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CONFERENCE SUMMARY

A Summary of the Fourth Annual Virology Education HIV Microbiome Workshop

Brett Williams,[1,*] Mimi Ghosh,[2,*] Charles Boucher,[3] Frederic Bushman,[4] Stacy Carrington-Lawrence,[5] Ronald G. Collman,[6] Satya Dandekar,[7] Que Dang,[8] Angela Malaspina,[8] Roger Paredes,[9] Cara Wilson,[10] Sandra Pinto Cardoso,[11] Laurel Lagenaur,[12] Jessica Santos,[13] Christopher Joy,[2] and Alan Landay[14]

Abstract

Each year, a growing international collection of researchers meets at the NIH to share and discuss developments in the microbiome HIV story. This past year has seen continued progress toward a detailed understanding of host–microbe interactions both within and outside the field of HIV. Commensal microbes are being linked to an ever-growing list of maladies and physiologic states, including major depressive disorder, chronic kidney disease, and Parkinson disease. PubMed citations for ''microbiome'' are growing at an exponential rate with over 11,000 in 2018. Various microbial taxa have been associated with HIV infection, and some of these taxa associated with HIV infection have also been associated with systemic markers of inflammation in HIV infected individuals. Causality remains unclear however as environmental and behavioral factors may drive HIV risk, inflammation, and gut enterotype. Much of the work currently being done addresses potential mechanisms by which gut microbes influence immune and inflammatory pathways. No portion of the microbiome landscape has grown as rapidly as study of the interplay between gut microbes and response to cancer immunotherapy. As Dr. Wargo discussed in her keynote address, this area has opened the door to better understanding on how commensal microbes interact with the human immune system.

Keywords: HIV, microbiome, mucosal immunology, microbial translocation, immune activation, comorbidities

Introduction

EACH YEAR, A growing international collection of researchers meets at the NIH to share and discuss developments in the microbiome HIV story. This past year has seen continued progress toward a detailed understanding of host–microbe interactions both within and outside the field of HIV. Commensal microbes are being linked to an ever-growing list of maladies and physiologic states, including major depressive disorder, chronic kidney disease, and Parkinson disease.[1–3] PubMed citations for ''microbiome'' are growing at an exponential rate with over 11,000 in 2018.

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[1]Division of Infectious Diseases, Rush University Medical Center, Chicago, Illinois, USA.
[2]Department of Epidemiology and Biostatistics, The George Washington University, Washington, District of Columbia, USA.
[3]Department of Virosciences, Erasmus Medical Center, Erasmus University Rotterdam, Rotterdam, the Netherlands.
[4]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.
[5]Office of AIDS Research, Division of Program Coordination, Planning, and Strategic Initiatives, Office of the Director, U.S. National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, Maryland, USA.
[6]Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.
[7]Department of Medical Microbiology and Immunology, University of California, Davis, Davis, California, USA.
[8]Vaccine Research Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA.
[9]Institut de Recerca de la SIDA IrsiCaixa i Unitat VIH, Universitat Autònoma de Barcelona, Universitat de Vic, Catalonia, Spain.
[10]Department of Medicine, University of Colorado Denver, Denver, Colorado, USA.
[11]Center for Research in Infectious Diseases, National Institute of Respiratory Diseases, Mexico City, Mexico.
[12]Osel, Inc., Mountain View, California, USA.
[13]Columbus Technologies and Services, Inc., NIAID/NIH, Bethesda, Maryland, USA.
[14]Division of Gerontology, Department of Internal Medicine, Rush University Medical Center, Chicago, Illinois, USA.
*These authors contributed equally to this work.

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Keynote Address

Dr. Jennifer Wargo from MD Anderson Cancer Center focused on this highly active area. Cancer therapeutics is a fast-moving field in general, and survival rates for many types of cancer have improved dramatically over the past 10 years. Responses to immunotherapy vary significantly from person to person with some tumors disappearing entirely and others seemingly unaffected. Gut microbiota, along with the tumor microenvironment, appears to explain much of this variability.

Microbes may also alter responses to traditional cytotoxic chemotherapy. Dr. Wargo, in collaboration with others, found that Gammaproteobacteria can metabolize the chemotherapeutic agent gemcitabine to an inactive form. Samples from human pancreatic tumors, which are notoriously chemoresistant, frequently contained Gammaproteobacteria.[4] Microbes residing within pancreatic ductal adenocarcinoma appear to generate a tolerogenic local environment, which can be reversed with bacterial ablation leading to increased TH1 differentiation, T cell activation, and PD-1 expression.[5] There is no published data to date on how the gut microbiome affects antiretroviral drug levels, although Klatt *et al.* showed that vaginal bacteria metabolize tenofovir and reduce its protective effect.[6] Such metabolism may dramatically impact pre-exposure prophylaxis (PrEP) efficacy, but this is yet to be investigated.

Gut resident microbes also seem to be significant in determining immune responses to malignancy. Dr. Wargo's group performed a longitudinal study on individuals starting therapy for metastatic melanoma and found that individuals with high baseline alpha diversity and high relative abundance of Ruminococcaceae had significantly better response to anti-PD-1 therapy.[7] Metagenomic analyses demonstrated enrichment for anabolic pathways in responders compared to nonresponders.

Several human cohorts have verified the link between gut microbiota and response to immunotherapy; however, the taxa associated with responses have differed between cohorts,[8,9] the reasons for which are currently unclear. Dr. Wargo argued for standardization of collection, DNA extraction, amplification, and analytical methods across the field. Microbiome research in HIV positive cohorts has suffered from similar inconsistencies and would also benefit from standardization. The greatest question which remains unanswered is whether we can modify therapy responses by manipulating the gut microbiome; the studies that will attempt to answer this question are currently in the planning stages. Developing a mechanistic understanding of these interactions between microbes and our immune cells will be of vital importance to the development of microbiome based therapeutics for both cancer and HIV.

Pathogenesis

Various gut bacterial taxa have been associated with inflammation in various disease states and health.[10] Much of the difference in gut microbiota seen between HIV infected and uninfected populations is driven by sexual behavior.[11,12] Sam Li from the University of Colorado presented his work on the inflammatory nature of the gut enterotype associated with men who have sex with men (MSM). He inoculated gnotobiotic mice with stool bacteria from men who were HIV uninfected and sexually active with women (MSW), HIV uninfected MSM, and HIV infected MSM. Engraftment of the bacteria in the mice was relatively similar across the groups. Mice receiving stool from either HIV infected or uninfected MSM developed more immune activation, as measured by CD69 and CD103 positive CD4 and CD8 cells in gut mucosa than from MSW. Immune activation levels in human donors correlated with that of recipient mice.

Dysbiosis has been defined by a variety of different microbial community signatures and is generally described by a specific enterotype associated with a disease of interest. Expansion of the phylum Proteobacteria and family Enterobacteriaceae does appear to be a common thread in dysbiotic states, particularly HIV.[10] Enterobacteriaceae are facultative anaerobes, which are usually outcompeted in the hypoxic gut environment. As Dr. Andreas Baumler discussed, disruptions in butyrate availability may drive intestinal epithelial cells to switch from using butyrate as an energy source to using the glycolytic pathway, which does not consume oxygen. In a mouse model, streptomycin treatment decreased colonic abundance of obligate anaerobes and butyrate producers Clostridia, Lachnospiraceae, and Ruminococcaceae.[13]

Butyrate works against facultative anaerobes using at least two other mechanisms. It signals through peroxisome proliferator-activated receptor (PPAR)-gamma to downregulate nitric oxide synthase (inducible nitric oxide synthase [iNOS]), thereby reducing mucosal levels of nitrogen which facultative anaerobes can also use as an electron acceptor. Mesalamine (5-ASA), a PPAR-gamma agonist, likely decreases colonic inflammation through this mechanism.

T regulatory (Treg) expansion appears to be dependent on short chain fatty acids (SCFAs) such as butyrate, signaling through G protein coupled receptors (GPCRs). Mice treated with streptomycin had a colonic mucosal Treg pool one third its normal size, which produced the same effect size as treatment with an anti-CD25 antibody. Treg cells exerted an independent effect from PPAR-gamma signaling in reducing mucosal oxygen concentration. Dr. Baumler's work has illuminated the mechanisms of SCFA activity in the gut and provided several potential new avenues of inquiry regarding the role of SCFAs in HIV infection.

The vast complexity of the host-microbiome relationship demands multifaceted approaches to build system based models. Dr. Robert Quinn of Michigan State University discussed using tandem mass spectrometry to identify the metabolites originating from microbes. A novel approach to mass spectrometry called meta-mass shift chemical (MeMSChem) profiling can determine the relatedness of molecules in molecular networks based on differences in mass.[14] Molecular networks are built by looking at partial signal overlaps between molecules.

Using MeMSChem, his group analyzed gastrointestinal metabolites in germ free vs normal mice. They found that about 14% of chemicals are unique to normal mice (with a microbiome) and about 10% are unique to germ-free mice. Bile acid metabolism seemed to be dramatically affected by the presence of gut bacteria. *Clostridium bolteae* produces bile acids that cannot be de-conjugated by bacteria and antagonize the host farnesoid X receptor leading to decreased bile acid secretion by the host. These bile acids are also found in humans.

Currently only a small fraction of the chemical signatures seen on mass spectrometry can be identified but the library is growing quickly. Because microbes differentially produce metabolites dependent on their microenvironment, connecting microbial community structure to disease states has been difficult. MeMSChem may allow us to directly correlate microbial metabolites to disease states.

SIV macaque models have been particularly helpful in unfolding the pathogenesis of HIV. Clarissa Santos Rocha of the University of California Davis discussed her work on a ligated ileal loop live macaque model in the Dandekar laboratory. This model allows for assessment of gut mucosal effects of multiple bacterial species in the same animal maintaining an anaerobic microenvironment.

Bacteria can be incubated for up to 8 h in a living animal. Injection of *Bifidobacterium infantis* and *Lactobacillus plantarum* lead to rapid (<5 h) recovery of mucosal zonulin-1 independent of CD4 recovery. This recovery was not seen with antiretroviral therapy solely. *B. infantis* and *L. plantarum* appear to downregulate inflammatory pathways and repair gut mucosa by driving tryptophan metabolism toward production of indolelactate which in turn induces IL-22 production. Further work will focus on the identification of potential targets to improve gut barrier integrity.

Geography has a dramatic effect on gut microbiome for a number of reasons, including environmental flora, diet, and cultural practices. David Gootenberg from the Ragon Institute discussed the interaction between HIV status, geography, and the gut microbiome. Changes in the genus *Prevotella* have frequently been associated with HIV, but the directionality of those changes seems to be dependent on location of residence. Prevotella is a gram negative anaerobe, which constitutes a large proportion of the gut microbiome in most human populations. *Prevotella* abundance was increased in HIV positive individuals in Boston, MA compared to HIV negative, but the opposite was seen when comparing HIV positive and negative individuals in Uganda and Botswana.

Bacterial taxa from each locale, particularly *Prevotella* species, including *Prevotella stercorea* and *Prevotella copri*, were associated with changes in sCD14 (an indirect marker of microbial translocation), but none of the taxa associated with increased sCD14 was shared between locales. These findings suggest that the interaction between microbiome and host has complexities that are not well accounted for by current studies and that we may be unfairly labeling the genus *Prevotella* as harmful, the impact of which may be context dependent.

Mode of HIV acquisition also appears to impact immune activation and risk of ensuing cardiovascular disease with perinatally infected individuals at particularly high risk.[15] Libera Sessa from the University of Rome discussed findings from her study on the fecal microbiota of 61 perinatally infected individuals and healthy controls. Perinatally infected individuals had significantly higher alpha diversity than healthy controls. There appeared to be two distinct microbial signatures among the perinatally infected group defined by high vs low abundance of *Akkermansia muciniphila.* The group with the higher abundance of *A. muciniphila* had significantly higher systemic markers of inflammation (IL-6), endothelial activation (ICAM-1, VCAM-1, E-sel), and microbial translocation (sCD14). Further work using *in vitro* studies will focus on the metabolomics of *A. muciniphila*, while *in vivo* studies will address other cardiac biomarkers.

While many studies have been conducted on the gut bacterial microbiome, the composition of gut commensal viruses (virome) is still essential at its infancy. The enteric virome community includes the eukaryotic virome, endogenous viral elements, and bacteriophages and is distinct between healthy adults living in industrialized nations versus those living in developing countries.[16] Analysis of the virome is more challenging due to the lack of a conserved target sequence, and DNA and RNA viruses require different methodologies. In addition, reference databases are not as curated as bacterial ones, and many reads do not align to known viral sequences, further adding to the challenges of studying the virome.

Dr. Scott Handley from Washington University School of Medicine presented three studies on the gut virome. One study observed significant divergence in enteric virome between healthy individuals and those diagnosed with Crohn's disease or ulcerative colitis. In addition, viral diversity was found to be inversely associated with bacterial diversity. In another study focused on dysbiosis of enteric bacteria in AIDS, Dr. Handley's group reported gut virome expansion associated with SIV infection and GI pathology.[17–19] In a macaque SIV vaccine challenge study, lack of protection correlated with increased Circoviridae and Adenoviridae. Studies in humans have also shown expansion of adenoviruses in the HIV-infected population with CD4 < 200.[20]

More studies are needed to understand the complex interrelations between bacteriophages and bacteria in the context of HIV infection and host responses. In addition, it is important for fecal microbiome transplant (FMT) or probiotic engraftment, since donors used to provide feces for these targeted interventions are not screened for bacteriophages.

Finally, Dr. Bryan Brown from University of Washington, School of Medicine, described a method called Pico (penalized isometric log ratio transformation, available as an R package in GitHub?), which determines ratios of microbial taxa and builds compositional balances that maximize distance (variance) between patient samples. This approach amplifies dissimilarity (noise reduction), thereby allowing detection of small yet meaningful changes. Microbiome data are commonly reported with relative abundance rather than absolute abundance. To illustrate how this method allows to circumvent, it was applied to a microbiome dataset of Nigerian infants, HIV-exposed uninfected infants (HEU), and HIV-unexposed infants (HU), who were administered the oral polio vaccine. Multiple balances (ratios between bacterial species) were obtained from stool samples at weeks 1 and 4 and allowed to segregate the HEU and HU groups. This is a promising approach that complements current analysis methodologies.

Metabolic Comorbidities

Andrew Gewirtz of Georgia State University described how bacteria invading through the gut epithelial barrier

trigger toll-like receptors (TLRs), particularly TLR-5 leading to immune cell recruitment and rapid bacterial clearance. TLR-5 knockout (T5KO) mice develop obesity, hyperglycemia, and insulin resistance, and a subset develops chronic colitis.[21] T5KO mice also have an altered gut microbiome as their ability to select against flagellated organisms is diminished.[22] Germ-free T5KO mice do not exhibit the colitic and metabolic syndrome phenotypes and restoring the gut microbiome in these mice reinstates the metabolic syndrome phenotype. Furthermore, transfer of the gut microbiome from a T5KO mouse to a germ-free wild type mouse is sufficient to cause metabolic syndrome in wild type mice suggesting that flagellated bacteria play a major role in the development of this syndrome.

In humans, metabolic syndrome is associated with microbial encroachment on the epithelial barrier.[23] Loss of TLR-5 appears to allow bacteria to penetrate further through the mucous layer and encroach on the epithelial barrier.[24] Use of emulsifiers in food has increased due to the growth in the processed food industry. For example, addition of emulsifiers to chow allows bacteria to encroach on the gut epithelium leading to metabolic syndrome in conventional wild type but not germ free mice.[25] High fat diets also promote microbial encroachment in mice, an effect that is blocked by the addition of inulin, a dietary soluble fiber to chow.[26]

Inhibition of SCFA production with beta acids had no effect on the capacity of inulin to block microbial encroachment, suggesting that inulin restores the mucous barrier independent of SCFAs. Dr. Gerwitz's work may explain some of the epidemiological links between processed foods and metabolic morbidity. Dietary interventions aimed at reducing chronic immune activation in HIV may still hold great promise, whereas the potential for pharmaceutical or probiotic interventions may be becoming less popular.

While the notion that the lungs are sterile has been challenged recently, it is nevertheless true that the biomass is considerably lower compared to other anatomical sites, in particular compared to the gut lumen. Given the low abundance of lung resident microorganisms and the inability to sample the lung without passing through the rich oral environment, it has been difficult to truly identify the lung microbiome from the oral microbiome and from other contaminants. Recent work appears to indicate that the lung microbiome originates from the oral microbiome.[27]

Dr. Alison Morris, chair of the University of Pittsburgh HIV Lung Research Center, discussed challenges associated with studying the lung microbiome and shared findings from the lung HIV microbiome project. HIV infected individuals have long been known to be at higher risk of developing chronic obstructive pulmonary disease (COPD), so it was surprising to find that their lung microbiome, sampled through bronchoalveolar lavage (BAL), was similar to HIV negative and HIV infected on antiretroviral therapy.[28–30] *Tropheryma whipplei* was however found to be in higher abundance in individuals with HIV and this abundance decreased following antiretroviral therapy.[31]

Bacterial composition of the lung does vary dependent on lung function in HIV, but *T. whipplei* does not appear to explain that variation.[32] Ultimately longitudinal studies which include assessment of metabolites in addition to taxonomy will likely be necessary to further define microbe–host interactions in the lung.

Although the overall lung microbiome does not appear to differ by HIV status, differential immune responses to these lung commensals may drive the increased risk of COPD in HIV infection.[30] Progression of COPD is associated with accumulation of immune cells, particularly B cells, in the walls of airways.[33] Indeed, mice models have shown that immune responses play a role in the development of COPD, as evidenced by the fact that depletion of B cells protects mice against the development of COPD following exposure to cigarette smoke.[34]

Dr. Daniel Dunlap of the University of Pittsburgh shared his proposed mechanism of action on how B cells may contribute to lung tissue damage through inflammation caused by antibody opsonization of commensal bacteria. Dr. Dunlap used magnetic cell sorting to identify lung bacteria, which were bound by IgG. He found that IgG bound bacteria were more abundant in the lungs of HIV-infected individuals than HIV negative controls. The population of IgG bound bacteria from HIV infected individuals also differed significantly from the IgG bound population in HIV negative controls based on principle coordinate analysis. In HIV-infected individuals, Pseudomonas was the most abundant IgG bound bacteria, and this bacterium was more abundant compared to HIV negative controls.

In HIV-infected individuals, IgG bound bacterial populations differed when considering the lung disease and its severity as measured by diffusing capacity. Abnormal immune responses to commensal organisms may contribute to COPD in HIV and could be a potential target for therapeutics.

Dr. Ronald Collman of the University of Pennsylvania discussed his work suggesting that viruses may also play a role in lung pathology in immunocompromised individuals.

Anelloviruses, which are small, single stranded, enveloped DNA viruses, dominate the lung virome and are found in high percentages of adults around the world.[35] Plasma and lung anellovirus levels increase following immunosuppression in lung transplantation patients, suggesting that anelloviruses are at least partially under immune surveillance and control.[36] The rise in anellovirus levels from pre to post lung transplantation correlated with risk of primary graft dysfunction.[37] Although anellovirus load appeared to increase with declining $CD4^+$ count in HIV, it did not appear to drive systemic T cell activation in treated HIV.[38,39] Further studies will hopefully unravel the possible link between decline in lung function and the abundance and load of anelloviruses in HIV.

Transmission and Prevention

Commensal microbes appear to modulate risk of HIV acquisition, particularly in the female reproductive tract, which has been extensively studied. Presenters in this session discussed the potential for modulating the microbiome to reduce HIV acquisition risk.

In 2017 the Kwon lab at the Ragon Institute published a sentinel paper demonstrating that specific vaginal microbiome signatures strongly correlated with HIV acquisition risk.[40] Young South African women with a *Lactobacillus crispatus*-dominant vaginal microbiome had a dramatically lower incidence of HIV infection compared to women with polymicrobial vaginal microbiomes. Matthew Hayward of the Ragon Institute discussed some of his high-resolution analyses of the vaginal microbiome and potential links with HIV acquisition. Using shotgun metagenomics and simulated metagenomes, he was able to identify individual strains of

Gardnerella vaginalis, which are linked to bacterial vaginosis and the predominant anaerobe found in polymicrobial vagina microbiomes. He was able to identify four different *G. vaginalis* gene profiles, which could segregate into four different cervicotypes. These 4 *G. vaginalis* cervicotypes varied significantly in their association with levels of inflammatory chemokines as measured in cervicovaginal lavage fluid. Further work involves determining if these cervicotypes are associated with different HIV acquisition risks in women who harbor these vaginal cervicotypes and potentially design specific targeted approaches to reduce the risk of HIV infection in these women.

Commensal bacteria affect multiple parameters that are associated with HIV transmission. Dr. Ian McGowan from Orion Biotechnology discussed which factors are important and need to be considered when studying different anatomical sites. Dr. McGowan pointed out that the microbiome sampled from anal versus rectal swabs can be remarkably distinct.[41]

Interestingly, the microbiome of the neovagina of transgender women has been found to resemble that of the rectal microbiome rather than the vaginal microbiome in cis-gender women, particularly regarding the abundance of *Prevotella* species. Sexual preference has also been found to influence the gut microbiome, with MSM being mostly dominated by *Prevotella* species, whereas non-MSM tend to be dominated by *Bacteroides* species, and this is independent of HIV status.

Other factors that can impact the rectal/anal microbiome are douching, anorectal sexually transmitted infection (STI), recreational drug use, and the use of PrEP.[42,43] Further studies are urgently needed to understand how these factors affect HIV acquisition/transmission. Studies in nonhuman primates have shown that increased HIV acquisition risk is associated with lower levels of *Firmicutes* and reduced ratio of *Bacteroides* to *Prevotella*, along with an increase in activated $CD4^+$ $CCR5^+$ $Ki67^+$ T cells in the rectal mucosa.[44] Future goals to prevent microbial dysbiosis include reducing mucosal damage incurred during sexual/parasexual behavior and testing for and treatment of STIs. Potential approaches include diet, use of probiotics, prebiotics, and symbiotics, as well as FMT.

Dr. Ami Bhatt from Stanford University discussed new approaches and technologies to study the microbiome that is applicable in clinical settings. Using a bioinformatics tool called StrainShifter, her group is able to identify highly concordant microbial strains between samples by comparing metagenomes to bacterial isolate sequencing. Using this method, they are able to identify the origin of a bacterial infection. Deep metagenomics is another technique that was developed by the Bhatt lab to obtain high quality microbial genome drafts where microbe isolation and culture are not possible.[45]

Use of a customized software Athena, a *de novo* assembler that uses read clouds and creates metagenomic assemblies, allowed the group to obtain high quality comprehensive genomic data comparable to existing short-read and synthetic long-read metagenomic sequencing techniques. Another concept introduced was that of a ''translatome'' which defines what is being translated in the cell (indirect measurement of protein abundance) using a technique known as metaRiboseq. This methodology offers different and additional information compared to metatranscriptomics because it is focused on structured RNA and not just RNA sequence. Finally, Dr, Bhatt discussed an often-overlooked component of the microbiome, the bacteriophages or ''Phageome.'' Bacteriophages are abundant in human gut but have been so far not or very little studied. CrAssphage (cross-Assembly phage) was discovered *in silico* and is an abundant double stranded DNA (dsDNA) phage that is highly gut specific and transmitted from mother to child, by FMT, through bone marrow transplantation and possibly through the environment (such as same hospital room). Interestingly the genome structure of CrAssphage seems to be conserved across human populations.

A polymicrobial, anaerobe-dense vaginal microbiome is associated with increased risk for HIV acquisition and transmission but also with preterm birth and human papillomavirus (HPV) persistence.[46] Dr. Kelly Hsu from the Bill and Melinda Gates Foundation discussed the goals of the Foundation in regards to microbiome studies.

As previously mentioned, women with a *Lactobacillus*-dominant vaginal microbiome have a low risk of HIV acquisition.[40,47] The major goals of the Foundation are to develop a product that could promote the permanent colonization of Lactobacillus-dominant vaginal microbiome (*L. crispatus*) with the end result of promoting maternal and child health. Dr. Hsu also stressed the importance of considering the end users when designing such a product. Specifically, taboos around menstruation in different cultures significantly affect the success of implementing this type of product. For example, certain menstruation products, like tampons, are used in the developed world and not necessarily accepted in other countries or cultures.

In recent years, rates of STIs have increased significantly in older women, both with and without HIV. Previous studies have shown immunobiological alterations in the genital tract of postmenopausal women that can enhance susceptibility to HIV.[48–53]

In her cross-sectional study of women recruited from the Bronx and Brooklyn WIHS cohort and Montefiore/Jacobi Medical center clinics, Dr. Kerry Murphy from Montefiore medical center studied immune mediators in HIV-infected and HIV-uninfected postmenopausal women compared to premenopausal groups (HIV+ and HIV−). She found significantly lower levels of immune mediators human beta defensin (HBD) HBD-2, HBD-3 and secretory leukocyte protease inhibitor (SLPI) in postmenopausal women compared to premenopausal. Serine protease inhibitor of the Kazal type 5 (SPINK5) and IL-6 were significantly reduced in the HIV infected group.

Genital samples from HIV-infected postmenopausal women showed significantly lower *Escherichia coli* inhibitory activity but significantly higher herpes simplex virus (HSV) inhibitory activity compared to the infected premenopausal group. However, HIV inhibitory activity was significantly higher in uninfected postmenopausal women. Within the HIV-infected group, many cervicovaginal lavage samples showed enhancement of HIV infectivity *in vitro*.

A subgroup analysis indicated that these women were more likely to be on protease inhibitor-based antiretroviral therapy (ART), had higher detectable plasma loads, and higher median Nugent scores (gram-staining scoring system to diagnose bacterial vaginosis). Taxonomic composition of the vaginal microbiome varied by reproductive age (premenopausal vs postmenopausal), *E. coli* inhibitory activity, and HIV inhibitory activity. While alpha diversity was higher in the postmenopausal women, especially in the HIV-infected population, there was no clear clustering of the vaginal microbiome by reproductive age. These findings indicate that aging may impact

genital health of women, most probably due to hormonal changes. The impact of aging on the vaginal microbiome is more pronounced in HIV-infected women, which may have implications for HIV risk acquisition.

Effects of the Microbiome on Vaccine Responses

The immunomodulatory properties of probiotics offer exciting possibilities for vaccine studies and have recently been an area of active research in the field of HIV. Dr. Jennifer Manuzak from the University of Miami discussed the impact of probiotic/prebiotic supplementation on gut immunity using nonprimate models.[54,55] Her previous work showed that probiotic/prebiotic supplementation increased the frequency of IgA^+ B cells, T follicular helper cells, and IL-23^+ antigen presenting cells in colon and lymph nodes (LN).[55]

Probiotic therapy also dampened TLR signaling and decreased frequency of activated and proliferating $CD4^+$ T cells in the colon thereby showing an overall improvement in mucosal health. Her current work aimed at evaluating the effects of probiotics (Visbiome) on vaccine responses. Four groups of macaques were used: untreated, vaccinated with a DNA-protein vaccine (SIV Gag-HIV Env-HIVgp140 trimer), orally dosed with the probiotic, or given a combination of vaccine and probiotic. Probiotic treatment increased the frequency of $CD4^+$ T cells in the colon and LN and decreased the frequency of intestinal $CCR5^+$ $CD4^+$ T cells and colonic $CCR6^+$ $CD4^+$ T cells.

However, upon SIV challenge, the rate of SIV acquisition and plasma viral load remained similar between all groups. Therefore, although probiotic treatment did improve overall mucosal health, it did not significantly enhance vaccine response upon SIV challenge. Further studies must be conducted to understand the role of the gut microbiome and its modulation on vaccine response.

Microbiome Based Therapeutics

The last session of the fourth HIV microbiome workshop focused on the potential use of commensal organisms for the diagnosis and treatment of human disease.

Dr. Matthew Henn from Seres therapeutics discussed clinical trials using microbiome-based therapeutics. He suggested that consortia of microbes may be able to target multiple pathways simultaneously, whereas drugs typically only target single pathways. Because microbiome-based therapeutics are generally well-tolerated, they might be used in conjunction with traditional drugs. Seres therapeutics has several microbial ''cocktails'' in development for the treatment of serious intestinal conditions, including *Clostridium difficile* infection, colitis, and inflammatory bowel disease.

For example, patients with mild-to-moderate ulcerative colitis who did not respond to standard of care treatment were included in a Phase 1b study trial of SER-287, an oral microbiome therapeutic candidate composed of a diverse bacterial spore ecology (SERES-101 study). The Phase 1b study had four arms, a placebo followed by placebo, a placebo followed by SER-287, oral vancomycin followed by weekly SER-287, and oral vancomycin followed by daily SER-287. The proportion of patients achieving remission was highest in the vancomycin/daily SER-287 arm (40%) followed by the vancomycin/weekly SER-287 (17.7%) and the placebo/weekly SER-287 (13.3%) arms. No patients in the placebo/placebo arm achieved remission. SER-287 had a similar safety profile (tolerability) compared to the placebo. Engraftment of SER-287 was dose dependent and was enhanced by oral vancomycin. Patients who achieved remission had microbial and metabolomic signatures distinct from those who did not. Several metabolic pathways associated with gut inflammation were modified, including those involved with SCFAs, tryptophan, and bile acids. Understanding the mechanisms and pathways involved in how changing the microbiome impacts human health and disease is at the forefront of microbiome research.

Conclusion

Our understanding of how the microbiome is influencing health and disease is increasing. This is an exciting time to delve deep into the mechanisms at the heart of how microbes shape our immune system and responses to vaccines and how modulating the microbiome can influence inflammation, host response, and disease. By including experts in fields other than HIV infection and learning from their approaches and struggles teaches us the need to have a global approach to studying the microbiome and bring together a community of researchers from different fields: immunology, chemistry, to advance the field of HIV, and microbiome.

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Address correspondence to:
Brett Williams
Division of Infectious Diseases
Rush University Medical Center
600 South Paulina No.143
Chicago, Illinois 60612
USA

E-mail: brett_williams@rush.edu