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Human Microbiome and HIV/AIDS

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Abstract

Understanding of the human microbiome continues to grow rapidly; however, reports on changes in the microbiome after HIV infection are still limited. This review surveys the progress made in methodology associated with microbiome studies and highlights the remaining challenges to this field. Studies have shown that commensal oral, gut, vaginal, and penile bacteria are vital to the health of the human immune system. Our studies on crosstalk among oral and gastrointestinal soluble innate factors, HIV, and microbes indicated that the oral and gut microbiome was altered in the HIV-positive samples compared to the negative controls. The importance of understanding

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the bacterial component of HIV/AIDS, and likelihood of “crosstalk” between viral and bacterial pathogens, will help in understanding the role of the microbiome in HIV-infected individuals and facilitate identification of novel antiretroviral factors for use as novel diagnostics, microbicides, or therapeutics against HIV infection.

Keywords

HIV; Oral microbiome; Oral manifestation; Innate immunity; 454 pyrosequencing; Gut microbiome; Highly Active Antiretroviral Therapy; Crosstalk

Introduction

A recent PubMed search of the term “microbiome” revealed over 1,400 publications since the year 2000, with 400 of these published already in 2011. Despite this high level of interest, only 12 publications dealt with the relationship of the microbiome to HIV/AIDS and these papers deal mostly with the genital microbiome (vagina, penis). Considering that HIV infection is associated with a wide range of microbial co-infections (Table 1), it is somewhat surprising that more attention has not been focused on the microbial flora in this disease. One area that has recently attracted attention is increased microbial translocation following HIV infection. Microbial translocation is defined as the translocation of microbes and/or microbial products without overt bacteremia, occurring after damage to the gastrointestinal tract. This suggests that injury to the immune component of the gastrointestinal mucosal surface, along with damage to the intestinal epithelial microenvironment with its antimicrobial functions, may effect systemic immune activation during the chronic phase of HIV infection through the increased translocation of luminal microbial products [1, 2]. Studies have shown that commensal gut and other mucosal bacteria are vital to the health of the human immune system [3]. Normal commensal flora induce anti-inflammatory events in the gut which protects epithelial cells from pathogens through Toll-like receptors (TLRs) and other pathogen-recognition signaling [4, 5••]. When the gut is depleted of normal commensal flora, the immune system becomes abnormal, with loss of T-helper type 1 (T_H1) T-cell function. Restoration of the normal gut flora can re-establish the balance in T-helper cell ratios characteristic of the normal immune system. However, there is limited understanding about the bacterial diversity in the gut microenvironments [3].

The Human Microbiome

Over 90% of the cells within and on the human body are of microbial origin [6–12]. These microorganisms, which live within and atop humans, are collectively known as microbiota. The genomes of these microbiota act together as a living system known as the microbiome (i.e., the collection of genes in the microbiota) [12, 13]. The majority of these commensal bacteria are anaerobes, meaning they survive in an environment with no oxygen. These commensal bacteria or normal flora bacteria can act as opportunistic pathogens in immuno-suppressed individuals. On a basic level, the human genome can be seen as having roughly the same number of genes as the common fruit-fly; however, when microbiota are added to the mix and considered as part of the human genome, this figure quickly changes because

fruit-flies, like many lower organisms, do not possess the complex microbiome that humans possess [6, 10, 12]. If we consider humans as supra-organisms encompassing these microbial symbionts [14], by far the majority of genes in the system are microbial. In this sense, completing the human genome requires us to also characterize the microbiome.

To understand the range of human genetic and physiological diversity, it is important to understand the factors that influence the distribution and evolution of the microbiome. The goal of the Human Microbiome Project (HMP: https://commonfund.nih.gov/hmp/) is to understand and expand on the connection these organisms have with their human hosts. It is felt that this knowledge can be used to better treat and diagnose disease, and perhaps find ways to prevent some diseases by elucidating the link(s) between microbiota and the disease [13].

Human Microbiome and Challenges

Culture Versus Culture: Independent Techniques

The classical method of culturing bacteria from human subjects excludes a large number of non-cultivable or not-yet-cultivated bacteria, and also under-represents the abundance of some species due to selection by the culture conditions. These non-cultivable and sometimes dormant bacteria occupy different ecological microniches and may be involved in latent infections [15–17]. To assess the role of bacteria in the development of disease, it is necessary to identify both cultivable and non-cultivable organisms as these non-cultivable have the potential to cause inflammation that may in turn support disease progression. Non-cultivable microbes play an uncertain role in human disease, but they can now be characterized by culture-independent methods, such as polymerase chain reaction-16S based degenerating gradient gel electrophoresis (PCR-DGGE) and 16S ribosome sequencing technologies. PCR-DGGE surveys species profiles by differentiating PCR-amplified 16S rRNA gene segments of complex bacterial samples without cultivation. The approach provides qualitative and semi-quantitative assessment of the bacterial community but lacks the ability to identify the phylotypes [18–21]. Pyrosequencing technology, which is also based on 16S rRNA gene segments, allows one to obtain broad views simultaneously into hundreds of communities of both the cultivable and non-cultivable bacterial population [21–24]. The technique uses small fragments of the 16S rRNA gene which are of sufficient size to serve as a proxy for the full length sequence [25••].

Read Length

The introduction of high-throughput pyrosequencing has dramatically increased the resolution at which microbial communities can be analyzed. The main drawback of pyro-sequencing is the current read length restriction of sequences obtained (100–400 bp). The method cannot be used to produce full-length 16S rDNA sequences, traditionally used for microbial taxonomic studies. However, the method does allow for the analysis of small hypervariable regions of the 16S rDNA gene. This approach is a challenge to the accurate assignment of the bacterial taxonomic groups and to estimation of the microbial richness due to the use of short sequences. New developments in pyrosequencing are anticipated that will increase read length from 400 to 1,000 bp [26]. The number of sequences required to

characterize a sample depends on the goal of the study, the diversity of species in the samples, the read length, and the choice of the gene and region for sequencing [27, 28]. The data generated are analyzed by various online bioinformatics programs (e.g., http://ab.inf.uni-tuebingen.de/software/megan/, greengenes.lbl.gov/, qiime.sourceforge.net/) [29–31].

Primer Design

Currently, there is no consensus on the single best region of 16S rRNA for pyrosequencing. As both the choice of the region and the design of the primers are critical, a poor choice of primers can lead to radically different conclusions [25]. Recently, Nossa et al. [27] reported that 347F/803R can increase the universality among foregut species and, therefore, is the most suitable pair of primers for classification of foregut 16S rRNA genes as well as for analysis of other complex microbiomes. It is important to use similar primers if the study requires the detailed comparison of relative abundance and species identification of all bacteria present in the sample. However, if the study question is based on only presence or absence of bacteria then even different primers will be useful in identifying the broad patterns in the microbiome.

Data Analysis

Analysis of the microbiome based on high-throughput sequencing generates vast amounts of data, so new procedures are required for depositing, storing, and mining different data types. There are two main issues with pyrosequenicing: low quality sequences and chimeras [21, 25••, 27, 28, 32]. To overcome these problems there are a few downstream analysis pipelines available online. QIIME [29] is one of the most commonly used sequence analysis pipelines for microbial community analysis. It integrates many third party tools and has become a standard in the sequencing field.

16S Ribosomal Database

The biggest challenge to microbiome studies is the lack of curated 16S rRNA sequence database. The nucleotide sequences deposited in public databases are questionable, since many depositions were of poor quality [33, 34]. Much of this misinformation that was originally present in such databases was thought to have been corrected; however, there is still ambiguity about the quality of sequences and applying the correct “label” to each sequence [35]. The most commonly used databases are The National Center for Biotechnology Information GenBank nr/nt and The Ribosomal Database Project (RDP), which provide ribosome-related data and services to the scientific community, including data analysis, aligned, and annotated sequence for bacterial and archaeal small-subunit 16S rRNA [36]. New site-specific 16S databases like CORE and HOMD are being developed [37, 38•, 39], but still there is a lack of one comprehensive universal curated database which can be used for identification of rare sequences in all microbiome studies.

Human Microbiome and HIV/AIDS Infection

Oral Microbiome

The presence of HIV-associated oral lesions is strongly linked to immune deterioration with decreased CD4 cell counts [40–43]. HIV salivary gland disease, human papilloma virus (HPV)–associated oral lesions including papilloma, condylomas, and focal epithelial hyperplasia (oral warts), xerostomia, and recurrent oral ulceration appear to have increased since the advent of HAART [43, 44]. On the other hand, oral candidiasis, oral hairy leukoplakia, Kaposi's sarcoma, and HIV-associated periodontal diseases have decreased after HAART [40–43]. Some studies have associated these variations with differences in access to oral health care, demographic and social factors, mode of HIV transmission, types of co-infections, disease stages, immune reconstitution, and the composition of the oral microbiome.

Oral infections associated with immunosuppression have been used as clinical indicators of HIV infection and disease progression [45–48], and they significantly affect the quality of life of HIV-infected individuals. The oral HIV/AIDS Research Alliance (OHARA), as part of the AIDS Clinical Trial Group (ACTG), is the largest HIV clinical trials organization in the world [48]. One of OHARA's main objectives is to investigate oral complications associated with HIV/AIDS, in particular the effects of antiretrovirals on oral mucosal lesion development and associated fungal and viral pathogens. OHARA has two ongoing studies focusing on oral HPV, and five protocols in development. Several protocols pertain to the use of oral fluids to monitor HIV RNA, KSHV DNA (and other herpes viruses), and HPV DNA shedding in relation to specific HIV-related covariates [49••]. To date, only a few studies have examined dental caries, periodontal diseases, and the oral microbiome in the HIV-infected population. Phelan et al. [47] examined the correlation between dental caries in HIV-infected women as compared to uninfected controls. The cohort (Woman's Inter-agency HIV Study [WIHS]) included 538 HIV-positive and 141 HIV-negative women. The coronal caries examination revealed a 1.2-fold higher prevalence in HIV-positive women. The severity of dental caries was also correlated with decreased saliva secretion. Whether increased oral diseases are associated with salivary hypofunction as seen in most immunocompromised individuals or due to changes in the oral microbiota remains unclear.

Lung Microbiome

Since the beginning of the HIV epidemic in the 1980s, it has been recognized that HIV-infected individuals are extremely susceptible to pneumonia caused by opportunistic and non-opportunistic microorganisms. Respiratory infections frequently cause morbidity, and may also increase the rate of HIV replication and accelerate the progression to HIV/AIDS. HIV-infected individuals experience loss of lung function following pneumonia, which is not observed in HIV-uninfected populations. Virtually nothing is known about the microbiome of the normal lung, and so characterization of the microflora of the lung is an area of considerable scientific and clinical relevance. Ongoing studies by James Beck, of University of Michigan, Ann Arbor Health System (http://lunghivmicrobiome.org/), are designed to characterize the microbiome of the lung and will provide insights into the development of lung disease in HIV-infected and uninfected populations.

Penis Microbiome

It is now widely accepted that male circumcision leads to a decrease in the transmission of a number of viral pathogens including HIV, HSV-2, and HPV [50–52, 53••, 54••, 55]. In the case of HIV, circumcision decreases the rate of viral transmission by approximately 50% [50]. One possible mechanism for this effect is alterations in the penile microbiome. Price et al. [53••] evaluated changes in the penile microbiome before and after circumcision in 12 HIV-negative individuals. In general the pre-circumcision microbiome was more heterogeneous prior to foreskin removal. Specifically, they identified a decrease in two anaerobic families (Clostridia and Prevotella) that initially were abundant but then significantly decreased after circumcision. The investigators suggested that the anoxic environment under the foreskin could lead to proinflammatory responses that in turn activate Langerhans cells. This could account for the beneficial effects of circumcision in decreasing HIV transmission. Anderson et al. [56] reviewed studies linking HIV infection to alterations of the immune defenses of the penis. They reported that *Treponema pallidum*, *Haemophilus ducreyi*, and *Neisseria gonorrhoeae* infections enhance HIV transmission, and all of these pathogens are decreased after circumcision [56].

Vaginal Microbiome

A significant amount of work has been done on the bacterial colonization of vagina; however, most of these studies were focused on one specific organism or specific disease (e.g., bacterial vaginosis [BV]). There is lack of data on the vagina full microbiome. A healthy vaginal microbiome is normally dominated by *Lactobacillus* species (*Lactobacillus iners, L. crispatus, L. gasseri, or L. jenseniiacillus*), but one sees a dramatic increase in the diversity of the vaginal microbiome in BV [57, 58••]. Spear et al. [59] studied the microbiome of the genital track of the rhesus macaque, obtaining samples of cervical vaginal lavage from 12 macaques and compared this to cervical vaginal lavage samples from women. The taxa found included many associated with bacterial vaginosis in women. In contrast, *Lactobacillus* sequences were found in only four of the macaques, and were not the predominant bacteria in any of the animals. The authors concluded that while macaques could be used for studies of BV in women, they might not be an ideal model for HIV infection studies, since the genital microbiome in HIV-positive and HIV-negative women is predominately *Lactobacillus* species [59]. However, it has been demonstrated that a significant proportion (7%–33%) of healthy women lack appreciable numbers of *Lactobacillus* species in the vagina, which may be replaced by other lactic acid–producing bacteria such as *Atopobium vaginae*, *Megasphaera*, and *Leptotrichia* species [57, 58••, 60•]. Although the structure of the communities may differ between bacterial populations, a healthy environment can be maintained by the ability of these communities to produce lactic acid [57]. These studies do not address whether some proportion of “healthy” women are patients in transition to or from BV, or whether they have asymptomatic BV, i.e., abnormal flora but no symptoms because of genetic or other factors. In immune-compromised women differences in the microbiome may be significant. There is emphasis in BV studies in understanding the shift in the vaginal microbiome [57, 58••, 60•, 61••, 62•, 63]. The bacterial diversity associated with BV may cause a functional imbalance with respect to mucosal permeability and lead to detrimental health effects. It has been observed that

patients with BV have a higher incidence of heterosexual transmission of HIV, indicating some interaction between the microbes and the human mucosal barrier. Recently, Spear et al. [63] showed that *Lactobacillus iners* sequences were present in 66% of HIV-positive women and 90% of HIV-negative women. Using high-throughput 16S sequencing and vaginal swab samples from 132 HIV-positive Tanzanian women, Hummelen et al. [60•] determined that the relative abundance of *Prevotella bivia* or a member of the order Clostridiales and family Lachnospiraceae was much higher in BV as compared to healthy women. Similarly in another study using pyrosequencing of a cohort of healthy American women including African Americans, *Prevotella* genus was found to be the most abundant [62•]. However, previous studies using culture-based techniques found that in Caucasian and black women from North America, *Atopobium vaginae* and genera of the order Clostridiales, such as *Megasphaera* species, were dominant. These differences in the abundance of *Prevotella* genus in different ethnic groups are significant as *Prevotella bivia* is a well-known pathogen reported to invade epithelial cells, cause inflammatory responses, endometritis, pelvic inflammatory disease, and perirectal abscesses [60•, 64].

Limited studies of the vaginal microbiome indicated that composition of the flora can be affected by HIV infection. These assumptions were further supported by results from microbicides studies where some agents led to higher HIV transmission rates than seen in subjects using placebos [57, 58••, 60•]. There is no clear explanation for these failures, but one hypothesis holds that microbicides alter the vaginal microbial flora in ways that increase inflammation or activate potential HIV host cells, thus enhancing transmission. Alternatives to condom use in these inherently high-risk encounters have the potential to increase the risk for HIV [61••]. The human vaginal microbiota plays an important role in the maintenance of health of women, their partners, and newborn infants. The inherent differences within and between women in different ethnic groups argue for a more refined description of the normal and HIV-infected vaginal bacterial communities. There is no doubt that more studies on the vaginal microbiome in HIV patients are warranted to better understand the relationships between bacteria, host, and viral transmission.

Crosstalk among Oral Gastrointestinal Soluble Innate Factors, HIV, and Microbes

Our group began a clinical study 4 years ago (Crosstalk among oral and gastrointestinal soluble innate factors, HIV, and microbes; Grant U19 DE018385; http://www.nyu.edu/projects/crosstalk/) to examine a population of HIV-infected, antiretroviral-naïve subjects, in comparison to HIV-uninfected individuals. We are sampling the entire gastrointestinal track from mouth to anus and monitoring changes in the proteome, microbiome, and innate immune system. Our preliminary studies have made the following intriguing observations.

Oral Microbial Profile and HIV Infection

In an analysis of 31 HIV-positive individuals and 40 age-, gender-, and race/ethnicity-matched HIV-negative controls, we examined the overall oral microbial population. The comparison was performed based on bacterial 16S rDNA fingerprints generated by PCR and DGGE methods and analyzed using the BioNumerics Program. Our preliminary results

suggest a different bacterial 16S rDNA profile between the two groups of individuals, and a greater microbial diversity in HIV-positive individuals compared to HIV-negative controls. The findings further support the hypothesis that HIV infection may have significant effects on total bacterial colonization and overall composition of bacterial population in saliva.

Oral Microbiota Varies with HIV

In addition, conventional bacterial cultivation methods were used to quantitatively and qualitatively evaluate the colonization of *S. mutans*, *S. sobrinus*, and total *Lactobacillus* species, total *Candida* species, total oral streptococci, and total cultivable bacteria in the saliva of all subjects. We observed that the levels of *S. mutans*, total *Lactobacillus* species, and total *Candida* species in saliva were higher in HIV-positive individuals as compared to HIV-negative controls (r^2=0.448; P<0.001).

Periodontal Pathogens and HIV Infection

Quantitative real-time PCR was performed with species-specific primers to evaluate the colonization of major periodontal pathogens, namely *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Aggregatibacter actinomycetemcomitans*, and *Treponema denticola*, in the plaque samples. We observed that the frequency of detection of *P. gingivalis*, *T. forsythia*, and *A. actinomycetemcomitans* was decreased in the saliva of HIV-positive individuals. These preliminary findings suggest that there are alterations in periodontal pathogens in HIV-infected individuals.

Pyrosequencing

We examined saliva samples from eight HIV-positive and eight HIV-negative subjects to determine the total bacterial diversity by pyrosequencing. The initial sequence analysis indicated that there were major differences in the phyla of *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and TM7 between the two groups. Phylum *Tenericutes* was only seen in saliva obtained from HIV-positive individuals. Further, at the genus level we identified difference in *Streptococcacea*, *Prevotellaceae*, *Porphyromonadaceae*, and *Neisseriaceae*. The cluster analysis at the genus level showed that the two groups had unique oral microbiomes and HIV-positive and all HIV-negative individuals clustered separately. These preliminary data suggested that there was a shift in the oral microbiome that may be associated with HIV infection and associated oral manifestations.

Gut Microbiome and its Alteration in HIV Infection

Bacterial mutualism in the gastrointestinal tract aids digestion, promotes development of the gut immune system, and provides competitive barriers to pathogen invasion [65]. This complex microbial population influences an estimated 10% of all metabolites in our body [66]. The host, in return, provides bacteria with safe habitats and nutrients during lean times. The host immune system has to balance permissive, tolerogenic responses to food antigens and commensal microbes with potentially damaging, and inflammatory responses to ward off pathogens. This delicate balance is maintained by the constant interplay among the microbiome, the intestinal barrier, and the mucosal immune system, which is a prerequisite

for normal gut homeostasis. Imbalance of this system may lead to autoimmune inflammation or infectious pathology [67].

Recently, it has been reported that the gut microbiome is involved in the development of inflammation in HIV infection and there is an interaction between the microbiome and systemic immune activation [68]. The hypothesis is that dysfunction of the mucosal immune response due to preferential depletion of intestinal mucosal immune cells, including effector CD4+ cells and DCs [68], may affect systemic immune activation through the increased translocation of microbes and bacterial products from the intestinal tract. If HIV infection itself impaired the GI barrier and the composition of the gut microbiome, the breakdown of the GI mucosa would cause acute and chronic exposure of peripheral lymphocytes to an abnormal intestinal microbiome, resulting in the increased translocation of gastrointestinal microbial products, such as lipopolysaccharide, directly contributing to systemic immune activation. In the chronic phase of HIV infection translocation may ultimately play a role in the rate of progression to AIDS [1]. However, antibody responses to gut commensal bacteria appear not to be affected by chronic HIV-1 infection and do not contribute to hypergammaglobulinemia [69]. While chronic immune activation and inflammation have long been described as characteristic features of progressive HIV disease, the source of inflammation, infection, and the component(s) of the microbiome responsible for disease progression in opportunistic infections and AIDS are still unidentified.

In our ongoing studies the gut microbiome was characterized using a high-throughput 16S rRNA gene survey in 48 mucosal samples (cytobrush) collected from six HIV-positive and six negative controls at multiple anatomical sites, including the esophagus, stomach, duodenum, and colon. Eighteen phyla, 1110 OTU (operational taxonomic unit) at the genus level were detected in HIV-positive patients and 17 phyla, 871 OTU were detected in healthy controls. The five most abundant phyla (Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Fusobacteria) that comprise 99% of the gut microbiome were moderately altered in the HIV-positive samples compared to the negative controls. Similarly, slight changes in the relative abundance were observed in the major genera (*Streptococcus*, *Prevotella*, *Veillonella*, *Faecalibacterium*, *Bacteroides*, and *Gemella*) that constitute 52% of the gut microbiome. These preliminary data suggest that HIV infection may diversify the gut microbiome at the genus levels, but may have limited effect on the total microbiome.

Although the crosstalk studies revealed general trends in the distribution of the gut microbiome in HIV-positive patients, statistical interpretation of the ongoing study has not yet been performed. We anticipate completing subject enrollment this year and will perform a more comprehensive analysis by the end of 2012.

Conclusions

Our survey of the literature on the microbiome has uncovered several intriguing observations. The vaginal and penile bacterial flora clearly influences HIV transmission. Ongoing studies on GI microbiome have identified shifts in diversity and changes in specific bacterial composition. At this point it is not clear if these are direct effects of HIV infection,

or rather result from immunosuppression. In either case, interactions between bacteria and HIV are likely to influence HIV transmission and result in a wide range of opportunistic infections.

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Table 1

Microbial manifestations of HIV infection

Site	Microorganism	Disease
Oral	*Porphyromonas gingivalis, Tannerella forsythia, Dialister pneumosintes, Actinobacillus actinomycetemcomitans, Fusobacterium* species, *Eubacterium* species, *Streptococcus mutans*, hemolytic streptococci, *Treponema* species, staphylococci, *Enterococci, Pseudomonas, Veillonella*, and *Candida*	Necrotizing ulcerative gingivitis, necrotizing ulcerative periodontitis, linear gingival erythema, caries, candidiasis
GI	*Salmonella, Shigella, Campylobacter, Isospora, Rochalimaea, Mycobacterium avium, M. xenopi, Entamoeba histolytica, Chlamydia, Candida, Cryptosporidium, Bacillus cereus, Clostridium perfringens*	Acute or persistent diarrhea, esophagitis, other enteric infections
Lung	*S. pneumonia, Haemophilus influenza, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, M. tuberculosis, M. kansasii, M. avium*	Upper respiratory tract infections such as sinusitis, pharyngitis, and acute bronchitis, Pneumocystis pneumonia, bacterial pneumonia, tuberculosis, pulmonary Kaposi sarcoma
Penis	*Treponema pallidum, Neisseria gonorrhoeae, Chlamydia trachomatis*	Sexually transmitted diseases
Vagina	*Gardnerella vaginalis, Mobiluncus species, Prevotella species, Mycoplasma hominis Atopobium vaginae, C. trachomatis, N. gonorrhoeae, M. genitalium, Bifidobacterium, Megasphaera elsdenii, Dialister, Leptotrichia, and Prevotella*	Bacterial vaginosis, ulcerative and nonulcerative sexually transmitted diseases