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The respiratory microbiome of HIV-infected individuals

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Abstract

Introduction—The respiratory tract is constantly exposed to various environmental and endogenous microbes; however, unlike other similar mucosal surfaces, there has been limited investigation of the microbiome of the respiratory tract.

Areas Covered—In this review, we summarize the current state of knowledge of the bacterial, fungal, and viral respiratory microbiomes during HIV infection and how the microbiome might relate to HIV-associated lung disease.

Expert Commentary—HIV infection is associated with alterations in the respiratory microbiome. The clinical implications of lung microbial dysbiosis are however currently unknown. Mechanistic studies are needed to establish causality between shifts in the respiratory microbiome and pulmonary complications in HIV-infected individuals.

Keywords

HIV infection; lung microbiome; mycobiome; virome; pulmonary disease; bronchoalveolar lavage

1. Introduction

The human microbiome is an aggregate of microorganisms—bacteria, fungi, viruses and archaea that co-evolved with human cells. Microbial genes outnumber those of the host, and microbes have important roles in immune modulation, metabolism and vitamin synthesis [1]. On the bases of culture-dependent techniques [2–4], the lung was originally considered sterile and excluded from initial efforts to characterize the human microbiome [5]. With the application of culture-independent techniques, emerging data [6–8] challenged the paradigm of lung sterility in human and murine models. Thus, the lung microbiome has recently become an area of active research.

The clinical profile of HIV infection has changed dramatically since the advent of highly active antiretroviral therapy (HAART). While HAART administration does not constitute an HIV cure, the resultant viral control and health gains have improved the longevity of HIV-

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Declaration of interest

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infected individuals. Infectious pulmonary diseases have historically been a major co-morbidity in HIV infection, with *Pneumocystis* pneumonia (PCP) and *Mycobacterium tuberculosis* (TB) being the most common HIV-associated pulmonary complications [9]. In the post-HAART era, non-infectious pulmonary conditions including chronic obstructive pulmonary disease (COPD) have increased in the HIV-infected population [10–12]. It has been proposed that lung microbial dysbiosis contributes to inflammation in HIV-associated chronic lung diseases [13], and comprehensive studies are ongoing to establish causality.

Much of what is currently known about the lung microbiome in HIV has been reported by members of the Lung HIV Microbiome Project (LHMP), a multi-center research consortium that investigated the microbiome of the upper and lower respiratory tract in various populations of HIV-infected individuals [6, 13, 22–26]. In this review, we summarize the immune changes in HIV infection that may alter the lung microbiome, discuss existing data from the LHMP and other groups on the microbiome of the various components of the respiratory tract, and speculate on how these changes may influence disease.

2. Lung Immunity in HIV-infected Individuals

HIV infection alters host immune responses which may lead to alterations in the microbiome. HIV modifies local pulmonary immune regulation and may also impact other organ systems, particularly the gut which may indirectly shift the lung immune response. Although immune responses are improved with HAART, altered immunity and immune activation persist even in treated individuals.

2.1 Alterations in Lung Immunity with HIV

HIV infection impacts both innate and adaptive pulmonary immune responses in ways that may lead to alterations in the lung microbiome. HIV preferentially infects CD4 T cells, but due to the high frequency of HIV-specific CD4 T cells and robust alveolar Th17 responses in the lung mucosa, HIV-mediated CD4 T cell depletion is initially delayed [27]. Cytopathic effects of HIV infection eventually cause CD4 T cell depletion, which drives infiltration of interferon-gamma (IFN-γ)-producing CD8 T cells into the alveolar space resulting in lymphocytic alveolitis [28]. Persistent IFN-γ chronically activates alveolar macrophages (AM), increases permissiveness to HIV infection and promotes further CD4 T cell loss. AM dysfunction and impaired epithelial integrity shift T cell differentiation from Th17 cells towards immunosuppressive regulatory T cell (T_{reg}) phenotype [28–30]. Ensuing immunosuppression results in poor pathogen control, increasing susceptibility to opportunistic lung infections [28]. Additionally, HIV infection impairs B cell and antibody function which also mediate poor pathogen control [28]. Cross-talk with the gut mucosa and further exposure to microbial products activates plasmacytoid dendritic cells in the lung which produce IFN-α and indolamine 2, 3-dioxygenase (IDO), and thereby promote further T cell apoptosis, T_{reg} recruitment and perpetuate the cycle of chronic immune activation [27, 31]. These conditions may contribute to alterations in lung microbial composition.

2.2 Alterations in Gut Immunity that Impact the Lung

HIV-mediated damage to the gut mucosa has consequences on both gut and lung microbiota. HIV targets the gut associated lymphoid tissue (GALT), which contains the largest pool of CD4 T cells in the body. CD4 T cell depletion from the gut mucosa increases permeability and allows transmucosal passage of microbes and microbial products into systemic circulation [32]. Gut microbial translocation is the primary driver of the characteristic immune activation in HIV infection [33].

The gut microbiome has been studied extensively in health and in HIV infection [13–16]. Gut microbial diversity is lower in HIV-infected persons relative to HIV-uninfected individuals [17, 18] and appears to partially normalize with increasing duration on HAART [18]. Additionally, depletion of protective bacteria is more pronounced in HIV infection and improves with prebiotic treatment in HAART-naïve HIV-infected individuals [19]. Likewise, changes in the rectal microbiome with HIV infection have been reported [20]. HIV infection reduces rectal microbial diversity and enriches *Fusobacteria*; however, HAART effectively reverses dysbiosis [21].

The gut and lung mucosa are hypothesized to constitute an immune organ with shared functions in immunosurveillance and host immune response [34]. Cross-talk between the two mucosal sites is speculated to occur through the following potential mechanism: gut microbiota mediate antigen-specific T cell priming through toll-like receptor (TLR) signaling [35]. Upon insult to the airways, primed memory T cells may home to the lung mucosa and enhance protective anti-inflammatory responses [36]. In response to the influence of microbes or microbial elements on gut or lung mucosal epithelial cells, TLR-mediated NFκB (nuclear factor kappa-light-chain enhancer of activated B cells) signaling could lead to pro-inflammatory cytokine production and mediate local or distal inflammatory responses. Thus, gut TLR signals could mediate lung mucosal inflammatory responses [35]. Similarly, respiratory infections can alter gut microbiota, leading to intestinal inflammation and injury [37].

HIV-mediated microbiota dysbiosis or aberrant TLR signaling can result in systemic and local inflammation [32]. Inflammation, as well as alterations in microbiome composition, has been associated with inflammatory lung diseases including COPD [38–40]. Additionally, inflammation driven by microbial translocation causes fibrosis of lymphoid tissues throughout the body, resulting in end-organ disease [33]. Bronchial associated lymphoid tissue (BALT) is lymphoid tissue located in the submucosa of bronchioles. BALT is autologous to GALT (gut associated lymphoid tissue) and can be induced by chronic respiratory infections and immunodeficiency [41]. BALT induction is a marker of inflammation and has been described in certain chronic inflammatory phenotypes [42]. Thus, there might be a link between HIV infection, microbial dysbiosis and altered immunity in lung disease [36, 43].

3. Terminology and Methods

A comprehensive overview of the data analysis, sampling and molecular techniques applied in microbiome research is beyond the scope of this review and has been previously discussed

[13, 44]. A glossary of basic terminology and methods are referenced in Table 1 and Figure 1.

4. The Bacterial Microbiome

Microbial dynamics in the healthy respiratory tract provide insights to the natural history of lung diseases. Bacteria enter the lung largely by microaspiration [45] and the constitution of the healthy lung microbiome is determined by equilibrium between factors that govern microbial immigration and clearance [46]. Disruption of this balance is thought to create selective pressures, which drive microbial shrifts (and permit colonization) associated with disease states [46]. Thus the conventional notion of a single predominant causative pathogen in lung disease has expanded to include the effects of perturbing lung microbial homeostasis. HIV infection can independently drive imbalances in lung homeostasis, thus it is clinically relevant to determine whether microbial shifts are more prominent in HIV-associated lung diseases.

Subject-subject variations in the lung microbiome are greater than intrasubject variations during health [46] and the converse it true of disease states [47]. The sputum microbiome of COPD [48] and CF [49, 50] patients is reportedly relatively stable although can shift around the time of exacerbations, and biodiversity does decrease with disease severity in both conditions [48–52]. Additional studies are needed to translate these findings into improving therapy for lung diseases.

4.1 The Upper Respiratory Tract

HIV-infected persons run a disproportionate risk of developing oral and periodontal diseases, yet associated microbial shifts have been understudied in HIV infection. Li *et al* demonstrated decreased oral biodiversity in HIV-infected persons relative to uninfected controls, likely due to overgrowth of opportunistic microbes [53]. Distinct bacterial genera were also detected in HIV-uninfected, but not HIV-infected persons, suggesting HIV-mediated dysbiosis of core healthy oral microbiota. Further, the outgrowth of commensals such as *Aggregatibacter* appeared to be ameliorated with six months of HAART therapy relative to the pre-HAART baseline [53]. Thus, HAART reverses, at least partially, HIV-mediated selective pressure. Interestingly, neither HIV status nor HAART status impacted the predominance of *Streptococcus* and *Veillonella* [53]. This finding is in contrast to reports of increased lingual *Streptococcus* colonization six months post-HAART in HIV-infected individuals [54]. These differences suggest that the oral and lingual microbiomes might be distinct in HIV infection or that different sampling methods may contribute to these observations. While Li *et al* examined saliva samples [53], tongue scrapings were sampled in the lingual microbiome experiments [54]. Both were small studies, and larger longitudinal studies would clarify HIV and HAART-mediated effects on the upper airway microbiome.

A recent LHMP study compared the upper airway microbiome in a cohort of HIV-uninfected individuals, HAART-naïve HIV-infected individuals and HAART-treated HIV-infected individuals [23]. In this cohort, microbial communities in the upper respiratory tract (URT) varied with both HIV and HAART status. *Atopobium* was more prevalent in the HIV-infected groups. *Streptococcus* and *Actinomyces* were more prevalent in the HAART-naïve

HIV-infected group, while *Rothia* was most abundant in the HAART-treated HIV-infected group [23]. *Streptococcus* causes opportunistic infections and is associated with multiple HIV-associated diseases, thus abundant *Streptococcus* in the HAART-naïve HIV-infected group suggests it may have a pathogenic role. Additionally, CD4 counts did not correlate with the URT microbial composition [23]. However, CD4 counts were relatively preserved in the cohort and similar between the HAART naïve HIV-infected and HAART-treated HIV-infected groups—median CD4 count 668 and 618 counts/μL respectively. Other cohorts with a wider range of CD4 cell counts might demonstrate more significant differences in the URT microbiome with HIV infection. Of note, correlations between CD4 counts and gut microbial profiles have yielded conflicting results in HIV-infected individuals. Consistent with the above findings in the URT, certain studies have reported a lack of association between CD4 counts and gut microbial composition [55, 56], whereas others have reported associations between CD4 counts and enrichment of certain bacterial taxa [57, 58]. Thus in addition to CD4 counts, other markers of HIV progression should be included in analyses for more robust results.

Overall, these studies suggest that the upper respiratory tract microbial composition is influenced by HIV infection. Large cohorts with varying degrees of immune recovery (for example, broader ranges of CD4 counts and markers of immune activation and inflammation) might be beneficial in dissecting overall effects of HAART on the URT microbiome. Studies that assess the effects of concomitant antibiotic use and illicit drug use in the URT of HIV-infected individuals might provide clearer definition of URT microbial diversity in the HIV-infected population.

5. The Lower Respiratory Tract

5.1 HIV Infection Status

Recent studies have reported the existence of a distinct lower respiratory tract (LRT) microbiome in both health [6, 25] and disease [6, 24]. One of the initial findings of the LHMP was the detection of *Tropheryma whipplei* in HIV-infected individuals. *T. whipplei* had previously been detected in a small number of HIV-uninfected controls [25, 59], but the LHMP reported significantly higher abundance of *T. whipplei* in the lower respiratory tract (LRT) of HIV-infected individuals compared with HIV-uninfected controls, as well as decreased carriage with effective HAART [24]. Notably, despite its enrichment in the lung, *T. whipplei* was not detected in the oral cavity of either HIV-infected or uninfected individuals, suggesting a true niche for *T. whipplei* in the lower airways. This phenotype was unexpected because *Tropheryma whipplei* is the cause of Whipple's disease, which classically manifests in the gastrointestinal (GI) tract. Pulmonary involvement including pleural effusion and granulomatous mediastinal adenopathy has been reported and only rarely presents [60, 61]. *T. whipplei* infections are also not increased in HIV infection [61]. Interestingly, none of the participants with high *T. whipplei* burden presented with serious pulmonary manifestations at the time of sampling [24], but more subtle effects on the lung inflammatory response have not been evaluated. The [51][50] clinical implications of asymptomatic *T. whipplei* colonization in the lung, as well as its functional interactions with other lung microbiota in the HIV-infected host are currently unknown.

Two recent LHMP studies characterized the lower respiratory tract microbiome at different stages of HIV disease [23, 26]. Beck and colleagues evaluated a cohort of HIV-uninfected individuals, HAART naïve HIV-infected individuals and HAART-treated HIV-infected individuals [23]. In this cohort, BAL samples showed similarities in microbial community structure between the HIV-uninfected and infected groups as well as between the HAART-naïve and treated groups. Thus, neither HIV nor HAART status appeared to be important factors in determining the lower airways microbial structure in this cohort, but CD4 cell counts were relatively well-preserved which may have prevented shifts in the microbiome. In addition, there may still be functional differences between the lung microbiota in HIV infection that are not captured in taxonomic descriptions.

The authors also compared the URT and LRT microbiome within the cohort and reported microbiome differences in anatomic site by HIV and HAART status. In the HIV-uninfected group, *Streptococcus, Veillonella, Prevotella, Fusobacterium* and *Rothia* were more abundant in the URT than the LRT. In the HAART-naïve, HIV-infected group, *Rothia, Veillonella, and Granulicatella* were more abundant in the URT than the LRT. This study was one of the first to describe the lung microbiome in HIV-infected individuals and lays the groundwork for studies examining function of the microbiome and impact on the host in HIV.

Twigg and colleagues recently examined the lung microbiome in 20 HIV-uninfected persons and 26 HIV-infected persons sampled by BAL at baseline (pre-HAART), 1 year and 3 years post-HAART initiation [26]. In contrast to the LHMP study above, the median CD4 count at baseline was 280 cells/μL and 375 cells/ μL at 3 years. At baseline, the lower airways of HIV-infected participants were characterized by decreased richness (α diversity), but increased β diversity of microbial communities. Taxonomic differences at baseline included elevated abundance of *Streptococcus* in HIV-infected subjects and increased abundance of *Flavobacterium* in uninfected controls [26]. Although these findings are in contrast with results from Beck *et al* [23], the differences are likely due to differences in the immune status of the cohorts.

The microbiome in these individuals also changed during the course of HAART use. After 1 year of HAART, the dissimilarities in α and β diversity between HIV-infected and uninfected groups were less pronounced, although they persisted to some degree. The differences in relative abundance of *Streptococcus* and *Flavobacterium* persisted at pre-HAART levels. Interestingly, the frequency of *Veillonella* and *Prevotella* increased in the HIV-infected group at this time point [26]. At the 3-year time point, the differences in abundance of *Streptococcus* and *Flavobacterium* were less pronounced. The differences in *Prevotella* and *Veillonella* were not significant between the groups; however, *Veillonella* was still higher at 3 years post-HAART, relative to baseline. *Prevotella* and *Veillonella* outgrowth has been associated with subclinical inflammation in individuals with stable COPD [62]. Thus the dynamics of these genera might be indicative of the kinetics of ART in regulating inflammation and resultant dysbiosis in the context of HIV. How these dynamics vary in HIV-associated COPD remains to be clarified. Overall, the differences in diversity and abundance of bacterial communities improved with increasing time on HAART.

Together, these studies suggest that HIV infection alters the lung microbiome, and that HAART may partially normalize the lung microbial communities. Measures of immune recovery (including CD4 count, inflammation and immune activation markers) may be important predictors of microbial dysbiosis in the lung of HIV-infected persons. Additional studies are required to identify peripheral biomarkers of alteration in the lung microbiome. Further mechanistic investigations into the functional correlates of lung microbial dysbiosis in HIV-infected individuals might enhance understanding of pulmonary disease pathogenesis in the HIV-infected population.

5.2 Acute Pneumonia

HIV-infected individuals, regardless of CD4 reconstitution and viral suppression, remain at increased risk for recurrent pneumonia [63]. Whether HIV infection and/or therapy mediated alterations in the microbiome can be used to predict recurrent pneumonia is currently being investigated. Dynamics of respiratory microbiota vary with therapeutic modalities and may influence treatment outcomes. In COPD for instance, antibiotics decrease sputum biodiversity, but corticosteroids tend to increase biodiversity, and the effects of these therapies extend beyond clinical recovery [48]. Microbial structure has also been shown to vary with stages of TB infection. Interestingly *Pseudomonas* was more abundant in treatment failure compared with recurrent or cured TB patients, suggesting a role of the microbiome in treatment outcomes. Defining the role of the microbiome, together with mechanistic studies are necessary to guide effective treatment when either disease is concurrent with HIV.

In two independent studies, Iwai and colleagues investigated respiratory bacterial communities associated with acute pneumonia in HIV-infected persons [64, 65]. In the first study [64], paired oral (combined lingual scrapings and oropharyngeal wash) and airway (BAL) samples were compared in a cohort of HIV-infected persons during the course of antimicrobial therapy for acute pneumonia. Airway microbiota were also compared between this cohort and HIV-uninfected acute pneumonia patients on antimicrobial therapy. Within the HIV-infected acute pneumonia group, bacterial burden was lower in the lower respiratory tract (LRT) compared to the upper respiratory tract (URT) [64]. The URT and LRT microbiota structure were similar in HIV-infected pneumonia patients who were using HAART, indicating HAART-mediated selective pressure on both oral and airway microbiota, despite concomitant anti-microbial use [64].

Interestingly, the baseline LRT microbial profile was found to predict the cause of recurrent pneumonia in a small number of the HIV-infected study participants [64]. This finding suggests a diagnostic potential of the microbial species present in the LRT despite antimicrobial therapy. Similar prognostic utility of microbial community structure has been demonstrated in certain chronic lung diseases. For example, bacterial community structure is reportedly the primary predictor of exacerbation frequency in bronchiectasis [66]. Larger longitudinal studies would be beneficial in evaluating the diagnostic potential of persistent microbes in the LRT of HIV-infected persons.

The study further investigated associations between airway microbial structure and acute pneumonia in HIV-infected and uninfected persons. HIV-infected persons with acute

pneumonia had greater airway diversity than HIV-uninfected pneumonia patients [64]. HIV-mediated immunosuppression and low CD4 count in the HIV group may permit bacterial outgrowth that could account for these observations. While acute pneumonia in HIV-uninfected persons was characterized by higher abundance of *Bacteriodes (Pseudomonas)* and *Proteobacteria*, acute pneumonia in the HIV-infected group was characterized by *Actinobacteria, Chloroflexi, Cyanobacteria, Bacteroidetes (Prevotellaceae*) and *Firmicutes (Clostridiaceae)* by 16S rRNA analyses. The distinct LRT microbiota in the two pneumonia groups suggests different pathogenesis of pneumonia, with the latter strains increasing susceptibility to recurrent HIV-associated pneumonia [64].

African HIV-infected individuals have higher mortality rates due to respiratory tract infections than HIV-infected individuals in developed countries. Having demonstrated the existence of distinct bacterial communities in the respiratory tract of HIV-infected individuals with acute pneumonia [64], Iwai and colleagues subsequently studied the LRT in HIV-infected Ugandan patients, within two weeks post-antimicrobial therapy for acute pneumonia. *Streptococcus pneumonia*—the most common agent of HIV-associated pneumonia in westernized countries was absent from the lower airway of Ugandan HIV-infected persons with pneumonia (by 16SrRNA quantification of BAL samples). Instead, *Pseudomonas aeruginosa* was the most frequent etiology of bacterial pneumonia in the Ugandan cohort [65]. Clinical factors in the Ugandan cohort, such as antimicrobial therapy and advanced HIV as indicated by low CD4 counts were associated with *Pseudomonas aeruginosa* [67] infections and were thought to contribute to these findings [65].

The lung microbiome may be important because it impacts the inflammatory response in the lung. Correlations were demonstrated between LRT microbial composition and pro-inflammatory gene expression from BAL samples in the Ugandan HIV-infected cohort [65]. TNF-α expression was directly correlated with bacterial burden and inversely correlated with phylogenetic diversity. Matrix metalloproteinase-9 (MMP-9), which is associated with airway remodeling, was inversely correlated with phylogenetic diversity and richness [65].

Interactions between microbial communities may be important in defining the LRT microbial composition in HIV-associated acute pneumonia. The ability of microbiota to regulate outgrowth of commensal bacterial species has been recently reported in the gut [68–70] as well as in the URT of a murine model [71]. In the Iwai study, LRT microbiome richness and phylogenetic diversity were inversely correlated with bacterial burden [65]. The lung microbiome of individuals with low bacterial burden was enriched for *Lachnospiraceae, Deslfuromonadacea and Alicyclobacillaceae* [65]. Importantly, these bacteria have been shown to function in immunomodulation [70] by inducing anti-inflammatory T_{regs} [72, 73] and producing hydrogen sulfide [74], each of which are protective to the lung. The predominance of these ‘lung protective’ phyla may account for the observed decreased LRT bacterial burden [65].

Overall, these results indicate the presence of distinct polymicrobial communities in the LRT of antimicrobial treated HIV-infected persons with acute pneumonia. LRT microbial structure influences local inflammatory responses and could impact clinical outcomes in HIV-associated pneumonias. Further evaluation of the impacts of different antimicrobial

classes and their effects on both lung microbiome and inflammation may provide beneficial insights to improved treatment regimens for HIV-associated pneumonias.

5.3 Geographic Effects

Given the interaction of the lung with the environment, it is possible that individuals in different geographic locations could have different lung microbiota. For example, variations in bacterial diversity and structure have been shown in sputum samples isolated from CF patients in the US compared with those from the UK [75]. Geographic effects on the lung microbiome in the HIV-infected population have been studied in a recent comparison of the respiratory microbiome in HIV-infected persons with acute pneumonia from San Francisco (US) and Uganda (east Africa) [65]. While lower airway bacterial burden was similar between the two cohorts, bacterial richness and phylogenetic diversity were significantly higher in the Ugandan population [65]. The San Franciscan LRT appeared to be primarily enriched with *Firmicutes* and *Actinobacteria*, while the Ugandan LRT was enriched in *Proteobacteria*.

Functionally, bacterial community enrichment was associated with glycan synthesis, polyketide degradation and lactose phosphotransferase pathways in the San Franciscan cohort. In the Ugandan cohort, associations were seen in pathways involved in flagellar assembly, lipopolysaccharide (LPS) and peptidoglycan synthesis as well as sugar metabolism [65]. Given that the pathways enriched in the Ugandan cohort are associated with pathogenesis of pneumonia, the authors conclude that enrichment for these pathways contribute to the higher rate of mortality in the Ugandan cohort [65].

While socioeconomic factors, compliance with HAART and other antimicrobial regimens may also influence the observed differences in the Ugandan and San Franciscan cohorts, there might be some underlying effects of geographical differences. Of note, between different cities within the US, the LHMP did not find any geographic differences in the lung microbiome of HIV-infected persons [24]. Aside from geographic effects, host genetics reportedly influences lung [76] and gut [77] microbiome structure. Though studies are limited, recent data from concordance studies suggest that host genetics affects the composition of the sputum microbiota in healthy individuals [76]. It is likely that a combination of lifestyle and environment might influence microbiome diversity differentially in genetically predisposed hosts. Additional studies are required to identify underlying genetic and molecular mechanisms in disease models. With the push towards personalized medicine, geographic and ethnic (genetic) effects on the respiratory microbiome require particular consideration.

5.4 Effects of Smoking

Cigarette smoking is common in HIV-infected individuals and may have multiple effects on mucosal surfaces. Smoking has been shown to modulate the microbial composition of the gut mucosa [78, 79]. Given that cigarette smoking has a direct impact on lung mucosal cells, it is likely that smoking could also alter the lung microbiome. Smoking promotes bacterial colonization of the upper airways by facilitating mucosal epithelial binding and by disrupting mucociliary clearance [80]. Additionally, smoking disrupts homeostasis of

commensal microbial communities in the upper airways and is associated with oral diseases in healthy individuals [81]. Despite these effects of smoking on the lung, the LHMP found no significant differences in lung bacterial communities by smoking status in HIV-uninfected individuals [25].

In HIV-infected individuals, the effects of smoking may be even more pronounced, but the direct effects of smoking on the lower airway microbiome in HIV are currently unknown. Smoking impairs immune responses to HAART [9]. It also enhances the risk of HIV-associated pneumonias [82]. COPD is one of the most common smoking related chronic lung diseases, and HIV infection is an independent risk factor for COPD [10–12]. There is evidence to suggest bidirectional cross-talk between airway microbiota and antigens from cigarette smoke in the pathogenesis and exacerbation of HIV-associated COPD. The 'vicious circle' hypothesis [38–40] posits that impaired lung immunity following an initial insult such as smoking, causes shifts in lung microbial colonization. The altered microbial antigens and other microbial products then perpetuate a cycle that contributes to chronic airway inflammation. It is therefore possible that smoking has direct effects on the lower respiratory microbiome in HIV-infected individuals.

6. The Fungal Microbiome (Mycobiome)

The microbiome is not solely composed of bacteria. Fungi and viruses are also present and may have important biological effects. Study of the fungal microbiota in the lung, or the lung mycobiome, lags behind the bacterial microbiome in studies relating to respiratory health and disease. Yet the mycobiome is an important component of the lung microbiome that interacts with bacterial communities and influences host immune responses [83, 84]. The mycobiome has been investigated in cystic fibrosis (CF) with findings of decreased fungal diversity in CF patients [85].

Effects of the mycobiome in HIV and associated lung diseases are only just emerging. Cui *et al* recently analyzed oral wash (OW), induced sputum (IS) and BAL from HIV-infected and uninfected individuals with or without COPD [86]. Regardless of HIV or COPD status, distinct fungal clusters were shown in each compartment suggesting niche specificity along the respiratory tract [86]. The relative abundance of *Candida* was highest in the OW and progressively decreased from IS to BAL. As in the bacterial microbiome analysis, this overlap likely results from anatomic contiguity and suggests that the mouth is the source of some fungi in other respiratory compartments. Taxonomic analyses also showed predominance of *Ceriporia lacerata*, *Saccharomyces cerevisaiae and Penicillium brevicompactum* in the BAL compared to the OW. These fungi are implicated in opportunistic infections and may represent lung-specific commensals [86].

The investigators then examined the relationship of fungal communities to HIV and to HIV-associated COPD. Unique fungal communities were identified with HIV and with COPD in HIV-infected individuals. *Pneumocystis jirovecii* was the most dominant organism that distinguished HIV-infected from uninfected persons and was found in HIV-infected individuals with COPD [86]. The lung mycobiome also varied with factors of immune

recovery in HIV infection. HIV-infected persons with lower CD4 counts showed outgrowth of *Zasmidium nocoxi* and *Teratosphaeria jonkershoekensis* [86].

Pneumocystis detection by polymerase chain reaction (PCR) has previously been reported both in HIV-infected and HIV-uninfected individuals with COPD [87, 88]. Subclinical *Pneumocystis* infections are associated with accelerated COPD [87] and predict airflow obstruction in both HIV-infected and uninfected smokers with COPD [88]. Additionally, airway remodeling in COPD has been linked to matrix metalloproteinases (MMPs), and MMP-12 has been found to be increased in *Pneumocystis*-colonized HIV-infected individuals with COPD [87]. Thus, synergistic pathways could mediate accelerated COPD in HIV-infected persons.

The association of *Pneumocystis* with COPD has been demonstrated in animal models. Shipley *et al* [42] provided additional insights into functional outcomes of host-*Pneumocystis* interactions in a simian human immunodeficiency virus (SHIV) model. *Pneumocystis*-colonized SHIV macaques developed airflow obstruction and anatomic evidence of emphysema—increased lung and airway volume with decreased lung weight [42] compared to SHIV-infected animals that did not become *Pneumocystis*-colonized. In a rodent model of cigarette smoke-induced COPD, Christensen and colleagues demonstrated that mice colonized with *Pneumocystis* and exposed to cigarette smoke developed greater airway obstruction and lung inflammation than animals exposed to cigarette smoke only [89].

These studies suggest that fungal community structure is important in HIV and in COPD. The effects of antimicrobials, corticosteroid treatments and HAART on alterations in the fungal microbiome of HIV-infected persons are currently unknown. Further analysis of fungal-bacterial interactions in HIV infection as well as functional correlates are other knowledge gaps that require further study.

7. The Viral Microbiome (Virome)

The viral microbiome, or virome, is the most unstudied component of the lung microbiome. Studies have indicated a role of the virome in respiratory diseases including COPD, asthma and cystic fibrosis [90, 91]; however, the respiratory virome has not been studied in HIV infection. There is limited evidence to suggest that the presence of particular bacterial communities can enhance respiratory viral infections [92]. For instance, proteolytic activity of *Staphylococcus aureus* has been shown to enhance influenza A viral replication and infection [93, 94]. Additionally, respiratory viral-induced metabolites are thought to alter host immune responses and interactions between gut and respiratory microbiome influences local lung responses to viral infections [92]. Although current data are limited, certain gut viruses may play a role in HIV-associated chronic lung diseases. Thus, exploring gut-lung microbiome interactions as potential therapy for respiratory infections is an emerging concept in microbiome research [92]. Together, these findings provide a premise for further investigations into host-virome and bacterial-virome interactions in the HIV-infected population.

8. Metabolomics

Metabolomics, the systematic study of unique chemical fingerprints from cellular metabolism [95], is gaining prominence in microbiome research. Metabolites may be host- or microbiome-derived, and metabolic profiles vary in health and disease states. Much lung microbiome research has focused on characterizing microbial communities associated with disease states. While these studies have advanced the field significantly, functional description of host-microbiome interaction and their pathophysiologic implications at a more granular level are lacking. Metabolomic research begins to address some of these knowledge gaps; in the gastrointestinal tract, metabolomic approaches are being used to establish mechanistic links between microbial profiles and metabolism with the aim of developing target therapies and for disease monitoring [96, 97]. Similar studies have been few in the respiratory microbiome of HIV-infected hosts.

One of the first studies of the lung metabolome evaluated the ability to distinguish HIV-infected persons from healthy controls based on specific metabolic profiles of BAL fluid [98]. *Pyochelin*—a siderophore (iron carrier) excreted by *Pseudomonas aeruginosa* when iron is depleted, was elevated in the BAL of HIV-infected individuals relative to controls. HIV-infected participants were otherwise healthy and had no previous history of pneumonia [98]. *P. aeruginosa* colonization and resultant oxidative stress increases susceptibility to infection. Thus, the authors suggest early *pyochelin* detection may be a potential prognostic marker of lung immune health [98].

Recently, the metabolic signatures representative of host-microbiome interactions were investigated in the lower airways of HIV-infected individuals [99]. Similar to previous reports [23], the lung microbiota was indistinguishable between HIV-infected subjects and HIV-uninfected controls [99]. In contrast, the metabolome differed significantly by HIV status [99]. Particular metabolites were found to be associated with specific bacteria found by microbiome sequencing. For example, lineolate, glycerophospholipid and fatty acid metabolism pathways associated with *Caulobacteracea, Staphlococcaceae* and *Nocardiodaceae*. Importantly, these bacteria are clinically relevant etiologies of pneumonia in HIV-infected individuals.[99].

Further metabolite analysis demonstrated that four features were significantly over-represented in the BAL of HIV-infected compared to HIV-uninfected individuals, including cystine and two complex carbohydrates. Cystine was particularly interesting as it is a product of oxidation of cysteine [100], with increased abundance generally indicating increased oxidative stress. Cysteine is a rate-limiting component of glutathione (GSH) synthesis (the principal anti-oxidant in the lung) and plays an important role in maintaining the detoxification of free radicals and reactive oxygen species (ROS) [100]. It is also an integral part of fatty acid synthesis. Cysteine supply is limiting for important lymphocyte functions and impaired in HIV [101, 102]. HIV infection is also known to cause oxidative stress [103–105]. Thus, higher cystine levels likely reflect decreased protective cysteine, which could be particularly important in HIV. In fact, treatment with N-acetylcysteine has been studied in ART-naïve HIV-infected patients [106, 107] with relative increases in CD4+ T cell numbers.

Based on these metabolomic associations, the authors proposed the following cascade for lung microbiome-host interactions in the HIV-infected host: HIV infection causes inflammation and oxidative stress, which alter inflammatory and oxidative metabolic pathways in the lung. The resultant shift in lung microbiota and/or host responses impair airway immunity, increasing the risk of pneumonia as well as other HIV-associated chronic pulmonary diseases [99]. Metabolomics is also being explored in diagnostic and therapeutic platforms to identify biomarkers of inflammation in respiratory diseases including COPD, CF and asthma.[108,109]. Larger, longitudinal studies are necessary to examine the applicability and clinically utility of metabolomics; however, the metabolomic approach presents a promising avenue for evaluating the respiratory microbiome in HIV infection.

9. Expert Commentary

The dogma of lung sterility has been supplanted by the notion of the existence of niche-specialized microbiomes along the respiratory tract. Host-microbiome interactions are no longer encompassed in a single view of microbes as pathogens. Instead, emerging roles of lung microbiota in local and systemic immune modulation in both health and disease states are widely appreciated. Culture-independent techniques have revolutionized the ability to study rare, but clinically relevant microbes. However, interpreting lung microbiome data across studies is often limited by differences in sampling, molecular and analytic methods as well by experimental controls and reference groups. To avert some of these issues, optimization and standardization of existing methodologies will be necessary. Additionally, technological advancements along with enhanced reference databases will be particularly necessary to facilitate research in understudied areas such as the mycobiome and virome. As the field develops, functional correlates of microbial communities and mechanistic studies will become increasingly important. The clinical implications of the lung microbiome also require further investigation. A summary of the current understanding of lung microbiome interactions and the development of lung disease in the HIV-infected host is referenced in Figure 2. Investigations of the lung microbiome in healthy HIV-infected individuals, in those with advanced immunosuppression, and in HIV-infected individuals with acute and chronic lung diseases may provide insight into microbial dysbiosis and pulmonary complications in these cohorts as well as in other diseases. Overall, the field is rapidly growing and promises to profoundly impact current understanding and management of respiratory diseases, particularly in the HIV-infected host.

10. Five-year View

Building upon current work and the view of the respiratory tract as a polymicroorganism, future investigations will integrate bacterial, fungal and viral biomes in defining the pathogenesis of respiratory diseases. Additionally, by understanding the protective effects of core microbiota, therapeutic interventions can be developed. An assessment of antimicrobial and antiretroviral effects on the core microbiome would be beneficial in augmenting therapeutic strategies which permit optimal microbial composition. The respiratory microbiome should not be studied in isolation, studies that elucidate interactions between microbiota in the respiratory mucosa with other mucosal sites would be beneficial: Cross-talk between gut and respiratory microbiomes in immunomodulation require further

investigation in health and disease. Further insights into respiratory symptoms resulting from vertical transmission of HIV require further assessment of interactions between vaginal and respiratory mucosa. Finally, longitudinal studies that ensure environmental and geographic diversity would not only provide globally applicable, data but will enhance personalized medicine.

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* of interest and

** of considerable interest

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11. Key Issues

- The lung is not sterile. Distinct respiratory microbiomes exist in both health and disease states.
- The bacterial microbiome of the lung might be altered in HIV infection and other states of pulmonary disease. Factors such as HAART use, anti-microbial therapy, and immune health— as measured by CD4 counts are associated with shifts in microbial communities. Further studies are required to elucidate HIV-specific effects on lung microbial dysbiosis.
- Mycobiome-bacteria interactions influence species outgrowth, susceptibility to infection and exacerbation of inflammatory lung disorders.
- The virome has been understudied, but co-infections with multiple latent viruses are particularly important in HIV persistence and resultant lung complications.
- Metabolomic signatures of host-microbe and microbe-microbe interactions can elucidate functional and mechanistic pathways involved in HIV-associated pulmonary complications.

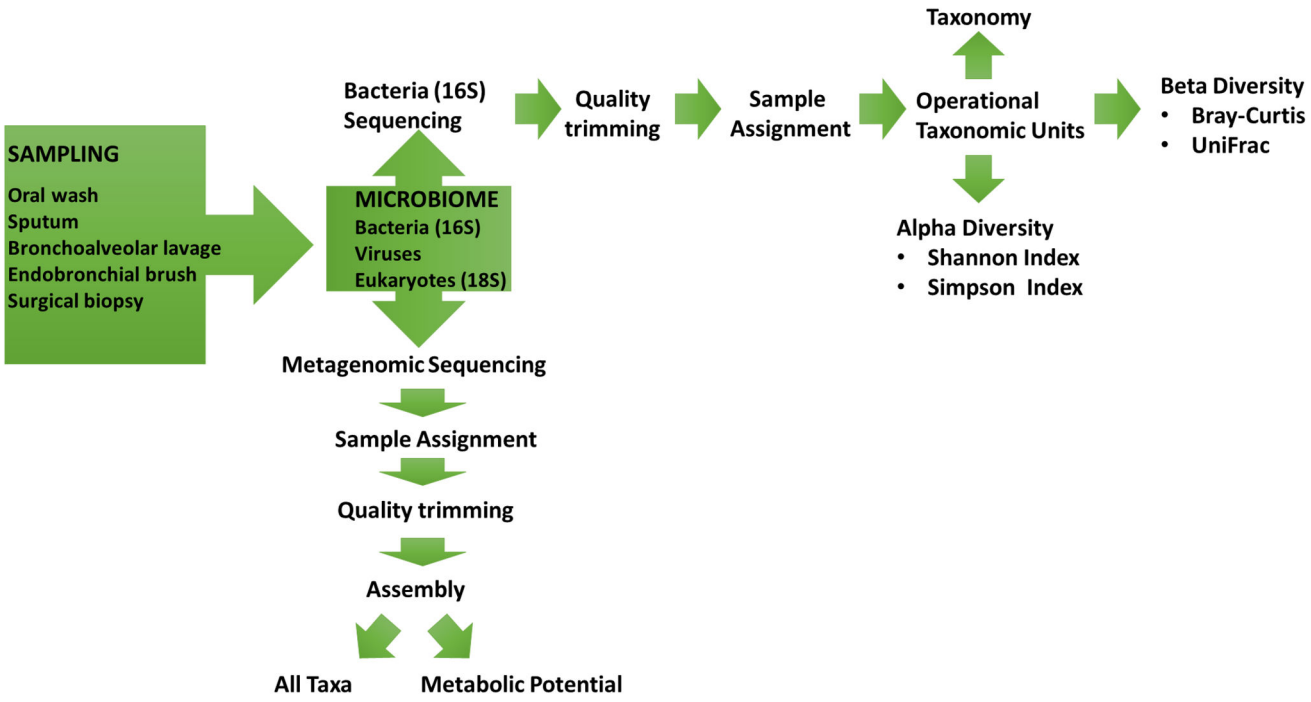

Figure 1.
Schematic of microbiome data acquisition, processing and data analysis.

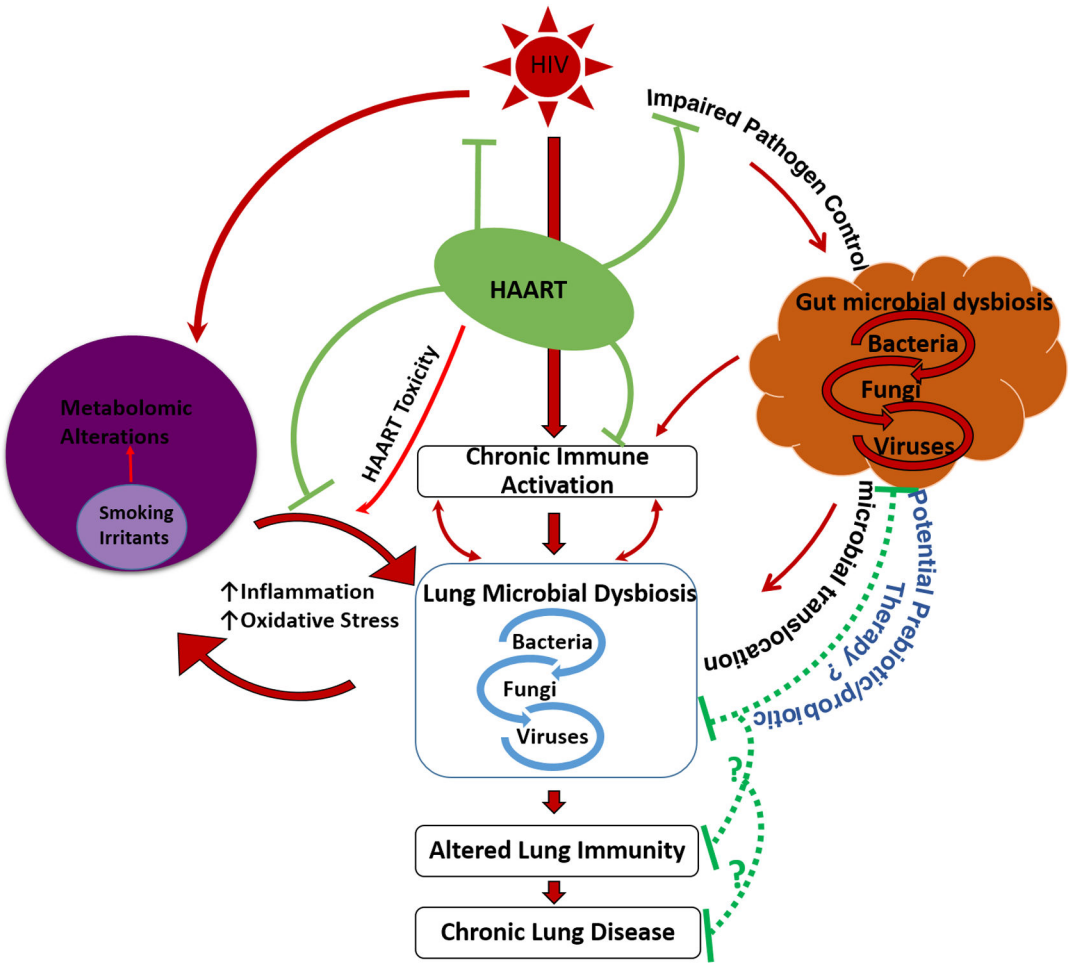

Figure 2.

Schematic of the relationships between HIV infection, alterations in host metabolic profiles (metabolome), alterations in gut and lung microbiomes and the development of chronic lung diseases.

Table 1

Terminology and Methods Used in Microbiome Research

Terminology	
Microbiota	The microorganisms of a particular site
Microbiome	Combined genetic material of all the microorganisms in a particular site
Mycobiome	Collection of the genomic contents of fungi within the microbiota
Virome	Collection of the genomic contents of viruses within the microbiota
Metabolomics	Study of unique chemical fingerprint from cellular metabolism includes both host- and microbe-associated metabolites
Processing	
Next generation sequencing	High throughput parallel DNA sequencing technologies. Includes Illumina, 454 Pyrosequencing, Polony sequencing etc.
Metagenomic shotgun sequencing	Method of determining nucleic acid sequence from a random mixed population of genomes.
16S rRNA gene sequencing	Method of determining DNA sequence of variable regions of the highly conserved bacterial 16S ribosomal gene
RNA-seq	Method of analyzing gene expression patterns from sequencing.
Analysis	
Alpha diversity	Measure of diversity within a sample
Beta diversity	Measure of uniqueness between populations
Richness	The number of species types in the microbiota
Evenness	Relative abundance or frequency of distribution of different communities
Chao1 richness index	Expression of numbers of different items in a population. Method of measuring α diversity.
Operational Taxonomic Unit (OTU)	Method of grouping similar taxa based on 16S rRNA sequence similarity. Used as a surrogate for species when sequences are 97% identical.
Shannon Index	Method of measuring diversity evenness. Lower number indicates lower diversity.
Simpson Index	Method of measuring diversity evenness. Lower number indicates higher diversity.
UniFrac	Method of determining phylogenetic distances between different populations.