The contribution of infection and the respiratory microbiome in acute exacerbations of idiopathic pulmonary fibrosis

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Microbial dysbosis plays a role in the pathogenesis of IPF and further changes occur during acute exacerbations despite exclusion of overt infection. http://bit.ly/2MQkfBN

Cite this article as: Invernizzi R, Molyneaux PL. The contribution of infection and the respiratory microbiome in acute exacerbations of idiopathic pulmonary fibrosis. *Eur Respir Rev* 2019; 28: 190045 [https://doi.org/10.1183/16000617.0045-2019].

ABSTRACT Idiopathic pulmonary fibrosis (IPF) arises in genetically susceptible individuals as a result of an aberrant wound-healing response following repetitive alveolar injury. The clinical course of the disease remains both variable and unpredictable with periods of more rapid decline, termed acute exacerbation of IPF (AE-IPF), often punctuating the disease trajectory. Exacerbations carry a significant morbidity and mortality, and their exact pathogenesis remains unclear. Given the emerging evidence that disruption and alteration in the lung microbiome plays a role in the pathogenesis and progression of IPF, this review discusses the current knowledge of the contribution of infection and the respiratory microbiome to AE-IPF.

Introduction

Although the pathogenesis of idiopathic pulmonary fibrosis (IPF) remains poorly understood the condition is thought to arise in genetically susceptible ageing individuals as a consequence of repeated microinjuries to the alveolar epithelium by environmental triggers [1]. Over time, this process results in an aberrant wound-healing response, which leads to fibrosis and causes the progressive structural destruction of the lung [2, 3]. This suggests both environmental and host factors play a role in IPF, likely with bidirectional interaction between the two. The clinical course of the disease is both variable and unpredictable with significant heterogeneity among individual patients [4]. Whatever trajectory the disease adopts it can be punctuated by unpredictable periods of acute deterioration; episodes termed acute exacerbations of IPF (AE-IPF). These are defined as acute, clinically significant respiratory deterioration with no identifiable cause, characterised by new, widespread alveolar abnormalities [5]. The exact incidence of acute exacerbations is unclear but seems to range between 4–20% per year with these episodes conferring a substantial morbidity and mortality (often >50%) [6, 7]. Known triggers for AE-IPF include surgical lung biopsy, lung resection and drugs [8–11]. Nonetheless, our current understanding of the pathophysiology of AE-IPF is limited and it remains unclear whether AE-IPF represent an accelerated

Submitted article, peer reviewed.

Received: April 24 2019 | Accepted after revision: June 16 2019

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phase of the underlying fibrotic condition or a response to an occult trigger. Given the growing evidence for the role of infection in the pathogenesis and progression of IPF [12, 13], in this review we seek to explore the potential contribution of infection and the microbiome in acute exacerbations.

The lung microbiome: a complex and dynamic community of microbes

The epithelial surfaces of the human respiratory tract have historically been described as sterile. More recently, novel culture-independent techniques have debunked this notion by demonstrating that the respiratory tract harbours a complex and dynamic community of microbes which play a role both in health and disease [14]. High-throughput sequencing of the 16S rRNA gene is the most commonly used approach to study bacterial communities. Groups of bacteria sharing similar gene sequences are clustered together and identified by comparison to 16S rRNA reference databases. The commensal, symbiotic and pathogenic microorganisms which inhabit the lower airways are collectively defined as the "lung microbiome". In health, the primary routes of microbial immigration to the lungs include microaspiration, inhalation of air-borne bacteria and direct dispersion along mucosal surfaces [15]. Of these, microaspiration is probably the dominant mechanism, as the composition of the oral microbiome most closely resembles the lung microbiome. It is also expected to be the most relevant to IPF given the strong association with reflux [16]. Elimination is achieved through mucociliary clearance, coughing, and innate and adaptive immune defences which selectively recognise, kill and clear the microbes [17]. The quantity and composition of the microbiome is influenced by the balance between the rate of immigration and clearance. The discovery of the lung microbiome has prompted the exploration of its role in a variety of respiratory conditions, including IPF. These molecular techniques have not only enabled researchers to characterise niche-specific microbial communities in the respiratory tract of patients and healthy individuals, but have also demonstrated associations which point towards the interaction between the microbiome and the host as key in the aetiology and progression of lung disease [15, 18–20].

The role of infection and the microbiome in AE-IPF

Historically, research investigating the role of infections in the pathogenesis of IPF and as triggers of AE-IPF has focused primarily on viruses. When considering the role of infection in AE-IPF, it is important to remember these are events that, prior to the latest revised diagnostic criteria [5], specifically required the exclusion of any infective trigger [21]. Therefore, the following findings are all in cohorts of patients where overt clinical infection has been excluded.

It has been hypothesised that chronic viral infection may act as a persistent antigenic stimulus or co-factor in susceptible hosts, triggering fibrosis, and there is growing evidence both from human and animal studies to support this [22, 23]. The human herpes virus (HHV) family has received the most attention as either an aetiologic or exacerbating agent of IPF [24, 25], but a number of other viral infections have been consistently associated with IPF and AE-IPF (table 1).

There is growing evidence, albeit associative, suggesting that subclinical or occult viral infections may play a pathogenic role in the development of AE-IPF [32]. Molecular analysis of viral particles and bronchoalveolar lavage (BAL) revealed infection in 19 out of 43 cases of AE-IPF, including 12 cases of torque teno virus (TTV) in the exacerbation cohort with no viruses detected in the stable IPF group [26]. Supporting this observation, IPF subjects who were found to be seropositive for TTV-DNA demonstrated a worse survival in an independent cohort [30]. More common respiratory viral pathogens have also been detected using molecular techniques during exacerbations, including respiratory syncytial virus and cytomegalovirus [27]. Having excluded infection clinically, a cohort of 37 IPF patients experiencing an exacerbation underwent surgical lung biopsy and over one-third exhibited evidence of viral infection based on immunohistochemistry or microarray [28]. More recently, viral sequences were profiled in nasopharyngeal swabs of 30 stable IPF and 30 AE-IPF patients which revealed higher virus-positive rates in the AE-IPF cohort (60%) compared to stable disease (43%) [29]. HHV was the most prominent virus in the AE-IPF group as well as influenza A [29]. As AE-IPF have been reported following influenza A vaccination, its presence suggests that cold-associated infection may potentially trigger these events [31]. Overall these observational studies highlight a potential role for viral infections, although the supporting mechanistic role for airborne viruses is insufficient to prove their causal role in AE-IPF.

While the role of viruses has been extensively explored, the long-held, but incorrect, theory of sterility of the lung outside of clinical infection means historically little work has evaluated the role of bacteria in AE-IPF. Using quantitative methods, the first study to investigate the presence of bacterial growth in the lower airways of 22 stable IPF patients confirmed the presence of known pathogens including *Haemophilus*, *Pseudomonas* and *Streptococcus* in 36% of the cases [33]. More recently, molecular culture-independent techniques have been used to characterise the microbial composition in the lower

TABLE 1 Summary of studies linking viruses with the pathogenesis, progression and acute exacerbation of idiopathic pulmonary fibrosis (IPF)

Virus	Main conclusions	[Ref.]
HHV	EBV detected in serum of 12 out of 13 subjects with IPF but not in patients with other forms of ILD	[22]
	MHV-68 triggers an exaggerated fibrotic response in mice	[23]
	Increased incidence of EBV in BAL and lung biopsies of IPF subjects compared to controls	[24, 25]
	EBV detected in BAL of two out of 43 AE-IPF subjects	[26]
	CMV detected in BAL of two out of 43 AE-IPF subjects	[27]
	A total of 38% of AE-IPF subjects exhibited evidence of CMV infection	[28]
	HHV detected in nasopharyngeal swabs of 15 out of 30 AE-IPF subjects and four out of 30 individuals with stable IPF	[29]
TTV	TTV detected in BAL of 12 out of 43 AE-IPF subjects	[26]
	No evidence of TTV in BAL of stable IPF subjects	[30]
	Increased mortality in IPF subjects with presence of TTV-DNA in serum compared to IPF subjects with no TTV-DNA	
Influenza A	Influenza A detected in nasopharyngeal swabs of 12 out of 30 AE-IPF subjects but not in individuals with stable IPF	[29]
	A case of AE-IPF was reported following pandemic influenza A vaccination	[31]

HHV: human herpes virus; TTV: torque teno virus; EBV: Epstein–Barr virus; ILD: interstitial lung disease; MHV-68: murine γherpes virus-68; BAL: bronchoalveolar lavage; AE-IPF: acute exacerbation of IPF; CMV: cytomegalovirus.

airways of IPF patients. Recent studies suggest that microbial dysbiosis may be linked to disease outcome (table 2).

The Correlating Outcomes with Biochemical Markers to Estimate Time-Progression (COMET) study was the first to truly evaluate the lung microbiome in IPF patients [34]. The authors demonstrated an increased abundance of either *Streptococcus* or *Staphylococcus* was associated with a significant reduction in progression-free survival time [34]. The enrichment of these two bacteria was observed in less than half of the cohort; therefore, it remains unlikely that these organisms alone can explain the disease pathogenesis. Consistent with the COMET study, Molyneaux *et al.*[36] demonstrated that the lower airways of patients with IPF were not sterile and indeed were more likely to harbour potentially pathogenic *Haemophilus*, *Neisseria* and *Streptococcus* species than healthy controls. The authors also

TABLE 2 Summary of studies linking the respiratory microbiome with the pathogenesis, progression and acute exacerbation of idiopathic pulmonary fibrosis (IPF)

Diagnosis	Main conclusions	[Ref.]
IPF	Positive BAL cultures in eight out of 22 stable IPF subjects: *Haemophilus influenzae* (n=2), *Haemophilus parainfluenzae* (n=2), *Moraxella catarrhalis* (n=1), *Pseudomonas aeruginosa* (n=1), *Proteus mirabilis* (n=1), *Streptococcus pneumonia* (n=1)	[33]
	Increased abundance of *Streptococcus* OTU1345 and *Staphylococcus* OTU1348 is associated with a significant reduction in progression-free survival in IPF	[34]
	Streptococcus pneumoniae triggers progression of pulmonary fibrosis through pneumolysin in two different mouse models	[35]
	Increased bacterial burden in IPF subjects compared with COPD and healthy controls	[36]
	Higher bacterial burden at the time of diagnosis predicts disease progression in IPF	[37]
	Germ-free mice protected from mortality following bleomycin exposure	
AE-IPF	Four-fold increase in bacterial burden in AE-IPF subjects compared to stable IPF	[38]
	Increased abundance of *Campylobacter* and *Stenotrophomonas* and decreased abundance of *Veillonella* in AE-IPF compared to stable IPF	
	Positive sputum cultures in nine out of 48 AE-IPF subjects: *Klebsiella pneumoniae* (n=2), *Mycobacterium tuberculosis* (n=4), *Pseudomonas aeruginosa* (n=1), *Loffi Acinetobacter* (n=1), other (n=1)	[29]

AE-IPF: acute exacerbation of IPF; BAL: bronchoalveolar lavage.

identified the presence of a higher overall bacterial burden in IPF, which was associated with a reduced progression-free survival time in subjects with IPF. Having characterised the microbiome in stable IPF, the same authors then set out to examine for potential changes during acute exacerbations. Using a cohort of matched stable and exacerbation patients who underwent bronchoscopy, the authors were able to demonstrate a further increase in bacterial burden during an exacerbation, despite the presence of negative clinical cultures [38]. Subjects experiencing an AE-IPF exhibited a marked change in the respiratory microbiome with an increase in *Campylobacter* and *Stenotrophomonas* species compared to stable disease. Both of these organisms are potential respiratory pathogens but the presence of *Campylobacter* also leads the authors to suggest their findings support the role of reflux and aspiration with AE-IPF. One weakness of the study was that there were only two paired samples from the same individuals when stable and experiencing an exacerbation. The stark changes in these two pairs of samples is intriguing, especially as in one individual there was a marked outgrowth of streptococcal species from the baseline microbiota which then dominated the exacerbation state. This result is concordant with findings from two distinct mouse models, where infection with *Streptococcus pneumoniae* induced exacerbation of established lung fibrosis [35].

More recently, Weng *et al.* [29] have employed molecular techniques to examine sputum cultures of 170 patients experiencing an exacerbation. They detected 38 different bacterial strains with gram-negative bacteria accounting for 89% of pathogens: *Klebsiella pneumonia* (26%), *Mycobacterium tuberculosis* (21%), and *Acinetobacter baumannii* (10%) dominated the sputum samples [29]. Together these studies demonstrate that alterations in the respiratory microbiome occur during acute exacerbations but they do not provide any functional or mechanistic insights. In a recent study, O'Dwyer *et al.* [37] tried to overcome this issue in an attempt to move from correlation to causation. A germ-free mouse model of fibrosis was used to explore the role of bacteria on lung inflammation and fibrinogenesis. Following bleomycin exposure, germ-free mice were protected from mortality, even though they exhibited similar severity of pulmonary fibrosis when compared to conventional mice [37]. The authors postulate that this may reflect the clinical observation that subjects with IPF not only die from progressive fibrosis but also from distinct inflammatory causes of acute-on-chronic respiratory failure including respiratory infections and AE-IPF. While there are several limitations of animal models in IPF, collectively, this study shows how preclinical germ-free models are a useful tool to investigate potential mechanisms of host-microbiota interactions and provides the first causal evidence that the microbiome participates in the pathogenesis and mortality of fibrotic lung disease.

Conclusions and future directions

The role of the respiratory microbiome in the pathogenesis and progression of IPF is just beginning to be elucidated. Clinically, acute exacerbations are important events in the natural course of IPF which carry a significant morbidity and mortality. While there is a paucity of information regarding the aetiology, pathophysiology and clinical management of acute exacerbations, an increasing number of findings indicate that infection, both viral and bacterial might be involved. Recent advances in sequencing technologies have allowed the use of molecular microbial technologies to characterise the respiratory microbiota in these patients demonstrating a clear change in the respiratory microbiome, as well as an increased bacterial burden in BAL compared to stable disease. However, the microbiome has not been fully characterised and further research should also investigate organisms other than bacteria and viruses, including fungi. It is unclear whether a higher bacterial load and an altered microbiome during an acute exacerbation reflect an active infection, increased aspiration or occur as a result of widespread diffuse alveolar damage. As sequencing DNA from BAL provides a snapshot in time of the microbial diversity, future research should incorporate longitudinal sampling before, during and after an AE-IPF. Furthermore, there is a pressing need to move from correlation to causation [39]. In order to do so, functional studies examining host-microbiome interactions as well as better models of AE-IPF are needed, given existing models fail to fully recapitulate physiologic findings in IPF. Current consensus is that all patients experiencing an exacerbation should receive broad spectrum empirical antibiotics even if no overt infection can be identified [40]. While this is likely driven by the use of immunosuppressive agents, the molecular microbial data may support this empiric therapy. Indeed, there is already some evidence that azithromycin may be beneficial in AE-IPF [41]. Ultimately, understanding the role of the microbiome in AE-IPF will allow its manipulation and may provide an opportunity for targeted therapeutic intervention.

Conflict of interest: R. Invernizzi has nothing to disclose. P.L. Molyneaux has, *via* his institution, received speaker's fees from Roche and Boehringer Ingelheim.

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