LUNG MICROBIOME

Lung Microbiome as a Treatable Trait in Chronic Respiratory Disorders

Filippo Scialò[1,2] · Maria Vitale[2] · Vito D'Agnano[1] · Domenica Francesca Mariniello[1] · Fabio Perrotta[1] · Alice Castaldo[3] · Susan F. M. Campbell[1] · Lucio Pastore[2,4] · Mario Cazzola[5] · Andrea Bianco[1]

Abstract

Once thought to be a sterile environment, it is now established that lungs are populated by various microorganisms that participate in maintaining lung function and play an important role in shaping lung immune surveillance. Although our comprehension of the molecular and metabolic interactions between microbes and lung cells is still in its infancy, any event causing a persistent qualitative or quantitative variation in the composition of lung microbiome, termed "dysbiosis", has been virtually associated with many respiratory diseases. A deep understanding of the composition and function of the "healthy" lung microbiota and how dysbiosis can cause or participate in disease progression will be pivotal in finding specific therapies aimed at preventing diseases and restoring lung function. Here, we review lung microbiome dysbiosis in different lung pathologies and the mechanisms by which these bacteria can cause or contribute to the severity of the disease. Furthermore, we describe how different respiratory disorders can be caused by the same pathogen, and that the real pathogenetic mechanism is not only dependent by the presence and amount of the main pathogen but can be shaped by the interaction it can build with other bacteria, fungi, and viruses present in the lung. Understanding the nature of this bacteria crosstalk could further our understanding of each respiratory disease leading to the development of new therapeutic strategies.

Keywords Lung microbiome · Respiratory disorders · Microbiome · Pathological mechanisms

Abbreviations

URT	Upper respiratory tract
LRT	Lower respiratory tract
BE	Bronchiectasis
CF	Cystic fibrosis
COPD	Chronic obstructive pulmonary disease
PDE	Phosphodiesterase
CFTR	Cystic fibrosis transmembrane regulator
AMP	Antimicrobial peptide
BAL	Bronchoalveolar lavage
SCLC	Small cell lung cancer
NSCLC	Non small cell lung cancer
EBC	Exhaled breath condensate

Filippo Scialò and Maria Vitale have contributed equally to this work.

✉ Andrea Bianco
andrea.bianco@unicampania.it

1 Department of Translational Medical Science, University of Campania Luigi Vanvitelli, Naples, Italy

2 CEINGE-Biotecnologie Avanzate-Franco Salvatore, Naples, Italy

3 Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy

4 Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Naples, Italy

5 Dipartimento di Medicina Sperimentale, University of Rome "Tor Vergata", Rome, Italy

Introduction

The term microbiome is used to define the genetic content of the microorganisms (bacteria, fungi, yeast, viruses, and others) that can be found in a specific site, such as a particular terrain or organ of our body; the actual collection of the microbial species that corresponds to a specific microbiome is called microbiota [1]. Microorganisms have co-evolved with all animal species including humans for millions of years becoming crucial players in different physiological processes such as the regulation of the immune system, the production of short-chain fatty acids/vitamins and other nutrients, and digestion. Although for a long time the lungs were thought to be sterile organs, microbes can be found everywhere in our body and lungs are not an exception [2,

3]. Lungs are continuously exposed to the environment and, therefore, to a variety of microbes that can colonize both the upper (URT) and lower respiratory tract (LRT) [4, 5]. The pulmonary microbiome is different from the gut or skin microbiome in terms of bacterial abundance and composition but is similar to the mouth and URT. The main bacteria present in healthy adult lungs include *Firmicutes, Proteobacteria, Bacteroitedes, and Actinobacteria; the main genera are Prevotella, Veillonella, Streptococcus, Neisseria, Haemophilus, Fusobacterium, Sphingomonas, Pseudomonas, Acinetobacter, Megasphaera, Staphylococcus, and Corynebacterium* [6–8]. Environment influences lung microbial composition as early as neonate delivery: in fact, LRT is colonized by vaginal/colon microbiome in case of vaginal delivery, whereas *Staphylococcus* is predominant after C-section delivery [9, 10]. Furthermore, URT microbiota is characterized by higher and very dynamic biomass with a prevalence of *Streptococcus, Moraxella, Corynebacterium, Staphylococcus, Prevotella, Veillonella,* and *Rothia* species amongst others [11]. In contrast, the LRT is more static and is characterized by lower biomass, controlled by both mechanical components, such as cough and mucociliary clearance, and adaptive/innate host immune response [11, 12]. Maintaining low bacterial biomass in the lungs is crucial to preserve oxygen and carbon dioxide exchange, their main physiological function. In fact, as we will discuss below, both qualitative and quantitative changes in bacterial composition can result in "dysbiosis" associated with several respiratory diseases [13]. However, in many pathologies such as chronic obstructive pulmonary disease (COPD), asthma, bronchiectasis, and lung cancer, it is still not clear whether lung microbiome dysbiosis is implicated in initiating events that will eventually lead to the disease or represent a consequence of the pathology itself. Furthermore, it is also relevant to understand whether lung dysbiosis is a cause or a consequence of clinical and pathophysiological disease exacerbation/progression (Fig. 1). This is a critical aspect to address, especially because the lung microbiome plays a key role in shaping the adaptive and innate immune response from the first week of life and, therefore, its modulation can have a significant impact on lung health [11]. In physiological conditions, the immune response leads to pathogens elimination and clearance; however, genetic mutations such as in the case of Cystic Fibrosis (CF) or constant exposure to air pollution, airborne organisms, or cigarette smoke [14] can modulate the lung environment making it more suitable for pathogens colonization and increasing the susceptibility to infection. Ultimately, this mechanism drives an inflammatory response that could impair lung function, compromise the immune response and lead to a respiratory pathology or trigger the exacerbation of existing pulmonary pathologies [12]. Inflammation in chronic lung diseases can be caused by an impaired barrier function; indeed, epithelial cells lose tight junctions resulting in an increase in permeability that promotes the entry of pathogens and toxic particles [15, 16]. In this review, we will focus on the association between several chronic lung diseases and microbiome dysbiosis highlighting the prevalence of certain bacteria shared amongst chronic lung pathologies including lung cancer. We will describe the mechanisms by which these lung microbiome dysbiosis can cause or participate in triggering respiratory disorders and their exacerbations, highlighting the importance of crosstalk mechanisms normally established amongst different pathogens and that are pivotal in establishing a pathological condition.

Lung Microbiome Dysbiosis in COPD

COPD is a chronic inflammatory disorder affecting the lungs and it is characterized by non-reversible airflow limitation [17]. The main cause of COPD is exposure to tobacco, that as well as causing damage to the airway through the action of toxins and carcinogen molecules contained in the tobacco mixture, can facilitate bacterial colonization thus interfering with normal defence mechanisms such as mucociliary clearance, damage to nasal cilia, depletion of the airway surface liquid and interfering with the action of the immune systems [18]. Bacterial infections and colonization are also frequently associated with the episodes of exacerbations that characterize COPD [19]. Fifty percent of patients affected by COPD harbour pathogens such as S*treptococcus pneumoniae* (Sp)*, Haemophilus influenzae* (Hi), and *Moraxella catarrhalis,* and their presence promotes a state of chronic inflammation leading to COPD and COPD exacerbations [20, 21]. This inflammation state is promoted by the High Mobility Group Box 1 (HMGB1), a prototypical damage-associated molecular pattern (DAMP) protein, that through the activation of Receptor for advanced glycation end products (RAGE) and/or Toll-like receptor 4 (TLR4) activates NF-κB transcription factor promoting the production of chemokines and inflammatory molecules [22]. This is responsible for the pathological changes that can be seen in COPD patients [23]. Interestingly, HMGB1 has been shown to correlate with pneumococcal bacteremia in the sputum of patients with acquired pneumonia [24]. Cigarette smoke can also promote a hyper-activation of platelets through the induction of an activating factor receptor that enhances the adherence of Hi and Sp to epithelial cells of bronchi. Furthermore, susceptibility to bacterial colonization also leads to the reduction of both macrophage phagocytosis and secretion of IgA [25]; in turn, bacterial colonization increases neutrophilic-mediated airway inflammation leading to lung tissue injury [26]. In patients with very severe COPD, an increase in *Proteobacteria* phylum and a decrease in *Firmicutes* have been found [27]. An interesting study
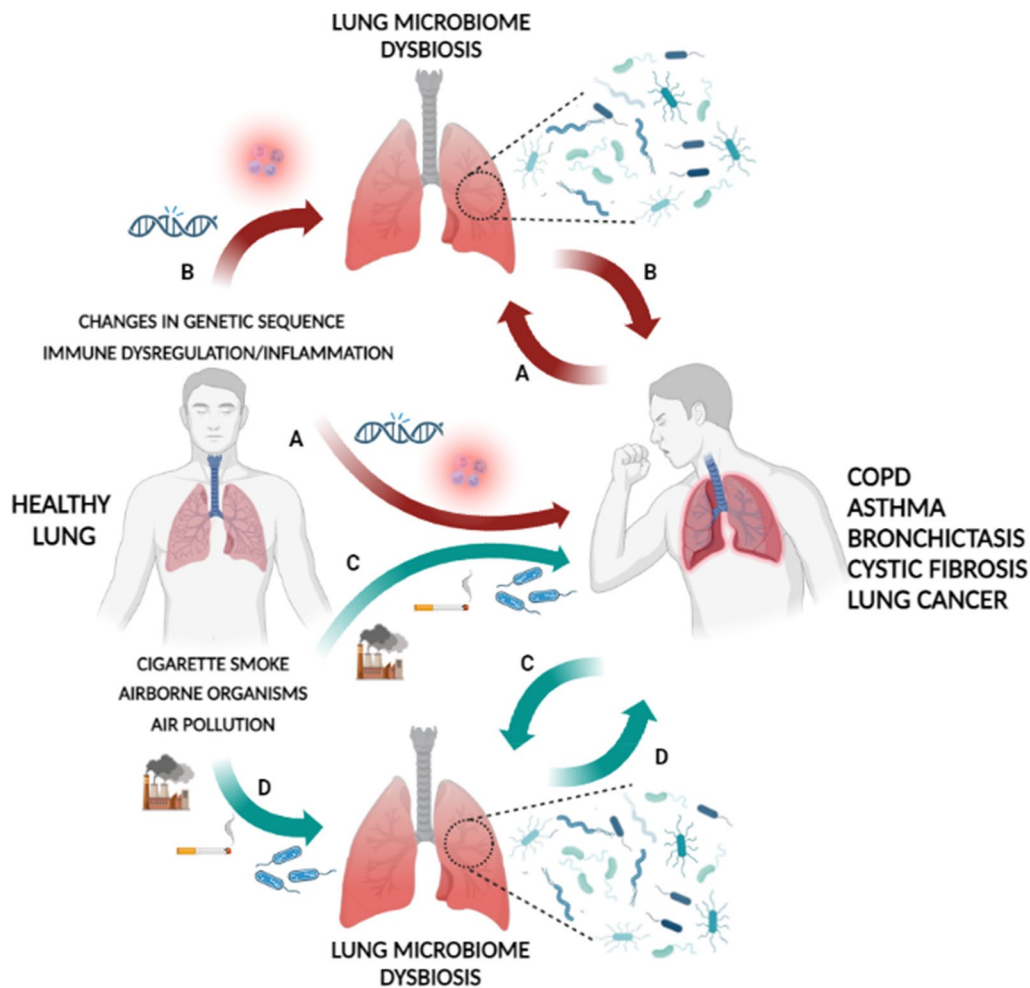
Fig. 1 Lung microbiome dysbiosis and respiratory pathologies. Here are described different hypothetical scenarios where lung microbiome dysbiosis can represent a driver or a consequence of a respiratory pathology. In the first hypothesis (red arrows), changes in genetic sequences, immune system dysregulation and chronic inflammation can cause direct damage to lung structure that will eventually lead to a lung pathology. This pro-inflammatory state provides a fertile substrate for pathogen colonization leading to dysbiosis and a subsequent exacerbation of the disease state (A). Alternatively, the genetic instability, immune system dysregulation and chronic inflammation will first promote pathogens colonization and dysbiosis that will be responsible to drive disease development and progression (B). In a second hypothesis (blue arrows) cigarette smoke, air pollution and airborne organism can cause a direct damage to lung structure leading to lung pathology. This will create the optimal conditions for pathogens colonization resulting in lung microbiome dysbiosis (C). Differently, an early colonization of pathogens due to changes in lung environment (decreased pH level, oxygen availability, and altered defences mechanism) caused by external insults such as cigarette smoke and air pollution promote pathogens colonization and lung microbiome dysbiosis that as in (A) would drive disease development and progression (D)

from Molyneaux et al., reveals that another possible cause of exacerbation in COPD patients is viral over-infection that leads not only to a change in bacterial load but even to a qualitative alteration of the phyla, with an increase in Proteobacteria. In addition, significant interplay between intestinal and respiratory microbiota prompting modifications of the mucosal immunity in bidirectional mode has been documented [28]. In the murine model, the absence of an intestinal microbiota is associated with a blunted phagocytic activity and bacterial clearance of alveolar macrophages favouring an increased susceptibility to pneumonia [29, 30]. On the other hand, clinical data showed that during an acute exacerbation of COPD (AECOPD) there is a significant increased permeability of small intestine, with potential negative impact on systemic inflammation for potential spillover of pro-inflammatory mediators [31, 32].

Focussing on upper gastrointestinal tract, gastroesophageal reflux disease (GERD), defined as the abnormal reflux of gastric contents into the oesophagus with consequent oesophageal mucosal injury, represents one of the most common comorbidity in patients with COPD [33]. Whilst GERD is related to a reduction of lung function, COPD symptoms such as cough and β2-agonists use worsen reflux, promoting a vicious circle [34]. In a recent meta-analysis of

observational studies, it has been demonstrated that GERD increased the risk of exacerbation of patients with COPD [35]. In this regard, data on impact of proton pump inhibitors (PPIs) are not homogeneous. However, a recent meta-analysis of randomized controlled trials suggested that PPIs treatment in COPD patients may reduce the case fatality rate, incidence of gastrointestinal bleeding, and other adverse reactions along with the number of acute exacerbations [36]. Large cohort clinical trials and preventive strategies for GERD in patients with COPD are however warranted. Finally, concerns exist about the selective pressure on microbiota from chronic therapies altering host-microbial homeostasis. Pharmacological treatment of COPD mainly consists of bronchodilators [37]. Contoli and coll. originally investigated the effects on sputum bacterial load of 12-month treatment with either salmeterol or salmeterol/ fluticasone propionate. They found that the combination therapy resulted in a significant increase in sputum bacterial load, modification of the microbiome composition and increased airway load of potentially pathogenic bacteria. When comparing salmeterol/FP versus salmeterol alone, there was an increased proportion of Firmicutes and Candida species, with a significant reduction in Proteobacteria [38].

In COPD patients who experience frequent exacerbations despite optimal inhaled treatment prolonged azithromycin treatment can be considered. However, antibiotic prophylaxis therapy was associated with a decreased microbial α-diversity of the airways. Carrera-Salinas et al. observed that long-term use of this antibiotic favours differences in lung colonization by bacterial pathogens. Before therapy, *M. catarrhalis* and *H. influenzae* were the most frequently isolated species during both stable phases and exacerbations. However, when azithromycin treatment was initiated, *H. influenzae*, *P. aeruginosa*, *S. maltophilia* and, in particular, *H. parainfluenzae* became the most frequently isolated bacteria [39].

Lung Dysbiosis in Asthma

Asthma is a heterogeneous and multi-factorial airway disease characterized by inflammation, lung-associated smooth muscle hyperplasia, and intermittent wheezing [40]. Asthma affects around 300 million people in the world and, despite the causes not being entirely clear, evidence suggests an important role for lung microbiome alteration as a causative agent. This disease affects mainly children but can progress to chronic forms in adults, potentially leading to chronic obstructive pulmonary disease (COPD). Asthma patients exhibit two main phenotypes; T2 high (allergic or non-allergic) and T2-low (mainly neutrophilic or paucigranulocytic) [41]. The association between asthma and the environment is important; several studies have described that exposure to

an environment with a heavy bacterial load reduces asthma manifestations. This is described as the ''farm effect'' and it is probably caused by the interaction between bacteria and the immune system. In fact, bacterial colonization during the first years of life is fundamental in educating the immune system to maintain the balance between innate and adaptive responses leading to proper tolerance and avoiding immune overreactions [42, 44]. Although viral infections cause seasonal exacerbation of asthma, bacterial infections have an additional effect. Healthy neonates with the upper respiratory tract colonized by *Streptococcus pneumonia*, *Haemophilus influenzae* or *Moraxella catarrhalis* show an increased risk of developing recurrent wheezing and asthma. In children, the bacterial community depends also on the delivery mode of birth; in fact, during vaginal delivery, neonates are colonized by maternal vaginal and colon microbiota, whereas after C-section a prevalence of epidermal bacteria colonizes the neonate, usually with a lower bacterial diversity [9, 45]. Several studies showed that children with a high presence of *Corynebaterium* and *Dolosigranulum* have a lower risk to develop loss of asthma control, compared to children with pathogen dominance such as *Staphylococcus, Streptococcus,* and *Moraxella.* Furthermore, the relative abundance of *Corynebaterium* has been inversely correlated with the development of severe exacerbations; this bacterial genus is the most abundant in the upper airways of healthy adults, as described in Human Microbiome Project, whilst is less represented in asthmatic adults, suggesting its possible protective role [46]. It is generally believed that a possible protective role of commensal bacteria is due to their competition with pathogens for the colonization of lung niches. Genera such as *Corynebacterium* and *Dolosigranulun* could inhibit colonization and spread of *Streptococcus* probably through the release of inhibitory bacterial metabolites [47]. Generally, bacterial or viral colonization alters airway epithelium leading to the activation of innate signalling receptors. The activation of these receptors induces the airway epithelial secretion of chemokines triggering the recruitment of immature Dendritic cells (DCs) to the mucosal epithelium. The DCs respond to stress signals through pattern recognition receptors (PRRs) that promote the maturation into competent antigen-presenting myeloid-type DCs. The activated DCs can drive T cell differentiation and migration to local lymph nodes and in turn, activated T cells, interact with naïve T cells through T cell receptors, major histocompatibility complex (MHC) II, and co-stimulatory molecules. The naïve T cells differentiate into TH2-type T cells that secrete several pro-allergic cytokines such as IL-3, IL-4, IL-5, IL-9, IL-13, and granulocyte–macrophage colony-stimulating factor (GM-CSF), leading to the production of IgE, mast cells and eosinophilic response, typical of asthma [48, 49]. Altogether, these observations suggest that microbiome dysbiosis causes colonization of pathogenic

bacteria, compromising the maintenance of healthy airways, whilst the switch from high levels of *Corynebaterium* and *Dolosigranulun* to *Moraxella* or *Streptococcus* is associated with a higher risk to loss of control of asthma and exacerbation [50]. In addition, *Moraxella* genus forms biofilms that protect bacteria from antibacterial drugs and promotes the colonization of other pathogens such as *S. pneumoniae* and *H. influenzae* [47]. Lastly, the influence of chronic inhaled treatment with corticosteroids on lung microbiota should be considered. Denner et co. demonstrated significant differences based on corticosteroid treatment, particularly when ICSs were combined with oral CSs; this leads to altered α- and β-diversity, with an increased abundance of Proteobacteria and the genus Pseudomonas, and decreased abundance of Bacteroidetes, Fusobacteria, and Prevotella species [51].

Lung Dysbiosis in Bronchiectasis

Bronchiectasis (BC) is a common chronic respiratory disease affecting the lower airways that ultimately results in an abnormal dilatation and distortion of bronchi and consequent airway destruction. This disorder is mainly associated with chronic inflammation, cough, and sputum production with structural damage to the airways. The prevalence in Europe and North America ranges from 67 to 566 per 100,000 inhabitants [52–54]. Pathogenesis and progression of the disease are still not clear: indeed, it can be caused by several mechanisms such as post-infection damages [55], obstruction, as a consequence of genetic disorders like cystic fibrosis (CF), autoimmune disease, and abnormal host response [53]. Although different causes have been identified, it is well recognized that infections and consequent lung microbiome dysbiosis is pivotal in its pathogenesis. Studies have shown that individuals with bronchiectasis have a distinct lung microbiome compared to healthy individuals, with an excess of certain bacteria and a reduction in the diversity of the overall microbiome. The alteration of the microbiome can contribute to chronic inflammation and airway destruction observed in bronchiectasis [56]. The high bacterial load establishes a vicious cycle in which the airway epithelium release antimicrobial peptide and pro-inflammatory cytokines and chemokines. Chemokines such as IL-8 are released in the sub-mucosa of airway epithelium initiating the inflammation and the recruitment of phagocyte population such as neutrophils, monocytes, macrophages, DCs, and lymphocytes. In bronchiectasis neutrophil-dominant inflammation is predominant, indeed these cells aggregate in the airway epithelium releasing a variety of enzymes, host defence protein and signalling protein that mediate bacterial elimination. The excessive neutrophilic degranulation and action of cytolytic enzymes are implicated in host tissue damage. Furthermore, to facilitate bacterial elimination,

neutrophils release Reactive oxygen species (ROS) although an uncontrolled ROS release contributes to damage to the surrounding tissue, worsening the lung disease process [57, 58]. Bacterial proteins are also recognized by Toll-Like receptors (TLRs), a cell surface protein expressed on T-cells, involved in either innate or adaptive immune responses. TLRs are able to recognize peptidoglycan, lipoprotein, and lipopolysaccharide (LPS) and activate innate host defence mediating the release of pro-inflammatory cytokines. For instance, the LPS of *P. aeruginosa* or *Haemophilus*, the prevalent pathogens in bronchiectasis, triggers the lung inflammation [59–62]. Patients culture-negative for these pathogens showed a milder disease, whilst the presence of *Pseudomonas* spp. and *Haemophilus* correlates with a severe form of the disease [63]. Bronchiectasis exacerbations are commonly defined as a deterioration of respiratory symptoms amongst which are an increase in cough, fatigue, breathlessness, and sputum purulence that lasts more than 48 h [64]; they are thought to be associated with the presence of or increase in a particular bacterial species [60]. Unfortunately, although a clear difference has been described in lung microbiome composition between healthy and bronchiectasis patients, the causes of exacerbations in these patients are still not associated with the presence or increase of a certain type of bacteria. Cox et al. demonstrated that there was no difference in terms of bacteria load and diversity between baseline and exacerbation of bronchiectasis [65]. However, they suggest that the microbiome can still be implicated in the exacerbations and that other unidentified bacteria may be involved in this process. On the other hand, another study demonstrated that the presence or increase in *Pseudomonas* spp. correlates with the high-frequency bronchiectasis exacerbation group; however, the authors speculate that the exacerbation phenotype is caused mostly by a network of positive and negative interactions of pathogens and other bacteria and/or fungi such as *Aspergillus, Haemophilus, Streptococcus, Prevotella, Veillonella*, and *Neisseria* [66]. This hypothesis can also explain why the use of antibiotic therapy with amoxicillin or macrolides, which do not target the predominant pathogen *Pseudomonas* spp., improves the clinical outcomes by reducing exacerbation risk [67]. With respect to gut-lung axis, Narayana et al. have lately identified two potential cluster of patients, according to composition of gut and lung microbiome. Interestingly, authors reported that subjects with high gut-lung interaction, characterized by gut Bacteroides and Saccharomyces and lung Pseudomonas have significantly more severe radiologically bronchiectasis as well as increased exacerbation when compared with patients with low gut-lung interaction, characterized by an overrepresentation of lung commensals, including Prevotella, Fusobacterium, and Porphyromonas with gut Candida [68, 69]. How manipulation of both gut microbiome and gut-lung axis

may play a significant role in the therapeutic management of patients with bronchiectasis urges to be clarified.

Furthermore, a relevant percentage of patients with bronchiectasis has been reported to complain acid regurgitation, one of the main symptoms of GERD. In a cohort of 58 patients with nodular bronchiectasis due to nontuberculous mycobacteria (NTM), it has been reported that subjects with GERD were more likely to have a positive sputum smear for acid-fast bacilli, higher risk of bronchiolitis and bronchiectasis in more lobe when compared with patients without GERD [70]. Therefore, PPIs have been investigated in patients with coexisting bronchiectasis and GERD. Although no impact of PPIs has been demonstrated in terms of lung function improvement in this cohort, significant improvement of both FEV1 and FVC has been reported amongst patients with GERD and high BMI, supporting a potential role in this subgroup of patients [71]. Lung-gut axis in bronchiectasis patients could be also modulated from maintenance treatments. Current recommendations suggest the long-term antibiotic use for patients with 3 or more exacerbations per year in absence of P. aeruginosa from cultures. In the multicentre BAT (Bronchiectasis and Long-term Azithromycin Treatment) trial, patients received either oral azithromycin (250 mg once per day) or placebo for 1 year. The microbiological profile of sputum samples didn't differ significantly between azithromycin-treated and placebo-treated patients at baseline and after 1 year of treatment [72]. However, concerns about microbiota diversity as reported in COPD paragraph remains.

Lung Dysbiosis in Cystic Fibrosis

Cystic fibrosis is a genetic disorder caused by a dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR) channel, commonly due to an F508del mutation in *CFTR* gene, but more than 2,000 mutations have been described so far [73, 74]. Although CF is a systemic disease, the main morbidity and mortality are caused by lung infections. Indeed, pathogens and opportunistic infections and chronic colonization in the lungs of CF patients are frequent and are caused by the impaired secretion of chloride ions that induces an accumulation of mucus resulting in an ideal environment for bacterial colonization [75]. Furthermore, the impaired function of the CFTR channel reduces the bicarbonate production in the airway lumen resulting in acidification and altered salt composition of the airway surface. The altered environment of the airway surface decreases the action of cationic antimicrobial peptides (AMPs), small proteins secreted by leukocytes with broad-spectrum antimicrobial activity, leading to a loss of immunomodulatory function [73, 76, 77]. Amongst these, the antimicrobial activity of beta-defensins is impaired by

the altered salt concentration [78] Furthermore, in the airway of CF patients, there is enhanced production of pro-inflammatory modulators that can be assessed in sputum and saliva [79] and relates to the severity of the disease [80]. Such cytokines trigger the recruitment of inflammatory cells like neutrophils, which release multiple products such as proteases that can degrade AMPs and components of the complement system contributing to bacterial infections [76]. However, the pathogenesis of respiratory infections is more complex and heterogeneous amongst patients with CF, and factors such as the variable activity of immunity peptides encoded by polymorphic modifier genes of CF phenotype like beta-defensin [81], mannose-binding lectin [82], and taste receptor T2R38 [83] are involved. In physiological conditions, the lung bacterial population is abundant in anaerobic bacteria such as *Prevotella*, *Fusobacterium*, *Veillonella,* and *Porphyromonas* and the upper and lower airway microbiomes show similarity [75]. Conversely, in CF patients upper and lower airways show pivotal differences, and analysis of sputum and bronchoalveolar lavage (BAL) discloses a complex and variable microbial community [84–86] with a deep variation from physiological conditions with the colonization of taxa such as *Streptococcus, Rothia, Actinomyces, Gemella, Granulicatella, Neisseria, and Atopobium* [87–89]. During childhood, patients affected by CF show a high microbiome diversity; however, in adulthood, diversity is lost and colonization by *Pseudomonas aeruginosa,* the major pathogen of CF patients, becomes predominant [90]. The main cause of biodiversity loss in adulthood is the massive antibiotic treatment and it could be considered a marker of lung function. In fact, the maintenance of microbiome diversity correlates with less severe lung affection, whilst, the colonization of *P. aeruginosa* often as biofilm, correlates with decreased lung function [91, 92]. Furthermore, the spread of *P. aeruginosa* was associated with the colonization of bacteria such as *Staphylococcus, Haemophilus, and Burkolderia* [93]. Despite the interactions between the pathogens bacteria being complex, it is known that the major causes of exacerbation are (i) increase of a specific family of pathogens, (ii) variation in the metabolic activity of specific bacteria, and (iii) transition of ''friendly'' bacteria into pathogens (including the appearance of bacterial biofilms) under altered environment conditions including antibiotic treatment which selects multi-resistant strains [94]. Furthermore, although CFTR modulators are now available for CF patients, antibiotic treatments are still considered a necessary therapeutic intervention to control pathogens colonization [91, 95]. In patients with CF, the lung microbiota may be used as a biomarker to predict patient responsiveness to therapy; in fact patients with higher abundance of Staphylococcus and anaerobic organisms including Prevotella and Fusobacterium were less likely to respond to CF therapy. In addition, similarly to other chronic respiratory conditions,

CF treatments may potentially alter lung microbiome though no significant changes in alpha diversity was reported in CF patients with P. aeruginosa infection treated with Aztreonam lysine for inhalation (AZLI) [96].

Lung Microbiome and Lung Cancer

Lung cancer is the first cause of cancer-related death worldwide. At molecular levels, lung cancer is caused by a wide range of genetic mutations and environmental factors, highlighting the heterogeneity of this disease [97, 98]. Small cell lung cancer (SCLC) represents 10–15% of lung cancer cases, whilst non-small cell lung cancer (NSCLC) is the most common type affecting up to 85% of

lung cancer patients [99]. Lung cancer is a multi-factorial disease caused mainly by both genetic and environmental factors such as mutations in tumour-suppressor genes and cigarette smoking. In the last 10 years, it has also become clear that microbiome dysbiosis may have a central role in initiating or the proliferation of lung cancer [100]. Recent studies emphasized the tight relationship between lung microbiome dysbiosis and the development or worsening of lung cancer [3, 101, 102]. Although in patients with lung cancer bacterial infections are common, the interaction between certain bacteria and cancer development and progression remains unclear. Some studies described a role for microbiome dysbiosis in lung cancer initiation and progression through processes that can (i) promote an unbalanced adaptive immune response
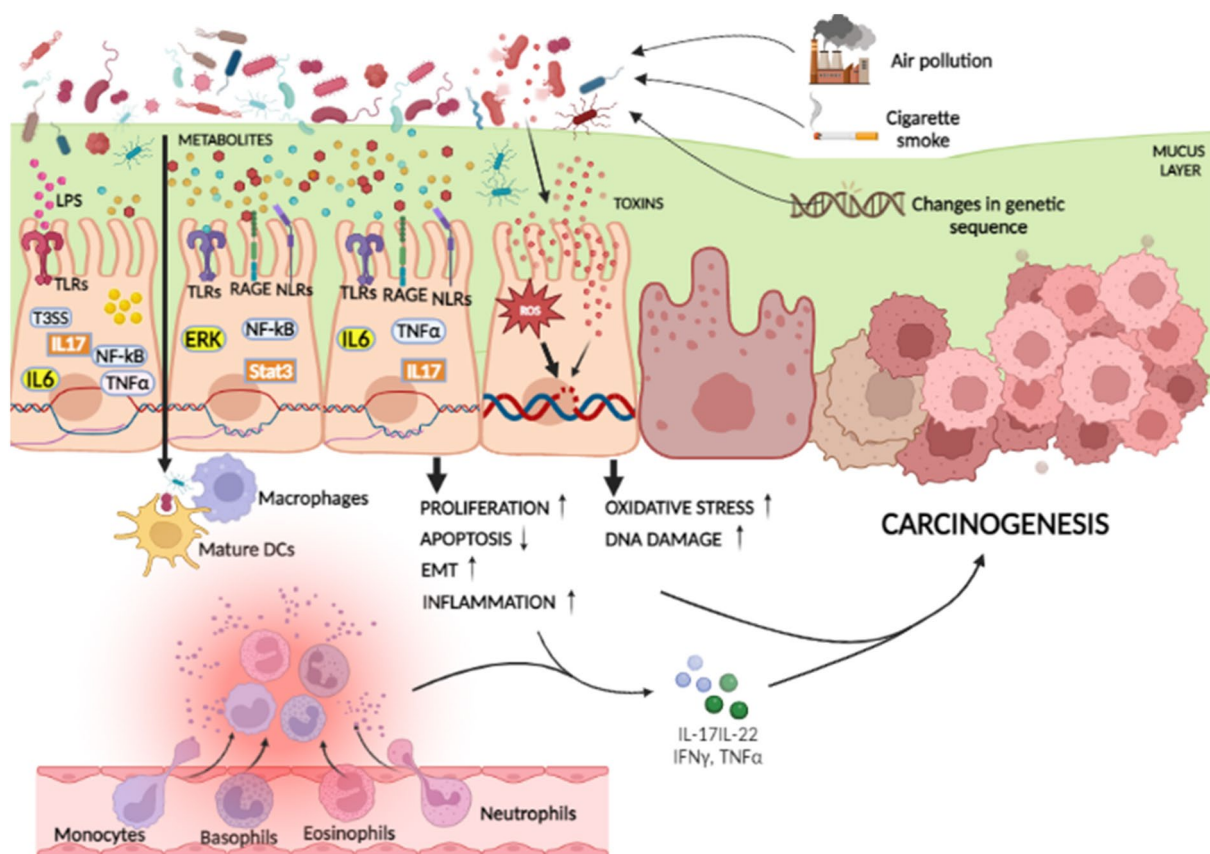

Fig. 2 Mechanisms of microbiome induced lung damage. Genetic predisposition, or environmental insults such as air pollution or cigarette smoking can alter the normal mechanisms of defence allowing pathogen colonization and proliferation, resulting in lung microbiome dysbiosis. Pathogens and their metabolites/toxins can activate TLRs, NLRs, and RAGE receptors promoting a series of downstream events that culminate in the transcription of chemokines and cytokines inducing an inflammatory state. Production of inflammatory molecules induce the activation and recruitment of immune cells such as Neutrophils, Eosinophils, Basophils and Monocytes. Whilst immune cells recruitment is essential for host defence, an excessive infiltration of activated immune cells can cause tissue damage worsening

lung function resulting in the development of lung pathologies such as COPD, Asthma, and Bronchiectasis or inducing exacerbation of an existing lung condition. Furthermore, pathogens and their cytotoxic molecules can induce DNA damage directly or by increasing oxidative stress inhibiting apoptosis and promoting proliferation that coupled with a compromised immune response can lead to carcinogenesis. *EMT* epithelial to mesenchymal transition, *TLR* toll-like receptor, *NLR* NOD-like receptor, *RAGE* receptor for advanced glycation end-products, *IL17* interleukin 17, *DC* dendritic cell, *Nf-kB* nuclear factor kappa-light-chain-enhancer of activated B cells, *TNFα* tumour necrosis factor alpha, *T3SS* type III secretion system, *STAT3* signal transducer and activator of transcription 3

by dysregulating cytotoxic CD8-T cells activation which stimulates tumour escape and progression [103, 104], (ii) produce metabolites that directly induce DNA damage of host cells or indirect activation of the innate immune system resulting in downstream inflammatory mediators to trigger downstream signalling pathways [105], (iii) activate proliferation (Fig. 2). The altered lung microbiome modulates specific oncogenic pathways, inducing cancer initiation and production of several bacterial metabolites in the tumour microenvironment that could modify cancer metabolism promoting oncogenic signalling. In addition, metabolites can produce direct DNA damage and, in turn, DNA damage and genomic instability lead to the production of reactive oxygen species promoting proliferation, angiogenesis, and tumour development [106]. It is important to underline that bacterial metabolites can up-regulate the expression of genes involved in the PI3K and ERK1/2 pathways. Upregulation of PI3K and ERK1/2 promotes transcriptomic changes that were observed only in lung cancer patients linking the process of tumourigenesis with disruption of the lung microbiome [107]. Another characteristic aspect of the interaction between lung cancer and the microbiome is the association between specific lung microbes and different histological types of lung cancer. For instance, Yu et al., and Dickoson et al., described bacteria populations in the lung of patients affected by squamous cell carcinoma tumours are different from the bacteria found in patients with adenocarcinoma [108–110]. For example, in patients affected by squamous cell carcinoma cancer with TP53 mutation an abundance of *Acidovorax* is described whilst, in small cell carcinoma, the main genera were *Klebsiella, Comamonas, Acidovorax, Polarmonas, and Rhodoferax* and this association is not found in adenocarcinoma [111]. These observations are interesting because they suggest the possibility of using specific genera as biomarkers to predict the type of lung cancer. This possibility needs to be further investigated. For this reason, it is extremely relevant to establish unified standard sampling methodologies because different bacterial species are found in lung tissue, sputum, BAL, or bronchoscopic samples, and further large-scale studies are required to identify and validate microbial biomarkers associated with specific lung tumour types [112].

Discussion

In this review, we extensively explore links between chronic airway inflammation, immune system dysregulation, changes in genetic sequence, and lung microbiome dysbiosis. It is being increasingly acknowledged that alteration in composition of lung microbiome may be associated with predisposition, development, progression, and exacerbation of lung disorders such as Asthma, COPD, bronchiectasis, Lung cancer [4, 7, 11]. Whilst microorganisms such as *Streptococcus*, *Pseudomonas,* and *Haemophilus,* are recognized pathogens affecting the course and severity of diseases like Bronchiectasis, Asthma, COPD, lung cancer, the extent

Table 1 Lung microbiome and their association with chronic lung disease and lung cancer

Types	Bacterial taxa	BE	Asthma	COPD	CF	LC
Gram− Aerobes	*Pseudomonas*	ᵛ	–	–	ᵛ	ᵛ
Gram + Anaerobes facultative	*Streptococcus*	ᵛ	ᵛ	ᵛ	ᵛ	ᵛ
Gram−Anaerobes	*Prevotella*	ᵛ	–	–	ᵛ	ᵛ
Gram−Anaerobes	*Fusobacterium*	–	–	–	ᵛ	–
Gram−Anaerobes	*Veillonella*	ᵛ	–	–	ᵛ	ᵛ
Gram−Anaerobes	*Porphyromonas*	–	–	–	ᵛ	–
Gram−Anaerobes	*Neisseria*	–	–	–	ᵛ	ᵛ
Gram−Anaerobes Facultative	*Haemophilus*	ᵛ	ᵛ	ᵛ	ᵛ	–
Gram−Aerobes	*Sphinogomonas*	–	–	–	–	ᵛ
Gram−Aerobes	*Acinetobacter*	–	–	–	–	–
Gram + Anaerobes Facultative	*Staphylococcus*	–	ᵛ	–	–	ᵛ
Gram−Anaerobes or Aerobes	*Corynebacterium*	–	ᵛ	–	–	–
Gram−Aerobes	*Moraxella*	–	ᵛ	ᵛ	–	–
Gram− Anaerobes or Aerobes	*Actinobacillus*	–	–	–	–	–
Gram + Anaerobes Facultative	*Propionibacterium*	–	–	–	ᵛ	–
Gram−Aerobic or Anaerobic	*Megasphaera*	–	–	–	–	ᵛ
Gram−Aerobes	*Acidovorax*	–	–	–	–	ᵛ

The table reported the main bacteria taxa associated with bronchiectasis (BE), asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and lung cancer (LC). For each bacterial taxa are indicated the type and metabolism

to which non-pathogenic bacteria may contribute to disease processes by modifying their metabolism needs to be established (Table 1). In addition, not only bacterial pathogens colonization but also lung microbiome disequilibrium in terms of abundance or diversity could be implicated in disease progression, exacerbation, and response to treatments [113]. There is now evidence to support the concept that in lung microbiome "more diversity leads to less pathology". It would be also interesting to establish whether a specific lung microbiome composition may predispose to respiratory tract viral infection including Sars-CoV-2 [114]. To date, there is still a lack of consistent and homogeneous data to define the direct correlation between lung dysbiosis and chronic lung disease or lung cancer [5, 99]. Dysbiosis may be caused by several mechanisms which include: (i) destruction of lung barriers that are involved in the elimination of bacteria, as in CF, (ii) modality of birth delivery [115], (iii) ''farm effect'' [43, 44], (iv) lifestyle [11], (v) frequent use of antibiotics [65], and (vi) gut microbiome modification [101]. Dysbiosis may contribute to stimulation and overreaction of the immune system leading to an inflammatory state priming disease development, progression, and exacerbation. Extensive studies are required to better understand the relationship between lung microbiome-host interactions which may lead to therapeutic breakthroughs in terms of modulation of microbiome in pulmonary diseases.

Declarations

Competing interests The authors declare no competing interests.

References

1. Zitvogel L, Daillère R, Roberti MP, Routy B, Kroemer G (2017) Anticancer effects of the microbiome and its products. Nat Rev Microbiol 15(8):465–478

2. Huffnagle GB, Dickson RP, Lukacs NW (2017) The respiratory tract microbiome and lung inflammation: a two-way street. Mucosal Immunol 10(2):299–306

3. Yagi K, Huffnagle GB, Lukacs NW, Asai N (2021) The lung microbiome during health and disease. Int J Mol Sci 22(19):1–13

4. Chotirmall SH, Gellatly SL, Budden KF, Mac Aogain M, Shukla SD, Wood DLA et al (2017) Microbiomes in respiratory health and disease: an Asia-Pacific perspective. Respirology 22(2):240–250

5. Budden KF, Shukla SD, Rehman SF, Bowerman KL, Keely S, Hugenholtz P et al (2019) Functional effects of the microbiota in chronic respiratory disease. Lancet Respir Med 7(10):907–920

6. Erb-Downward JR, Thompson DL, Han MK, Freeman CM, McCloskey L, Schmidt LA et al (2011) Analysis of the lung microbiome in the "healthy" smoker and in COPD. PLoS ONE 6(2):e16384

7. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C et al (2010) Disordered microbial communities in asthmatic airways. PLoS ONE 5(1):e8578

8. Gomes S, Cavadas B, Ferreira JC, Marques PI, Monteiro C, Sucena M et al (2019) Profiling of lung microbiota discloses differences in adenocarcinoma and squamous cell carcinoma. Sci Rep 9(1):1–11

9. Valverde-Molina J, García-Marcos L (2023) Microbiome and asthma: microbial dysbiosis and the origins, phenotypes, persistence, and severity of asthma. Nutrients 15(3):486

10. Hu T, Dong Y, Yang C, Zhao M, He Q (2021) Pathogenesis of children's allergic diseases: refocusing the role of the gut microbiota. Front Physiol 12:1–10

11. Natalini JG, Singh S, Segal LN (2023) The dynamic lung microbiome in health and disease. Nat Rev Microbiol 21:222–235

12. O'Dwyer DN, Ashley SL, Gurczynski SJ, Xia M, Wilke C, Falkowski NR et al (2019) Lung microbiota contribute to pulmonary inflammation and disease progression in pulmonary fibrosis. Am J Respir Crit Care Med 199(9):1127–1138

13. Dickson RP, Erb-Downward JR, Huffnagle GB (2013) The role of the bacterial microbiome in lung disease. Expert Rev Respir Med 7(3):245–257

14. Esposito V, Lucariello A, Savarese L, Cinelli MP, Ferraraccio F, Bianco A et al (2012) Morphology changes in human lung epithelial cells after exposure to diesel exhaust micron sub particles (PM1.0) and pollen allergens. Environ Pollut 171:162–167

15. Brune K, Frank J, Schwingshackl A, Finigan J, Sidhaye VK (2015) Pulmonary epithelial barrier function: some new players and mechanisms. Am J Physiol-Lung Cell Mol Physiol 308(8):L731–L745

16. Boccia M, Aronne L, Celia B, Mazzeo G, Ceparano M, D'Agnano V et al (2020) COVID-19 and coagulative axis: review of emerging aspects in a novel disease. Monaldi Arch Chest Dis. https://doi.org/10.4081/monaldi.2020.1300

17. Roca J, Vargas C, Cano I, Selivanov V, Barreiro E, Maier D et al (2014) Chronic obstructive pulmonary disease heterogeneity: challenges for health risk assessment, stratification and management. J Transl Med 12(Suppl 2):S3

18. Prasetyo A, Sadhana U, Budiman J (2021) Nasal mucociliary clearance in smokers: A systematic review. Int Arch Otorhinolaryngol 25(1):160–169

19. Sethi S, Maloney J, Grove L, Wrona C, Berenson CS (2006) Airway inflammation and bronchial bacterial colonization in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 173(9):991–998

20. Hirschmann JV (2000) Do bacteria cause exacerbations of COPD? Chest 118(1):193–203

21. Pragman AA, Kim HB, Reilly CS, Wendt C, Isaacson RE (2012) The lung microbiome in moderate and severe chronic obstructive pulmonary disease. PLoS ONE 7(10):e47305

22. Lin L, Li J, Song Q, Cheng W, Chen P (2022) The role of HMGB1/RAGE/TLR4 signaling pathways in cigarette smoke-induced inflammation in chronic obstructive pulmonary disease. Immunity, Inflamm Dis 10(11):e711

23. Gangemi S, Casciaro M, Trapani G, Quartuccio S, Navarra M, Pioggia G, et al (2015) Association between HMGB1 and COPD: a systematic review. Mediat Inflamm

24. Alpkvist H, Athlin S, Mölling P, Norrby-Teglund A, Strålin K (2018) High HMGB1 levels in sputum are related to

pneumococcal bacteraemia but not to disease severity in community-acquired pneumonia. Sci Rep 8(1):1–9

25. Polosukhin VV, Cates JM, Lawson WE, Zaynagetdinov R, Milstone AP, Massion PP et al (2011) Bronchial secretory immunoglobulin a deficiency correlates with airway inflammation and progression of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 184(3):317–327

26. Baraldo S, Turato G, Badin C, Bazzan E, Beghé B, Zuin R et al (2004) Neutrophilic infiltration within the airway smooth muscle in patients with COPD. Thorax 59(4):308–312

27. Sze MA, Dimitriu PA, Hayashi S, Elliott WM, McDonough JE, Gosselink JV et al (2012) The lung tissue microbiome in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 185(10):1073–1080

28. Dang AT, Marsland BJ (2019) Microbes, metabolites, and the gut-lung axis. Mucosal Immunol 12(4):843–850

29. Chen L-W, Chen P-H, Hsu C-M (2011) Commensal microflora contribute to host defense against *Escherichia coli* pneumonia through Toll-like receptors. Shock 36(1):67–75

30. Schuijt TJ, Lankelma JM, Scicluna BP, de Sousa e Melo F, Roelofs JJTH, de Boer JD et al (2016) The gut microbiota plays a protective role in the host defence against pneumococcal pneumonia. Gut 65(4):575–583

31. Sprooten RTM, Lenaerts K, Braeken DCW, Grimbergen I, Rutten EP, Wouters EFM et al (2018) Increased small intestinal permeability during severe acute exacerbations of COPD. Respiration 95(5):334–342

32. Qu L, Cheng Q, Wang Y, Mu H, Zhang Y (2022) COPD and gut-lung axis: how microbiota and host inflammasome influence COPD and related therapeutics. Front Microbiol 13:868086

33. Zou M, Zhang W, Xu Y, Zhu Y (2022) Relationship between COPD and GERD: a bibliometrics analysis. Int J Chron Obstruct Pulmon Dis 17:3045–3059

34. Crowell MD, Zayat EN, Lacy BE, Schettler-Duncan A, Liu MC (2001) The effects of an inhaled beta(2)-adrenergic agonist on lower esophageal function: a dose-response study. Chest 120(4):1184–1189

35. Huang C, Liu Y, Shi G (2020) A systematic review with meta-analysis of gastroesophageal reflux disease and exacerbations of chronic obstructive pulmonary disease. BMC Pulm Med 20(1):2

36. Yu F, Huang Q, Ye Y, Zhang L (2022) Effectiveness of proton-pump inhibitors in chronic obstructive pulmonary disease: a meta-analysis of randomized controlled trials. Front Med 9:841155

37. Agustí A, Celli BR, Criner GJ, Halpin D, Anzueto A, Barnes P et al (2023) Global initiative for chronic obstructive lung disease 2023 report: GOLD executive summary. Eur Respir J 61(4):2300239

38. Contoli M, Pauletti A, Rossi MR, Spanevello A, Casolari P, Marcellini A et al (2017) Long-term effects of inhaled corticosteroids on sputum bacterial and viral loads in COPD. Eur Respir J 50(4):1700451

39. Carrera-Salinas A, González-Díaz A, Ehrlich RL, Berbel D, Tubau F, Pomares X et al (2023) Genetic adaptation and acquisition of macrolide resistance in *Haemophilus* spp. during persistent respiratory tract colonization in chronic obstructive pulmonary disease (COPD) Patients receiving long-term azithromycin treatment. Microbiol Spectr. 11(1):e0386022

40. Nigro E, Daniele A, Scudiero O, Monaco ML, Roviezzo F, D'Agostino B, Mazzarella G, Bianco A (2015) Adiponectin in asthma: implications for phenotyping. Curr Protein Pept Sci 16(3):182–187

41. Scherzer R, Grayson MH (2018) Heterogeneity and the origins of asthma. Ann Allergy Asthma Immunol 121(4):400–405

42. Martinez FD, Guerra S (2018) Early origins of asthma role of microbial dysbiosis and metabolic dysfunction. Am J Respir Crit Care Med 197(5):573–579

43. Durack J, Boushey HA, Lynch SV (2016) Airway microbiota and the implications of dysbiosis in asthma. Curr Allergy Asthma Rep 16(8)

44. Loss GJ, Depner M, Hose AJ, Genuneit J, Karvonen AM, Hyvärinen A et al (2016) The early development of wheeze environmental determinants and genetic susceptibility at 17q21. Am J Respir Crit Care Med 193(8):889–897

45. Martín R, Heilig GHJ, Zoetendal EG, Smidt H, Rodríguez JM (2007) Diversity of the Lactobacillus group in breast milk and vagina of healthy women and potential role in the colonization of the infant gut. J Appl Microbiol 103(6):2638–2644

46. Losol P, Park HS, Song WJ, Hwang YK, Kim SH, Holloway JW et al (2022) Association of upper airway bacterial microbiota and asthma: systematic review. Asia Pac Allergy 12(3):1–15

47. Teo SM, Mok D, Pham K, Kusel M, Serralha M, Troy N et al (2015) The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development. Cell Host Microbe 17(5):704–715

48. Jacquet A (2011) Interactions of airway epithelium with protease allergens in the allergic response. Clin Exp Allergy 41(3):305–311

49. Holgate ST, Wenzel S, Postma DS, Weiss ST, Renz H, Sly PD (2015) Asthma. Nat Rev Dis Prim 1:1–22

50. Petersen C, Round JL (2014) Defining dysbiosis and its influence on host immunity and disease. Cell Microbiol 16(7):1024–1033

51. Denner DR, Sangwan N, Becker JB, Hogarth DK, Oldham J, Castillo J et al (2016) Corticosteroid therapy and airflow obstruction influence the bronchial microbiome, which is distinct from that of bronchoalveolar lavage in asthmatic airways. J Allergy Clin Immunol 137(5):1398–1405

52. Richardson H, Dicker AJ, Barclay H, Chalmers JD (2019) The microbiome in bronchiectasis. Eur Respir Rev 28(153):190048

53. Purcell P, Jary H, Perry A, Perry JD, Stewart CJ, Nelson A et al (2014) Polymicrobial airway bacterial communities in adult bronchiectasis patients. BMC Microbiol 14(1):1–11

54. King P (2011) Pathogenesis of bronchiectasis. Paediatr Respir Rev 12(2):104–110

55. Pasteur MC, Helliwell SM, Houghton SJ, Webb SC, Foweraker JE, Coulden RA et al (2000) An investigation into causative factors in patients with bronchiectasis. Am J Respir Crit Care Med 162(4I):1277–1284

56. Flume PA, Chalmers JD, Olivier KN (2018) Advances in bronchiectasis: endotyping, genetics, microbiome, and disease heterogeneity. Lancet 392(10150):880–890

57. Tung JP, Fraser JF, Wood P, Fung YL (2009) Respiratory burst function of ovine neutrophils. BMC Immunol 10:1–11

58. Whitters D, Stockley R (2012) Immunity and bacterial colonisation in bronchiectasis. Thorax 67(11):1006–1013

59. Martínez-García MA, Soler-Cataluña JJ, Perpiñá-Tordera M, Román-Sánchez P, Soriano J (2007) Factors associated with lung function decline in adult patients with stable non-cystic fibrosis bronchiectasis. Chest 132(5):1565–1572

60. Byun MK, Chang J, Kim HJ, Jeong SH (2017) Differences of lung microbiome in patients with clinically stable and exacerbated bronchiectasis. PLoS ONE 12(8):1–18

61. Rogers GB, Van Der Gast CJ, Cuthbertson L, Thomson SK, Bruce KD, Martin ML et al (2013) Clinical measures of disease in adult non-CF bronchiectasis correlate with airway microbiota composition. Thorax 68(8):731–737

62. Tunney MM, Einarsson GG, Wei L, Drain M, Klem ER, Cardwell C et al (2013) Lung microbiota and bacterial abundance in patients with bronchiectasis when clinically stable and during exacerbation. Am J Respir Crit Care Med 187(10):1118–1126

63. Finch S, McDonnell MJ, Abo-Leyah H, Aliberti S, Chalmers JD (2015) A comprehensive analysis of the impact of pseudomonas aeruginosa colonization on prognosis in adult bronchiectasis. Ann Am Thorac Soc 12(11):1602–1611

64. Hill AT, Haworth CS, Aliberti S, Barker A, Blasi F, Boersma W et al (2017) Pulmonary exacerbation in adults with bronchiectasis: a consensus definition for clinical research. Eur Respir J 49(6)

65. Cox MJ, Turek EM, Hennessy C, Mirza GK, James PL, Coleman M et al (2017) Longitudinal assessment of sputum microbiome by sequencing of the 16S rRNA gene in non-cystic fibrosis bronchiectasis patients. PLoS ONE 12(2):1–17

66. Mac Aogáin M, Narayana JK, Tiew PY, Ali NABM, Yong VFL, Jaggi TK et al (2021) Integrative microbiomics in bronchiectasis exacerbations. Nat Med 27(4):688–699

67. Vanfleteren LEGW, Spruit MA, Wouters EFM, Franssen FME (2016) Management of chronic obstructive pulmonary disease beyond the lungs. Lancet Respir Med 4(11):911–924

68. Narayana JK, Aliberti S, Mac Aogáin M, Jaggi TK, Ali NABM, Ivan FX et al (2023) Microbial dysregulation of the gut-lung axis in bronchiectasis. Am J Respir Crit Care Med 207(7):908–920

69. Narayana JK, Aliberti S, Aogáin MM, Jaggi TK, Ali NABM, Xaverius IF et al (2022) Dysregulation of the microbial 'gut-lung' axis in bronchiectasis. Eur Respir J 60(Suppl 66):1646

70. Koh W-J, Lee JH, Kwon YS, Lee KS, Suh GY, Chung MP et al (2007) Prevalence of gastroesophageal reflux disease in patients with nontuberculous mycobacterial lung disease. Chest 131(6):1825–1830

71. Ahn B, Lee DH, Lee CM, Hwang JJ, Yoon H, Shin CM et al (2016) Effect of proton pump inhibitors in bronchiectatic patients with gastroesophageal reflux disease. Korean J Gastroenterol 68(1):10–15

72. Altenburg J, de Graaff CS, Stienstra Y, Sloos JH, van Haren EH, Koppers RJ et al (2013) Effect of azithromycin maintenance treatment on infectious exacerbations among patients with non-cystic fibrosis bronchiectasis: the BAT randomized controlled trial. JAMA 309(12):1251–1259

73. Malhotra S, Hayes D, Wozniak DJ (2019) Cystic fibrosis and pseudomonas aeruginosa: the host-microbe interface. Clin Microbiol Rev 32(3):1–46

74. Françoise A, Héry-Arnaud G (2020) The microbiome in cystic fibrosis pulmonary disease. Genes 11(5):536

75. Scialo F, Amato F, Cernera G, Gelzo M, Zarrilli F, Comegna M et al (2021) Lung microbiome in cystic fibrosis. Life 11(2):1–7

76. Shah VS, Meyerholz DK, Tang XX, Reznikov L, Alaiwa MA, Ernst SE et al (2016) Airway acidification initiates host defense abnormalities in cystic fibrosis mice. Science 351(6272):503–507

77. Pezzulo AA, Tang XX, Hoegger MJ, Abou Alaiwa MH, Ramachandran S, Moninger TO et al (2012) Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. Nature 487(7405):109–113

78. Scudiero O, Galdiero S, Cantisani M, Di Noto R, Vitiello M, Galdiero M et al (2010) Novel synthetic, salt-resistant analogs of human beta-defensins 1 and 3 endowed with enhanced antimicrobial activity. Antimicrob Agents Chemother 54(6):2312–2322

79. Carnovale V, Castaldo A, Di Minno A, Gelzo M, Iacotucci P, Illiano A et al (2022) Oxylipin profile in saliva from patients with cystic fibrosis reveals a balance between pro-resolving and pro-inflammatory molecules. Sci Rep 12(1):1–10

80. Castaldo A, Iacotucci P, Carnovale V, Cimino R, Liguori R, Comegna M et al (2020) Salivary cytokines and airways disease severity in patients with cystic fibrosis. Diagnostics 10(4):222

81. Crovella S, Segat L, Amato A, Athanasakis E, Bezzerri V, Braggion C et al (2011) A polymorphism in the 5' UTR of the DEFB1 gene is associated with the lung phenotype in F508del

82. Tomaiuolo R, Ruocco A, Salapete C, Carru C, Baggio G, Franceschi C et al (2012) Activity of mannose-binding lectin in centenarians. Aging Cell 11(3):394–400

83. Castaldo A, Cernera G, Iacotucci P, Cimbalo C, Gelzo M, Comegna M et al (2020) TAS2R38 is a novel modifier gene in patients with cystic fibrosis. Sci Rep 10(1):5–10

84. Hogan DA, Willger SD, Dolben EL, Hampton TH, Stanton B, Morrison HG et al (2016) Analysis of lung microbiota in bronchoalveolar lavage, protected brush and sputum samples from subjects with Mild-To- Moderate cystic fibrosis lung disease. PLoS ONE 11(3):1–23

85. Rieber N, Hector A, Carevic M, Hartl D (2014) Current concepts of immune dysregulation in cystic fibrosis. Int J Biochem Cell Biol 52:108–112

86. Klepac-Ceraj V, Lemon KP, Martin TR, Allgaier M, Kembel SW, Knapp AA et al (2010) Relationship between cystic fibrosis respiratory tract bacterial communities and age, genotype, antibiotics and *Pseudomonas aeruginosa*. Environ Microbiol 12(5):1293–1303

87. Keravec M, Mounier J, Guilloux CA, Fangous MS, Mondot S, Vallet S et al (2019) Porphyromonas, a potential predictive biomarker of *Pseudomonas aeruginosa* pulmonary infection in cystic fibrosis. BMJ Open Respir Res 6(1):1–5

88. Van Der Gast CJ, Walker AW, Stressmann FA, Rogers GB, Scott P, Daniels TW et al (2011) Partitioning core and satellite taxa from within cystic fibrosis lung bacterial communities. ISME J 5(5):780–791

89. Keravec M, Mounier J, Prestat E, Vallet S, Jansson JK, Burgaud G et al (2015) Insights into the respiratory tract microbiota of patients with cystic fibrosis during early *Pseudomonas aeruginosa* colonization. Springerplus 4(1):1–8

90. Goss CH, Burns JL (2007) Exacerbations in cystic fibrosis·1: epidemiology and pathogenesis. Thorax 62(4):360–367

91. Coburn B, Wang PW, Diaz Caballero J, Clark ST, Brahma V, Donaldson S et al (2015) Lung microbiota across age and disease stage in cystic fibrosis. Sci Rep 5:1–12

92. Zhao J, Schloss PD, Kalikin LM, Carmody LA, Foster BK, Petrosino JF et al (2012) Decade-long bacterial community dynamics in cystic fibrosis airways. Proc Natl Acad Sci USA 109(15):5809–5814

93. Goddard AF, Staudinger BJ, Dowd SE, Joshi-Datar A, Wolcott RD, Aitken ML et al (2012) Direct sampling of cystic fibrosis lungs indicates that DNA-based analyses of upper-airway specimens can misrepresent lung microbiota. Proc Natl Acad Sci USA 109(34):13769–13774

94. Whelan FJ, Surette MG (2015) Clinical insights into pulmonary exacerbations in cystic fibrosis from the microbiome what are we missing? Ann Am Thorac Soc 12(6):S207–S211

95. Fonseca C, Bicker J, Alves G, Falcão A, Fortuna A (2020) Cystic fibrosis: physiopathology and the latest pharmacological treatments. Pharmacol Res 162:105267

96. Heirali AA, Acosta N, Storey DG, Workentine ML, Somayaji R, Laforest-Lapointe I et al (2019) The effects of cycled inhaled aztreonam on the cystic fibrosis (CF) lung microbiome. J Cyst Fibros 18(6):829–837

97. Perrotta F, Rocco D, Vitiello F, De Palma R, Guerra G, De Luca A et al (2019) Immune checkpoint blockade for advanced NSCLC: a new landscape for elderly patients. Int J Mol Sci 20(9):2258

98. Nigro E, Perrotta F, Monaco ML, Polito R, Pafundi PC, Matera MG et al (2020) Implications of the adiponectin system in non-small cell lung cancer patients: a case-control study. Biomolecules 10(6):926

99. Stella GM, Scialò F, Bortolotto C, Agustoni F, Sanci V, Saddi J et al (2022) Pragmatic expectancy on microbiota and non-small cell lung cancer: a narrative review. Cancers 14(13):3131

100. Nigro E, Perrotta F, Scialò F, D'Agnano V, Mallardo M, Bianco A et al (2021) Food, nutrition, physical activity and microbiota: which impact on lung cancer? Int J Environ Res Public Health 18(5):2399

101. Dong Q, Chen ES, Zhao C, Jin C (2021) Host-microbiome interaction in lung cancer. Front Immunol 12:1–9

102. Mao Q, Jiang F, Yin R, Wang J, Xia W, Dong G et al (2018) Interplay between the lung microbiome and lung cancer. Cancer Lett 415:40–48

103. Bianco A, Malapelle U, Rocco D, Perrotta F, Mazzarella G (2018) Targeting immune checkpoints in non small cell lung cancer. Curr Opin Pharmacol 40:46–50

104. Bachem A, Makhlouf C, Binger KJ, de Souza DP, Tull D, Hochheiser K et al (2019) Microbiota-derived short-chain fatty acids promote the memory potential of antigen-activated CD8+ T cells. Immunity 51(2):285–329

105. Holmes E, Li JV, Marchesi JR, Nicholson JK (2012) Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. Cell Metab 16(5):559–564

106. McLean AEB, Kao SC, Barnes DJ, Wong KKH, Scolyer RA, Cooper WA et al (2022) The emerging role of the lung microbiome and its importance in non-small cell lung cancer diagnosis and treatment. Lung Cancer 165:124–132

107. Tsay JCJ, Wu BG, Badri MH, Clemente JC, Shen N, Meyn P et al (2018) Airway microbiota is associated with upregulation of the PI3K pathway in lung cancer. Am J Respir Crit Care Med 198(9):1188–1198

108. Yu G, Gail MH, Consonni D, Carugno M, Humphrys M, Pesatori AC et al (2016) Characterizing human lung tissue microbiota and its relationship to epidemiological and clinical features. Genome Biol 17(1):1–12

109. Dickson RP, Martinez FJ, Huffnagle GB (2014) The role of the microbiome in exacerbations of chronic lung diseases. Lancet 384(9944):691–702

110. Dickson RP, Erb-Downward JR, Freeman CM, McCloskey L, Beck JM, Huffnagle GB et al (2015) Spatial variation in the healthy human lung microbiome and the adapted island model of lung biogeography. Ann Am Thorac Soc 12(6):821–830

111. Goto T (2020) Airway microbiota as a modulator of lung cancer. Int J Mol Sci 21(9):3044

112. Yan X, Yang M, Liu J, Gao R, Hu J, Li J et al (2015) Discovery and validation of potential bacterial biomarkers for lung cancer. Am J Cancer Res 5(10):3111–3122

113. Erb-Downward JR, Falkowski NR, D'souza JC, McCloskey LM, McDonald RA, Brown CA et al (2020) Critical relevance of stochastic effects on low-bacterialbiomass 16s rrna gene analysis. MBio 11(3):1–12

114. D'Agnano V, Scialò F, Perna F, Atripaldi L, Sanduzzi S, Allocca V et al (2022) Exploring the role of Krebs von den Lungen-6 in severe to critical COVID-19 patients. Life 12:1141

115. Pattaroni C, Watzenboeck ML, Schneidegger S, Kieser S, Wong NC, Bernasconi E et al (2018) Early-life formation of the microbial and immunological environment of the human airways. Cell Host Microbe 24(6):857–865