54,58] and *Fusobacteria*[60,70]. Despite being considered a commensal genus, the relationship between *Streptococcus* and COPD is inconsistent based on various studies. Several studies have observed an increased abundance of *Streptococcus* in COPD[41,59,69,70,73], which has been linked to decreased lung function[73,74] and could be associated with infection by the potentially pathogenic *S. pneumoniae*[36,72]. In contrast, other studies reported a decline in the abundance of *Streptococcus* in COPD[62,68]. A plausible explanation for this discrepancy might be attributed to the divergent roles performed by distinct species within the *Streptococcus* genus. This is fundamentally because 16S rRNA analysis is limited to differentiation at the genus level and is therefore incapable of distinguishing between individual species and their respective functionalities. Another possible reason is that the specific microbiota itself can develop contradictory functionalities; for example, *S. pneumoniae is* a highly adapted commensal but has the ability to cause severe disease according to specific bacterial and host factors.[75] This complex and dynamic relationship between the host and its microbiota is a key focus of current research to better understand and manage various diseases.

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

2.3 Relationship between the respiratory microbiome and COPD exacerbations

The common feature of the respiratory microbiome during COPD exacerbations is decreased α-diversity and Proteobacteria dominance. When compared with stable COPD, decreased α-diversity was found in COPD exacerbations using 16S rRNA sequencing[26,33,66,76] and metagenomic sequencing[77]. A meta-analysis found that there was a slight trend toward decreased α-diversity in COPD exacerbations compared to stable COPD.[64] In accordance with β-diversity, contradictory results also exist.[26,34,37,49,66,67] A decrease in α-diversity was correlated with higher frequency in exacerbations[76,77] and higher mortality[78], whereas deviation of microbial composition was linked to reduced FEV_1 and FVC during exacerbations[37]. Notably, remodeling of the respiratory microbiome is not commonly seen in all exacerbation cases but only in a subgroup[33,34,37,76]; for example, a study found that significant deviation of pulmonary microbial composition was only present in 41% of all patients with exacerbations[37]. In fact, great heterogeneity in the respiratory microbiome usually exists within COPD patients during exacerbations[34,35], indicating the existence of various triggers of AECOPD.

Consistent with findings in stable COPD, Proteobacteria dominance (especially *Haemophilus* and *Moraxella*) and diminishment of commensal bacteria (especially *Veillonella*) were also present in AECOPD. (Table 2) Several studies reported a slight trend of increased Proteobacteria and decreased Actinobacteria and Firmicutes[26,33–35,77], of which Proteobacteria dominance was related to increased mortality.[56] The Proteobacteria dominance is possibly due to an elevation in both representative and nonrepresentative pathogenic taxa from the genera *Moraxella* (including *M. catarrhalis*)[26,33,39,46,47,50,76,77,79] and *Haemophilus* (including *H. influenzae*)[26,33,35,39,46,47,50,76,79], suggesting that synergic effects of bacteria from the same genus have contributed to AECOPD. The risk of exacerbations was elevated with the presence of *H.*

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

influenzae and *M. catarrhalis*.[45,46,76] During exacerbations, the colonization of the *Haemophilus* genus is often found concurrently with a reduction of other commensal bacteria[33,76] but an elevation in human rhinovirus (HRV)[39,46]. In contrast, the Moraxella genus tends to trigger exacerbations in an independent manner without viral effects.[39] In agreement with these results, a diminishment of commensal bacteria was reported in exacerbations, especially the genus *Veillonella*.[37,77] A study found that the decreased abundance of *Veillonella* and increased abundance of *Staphylococcus* were independent predictors of mortality in AECOPD, elevating the risk by 13.5- and 7.3-fold, respectively.[78] As previously mentioned, the respiratory microbiome of patients with AECOPD exhibits great heterogeneity, with different studies reporting diverse altered taxa (including *Acinetobacter*, *Actinomyces*, *Ehrlichia*, *Fusobacteria*, *Perlucidibaca*, *Prevotella*, *Pseudomonas* and *Sph*ingomonas).[26,34,76–79] This implies that the respiratory microbiome in AECOPD may undergo varying degrees of change and remodeling. Overall, increases in the abundance of *Moraxella*, *Haemophilus* and HRV, as well as decreases in α-diversity and the abundance of *Veillonella,* are associated with COPD exacerbation frequency, severity and mortality.

2.4 Relationship between the respiratory microbiome and host immunity in COPD

Several studies have shown that dysbiosis in the respiratory microbiome is linked to pulmonary inflammation pathways. Overall, the diversity and community organization of the respiratory microbiome were strongly correlated with sputum CXCL8/IL-8.[33] Host gene expression analysis found that decreased microbial diversity was related to emphysematous destruction, remodeling of the bronchiolar and alveolar tissue, and infiltration of the tissue by $CD4^+$ T cells.[80] The genera related to pathogenic states, *Haemophilus* and *Moraxella*, have been

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

shown to correlate with exacerbated lung inflammation in COPD. Specifically, *Haemophilus* is more commonly associated with stable COPD, whereas *Moraxella* is more commonly associated with AECOPD. Both *Haemophilus* and *Moraxella* were found to be related to the excessive production of several chemokines in sputum samples, including IL-1β, IL-8, IL-10 and TNF-α.[33,42,44,50,59,72,81] The bacterial load of *H. influenzae* was found to be an independent predictor of sputum IL-1β and TNF-α in stable COPD, while the bacterial load of *M. catarrhalis* was correlated with elevated sputum IL-1β and TNF-α concentrations in COPD exacerbations.[41,72] In addition to proinflammatory mediators, the abundance of *Moraxella* and *Haemophilus* was also found to correlate with neutrophil counts in sputum[33,36,38] or blood[38] in both cross-sectional and longitudinal studies.

The type of inflammation is linked to particular microbial communities. Neutrophilic inflammation is associated with Proteobacteria dominance, particularly *Haemophilus* and *Moraxella*, which is correlated with host interferon and proinflammatory signaling pathways, Eosinophilic inflammation, on the other hand, may be related to a diverse microbial profile dominated by Firmicutes or Bacteroidetes.[41,56] Additionally, the dominance of Firmicutes, especially *Streptococcus,* has a positive relationship with peripheral eosinophil counts.[56] Another study found that peripheral eosinophil levels ≥2% were correlated with a higher diversity in the respiratory microbiome[67], which can partially explain the negative correlation between Proteobacteria and eosinophil counts in blood[56], as the colonization of *Haemophilus* has been linked to decreased microbial diversity in the respiratory microbiome.[33,76] Consistently, a study classified three clusters of COPD and asthma patients: cluster 1 was characterized by Proteobacteria dominance, increased proinflammatory mediators (IL-1β, IL-6, TNF-α, vascular endothelial growth factor (VEGF), etc.) and increased neutrophil counts in both blood and sputum. Cluster 2 was characterized by Bacteroidetes dominance, increased type 2 mediators (IL-

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

5, IL-13, CCL13, CCL17 and CCL26) and increased eosinophil counts. The features of cluster 3 were Actinobacteria and Firmicutes dominance and increased type 1 mediators (CXCL10, CXCL11 and interferon gamma (IFN-γ)).[82]

The clinical decision-making process is directly influenced by the type of inflammation present. For instance, genomic markers of type 2 inflammation are associated with elevated eosinophils (one of the main effector cells of type 2 inflammation) and a favorable response to inhaled corticosteroids (ICS), As a result, eosinophil counts were the first blood biomarker incorporated into the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines. This addition was based on evidence that eosinophil counts aid in determining the role of ICS treatment in patients prone to symptom exacerbation.[1] Therefore, the integration of microecological data will contribute to our understanding of the molecular mechanisms and characterization of various COPD subtypes. Alterations in chemokines and inflammatory cells found in sputum samples have the potential to act as biomarkers, enabling the identification of subsets of COPD with distinctive features in pathogen growth and inflammatory pathways.

It is plausible that activation of neutrophilic inflammatory pathways by Proteobacteria contributes to pathogenic phenotypes in COPD. Toll-like receptors (TLRs) have the ability to recognize and modify the inflammatory response of the host by detecting conserved microbial-associated molecular patterns.[83] The surface structures of gram-negative bacterial colonies, such as lipopolysaccharide (LPS) and polysaccharide A (PSA), can develop either beneficial or harmful effects. In terms of beneficial effects, it has been shown that PSA generated by colonies of gram-negative bacteria holds the capacity to rectify immune deficiencies in gnotobiotic mice and inhibit the progression of experimental colitis, although the underlying molecular processes that enable these effects require further research.[84] On the other hand, proinflammatory LPS interacts with the host myeloid-differentiation-2/Toll-like receptor 4 (MD-2/TLR4) receptor

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

complex, hence triggering a cascade of events that result in the activation of the transcription factor NF-κB, which then leads to the expression of genes responsible for producing proinflammatory cytokines and chemokines.[85] Similar proinflammatory effects can also be induced in a TLR3- or TLR4-dependent manner, which are associated with COPD aggravation.[86] (Figure 2) Proinflammatory LPS challenge has been linked to bacterial exacerbations of COPD characterized by elevated pulmonary and systemic inflammation.[87] In an in vivo study in mice, *H. influenzae* caused severe COPD-like inflammation in a TLR2-independent manner, which was characterized by airway neutrophilia, neutrophilic cytokine/chemokine profile and lung immunopathology.[88] Furthermore, enhanced expression of TLR4 and NOD-like receptors (NLRs) in the bronchial epithelium of severe COPD patients has been observed to correlate with exacerbated bronchial inflammation and an increased *P. aeruginosa* bacterial load. This relationship may potentially be involved in the fundamental pathogenesis of COPD.[89] These results are consistent with the aforementioned findings of increased abundance in gram-negative bacteria such as *Haemophilus*, *Moraxella* and *Pseudomonas* in the respiratory microbiome of COPD patients. The link between lung pathology and microbiome alterations has also been observed in clinical studies. For instance, deviation of the respiratory microbiome was found to be related to severe subtypes determined by structural alterations (either airway or emphysema type changes) in CT scans.[70] Similarly, colonization of *H. influenzae* was found to be associated with bronchiectasis in COPD, and the degree of emphysema was correlated with the IL-8 concentration in sputum samples.[43]

The diminishment of commensal bacteria suggests a loss of protective effects provided by these microorganisms, which may contribute to COPD. Contrary to the effect of *Proteobacteria*, several commensal bacteria were found to have anti-inflammatory effects. The most mentioned commensal genus that is altered in COPD, *Prevotella*, was found to be associated with mild

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

neutrophilic airway inflammation in an in vivo study.[88] Another study discovered that protection against *S. pneumoniae* generated by *Prevotella* was achieved by identification of *Prevotella* lipoproteins through TLR2, which triggered TNF-α production and elevated neutrophils.[90] *Prevotella* is thought to cause only a low to moderate level of inflammation, which could be the reason why it is tolerated by the respiratory immune system and develops protective effects. However, further research is needed to fully understand the intricate relationship between *Prevotella* and the immune system in the respiratory tract.[88,90] *S. pneumoniae* has a similar ability to trigger TLR2-related responses[75], yet it is worth mentioning that the effect of TLR priming varies depending on the infection. For example, TLR4 priming with LPS derived from *Escherichia coli* enhances protection against lung infection caused by *Klebsiella pneumoniae* and influenza A virus. However, this is not the case with *S. pneumoniae*. To the best of our knowledge, proliferation of the genus was linked to reduced lung function[73,74]. The intrinsic mechanism of various effects induced by these bacterial colonies requires further investigation.

2.5 Relationship between clinical therapy and the respiratory microbiome in COPD

Antibiotic treatment in cases of acute exacerbations of COPD has demonstrated a beneficial impact, leading to a reduction in the abundance of Proteobacteria and facilitating the restoration of microbial diversity. A study by Wang et al. highlighted that steroid treatment alone reduced respiratory microbial diversity and increased the Proteobacteria:Firmicutes ratio, whereas antibiotic treatment had the opposite effect in AECOPD patients.[33] Similarly, Huang et al. observed a decrease in Proteobacteria within AECOPD patients treated with antibiotics alone.[35] However, the beneficial impacts are not necessarily sustained, especially when patients require prolonged antibiotic use for conditions like recurrent acute exacerbations. In patients with

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

cystic fibrosis, antibiotic use has been associated with a decline in bacterial diversity in the respiratory microbiome over a decade, independent of age and lung function.[91] In addition, different types of antibiotics, pathogenic bacterial strains and bacterial loads can influence sequencing results, and the long-term impact of antibiotics on respiratory microecology of COPD patients with recurrent exacerbations remain unclear. Further accumulation of long-term clinical data is necessary.

3. Gut-lung axis in COPD

3.1 Evidence of gut-lung crosstalk in COPD

Emerging evidence has indicated the possible interaction between the gut microbiome and respiratory microbiome. The respiratory microbiome is thought to be partly shaped through microaspiration from the oropharynx[20,92], indicating that it can be affected by both the upper respiratory tract and gastrointestinal tract. While there is currently no evidence supporting direct transfer between the gut and respiratory microbiomes, the correlation between the gut and respiratory microbiome may be attributed to the potential transfer of metabolites between these sites. An analysis of cystic fibrosis in infancy found a strong correlation between the changes in bacterial composition over time in both the gut and respiratory tract and that microbial colonization of the respiratory tract can be anticipated by prior colonization of the gut.[93] In regard to COPD, the genus *Streptococcus* proliferated in both the lung and gut, indicating a transfer of microbiota through the oropharynx.[14] In contrast, the genus *Prevotella* presented contradictory abundance and correlation with lung function depending on its location. The genus proliferated in the guts of COPD patients and healthy smokers[15,94] but was reduced in the lungs.[58–60,70] The varying microenvironments and metabolite transfer across the body may account for these

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

differences. Examining the intrinsic mechanisms that allow the same microbiota to carry out various abundances and functions depending on the local microenvironment may aid in better understanding the gut-lung axis.

Recent studies are starting to reveal a potential link between the environments of the lungs and gut COPD. This suggests that these two organ systems might interact or influence each other's health and disease states. In an in vivo study, mice that received transplantation of feces from COPD patients developed inflammation in their lungs.[6] Another study revealed that the transplantation of normal fecal microbiota could alleviate COPD pathogenesis by reducing lung and intestinal inflammation, as well as restoring abnormal amino acid metabolism in sera.[5] On the other hand, patients with COPD had considerably higher occurrences of IBD, and the risk rose along with the severity of COPD.[95,96] Consistently, reduced integrity and function of the intestinal barrier were observed in COPD patients[7,8], suggesting that chronic lung inflammation has a systemic effect on the gut ecosystem.

3.2 The intrinsic mechanisms of gut-lung crosstalk in COPD

Colonization of potentially pathogenic microorganisms in the lungs plays an important role in the advancement and deterioration of COPD. As mentioned before, gram-negative bacterial colonies can trigger proinflammatory TLR signaling through surface structures, including LPS, which leads to elevated pulmonary and systemic inflammation.[85,87,88] Proinflammatory LPS may have further effects on intestinal homeostasis, as decreased fecal secretory IgA levels and enlargement of Peyer's patches were observed in cigarette smoke- and LPS-induced murine models.[97] This suggests that exacerbated systemic inflammation induced by

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

the lung microbiota may have disrupted the balance of the intestinal environment, including its microecology.

The proper balance of microorganisms in the gut has a significant impact on regulating innate and acquired immunity. Harmful factors such as cigarette smoke, pollution particles and unbalanced diet cause dysbiosis of both the gastrointestinal and respiratory microbiome, depriving patients of the protective benefits of a healthy microecology.[98] Loss of these modifications may further aggravate systemic and lung inflammation, creating a vicious cycle. The modifications of host immunity by gut microbiota are achieved primarily through the secretion of SCFAs and recognition of microbe-associated molecular patterns. (Figure 1) Evidence has shown that COPD patients experience a loss of protective effects from SCFAs produced by gut microbiota.[27,28] Since SCFAs are present in human lungs, the absence of the substrates needed for SCFA synthesis by fermenting bacteria indicates that gut microbiota-produced SCFAs can transfer into the lungs through systemic circulation.[28] The bacterial phylum that is less abundant in the gut microbiome of COPD patients, Bacteroidetes, was associated with higher levels of SCFAs[29], which is consistent with lower levels of SCFAs in COPD.[6,99] SCFAs have been found to develop anti-inflammatory properties by modifying the Th2 response, modulating pathways of pulmonary ILC2s and inhibiting M2 macrophage polarization in airway hyperreactivity and airway inflammation models.[28] In regard to recognition of microbial surface structures, the capsular polysaccharide component PSA produced by members from the genus *Bacteroides* in the gut has developed anti-inflammatory properties and the ability to correct immune defects.[84] Thus, the decline in *Bacteroides* in stable COPD may represent a loss of PSA-mediated protective effects. An increased ratio of Firmicutes/Bacteroidetes, which was observed in stable COPD, has previously been associated with elevated lung IL-17 and IL-22 responses.[100]

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

Further research is necessary to determine whether the above anti-inflammatory responses are compromised in COPD patients.

Conclusions and perspectives

In conclusion, specific patterns in the gut and respiratory microbiome were discovered in both stable COPD and COPD exacerbations. The commensal microorganisms associated with protective effects were diminished, whereas potentially pathogenic microorganisms proliferated, leading to activated systemic and pulmonary inflammation. As a consequence, deteriorated pulmonary function, enhanced severity, increased onset of exacerbations and elevated mortality were observed. Further investigations on the relationship between the gut microbiome and clinical manifestations of COPD, the intrinsic mechanisms of protective or harmful effects induced by certain genera and the transformation from current knowledge on gut and respiratory microbiome dysbiosis to therapeutic strategies are warranted.

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

Acknowledgements

Authors' contributions

ZXC wrote the main manuscript text and prepared figures 1-2. JZ reviewed the manuscript.

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

Declaration of interest

The author reports no conflicts of interest in this work.

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

References

1. Christenson SA, Smith BM, Bafadhel M, Putcha N. Chronic obstructive pulmonary disease. *The Lancet*. 2022;399(10342):2227-2242. doi:10.1016/S0140-6736(22)00470-6

2. Stolz D, Mkorombindo T, Schumann DM, et al. Towards the elimination of chronic obstructive pulmonary disease: a Lancet Commission. *The Lancet*. 2022;400(10356):921-972. doi:10.1016/S0140-6736(22)01273-9

3. He Y, Wen Q, Yao F, Xu D, Huang Y, Wang J. Gut–lung axis: The microbial contributions and clinical implications. *Critical Reviews in Microbiology*. 2017;43(1):81-95. doi:10.1080/1040841X.2016.1176988

4. Budden KF, Gellatly SL, Wood DLA, et al. Emerging pathogenic links between microbiota and the gut–lung axis. *Nat Rev Microbiol*. 2017;15(1):55-63. doi:10.1038/nrmicro.2016.142

5. Lai HC, Lin TL, Chen TW, et al. Gut microbiota modulates COPD pathogenesis: role of anti-inflammatory *Parabacteroides goldsteinii* lipopolysaccharide. *Gut*. 2022;71(2):309-321. doi:10.1136/gutjnl-2020-322599

6. Li N, Dai Z, Wang Z, et al. Gut microbiota dysbiosis contributes to the development of chronic obstructive pulmonary disease. *Respir Res*. 2021;22(1):274. doi:10.1186/s12931-021-01872-z

7. Rutten EPA, Lenaerts K, Buurman WA, Wouters EFM. Disturbed Intestinal Integrity in Patients With COPD. *Chest*. 2014;145(2):245-252. doi:10.1378/chest.13-0584

8. Kirschner SK, Deutz NEP, Jonker R, et al. Intestinal function is impaired in patients with Chronic Obstructive Pulmonary Disease. *Clin Nutr*. 2021;40(4):2270-2277. doi:10.1016/j.clnu.2020.10.010

9. Schmidt TSB, Raes J, Bork P. The Human Gut Microbiome: From Association to Modulation. *Cell*. 2018;172(6):1198-1215. doi:10.1016/j.cell.2018.02.044

10. Combrink L, Humphreys IR, Washburn Q, et al. Best practice for wildlife gut microbiome research: A comprehensive review of methodology for 16S rRNA gene investigations. *Front Microbiol*. 2023;14:1092216. doi:10.3389/fmicb.2023.1092216

11. Knight R, Vrbanac A, Taylor BC, et al. Best practices for analysing microbiomes. *Nat Rev Microbiol*. 2018;16(7):410-422. doi:10.1038/s41579-018-0029-9

12. Krautkramer KA, Fan J, Bäckhed F. Gut microbial metabolites as multi-kingdom intermediates. *Nat Rev Microbiol*. 2021;19(2):77-94. doi:10.1038/s41579-020-0438-4

13. Ruan W, Engevik MA, Spinler JK, Versalovic J. Healthy Human Gastrointestinal Microbiome: Composition and Function After a Decade of Exploration. *Dig Dis Sci*. 2020;65(3):695-705. doi:10.1007/s10620-020-06118-4

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

14. Bowerman KL, Rehman SF, Vaughan A, et al. Disease-associated gut microbiome and metabolome changes in patients with chronic obstructive pulmonary disease. *Nat Commun*. 2020;11(1):5886. doi:10.1038/s41467-020-19701-0

15. Chiu YC, Lee SW, Liu CW, et al. Comprehensive profiling of the gut microbiota in patients with chronic obstructive pulmonary disease of varying severity. Singanayagam A, ed. *PLoS ONE*. 2021;16(4):e0249944. doi:10.1371/journal.pone.0249944

16. Chiu YC, Lee SW, Liu CW, Lan TY, Wu LSH. Relationship between gut microbiota and lung function decline in patients with chronic obstructive pulmonary disease: a 1-year follow-up study. *Respir Res*. 2022;23(1):10. doi:10.1186/s12931-022-01928-8

17. Oelsner EC, Balte PP, Bhatt SP, et al. Lung function decline in former smokers and low-intensity current smokers: a secondary data analysis of the NHLBI Pooled Cohorts Study. *Lancet Respir Med*. 2020;8(1):34-44. doi:10.1016/S2213-2600(19)30276-0

18. Anthonisen NR, Connett JE, Murray RP. Smoking and Lung Function of Lung Health Study Participants after 11 Years. *Am J Respir Crit Care Med*. 2002;166(5):675-679. doi:10.1164/rccm.2112096

19. Kotlyarov S. Role of Short-Chain Fatty Acids Produced by Gut Microbiota in Innate Lung Immunity and Pathogenesis of the Heterogeneous Course of Chronic Obstructive Pulmonary Disease. *IJMS*. 2022;23(9):4768. doi:10.3390/ijms23094768

20. Wypych TP, Wickramasinghe LC, Marsland BJ. The influence of the microbiome on respiratory health. *Nat Immunol*. 2019;20(10):1279-1290. doi:10.1038/s41590-019-0451-9

21. Jang YO, Kim OH, Kim SJ, et al. High-fiber diets attenuate emphysema development via modulation of gut microbiota and metabolism. *Sci Rep*. 2021;11:7008. doi:10.1038/s41598-021-86404-x

22. Jang YO, Lee SH, Choi JJ, et al. Fecal microbial transplantation and a high fiber diet attenuates emphysema development by suppressing inflammation and apoptosis. *Exp Mol Med*. 2020;52(7):1128-1139. doi:10.1038/s12276-020-0469-y

23. Szmidt MK, Kaluza J, Harris HR, Linden A, Wolk A. Long-term dietary fiber intake and risk of chronic obstructive pulmonary disease: a prospective cohort study of women. *Eur J Nutr*. 2020;59(5):1869-1879. doi:10.1007/s00394-019-02038-w

24. Corrêa RO, Castro PR, Moser R, et al. Butyrate: Connecting the gut-lung axis to the management of pulmonary disorders. *Front Nutr*. 2022;9:1011732. doi:10.3389/fnut.2022.1011732

25. Wu Y, Luo Z, Liu C. Variations in fecal microbial profiles of acute exacerbations and stable chronic obstructive pulmonary disease. *Life Sciences*. 2021;265:118738. doi:10.1016/j.lfs.2020.118738

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

26. Sun Z, Zhu QL, Shen Y, Yan T, Zhou X. Dynamic changes of gut and lung microorganisms during chronic obstructive pulmonary disease exacerbations. *Kaohsiung J Med Sci*. 2020;36(2):107-113. doi:10.1002/kjm2.12147

27. He J, Zhang P, Shen L, et al. Short-Chain Fatty Acids and Their Association with Signalling Pathways in Inflammation, Glucose and Lipid Metabolism. *Int J Mol Sci*. 2020;21(17):6356. doi:10.3390/ijms21176356

28. Ney LM, Wipplinger M, Grossmann M, Engert N, Wegner VD, Mosig AS. Short chain fatty acids: key regulators of the local and systemic immune response in inflammatory diseases and infections. *Open Biol*. 2023;13(3):230014. doi:10.1098/rsob.230014

29. Simpson HL, Campbell BJ. Review article: dietary fibre–microbiota interactions. *Aliment Pharmacol Ther*. 2015;42(2):158-179. doi:10.1111/apt.13248

30. Huang YJ, Kim E, Cox MJ, et al. A persistent and diverse airway microbiota present during chronic obstructive pulmonary disease exacerbations. *OMICS*. 2010;14(1):9-59. doi:10.1089/omi.2009.0100

31. Ditz B, Christenson S, Rossen J, Brightling C, Faiz A. Sputum microbiome profiling in COPD: Beyond singular pathogen detection. *Thorax*. 2020;75(4):thoraxjnl-2019-214168.

32. Whiteside SA, McGinniss JE, Collman RG. The lung microbiome: progress and promise. *J Clin Invest*. 2021;131(15):e150473. doi:10.1172/JCI150473

33. Wang Z, Bafadhel M, Haldar K, et al. Lung microbiome dynamics in COPD exacerbations. *Eur Respir J*. 2016;47(4):1082-1092. doi:10.1183/13993003.01406-2015

34. Huang YJ, Sethi S, Murphy T, Nariya S, Boushey HA, Lynch SV. Airway microbiome dynamics in exacerbations of chronic obstructive pulmonary disease. *J Clin Microbiol*. 2014;52(8):2813-2823. doi:10.1128/JCM.00035-14

35. Huang YJ, Boushey HA. The Sputum Microbiome in Chronic Obstructive Pulmonary Disease Exacerbations. *Ann Am Thorac Soc*. 2015;12 Suppl 2(Suppl 2):S176-180. doi:10.1513/AnnalsATS.201506-319AW

36. Bafadhel M, Haldar K, Barker B, et al. Airway bacteria measured by quantitative polymerase chain reaction and culture in patients with stable COPD: relationship with neutrophilic airway inflammation, exacerbation frequency, and lung function. I*nt J Chron Obstruct Pulmon Dis.* 2015;10:1075-83. doi:10.2147/COPD.S80091

37. Wang Z, Singh R, Miller BE, et al. Sputum microbiome temporal variability and dysbiosis in chronic obstructive pulmonary disease exacerbations: an analysis of the COPDMAP study. *Thorax*. 2018;73(4):331-338. doi:10.1136/thoraxjnl-2017-210741

38. Beech AS, Lea S, Kolsum U, et al. Bacteria and sputum inflammatory cell counts; a COPD cohort analysis. *Respir Res*. 2020;21(1):289. doi:10.1186/s12931-020-01552-4

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

39. Wilkinson TMA, Aris E, Bourne S, et al. A prospective, observational cohort study of the seasonal dynamics of airway pathogens in the aetiology of exacerbations in COPD. *Thorax*. 2017;72(10):919-927. doi:10.1136/thoraxjnl-2016-209023

40. Damera G, Pham TH, Zhang J, et al. A Sputum Proteomic Signature That Associates with Increased IL-1β Levels and Bacterial Exacerbations of COPD. *Lung*. 2016;194(3):363-369. doi:10.1007/s00408-016-9877-0

41. Wang Z, Maschera B, Lea S, et al. Airway host-microbiome interactions in chronic obstructive pulmonary disease. *Respir Res*. 2019;20(1):113. doi:10.1186/s12931-019-1085-z

42. Desai H, Eschberger K, Wrona C, et al. Bacterial Colonization Increases Daily Symptoms in Patients with Chronic Obstructive Pulmonary Disease. *Annals ATS*. 2014;11(3):303-309. doi:10.1513/AnnalsATS.201310-350OC

43. Tufvesson E, Markstad H, Bozovic G, Ekberg M, Bjermer L. Inflammation and chronic colonization of Haemophilus influenzae in sputum in COPD patients related to the degree of emphysema and bronchiectasis in high-resolution computed tomography. *COPD*. 2017;Volume 12:3211-3219. doi:10.2147/COPD.S137578

44. Tufvesson E, Bjermer L, Ekberg M. Patients with chronic obstructive pulmonary disease and chronically colonized with Haemophilus influenzae during stable disease phase have increased airway inflammation. *Int J Chron Obstruct Pulmon Dis.* 2015;10:881-9. doi:10.2147/COPD.S78748

45. Malvisi L, Taddei L, Yarraguntla A, Wilkinson TMA, Arora AK, the AERIS Study Group. Sputum sample positivity for Haemophilus influenzae or Moraxella catarrhalis in acute exacerbations of chronic obstructive pulmonary disease: evaluation of association with positivity at earlier stable disease timepoints. *Respir Res*. 2021;22(1):67. doi:10.1186/s12931-021-01653-8

46. Taddei L. Airway pathogens detected in stable and exacerbated COPD in patients in Asia-Pacific. *ERJ Open Res.* 2022;8(3):00057-2022. doi: 10.1183/23120541.00057-2022

47. Garcha DS, Thurston SJ, Patel ARC, et al. Changes in prevalence and load of airway bacteria using quantitative PCR in stable and exacerbated COPD. *Thorax*. 2012;67(12):1075-1080. doi:10.1136/thoraxjnl-2012-201924

48. Jacobs DM, Ochs-Balcom HM, Zhao J, Murphy TF, Sethi S. Lower Airway Bacterial Colonization Patterns and Species-Specific Interactions in Chronic Obstructive Pulmonary Disease. Forbes BA, ed. *J Clin Microbiol*. 2018;56(10):e00330-18. doi:10.1128/JCM.00330-18

49. Tangedal S, Nielsen R, Aanerud M, et al. Sputum microbiota and inflammation at stable state and during exacerbations in a cohort of chronic obstructive pulmonary disease (COPD) patients. Singanayagam A, ed. *PLoS ONE*. 2019;14(9):e0222449. doi:10.1371/journal.pone.0222449

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

50. Xue Q, Xie Y, He Y, et al. Lung microbiome and cytokine profiles in different disease states of COPD: a cohort study. *Sci Rep*. 2023;13(1):5715. doi:10.1038/s41598-023-32901-0

51. Eisenhofer R, Minich JJ, Marotz C, Cooper A, Knight R, Weyrich LS. Contamination in Low Microbial Biomass Microbiome Studies: Issues and Recommendations. *Trends Microbiol*. 2019;27(2):105-117. doi:10.1016/j.tim.2018.11.003

52. Kalema N, Boon SD, Cattamanchi A, et al. Oral antimicrobial rinse to reduce mycobacterial culture contamination among tuberculosis suspects in Uganda: a prospective study. *PLoS One*. 2012;7(7):e38888. doi:10.1371/journal.pone.0038888

53. Peres RL, Palaci M, Loureiro RB, et al. Evaluation of oral antiseptic rinsing before sputum collection to reduce contamination of mycobacterial cultures. *J Clin Microbiol*. 2011;49(8):3058-3060. doi:10.1128/JCM.00541-11

54. Galiana A, Aguirre E, Rodriguez JC, et al. Sputum microbiota in moderate versus severe patients with COPD. *Eur Respir J*. 2014;43(6):1787-1790. doi:10.1183/09031936.00191513

55. Garcia-Nuñez M, Millares L, Pomares X, et al. Severity-related changes of bronchial microbiome in chronic obstructive pulmonary disease. *J Clin Microbiol*. 2014;52(12):4217-4223. doi:10.1128/JCM.01967-14

56. Dicker AJ, Huang JTJ, Lonergan M, et al. The sputum microbiome, airway inflammation, and mortality in chronic obstructive pulmonary disease. *Journal of Allergy and Clinical Immunology*. 2021;147(1):158-167. doi:10.1016/j.jaci.2020.02.040

57. Erb-Downward JR, Thompson DL, Han MK, et al. Analysis of the Lung Microbiome in the "Healthy" Smoker and in COPD. Bereswill S, ed. *PLoS ONE*. 2011;6(2):e16384. doi:10.1371/journal.pone.0016384

58. Einarsson GG, Comer DM, McIlreavey L, et al. Community dynamics and the lower airway microbiota in stable chronic obstructive pulmonary disease, smokers and healthy non-smokers. *Thorax*. 2016;71(9):795-803. doi:10.1136/thoraxjnl-2015-207235

59. Ramsheh MY, Haldar K, Esteve-Codina A, et al. Lung microbiome composition and bronchial epithelial gene expression in patients with COPD versus healthy individuals: a bacterial 16S rRNA gene sequencing and host transcriptomic analysis. *Lancet Microbe*. 2021;2(7):e300-e310. doi:10.1016/S2666-5247(21)00035-5

60. Mac Aogáin M, Lau KJX, Cai Z, et al. Metagenomics Reveals a Core Macrolide Resistome Related to Microbiota in Chronic Respiratory Disease. *Am J Respir Crit Care Med*. 2020;202(3):433-447. doi:10.1164/rccm.201911-2202OC

61. Pragman AA, Kim HB, Reilly CS, Wendt C, Isaacson RE. The Lung Microbiome in Moderate and Severe Chronic Obstructive Pulmonary Disease. Taube C, ed. *PLoS ONE*. 2012;7(10):e47305. doi:10.1371/journal.pone.0047305

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

62. Haldar K, George L, Wang Z, et al. The sputum microbiome is distinct between COPD and health, independent of smoking history. *Respir Res*. 2020;21(1):183. doi:10.1186/s12931-020-01448-3

63. Zakharkina T, Heinzel E, Koczulla RA, et al. Analysis of the Airway Microbiota of Healthy Individuals and Patients with Chronic Obstructive Pulmonary Disease by T-RFLP and Clone Sequencing. Hartl D, ed. *PLoS ONE*. 2013;8(7):e68302. doi:10.1371/journal.pone.0068302

64. Avalos-Fernandez M, Alin T, Métayer C, Thiébaut R, Enaud R, Delhaes L. The respiratory microbiota alpha-diversity in chronic lung diseases: first systematic review and meta-analysis. *Respir Res*. 2022;23(1):214. doi:10.1186/s12931-022-02132-4

65. Sze MA, Dimitriu PA, Hayashi S, et al. The Lung Tissue Microbiome in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2012;185(10):1073-1080. doi:10.1164/rccm.201111-2075OC

66. Wang J, Chai J, Sun L, Zhao J, Chang C. The sputum microbiome associated with different sub-types of AECOPD in a Chinese cohort. *BMC Infect Dis*. 2020;20(1):610. doi:10.1186/s12879-020-05313-y

67. Millares L, Pascual S, Montón C, et al. Relationship between the respiratory microbiome and the severity of airflow limitation, history of exacerbations and circulating eosinophils in COPD patients. *BMC Pulm Med*. 2019;19(1):112. doi:10.1186/s12890-019-0867-x

68. Park H, Shin JW, Park SG, Kim W. Microbial Communities in the Upper Respiratory Tract of Patients with Asthma and Chronic Obstructive Pulmonary Disease. Chu HW, ed. *PLoS ONE*. 2014;9(10):e109710. doi:10.1371/journal.pone.0109710

69. Garcia-Nuñez M, Marti S, Puig C, et al. Bronchial microbiome, PA biofilm-forming capacity and exacerbation in severe COPD patients colonized by *P. aeruginosa*. *Future Microbiology*. 2017;12(5):379-392. doi:10.2217/fmb-2016-0127

70. Engel M, Endesfelder D, Schloter-Hai B, et al. Influence of lung CT changes in chronic obstructive pulmonary disease (COPD) on the human lung microbiome. Wilson BA, ed. *PLoS ONE*. 2017;12(7):e0180859. doi:10.1371/journal.pone.0180859

71. Yang CY, Li SW, Chin CY, et al. Association of exacerbation phenotype with the sputum microbiome in chronic obstructive pulmonary disease patients during the clinically stable state. *J Transl Med*. 2021;19(1):121. doi:10.1186/s12967-021-02788-4

72. Barker BL, Haldar K, Patel H, et al. Association Between Pathogens Detected Using Quantitative Polymerase Chain Reaction With Airway Inflammation in COPD at Stable State and Exacerbations. *Chest*. 2015;147(1):46-55. doi:10.1378/chest.14-0764

73. Opron K, Begley LA, Erb-Downward JR, et al. Lung microbiota associations with clinical features of COPD in the SPIROMICS cohort. *npj Biofilms Microbiomes*. 2021;7(1):14. doi:10.1038/s41522-021-00185-9

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

74. Madapoosi SS, Cruickshank-Quinn C, Opron K, et al. Lung Microbiota and Metabolites Collectively Associate with Clinical Outcomes in Milder Stage Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2022;206(4):427-439. doi:10.1164/rccm.202110-2241OC

75. Weiser JN, Ferreira DM, Paton JC. Streptococcus pneumoniae: transmission, colonization and invasion. *Nat Rev Microbiol*. 2018;16(6):355-367. doi:10.1038/s41579-018-0001-8

76. Pragman AA, Knutson KA, Gould TJ, Isaacson RE, Reilly CS, Wendt CH. Chronic obstructive pulmonary disease upper airway microbiota alpha diversity is associated with exacerbation phenotype: a case-control observational study. *Respir Res*. 2019;20(1):114. doi:10.1186/s12931-019-1080-4

77. Li W, Wang B, Tan M, Song X, Xie S, Wang C. Analysis of sputum microbial metagenome in COPD based on exacerbation frequency and lung function: a case control study. *Respir Res*. 2022;23(1):321. doi:10.1186/s12931-022-02246-9

78. Leitao Filho FS, Alotaibi NM, Ngan D, et al. Sputum Microbiome Is Associated with 1-Year Mortality after Chronic Obstructive Pulmonary Disease Hospitalizations. *Am J Respir Crit Care Med*. 2019;199(10):1205-1213. doi:10.1164/rccm.201806-1135OC

79. Millares L, Ferrari R, Gallego M, et al. Bronchial microbiome of severe COPD patients colonised by Pseudomonas aeruginosa. *Eur J Clin Microbiol Infect Dis*. 2014;33(7):1101-1111. doi:10.1007/s10096-013-2044-0

80. Sze MA, Dimitriu PA, Suzuki M, et al. Host Response to the Lung Microbiome in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2015;192(4):438-445. doi:10.1164/rccm.201502-0223OC

81. Simpson JL, Baines KJ, Horvat JC, et al. COPD is characterized by increased detection of *H aemophilus influenzae* , *S treptococcus pneumoniae* and a deficiency of *B acillus* species: Airway bacteria in COPD and controls. *Respirology*. 2016;21(4):697-704. doi:10.1111/resp.12734

82. Ghebre MA, Pang PH, Diver S, et al. Biological exacerbation clusters demonstrate asthma and chronic obstructive pulmonary disease overlap with distinct mediator and microbiome profiles. *Journal of Allergy and Clinical Immunology*. 2018;141(6):2027-2036.e12. doi:10.1016/j.jaci.2018.04.013

83. Adak A, Khan MR. An insight into gut microbiota and its functionalities. *Cell Mol Life Sci*. 2019;76(3):473-493. doi:10.1007/s00018-018-2943-4

84. Wexler AG, Goodman AL. An insider's perspective: Bacteroides as a window into the microbiome. *Nat Microbiol*. 2017;2:17026. doi:10.1038/nmicrobiol.2017.26

85. Lin TL, Shu CC, Chen YM, et al. Like Cures Like: Pharmacological Activity of Anti-Inflammatory Lipopolysaccharides From Gut Microbiome. *Front Pharmacol*. 2020;11:554. doi:10.3389/fphar.2020.00554

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

86. Arora S, Ahmad S, Irshad R, et al. TLRs in pulmonary diseases. *Life Sci*. 2019;233:116671. doi:10.1016/j.lfs.2019.116671

87. Kobayashi S, Fujinawa R, Ota F, et al. A Single Dose of Lipopolysaccharide into Mice with Emphysema Mimics Human Chronic Obstructive Pulmonary Disease Exacerbation as Assessed by Micro-Computed Tomography. *Am J Respir Cell Mol Biol*. 2013;49(6):971-977. doi:10.1165/rcmb.2013-0074OC

88. Larsen JM, Musavian HS, Butt TM, Ingvorsen C, Thysen AH, Brix S. Chronic obstructive pulmonary disease and asthma-associated Proteobacteria, but not commensal *Prevotella* spp., promote Toll-like receptor 2-independent lung inflammation and pathology. *Immunology*. 2015;144(2):333-342. doi:10.1111/imm.12376

89. Di Stefano A, Ricciardolo FLM, Caramori G, et al. Bronchial inflammation and bacterial load in stable COPD is associated with TLR4 overexpression. *Eur Respir J*. 2017;49(5):1602006. doi:10.1183/13993003.02006-2016

90. Horn KJ, Schopper MA, Drigot ZG, Clark SE. Airway Prevotella promote TLR2-dependent neutrophil activation and rapid clearance of Streptococcus pneumoniae from the lung. *Nat Commun*. 2022;13(1):3321. doi:10.1038/s41467-022-31074-0

91. Zhao J, Schloss PD, Kalikin LM, et al. Decade-long bacterial community dynamics in cystic fibrosis airways. *Proc Natl Acad Sci USA*. 2012;109(15):5809-5814. doi:10.1073/pnas.1120577109

92. Gleeson K, Eggli DF, Maxwell SL. Quantitative aspiration during sleep in normal subjects. *Chest*. 1997;111(5):1266-1272. doi:10.1378/chest.111.5.1266

93. Madan JC, Koestler DC, Stanton BA, et al. Serial Analysis of the Gut and Respiratory Microbiome in Cystic Fibrosis in Infancy: Interaction between Intestinal and Respiratory Tracts and Impact of Nutritional Exposures. Ausubel FM, ed. *mBio*. 2012;3(4):e00251-12. doi:10.1128/mBio.00251-12

94. Benjamin JL, Hedin CRH, Koutsoumpas A, et al. Smokers with active Crohn's disease have a clinically relevant dysbiosis of the gastrointestinal microbiota. *Inflamm Bowel Dis*. 2012;18(6):1092-1100. doi:10.1002/ibd.21864

95. Ekbom A, Brandt L, Granath F, Löfdahl CG, Egesten A. Increased risk of both ulcerative colitis and Crohn's disease in a population suffering from COPD. *Lung*. 2008;186(3):167-172. doi:10.1007/s00408-008-9080-z

96. Lee J, Im JP, Han K, et al. Risk of inflammatory bowel disease in patients with chronic obstructive pulmonary disease: A nationwide, population-based study. *World J Gastroenterol*. 2019;25(42):6354-6364. doi:10.3748/wjg.v25.i42.6354

97. Wang L, Pelgrim CE, Peralta Marzal LN, et al. Changes in intestinal homeostasis and immunity in a cigarette smoke- and LPS-induced murine model for COPD: the lung-gut axis.

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

Am J Physiol Lung Cell Mol Physiol. 2022;323(3):L266-L280. doi:10.1152/ajplung.00486.2021

98. Cheng WL, Chang CC, Luo CS, et al. Targeting Lung-Gut Axis for Regulating Pollution Particle-Mediated Inflammation and Metabolic Disorders. *Cells*. 2023;12(6):901. doi:10.3390/cells12060901

99. Li N, Yang Z, Liao B, et al. Chronic exposure to ambient particulate matter induces gut microbial dysbiosis in a rat COPD model. *Respir Res*. 2020;21(1):271. doi:10.1186/s12931-020-01529-3

100. McAleer JP, Kolls JK. Contributions of the intestinal microbiome in lung immunity. *Eur J Immunol*. 2018;48(1):39-49. doi:10.1002/eji.201646721

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

Table 1 The relationship between gut and respiratory microbiome dysbiosis and stable COPD manifestation

Phylum	Genus	Abundance	Clinical manifestations	Inflammation
Bacteroidetes	Nonspecific	(Gut) ↓ [6,15,16]	Lung function↓ [15,16]	/
		(Lung) ↑	N/A	IL-5, IL-13, CCL 13, CCL17 and CCL26; Eosinophils↑ [82]
	Bacteroides	(Gut) ↓ [14]	Lung function↓	Eosinophils↑ [15]
	Prevotella	(Gut) ↑ [6,16]	Lung function↓ [16]	N/A
		(Lung) ↓ [58–60,70]	Lung function↓ [59,73,74]; Alleviated symptoms [74]	N/A
Actinobacteria	Nonspecific	(Lung) ↑	N/A	CXCL10, CXCL11 and IFN-γ [82]
Firmicutes	Nonspecific	(Gut) ↑ [6,16]	Lung function↓ [16]	
	Nonspecific	(Lung) ↑	N/A	CXCL10, CXCL11 and IFN-γ [82]
	Streptococcus	(Gut) ↑ [14]	Lung function↓ [14,16]	N/A
		(Lung) ↑ [59,73,74]	Lung function↓ [73,74]; Aggravated symptoms [42,74]	IL-8 [33]; Eosinophils↑ [56]
Proteobacteria	Nonspecific	(Lung) ↑ [62]	Lung function↓ [56,71]	IL-1β, IL-6, TNF-α and VEGF; Neutrophils↑; Eosinophils ↓ [41,56]
	Haemophilus	(Lung) ↑ [41,60,62]	Lung function↓ [36,44] Aggravated symptoms [42]	IL-1β, IL-8, IL-10 and TNF-α; Neutrophils↑ [33,36,38,41,42,44,72,81]
	Moraxella	(Lung) ↑ [41,59,62]	Aggravated symptoms [42]	IL-1β, IL-8, IL-10 and TNF-α; Neutrophils↑ [33,42,59,62,72]
	Pseudomonas	(Lung) ↑ [58,60,67–69]	Lung function↓ [67] Aggravated symptoms[42]	IL-8[42]

N/A: Currently no evidence available

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

Table 2 The relationship between gut and respiratory microbiome dysbiosis and AECOPD manifestation

Phylum	Genus	Abundance	Clinical manifestations	Inflammation
Actinobacteria	Nonspecific	(Gut) ↓ [25]	Lung function ↓ [25]	IL-6, IL-8, TNF-α, PCT and CRP [25]
		(Lung) ↓ [26,33–35,77]	N/A	N/A
Firmicutes	Nonspecific	(Gut) ↓ [25]	Lung function ↓ [25]	IL-6, IL-8, TNF-α, PCT and CRP [25]
		(Lung) ↓ [26,33–35,77]	N/A	N/A
	Veillonella	(Lung) ↓ [37,77]	Mortality↑ [78]	N/A
Proteobacteria	Nonspecific	(Lung) ↑ [26,33–35,77]	Mortality↑ [56]	N/A
	Haemophilus	(Lung) ↑ [26,33,35,39,46,47,76,79]	Risk of exacerbations↑ [45,46,76]	N/A
	Moraxella	(Lung) ↑ [26,33,39,46,47,76,77,79]	Risk of exacerbations↑ [45,46,76]	IL-1β, IL-8, IL-10 and TNF-α Neutrophils↑ [33,41,72]

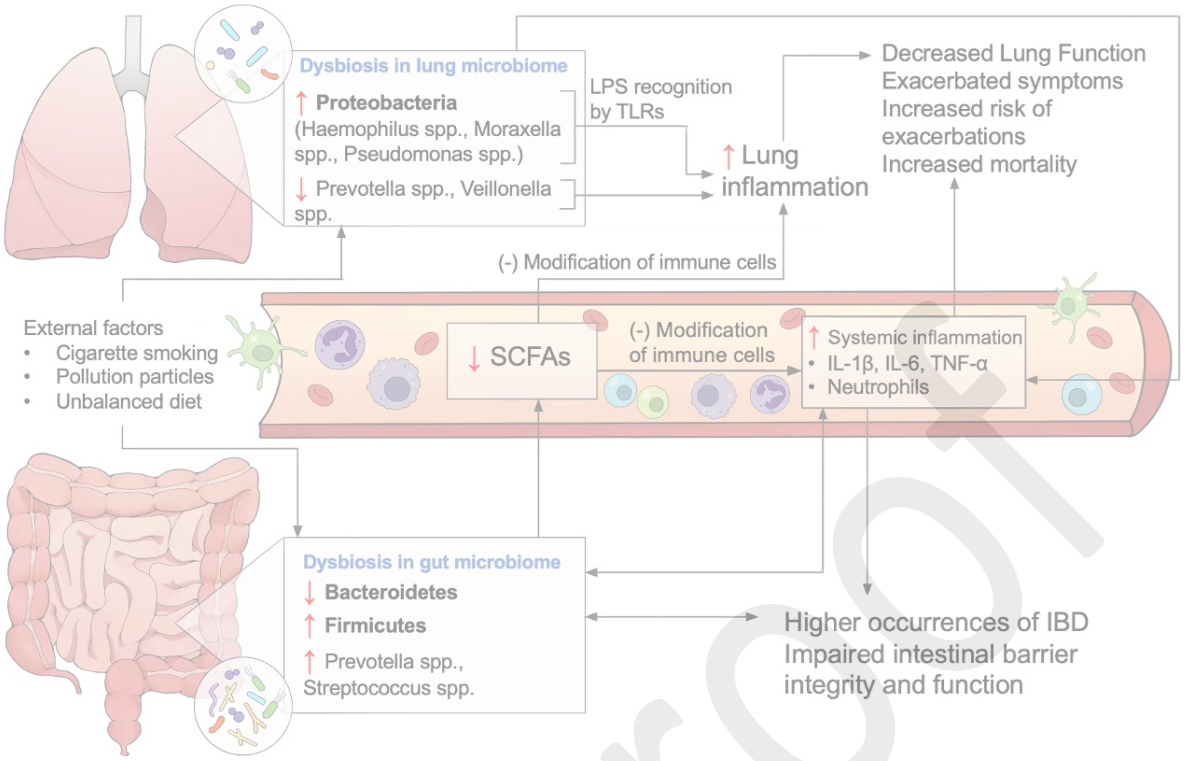
N/A: Currently no evidence available

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

Figure 1 Role of gut and respiratory microbiome dysbiosis in the pathogenesis of COPD

Gut and respiratory microbiome dysbiosis can be triggered by external factors such as smoking, pollution particles, and an unbalanced diet. When Proteobacteria (including *Haemophilus*, *Moraxella* and *Pseudomonas*) become dominant in the lung, the bacterial surface structure lipopolysaccharide (LPS) can intensively activate proinflammatory responses by binding with Toll-like receptors (TLRs), hence worsening inflammation in the lungs and throughout the body. This leads to the development of COPD-related symptoms in both the lungs and intestines and affects the gut microbiota. Changes in the gut microbiome (particularly decreased Bacteroidetes) can lower the production of protective short-chain fatty acids (SCFAs) that normally modify systemic and lung immunity, creating a vicious cycle of inflammation. It is worth noting that the abundance of *Prevotella* varies depending on its location and has contradictory correlations with lung function, suggesting that there are diverse host-microbial interactions and potential transfers of microbiota.

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

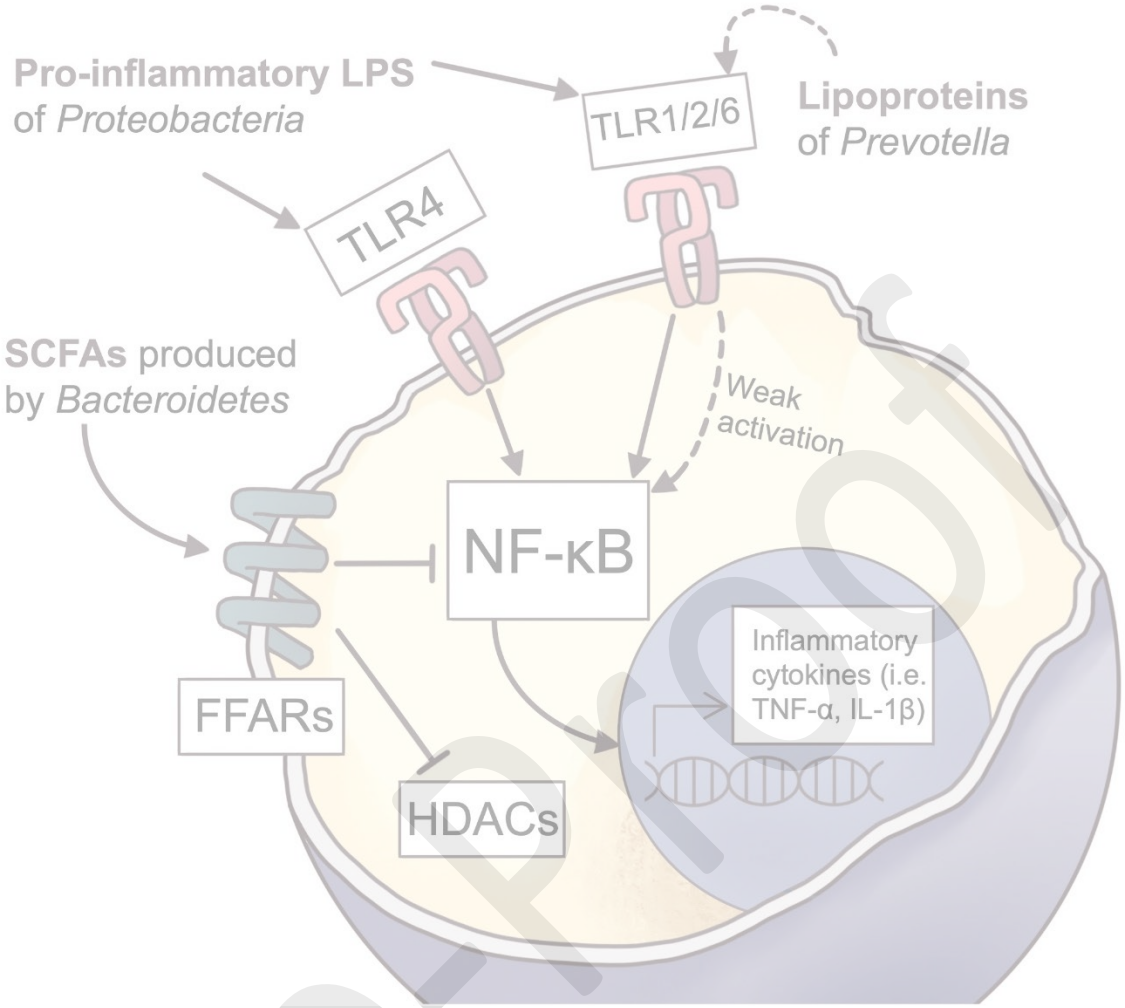


Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442

Figure 2 The epigenetic regulation of immune cells by gut-lung microbiota

(1) Bacteroidetes generate short-chain fatty acids (SCFAs) that interact with free fatty acid receptors (FFARs) present on immune cells. This interaction results in the inhibition of histone-deacetylase complexes (HDACs) and the suppression of nuclear factor-kappa B (NF-KB), thereby eliciting anti-inflammatory responses. (2) Conversely, the proinflammatory lipopolysaccharide (LPS) produced by Proteobacteria can stimulate toll-like receptors (TLRs), triggering the activation of the transcription factor NF-KB. This activation subsequently induces the expression of genes that orchestrate the production of proinflammatory cytokines, culminating in systemic and pulmonary inflammation. (3) Lipoproteins of *Prevotella* exhibit the capacity to bind with TLR2, albeit inducing only mild activation of NF-KB. This subdued response prevents lung pathology, allowing the host immune system to tolerate the presence of this genus and potentially reap beneficial effects.

Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442



Published online April 1, 2024 https://doi.org/10.15326/jcopdf.2023.0442