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LUNG MICROBIOME IN HIV INFECTION

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Abstract

The lung microbiome plays a significant role in normal lung function and disease. Since microbial colonization is likely influenced by immunodeficiency, one would speculate that infection with Human Immunodeficiency Virus (HIV) alter the lung microbiome. Furthermore, how this alteration might impact pulmonary complications now seen in HIV-infected patients on antiretroviral therapy (ART), which has shifted from opportunistic infections to diseases associated with chronic inflammation, is not known. There have been limited publications on the lung microbiome in HIV infection, many of them emanating from the Lung HIV Microbiome Project. Current evidence suggests that the lung microbiome in healthy HIV-infected individuals with preserved CD4 counts is similar to uninfected individuals. However, in individuals with more advanced disease there is an altered alveolar microbiome characterized by a loss of richness and evenness (alpha diversity) within individuals. Furthermore, as a group the taxa making up the HIV-infected and uninfected lung microbiome are different (differences in beta diversity), and the HIV-infected population is more spread out (greater dispersion) than the uninfected population. These differences decline with ART, but even after effective therapy the alveolar microbiome in HIV-infected individuals contain increased amounts of signature bacteria, some of which have previously been associated with chronic lung inflammation. Furthermore, more recent investigations into the lung virome in HIV infection suggest that perturbations in lung viral communities also exist in HIV infection, and that these too are associated with evidence of lung inflammation. Thus it is likely both microbiome and virome alterations in HIV infection contribute to lung inflammation in these individuals, which has important implications on the changing spectrum of pulmonary complications in patients living with HIV.

Introduction

While the lung has traditionally been thought of as a sterile organ, the use of culture independent microbial detection methods such as 16S ribosomal RNA (rRNA) gene

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sequencing has strongly suggested that a lung microbiome is present, both in healthy (1-3) and diseased (1, 4, 5) populations. Recognition of the potential impact of the human microbiome on health and disease led the National Institutes of Health to add the Human Microbiome Project to the NIH Roadmap in 2007. In 2009 the National Heart, Lung, and Blood Institute created the Lung HIV Microbiome Project (LHMP) to better define the lung microbiome, both in healthy individuals and those with HIV infection. This project was driven by the recognition that pulmonary complications continued to be a major causes of morbidity in HIV-infected individuals even in the era of highly active antiretroviral therapy (ART) (6). Given the significant immune defects found in HIV infected individuals, the fundamental question proposed by all sites in the LHMP consortium was whether HIV infection altered the respiratory microbiome. Almost all that is now known about the lung microbiome in HIV infection arose from work performed by investigators in this consortium, some as manuscripts from the entire group, others as individual site research projects. In this review we will describe what is known about the respiratory microbiome in HIV infection to date. This will include descriptions of various diversity indices in HIV-infected individuals and uninfected controls, as well as the presence of overrepresented or signature bacteria in the HIV-infected population. We will speculate on potential models that may explain differences in various diversity models, both between HIV-infected and uninfected individuals and changes that occur in infected individuals on ART. Finally, we will discuss how perturbations in the lung microbiome might contribute to the changing spectrum of lung complications in HIV infection in the ART era.

The Lung Immune and Inflammatory Environment in HIV Infection

Untreated HIV infection impacts all components of the pulmonary immune response (7). In general, the alveolar environment in HIV infection is characterized by chronic alveolar macrophage (8, 9) and T cell (10, 11) activation, increased concentrations of most macrophage and lymphocyte cytokines (12), an inverted CD4:CD8 T cell ratio in the alveolar space due mostly to an increase in HIV-specific CD8+ cells resulting in a lymphocytic alveolitis (13-15), early preferential loss of antigen-specific memory CD4 T cells (16-18), and high immunoglobulin concentrations (19, 20) but with poor opsonic activity (21, 22).

Many of these findings are felt to be driven by the presence of HIV in the lung driving local immune and inflammatory responses. Since ART is associated with a significant decline in the lung HIV load (23), one would expect it to have a significant impact on lung inflammation and immunity. Indeed, this is true and has been described in detail previously (24, 25). In general, ART is associated with a decrease in CD8+ lymphocytes in the alveolar space leading to a more normal CD4:CD8 ratio (23), reduced lung T cell activation (26), and reduced concentrations of inflammatory cytokines and chemokines in bronchoalveolar lavage (7, 27, 28). However, despite improvements in immune function, subtle defects can still be detected in HIV-infected patients on ART (25).

The Lung Microbiome in HIV Infected Patients with Pneumonia

Given the effect of HIV infection on pulmonary immunity, it is not surprising these individuals were susceptible to lung infections in the pre-antiretroviral era. In general, despite evidence of chronic macrophage and lymphocyte activation, the ability to respond appropriately to microbial challenges is impaired. As a result, prior to the ART era opportunistic infections were the predominant pulmonary complication in HIV-infected individuals (29-31). The predominance of infections in HIV-infected individuals led early lung microbiome investigators to focus on individuals with clinical pneumonia. One of the first studies compared the lung microbiome between HIV-infected individuals and uninfected controls with pneumonia undergoing diagnostic bronchoscopy with BAL (32). Interesting findings from this study included an increased abundance of Proteobacteria in the uninfected group and increased Actinobacteria, Bacteroidetes, and Firmicutes in the HIV-infected population. Also notable was a significant increase in Prevotella in the HIV-infected population (32), a finding repeatedly found in later studies. This same investigative team subsequently compared the lung microbiome in HIV-infected patients in Ugandan with pneumonia to similar patients in San Francisco (33). The major finding from this work was that the lung microbiome differed in the two populations, not surprising given the likely major influence of environment on the microbiome at mucosal surfaces (34). The San Francisco cohort lung microbiome was enriched for Firmicutes and Actinobacteria. In contrast, the Ugandan cohort was enriched for Proteobacteria. *Pseudomonas aeruginosa* was the most frequently detected pathogen in the Ugandan cohort. Not stressed was the observation that of 2,671 taxa found in the Ugandan cohort, only 33 were found in all 60 individuals. Many of these taxa belonged to the Prevotellaceae, the second time this family of bacteria was found to be enriched in an HIV-infected population.

The Lung Microbiome in HIV Infected Patients Without Pneumonia

The next series of papers examining the lung microbiome in HIV infection focused on clinically stable individuals without obvious pneumonia. Even though these were clinically healthy individuals, based on the known immunodeficiency in HIV infection most investigators in the LHMP anticipated finding unusual organisms in the lung microbiome of these individuals. Indeed, one of the first observations made by multiple independent investigators in the LHMP consortium was an unusual number of clinically asymptomatic HIV-infected individuals whose lung microbiome measured in bronchoalveolar lavage (BAL) contained *Tropheryma whipplei*, an organism historically associated with the gut microbiome (4). In many of the individuals *T. whipplei* was the predominant organism, and, in subjects with longitudinal data, ART was associated with a decline in the relative abundance of this organism in BAL (4). Potential mechanisms leading to increased *T. whipplei* in the lung of HIV-infected individuals include lung immunodeficiency leading to a favorable ecological environment for the organism in the lower respiratory tract and the known breakdown of the gut mucosal barrier in HIV infection leading to increased translocation of gut microbes into the vascular compartment (35).

The second major paper from the LHMP consortium was a large study directly comparing the lung microbiome in whole BAL between HIV-infected individuals and uninfected

controls (36). This study included treatment naïve HIV-infected individuals as well as those on ART. The primary finding in this work was that in a large relatively healthy HIV cohort (median CD4 count in treatment naïve individuals 668 cells/mm³ and in individuals on highly active antiretroviral therapy 618 cells/mm³), the respiratory microbiome measured in whole bronchoalveolar lavage was indistinguishable between an HIV infected and uninfected population. These findings held for both individuals on antiretroviral treatment as well as treatment naïve individuals. Another multicenter study of HIV-infected individuals with preserved CD4 counts (median 600 cells/mm³) found identical results (37). Thus, in a clinically healthy HIV-infected population with relatively preserved peripheral blood CD4 counts the lung microbiome is similar to that found in a healthy control population.

While the above work was encouraging for HIV-infected individuals, it did not address the lung microbiome in individuals with more advanced disease, where immunologic and inflammatory perturbations were likely to be more pronounced. Recent work compared the lung microbiome between treatment naïve HIV-infected individuals with more advanced disease (mean CD4 count of 262 ± 198 cells/mm³) and an uninfected control population (38). Furthermore, the lung microbiome was examined longitudinally up to three years after beginning ART. Several interesting patterns were identified. First, alpha diversity richness (a measure of the number of different taxa in a sample, in this case an individual BAL) as assessed by Chao 1 and ACE richness indices was lower in HIV-infected individuals with advanced disease compared to uninfected individuals. Similarly, alpha diversity evenness (a measure of the distribution of taxa in a sample) as assessed by the Shannon diversity index and Simpson's index of diversity was lower in untreated HIV-infected individuals compared to uninfected individuals. This pattern of decreased individual or alpha diversity in advanced disease states has been described in other pulmonary processes, including COPD (1) and cystic fibrosis (5) and may reflect the tendency for a particular organism to gain a survival advantage in the altered lung environment. Second, beta-diversity (a measure of differences in taxa composition between two populations) analysis demonstrated there were significant differences between the untreated HIV-positive group as a whole and uninfected controls (**Figure 1**), suggesting that as a group HIV-infected patients were different from the uninfected population. Finally, the beta diversity pattern in the untreated HIV-infected population demonstrated significantly larger population dispersion compared to uninfected controls, with marked differences between various HIV-infected individuals (see figure legend for details). This large beta diversity dispersion in the HIV-infected population has been noted by other investigators as well (37). These data demonstrate there is significant heterogeneity in the lung microbiome within the HIV population as a whole.

How might this dichotomy between lung microbiome alpha and beta diversity in the HIV-infected population be explained? Delving into details of what constitutes each individual's lung microbiome is revealing. **Figure 1** shows individual subject identifiers in a population beta diversity plot at the Operational Taxonomic Units (OTU) level using weighted UniFrac analysis of previously reported antiretroviral naïve HIV-infected individuals and uninfected controls (38). It is readily apparent that the HIV population is more spread out, leading to greater beta diversity in the HIV-infected population as a whole. Specific analysis of plot outliers reveals that some individuals are disproportionately colonized with organisms not

found in the uninfected population. For example, individuals 001 and 003 contained in circle A are two HIV-infected individuals whose lung microbiome was dominated by Tropheryma (relative abundance 84% and 96%, respectively). The lung microbiome of the four HIV-infected individuals clustered within circle B are heavily weighted towards Streptococcus (subject 002 82%, subject 005 67%, subject 007 86%, subject 028 71%). It was uncommon for a non-HIV infected subject to have greater than 20% Streptococcus. Finally, the lung microbiome from individuals 014 and 012 in circle C were dominated by Burkholderia, Lactobacillus, and Propionibacterium, organisms again uncommon in the HIV uninfected population. Having a disproportionately large relative abundance of a particular taxa decreases alpha diversity within a subject. Having different organisms dominate particular individuals in a population increases group beta diversity. In summary, the lung microbiome in many asymptomatic HIV-infected individuals appears to demonstrate an overgrowth of both unusual (Tropheryma, Burkholderia) and usual (Streptococcus, Lactobacillus) organisms.

After 1 year of therapy, while differences in lung microbiome alpha diversity persist between uninfected and HIV-infected individuals, in most instances the difference is less pronounced. This is especially true for evenness assessments using Shannon or Simpson Indices (**Figure 2**). Differences in beta diversity between uninfected BAL and BAL from treated HIV-infected individuals as visualized by UniFrac also persist after 1 year on therapy (**Figure 3**). However dispersion analysis shows that the population spread in HIV-infected individuals after 1 year of ART is significantly less than in the untreated HIV-infected population and in fact is no longer significantly different from uninfected controls (see figure legend for details). Notable examples include individuals 001 and 003, whose baseline lung microbiome was dominated by Tropheryma. These taxa are markedly less abundant after one year of treatment, resulting in these individuals now falling within the center of the HIV cluster.

In summary, the lung microbiome in HIV-infected individuals with advanced disease demonstrates decreased alpha diversity (richness and evenness) and differences in beta diversity compared to uninfected BAL. Differences are reduced following ART, but still persist up to three years after starting therapy. Population dispersion in the HIV-infected group is significantly greater than in uninfected individuals, but declines with treatment. Taken together with the LHMP consortium findings, these data suggest that in healthy HIV-infected individuals, even those who have low CD4 counts before starting antiretroviral therapy, it is possible to attain a more normal appearing lung microbiome as assessed by various diversity indices and the presence of signature bacteria. These findings support the evolving practice of starting HIV-infected patients on antiretroviral therapy at earlier time points to try and prevent more severe immunologic and inflammatory perturbations and limit HIV-related complications (39, 40).

The Lung Microbiome in HIV Infection – Signature Bacteria

The initial LHMP manuscript suggested it would be easy to identify signature bacteria in the lung microbiome of HIV-infected individuals as a group, in this case Tropheryma whipplei (4). However, this has not been borne out in subsequent studies. The large LHMP

consortium paper comparing the lung microbiome from HIV infected individuals and uninfected controls failed to identify any signature bacteria in the HIV group (36). Even in HIV-infected individuals with more advanced disease, it was difficult to find evidence of increased abundance of a particular bacterial genus in the group as a whole. In untreated HIV-infected individuals with advanced disease, only *Streptococcus* was found in significantly higher abundances in the HIV microbiome (38). While interesting given the known high incidence of streptococcal pneumonia in HIV-infected individuals (41), it was none-the-less surprising that more signature bacteria were not seen. However, since impaired immune defenses in untreated HIV-infected individuals could allow carriage of a broad range of taxa leading to significant heterogeneity in this group (as seen in Figure 1), it is understandable that, as a group, the HIV population does not have average abundances of specific bacteria which differ from an uninfected population. Consistent with this speculation, after one year of ART when this heterogeneity has decreased, there were significantly more unique taxa found in greater abundance in the HIV population compared to an uninfected population. This included the persistent increase in the relative abundance of *Streptococcus* and now evident increases in the relative abundance of *Prevotella*, *Veillonella*, and *Actinomyces*. These genera are all common in the oral cavity (42), which may be a source of colonizing taxa for both the uninfected and HIV lung microbiome. In summary, we speculate that the large dispersion in an untreated HIV-infected population makes comparisons of specific bacterial abundances between this group and an uninfected population problematic. This could explain why after one year of treatment, at a time when dispersion has declined and the HIV-infected population has become more uniform, a greater number of differences in relative abundant bacterial taxa are now found between the HIV-infected and uninfected population comparison.

When examining which taxa are overrepresented in the HIV population after one year of therapy several intriguing findings emerge. The relationship between increased *Streptococcus* abundance and pneumonia has already been mentioned (41). Equally intriguing is the increased relative abundance of *Prevotella* and *Veillonella*. Once again we see the increased presence of *Prevotella* in the HIV-infected population, similar to other investigators (32, 33). The presence *Prevotella* and *Veillonella* in BAL has been used to define a lung microbiome pneumotype_{SCT}, a distinct lung microbiome defined by the enrichment with supraglottic characteristic taxa. Many feel this pneumotype is the most reflective of a true lung microbiome from chronic microaspiration (43). Furthermore, the presence of these organisms has been associated with markers of lung inflammation, including increases in BAL lymphocytes and neutrophils as well as elevated levels of exhaled nitric oxide (43). Persistence of lung inflammation is likely critical for the development of pulmonary complications in HIV-infected individuals in the ART era as discussed below.

The Lung Microbiome and Chronic Lung Inflammation

Antiretroviral therapy has greatly influenced the morbidity and mortality of HIV infection, and HIV-infected individuals are now living near normal life spans (44). This has generally been attributed to improvements in immunologic function, either by preventing the progressive loss of immunity in HIV infection or by actually promoting immune

reconstitution. However, despite the improved outlook in HIV infection, pulmonary complications continue to plague these individuals (6). Many of these complications occur on the background of, or result in, chronic lung inflammation. Numerous studies have demonstrated persistence of chronic lung inflammation even in HIV-infected individuals on ART with documented good viral control (7, 14, 28). It is tempting to speculate that this chronic inflammation may contribute to the lung complications seen in the HAART era, which have moved away from classic opportunistic infections towards more chronic lung diseases such as COPD, pulmonary hypertension, and lung cancer (45).

How might alterations in the lung microbiome lead to chronic inflammation in HIV-infected individuals? The microbiome field is now moving beyond simple description of microbial communities into arenas such as metabolomics, transcriptomics, and whole genome sequencing, as investigators try to link microbial communities to host responses leading to inflammation. In patients with pneumonia lung gene expression of tumor necrosis factor- α was positively correlated with bacterial burden and negatively with richness and diversity (33). Conceptually this makes sense as one would predict that overgrowth of a particular organism, as seen in pneumonia, would reduce microbial diversity in an ecological niche and at the same time result in a more vigorous host response to ward off infection.

Equally intriguing is the effect of the lung microbiome in otherwise healthy HIV-infected individuals. Up to now we have stressed the potential linkage between Prevotella in the HIV lung microbiome and various parameters of lung inflammation (43). Others have linked bacteria to evidence of activated metabolic pathways in the lower respiratory tract. For example, recent work by Cribbs and colleagues demonstrated significant alterations in several lung metabolic pathways in HIV-infected individuals, including alterations in lineolate, glycerophospholipid, and fatty acid metabolism (37). Alterations in these pathways were correlated with the presence of Caulobacteraceae, Staphylococcaceae, Nocardioideae, and Streptococcus in the BAL microbiome. The end result of these perturbations was elevated cystine concentrations in the BAL of HIV-infected individuals, a marker of oxidative stress. The authors speculate that HIV itself altered the metabolic pathways leading to oxidative stress which subsequently affected lung immunity and allowed some of these bacteria to be overexpressed. It is similarly plausible that an altered microbiome itself can lead to changes in oxidative stress. These provocative data provide another example of how the lung microbiome can lead to lung inflammation.

The discussion above has focused on how specific organisms can directly induce inflammation in the lung. A second potential mechanism for the chronic inflammation seen in the lungs of HIV-infected individuals is immunosenescence. It is now recognized that HIV-infected individuals, including those on HAART, demonstrate accelerated immunologic aging characterized by accumulation of terminally differentiated lymphocytes with reduced proliferative and immunologic function (46-48). Paradoxically, senescent lymphocytes secrete an inflammatory cytokine profile (49, 50) As a result, tissues containing abundant senescent immune cells are characterized by chronic inflammation. Thus immunosenescence not only results in an impaired ability to respond to new infectious challenges, it is also creates a chronic inflammatory background in affected tissues.

The major driving factor in immunosenescence is chronic antigenic stimulation. With each replicative cycle lymphocytes demonstrate telomere shortening, ultimately resulting in a limited ability to proliferate (50, 51). In the presence of immunodeficiency, many persistent pathogens could promote chronic antigenic stimulation. Viruses, including HIV itself, are the most likely candidates because of their capacity to establish a latent infection in the host. In this vein, study of the lung virome in HIV-infected individuals is an emerging area of investigation.

The Lung Virome and Mycobiome in HIV Infection

Several studies have examined targeted viruses in the lungs of HIV-infected individuals. While ART is associated with a rapid decline in the lung HIV viral load, in a significant number of individuals HIV RNA and DNA remains readily detectable in lung cells after 6 months of therapy (52). It remains controversial whether the HIV reservoir in the lung resides primarily in the alveolar lymphocyte population (14, 52) or in the alveolar macrophage compartment (53, 54). Never-the-less, persistent HIV expression in the lung, either intact virus or viral proteins, could lead to chronic antigenic stimulation, immunosenescence, and chronic inflammation. Other persistent viral infections are equally well described in HIV infected patients on ART, including human herpesvirus (55), Epstein-Barr virus (56), and cytomegalovirus (57, 58).

Newer approaches are using whole genome shotgun sequencing to more fully characterize the entire lung virome in HIV infection. Much virome work has used stool samples which have low amounts of human DNA (59), but host contamination poses a major challenge to lung virome studies where over 90% of the DNA can come from the host. Published data on the lung virome in HIV infection are very limited to date. In a study examining the virome in lung transplant recipients, three HIV-positive individuals were included as control subjects (60). The primary viruses detected in BAL from all the individuals (lung transplant recipients as well as HIV-positive and uninfected controls) were anelloviruses, with the major difference being a much higher abundance of these viruses in the lung transplant recipient group. Anellovirus has been also been well described in a normal population in multiple body sites, including nose, skin, mouth, vagina, and stool (61). The other major contributors to the lung virome were bacteriophages. In lung transplant patients, high anellovirus loads were associated with an aberrant lung microbiome, though linkage with specific bacterial taxa was not seen, likely due to the small number of individuals in the study.

We have similarly begun to examine the lung virome in HIV-infected individuals. In very early preliminary data, using shotgun sequencing on DNA isolated from BAL we found evidence of lymphocryptovirus in the lung of 2 out of 3 HIV positive individuals, compared to essentially none of 6 uninfected controls (**Table 1**). The genus Lymphocryptovirus includes Epstein-Barr virus, known to be capable of establishing latent infections in humans. In more recent work we have performed shotgun sequencing on DNA and RNA preps from lung BAL cells and peripheral blood mononuclear cells from ten HIV-infected individuals who have been on antiretroviral therapy for over three years with good viral control and significant improvements in their peripheral blood CD4 count (mean CD4 count 744

cells/mm³). Similar to published work (60), bacteriophages were highly prevalent in the lung and blood compartments. However, a variety of RNA and DNA viruses were also detectable. **Table 2** shows the number of reads of various viral families in the blood and lung compartments of these individuals. Several interesting findings are noted. First, several DNA viruses are found in RNA preparations, suggesting active replication of these viruses. Second, similar to the Penn group (60), we also detected Anellovirus in the HIV samples, though this was confined to the blood compartment. The lack of Anellovirus in the lung compartment may reflect the fact we were looking at a healthier HIV positive population. Third, Herpesviridae were detected easily in the lung and blood of these individuals, including a large amount in RNA preparations, again suggesting this DNA virus was undergoing replication. Note that Lymphocryptovirus, found in earlier preliminary work, would fall under this family. Fourth, Retroviridae were easily detected. However, this was mostly due to Human Endogenous Retrovirus. HIV was detected in only one of our ten individuals. Finally, Parvoviridae were detected in many lung and blood samples, again in the RNA preparation suggesting active infection. Thus, many viral families can be found in the lungs of HIV-infected individuals, including some demonstrating evidence of active replication.

Finally, the role of the lung mycobiome in health and disease is an emerging area of investigation. Recent work in the field has emphasized the importance of fungi in diseases such as asthma, COPD, and cystic fibrosis (62-64). Given the defects in cellular immunity seen in HIV infection, it would be surprising if the mycobiome in HIV infection was not affected. Certainly HIV-infected individuals are at risk for infections with fungi (*Candida* species, *Cryptococcus*, *Pneumocystis* species). Recent studies in fact confirm that the mycobiome identified in BAL from HIV-infected individuals differs from that in uninfected individuals (65). This includes not only the expected increase in *Pneumocystis jirovecii*, but also increases in 8 other taxa, including *Ceriporia lacerata*, another potential pathogen in HIV infection. The impact of increases in the other “non-pathogenic” fungi in the lungs of HIV-infected individuals is completely unknown at this time.

Linking the Lung Microbiome and Virome to Inflammation Clinical Outcomes

Ultimately the importance of the lung microbiome and virome lies in its potential to influence lung immunity, inflammation, and disease. We have already addressed the potential impact of an altered HIV lung microbiome, focusing on the association of *Prevotella* and *Veillonella* with chronic inflammation (43). It is equally likely that the lung virome is impacting the lung inflammatory environment. Indeed, BAL chemokine and cytokine analysis for individuals shown in Table 1 demonstrated that the HIV positive individuals who had Lymphocryptovirus detected contained the highest concentrations of CXCL10 and CXCL9 (**Table 3**), two interferon- γ inducible chemokines which recruit inflammatory cells to the lung.

In more recent work we have compared the BAL inflammatory and immunologic environment in 32 HIV infected individuals on long term ART (over three years) and 10

uninfected controls. No differences in average chemokine concentrations, cytokine concentrations, lymphocyte activation markers, or markers of lymphocyte immunosenescence were seen between the two groups. However, there were significant outliers in the HIV infected population, with very high chemokine and cytokine concentrations associated with increases in cellular activation markers and markers of immunosenescence. One of the outliers (subject 10) was one of the 10 individuals who had lung and blood virome analysis performed shown in Table 2. Intriguingly, this subject had the largest amount of virus detected in lung cells, in this case consisting primarily of Parvoviridae (**Figure 4**). Furthermore, this was largely confined to an alveolar macrophage enriched population. Similar increases were not seen in the blood compartment. Finally, since Parvovirus was detected in an RNA preparation, it implies this DNA virus was undergoing active replication.

These data suggest a potential linkage between the presence of a replicating virus and lung inflammation. Even more interestingly, we have associated pulmonary function testing and chest CT scanning on these individuals. Subject 10 had the lowest Forced Expiratory Volume in one second/Forced Vital Capacity (FEV-1/FVC) ratio (51%) and lowest diffusion capacity (35% predicted normal) of any of the ten individuals with associated virome data. This subject's chest CT scan showed moderate emphysema. While these data do not prove causality (it is certainly possible that COPD itself can alter the lung virome), it does provide an intriguing association between the presence of replicating virus in the lung, chronic inflammation, and disease phenotype.

Conclusion

The human microbiome is influenced by numerous host and environmental factors. Furthermore, it is likely that our microbiome helps define human phenotypes, both in health and disease. Compared to other body sites, the study of the lung microbiome is relatively new. Never-the-less, accumulating evidence suggests that the lung microbiome can play a significant role in normal lung function and in various pulmonary diseases. Given the known significant immunodeficiency in HIV infection, it is not surprising that one can find alterations in the lung microbiome in these individuals.

To date, evidence suggests that the lung microbiome in healthy HIV-infected individuals with preserved CD4 counts is similar to uninfected individuals. However, in individuals with more advanced disease there is an altered alveolar microbiome characterized by a loss of richness and diversity within individuals, but an increase in beta diversity differences between individuals. These differences decline with ART, but even after effective therapy the alveolar microbiome in some HIV-infected individuals contain increased amounts of signature bacteria, some of which have been previously associated with chronic lung inflammation. Furthermore, more recent investigations into the lung virome in HIV infection suggest that perturbations in lung viral communities also exist in HIV infection. It is likely both microbiome and virome alterations in HIV infection contribute to chronic lung inflammation in these individuals, which have important implications given the changing spectrum of pulmonary complications in patients living with HIV.

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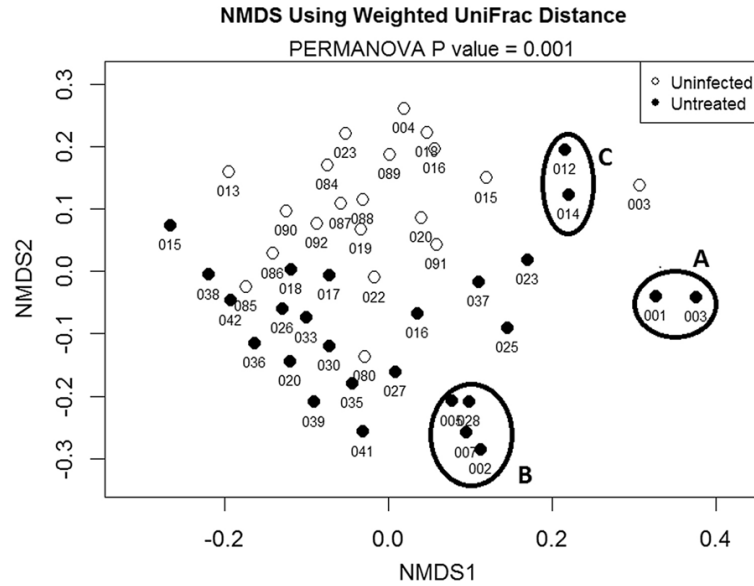


Figure 1. Comparison of beta diversity in BAL at the OTU level between uninfected and untreated HIV-infected individuals using UniFrac Principal Coordinate Analysis. Individual subject identifiers are shown under each circle. The HIV-infected population (black circles) is significantly different compared to BAL from uninfected individuals (white circles). Furthermore, when dispersion of the two communities is analyzed, the untreated HIV-infected population is significantly more spread out compared to the normal population (average weighted UniFrac distance to centroid at the OTU level: 0.167 for the uninfected group, 0.204 for the untreated HIV infected group, $p = 0.041$). Circles A, B, and C represent outliers in the HIV-positive group which contain either unique or increased abundances of particular bacteria compared to the uninfected population, which is leading to increased dispersion in this group (see text for details).

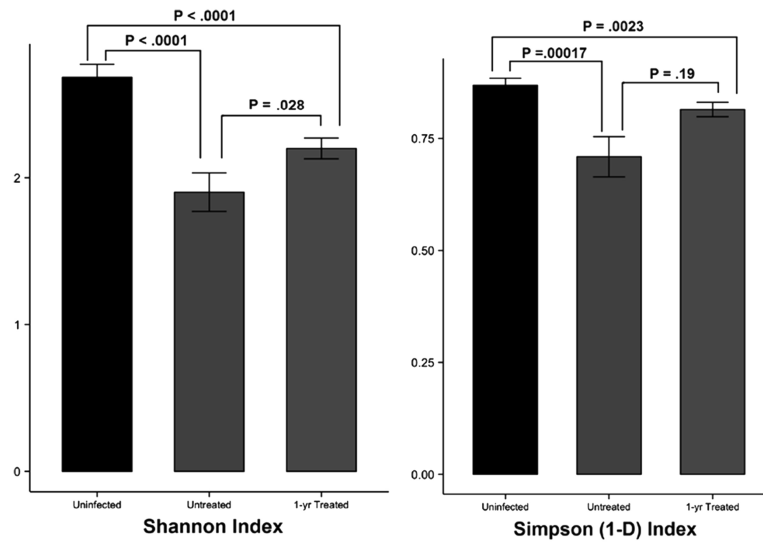


Figure 2. Comparison of alpha diversity in acellular BAL fluid at the OTU level between uninfected, untreated HIV-infected individuals, and HIV-infected treated with HAART for one year. Alpha diversity is significantly greater in the uninfected population compared to a treatment naïve HIV-infected population. However, differences between the HIV infected population and uninfected controls is less after 1 year of treatment.

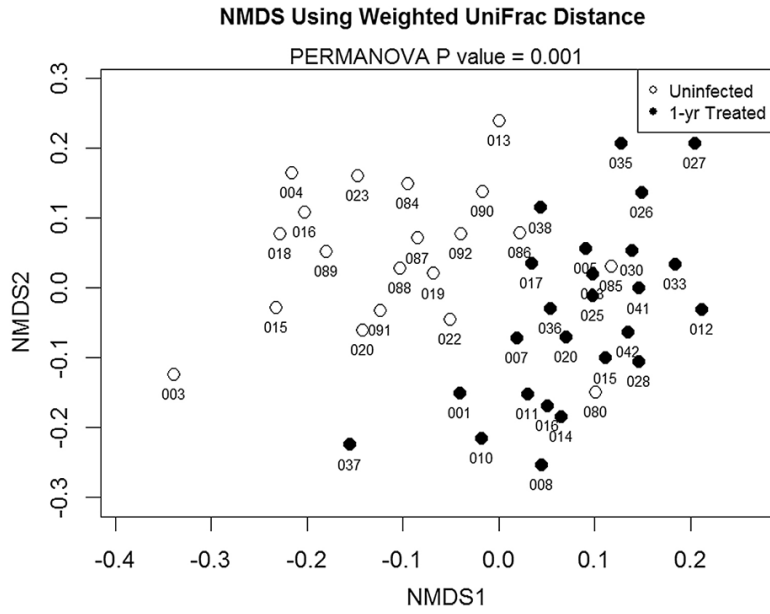


Figure 3. Comparison of beta diversity in BAL at the OTU level between uninfected and HIV-infected individuals treated for one year with ART using UniFrac Principal Coordinate Analysis visualized by non-metric multidimensional scaling (NMDS). The HIV-infected population (black circles) remain significantly different compared to BAL from uninfected individuals (white circles) even after 1 year on therapy. However, the population spread in HIV-infected individuals after 1 year of ART is no longer significantly different from uninfected controls (average distance to centroid: 0.167 for the uninfected group, 0.157 for the 1 year treated group; $p = 0.48$).

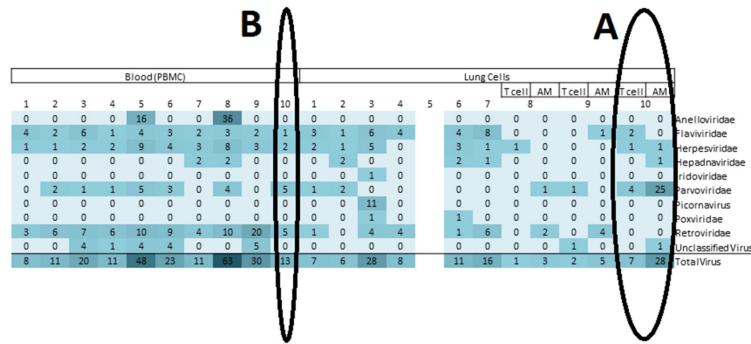


Figure 4. Viral families detected in RNA samples from lung and blood cells of 10 HIV positive individuals. Blood cells on the left are peripheral blood mononuclear cells. Lung cells on the right consist of total lung BAL cells except for subjects 8, 9, and 10, in which total lung BAL cells were enriched for either T cells or alveolar macrophages (AM). Lung cells from subject 5 did not amplify and thus no lung data is show for that individual. In addition to other viruses, Parvoviridae are found in both the lung and blood of these individuals. Subject 10 in particular had a lot of Parvoviridae in alveolar macrophage enriched lung cells (A) compared to lymphocyte enriched lung cells or peripheral blood mononuclear cells (B). This subject had simultaneous evidence of lung inflammation on immunologic analysis and moderate emphysema on pulmonary function testing and chest CT scanning.

Table 1

Viral genera in BAL from HIV positive and negative individuals (total number of reads).

Subject	HIV	VL	Lenti	Betap	Masta	Lymph	Roseo	Erythr	Alpha	Circo
1	Pos	102,093	2	0	0	0	0	0	0	1
2	Pos	178	0	0	0	24	0	0	2	0
3	Pos	302	0	0	0	16	0	0	0	0
<hr/>										
4	Neg		0	0	0	0	0	0	0	0
5	Neg		0	0	0	0	0	0	0	0
6	Neg		0	22	0	0	0	0	0	0
7	Neg		0	0	0	0	0	0	0	0
8	Neg		0	0	0	0	0	0	0	0
9	Neg		0	0	4	2	6	0	0	0

Lenti = Lentivirus, Betap = Betapapillomavirus, Masta = Mastadenovirus,

Lymph = Lymphocryptovirus, Roseo = Roseolovirus, Erythr = Erythrovirus,

Alpha = Alphatorquevirus, Circo = Circovirus

VL = Blood viral load

Table 2

Viral families in blood and lung cells from ten HIV-infected individuals (total number of reads).

	DNA Prep		RNA Prep	
	Blood	Lung	Blood	Lung
DNA viruses				
Anellovirus	15	0	52	0
Hepadnaviridae	0	0	2	8
Parvoviridae	0	0	19	36
Herpesviridae	13	7	31	19
Papillomaviridae	2	1	0	0
RNA viruses				
Flaviviridae	0	0	24	33
Picornavirus	0	0	0	11
Retroviridae	106	92	81	32

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Table 3

Chemokine and cytokine concentrations (pg/ml) in BAL of HIV positive and negative individuals corresponding to virome data in Table 1.

Subject	HIV	VL	CXCL10	MCP-1	CXCL9	IL-8	IL-6	IL-17a
1	Pos	102,093	60.2	62.2	13.0	61.0	3.8	3.6
2	Pos	178	72.7	23.5	32.0	21.8	1.6	3.2
3	Pos	302	78.7	11.0	70.3	15.4	0.5	3.2
4	Neg		9.1	92.8	4.2	16.1	0.7	2.3
5	Neg		10.7	67.5	5.0	85.3	2.0	2.1

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