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Review

The Gut–Lung Axis, Epigenetics and Respiratory Disease

Alex Seungyeon Byun[1], Philip Chi Lip Kwok[1], Hak-Kim Chan[1], Luis Vitetta[1,*]

[1]School of Pharmacy, Faculty of Medicine and Health, University of Sydney, Sydney, NSW 2006, Australia
*Correspondence: luis.vitetta@sydney.edu.au (Luis Vitetta)
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Abstract

The first and second phases of the human microbiome project provided a view of mucosal surfaces and the skin of humans that mapped an abundant and complex ecosystem (microbiota) that is composed of bacteria (bacteriobiota), fungi (mycobiota), viruses (virobiota), enteric phages, archaea, protists, and helminths. Intestinal dysbiosis describes an adverse shift in microbial homeostasis in the gut that enhances intestinal epithelial permeability, translocating toxins that may lead to endotoxemia. Numerous intestinal and extra-intestinal illnesses have been linked to gut dysbiosis, including inflammatory bowel disease, infections, food allergies, asthma, diabetes, obesity, multiple sclerosis, autism, periodontitis, and colorectal cancer. The gut-lung axis is a bidirectional communication network between the lungs and the intestines mediated by bacterial elaborated products (e.g., butyrate), immune cells and neural pathways influencing health and disease at both sites. This review has focused on the gut-lung axis and the role that probiotics, prebiotics and postbiotics may play on the amelioration of respiratory symptoms that may result from viral and/or bacterial lung infections. Clinicians have for some time focused on treating inflammatory lung disorders such as asthma and chronic obstructive pulmonary disease by encouraging beneficial effects on the intestinal microbiome through the gut–lung axis with orally administered probiotics and pre- and/or postbiotics. The purpose is to restore gut microbial homeostasis. Developing novel delivery platforms to administer probiotics directly to the airways or as adjunctive systemic modulators is a plausible and increasingly supported hypothesis, with careful strain selection, formulation to preserve viability, targeted delivery, and rigorous safety and efficacy testing before clinical use. It is posited that such adjunctive treatments may significantly influence the lung microbiota epigenome by positively impacting the balance of microorganisms within the lung, restoring eubiosis and consequently health.

Keywords: microbiota; dysbiosis; probiotics; prebiotics; butyric acid/short-chain fatty acids; respiratory tract infections; asthma; chronic obstructive pulmonary disease; cystic fibrosis; intestinal permeability; immunology

1. Introduction

The intestinal and lung mucosal surfaces share structural similarities, a consequence of their embryonic origin [1]. The initial phases of the human microbiome project (HMP) revealed a diverse and intricate system of bacteria, fungi, viruses, phages, archaea, protists and helminths inhabiting human mucosal surfaces and skin [2,3]. Consequently the "microbiota" ecosystem has progressed research in microbiome-host interactions in health and disease [4], that has focused on the gut-brain axis [5], gut-skin axis [6], gut-liver axis [7], gut-lung axis [8] and gut-joint axis [9].

Genome investigation technologies such as high-throughput sequencing have demonstrated substantial intra-individual microbiome variation (i.e., at the phylum level) at different anatomical sites (Fig. 1, Ref. [2,10]). In addition, studies have shown inter-individual variations at the same anatomical sites as reported by different investigative groups [2,11–14]. This has been achieved by employing a competitive response with pathogens (e.g., *Clostridium perfringens*), through niche exclusion [15], the production of antimicrobial peptides, participation in metabolism and energy production [16], bile salt metabolism, short chain fatty acids (SCFAs) production and utilization, synthesis of vitamins, neurotransmitters, as well as xenobiotic degradation [17]. Accordingly, the intestinal microbiome has been reported to exert significant influence on the host's health [18] from birth prompting the development of the immune system to achieve immunological and metabolic tolerance [19].

Therefore, a posit has been advanced that there are a multitude of intestinal and extra-intestinal illnesses linked to gut dysbiosis [20–22] related to an overgrowth of pathobionts [23]. Pathobionts are commensal microbes that may cause disease when subject to environmental or host pressure [23]. These can include inflammatory bowel diseases (IBD), infections, food allergies, asthma, diabetes, obesity, multiple sclerosis, autism, periodontitis, and colorectal cancer [24]. The narration for this review will focus on the gut-lung axis, bacteria-based epigenetics, and the role that functional foods (e.g., probiotics, prebiotics) may have on the mitigation of respiratory symptoms that may ensue from viral and or bacterial infections of the lung. Furthermore, the exploration of a novel delivery platform for the administration of heat-killed probiotics as adjunctive medicines for the management of respiratory infections is advanced.

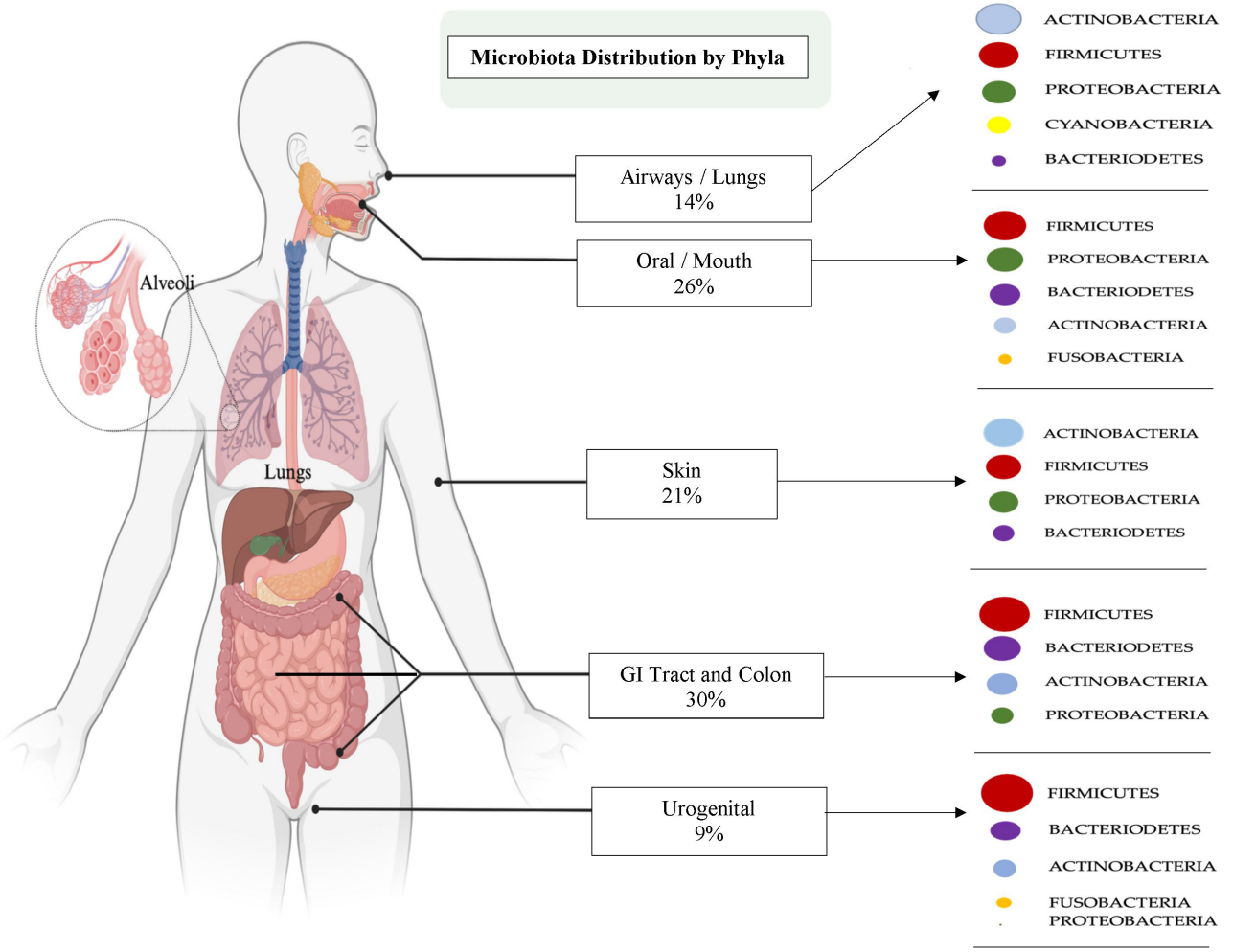

Fig. 1. Human microbiota/microbiome distribution across anatomical sites displaying microbial phyla with percentage allocations in different structural sites. [Adapted from Cho and Blaser (2012)] [2] and Ziki Ma *et al.* [10]. Figure created in BioRender.com by A Byun and L Vitetta 2025.

2. Epigenetics and the Microbiota

Epigenetic regulation encompasses multiple mechanisms that includes DNA modifications that describe stable changes to cellular functions [25]. The biochemical signals that control DNA-protein interactions progress phenotypic changes without producing genetic mutations in the infrastructure of the nucleotide sequence [26–28]. Epigenetic marks are superimposed on the genome that then shapes transcriptional actions and the cellular identity [28,29]. Epigenetic modifications can also include acetylation, methylation, phosphorylation, and ubiquitination and constitute a dynamic regulatory layer that controls chromatin structure and thereby gene expression. Histone acetylation is a central and well-characterized epigenetic event governed by the opposing activities of histone acetyltransferases (HATs), which add acetyl groups to lysine residues on histone tails, and histone deacetylases (HDACs), which remove the acetyl groups. Acetylation neutralizes positive charges on histones, relaxes nucleosome packing and increases accessibility of the transcriptional machinery, promoting gene activation. Conversely, deacetylation restores chromatin compaction and contributes to transcriptional repression. Moreover, the HATs–HDACs balance is highly context dependent and integrates upstream signaling, metabolic state, and developmental cues to produce cell-type specific transcriptional programs. Perturbations of this balance are implicated in diverse lung pathologies including cancer and immune disorders because they can broadly rewire gene networks controlling proliferation, differentiation, apoptosis, and inflammatory responses [25,28] (Fig. 2).

Clinical knowledge of epigenetics in lung diseases is further complicated through the reduced activity of histone deacetylases (HDACs) [30] the enzymes that remove acetyl groups from histone proteins, increasing chromatin compaction and reducing accessibility to the transcriptional machinery [31]. It is reported that by tightening DNA–histone interactions, HDAC activity represses gene tran-

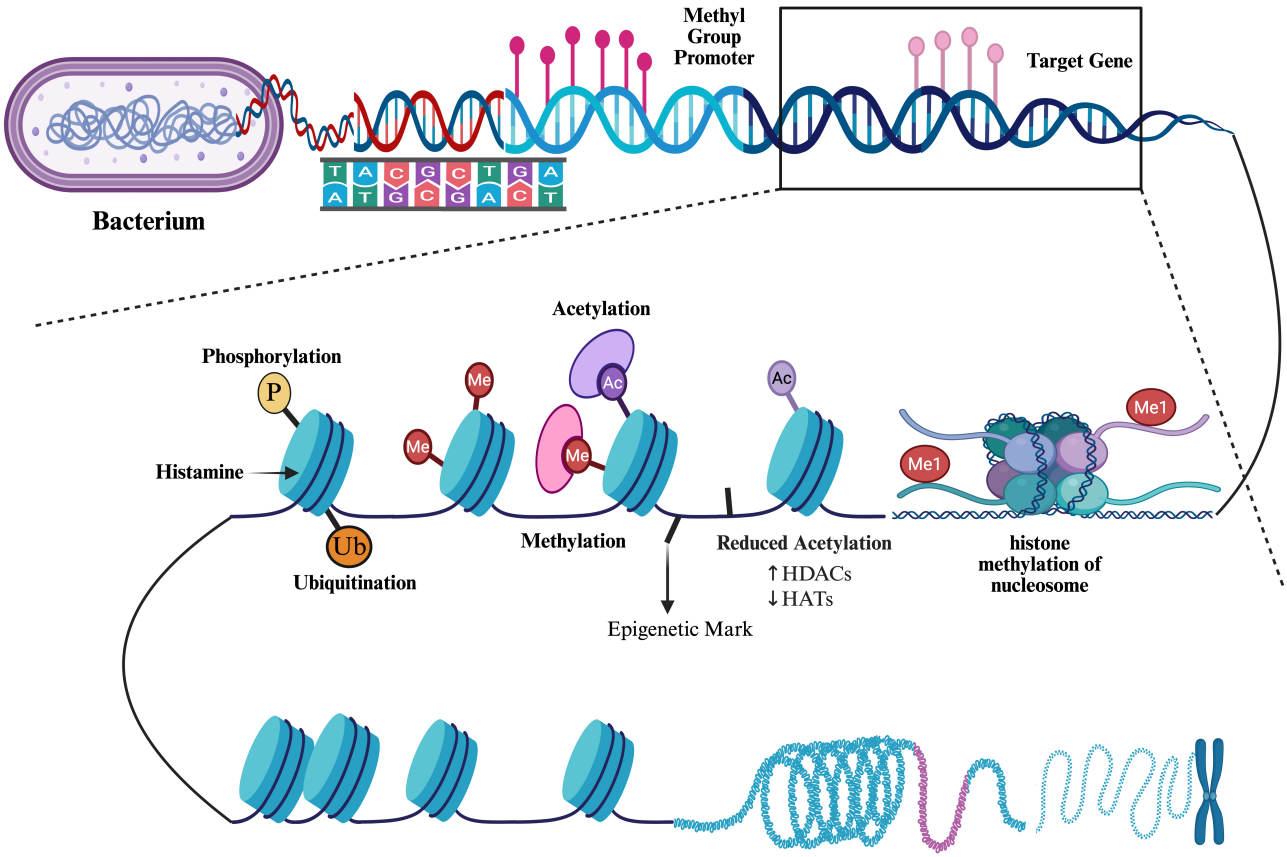

Fig. 2. Diagrammatic representation of bacterial regulatory epigenetic modification mechanisms. [Created in BioRender.com by Vitetta L 2025].

scription and thereby influences fundamental cellular processes such as proliferation, differentiation, apoptosis, and stress responses [31]. Furthermore, HDACs are implicated in numerous diseases, notably cancer, where altered acetylation dynamics contribute to dysregulated growth and survival pathways [32]. Whereas, histone acetyltransferase (HAT) activity acetylates histone lysine, relaxing the chromatin structure and facilitating transcription of proinflammatory genes in the airways [33]. In asthma, upregulated HAT activity skews the epigenetic balance toward enhanced expression of cytokines, chemokines, and other mediators that drive airway inflammation and hyperresponsiveness [33]. Inhaled corticosteroids exert part of their anti-inflammatory effect by restoring acetylation balance, in part through suppression of HAT-driven transcriptional activation. Additional therapeutic strategies therefore aim either to restore physiological acetylation dynamics (e.g., employing HDAC inhibitors or HAT modulators) or to target specific acetylation-dependent pathways, with the goal of achieving selective modulation of pathogenic transcriptional states while minimising off-target effects.

Notwithstanding, DNA methylation comprises the most pervasive active mechanism serving as a fundamental biochemical action that controls gene regulation [34,35]. Several epigenetic mechanisms influence genetic processes such as DNA modifications (e.g., CpG methylation and demethylation), histone modifications (e.g., acetylation and deacetylation), and RNA that integrate gene regulatory networks with environmental cues [35]. Of these DNA methylation comprises the most stable modification that can persist following cell divisions to the next generation. DNA methyltransferases (DNMTs) are indispensable enzymes whose activities establish and maintain methylation biochemical processes that overall ensure genome fidelity and continuance of epigenetic regulation [35,36].

Epigenetic-like DNA, RNA/microRNA methylations and histone modifications significantly influence gut bacterial cellular functions such as metabolism, bacterial persistence, replication cycles, bacteriophage susceptibility, virulence and genome plasticity [37–41]. Methylation reactions in bacteria have been reported to commonly target N6-adenine and C5-cytosine residues [40,41], and with N4-cytosine methylation also observed in many bacterial taxa [40]. Beyond classical methylation reactions reported, novel DNA modifications such as phosphonothioate based alterations [42] such biochemical reactions influence cellular redox potential status [43] thereby expanding the repertoire of heritable, non-sequence-based regulatory entities [42]. These modifications, together with RNA methylation, can produce stable, heritable phenotypes that alter gene ex-

pression without changes to the genome sequence of bacterial species. Moreover, these post-replicative modifications [43] enable rapid, reversible tuning of gene expression in response to local environmental pressures. Such responses allow bacteria to adapt growth, survival/persistence, and pathogenic strategies on a timescale that is more rapid than those through *de novo* mutations. This adaptive plasticity links bacterial DNA/RNA modification systems to organismal functions and, increasingly to disease processes in mammalian hosts.

Epigenetic changes can have a significant impact on the balance of microorganisms in any anatomical site. The interplay between the microbiome and the epigenome is intricate and mutually influential, especially in the gut [44]. In the lungs a dysbiotic microbial environment may contribute to the development and progression of lung diseases [45]. Modulation of the microbiota in the intestines through epigenetic changes that can be provoked by probiotics, prebiotics, synbiotics SCFAs, and micronutrients [44,46] have been postulated to represent potential therapeutic strategies that protect against lung diseases [44,45].

Many bacterial species are subject to epigenetic changes describing DNA modifications as gene biochemical regulatory actions. That is where epigenetic signals control DNA–protein interactions and can cause phenotypic changes in the absence of a mutation. A recent review [47] appropriately summarized how epigenetic DNA methylations in bacteria can protect bacterial genomes, promote chromosome replication and segregation, and nucleoid organization, and control bacterial cell cycles as well as repair bacterial genome DNA and regulate transcriptional activities. Moreover, DNA methylation has been shown to control the reversible switching of gene expression [48]. This versatile action is a phenomenon that generates phenotypic cell variants [49]. Hence, the development of epigenetic bacterial lineages is important as it facilitates the adaptation of bacterial populations to severe or changing environmental conditions and modulates the interaction of prokaryotic pathogens with their eukaryotic hosts [34,49].

Reports cite epigenetic regulation and control as being increasingly recognized as a potent mechanism through which the microbiota influence host physiology occurring through multiple potential mechanisms [44,47]. That is, in addition to environmental inputs (e.g., nutrition), epigenetic control includes (i) microbial biosynthesis or metabolism which influence the availability of chemical donors for DNA methylation, histone modifications or chromatin remodelling; (ii) regulation of epigenetic-modifying enzyme expression and/or activity; or (iii) activation of host cell intrinsic processes that direct epigenetic pathways such as microRNA pathways [47].

Research on the benefits of probiotic bacteria from major genera such as *Lactobacillus* has progressed from observational and interventional studies to the identification of the underlying molecular mechanisms that exist [34]. A regulatory mechanism of chromatin structure and gene expression is histone acetylation. Recent *in vitro* studies that have explored gene expression reported that probiotic bacteria such as *Lactobacillus rhamnosus* and *Lactobacillus fermentum* modulate host epigenetic signatures of intestinal epithelial cells through global histone acetylation independent of the recruitment of transcriptional activators and via *Escherichia coli* challenge [50].

A recent comprehensive screening investigation of existing datasets with chronic lung diseases utilising data from biological samples has further emphasised the importance in deciphering the relationship between the lung microbiome, epigenetics and its modifications in the prevention of chronic pulmonary disease or its progression [51]. An appreciation of the role of the lung microbiome in maintaining local eubiosis is recognized. Epigenetic microbial alterations can impact and skew the lung microbial cohort toward dysbiosis, significantly contributing to the development and progression of lung diseases [51].

Pseudomonas is a bacterial pathobiont that can cause respiratory infections in individuals with compromised immune systems [52]. As it occurs in severe asthma, cystic fibrosis or chronic obstructive pulmonary disease (COPD). *P. aeruginosa* is an opportunistic pathogen that frequently infects the lungs, particularly in immunocompromised patients. In individuals with cystic fibrosis, it colonises the thickened mucus lining of the airways. In the lungs the mucus provides a supportive, nutrient-rich environment for the bacterium's survival without disturbing the local mucosal airway epithelial cell scaffold [53]. The persistent presence of this pathobiont in the respiratory tract of the lungs can lead to disruption of the composition of the respiratory microbiome. This disturbance results in changes to its normal functionality increasing susceptibility to respiratory infections [54]. In cystic fibrosis, changes in the α-diversity of the lung microbiota are consistently linked to lung function [55]. *Pseudomonas* can impact the epigenetic regulation of host cells [56,57]. The *in vitro* study by Kyung Lee *et al.* [56] demonstrated that bacteria can alter DNA methylation of the host epigenome. The study concluded that changes in DNA methylation in distal DNA regulatory regions could lead to modulating cellular gene expression and potential downstream cellular processes, contributing to the development and progression of respiratory disease.

3. Crosstalk in the Gut–Lung Axis

It is generally accepted that the lungs and the gut are two independent systems that originated from one common embryonic organ, the foregut [58]. These two distinct anatomical sites share an embryological origin that structurally contain mucosa-lined luminal surfaces that show specific anatomic and biochemical features [59], resulting in markedly different microbial communities and immune system interactions [60]. Currently emerging research shows that there is a gut-lung axis that very much

allows for bidirectional crosstalk interactions between the intestinal microbiota and the lung microbiota. The lungs and intestines, with large surface areas exposed to the external environment, are subject to immune homeostasis that determines continued health [61]. This proposition is dependent on a varied and balanced microbiome that includes microbial components and metabolites (e.g., SCFAs) that are important for the priming, maturation, and metabolic equilibrium of the immune system in both anatomical sites [62].

Environmental factors such as diet, psychosocial stressors, physical activity, and the use of prescribed antibiotics significantly affect the stability and composition of the gut and lung microbiomes [63–65]. Dysbiosis of the intestinal microbiome has been extensively studied and the data from association studies show that changes in the abundance and evenness of the gut microbiome can affect the susceptibility to diseases in distant organs, as so happens with pulmonary diseases. Inflammatory diseases of the gut (e.g., IBD), especially in patients diagnosed with ongoing IBD and irritable bowel syndrome (IBS), present a higher prevalence of pulmonary disease. Intestinal microbiome adverse changes have been reported to occur in various chronic lung disorders, such as asthma [66], COPD [67], and cystic fibrosis (CF) [68]. Moreover, the prescription of antibiotics in early life has been implicated [69] and reported to significantly increase the risk of developing atopy and specifically exacerbating asthma [70], and subsequently correlated to a reduced abundance of gut bacteria [71]. The intestinal microbiota also participates in a protective role against common viral and pulmonary infections [72], through the regulation of the innate and adaptive immune responses [73].

The relationship between the intestinal cohort of bacteria and common respiratory diseases involves gut derived metabolites such as SCFAs that stimulate protective mechanisms by increasing macrophage/dendritic cell progenitor cells [46]; as well as being directly involved in modulating the production of lipopolysaccharides (LPS). The bacterial metabolites SCFAs have key roles in the migration of immune cells via the systemic circulation, priming myeloid cells in the bone marrow promoting haematopoiesis, thereafter, migrating to the lungs supporting an anti-inflammatory environment [46].

The lungs are populated with alveolar macrophage progenitor cells that activate Th2 effector cells, which mediate immune responses through the production of local cytokines [46]. In essence, the changes in the abundance of intestinal bacteria that affects gut bacterial metabolites is linked to changes in immune responses that progress pro-inflammatory sequelae.

The host–microbe connections underpin the bidirectional crosstalk that exists between the intestines and the lungs involving physiological and pathological connections [74]. The pulmonary cohort of bacteria has been reported to modulate microbial communities in the intestines with a subsequent influence on intestinal signaling [75]. In the lungs, infections such as *Mycobacterium tuberculosis* have been reported to dysregulate the immune system, resulting in alteration in the intestinal microbiome. Studies with patients diagnosed with tuberculosis (TB) versus healthy controls the phyla of *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia* were reduced in abundance whereas the *Actinobacteria*, *Bacteroidetes*, and *Fusobacteria* phyla expressed an increased abundance [76]. In patients with *de novo* infections or recurrent TB infections the changes in gut bacterial cohort showed decreased abundance in *Bacteroidetes*, the genus *Prevotella* and *Lachnospira* and enrichment in Actinobacteria and Proteobacteria [77]. The dysbiosis reported provides evidence for a disequilibrating in the production of microbial elaborated metabolites such as SCFAs. In a reverse intestine to lung cross-talk study it was shown that lung colonisation in the intensive care unit was driven by the translocation of *Pseudomonas aeruginosa* from the intestines [78].

3.1 Asthma

Alterations in the gut microbiome in patients with asthma have been observed. Current evidence points to the existence of the gut-lung axis from birth as the microbial composition affects the risk of developing asthma in infants [79]. In the infant gut microbiome, a significant increase in α-diversity and the greatest change in β-diversity was observed from the period between one month to one year of life [79]. Alpha-diversity describes the variety and distribution of the microbiome within a single sample whilst β-diversity compares the composition between different samples or communities [80]. Although the α-diversity was not associated with an increase in asthma risk at 5 years of age, β-diversity at one year was significantly different between children with asthma at five years compared to those without asthma [79]. Such differences in the composition of the gut microbiome may affect the balance of SCFAs that have a protective effect against asthma in childhood [81]. This outcome supports the finding in the Protection against Allergy: Study in Rural Environments (PASTURE) study where asthma in children at school age was inversely associated with the levels of faecal butyrate, bacterial taxa that predict butyrate production, and the relative abundance of gene encoding butyryl-coenzyme A:acetate-CoA-transferase [82]. However, the mechanisms in which the gut microbiome influences the disease outcomes have been insufficiently understood. A recent study by Burrows *et al*. [83] suggests that a gut commensal protozoan, *Tritrichmonas musculis* (*T.mu*), remotely modifies the pulmonary immune system through the activation of a tripartite network of Group 2 innate lymphoid cells, B cells, and T cells. This activation promotes eosinophilia, consequently exacerbating asthma and hence could act as a future biomarker for assessing disease severity of therapeutic efficacy [83].

In adult patients with mild-moderate asthma with well-controlled symptoms, strong associations between the forced expiratory volume in 1 second (FEV_1) and the differences in gut microbiota compositions at the phylum level were observed [84]. The ratio of *Bacteroidetes* to *Firmicutes* relative abundance was lower in subjects with asthma [84] which was in contrast to the findings by Wang *et al.* [85], who reported no significant differences at the phylum level between non-severe asthma, severe asthma, and healthy patients. These differences may be due to variation in the environmental exposure of the patients in different geographical locations. Unlike the intestines, the lungs are anatomically exposed to the external environment as it is the passage for continuous breathing. An analysis of the lung microbiota of 162 patients with chronic respiratory conditions across different care centres in the United Kingdom revealed that there were significant differences in the β-diversity [86]. This highlights that variation in the geographical location, even within the same country can uniquely influence the composition of the lung microbiota. Participants recruited in Michigan [84] and Guangzhou [85] may also be exposed to different levels and composition of air pollutants which can affect the abundance and diversity of the lung [87] and gut microbiome [88].

Despite the discrepancies in the current studies, the data suggests that the differences in the microbiota gut compositions were associated with specific clinical respiratory presentations [84,85]. Both studies [84,85] have found that the asthmatic group had a higher proportion of bacteria from the *Lachnospiraceae* family than the non-asthmatic group. Furthermore, separating asthmatic patients according to their gut bacterial community structures revealed that the microbiota compositions were associated with lung function [84]. One particular group with the lowest community diversity but with high *Prevotella* abundance presented low levels of lung function [84]. Higher relative abundances of *Prevotella* in the gut may be associated with a more severe clinical presentation of asthma [85]. Again, contradicting results were reported by Sampaio *et al.* [89] where *Prevotella* was more abundant in patients without asthma compared to those with type 2 inflammatory asthma. However, it should be noted that the sample size of this study was very small (n = 28 asthma, n = 29 control) and consisted of children and young adults [89].

The transient nature of the lung microbiota due to constant environmental exposure may inevitably affect the gut and hence it is difficult to conclusively link the phenotypic symptoms of asthma with the diversity of the gut microbiota. It is well recognised though that intestinal dysbiosis has been significantly linked to asthma development, with severity linked to the gut-lung axis, affecting immune system maturation, promoting inflammation, and increasing allergies [90]. Moreover, low diversity of specific beneficial bacteria (i.e., *Bifidobacterium*, *Faecalibacterium*, *Roseburia*) may be linked to protective effects against asthma outcomes [90], while certain microbial imbalances increase asthma risk [91]. This connection suggests that early-life microbial exposure and restoring the intestinal microbial balance with probiotics or other interventions could better help manage asthma.

3.2 Chronic Obstructive Pulmonary Disease (COPD)

Pulmonary involvement in IBD is often reported in the literature [67,92–95] and possible reasons include the presence of chronic inflammation in the intestines which in turn affect the lungs, possible due to the common embryonic origins that the lungs and intestines share [67]. An association between new onset IBD and COPD reflects microbiome dysbiosis and intestinal endothelial barrier dysfunction that may occur in the gut of patients with COPD [93]. However, despite the bidirectional nature of the gut-lung axis, no reciprocal association of increased risk of COPD in IBD patients was observed as reported in a systematic review [93]. When patients were assessed for a more general obstructive lung disease (OLD), there was a 60% increased risk for individuals with new onset IBD [92]. The risk of OLD diagnosis further increased by 40% after IBD diagnosis for all age groups [92]. These results support the outcome that induced colitis in mice was causal for lung damage [94].

To determine whether a dysfunction in the gut microbiota affects the lungs, mice were treated with specific antibiotics, such as vancomycin and ampicillin, two weeks prior to COPD induction [96]. Antibiotic treatment reduced the extent of COPD pathogenesis, indicating that the composition of the gut microbiota may result in COPD development [96]. To further explore this implication, the gut microbiota of patients with different severity of COPD were compared [97]. Over the course of one year, patients with declining lung function (reduction in FEV_1) were more abundant with *Firmicutes* [97]. Upon comparing patients with and without COPD, no significant differences in the relative abundances of the 20 most prevalent genera were observed [98]. However, COPD patients had differences in some of the least abundant taxa where there was a reduction in *Veillonella* genus and an increase in an unclassifiable genus from the *Clostridia* class [98]. Separately, species such as *Streptococcus sp000187445*, *S. vestibularis* were found to negatively correlate with FEV_1 [99]. Furthermore, COPD-associated members of *Lachnospiraceae* was inversely related to the predicted forced vital capacity (FVC) and FEV_1, whilst *Desulfovibrio piger_A*, *CAG-302sp001916775* positively related to the lung function [99].

COPD often involves emphysema and in patients with computed tomography-verified emphysema had significantly reduced bacteria from the *Lachnospiraceae ND3007* group and *Eubacterium halli* [98]. These groups produce SCFAs which progress and maintain intestinal homeostasis [100]. It is possible that a decrease in SCFA production led to the dysregulation of inflammation. SCFAs, especially

propionate, can inhibit IL-17 production by intestinal $\gamma\delta$ T cells [101] which is an interleukin often overexpressed in patients with IBD [102]. Unexpectedly, acute exacerbations in COPD did not affect the α-diversity of the gut microbiota compared to COPD patients without acute exacerbations [103]. However, this result may be due to the treatment of COPD exacerbations with antibiotics which affect the richness of the gut microbiota. Prophylactic antibiotics treatment led to a decrease in α-diversity but not β-diversity in the lung microbiome in COPD patients [104]. However, β-diversity was shifted in the acute exacerbations group, represented by an increase in the relative abundance of *Firmicutes* and *Bacteroidetes* due to increases in *Lachnoclostridium*, *Alistipes*, *Streptococcus* and *Prevotella* compared to the non-exacerbation group [103]. These distinctive increases in the aforementioned genera may be used as an early indication for acute exacerbation in COPD [103]. However, currently, there is difficulty in establishing the causality relationship between COPD and gut microbiota due to the interpersonal variability between the analysed datasets [99].

What is of clinical importance with COPD is intestinal dysbiosis [105]. An imbalance of intestinal microbes is closely linked to COPD through the gut-lung axis where gut bacteria and their metabolites (e.g., butyrate) influence lung inflammation, potentially improving or worsening COPD. Furthermore, lung issues can alter the intestinal microbiota exacerbating lung diseases. In patients diagnosed with COPD patients that express intestinal dysbiosis often exhibit changes such as lower levels of *Bacteroidetes* and higher levels of *Firmicutes*, that result in increased gut permeability and inflammatory sequelae that affects the lungs via immune cell activities and circulating substances [105,106]. These results stress the importance of dietary intake (e.g., fiber, fermented foods) and the administration of probiotics as potential management strategies.

3.3 Cystic Fibrosis

Gastrointestinal (GI) symptoms and gut microbiota dysbiosis are observed in patients with cystic fibrosis (CF). These differences in the composition of the intestinal microbial communities are observed from early childhood [107,108]. The diversity of the microbiome composition plateaus at around 2 years of age for children with CF (cwCF) [108]. Generally, decreased relative abundance and diversity of GI microbiome in children [107,108] is continued as adults [109,110]. Significant increases in *Firmicutes* and *Actinobacteria* were observed in the gut of adults with CF (pwCF) whilst there was a decrease in *Faecalibacterium*, *Roseburia*, and *Bifidobacterium*, groups considered to be 'healthy gut bacteria' [109]. Enrichment of *Adlercreutzia*, *Ruminococcaceae*, *Lachnospiraceae*, *Tyzzerella*, and *Candidatus soleaferrea* in paediatric CF gut microbiota were directly associated with FEV_1 [107]. CF inflammatory marker calprotectin positively correlated with *Acidaminococcus*, *Allisonella*, *Eubacerium coprostanoligenes* group, *Howardella*, *Lachnospiraceae UCG-010*, *Mogibacterium*, *Olsenella*, *Sutterella*, uncultured *Lachnospiraceae*, and uncultured *Porphyromonadaceae* [107]. *Fusobacterium*, which has been linked to colorectal cancer (CRC) [111–113] was more abundant in cwCF [107] but not in pwCF [114]. A possible reason for its absence in adults may be due to the continual development of the infant microbiome with aging. Price *et al*. [108] reported that that majority of the taxa changed in relative abundance to shift towards "a healthy-like" microbiome with age. However, genera such as *Prevotella_7*, *Akkermansia*, *Bifidobacterium* and *Blautia* increased to resemble "a CF-like" microbiome [108].

Nonetheless, the risk of colorectal cancer in pwCF was elevated [115], despite the cluster distinction of the gut microbiome of pwCF from CRC and healthy samples [114]. In addition to CF-driven microbiota changes, the greater exposure to antibiotics for pwCF may further account for these differences. Early antibiotic exposure can have a significant long-term impact on the gut microbiota [108]. Within the cwCF, as expected, children who used antibiotics had a decreased α-diversity 1-month after antibiotic use [116]. Furthermore, as the frequency of antibiotic use increased, an increase in pathobionts, such as *Enterococcus*, *Clostridium XIVa*, and *Neisseria*, was observed whilst commensals bacterial from *Bifidobacterium*, *Rumicoccus*, and *Akkermansia* groups were decreased [116]. A challenge in treating CF lies in minimising antibiotic-induced gut dysbiosis. The use of the recently approved triple modulator therapy with elexacaftor/tezacaftor/ivacaftor (ETI), significantly reduced the number of antibiotic days from 22.5 days per 6 months to 0 antibiotic days per 6 months in cwCF [117]. This consequently improved the microbiota diversity to resemble that of a healthy gut [117,118]. ETI treatment led to significant improvements in the lung function [119,120], but whether this improvement was related to the changes in the gut, or the lung microbiota has not been reported yet. An exploratory treatment option to limit disturbances to the gut microbiota, is to supplement *Bacterioides* to significantly increase propionate levels and ultimately downregulate the inflammatory response of CFTR-/- Caco-2 intestinal epithelial cells [121].

As with asthma and COPD, cystic fibrosis has also been linked to intestinal dysbiosis as a causative factor [122]. Mechanistically a defective Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein progresses thick mucus, dehydration and adverse changes in the intestinal environment with overall reductions in beneficial bacteria (e.g., *Bacteroides*) and increased abundance of dysbiosis triggering bacteria (e.g., *E. coli*, *Staphylococcus*). These adverse microbiome changes are known to drive inflammatory processes, disrupting the intestinal epithelial barrier with links to issues such as small intestinal bacterial over growth (SIBO), distal intestinal obstruction syndrome

(DIOS), and an increase in cancer risk [123]. It is reported that new therapies such as CFTR modulators and probiotics show promise for restoring gut balance and as such improving cystic fibrosis outcomes [124].

4. Immunomodulatory Interactions in the Lungs

The intestinal microbiota has been extensively studied, with first reports appearing in the Western literature circa the 1840s [125]. The lung microbiota has alternatively had a relatively recent history, established in 2010 by Hilty *et al*. [126], and successfully challenged the then-established misconception of a "sterile lung". However, pulmonary manifestations have been reported over the last 50 years in patients with IBD [127,128]. Connections between the intestines and the lungs have only now been recognised and the establishment of a gut-lung axis has only recently been addressed.

Reports from studies on healthy individuals and those with disordered microbial communities in the lungs, detail a core microbiome in the lungs that includes bacteria from the *Pseudomonas*, *Streptococcus*, *Proteus*, *Clostridium*, *Haemophilus*, *Veillonella*, and *Porphyromonas* genera [126,129]. In the lungs, the greater proportion of bacteria are aerobic or obligate anaerobes such as those from the *Clostridium*, *Veillonella* and *Porphyromonas* genera [59].

The ecological habitat and bacterial niches that exist in the lungs express a dynamic environmental flux that is very much dependent on bacterial migratory movements from the nasal cavity, paranasal sinuses, pharynx, and supraglottic portion of the larynx. Notwithstanding the upper respiratory tract displays significant topographical differences in microbial composition of dominant taxa that inhabit different anatomical locales, such as the nasal cavity and nasopharynx (i.e., species from *Moraxella*, *Staphylococcus*, *Corynebacterium*, *Haemophilus*, and *Streptococcus* groups), and the oropharynx with a high abundance of species from the *Prevotella*, *Veillonella*, *Streptococcus*, *Leptotrichia*, *Rothia*, *Neisseria*, *and Haemophilus* groups [130]. As so happens with colonisation of the infant gut after birth [131], commensal bacteria in the lungs are essential for the regulated development of local immune homeostasis. In the lungs, as in the intestines [132], the recognition of microorganisms by the innate immune system initiates a signalling cascade in the respiratory tract from microbial encounters with amniotic fluid, placenta and the vagina that progresses the development of the lung microbiota, and related responses toward immune tolerance, equilibrium and maturation.

Eubiosis to dysbiosis in the intrapulmonary microbiota significantly alters the structure, abundance, and diversity of the commensal cohort increasing susceptibility to infections by pathobionts with disease progression [73]. Disruptive interactions between lung microbes and immune mucosal barriers can result in irregular local inflammatory responses (e.g., bronchopulmonary dysplasia). Innate immune response signalling can be elicited by internalising particles from ambient air such as toxins, allergens, microbes and endogenous debris [132]. As with the intestinal epithelia, the lung epithelial cells are also part of multiple mechanisms of non-haematological and haematological interactions with the intrapulmonary microbiota primarily acting as a permeability barrier that continually senses microbes and responds to their migratory effects that may lead to infections [133]. Effective clearance of endogenous particles and bacteria is the first site of interplay between the non-haematological structures of the lung (i.e., epithelia and mucus cilia) and the commensal lung cohort. Chronic infections induce the production of increased mucus levels by lung epithelial cells. High levels of mucus can facilitate the growth of bacteria by eliciting low oxygen concentrations and elevating temperatures in lower respiratory tract areas, promoting selective stability of specific bacterial species that shape intrapulmonary immunity [134].

In the intestines, macrophages that encounter resident bacteria generate signals that lead to changes in the phenotype of macrophages corresponding to adaptive immunity effects. Similarly in the lungs, there exists a connection between adaptive immunity and the intrapulmonary microbiome that drives adaptive immunity associated cell involvement [75]. Murine models with neutrophil infiltration due to bacterial infections show high levels of IL-6 and TNF-α and moderate levels of CD4+ T-cell-derived IFN-γ and IL-17 consequent to *Proteobacterium catarrhalis* infection. Further, the inhalation of oral commensals by healthy mice induces a prolonged immune response, that includes CD4+/CD8+ T-cell activation, Th17/$\gamma\delta$ T-cell recruitment including counter regulatory immune responses with elevated concentrations of Treg cells and immune checkpoint inhibitor markers expressed on T-cells [135]. Investigations with acellular bronchoalveolar lavage samples from healthy adults, showed that enrichment of the oropharyngeal microbiome with species from the *Veillonella* and *Revotella* genera correlated with phenotypes of inflammation. The inflammatory response presented in alveolar macrophages elevated levels of Th17 lymphocytes, increased expression of inflammatory cytokines, and reduced expression of the inflammatory cytokine Toll-Like-Receptor 4 (TLR4) [136]. Certain pulmonary microorganisms such as *Staphylococcus*, produce SCFAs that enables regulatory changes in microorganisms' resident in the oral cavity [75]. In the epithelial lining of immunocompromised patients, SCFAs production is correlated with increased levels of *Mycobacterium tuberculosis* Treg cells that have been antigen-induced [136]. Other murine studies have shown that respiratory viruses such as influenza A can modify host adaptive immune responses. The mechanism is linked to the suppression of Th17-induced production of antimicrobial peptides where the influenza A virus exacerbates *S. aureus* colonization and infection. In a similar mechanistic man-

ner, a murine model has shown that impaired Treg cell function was caused by early infection with respiratory syncytial virus increasing susceptibility to allergic asthma [137].

A healthy intrapulmonary microbiome is in a transient state of flux and is influenced by adjacent body parts and the external environment [138]. As reported for a dysbiotic intestinal microbiome, abundance and diversity changes in the status of the intrapulmonary microbiome from eubiosis to dysbiosis has links associated with disease progression [138]. In respiratory diseases, the microbiota is much more likely to be long-lasting and reside in the respiratory tract and lungs, further confirming that the lungs can also harbour an unstable infective disease state of the pulmonary microbiota. Several studies indicate the existence of a relay network between the intestinal microbiome, metabolites such as SCFAs and the intrapulmonary microbiota suggestive of a microbiota-gut-lung-microbiota axis. Perturbations originating in the composition of the intestinal microbiota can induce acute and chronic effects on the pathophysiology of lung diseases [138].

5. Interactions Between the Lungs and Intestines: Lung Infections

5.1 Viral Infections

5.1.1 Coronavirus Disease

Chronic inflammatory lung diseases have a bidirectional impact on the gut microbiome. As respiratory viral infections exert an inflammatory response from the immune system, this may also indirectly affect the gut. Viral respiratory infections post-allogeneic haematopoietic stem cell transplantation is more likely to result in a lower respiratory tract infection (LRTI) [139]. However, patients with higher abundance of butyrate-producing bacteria were inversely associated with LRTI development from the initial infection [139]. Similar observations were made in patients with coronavirus disease (COVID-19) [140–142]. Differences in the gut microbiome composition was observed upon comparison of the gut microbiome of antibiotic-naïve patients with COVID-19 and patients without COVID-19 [142]. This difference was significant when the patients were at a severe stage of infection, with depletions in *Bifidobacterium adolescentis*, *Ruminococcus bromii*, *F. prausnitzii* and an enrichment of *Bacteroides ovatus*, *Bacteroides dorei* and *Bacteroides thetaiotaomicron* was noted [142]. As a result, these patients had lower levels of SCFAs, such as butyric acid [142]. A cross-sectional study found that patients with COVID-19 or H1N1 influenza had significantly reduced gut microbiota richness and diversity compared to healthy controls [143]. Patients with COVID-19 presented a discernible reduction in the abundance of the *Ruminococcaceae* family and several genera within the *Lachnospiraceae* family compared to healthy controls [143]. These alterations in butyrate-producing bacteria observed in COVID-19 patients differed from those reported in individuals with H1N1 infection [143]. Furthermore, the reduction in butyrate-producing bacteria negatively correlated with proinflammatory biomarkers, C-reactive protein (CRP), procalcitonin or D-dimer levels [143]. Similarly, patients with elevated D-dimer levels experienced more severe COVID-19 and had significant depletion of SCFAs [144]. The severity of the COVID-19 infection was negatively correlated with the abundance of *Faecalibacterium prausnitzii* [140,141], which is a well-known butyrate producing species [145]. These observations were not limited to lung infections, but was also noted in patients hospitalised due to abdominal, urinary tract, and skin infections [146]. However, whether these infections were viral or bacterial was not specified [146]. Additionally, amongst post-acute COVID-19 syndrome (PACS) patients, those with significantly decreased lung function, longer hospital stays, and higher rates of intensive care unit admissions had reduced α-diversity and lower proportion of butyrate-producing bacteria in the gut [141]. Furthermore, population-level analyses of gut microbiota in China revealed a significant negative correlation between *E. rectale* (a butyrate producer) abundance and mortality outcomes associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants [147]. In Japan, patients with COVID-19 also presented significantly reduced levels of SCFA-producing gut bacteria [144]. It seems that the infections with SARS-CoV-2 results in an altered gut microbiome which negatively impacts the production of SCFAs. Notably, shifts in the microbiota composition mirrored changes in the pro- and anti-inflammatory cytokine levels during COVID-19, strengthening the link between gut microbiota and the immune response [144]. These associations suggest that administration of SCFA-producing probiotics or butyrate itself may alleviate the symptoms or even reduce the duration of COVID-19.

Vaccines were also shown to alter the composition of gut microbiota. COVID-19 vaccines shifted β-diversity and decreased α-diversity [148]. Recipients with a higher level of neutralising antibody after the vaccines had differing gut microbial community, indicating that the microbial composition may influence the immune response towards the vaccines [148]. High-responding CoronaVac (Sinovac) recipients had higher abundance of *B. adolescentis* whilst high-responding Comirnaty (Pfizer-BioNTech) recipients had higher abundances of *Roseburia faeci*, *Eubacterium rectale*, *B. thetaiotaomicron* and *Bacteroides* sp. OM05-12 [148]. Recipients with higher *Prevotella copri* and *Megamonas spp.* were less likely to experience vaccine-related side effects, suggesting that the two species may be anti-inflammatory [148]. Contrastingly, in a different study [149], *Eubacterium rectale*, a butyrate-producing bacterium was enriched in individuals that had a low immune response one year after receiving CoronaVac.

5.1.2 Respiratory Syncytial Virus

Respiratory infections, such as respiratory syncytial virus (RSV) infections and influenza, increase the relative abundance of *Bacteroidetes* whilst decrease *Firmicutes* in the gut [150]. Groves *et al*. [150] suggested that this change was driven by the loss of appetite as a result of the infection. Although the viral load was not directly related to the weight change, depletion of CD8+ T cells resulted in an increased viral load and weight loss [150]. CD8+ T cells are responsible for viral clearance in a respiratory viral infection [151] and is modulated by the gut microbiota [152]. This modulation by the microbiota may be through its metabolites as oral butyrate administration in mice increased IFN-γ+ by CD8+ T cells in a dose-dependent manner [153]. It is well documented that gut dysbiosis leads to immune dysregulation, which may affect distal sites such as the lungs. However, studies investigating the alterations in the gut microbiome during RSV infections in humans remain limited. A systematic review covering 2012 to 2022 only identified 3 articles [154–156] that have focussed on the changes in the gut [157]. Yagi *et al*. [157] reported that the available studies did not yield a conclusive trend in differences in the relative abundance of the commensal microbiota infants with RSV and the control. However, the composition of the gut microbiota of infants with RSV was significantly different to the control group [157]. Again, the possible reasons for the absence of a variability in significant taxa maybe due to differences in environmental exposures that may shape the gut microbiome [158–160]. Environmental exposures influencing the gut microbiome may extend beyond geographical location as two studies [154,161] conducted in Madrid, Spain reported dissimilar findings. Alba *et al*. [154] found that the gut microbiome was generally similar between infants with bronchiolitis due to RSV and controls except lower relative abundance of *Staphylococcus*, *Haemophilus* and higher relative abundance in *Eggerthella*. Altenratively, Cabrera-Rubio *et al*. [161] reported significantly lower gut microbial richness with *Bifidobacterium*, *Faecalibacterium*, *Escherichia/Shigella*, *Veillonella* and *Akkermansia* associated with severe bronchiolitis, viral infection and recurrent wheezing. Although the association with the bacterial genera and clinical symptoms was evident, the authors suggested that these could act as biomarkers, albeit inconsistencies in current clinical evidence limits their reliability and applicability.

The gut microbiota is not the only system to be altered in patients with respiratory viral infections [144,162–167]. Nasopharyngeal samples collected from infants with RSV in their first year of life had differing composition of the lung microbiota compared to infants without infection [168]. Increased abundance in *Haemophilus*, *Streptococus*, *Moraxella* genera and lower abundance of *Dolosigranulum*, *Corynebacterium* was associated with severe disease [168]. Similar increases in *Haemophilus* and *Streptococcus* was found in other studies that characterised the nasopharyngeal microbiota of infants under 2 years [169] and 1 year [161] of age with RSV infections. Interestingly, the study of the lung microbiota during RSV infection was more common than that of the gut and produced more consistent results as previously outlined [157,161]. The consistency of these findings suggests that the changes in the lung microbiota due to RSV infections may be more promising as future biomarkers.

5.1.3 Influenza Virus

Substantial evidence has long indicated that influenza infections can induce alterations in the gut microbiota. Sublethal infections with influenza A virus (IAV) led to minimal changes in either α or β-diversity in the lower respiratory tract of mice [170]. However, transient but significant reductions in microbial richness and changes to the β-diversity in the small intestines were observed [170]. Changes in the gut microbiota was also observed in humans with IAV (H7N9) [171]. In patients infected with IAV, *Eubacterium*, *Ruminococcus*, *Bifidobacterium*, *Roseburia*, *Faecaelibacterium* and *Haemophilus* were significant reduced compared to the control patients [171]. Similar to COVID-19 infections, *F. prausnitzii* was radically decreased in the IAV group treated with antibiotics [171]. However, whether this correlated with the severity of the IAV infection was not investigated. A Mendelian randomisation of confirmed H7N9 infected patients and healthy poultry workers resulted in negative correlations between the susceptibility of H7N9 infections with two known SCFA producers, *Clostridium hylemonae*, *F. prausnitzii* [172]. Similarly, patients with H1N1 infections showed a significant depletion of butyrate-producing bacteria belonging to the *Lachnospiraceae* and *Ruminococcaceae* families, with higher levels of *Enterococcus*, *Prevotella*, *Finegoldia* and *Peptoniphilus* [143]. These changes in the gut microbiota profile positively correlated with increased inflammatory cytokines IL-2, IL-4 and IL-6 [143] indicating that microbial dysbiosis may contribute to heightened systemic inflammation during respiratory infections.

In a separate study consisting of a larger sample size and assessing patients over 60 years old with influenza-like-illness (ILI), *Bacterioidetes* and *Proteobacteria* were more abundant compared to the control [166]. It was hence reported that the relative abundance of *Ruminococcus torques* was positively associated with ILI and proinflammatory responses, while the co-occurrence of *Echierichia* and *Shingella* was negatively associated with certain beneficial taxa such as butyrate producers [166]. *R. torques*, an intestinal mucous degrader [173] associated with IBD [174] was also enriched in patients with COVID-19 [144], suggesting a possible role in contributing to the gastrointestinal symptoms during respiratory infections. The impact that IAV has on the gut microbiota composition leading to damaged intestinal barrier and decreased production of SCFA

was also observed in a murine model [175]. SCFA supplementation 2 days post infection resulted in symptoms indicative of reduced inflammation and partially restored the damaged intestinal barrier [175]. Thus, IAV infection disrupts SCFA-producing commensals which may have clinically relevant implications as diminished SCFA levels affect immunomodulation and epithelial integrity. However, these clinical symptoms have not yet been thoroughly investigated in the context of IAV infections especially in humans.

5.2 Bacterial Infections

The gut microbiome modulates the host immune response against bacterial lung infections. Gut microbiota-depleted mice had higher bacterial load and higher mortality rate compared to control mice post intranasal *S. pneumoniae* infection [176]. Faecal microbiota transplantation (FMT) to the microbiota-depleted mice resulted in a comparable TNF-α and IL-10 levels and bacterial clearance rate to the control mice post infection, verifying the importance of the gut microbiome in lung infections [176]. Furthermore, the gut microbiota supports alveolar macrophage function to phagocytose *S. pneumoniae* indicating that a healthy gut microbiome can improve the outlook in a bacterial lung infection [176]. *S. suis* lung infection also resulted in tissue damage of the small intestines of healthy mice [177]. However, the extent of intestinal damage was more profound in gut microbiota-depleted mice [177]. Additionally, the concurrent lung damage was more severe with histopathology revealing thicker alveolar spaces, higher inflammatory cell infiltration and more alveolar haemorrhage in mice with gut dysbiosis [177]. The reason for exacerbated damage was thought to be due to the imbalance of Th1/Th2 due to gut dysbiosis [177]. This imbalance was also associated in COVID-19 patients having a higher mortality risk [178] and acute exacerbations of COPD [179].

6. Mucosal Surfaces of the Gut and Lungs and Inflammatory Sequelae

Observations that lung diseases, such as COPD and asthma, regularly occur with chronic inflammatory intestinal diseases, such as IBD or IBS, has progressed the posit that the gut–lung axis presents an intimate connectivity in health and disease. In support, epidemiological studies have reported that at least 50% of adults with IBD and 33% of patients with IBS have pulmonary inflammation involvement or with impaired lung function and this without a previous history of acute or chronic respiratory diseases. Notwithstanding, patients diagnosed with COPD were reported to be 2–3 times more likely to be diagnosed with IBD [180]. Furthermore, in patients diagnosed with asthma there were reported functional and structural alterations in the intestinal mucosa suggestive of gut dysbiosis [181]. Likewise, patients with COPD typically had an increase in intestinal permeability [97,182].

Intestinal dysbiosis not only describes adverse shifts in the intestinal microbiome but the consequent effects of increased intestinal permeability that is associated with numerous metabolic disorders and extra-intestinal diseases [183]. Increased intestinal barrier permeability has been associated with an exaggerated pro-inflammatory response as so happens with IBD. Pro-inflammatory cytokines and chemokines are present in elevated levels in mucosal tissue and/or in peripheral blood, suggesting a monocyte/macrophage stimulatory effects by enteric bacteria and/or their constituents (e.g., LPS) [184,185]. Studies with murine models of IBD have reported that organisms such as *Citrobacter rodentium* and *Helicobacter hepaticus* can progress increased intestinal barrier permeability and mucosal dysbiosis with translocation of bacterial endotoxins (e.g., LPS) that trigger local endotoxemia [24].

There are numerous mechanisms that have been reported associated with intestinal microbial dysbiosis [24]. An often-cited example is the overgrowth of *Enterobacteriaceae* from the phylum Proteobacteria that resides in the gut near the intestinal epithelial barrier. This is attributed to the relative higher oxygen tolerance by these bacteria due to gas diffusion across the intestinal epithelium. Under normal physiological conditions *Enterobacteriaceae* are present in low levels. An increased abundance of *Enterobacteriaceae* with pathogenic members from this family (e.g., *Escherichia coli*) signals significantly enhanced local inflammatory responses, as observed in IBD [186]. Moreover, studies have provided evidence that an inflammatory tissue environment is conducive to perturbations of the intestinal microbiota often characterized by bacterial species endowed genetically with the capability of utilizing nutrients more abundantly found in the inflamed gut. This causes significant disturbances observed in the intestinal bacteria community structure [24].

Recently, there has been an increased understanding and consequently research interest in the gut–lung axis and the link to respiratory diseases [187]. In an investigation of the intestinal microbiota make-up in infants and adolescents diagnosed with asthma it was reported that there was observed reduced levels of *A. muciniphila* and *F. prausnitzii* critical gut commensals that elaborate SCFAs associated with a healthy gut [188]. Consequently, the researchers also showed that the levels of inflammatory factors, including CRP, TNF-α, and IL-6 in peripheral serum of adolescents with asthma was significantly increased.

Probiotics have a long history of use and have provided contentious results for their anti-inflammatory and immunomodulatory effects in attenuating intestinal inflammation and atopic diseases. The first documented randomised double–blind placebo–controlled study with *Lactobacillus gasseri* demonstrated efficacy in school children with asthma and allergic rhinitis [189]. There was a significant increase on pulmonary function and peak expiratory flow rate, and the clinical symptom scores for asthma

and allergic rhinitis decreased in the probiotic-treated group as compared to the controls [189]. In addition, inflammatory markers TNF-α, IFN-γ, IL-12, and IL-13 production by peripheral blood mononuclear immune cells were significantly reduced following probiotic treatment. More recently an additional study continued to probe the efficacy of orally administered probiotics [190]. An 8-week double–blind randomised placebo–controlled supplementation study reported significant immunomodulatory effects by probiotics in attenuating asthma symptomatology with reduced Th2 cells-associated IL-4 and improved forced expiratory volume and forced vital capacity [190].

7. The Role of Prebiotics, Probiotics and Postbiotics in Respiratory Infections

7.1 Probiotics

The administration of probiotics that can induce positive effects on adverse intestinal microbiome profiles has a long history of use, that is underpinned by the fact that probiotics are safe yet not a panacea to ameliorate all metabolic diseases in the gut [191]. Probiotic formulations present as single or multiple bacterial species with variable concentrations expressed as colony forming units making it difficult to translate to clinical practice. Formulations usually contain bacterial species from the *Lactobacillus* and *Bifidobacterium* genera that have been studied in different scenarios in clinical studies [191]. Notwithstanding some clinical studies have reported efficacy in upper respiratory tract infections [192], reduced occurrence of allergies in children [193] and immune response to oral rotavirus vaccine [194], results that propounds the idea of adopting further investigations with probiotics in lung diseases.

There is therefore biological plausibility for investigating inhaled probiotic formulations delivered directly to the lungs, as a posited strategy for the treatment of respiratory infections. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [195]. Probiotic bacteria are conventionally administered orally to improve gut health, but their beneficial effects are not restricted to the gastrointestinal tract. Prophylactic oral *L. rhamnosus* GG (LGG) decreased non-infective lung inflammation and fibrosis in rodents by modulating immune responses and improving the diversity of the intestinal microbiota [196,197]. The lung function of rats improved with reduced pro-inflammatory cytokines (IL-1β, IL-6, IL-17A, TNF-α) in the bronchoalveolar lavage fluid (BALF) and increased anti-inflammatory cytokines (IL-10, TGF-β) [196]. These trends also followed a decrease in the bacteria in the gut linked to inflammation and an increase in those associated with good gut health [196]. Oral *Lactobacillus* also showed protective effects when given before pulmonary infections, such as increasing the clearance of *P. aeruginosa* from infected murine lungs and improved survival [198,199]. Alveolar macrophage phagocytic activity, as well as IgA and IgM levels in the BALF increased [198]. On the other hand, serum IL-6 decreased and IL-10 levels increased, indicating anti-inflammatory responses in the lungs [199]. Likewise, oral *L. rhamnosus* CRL1505 given to mice before inducing airway inflammation with polyinosinic:polycytidylic acid (poly(I:C)); a viral infection simulant, decreased IL-6 and increased IL-10 levels in the BALF [200]. It also reduced the lung influenza viral titre when the mice were infected by the actual virus [200].

Probiotics were administered to investigate whether these formulations could restore the immunomodulatory role of the gut microbiome. To model gut dysbiosis, antibiotic-treated mice were subsequently infected with IAV [201]. This intervention led to significantly reduced CD4 and CD8 T cell responses as well as lower levels of virus-specific antibody levels, resulting in elevated IAV titres 9 days post infections [201]. Specifically, oral treatment of neomycin led to a significant reduction in the gut microbial biomass which was associated with the absence of CD8 T cell responses in the lung [201]. Murine maternal supplementation with Gram positive probiotics such as *L. rhamnosus* and *B. animalis subsp. lactis* resulted in increased IAV clearance in neonates and improved survival [202]. Perinatal exposure to the two probiotics resulted in increased IAV antigen-experienced IFN-γ producing effector CD8 T cells in neonate mice and IAV-specific resident memory CD8 T cells in adulthood [202]. This suggests that probiotic supplementation may restore the regulation of the immune system caused by gut dysbiosis. Furthermore, commensal bacteria such as *B. longum* were associated with elevated anti-IAV IgG concentrations [203], suggesting that administering specific probiotic species may enhance immune responses attenuating respiratory infections.

Oral probiotic administration in humans has also demonstrated comparable efficacy, suggesting its potential to modulate immune responses similarly to the findings observed in animal models. A recent randomised controlled trial (RCT) by Lau *et al*. [204] studied whether the treatment with a symbiotic preparation consisting of *B. adolescentis*, *B. bifidum*, *B. longum* and galacto-oligosaccharides, xylo-oligosaccharides and resistant dextrin (SIM01) were able to promote growth of these strains in the gut and also increase SCFA levels to alleviate the symptoms of PACS. Patients reported that clinical symptoms such as fatigue, memory loss and difficulty in concentration were reduced without associated adverse events [204]. SIM01 administration was able to increase the bacterial diversity and richness compared to the placebo group at 6 months [204]. The targeted *Bifidobacterium* genus had significant increases in relative abundance in the SIM01 group along with *Roseburia intestinalis*, *R. hominis*, *F. prausnitzii* and *A. munciniphila* which led to consequent increases in SCFA production [204]. Oral LGG treatment in participants who had household-exposure to someone with confirmed COVID-19 reduced the risk of developing COVID-

19 symptoms [162]. However, the incidence of COVID-19 diagnosis had no difference to placebo groups [162]. In a RCT involving children with upper respiratory tract infection (viral or bacterial not specified), oral administration of a commercially available mixture of *B. breve* M-16V, *B. lactis* HN019, *L. rhamnosus* HN001 reduced fever duration by 2 days compared to the placebo group [205]. Adverse effects reported included diarrhoea, constipation and abdominal pain but its distribution was not significant between the treatment and placebo groups indicating the safety of oral probiotics in adolescents [205]. These results strongly support the oral administration of probiotics to alleviate the duration and or the symptoms of respiratory infections.

Although the mechanism of these oral-to-lung protective effects remain uncertain, they are attributed to the complex physiological and immunological network between the gastrointestinal and respiratory tracts. Translational interactions between gut-associated immune cells trigger immunomodulation indirectly in the lungs, resulting in local anti-inflammatory and antimicrobial responses.

Mechanistically intestinal-derived immune factors exert profound effects on pulmonary immunity through both direct and indirect pathways. Direct communication occurs via the migration of activated immune cells through the blood and lymphatic systems, enabling effector populations primed in the intestines to influence distal sites such as the lungs [206]. Exemplified by T helper 17 (Th17) cells and regulatory T (Treg) cells that are elaborated in the intestinal environment that can then traffic to the pulmonary mucosa, where modulation of the local immune responses occur [206]. Furthermore, the indirect communication is reported to be mediated by systemically circulated microbial components and metabolites such as short-chain fatty acids (e.g., SCFAs) [206,207]. Among these, SCFAs produced by gut commensal bacterial species (e.g., the *Clostridia* cluster IV and XIVa group of gut commensals produce butyrate) and lipopolysaccharides (LPS) derived from Gram-negative bacteria (e.g., *Escherichia coli*) are key modulators of the gut immune tone. SCFAs influence the differentiation and function of immune cells, including those within the bone marrow, thereby shaping both innate and adaptive responses. LPS, conversely, can act as a potent immunostimulant, priming inflammatory pathways that extend beyond the gut and in this instance to the lungs. This combined effort exemplifies the bidirectional nature of the gut–lung axis, whereby intestinal immune education and elaboration, and microbial metabolites orchestrate pulmonary homeostasis and the resolution of airway inflammatory responses [207].

However, using oral probiotics for respiratory conditions entails reliance on the yet unclear workings of the gut-lung axis. Furthermore, probiotics transiting through the gastrointestinal tract must survive in, or protected from, stomach acid to ensure the right dose can be delivered. Gastric food content also affects residence time of probiotics in the upper gastrointestinal tract. These factors may render the efficacy of oral probiotics slow and/or variable. A more efficient and rapid delivery of a probiotic to the lungs can be envisaged by the direct administration of a probiotic into the respiratory tract and to the lungs to induce local immunomodulation.

While inhaled probiotics have not been investigated as extensively as oral formulations, research has shown that administering probiotics directly to the respiratory tract before pathogen exposure can have beneficial effects. However, these findings come solely from animal studies. Intratracheally and intranasally instilled probiotics in mice protected against subsequent acute bacterial and viral infections, reduced pathogen load, and increased survival. Murine models were the most prevalent and intranasal instillation of the *Lactobacillus* species were common. Table 1 (Ref. [195,208–212]) highlights the anti-inflammatory and antibacterial effects of live and heat-killed probiotics.

The idea of combining nanomedicine technologies with dry powder inhalers into a loosely bound submicron sized microparticle (i.e., usually ~1 to 5 µm) is a feasible posit [213]. The idea is that on inhalation the particle agglomerates and disperses into the nanoparticle constituents in the airway surface liquid, enabling deep lung deposition. Delivering and accumulating the nanoscale drug carriers in the lung tissue would constitute a strategy that bridges the aerodynamic requirements for inhalation with the functional advantages of nanoparticles, given that otherwise the drug would be exhaled or poorly aerosolized [213].

There are a number of advantages with nanoparticles such as (i) enhanced solubility and dissolution with increases in particle surface area, carrying poorly soluble payloads in amorphous and solubilized matrices that can accelerate dissolution at the epithelial surface [214]. It is also reported that nanoparticles (ii) exhibit enhanced stability in dry form. This is especially important stabilizing labile molecules (e.g., peptides, proteins, nucleic acids), protecting the payload from hydrolysis and aggregation storage especially when compare to liquid aerosols [215]. Furthermore, nanoparticles as nanocarriers of lipid, polymeric or inorganic molecules can (iii) be target tissue specific for delivery with controlled release of the drug [214,215]. These functional gains have been demonstrated in preclinical and formulations investigations of inhalable nanopowders [214].

Direct respiratory delivery of probiotics is a promising scientific pursuit. However, it remains under-studied in humans. Advancing this research posit requires further rigorous safety testing, sensitive lung microbiome methods, and clinical studies that link local microbiome changes to beneficial immune and clinical outcomes. The challenges encountered raise numerous concerns around (i) low biomass and contamination risk that makes reliable lung microbiome profiling difficult; technical problems in distinguishing re-

Table 1. Main outcomes of some intratracheal/intranasal live and heat-killed probiotic animal studies.

Pathogen	Probiotics/Postbiotics	Route and Murine Model	Main outcomes	Reference
P. aeruginosa PAO1	Mixture of live *L. fermentum* *L. zeae* *L. paracasei* at dose 10^7 CFU/mouse at 18 h or 42 h and 18 h before infection.	Intratracheal C57BL/6	↓ bacterial load ↓ IL-6 ↓ TNF-α	[195]
P. aeruginosa PAO1	Mixture of live *L. rhamnosus* *L. fermentum* and *L. fermentum* or Mixture of live *L. paracasei* *L. salivarius* *L. brevis* at unspecified dose 18 h before infection.	Intranasal C57BL/6J	Improved survival ↓ bacterial load ↓ pro-inflammatory cytokines ↓ chemokines ↑ anti-inflammatory cytokine IL-10 in BALF	[208]
Influenza virus H1N1 A/PR8/34	Heat-killed *L. rhamnosus* GG unspecified dose to mice for 72 h consecutively before infection.	Intranasal BALB/c	Improved survival and respiratory immune responses ↓ infection symptoms	[209]
Influenza virus H1N1 A/PR8/34	Live or heat-killed *L. rhamnosus* at dose 10^8 CFU/mouse/day for 48 h consecutively before infection.	Intranasal BALB/c	Improved survival ↓ viral load ↓ tissue damage ↓ oedema in lungs ↓ pro-inflammatory cytokines ↓ chemokines ↑ anti-inflammatory cytokine IL-10 in BALF Improved antiviral response	[210]

Table 1. Continued.

Pathogen	Probiotics/Postbiotics	Route and Murine Model	Main outcomes	Reference
Influenza virus H1N1 A/PR8/34 or H3N2 A/Philippines/82	Live *L. plantarum* at dose 10^7 or 10^8 CFU/mouse for 96 h before infection or 10^8–10^9 CFU/mouse at infection.	Intranasal BALB/c	↓ weight loss and ↓ lung viral load ↓ IL-6 and TNF-α in BALF Modulated dendritic cells and macrophages	[211]
Influenza virus H1N1 A/PR/8 RSV SARS-CoV-2 (Omicron variant BA.1)	Heat-killed *Bacillus subtilis* DSM 3244 at dose 1.5×10^9 CFU/mouse for 21, 14, 7 days before infection.	Intranasal C57BL/6 (H1N1) BALB/c (RSV) K18-hACE2	IAV 100% survival when challenged with IAV ↑ neutrophils T cells recruitment ↑ IFNγ | TNF-α IL-1β | IL-6 CCL5 | CXCL10 in BALF RSV No obvious weight loss and complete clearance of RSV viral load ↑ CD4 | CD8 T cells SARS-CoV-2 Significantly decreased SARS-CoV-2 viral load by day 4 post infection ↑ serum IgG pulmonary IgA	[212]

Note: BALF, Bronchoalveolar Lavage Fluid; CCL5, CC motif Chemokine receptor 5; CD4, Cluster of Differentiation 4; CD8, Cluster of Differentiation 8; CXCL10, CXC motif Chemokine Ligand 10; CFU, Colony Forming Units; IAV, Influenza A Virus; IL, Interleukin; IFN, Interferon; RSV, Respiratory Syncytial Virus; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; TNF, Tumour Necrosis Factor; ↑, increased; ↓, decreased.

sident bacteria from transient colonizers with strict sampling and negative control requisites; (ii) further safety barriers relative to the introduction of live microbes into already inflamed and immunocompromised airways that would raise regulatory and ethical concerns; and (iii) formulation and delivery loopholes that relate to include keeping probiotic strains viable in aerosols, with the avoidance of bronchospasm that then achieves deposition of the drug payload in target airway regions.

At present, only a limited number of studies have explored the respiratory route for probiotic administration in humans as outlined in Table 2 (Ref. [216–219]). A notable study used a nasal spray of containing 5 billion of *Bacillus subtilis* ANA4 and *B. clausii* ANA39 suspended in 0.9% NaCl (LiveSpo Navax) was developed to be tested on children with RSV [216]. The children did not experience any negative side effects upon spraying the LiveSpo Navax spray such as choking, signs of nasal mucosa irritation, symptoms of local bacterial infection, vomiting or diarrhoea indicating the safety and suitability of the dosage form [216]. In terms of efficacy, patients using the LiveSpo Navax no longer had runny noses by day 6 compared to 17.5% of patients in the control group [216]. In a Phase 2 trial with children with severe pneumonia due to RSV and bacterial co-infections, significant reductions in RSV and bacterial co-infections were noted at day 3 of LiveSpo Navax treatment [217]. Similar to their previously reported study, these reductions were accompanied with notable decreases in pro-inflammatory cytokines such as IL-6, IL-8 and TNF-α and the restoration of the nasal microbiota [217]. Although these results are promising, interventions aimed at correcting dysbiosis should be approached with caution, as beneficial outcomes are not always assured. An example is a study by Mårtensson *et al*. [218] which utilised a nasal spray to deliver 1.9×10^{10} CFU/dose of *L. rhamnosus* SP1, *L. paracasei* 101/37, *Lactococcus lactis* L1A two times a day for 3 weeks to patients with seasonal allergic rhinitis. Mini Rhinoconjunctivitis Quality of Life Questionnaire (Mini-RQLQ), total nasal symptom score (TNSS), peak nasal inspiratory flow (PNIF) scores between probiotic-treated and control patients showed no differences indicating its lack of efficacy in improving nasal symptoms in patients with allergies [218]. No major adverse effects were recorded but two out of 13 patients complained of burning sensation, one patient reported itching, one patient reported pain in association with administration [218]. *Lactobacillus* genera used in the nasal spray are prevalent in a healthy upper respiratory tract microbiome [220]. The dose of 10^{10} CFU is commonly used in intranasal murine models and in oral probiotics for humans. However, the minor innate responses observed (Table 2) suggests that the strains of *Lactobacilli* and *Lactococcus* used may not have been optimal for the condition. In contrast, administration of *Lactococcus lactis* W136 at comparable dose and frequency has previously demonstrated improvements in sinonasal symptom score (SNSS), sino-nasal outcome test 22 item (SNOT-22) and peri-operative sinus endoscopy (POSE) score [219]. These findings highlight the importance of selecting appropriate probiotic strains tailored to specific clinical conditions to achieve meaningful therapeutic outcomes.

A recent review [221] has narrated a synthesis of the published research evidence based on the nature of the studies namely, clinical studies or human samples, *in vivo* or animal models, *in situ*, *in vitro* and/or *in silico* concluding that probiotics may have adverse effects. What is of importance is the administration of a probiotic formulation to an immune compromised patient with a chest infection and the risks that probiotic treatments may ensue. In such vulnerable patients, the modulation of the lung microbiota with probiotic formulations can present several potential risks to health and limitations to treatment efficacy. In particular opportunistic infections, excessive immune stimulation, transfer of antibiotic resistance genes, and challenges in effective delivery to the lungs [222]. Notwithstanding expert opinions do not recommend stopping research into probiotics, rather, there is encouragement for rigorous, targeted research to better understand probiotic specific effects, safety, and mechanisms of action [223]. Specifically current ideas focus on the effects of probiotics that are highly specific to strains and the individuals administered to [223,224].

7.2 Prebiotics

Prebiotics are "selectively fermented ingredient that allows specific changes both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" [225]. Common examples approved by the United States Food and Drug Administration include alginate and inulin [226] which are used to drug delivery. Alginate is frequently used for stabilising and enhancing pulmonary drug delivery [227–229]. Encapsulation with alginate is a promising approach as it does not compromise the antimicrobial effect of antibiotics and is not toxic to lung cells [227]. More recently, dry powder alginate oligosaccharide has been tested as a drug in patients with cystic fibrosis [229] as *in vitro* studies demonstrated the potential to modify the viscoelastic properties of CF sputum [230]. Though its desired effects were not observed in the Phase 2 clinical trial, the trial demonstrated the safety of inhaling alginate oligosaccharides [229]. Inulin is also popular in drug formulation and delivery as it stabilises and protects drugs against the acidic environment of the stomach, making them especially useful for oral delivery [231–233]. Interestingly, inulin-enriched diet for 15 days prior to lethal intranasal *P. aeruginosa* challenge increase the survival of mice by 33% through $\gamma\delta$ T cell activation [234]. It has been suggested that inulin induces the production of SCFA and other metabolites to modulate the level of T lymphocytes and enhance the anti-inflammatory

Table 2. The effect of intranasal delivery of probiotics in humans.

Disease	Treatment	Administration Route Patient profile n = number	Main outcomes	Reported adverse events	Reference
RSV	LiveSpo Navax: *Bacillus subtilis* ANA4 *B. clausii* ANA39 5×10^9 CFU/dose in 0.9% NaCl Dose: t.i.d./6 days	Intranasal (spray) Children Treatment Gp (n = 46) Placebo Gp (n = 40)	Day 3: ↓ percentage of patients with moist rales, fast pulse in treatment group RSV viral load ↓ ~600-fold ↓ IL-6 | IL-8 | TNF-α Day 6: No patients in treatment group with runny nose Treatment Gp recovered in 4 days compared to 5 days in Placebo Gp	No abnormal changes to —breath rate —heart rate —body temperature —No choking upon spraying —No signs of nasal mucosa irritation —No local bacterial infection —No emesis —No diarrhoea	[216]
Pneumonia due to RSV bacterial co-infection	LiveSpo Navax Dose frequency not specified	Intranasal (spray) Children Total recruited (n = 120)	Day 3: ↓ RSV and bacterial co-infection ↓ *H. influenzae* *S. pneumoniae* ↓ IL-6 | IL-8 | TNF-α ↑ IgA Restoration of nasal microbiota ↑ *Corynebacterium* *Bacillus* ↓ *Streptococcus* *Haemophilus* *Moraxella*	N/A	[217] (Abstract only)

Table 2. Continued.

Disease	Treatment	Administration Route Patient profile n = number	Main outcomes	Reported adverse events	Reference
Allergic rhinitis (Positive skin prick test to birch or grass pollen with wheal diameter ≥3 mm)	*L. rhamnosus* SP1 *L. paracasei* 101/37 *Lactococcus lactis* L1A 1.9×10^{10} CFU/dose Dose: b.i.d./21 days	Intranasal (spray) Adults Treatment Gp (n = 13) Placebo Gp (n = 11)	No significant differences in Mini-RQLQ | TNSS | PNIF No significant differences in FeNo | specific IgE and total IgE ↑ TNF-α | MIP-1α | MIP-1β | MCP-1 | IL-6 IL-8 | IL-10	—No severe adverse events reported Treatment group: —2 patients: burning sensation —1 patient: itching —1 patient: pain upon spraying	[218]
Chronic rhinosinusitis with previous endoscopic sinus surgery	*Lactococcus lactis* W136 1.2×10^{9} CFU/dose Dose: b.i.d./14 days	Intranasal sinus (irrigation in 0.9% saline) Adults Treatment Gp (n = 24) No Placebo Gp	SNSS: progressive improvement over treatment period Greatest improvement in nasal congestion post-nasal drip need to blow nose SNOT-22: ↑ in score over treatment period POSE: Improvement aligned with SNSS and SNOT-22 UPSIT-40: Stable no deterioration Nasal microbiome: no change in a-diversity ↓ *Dolosigranulum* *pigrum*	Well tolerated —No acute infections during Treatment Reported —headaches —migraines —nasal congestion —dental infection —throat pain —cold sore —gastroenteritis —nasal allergy —shoulder pain but… did not differentiate between before trial and during trial	[219]

Note: Gp, Group; b.i.d., twice per day; t.i.d., three times per day; SNSS, Total Sino Nasal Symptom Score; SNOT 22, Sino-Nasal Outcome Test-22; POSE, Perioperative Operative Sinus Endoscopy Score; UPSIT-40, University Pennsylvania Smell Identification Test-40; ↑, increased; ↓, decreased.

responses [234,235]. Oral inulin administration to maternal rats affected the level of pulmonary inflammation in their offspring by decreasing IFN-γ and increasing IL-4, IL-17 and IgE levels [236]. Upregulation of G-coupled protein receptor (GPR) 43 and GPR41 after inulin intake observed in the offspring rats [236] as well as individuals with asthma [237] are associated with improvement in lung function. A general high fibre diet consisting of cellulose and/or pectin was able to protect mice against emphysema induction [238]. Hence, it is likely that the inulin itself is not having an immunomodulatory effect as dietary fibre is metabolised by the gut microbiota to SCFA. The consequent production of SCFA can significantly reduce airway inflammation in stable asthma [237]. Unless the lung microbial community can readily metabolise these prebiotics, a direct role of prebiotics in the management of respiratory health may be limited.

7.3 Postbiotics

Postbiotics are not live bacteria, rather compounds produced by microorganisms during a growth phase or the fermentation of dietary fiber, the indigestible part of plant-based foods (e.g., vegetables, legumes) [239]. Furthermore, postbiotics are "formulations of inanimate microorganisms and/or their components that confers a health benefit on the host" [240]. These compounds include small molecules, peptides, enzymes, cell wall fragments, and other microbial products such as SCFAs (e.g., acetate, propionate, butyrate), bacteriocins, exopolysaccharides, enzymes and vitamins produced during microbial fermentation [241]. Moreover, postbiotics can also encompass larger molecules like polysaccharides and proteinaceous factors [241]. Hence heat-killed bacteria shown in Table 1 can be considered postbiotics. Interestingly, heat-killed probiotics were reported to be as good as their live counterpart in that regard [210]. Intranasal live and heat-killed *L. rhamnosus* CRL1505 were also effective against respiratory syncytial virus infection in BALB/c mice [242]. This phenomenon is well-documented for oral probiotics and is attributed to the immunomodulatory effects of bioactive intracellular or cell membrane components released upon the death of the probiotic [243]. Using non-viable probiotics is clinically safer because it eliminates the risk of live probiotics infecting or causing sepsis in immunocompromised patients. It would also facilitate the manufacture and storage of the formulations because there is no need to maintain a live probiotic load [243]. The immunomodulatory effects of non-viable probiotics administered via the respiratory route remain underexplored, underscoring the need for further investigation in this area.

SCFAs are a central subset of postbiotics and considered to be the most studied entity [241]. SCFAs can also be considered products of probiotic gut metabolism. SCFAs postbiotics have anti-inflammatory, intestinal barrier-supporting and metabolic-modulating effects in the gut [241]. Investigations with SCFAs are reported as safe, well-defined biotherapeutics or as supplements for chronic diseases, intestinal disorders, and immune modulation [241]. Strengthening factors such as standardization of products, doses, and administration without live organisms further improves the knowledge base for clinical practice approval. Reports on how actively mapping which specific postbiotic molecules produce what host response is observed, will correlate how the formulation, the dose, and the delivery route affect outcomes [241].

Although less extensively studied than probiotics in terms of their effects on the lungs, emerging research supports the direct use of postbiotics as a promising approach for managing common respiratory infections [244, 245]. Oral administration of a butyrate releaser, *N*-(1-carbamoyl-2phenyl-ethyl) butyramide (FBA) had a protective effect against SARS-CoV-2 in humans [246]. It reduced the number of SARS-CoV-2-infected cells and reduced *angiotensin-converting enzyme 2* (*ACE2*) and *transmembrane protease serine-2 TMPRSS2* expressions which were related to the pathogenicity of SARS-CoV-2 [246]. Emerging animal evidence from animal studies suggests that butyrate may have a therapeutic potential in treat pulmonary conditions, such as pulmonary fibrosis [247] and COPD [248]. However, pulmonary delivery of butyrate is yet to be explored.

In section 7.1., certain commensal microbiota were shown to be responsible for CD8 T cell regulation in mice [201]. Recently, the impairment in the immunoregulation observed in dysbiotic mice were also evident in infant humans with gut dysbiosis through the disruption of nuclear factor interleukin 3 (NFIL3)-dependent T cell programming [249]. Among the four genera that significantly distinguish healthy from dysbiotic human infant gut microbiota, *Bifidobacterium* is notable for its metabolite, inosine [249]. Oral administration of *B. pseudolongum* in mono-associated germ-free infant mice resulted in increased inosine levels in both lungs and faeces which subsequently led to 2-fold increase in effector CD8 T cells in the lungs [249]. Directly treating dysbiotic mice with IAV infection with inosine resulted a higher influenza-specific CD8 T cells levels and increased influenza-specific tissue resident memory CD8 cells [249]. The observed reduction in morbidity among inosine-treated dysbiotic mice suggests that similar immunomodulatory effects may be replicable in human infants, potentially leading to improved health outcomes.

SCFAs were found in millimolar concentrations in the cystic fibrosis airways due to hypoxia [250]. The immune responses vary depending on the type of SCFAs, its concentration and the cell line used in testing [250]. High concentrations of SCFAs (25–50 mM) reduced the levels of inflammatory cytokines such as GM-CSF, IL-6 in A549 and control cystic fibrosis bronchial epithelial (CFBE) cells [250]. In contrast, low acetate levels (0.5–2.5 mM) and IL-8 expression specifically in F508del-CFTR, suggests its

potential role in CF airway inflammation [250]. SCFA concentration also influenced the growth of a common CF pathogen, *P. aeruginosa* [250]. High SCFA levels inhibited the pathogenic growth whilst low levels promoted it under aerobic conditions [250]. However, under microaerobic conditions mimicking the CF lungs environment, SCFAs significantly suppressed *P. aeruginosa* growth, highlighting their vital role in maintaining lung homeostasis and health [250]. Currently, the application of SCFA-based interventions in human clinical settings for cystic fibrosis patients remain unexplored.

Vancomycin induces gut dysbiosis by reducing the overall abundance and diversity, especially decreasing the *Clostridia* class which produces butyrate [251]. Oral supplementation with a SCFA cocktail consisting of butyrate, acetate and propionate did not replace what is lost by vancomycin in the gut [251]. However, it reduced airway allergic inflammation [197] evident through significantly decreased IgE levels and histopathology during allergen exposure [251]. Similar anti-inflammatory observations were made with older mice with acute lung injury [252]. Oral SCFA treatment significantly reduced the pulmonary IL-6, CXCL1, and GM-CSF levels in old mice [252]. However, the anti-inflammatory effect was less profound in young mice and in fact IL-12 and IFN-γ levels were increased [252]. The possible reason for such differences may be due to the differences in the gut microbiome that come about with aging as *Bifidobacterium*, *Faecalibaculum*, *Lactobacillus* and *Limosilactobacillus* genera were significantly increased in older mice [252].

Interestingly, SCFAs failed to suppress TNF-α-induced proinflammatory responses in human lung mesenchymal cells [253]. Although treatment with individual SCFAs did not induce cytokine release, a combination of propionate or butyrate at a high concentration and TNF-α had an amplified effect in increasing IL-6 and CXCL8 levels than TNF-α alone [253]. These effects were limited to the lung fibroblasts and airways smooth muscle cells as high concentration (25 mM) of propionate reduced LPS induced cytokine release from THP-1 cells [253]. The observed discrepancies may be due to the differing concentration of SCFAs used in the studies as they can exert pro- or anti-inflammatory effects on intestinal epithelial cells depending on their concentration [250]. However, there are difficulties in directly comparing these concentrations as the level of SCFAs found in the lungs were not reported in studies that used oral supplementation in mice. Currently, only a limited number of studies investigate the effects of SCFAs on respiratory infections. There is a possibility that rather than the probiotics itself but the SCFAs may be responsible for the immunomodulatory effects observed in treating viral and bacterial infections listed in Table 1, warranting further investigations.

In multiple clinical trials, inosine pranobex (IP), a combination of p-acetamindo-benzoate salt of *N-N* dimethylamine-2-propanol and inosine in 3:1 molar ratio [254], demonstrated safe and efficacious effects in reducing influenza-like symptoms in adults [254,255]. In the phase 4 trial, though patients in the treatment group had their influenza symptoms ending 2 days earlier than the placebo group, the difference in time to resolution of all flu-related symptoms were not statistically significant [254]. However, for a subgroup of patients under 50 without related ongoing disease and non-obese, the time to symptom resolution was significantly shorter. Similar results were reported in the phase 3 trial that investigated the efficacy of IP against COVID-19 [255]. On day-6 of treatment in COVID-19 patients, patients on IP displayed a higher clinical response (2-point improvement or being asymptomatic on the modified WHO ordinal scale) and higher clinical cure (asymptomatic on the modified WHO ordinal scale) [255]. Both trials reported minimal adverse events indicating the safety of IP [254,255].

7.4 Bacterial Compounds

Reports show that microbiota derived compounds from the metabolism of dietary sources such as SCFAs and inositol can regulate host epigenetics that affect DNA methylation and histone acetylation [49,256]. SCFAs and inositol can also deliver enzymes that directly modify host proteins or trigger signaling pathways that can alter the host's epigenetic machinery, influencing gene expression related to immune response and metabolism [257].

SCFAs produced by intestinal bacteria (i.e., butyrate, propionate, acetate) can act as inhibitors of class I/II HDACs raising the global histone acetylation and promoting open chromatin at certain target loci [258]. SCFAs can also influence one-carbon metabolism and methyl-donor availability, biochemical reactions that indirectly affect DNA methylation patterns and gene expression that are linked to inflammation and metabolism [258]. In combination with the activity of SCFAs gut bacteria encode methyltransferases that can modify host substrates or when delivered to host cells, alter host methylation environment [259,260]. Moreover, bacterial methyltransferases can target nucleotides and proteins. Interactions with host chromatin or regulatory proteins can enact stable epigenetic changes that modify host transcriptional responses to infection or gut bacterial colonization [259].

In the gut intestinal pathogens influence host histone-modifying enzymes as a survival mechanism by delivering effectors that inhibit HDACs or alter HAT activity to suppress antimicrobial genes or skew inflammatory programs [261]. This epigenetic targeting pathogen survival mechanism is a documented immune-evasion strategy that can be exploited by both commensal bacteria for homeostasis and pathobionts for persistence [261].

Gut bacteria have also been reported to release outer membrane vesicles and deploy secretion systems specialized to translocate proteins, nucleic acids and small

molecules into host cells [259,261]. The net effect is that these compounds the host cell's nucleus proximal machinery and signaling centres and there alter the state of chromatin and transcriptional outputs in the recipient cells.

Bacterial epigenetic actions can ensue functional consequences. As in (i) immune modulation where epigenetic reprogramming can dampen or amplify innate and adaptive immune responses that can then affect tolerance, inflammatory dispositions, and susceptibility to infections and autoimmunity. (ii) Metabolic effects that reshape expression of metabolic genes in intestinal epithelia cells, liver and adipose tissue that in concert influence host energy balance and risk of metabolic disease (e.g., diabetes). Microbial driven epigenetic physiological and chemical signals are essential factors during early life and at stages of chronic colonization shifts in the gut as they can influence epithelial differentiation, gut barrier integrity and long-term tissue homeostasis [258–260].

8. Discussion

Given the constant migration of bacteria between the upper and lower respiratory tract sites, the lung microbiota is in a state of continuous flux. There is almost a consensus that by examining the diversity of the lung microbiome, new targets for therapeutic interventions can be identified. The gut-lung axis mediated by microbial components and metabolites such as postbiotics like SCFAs links intestinal health to respiratory health. Reviewing the clinical evidence of probiotics, fecal microbiota transplantation (FMT), and microbial metabolites modulating lung immunity and pathology in animal models and early clinical work, has provided technical challenges that help focus research on the lung microbiome. Given the complex nature of the interactions that exist between the microorganisms that inhabit the lung, host immunological defences and the environment, provide insight into the close physiological and pathological influences between the intestines and the lungs [74]. The reliance encountered is mainly associated with what the nature of the host-microbe cross-talk entails [74]. The cross-talk between intestinal and lung resident commensals necessitates an exchange of components and metabolites through the systemic circulation that indeed can then contribute significantly to the health of both sites [262].

The application of probiotics to influence the intestinal microbiome to manage disease processes has a long history [263], with formulations posited to have mutually beneficial relationships with humans pivotal in maintaining human health [263]. Consequently, members from the *Lactobacillus* genera have been advanced as natural immunobiotics [264]. Such immune-active actions have originated from the administration of *Lactobacillus* species in animal models and clinical trials that have reported alleviation of the symptoms of respiratory diseases such as asthma, respiratory tract infections, lung cancer and cystic fibrosis [264,265]. Of further interest has been the role that *Lactobacillus* species may have in regulating respiratory mucosal immunity. Murine models have shown that when the lungs were infected with *S. pneumoniae*, nasal administration of heat-killed *L. casei* increased resistance to infection, decreased pulmonary bacteria load, and increased survival [266].

The crosstalk between the gut and lung teaches that with microbiome modifications progressed with FMT interventions it may be possible to influence the condition of the lungs. In emphysema-induced mice, FMT coupled with diet modifications resulted in reduced lung inflammation, limiting alveolar destruction [238]. Interestingly FMT treatments resulted in increases in the relative abundance of bacterial families such as *Bacteroidaceae*, *Lachnospiraceae*, and *Ruminococcus* in the gut that are involved in the production and metabolism of SCFAs [238]. Improving the intestinal microbiome improved the tone of the immune system and reduced inflammation in the lungs.

Oral administration of SCFAs also decreased inflammation and alveolar destruction in emphysema mice [238].

Massalha and colleagues [267] have investigated FMT for improving lung immunity with checkpoint inhibitors. Currently, a Phase 2 clinical trial is investigating whether FMT can be used as an adjuvant therapy for metastatic lung cancer and also analyse its effect on the gut microbiome [268].

FMT using post-COVID microbiota obtained from patients was performed on mice to investigate whether post-COVID symptoms could be induced [269]. Lung histology revealed that post-COVID FMT resulted in an increase in inflammatory cells, and an increase in a-smooth muscle actin expression, indicating physiological dysfunction [269]. Gut microbiota modifications affect the lungs but whether it can also affect the lung microbiome has not been studied. The difficulty in studying this possible change exists as the biomass in the lungs is significantly smaller than that of the gut.

Exploring direct lung–targeted approaches with inhaled probiotics, prebiotics, synbiotics or postbiotics share the same goals as gut-delivered strategies. The aim being to modulate the local microbiota and host immunity. Differences in delivery, dosing, safety and tolerability present technical challenges for efficacious treatments. Hence, drug delivery for lung conditions such as asthma and COPD is achieved by inhalation devices such as nebulisers, dry powder inhalers, and pressurised metered dose inhalers. Nebulisers are an inexpensive and easy device to deliver drugs into the airways. A recent study explored the possibility of delivering LGG via a vibrating mesh and jet nebuliser [270]. Although only 10% of the loaded dose was deliverable to the lungs [270], the advantage of a nebuliser is that it can deliver doses larger than those from dry powder inhalers and pressurised metered dose inhalers. Hence

the lower delivery efficiency can be overcome by loading a higher dose to achieve the desired dose in the lungs.

Additionally, probiotic delivery to the lungs has employed dry powder formulations. The food industry has progressed dry processed powdered probiotics in a lyophilized form, that could be formulated to be inhalable. Numerous *in vitro* studies have reported promising inhalable results with spray dried powders of LGG, *L. plantarum* and *L. acidophilus*. Importantly also showing that they were individually safe against human lung-derived Calu-3 and A549 epithelial cell lines [271]. Moreover, *L. plantarum* in particular displayed antimicrobial properties by eradicating a common respiratory pathogen *P. aeruginosa* [271]. Similarly, a spray freeze dried *L. rhamnosus* GG powder was non-toxic against A549 human adenocarcinoma cell line whilst inhibiting *P. aeruginosa* growth [272].

An inhalable spray dried powder containing a blend of live *L. plantarum* BAA-793, *L. acidophilus* 4356, and LGG improved lung structure and function in murine models of COPD and bronchopulmonary dysplasia [273]. Nasal sprays is an alternative and promising drug delivery platform to the upper respiratory tract. A nasal spray containing *Bacillus* spores were successful in reducing RSV symptoms in children whilst demonstrating safety [216].

Currently, there is a paucity of human clinical studies investigating what constitutes the potential of direct respiratory delivery of probiotics to the respiratory tract. To therapeutically target the respiratory microbiome a precise requisite understanding of the respiratory microbiome is essential, the effects that clinical interventions may have on the equilibrium of the respiratory cohort of bacteria and what immunological effects the lung microbiome provides [274].

9. Conclusions

Respiratory infections of viral or bacterial origin are among the most common infectious diseases. The gut microbiota plays an important role in dictating the severity of these infections. The literature points towards a healthy gut equalling a healthy lung. Oral probiotics and postbiotics may be used to indirectly treat lung conditions.

The last two decades has been witness to an exponential increase in support and a rapid escalation in interest for the administration of probiotics, prebiotics, postbiotics and synbiotic (i.e., a probiotic + prebiotic) formulations that can act as mediators in health and disease [224]. The future challenges are extensive and complex. A recent report has advanced provisions into how industry and academia must adapt to probiotic research [224]. The posits are centred on how to maximize treatment success through targeted applications of probiotic strains such as narrated herewith with the clinical translation of inhaled probiotics to manage bacterial pathobiont and viral infections in the lungs. This being dependent on individual probiotic strain capabilities as well as the application of multiple advanced analytical technologies that can then further elucidate mechanistic understanding and fast-track the science of the microbiome in organ specific sites (e.g., the lungs).

The scientific pursuit of innovative treatments for lung diseases via the incorporation of nanoparticle technology of inhaled compounds is a plausible posit. Given that probiotics and postbiotics have shown high level of safety in the gut illustrates translational interest, albeit, without direct inhaled delivery. Direct pulmonary delivery would be the most efficient and effective delivery platform for postbiotic compounds and heat-killed probiotic bacteria to further investigate efficacy and safety in the management of lung infections.

Author Contributions

Conceptualization and design of the review framework, LV, AB, PK, H-KC ; interpretation of findings across studies, LV, AB, PK, H-KC; creating figures or tables that summarise key concepts or evidence, LV, AB, PK, H-KC; writing—original draft preparation, LV, AB, PK; writing—review and editing, LV, AB, PK, H-KC. All authors have read and agreed to the published version of the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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