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CONFERENCE SUMMARY

A Summary of the First HIV Microbiome Workshop 2015

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Abstract

The role of microbiota in the pathogenesis of HIV infection has become the subject of intense research in recent years. A rapidly growing amount of data suggest that microbial dysbiosis—in the gut or the genital tract—can influence HIV transmission and/or disease progression; however, a deeper understanding of the mechanisms involved is lacking. To better understand the relationship between the microbiome and HIV infection, investigators from a wide variety of disciplines, including those working in basic and clinical HIV studies, cardiovascular disease, reproductive health, and bioinformatics, gathered at the first International Workshop on Microbiome in HIV Pathogenesis, Prevention and Treatment, at NIH on 7 and 8 April, 2015.

Keywords: HIV, microbiome, immune activation, microbial translocation

Introduction

THE ROLE OF MICROBIOTA in the pathogenesis of HIV infection has become the subject of intense research in recent years. A rapidly growing body of data suggests that microbial dysbiosis—in the gut or the genital tract—can influence HIV transmission and/or disease progression; however, a deeper understanding of the mechanisms involved is lacking. To better understand the relationship between the microbiome and HIV infection, investigators from a wide variety of disciplines, including those working in basic and clinical HIV studies, cardiovascular disease, reproductive health, and bioinformatics, gathered at the first International Workshop on Microbiome in HIV Pathogenesis, Prevention and Treatment, at NIH on 7 and 8 April, 2015.

Pathogenesis

During chronic infection, HIV-infected people exhibit persistent immune activation, which is closely linked to clinical outcomes.[1,2] This immune activation appears to be driven at least, in part, by microbial products that translocate from the gastrointestinal tract.[3,4] Despite effective suppression of HIV replication with antiretroviral therapy (ART), many HIV-infected individuals have persistent immune activation, which is associated with excess morbidity and mortality and is a central focus of current research.[5–7] Presentations in this first session covered some of the mechanisms by which this immune activation may originate and persist, despite viral suppression.

Dr. David Wang from Washington University discussed his work regarding the enteric virome of SIV-infected nonhuman primates. The virome of primates demonstrated an expansion of eukaryotic viruses in macaques following SIV infection that was mostly due to picornaviruses and adenoviruses.[8] To validate their findings, they studied additional cohorts in other primate centers and confirmed eukaryotic virus expansion in SIV-positive macaques, which progress to AIDS. However, this expansion was not seen in African green monkeys, which do not exhibit immune activation

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during chronic infection and do not progress to AIDS. Questions remain as to whether this expansion of the virome occurs during HIV infection, if it contributes to persistent immune activation, and how it might be impacted by ART. Since the conclusion of this meeting, this group has demonstrated that indeed there is an expansion of enteric adenovirus in HIV infection that relates to low CD4 count.[9]

Dr. Cara Wilson from University of Colorado discussed her seminal work in identifying immune correlates with changes in the microbiome. Her working hypothesis is that dysbiosis in untreated HIV infection is associated with colonic mucosal inflammation and CD4 T-cell depletion. She described two studies by her group that demonstrated the nature of intestinal dysbiosis in untreated HIV infection and defined a linkage between dendritic cells (DCs) and gut bacterial diversity. At the phylum level, there was an increase in Proteobacteria and a decrease in Firmicutes in HIV-infected individuals. At the genus level, there was an increase in *Prevotell*a and decrease in *Bacteroides* in colonic mucosa. Changes were more marked in biopsy versus stool samples.[10] Several biomarkers linked to HIV disease progression, such as plasma LPS, colonic and blood CD4 and CD8 T-cell activation, as well as colonic DC activation, were positively associated with gut dysbiosis. Dysbiosis was negatively associated with colonic Th22 cells. *Prevotella* overabundance was most closely associated with immune activation in this study.[11]

These studies raise several questions. First, is HIV-associated pathology related to an increase in proinflammatory or decrease in regulatory bacteria? Second, is *Prevotella* a pathobiont? *Prevotella* has been associated with periodontal disease, is increased in the lingual microbiome of HIV-positive individuals, can degrade mucin, promotes DC activation and T-cell IFN-gamma production *in vitro*, and is associated with increased levels of atherogenic Trimethylamine N-oxide (TMAO).[10–15] Third, as discussed later by Dr. Lozupone, *Prevotella* is predominant in agrarian cultures and in those with carbohydrate heavy diets, so is the impact of *Prevotella* on inflammation dependent on diet?

Dr. Stephanie Dillon of University of Colorado discussed ''The Short Chain Fatty Acid Butyrate Reduces HIV-1 and Pathobiont-associated Increases in Intestinal Myeloid DC (mDC) and T-Cell Activation.'' Short chain fatty acids (SCFAs) are metabolic products of dietary fiber fermentation by anaerobic bacteria in the large intestine. Butyrate, mostly produced by Firmicutes (*Clostridia* class), has particularly been noted to have immune modulating properties. Butyrate also acts as a histone deacetylase inhibitor and can induce HIV expression in latently infected cells.

They also assessed the effects of SCFA on colonic T-cell and mDC function in the setting of HIV-1 and exposure to *Prevotella* in an *ex vivo* model. In recent unpublished studies, mDC activation also associated with the ratio of *Prevotella*:butyrate producing bacteria (BPB). Using the *ex vivo* lamina propria mononuclear cell model, they found that *Prevotella stercorea* enhanced T-cell-associated cytokine production, activation, and infection, as well as mDC activation. High doses of butyrate reduce *Prevotella*-induced T-cell activation, infection, cytokine production, and mDC activation. This effect was not seen with low-dose butyrate. Low-dose butyrate did however reduce IFN-gamma and IL-17 production. Dr. Dillon's data suggest that a lower abundance of intestinal BPB may exacerbate inflammatory mucosal immune responses to pathobionts and contribute to mucosal pathogenesis.[3,9] Dr. Piotr Nowak from The Karolinska Institutet Sweden presented data from his prospective observational study, which longitudinally analyzed stool microbiota and plasma markers of microbial translocation/inflammation in HIV-positive subjects.[16] Controls were household members. They found profound alterations in both diversity and composition of gut microbiota in HIV-1-infected patients. Alpha diversity, or species richness within an individual, correlated positively with CD4 T-cell counts and inversely with sCD14, LPS, LBP, and sCD163. Short-term ART did not restore gut microbiota to normal.

Several important questions remain regarding the role of the enteric microbiome in gut mucosal integrity, microbial translocation, and persistent immune activation. First, is the increased gut permeability noted in HIV caused by enteric viruses? Second, are the alterations in the microbiome secondary to other social or behavior factors rather than HIV? Third, is Prevotella a pathobiont?

Transmission and Prevention (Microbicides)

In this session, experts in the fields of reproductive immunology/microbiology and women's health discussed the relationship between vaginal microbiota and HIV risk in women. They also addressed some of the scientific and social challenges that persist in prevention of HIV transmission.

Dr. Marrazzo from the University of Washington discussed the role of the vaginal microbiome in microbicide research. She described the classical spectrum of vaginal bacteria consisting of *Lactobacillus* species, capable of producing lactic acid and H_2O_2. Indeed, an outgrowth of commensal anaerobes could result in bacterial vaginosis (BV), linked to increased risk of HIV acquisition and transmission (reviewed Mirmonsef et al.[17] and Mirmonsef and Spear[18]).

The alteration in the normal vaginal microflora and pH is thought to lead to subclinical inflammation, HIV target cell recruitment and activation, altered innate immune response, and disruption or weakening of the epithelium, resulting in increased susceptibility to mucosal HIV infection.[19–21] She cited studies demonstrating the presence of SCFAs in vaginal fluids in women with BV and how these compounds may also regulate immune responses in the lower female genital tract.[17,22]

She stressed that all components of metadata (such as the relationship of specific BV-associated bacteria to clinical features and innate immunity, sex, vaginal product use, and hormonal environment) were needed to fully understand their impact on risk of HIV transmission. The incorporation of the ''omics'' approach will also be necessary to better understand the role of various metabolites and proteins, as well as host genetics, in HIV infection and transmission. Moreover, frequent sampling of the female genital tract will be necessary to enhance our understanding of the dynamic nature of microbiota and its diversity and also to guide development of microbicides for women at risk for HIV infection/transmission.

The lack of suitable experimental models has been a major challenge in studying the underlying mechanisms by which vaginal dysbiosis could increase the risk of HIV acquisition. To this end, Dr. Nichole Klatt from The University of Washington presented data from her work developing a pigtail macaque (PTM) model of vaginal dysbiosis. Her group observed a *Lactobacillus*-dominant microbiome at ovulation in some of the PTMs, which was associated with decreased

innate inflammation in the vagina. Vaginal levels of inflammatory cytokines were higher in follicular and luteal stages, compared to the ovulatory stage. They also observed an increase in the frequency of vaginal neutrophils during the luteal stage. It should be noted that a recent study by Thurman *et al.* of two longitudinal clinical trials of healthy women found no differences in vaginal tissue CD45+ immune cell populations and activation status obtained in the follicular phase versus the luteal phase of the menstrual cycle, although they did not assess neutrophils.[23] Clearly, more work is needed to better understand the dynamics of bacterial communities and immune responses throughout the menstrual cycle.

Next, Dr. Richard Pyles from the University of Texas Medical Branch presented work in which immortalized human vaginal epithelial cell multilayers were utilized to study the impact of vaginal microbiota on host protein expression involved in the movement and processing of topically applied antiretroviral drugs. They found that the expression of several transporters, thought to be associated with drug transport, was suppressed by dysbiotic vaginal microbiota. Understanding these molecular mechanisms will aid in the development and testing of pre-exposure prophylaxis approaches.

Dr. Nyaradzo Mavis Mgodi from the University of Zimbabwe discussed the prevalence of intravaginal practices (IVPs) and their effects on vaginal microbiome, immune activation, and immunological biomarkers among women in Zimbabwe. Certain vaginal practices, such as douching, alter the microbiome and increase the risk of acquiring vaginal infections and HIV.[24,25] She stressed that understanding IVPs, promotion of effective interventions aimed at changing IVPs, as well as the development of biomedical HIV interventions that do not disrupt the vaginal microbiome, are all critical in HIV prevention in African women. The panelists agreed that in communities where IVPs are widely practiced, it might be unrealistic to eliminate their use entirely; however, it might be possible to encourage women to engage in healthier practices.

The session on Pathogenesis and Prevention was concluded by emphasizing the need for new and improved immunological approaches/models, controlling sexually transmitted infections, discouraging the use of the harshest IVPs, and the inclusion of social and behavioral scientists in these studies. Furthermore, understanding the molecular mechanisms by which ART and vaginal microbiota interact will be key in development and testing of pre-exposure prophylaxis approaches.

Comorbidities

Disturbances in the microbiome have been associated with a wide variety of comorbid conditions that are seen in HIV-infected people. Speakers in this session discussed some of these conditions and their pathogenesis, in studies based in HIV-uninfected as well as HIV-infected individuals.

Dr. Jack Gilbert from the University of Chicago gave an overview of the role of microbes in normal mammalian development, health, and disease. He presented data demonstrating that the reintroduction of clostridium to germ-free mice resolved peanut allergy.[26] He cited a study where introduction of *B. fragilis* to mice with a neurodevelopmental disorder leads to partial normalization of behavior.[27] Microbes have also been associated with modulation of body weight. An *Enterobacter*, which was closely associated with obesity in a human volunteer, was given to germ-free mice, which then developed obesity and insulin resistance on a high-fat diet, whereas animals not given the microbe did not.[28] In humans, lean individuals have more abundant *Christensenellaceae*. Germ-free mice given *Christensenellaceae minuta* gained less weight than those which did not receive this microbe.[29]

Dr. Wilson Tang of the Cleveland Clinic discussed the cross talk between microbiota and host and the influence of diet on metabolic pathways and mucosal immunity. Prokaryotes create TMAO from choline/phosphatidylcholine and L-carnitine. TMAO levels correlate with cardiovascular disease risk, and dietary choline/TMAO exposure contributes to renal dysfunction in humans.[30–32] The precise mechanisms of TMA/TMAO-induced pathogenesis remain unclear, but targets that might be modulated to minimize the consequences of TMAO, such as TMA lyases, are being identified.

Dr. Catherine Lozupone of the University of Colorado discussed ''Are Gut Microbiome Alterations a Factor in High Prevalence of Metabolic Co-Morbidity with HIV-1 infection?'' She suggested that profound CD4 depletion in GALT leads to dysbiosis, which then contributes to disease progression. Fecal microbiota was dramatically different in HIV-positive subjects in her study, and gut microbiota, like CD4 cells in the GALT, did not consistently normalize with ART. Similar to Dr. Wilson, she saw an increase in *Prevotella* and decrease in *Bacteroides* with HIV infection.[3,33]

Surveys of healthy individuals have found that the microbiome varies with geographic location and diet.[34] *Prevotella* was noted to be more prevalent in those with a more carbohydrate-based diet versus *Bacteroides* found in those with a more animal fat/protein-based diet.[35] Dr. Lozupone found that HIV-positive subjects often had microbiota similar to those in agrarian cultures.[13] The Western microbiota is less diverse and is associated with increased obesity, allergy, and inflammatory bowel/autoimmune diseases, disorders which are commonly associated with HIV. She suggested this might mean that an agrarian-like microbiome is not healthy for everyone and that the adaptive immune system matches the gut microbiota to the diet. For example, *Bacteroides uniformis*, which is significantly reduced in HIV and agrarian microbiomes, ameliorates metabolic and immune dysfunction in high-fat diet mice, but not in standard diet mice.[36] Also, *Prevotella*-rich communities can promote atherosclerosis through TMAO, but only in those who eat red meat,[15] and TMA-lyase producing bacteria appear to increase with HIV infection.[37]

As the discussed earlier, the intestinal microbiome can effect several different organ systems and commensal microbes can either drive or protect against comorbid conditions. Manipulating the intestinal microbiome may be a relatively high yield method of altering the development and course of these comorbid conditions, which now cause the majority of deaths in people with HIV.[38]

Microbiome and Vaccines

Emerging data, comparing vaccine responses in developed and developing countries, suggest that the microbiota plays a key role in vaccine efficacy.[39,40] Several groups have begun to address whether the microbiome might impact HIV-1 infection and vaccine immune responses. In this session, experts in the field of vaccine immunology discussed the potential influence of the microbiome in shaping the immune

repertoire before HIV-1 infection and vaccination and how that might affect vaccine efficacy.

Professor Sir Andrew McMichael from the University of Oxford, United Kingdom, reported on the existence of a HIV-1-reactive T-cell repertoire in HIV-1-unexposed subjects who may have been primed by microbiome-derived antigens.[41] HIV-1-specific T cells were present in both naive and memory CD4 and CD8 T-cell pools,[42] potentially influencing the specificity and magnitude of the postinfection and post-vaccination immune responses. Importantly, many of the HIV-1 epitope peptide sequences, recognized by the reactive pre-existing T cells, were also detected in natural HIV-1 infection and had near perfect sequence matches in the human microbiome. These data strongly suggest that microbiome-derived antigens may contribute to the stimulation of the preinfection HIV-1-cross-reactive memory T-cell repertoire.

Dr. M. Anthony Moody from Duke University School of Medicine investigated whether gut and other environmental antigens precondition B-cell responses to HIV-1 antigens. He found that anti-gp41 Abs isolated from blood and terminal ileum of acutely HIV-1-infected individuals were non-neutralizing and polyreactive with host and intestinal microbiota antigens.[43] By mass spectrometry sequencing, *Escherichia coli* RNA polymerase was identified as one of the possible antigens to cross-react with anti-gp41 Abs. Questions remain as to how post-HIV vaccination immune responses may be affected by the presence of microbiome cross-reactive memory T and B cells.

Dr. Wilton Williams (Duke University School of Medicine) presented data suggesting the microbiome may have affected HIV-1 vaccine responses in HIV-negative adult volunteers in the phase Ib (HVTN 082) and II (HVTN 204) immunogenicity trials, as well as the phase IIb (HVTN 505) efficacy trial that did not provide protection from HIV-1 acquisition.[44–47] The memory B-cell Ab response studied in the phase Ib and II trials indicated a dominant gp41-reactive Ab response, which was non-neutralizing, and had weak or no FcR-mediated anti-HIV-1 activity.[43] Furthermore, prevaccination B cells were found to be reactive with both Env gp41 and intestinal microbiota, as reported by Dr. Moody. Thus, the vaccines likely stimulated a pre-existing pool of HIV-1 cross-reactive B cells that did not protect against HIV-1 acquisition. These data suggest a previously unappreciated effect of the microbiome on diverting HIV-1 vaccine responses.

Finally, Dr. Bali Pulendran (Emory University School of Medicine) presented data supporting the role of the microbiota in affecting the ability of vaccines to establish immunity. He described a systems biology analysis of the immune response induced by trivalent inactivated seasonal influenza vaccine, which revealed a strong correlation between the early expression of toll-like receptor 5 (TLR5) and the magnitude of the protective hemagglutination inhibition titers 4 weeks after vaccination.[41] Flagellated intestinal microbiota-mediated TLR5 signaling directly promoted antibody responses to the viral vaccine. Remarkably, humoral responses to inactivated polio vaccine, another subunit unadjuvanted vaccine, were also observed to have TLR5-enhanced immunity, thus suggesting an important role of the microbiota in controlling immunity induced by subunit vaccines containing weak or no adjuvants.

In summary, the data presented in the Vaccine Session highlighted emerging evidence that the microbiome could not only precondition the immune repertoire to HIV infection and disease but may also be a critical determinant of HIV vaccine efficacy.

Treatment and Cure

Early investigations into microbiome-directed therapies for persistent immune activation as well as adjuncts for HIV cure are already under way. Speakers in this session discuss some of this pioneering work.

Dr. Jason Brenchley of NIAID/Division of Intramural Research discussed ''Dysbiotic microbes translocate in progressive SIV infection.'' In humans, there is incomplete reversal of microbial translocation and inflammation,[4] which in turn is associated with collagen deposition in the lamina propria,[48] poor reconstitution of CD4 cells in the GI tract,[49] decreased Th17 cells and homing of T cells to the lamina propria,[50] decreased expression of epithelial repair genes, and increased enterocyte apoptosis.[51]

Dr. Brenchley and Dr. Klatt's work with SIV-infected macaques has demonstrated improvements in mucosal CD4 reconstitution, lymphoid follicle fibrosis, and prognosis with probiotic supplementation of ARVs.[52] Dr. Klatt subsequently demonstrated that this same probiotic increased the number of IgA secreting B cells in the colon and lymph nodes, increased T follicular helper cells in lymph nodes, and IL-23+ APCs in the colon of healthy macaques.[53] Recently, a trial by Dr. Klatt's laboratory of fecal microbial transplantation (FMT) in SIV-infected macaques demonstrated lower CD4 activation post-FMT, suggesting that gut microbes may help prevent mucosal CD4 activation and loss.[54]

He also noted that unlike what has been seen in humans, the microbiome did not change substantially following SIV infection and hypothesized that standardized diet may account for lack of dysbiosis in laboratory animals with SIV.

Compared to their prevalence in the colon, Proteobacteria are overrepresented in the liver and mesenteric lymph nodes of SIV-infected animals, suggesting that they are responsible for much of the translocation. Why Proteobacteria preferentially translocate is not known, but Dr. Brenchley suggested their motility, high metabolic rate, and possession of immune evasion genes as possible reasons.[3] Correction of dysbiosis may help restore mucosal immunity and reduce systemic immune activation, and an ACTG protocol (A5350) is being developed to determine if a probiotic improves inflammation and mucosal immune reconstitution in treated HIV.

Dr. Angela Wahl of the University of North Carolina at Chapel Hill spoke on ''The Role of the Microbiome in Latent HIV Reservoirs.'' The primary obstacle in achieving a functional cure of HIV infection is the purging of cellular reservoirs because replication-competent HIV persists in infected cells, despite suppressive ART. TLR stimulation by bacteria may increase or suppress HIV expression. Two recent papers demonstrated that particular intestinal microbiota are important cofactors for initiating antitumor immune responses following administration of check point blocking antibodies.[55,56] This could potentially apply to cure research if these microbes are able to facilitate CD8 T-cell-driven apoptosis in latently infected CD4 cells.

Bone marrow/liver/thymus (BLT) humanized mice are a useful model for *in vivo* study of HIV latency. They are systemically reconstituted with human hematopoietic cells,

including the gut, so they are susceptible to HIV infection through multiple routes, and viral replication can be suppressed with typical ARVs.[57] These mice may be helpful in evaluating the role of the microbiome in HIV latency because the gut microbiome can be manipulated to yield germ-free, antibiotic-treated, or selectively colonized variants. Germ-free BLT mice (carrying a human immune system) can be used to generate mice colonized with the microbiota of different humans (HuM-BLT mice), and the gut microbiome is maintained human-like over time. Ongoing experiments are evaluating the effect of ART on the composition of the gut microbiome in HuM-BLT mice, the tissue distribution of latently infected cells in HuM-BLT mice, and quantification of latently infected cells in HuM-BLT mice, particularly in the gut.

The data presented in these two studies indicate that modifications or alterations to the gut microbiome through the use of probiotics and other therapeutic agents, including ARVs, can have a profound impact on HIV pathogenesis by stimulating or suppressing the natural immune response to infection.

Technologies and Methodologies

The study of microbiomics and metabolomics has been made possible by advances in DNA sequencing and statistical manipulation and presentation of data. The concluding session of the meeting addressed the challenges and future direction of this growing field.

Dr. Frederic Bushman of the University of Pennsylvania opened with observations on the challenges of microbiome research. He noted that research was improving with the use of discovery and validation cohorts that allow for a better understanding of correlation versus noise. All microbiome sequence recovery methods are biased because they are always looking at a slice, not all microbiota, and that needs to be recognized in microbiome studies. There are huge and underappreciated challenges in analyzing low microbial biomass samples. Other challenges include the need for absolute abundance measures, as well as microbiome longitudinal instability and frequent confounding by antibiotics. In studies of mice, there are strong cage effects—mice are coprophagic, so the gut bacteria in all mice in a cage come to be similar over time. Thus, studies need to be carried out over many cages, and cage history needs to be treated as a variable in statistical analysis.

Dr. Roger Paredes of the IrsiCaixa foundation added that the major challenge is trying to connect microbiomic data with the real world, including how to control for the extensive behavior-related potential confounding on the microbiome, the difficulties in consistently identifying genuine dysbiotic patterns for the different disease states, or to define specific targets for microbiome interventions in human populations.

Dr. John McGowan of NIAID discussed Nephele, the NIH Microbiome cloud pilot project currently in development, which aims to offer a centralized environment for analytic tools and facilitate access for those lacking infrastructure and/or expertise.

Conclusion

Microbiomics and metabolomics in HIV are a rapidly evolving field. Future discoveries may lead to advances in the prevention of HIV infection, including vaccine development; amelioration of comorbidities in cART-treated individuals; and efforts to cure HIV. Much work needs to be done to further define therapeutic targets and to determine whether modulation of these targets improves clinical outcomes. This work holds the promise of paradigm shifts in our understanding of health and disease, as well as fewer HIV infections and better longer lives for those living with HIV.

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Author Disclosure Statement

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