The lung microbiome in interstitial lung disease

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Shareable abstract (@ERSpublications)
We outline key features of the lung microbiome that associate with alveolar immunity, pulmonary physiology and clinical outcomes in ILD in addition to discussing the implications of the oral–lung and gut–lung axis in ILD. https://bit.ly/3WvhY0h

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Abstract

Interstitial lung disease (ILD) is a heterogeneous chronic form of lung disease. The pathogenesis of ILD is poorly understood and a common form of ILD, idiopathic pulmonary fibrosis (IPF) is associated with poor prognosis. There is evidence for substantial dysregulated immune responses in ILD. The microbiome is a key regulator of the immune response, and the lung microbiome correlates with alveolar immunity and clinical outcomes in ILD. Most observational lung microbiome studies have been conducted in patients with IPF. A consistent observation in these studies is that the bacterial burden of the lung is elevated in patients with IPF and predicts mortality. However, our understanding of the mechanism is incomplete and our understanding of the role of the lung microbiome in other forms of ILD is limited. The microbiomes of the oropharynx and gut may have implications for the lung microbiome and pulmonary immunity in ILD but require substantial further research. Here, we discuss the studies supporting a role for the lung microbiome in the pathogenesis of IPF, and briefly describe the putative role of the oral–lung axis and the gut–lung axis in ILD.

Educational aims

- To understand key features of the lung microbiome that associate with clinical outcomes in ILD.
- To understand the potential role of the gut and oral microbiome in ILD.

Introduction

Interstitial lung disease (ILD) is a heterogeneous group of pulmonary disorders characterised by varying degrees of inflammation and fibrosis within the lung parenchyma [1]. In a subset of approximately 40% of patients with ILD, pulmonary inflammation and fibrosis is progressive with unchecked collagen deposition within the lung interstitium resulting in loss of efficient gas exchange units, progressive hypoxemia, chronic symptoms of cough and dyspnoea, and ultimately respiratory failure [1]. This form of fibrotic ILD is termed progressive pulmonary fibrosis and is associated with a poor prognosis [2, 3]. In Europe, the incidence of ILD ranges between 20.0–42.5 cases per 10^5 person-years and the overall prevalence ranges between 72.1–164.2 cases per 10^5 persons, while the incidence of progressive pulmonary fibrosis ranges from 17.9–38.3 cases per 10^5 person-years and prevalence ranges from 66.8–152.6 cases, respectively [4]. There are several proposed methods to classify ILDs. Recent evidence based guidelines provide a consensus classification system based on potential aetiological associations and include 1) idiopathic interstitial pneumonias *i.e.* idiopathic pulmonary fibrosis (IPF); 2) autoimmune ILDs; 3) exposure related ILDs *i.e.* hypersensitivity pneumonitis (HP); 4) ILDs with cysts or air space filling; and 5) sarcoidosis [5]. These pulmonary disorders share many clinical features and are often indistinguishable based on clinical presentation alone. However, they display distinct histopathological appearance and radiographic correlates. These conditions also carry varied prognosis with the most common form of idiopathic interstitial pneumonia, namely IPF, carrying the worst prognosis.

The pathogenesis of ILD

The pathogenesis of ILD is incompletely understood. Our current understanding is centred on the presence of an aberrant and unchecked wound healing response in genetically susceptible individuals as a result of recurrent alveolar epithelial injury [1]. Most studies of ILD pathogenesis have focused on IPF. It is thought that interactions between genetic susceptibility, the effects of ageing and environmental risk associate to promote recurrent alveolar epithelial injury in IPF. The alveolar epithelium consists mostly of type 1 alveolar epithelial cells (AT1), while type 2 alveolar epithelial cells (AT2) act to regenerate AT1 populations in injury [6]. Studies have highlighted dysfunction of AT2, and other studies have identified aberrant airway basaloid cells that promote dysfunctional repair [7–9]. Immune cells also play a prominent role, with studies supporting a role for monocyte recruitment and transition to pro-fibrotic macrophages in the IPF lung [10, 11]. Fibroblasts are key cellular effectors of wound healing and elimination of fibroblasts limits excessive collagen and matrix deposition during this process [12]. However, in IPF fibroblasts are resistant to elimination by apoptosis [13], differentiate to highly contractile myofibroblasts which deposit excess collagen and extracellular matrix molecules under the influence of the canonical pro-fibrotic cytokine transforming growth factor-β (TGF-β) [14].

One recent emerging finding is the potential role of the lung microbiome in the pathogenesis of ILD [15]. The microbiome consists of the collection of all microbe associated genomes within an ecosystem, while microbiota refer to the microbes that collectively inhabit said ecosystem [16]. Humans are holobionts, a shared host and microbial ecosystem, and human-associated microbiota typically reside on mucosal surfaces such as the gut, lung and oropharynx [16–18]. From these locations microbiota exert profound influence on human physiology and immunity, and perturbations in communities are associated with pathology [19]. Studies of the lung microbiome were not included in the human microbiome project [20]. However, the importance of lung microbiota in lung immunity and disease was quickly recognised [21]. In IPF, the exact mechanism through which lung microbiota contribute to pathogenesis is unknown. It is possible that alterations in the lung microbiome may contribute to alveolar epithelial injury, alveolar macrophage responses, promote reprogrammed or aberrant crosstalk among key participating cell types or augment cell production of pro-fibrotic cytokines systems such as interleukin-17 (IL-17) [22–25]. In this review we will discuss the observational and pre-clinical studies completed to date that have defined a role for the lung microbiome in ILD and highlight future potential avenues of investigation and therapeutic strategies. We will also address the putative role of the oral microbiome in ILD and highlight several novel studies evaluating the role of the gut microbiome in pulmonary fibrosis.

The lung microbiome

The lung microbiome is a unique low-biomass community that exists in a postulated dynamic state [26]. However, research in the lung microbiome has rapidly expanded since the first paper by Hilty *et al.* [27] in 2010. We now know that lung microbiota are metabolically active [28, 29], culturable from human lung samples [30], associated with alveolar immunity and predict clinical outcomes in acute and chronic lung disease [18]. Major features of microbial communities include microbial diversity (or α-diversity, the richness and evenness of microbial communities), the compositional analysis of microbial communities (or β-diversity) and the bacterial burden (or biomass). These key features are determined by an ecological model often referred to as "the adapted island model" of lung microbial ecology [31]. The main components of this model include immigration to the lung, replication within the lung and elimination from the lung (figure 1). This ecological model has been reviewed in detail elsewhere for the interested reader [31]. Importantly, dysfunction of these key components of immigration, replication and elimination are implicated in the pathogenesis of lung disease including ILD [15].

This revolution in lung microbiome studies has stemmed from the introduction of culture independent microbiology platforms which has facilitated high throughput detection and identification of bacterial, viral and fungal communities in both health and disease [18]. This work has been largely based on pioneering studies of the 16S rRNA gene, a highly conserved locus of the bacterial genome. When the 16S rRNA sequence has been amplified, these sequences can be aligned with taxonomic databases to identify taxa accurately to the genus classification. Shotgun metagenomic sequencing is an approach where both host and microbial DNA are sequenced, reference-based metagenomic analysis of the microbial metagenomic reads then allows for high-level identification to the strain and even isolate level of taxonomic classification. Recently developed metatranscriptomic approaches involve sequencing all RNA transcripts and have been successfully used to characterise microbial gene transcription in the lung. This approach has been applied to integrated host studies to investigate putative host and microbiota related mechanistic pathways associated with deleterious outcomes in disease [28, 29]. These approaches however have limited validation pipelines and require further refinement but are exciting and represent the future of lung microbiome studies.

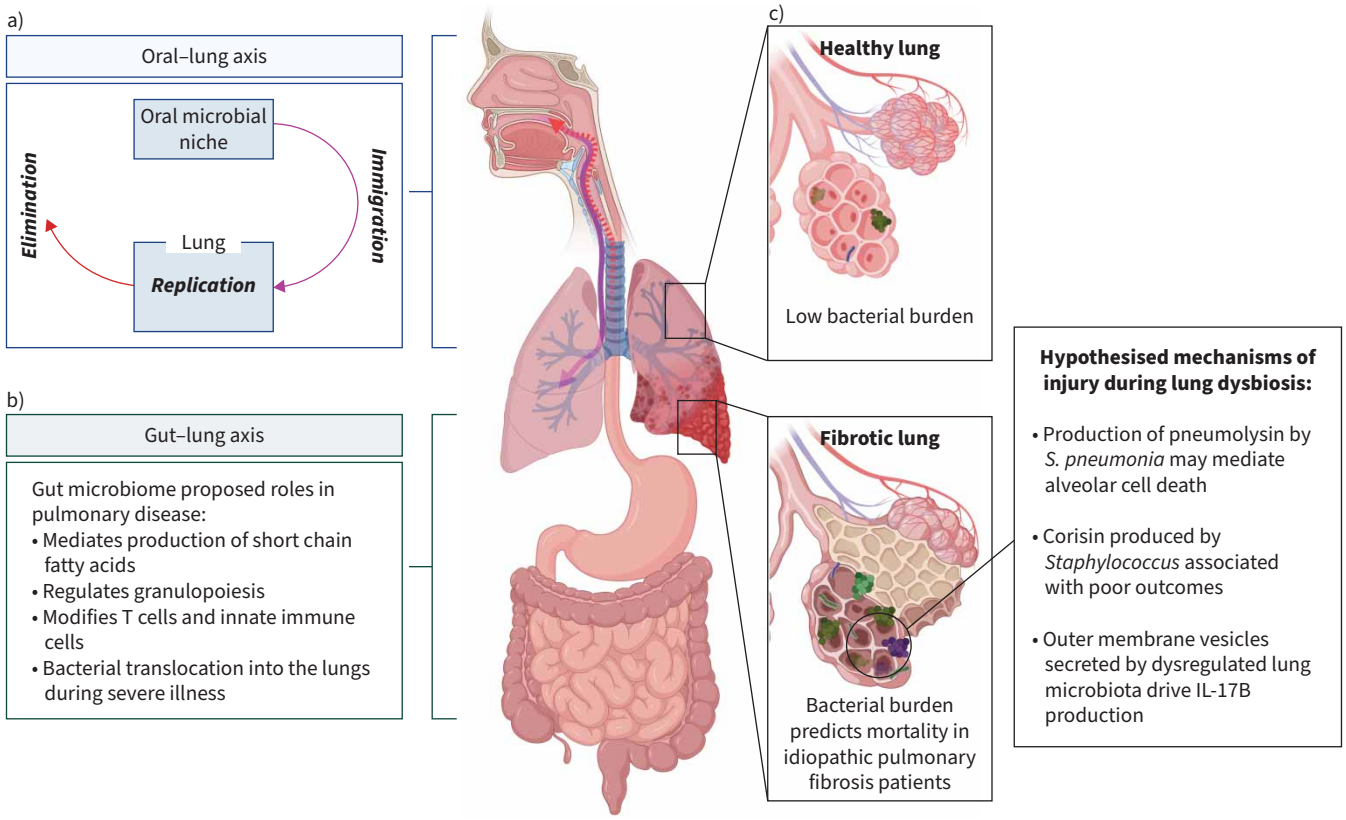

FIGURE 1 The lung microbiome in interstitial lung disease and the gut– and oral–lung axis. a) The oral–lung axis describes the immigration of oral microbiota to the lung across the respiratory tract by microaspiration. b) The gut–lung axis describes the potential impact of gut microbiota and its products on lung immunity and disease, including modification of regulatory T cells and innate lymphoid cells, the effect of circulating gut microbiota short-chain fatty acids and microbial ligands on bone marrow granulopoiesis, and the translocation of bacteria to the lung in addition to other mechanisms. c) The lung microbiome in idiopathic pulmonary fibrosis is characterised by an increased bacterial burden compared to healthy volunteers. Putative mechanisms associated with lung fibrosis include the production of bacteria-derived toxins such as pneumolysin (*Streptococcus pneumonia*) [32] and corisin (*Staphylococcus* genus) [33] and interactions between lung microbiota and interleukin (IL)-17 production by macrophages [22].

Over the past decade several well-designed studies have identified key changes in the lung microbiome that associate with clinical outcomes in IPF. As the bulk of the research has been conducted in patients with IPF, we will next highlight these studies and discuss the potential causal role for lung microbiota in IPF and other forms of ILD.

The lung microbiome in IPF

IPF is well-studied, associated with high morbidity and the diagnosis carries a very poor prognosis [34]. IPF results in severe architectural distortion of the lung whereby with progressive lung fibrosis, affected airways of the lung are subject to abnormal alveolar and airway remodelling with the development of subpleural reticulation, traction bronchiectasis and honeycomb cysts [5]. Conceivably, these changes would promote loss of local homeostasis and would significantly impair elimination of microbiota, replication of microbiota and possibly the immigration of microbiota to the fibrotic lung [15]. Thus, changes in the diversity, composition and burden of lung microbiota may play a key role in IPF pathogenesis.

The first examination of the lung microbiome in IPF was conducted in 2014. Molyneaux *et al.* [35] used 16S rRNA amplicon sequencing of bronchoalveolar lavage (BAL) fluid from patients with IPF, healthy age-matched volunteers and patients with COPD as a disease control. The authors measured bacterial biomass within BAL fluid using PCR of the 16S rRNA gene and found that bacterial biomass was significantly higher in patients with IPF compared to healthy and disease controls (figure 1). They reported an association between higher bacterial burden and an increased relative risk of death in this cohort. In addition, the authors found that patients with IPF with a greater than 10% decline in per cent predicted

forced vital capacity at 6 months had an increased bacterial burden compared to stable patients. Subsequent independent studies have validated this key observation showing that in cohorts of patients with IPF, a higher bacterial burden in BAL fluid is an independent predictor of disease progression [36–38]. A key question stemming from these observations was whether elevated bacterial burden represented a secondary phenomenon: was this simply the result of the considerable architectural distortion that occurs in the fibrotic lung? A subsequent follow up study confirmed that BAL bacterial burden associated with disease progression and this association was independent of the degree of architectural distortion or the presence of honeycombing and traction bronchiectasis [36]. These findings support a direct role for an increased bacterial burden in IPF pathogenesis. Acute exacerbations of IPF are acute to subacute worsening of symptoms with increased inflammatory changes on imaging and are associated with a poor prognosis [39]. Studies of the lung microbiome using 16S rRNA gene sequencing from BAL fluid suggest that bacterial burden is also elevated during these poorly understood acute inflammatory phases of the disease [40]. Strategies targeted at depleting bacterial burden in patients with IPF were considered to present a new therapeutic avenue. While initial studies of cotrimoxazole and azithromycin showed some promise [41, 42], two multicentre randomised control trials testing cotrimoxazole *versus* doxycycline to usual care (Clean-UP IPF) [43] and cotrimoxazole (EME-TIPAC) [44] were negative studies. However, these results require some pause, as our understanding of the regulation of bacterial burden in IPF is incomplete; the impact of long term antibiotics was assumed to reduce lung bacterial burden but the trials did not measure or confirm this, and previous studies have shown how long term systemic antibiotics can associate with dysregulated immunity and deleterious clinical outcomes [45, 46].

The microbiome is compositional, representing high dimensional bacterial communities which exist within niche environments in a shared host-microbial ecosystem. Changes in the abundance of one taxa may have key implications for other taxa and for the host. Recent work has identified a potential role for two key taxa in IPF, namely *Streptococcus* and *Staphylococcus*. Han *et al*. [47] initially identified an increased relative abundance of *Staphylococcus* and *Streptococcus* operational taxonomic units in BAL fluid associated with an increased risk of disease progression in the COMET observational study. However, these taxa were not present in the lungs of all patients. Work investigating the taxonomic composition of lung microbiota in patients with IPF and HP showed using clustering approaches that the HP and IPF lung microbiome were distinct and the major driver of difference in community composition was changes in the *Firmicutes* phyla (which includes the *Staphylococcus* and *Streptococcus* genera) [38]. This study also identified an increased relative risk of death with elevated *Streptococcus* and *Staphylococcus* [35]. However, again these taxa were not present in all study subjects and are therefore not directly attributable to all cases of mortality. *Streptococcus* has some important biological implications in pulmonary fibrosis. *S. pneumonia* produces a toxin, pneumolysin, which can mediate alveolar epithelial cell death in preclinical models [32]. The potential role of *Staphylococcus* has been further elaborated. The fibrotic lung is a salty microenvironment with higher concentrations of sodium ions observed in mouse models of lung fibrosis [33]. While the mechanisms are not fully understood, TGF-β is a negative regulator of sodium and chloride transport and may promote abnormal salt storage in fibrotic lung tissue [48]. A salty lung microenvironment in turn predisposes to salt loving or halophilic bacteria such as the *Staphylococcus* genera [49]. *Staphylococcus* produces a highly conserved peptide called corisin which is increased in the BAL fluid of patients with IPF and further increased during acute exacerbations [33]. D'Alessandro-Gabazza *et al*. [50] generated a corisin-targeted monoclonal antibody which ameliorated disease when delivered in preclinical models of lung fibrosis. These studies are supportive of a modifiable lung microbiome that can be leveraged to attenuate lung injury and fibrosis. However, large scale studies are required to refine our knowledge of the key taxonomic features of the lung microbiome that should be targeted and whether key microbial features can select patients who will respond to therapy or predict outcomes.

Microbial diversity is an important feature of microbial communities and a measure of the evenness and richness of community composition. Our understanding of diversity and its association with disease is derived from studies of the gut microbiome in which relatively lower diversity associates with disease and dysregulated immunity [16, 51]. We have previously shown that lower microbial diversity in BAL fluid from patients with IPF measured using the Shannon diversity index is associated with higher levels of pro-inflammatory pulmonary cytokines and a higher bacterial burden [37]. A second study suggests that lower diversity in BAL fluid from patients with IPF was associated with impaired pulmonary function with lower forced vital capacity and shorter survival [52]. Our understanding of how a less diverse lung microbial community may impact clinical outcomes in IPF is incomplete and will need further research.

The microbiome is a key regulator of immunity and is known to shape and tone immune responses within the lung microenvironment [24, 53]. Studies have shown key associations with immunity and lung

microbiota in IPF. Using pre-clinical models YANG *et al.* [22] identified associations between dysregulated commensal lung microbiota and Th17 inflammation which can be potently fibrogenic. Lung microbiota produced outer membrane vesicles that signalled through TLR-Myd88 innate immune system to drive IL-17B production and exacerbate lung fibrosis [22]. Innate immune receptors appear to play a considerable role in IPF, and previous work supported a signature of innate immune activation in peripheral blood tied to lung dysbiosis [54]. Using peripheral blood transcriptomic immune pathway analysis from patients enrolled in the COMET observational study, HUANG *et al.* [54] showed that pulmonary *Streptococcus* relative abundance associated with increased NOD-like receptor signalling in the peripheral blood. Several of these immune pathways were downregulated, correlated with lung microbial features and associated with progression-free survival. Further insightful work supported a host defence transcript signature in peripheral blood that correlates with lung microbiota and was persistently overexpressed in patients with progressive IPF [55]. We have also shown an association between lung microbiota and toll interacting protein, a downstream regulator of TLR2 and TLR4, key innate immune receptors for gram positive and gram-negative bacteria. Patients' heterozygote for the toll interacting protein singe nucleotide polymorphism rs5743890 demonstrated significantly dissimilar lung microbiota to wildtype patients, suggesting an interaction between genetic variation in innate immune response, lung microbiota and mortality in IPF [56, 57]. Taken together, lung microbiota in IPF correlate with both local and systemic immune responses suggesting that a proportion of the dysregulated immunity associated with IPF may be attributable to abnormal host–microbiota immune interactions.

Examining causation is regarded as broadly challenging in microbiome studies as it is unclear whether a microbial community produced the adverse outcome (*i.e.* disease) or whether the adverse outcome selected for the community [58]. Determining causal associations between lung microbiota and IPF have proven equally challenging [59]. Causal experiments using germ-free mice demonstrate that the absence of a microbiome is associated with attenuated lung fibrosis and reduced mortality compared to specific pathogen-free mice (with resident microbiota) [22, 37, 60]. However, in germ free mice aberrant immune responses develop in the absence of exposure to live bacteria and related antigens making direct comparison between systems difficult [61–65]. Given that lung bacterial burden is a validated and independent predictor of mortality in IPF, studies were designed to test for causal associations through modification of lung bacterial burden using antimicrobial therapy [43, 44]. While these studies were negative, several limitations are notable. In both CleanUP-IPF and EME-TIPAC, bacterial burden was not quantified pre and post therapy to confirm a therapeutic effect. In previous studies using long term antimicrobials, bacterial burden within the lung was not affected [66, 67]. In CleanUP-IPF both antimicrobial treatment arms were pooled in the trial analysis. However, when stratified by antimicrobial arm, doxycycline was associated with a trend towards benefit and cotrimoxazole associated with a trend towards harm [43]. It is conceivable that in an adequate sample size, doxycycline may have provided benefit. Azithromycin was shown to potentially reduce non-elective hospitalisation in patients with IPF in a small retrospective study [42]. A subsequent study examined the impact of azithromycin on the sputum microbiota of patients with IPF. The authors found that azithromycin decreased microbial diversity and altered community composition but did not decrease bacterial burden in sputum [68].

The lung microbiome is perturbed in IPF with changes in composition, burden and diversity associated with alveolar immune response and clinical outcomes. However, whether the lung microbiome represents a causal avenue for investigation and what features should be targeted to obtain clinical benefit remain unknown and require further work.

The lung microbiome in HP

HP is a form of ILD that is characterised by exaggerated pulmonary inflammation and injury on inhalation of specific antigens, most commonly fungal spores or avian proteins [69]. Exposure over time to a precipitating antigen promotes a fibrotic response resulting in a chronic fibrotic HP phenotype [70]. However, a risk factor for poor outcomes includes the inability to identify and/or remove the inciting antigenic exposure [71]. Several environmental microbes are considered inciting agents in HP including *Thermoactinomyces* spp. and *Acinetobacter* spp. [69]. As a result of an inciting environmental exposure which may be microbial, it is plausible that lung microbiota may be both causal and affected by progressive HP. However, the data examining the role of lung microbiota in HP is limited.

The largest study to date to evaluate lung microbiota in HP was conducted by INVERNIZZI *et al.* [38] in 2021. A cohort of 110 fibrotic HP patients underwent bronchoscopy and 16S rRNA gene sequencing of BAL fluid and microbial communities were compared to IPF and control cohorts. The composition of the HP lung microbiome varied significantly compared to IPF and controls. However, the bacterial burden in HP was lower than IPF and not associated with mortality. These interesting results suggested that while

fibrotic HP and IPF share many histopathological and radiographic findings, the immunobiology is likely significantly different and the role of the microbiome in this disorder requires further dedicated research [72]. However, a recent cross-sectional case control study design of both environment and human lung tissue samples identified a potential relationship between transfer of environmental microbiota and lung disease. Wu *et al.* [73] identified increased similarity of microbiota in paired environment and lung tissue samples in cases with occupational respiratory diseases compared to controls, suggesting a possible causal link between environmental microbiota and occupational lung disease. While these cases of respiratory disease exhibited histopathological changes that were not consistent with HP, the work remains relevant as a "proof-of-concept" example of how environmental microbiota transfer may contribute to lung disease. There is however, a paucity of large scale studies evaluating the role of the lung microbiome in HP.

The lung microbiome in connective tissue disease-related ILD

Connective tissue diseases (CTDs) are classified as a subgroup of autoimmune diseases where there is dysregulated immune responses to self-antigens resulting in chronic target-organ inflammation and dysfunction. In many cases, without intervention, progressive organ dysfunction and failure occur. While genetic predisposition and environmental exposures are implicated in the pathogenesis of CTD, several studies have highlighted potential causal associations between the microbiome and CTD [74]. A leading hypothesis for the causal association is the concept of "molecular mimicry", where structural similarity between pathogenic or commensal microbiota-derived products activates adaptive immunity with a cross-reacting host protein and self-antigen-promoted inflammation [75]. A key factor promoting increased morbidity and mortality in CTD is the development of ILD [76]. As an organ with an extensive vascular supply and embedded connective tissue, the lung is frequently a site of chronic inflammation in CTD [77]. However, studies of the role of the lung microbiome in CTD-ILD are limited.

In rheumatoid arthritis (RA) Scher *et al.* [78] used 16S rRNA gene sequencing of BAL fluid in early RA. Importantly, this patient cohort was naïve to immune modifying drugs. The authors found that in less severe RA microbial diversity was reduced compared to healthy controls and the lung microbiome was enriched with *Treponema* and *Porphyromonas*. In a study comparing BAL fluid from patients with dermatomyositis (DM) to RA, the investigators found no significant differences in microbial diversity across study groups including heathy controls [79]. Compositional analysis demonstrated significant enrichment of *Actinobacteria*, *Fusobacteria*, *Prevotella* and *Corynebacterium* in both DM and RA compared to controls. Data on the role of the lung microbiome in systemic sclerosis is limited and we are not aware of any observational human studies examining lung microbiota in systemic sclerosis related ILD. However, early life antimicrobial exposure in experimental models of systemic sclerosis was associated with aggravated lung fibrosis suggesting a potential role for microbiome disruption [80].

There is a need for robust data generation and clarity on the role of the lung microbiome in CTD-ILD as a growing body of evidence has established associations between lung microbiota and IPF. Given therapies that modify the immune system by inhibiting T cell proliferation (mycophenolate) are first line in CTD-ILD [81], and are harmful in IPF [82], an improved understanding of these host microbiota interactions will ensure further safety and refined therapeutic strategies.

The oral–lung axis in ILD

The oropharynx and the lower respiratory tract are anatomically contiguous, but, overall, there are significant differences in many factors within these ecological niches including air flow, host defence, surfactant production and pH, among others. Nevertheless, studies have consistently demonstrated significant overlap in oral and pulmonary microbial communities when sampling across both ecological habitats with a decreasing biomass from the oropharynx to the lower lobes in health [27, 83–85]. The adapted island model of lung ecology states that the lung microbiome is dependent on microbial immigration from the oropharynx (figure 1) [31]. The method through which oral microbiota populate the lung is considered to be subclinical microaspiration [31].

Microaspiration, the aspiration of small quantities of material from the oropharynx to the lung occurring during sleep or reduced states of consciousness, is common in humans even in health [86, 87]. We have recently shown that the microbial features of the buccal microbiome, a highly adapted oral microbial niche dominated by *Streptococcus mitis* spp., associate with IPF disease severity and predict mortality [88]. *S. mitis* dominates buccal communities and maintains a relatively lower buccal microbial diversity. Both a higher relative abundance of *S. mitis* and a lower microbial diversity were associated with improved survival in IPF [88]. The relative abundance of BAL *Streptococcus* (operational taxonomic units) in the COMET observational study was associated with an increased risk of disease progression and subsequently

validated in other studies [38, 47]. *Streptococcus* species, excluding *S. pneumonia*, can induce significant airway inflammation and injury on dispersion to the lower respiratory tract by aspiration [89]. Preclinical models of experimental aspiration (*Streptococcus*, *Prevotella*) demonstrate a persistent Th17 immune response after aspirated bacteria have been eliminated from the lung, a cytokine-based immune system with a known fibrogenic role [23, 90]. Taken together, the dispersion of commensal bacteria from the mouth to the lung can plausibly induce injury and pro-fibrotic immune responses in ILD. Furthermore, there is evidence of an increased frequency of oropharyngeal dysfunction in case series of patients with ILD suggesting that oral immigration to the lung is maladapted. Further work is required to integrate oesophageal dysmotility, aspiration and oropharyngeal dysfunction with the immunobiology of IPF and other ILDs [91, 92].

The gut–lung axis in ILD

The gut–lung axis is a poorly defined term that refers to the incompletely explored association between the gut microbiome and its functional role in pulmonary diseases. There are several postulated pathways including gut microbiome-meditated production of short-chain fatty acids and the regulation of granulopoiesis, modification of regulatory T cells and innate lymphoid cells [93–98] (figure 1). Translocation of gut bacteria to the lung because of damage or reduced integrity and barrier function of the gut has been associated with acute lung injury and acute respiratory distress syndrome [99]. Several studies have demonstrated phenotypic differences in response to lung injury and lung fibrosis in the bleomycin model that appears dependent on microbiota [60, 100, 101]. However, the differential contribution of compartmentalised microbiota (gut *versus* lung) has been challenging to elucidate accurately in pre-clinical models [60].

To our knowledge, to date there are no human observational studies published investigating the role of gut microbiota in idiopathic interstitial pneumonias including IPF. However, in CTD-ILD studies have examined the gut microbiome and metagenome to better understand differences across disease groups and healthy controls. A recent study in DM patients with ILD showed that DM patients had lower gut microbial diversity compared to controls [102]. Patients with DM associated ILD had altered gut microbiota composition with significant increases in Proteobacteria abundance compared to controls. In addition, functional metagenomic pathway analysis identified correlations between Proteobacteria and lipopolysaccharide synthesis and transport, suggesting low grade Gram-negative mediated gut injury. Patients with Sjogren's syndrome have lower microbial diversity than controls and altered composition with enrichment of *Lactobacillus salivarius* in ILD patients, increased abundance of the metabolic superpathway of I-phenylalanine biosynthesis and increased virulence genes involved in bacterial colonisation and invasion [103]. Another study of myositis patients demonstrated gut enrichment with *Streptococcus* and *Lactobacillus* [104]. The abundance of *Lactobacillus* correlated with serological markers of disease activity (erythrocyte sedimentation rate, anti-Ro52 antibodies) while the abundance of *Roseburia* was negatively correlated with the presence of ILD. In RA, studies have demonstrated significant gut dysbiosis which is partially mitigated by RA-directed treatment [105]. Further study in patients with RA and RA-ILD identified significant changes in bacteria but also virus and fungi present within the microbiome of patients with ILD, suggesting important multi-kingdom interactions that may aid in diagnostics and contribute to pathogenesis [106].

Overall, our understanding of the impact of changes in the composition and function of the gut microbiome in ILD is in its infancy. Considerable dedicated work, including well-designed observational and mechanistic studies, is required to build on the limited number of correlative studies already published to generate new potential therapeutic strategies in ILD.

Conclusion

The lung microbiome is considered an interesting putative treatment target in ILD. Robust observational and mechanistic data supports the role of bacterial burden in the airways and innate immune interactions as important contributors to IPF pathogenesis. However, our understanding of the functional implications of alterations in the diversity and composition of lung microbiota is limited in ILD and we know extraordinarily little of how these functional and compositional changes alter immune cell interactions in the fibrotic lung. While broad spectrum antibiotic trials have been completed, the lack of "a priori" strategies to randomise based on the microbiome and the lack of data on the impact of the long-term antibiotics on the composition and function of the microbiome limits the conclusions of these studies. Dedicated studies of the lung, gut and oral microbiome in ILD are necessary to determine in detail and with rigor the potential therapeutic avenues harboured by the microbiome.

Key points

- The lung microbiome consists of low-biomass metabolically active microbial communities in health.
- Key features of the lung microbiome include microbial diversity, composition and biomass or burden.
- The lung microbiome is thought to follow an adapted island model of microbial ecology whereby its key features are regulated by immigration of microbes to the lung, replication of microbes within the lung and elimination of microbes from the lung.
- Our understanding of the microbiome in ILD is largely based on studies of patients with IPF, which is the most common form of ILD and carries the worst prognosis.
- An increased lung bacterial burden occurs during exacerbations. In stable patients increased bacterial burden is an independent predictor of mortality in IPF.
- The composition of the lung microbiome is altered in IPF, and an increased relative abundance of *Staphylococcus* and *Streptococcus* associates with an increased relative risk of death in patients where these taxa are detectable in bronchoalveolar fluid.
- The oral–lung axis refers to the overlap in oral and pulmonary microbial communities that occurs because of dispersion or microaspiration across the respiratory tract.
- The gut–lung axis refers to the impact of gut microbiota (and related products including short-chain fatty acids) on pulmonary immunity.

Self-evaluation questions

1. In idiopathic pulmonary fibrosis, an elevated bacterial burden in bronchoalveolar fluid is associated with which of the following:
    a) Mortality
    b) Traction bronchiectasis
    c) Honeycomb cyst formation
    d) Extent of lung fibrosis on computed tomography of chest
2. In idiopathic pulmonary fibrosis, which of the following taxa, when detectable in bronchoalveolar fluid, demonstrate an association with an increased relative risk of death with increasing abundance (select all that apply):
    a) *Lactobacillus*
    b) *Corynebacterium*
    c) *Staphylococcus*
    d) *Streptococcus*
3. Is the following statement true or false? In hypersensitivity pneumonitis, an increased lung bacterial burden in bronchoalveolar fluid is associated with an increased relative risk of death.
4. Is the following statement true or false? Lung bacterial burden in bronchoalveolar fluid is elevated compared to healthy controls in both patients with idiopathic pulmonary fibrosis and hypersensitivity pneumonitis compared to healthy controls.

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Suggested answers

1. a. Mortality.
2. c. *Staphylococcus*, d. *Streptococcus*.
3. False.
4. True.