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Evaluation of the lung microbiome as a therapeutic target in the management of idiopathic pulmonary fibrosis: role of antioxidant/antibiotic combination therapy

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Abstract. – OBJECTIVE: Changes in the composition of the lung microbiome influence many lung diseases, including idiopathic pulmonary fibrosis (IPF), with a demonstrated association between the progression of IPF and the assessed pulmonary microbial community. A hypothesis to explain the pathogenesis of IPF is that an oxidant-antioxidant imbalance causes repeated epithelial cell injury and endogenous and exogenous antioxidants/redox modulators influence fibrogenesis, protect the lung against fibrosis, and prevent its progression.

MATERIALS AND METHODS: The present article is focused on Lung Microbiome in Idiopathic Pulmonary Fibrosis and the role of Antioxidant/Antibiotic Combination Therapy.

RESULTS: N-Acetylcysteine (NAC) at concentrations possibly achievable by nebulization showed an *in vitro* synergy with colistin against *S. maltophilia* isolates (a common coloniser of the respiratory tract of patients with chronic lung disease). Combined NAC plus colistin seems to have a beneficial role in restoring oxidant injury which may be related to its antioxidant effect. Progress has been made in the identification of the lung microbiome and the possible causal role of bacteria in the IPF pathogenesis. Recent studies suggest that antibacterial therapy in combination with antioxidant therapy may be a promising avenue for the treatment of this untreatable disease. Novel routes of administration are also an important area of research and studies assessing the use of inhaled NAC in patients with IPF could be considered.

Key Words:
Idiopathic pulmonary fibrosis, N-Acetylcysteine, Lung microbiome, Inhaled, Antioxidant.

Introduction

Although the history of gastric disturbances dates back to Hippocrates, when Marshall et al[1] put forward their theory in the early 1980s on the role of *Helicobacter pylori*, they met derision and disbelief. In an era when the general conviction was that bad diet, stress, and lifestyle were the major causes of gastric ulcers, proposing that they were caused by an infectious disease represented a seismic shift. Marshall et al[1] won the Nobel Prize over 20 years later for the discovery of the bacterium *H. pylori* and its causative role in severe gastric inflammatory disease. The Nobel Committee commented that "thanks to the pioneering discovery by Marshall et al[1], peptic ulcer disease is no longer a chronic, frequently disabling condition, but a disease that can be cured by a short regimen of antibiotics and acid secretion inhibitors". Subsequently, others showed that *Helicobacter pylori* plays a fundamental role in a range of gastric conditions[2]. Marshall et al[1] discovery represented a sea change in how the scientific establishment viewed the possible involvement of microbes in chronic inflammatory conditions and paved the way for investigation

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of the role of microflora in other organ systems. Therefore, the manipulation of the flora became a realistic therapeutic and prophylactic strategy for many infectious, inflammatory, and even neoplastic diseases[3]. In particular, if the intestinal barrier fails, the normal physiological barrier function is lost and immune cells come into direct contact with luminal antigens and the delicate balance is lost. This alteration is the basis for the pathogenesis of many intestinal and extraintestinal diseases, including infectious enterocolitis, inflammatory bowel disease, irritable bowel syndrome, small intestinal bacterial overgrowth, celiac disease, hepatic fibrosis, food intolerances, and also allergies[4-8].

The Lung Microbiome

In 2010 scientists began to question the long-held scientific wisdom that healthy lungs are sterile and devoid of resident microbes. Paradoxically, while 18 body sites, including the nose and oral cavity, were studied in the initial Human Microbiome Project (HMP), the lower respiratory tract (LRT) was not included[9,10]. The idea that the lung was devoid of microorganisms does not stand up to careful scrutiny as it is a dynamic environment constantly bombarded with debris and microbes that make their way from the mouth and nose through the trachea. We now know that although it is less populated than the mouth or gut, the respiratory tract is colonised by a persistent community of microorganisms. It is this community of microorganisms, the lung microbiota, that changes when the dynamic homeostasis between host and microbiome is disrupted[11] (Figure 1). Next, the LRT was included in the American HMP program as a body site and the lung microbiome has become a fast-growing field of research[12].

The introduction of independent culture, compared to standard microbiological techniques, was instrumental in showing that the respiratory tract of healthy subjects and patients with respiratory diseases contains a complex microbial community, including bacteria, fungi, phages, and viruses[13]. It should be remembered, however, that there are major differences in the gut microbiota and that of the LRT. Microbes have to adapt to the specific conditions in the lung environment (highly aerated organ, antimicrobial peptides,

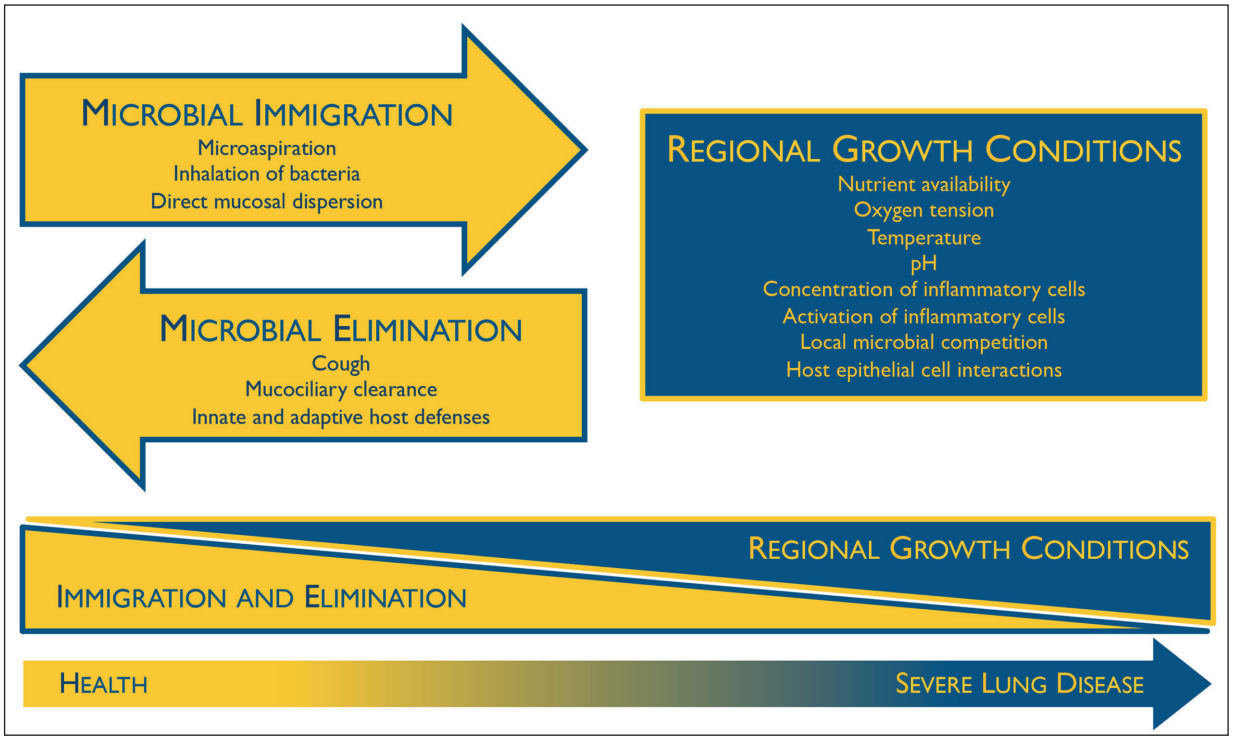

Figure 1. Ecological determinants of the respiratory microbiome. The constitution of the respiratory microbiome is determined by three factors: microbial immigration, microbial elimination, and the relative reproduction rates of its members. In health, the community membership is primarily determined by immigration and elimination; in advanced lung disease, the membership is primarily determined by regional growth conditions [Dickson and Huffnagle 2015[10]].

highly immune active mucosa, phospholipid-rich environment, mucociliary clearance) and thus specialised strains are likely to be present[13]. In the GI tract, there is a high biomass (10^{14} bacteria), well-known interactions, and broad modifications in disease states, while in the LRT the biomass is low (10-100 bacteria per 1000 human cells), there is high noise and changes in microbial composition and function/dysbiosis occur only at a late stage[14]. It may be that in the lung the dysbiosis signal is confused with noise until the disease progresses to an advanced stage[15]. Emerging evidence also suggests that the gut microbiome plays a role in lung diseases through the modulation of systemic immune responses. For example, dysbiosis of the gut microbiota can cause systemic inflammation and an increase in opportunistic pathogens which in turn lead to chronic inflammation in the lungs. Therefore, the lung microbiota could affect or be affected by microorganisms or immune response at a distal site[4,16]. So, while the precise mechanisms mediating the gut-lung cross-talk and the role of the gut/lung microbiotas in maintaining this cross talk remain to be elucidated, it is thought that the gut-lung axis does not occur in one direction but is a two-way street whereby stimuli to the gut are transferred to the lung and vice versa[17].

Current Molecular Techniques for Microbial Detection

The most widely used method to study bacterial communities involves high-throughput sequencing of amplicons of the 16s ribosomal RNA (rRNA) gene, a small and highly conserved locus of the bacterial genome which is present in all bacteria and allows genus/species identification. However, 16s rRNA sequencing may not be able to differentiate between species with different immunogenicity/pathogenicity and other methodologies have therefore been investigated[18]. For example, whole genome shotgun sequencing (microbial communities sampled directly from their natural environment, without prior culturing "metagenomics") has been investigated but a report comparing 16S rRNA gene sequencing and whole genome shotgun sequencing showed significant differences in the bacterial diversities of the same sample. The authors concluded that 16S rRNA gene sequencing can profile the bacterial communities in greater detail than metagenomics[19]. Nevertheless, the use of culture-based techniques is still useful in the determination of viability, in speciation, and in microbial phenotyping[20].

While there are now relatively reliable methods for the study of the bacterial communities in the lung, molecular methods for the analysis of other microorganisms – virus and fungi – are less advanced.

The Lung Microbiome and Respiratory Diseases

Just as alterations of the gut host-microbial equilibrium cause a range of gastrointestinal and other conditions, many pulmonary diseases including cystic fibrosis (CF), asthma, chronic obstructive pulmonary disease (COPD), and idiopathic pulmonary fibrosis (IPF) are associated with modifications in the lung microbiome. Lung disease changes microbial elimination (cough, mucociliary clearance, innate/adaptive host defences) and immigration (microaspiration, inhalation of bacteria, direct mucosal dispersion), and in turn regional growth conditions of the lung microbial system. The ability of the lung microbiota to modify local inflammatory responses may explain its role in the pathogenesis of chronic lung disease (Table I). In healthy people, the composition of the respiratory tract microbiota is varied and well balanced but the lung microbiota in patients with lung diseases shows distinct differences[21,22].

There is good evidence on the role of the microbiome in lung diseases but our specific focus in this review is the role played by the respiratory microbiome in idiopathic pulmonary fibrosis (IPF).

Lung Microbiome as Biomarker of Disease Progression in Idiopathic Pulmonary Fibrosis

IPF is an extremely complex disease with a highly variable clinical course characterized by a progressive and irreversible loss of lung function for which there is no effective therapy.

Progression involves either a slow worsening of the severity of dyspnoea and rapid progression to death or periods of relative stability punctuated with acute exacerbations contributing to the high disease-related morbidity and mortality[23]. Management of IPF is challenging and treatment options are limited to those that reduce the rate of functional decline[24]. It is proposed that repeated lung injury from a combination of host and environmental factors in people that are genetically susceptible, causes remodelling of the lung parenchyma with subsequent impaired gaseous exchange and ultimately respiratory fail-

Table I. Factors that influence the lung microbiota during acute and chronic disease.

Architectural	• Airway obliteration (lung transplant, IPF)
	• Terminal bronchiole destruction (COPD)
	• Honeycombing and fibrosis (IPF)
	• Impaired mucociliary clearance (COPD, asthma)
Immunologic	• Innate immune cell impairment
	• Altered PRR signaling
	• Release of anti-microbial peptides
	• Apoptosis/Autophagy
	• Inflammation
	• Cytokine alterations
Microbiologic	• Overgrowth of limited bacterial species (IPF, CF)
	• Antibiotic use (esp. in CF)
	• Lytic viral infection (COPD, asthma)
	• Latent viral infection (IPF?)
	• Biofilm formation (CF, COPD)
Pathologic	• Osmotic changes (CF)
	• Thickened mucus (CF)
	• Damaged cilia (COPD)
	• Changes in oxygen tension, ventilation and perfusion (IPF, COPD, CF, asthma)
	• Micro aspiration (IPF)

ure[25]. Despite years of research, we still do not know for sure what initiates the fibrotic process in genetically susceptible individuals but there is evidence that infection acts as a cofactor in fibrosis initiation/progression and as a trigger in exacerbations[26]. Han et al[27] retrospectively observed the microbiota of 55 patients with IPF who participated in the Correlating Outcomes with biochemical Markers to Estimate Time-progression (COMET) in IPF study. The most commonly detected bacteria were *Prevotella sp, Veillonella sp, and Escherichia sp* — all three make up the healthy lung microbiome. *Streptococcus sp* or *Staphylococcus sp* were associated with IPF disease progression and survival. Molyneaux et al[28] suggested that an altered lung microbiota might, "as has been shown in the gut, trigger a low-level antigenic stimulus resulting in aberrant activation of wound healing cascades". Two mouse models showed that *Streptococcus pneumoniae* triggered the progression of pulmonary fibrosis through pneumolysin (pneumococcus produced toxin, which mediates fibrotic progression in animal models *via* injury of the alveolar epithelium) and that antibiotic treatment stopped infection-induced fibrosis progression[29]. In a prospective case-controlled study, patients with IPF had a higher bacterial load in BAL and significant differences in the composition and diversity of their microbiota (increased presence of *Haemophilus, Streptococcus, Neisseria* and *Veillonella*)[30]. Patients with a higher bacterial load on diagnosis tended to have more rapidly progressing IPF and a higher risk of mortality. From these results, it is not possible to say definitely that an altered microbiome is the cause or result of destruction of the normal lung structure, but they provide a strong rationale for investigating antimicrobial therapy in IPF. A fact reinforced by Morris et al[31] who concluded that"antibacterial therapy may be a promising avenue for treatment of this currently untreatable disease".

Targeting the Lung Microbiome

We know that IPF is a devastating disease and that current therapies reduce the disease progression but not mortality. We also know that bacterial infections may play a greater role in IPF than previously thought. The utility of antibiotics in IPF patients is not a new phenomenon but was first observed in 1996, when a patient with advanced fibrotic lung disease showed clinical improvement following treatment with oral co-trimoxazole. Then, 14 patients with end-stage fibrotic lung disease also responded to oral co-trimoxazole. Encouraged by these observations, Varney et al[32] conducted a double-blind, randomized, placebo-controlled pilot study in 20 patients with progressive fibrotic lung disease. Treatment with co-trimoxazole improved exercise capacity, breathlessness, and symptom scores with significant improvements in objective and subjective parameters which fulfilled the ATS/ERS (2000) criteria of "a favourable response to treatment". In a double-blind follow-up, the multicentre study of 181 patients with IPF,for the protocol analysis

of patients who successfully took co-trimoxazole showed significant reductions in mortality (up to five times) with reduced frequency of respiratory tract infections, improved overall health-related quality of life, and fewer patients requiring an increase in oxygen therapy[33]. The intention-to-treat analysis for mortality (not the primary outcome) was negative since the treatment was not always well tolerated and drop-out rates were high in the treatment arm. In a study of 85 patients with acute exacerbations of IPF, mortality in patients treated with azithromycin was significantly lower than in those treated with fluoroquinolones - the hypothesis is that azithromycin has a direct effect on organizing pneumonia areas/diffuse alveolar damage by reducing the priming of alveolar macrophages[34].

Targeting the Oxidant-Antioxidant Imbalance

While the pathogenesis of IPF has yet to be fully elucidated, a hypothesis is that an oxidant-antioxidant imbalance causes a repeated epithelial cell injury, and endogenous and exogenous antioxidants/redox modulators influence fibrogenesis, protect the lung against fibrosis, and prevent its progression[35]. In addition, patients with IPF have been found to have reduced levels of reduced glutathione (GSH) in bronchoalveolar lavage (BAL) fluid[36]. Restoring the antioxidant capacity may, therefore, have a therapeutic role in IPF. N-acetyl-l-cysteine (NAC) is the antidote to acetaminophen overdose, acting as a precursor for L-cysteine in the synthesis of hepatic reduced glutathione (GSH) depleted through drug conjugation. It restores pulmonary glutathione levels and improves lung function in patients with fibrosing alveolitis. To exert its antioxidant activity there needs to be GSH depletion and the presence of functional metabolic pathways for the conversion of NAC to GSH. Evidence shows that NAC replaces GSH in deficient cells but is not effective in cells that are adequately supplied with GSH[37]. A novel antifibrotic mechanism has been suggested whereby NAC inhibits lysyl oxidase (LOX) activity *via* elevation of lung GSH in BLM-induced pulmonary fibrosis[38].

The IFIGENIA (Idiopathic Pulmonary Fibrosis International Group Exploring N-Acetylcysteine I Annual) trial demonstrated that high dose NAC (600 mg tid), added to standard therapy (prednisone and azathioprine), in patients with IPF preserved vital capacity and single-breath carbon monoxide diffusing capacity better than the standard therapy alone[39]. These results were not confirmed in PANTHER-IPF (Prednisone, Azathioprine, and N-acetylcysteine: study THat Evaluates Response in Idiopathic Pulmonary Fibrosis) which showed that NAC 600 mg tid was not associated with preservation of FVC compared to a matched placebo in patients with mild-to-moderate impaired pulmonary function. However, patients treated with NAC monotherapy reported a better mental wellbeing (SF-36 and ICECAP scores) over a 60-week period[40].

Oldham et al[41] demonstrated that NAC might reduce clinically meaningful endpoint risk in genetically predisposed individuals specifically those carrying an rs3750920 (TOLLIP) TT genotype. In a multicentre, prospective, randomized, controlled trial in patients with early-stage IPF, inhaled NAC monotherapy stabilized the decline in FVC in some patients without the use of immunosuppressive or anti-fibrotic agents[42].

Monotherapy vs. Combination Therapy

Landmark studies such ASCEND and INPULSIS have shown that pirfenidone and nintedanib are effective in slowing the decrease in FVC and may improve life expectancy compared to the best supportive therapy, but the survival curves still show high overall mortality in IPF patients. It is generally accepted that monotherapy will not be able to meet the significant unmet medical needs in IPF and that combination therapy is the way forward[43]. But which combination should be used? Survival advantages with novel antifibrotic agents have not definitely been established and researchers are turning their attention to other combinations[44].

Combination Therapy for the Management for Idiopathic Pulmonary Fibrosis: Role of Antioxidant/Antibiotic Combination Therapy

Given the evidence of the role of antibiotic and antioxidant therapies in IPF it is reasonable to propose a combination of NAC plus colistin**.** Sergio et al[45] reported a synergistic activity of N-acetylcysteine in combination with colistin against *Stenotrophomonas maltophilia***,** a common coloniser of the respiratory tract of patients with chronic lung disease. The NAC at concentrations that can be achievable by nebulization showed a remarkable *in vitro* synergy with colistin against *S. maltophilia* isolates. Combined treatment of colistin plus NAC seems to have a beneficial role in the restoration of the oxidant injury which may

be related to its antioxidant effect[46]. Of note, recently Zheng et al[47] demonstrated the preventive effect of NAC on intestinal dysbiosis with NAC reshaping the structure of the gut microbiota and improving the disturbance in glucose metabolism in high fat diet-fed mice.

Therapeutic Applications and Future Directions

IPF is a difficult disease with a depressing prognosis. Although major advances have been made, a curative therapy for this severe lung disease remains elusive. Progress has been made in the identification of the lung microbiome and the possible causal role of bacteria in IPF pathogenesis. Recent studies suggest that antibacterial therapy in combination with antioxidant therapy may be a promising avenue for the treatment of this untreatable disease. Novel routes of administration are also an important area of research and studies assessing the use of inhaled N-acetylcysteine in patients with IPF could be considered.

Who knows in time, the Nobel Committee may be commenting, as they did for Marshall et al[1] pioneering discovery, "IPF is no longer a devastating deadly disease, but one that can be cured by a regimen of antibiotics and antioxidants". We owe it to our patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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