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CONFERENCE SUMMARY

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A Summary of the Sixth International Workshop on Microbiome in HIV Pathogenesis, Prevention, and Treatment

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Abstract

In October of 2020, researchers from around the world met online for the sixth annual International Workshop on Microbiome in HIV Pathogenesis, Prevention, and Treatment. New research was presented on the roles of the microbiome on immune response and HIV transmission and pathogenesis and the potential for alterations in the microbiome to decrease transmission and affect comorbidities. This article presents a summary of the findings reported.

Keywords: HIV/SIV, microbiome, pathogenesis, therapeutics, comorbidities, transmission, prevention

Introduction

THIS YEAR MARKED the sixth meeting of the International Workshop on Microbiome in HIV Pathogenesis, Prevention, and Treatment.[1–5] The conference aims to bring together experts from across the field of microbiome research for a multidisciplinary discussion of the impact of the microbiome on HIV outcomes. Due to the COVID-19 pandemic, the conference was held virtually.

In spite of the difficulties, researchers came together on the virtual platform to discuss interesting findings on the role of the microbiome and inflammation in HIV-1 transmission, prevention, and comorbidities. The first keynote address by Dr. Elodie Ghedin described her work to identify changes in bacteriophage and host bacteria during viral infection, while the second keynote address by Dr. Cynthia Sears discussed her work identifying links between the gut microbiome and colorectal cancer.

First Keynote Address

The 2020 conference opened with a keynote presentation on the dynamics of phage–bacteria interactions in the respiratory tract from Dr. Elodie Ghedin from the National

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Institute of Allergy and Infectious Diseases. Phages have a multitude of effects on the microbiota and, in addition, can interact with immune cells directly.[6] Dr. Ghedin's team investigated households in Nicaragua with index cases positive for influenza and asked whether there was higher transmission of pathogenic bacteria and antibiotic resistance genes in households with higher rates of intrahousehold influenza transmission.[7] Metatranscriptome and metagenome analyses of nasal washes showed both differential abundance and differential expression of bacteria and phages that had some association with the influenza status of the subject.

The team also applied a novel method to investigate phage–bacteria interaction by matching the phages they observed to sequences detected in bacterial CRISPR arrays. The CRISPR system occurs in many bacterial species and serves as an acquired antiviral defense by incorporating DNA sequence from previous phage infections into a CRISPR array that is then used to guide cleavage of any future DNA matching that sequence.[8] Combining abundance-based[9] and CRISPR-based inference of phage–bacteria interactions enables high-resolution capture of dynamic interactions in the microbiome and is being used to further characterize transmission of bacteria within and between households.

Transmission

The progestin-based contraceptive depot medroxyprogesterone acetate (DMPA) has been reported to be associated with increased risk of HIV acquisition and transmission in multiple observational studies.[10] In contrast, the recent Evidence for Contraceptive Options and HIV Outcomes Trial[11] reported no increased risk of HIV acquisition in women using DMPA. However, the interpretation of these data remain controversial as the study did not have a ''no contraceptive'' control group and the study was designed to detect a 50% or greater increase in HIV acquisition risk.[12–15] Multiple studies have reported changes in genital immune parameters among DMPA users,[16] although, no major changes in microbiome have been described in the majority of these studies.[17,18] Increased risk of HIV acquisition has, however, been described in DMPA users who have a polymicrobial vaginal microbiome, a known risk factor for increased acquisition of HIV.[19,20]

To understand the mechanisms underlying this association, Dr. Adam Burgener from Case Western Reserve University used previously published microbiome data from the CAPRISA-004 trial[21] and compared the relative risk of HIV acquisition among those using DMPA, norethisterone enanthate (NET-EN), and combined oral contraceptives (COC). They found that in women whose vaginal microbiome was *Lactobacillus* dominant, HIV acquisition was threefold higher in women using DMPA relative to women using NET-EN or COC, whereas women whose microbiomes were not *Lactobacillus* dominant showed no significant difference.[22] Higher serum medroxyprogesterone acetate levels associated with increased molecular markers of inflammation in the vaginal mucosal fluid of *Lactobacillus*-dominant women while women who had non-*Lactobacillus*-dominant microbiomes displayed markers of inflammation regardless of contraceptive use.[22] Although limited by the nonrandomized *post hoc* design of the study, these findings nonetheless suggest an interaction among microbiome, hormonal contraceptives, and HIV susceptibility.

Dr. Colleen Kelley from Emory University discussed the immune environment of the rectal mucosa and the impact on HIV transmission. In the United States, men who have sex with men (MSM) are the group most highly affected by HIV-1 with the majority of transmission occurring through condomless receptive anal intercourse (CRAI).[23,24] The rectal epithelium, distinct from vaginal and penile epithelium, is composed of a single layer of cells that results in a fragile barrier that offers limited protection to viral entry. Furthermore, the gastrointestinal tract is rich in CD4+CCR5+ cells, the primary targets of HIV. Dr. Kelley and collaborators have previously reported distinct rectal mucosal transcriptomes, including enrichment in immune activation and mucosal injury and repair pathways, and changes in the microbiome, including an enrichment of *Prevotella*, in MSM who practice CRAI.[25] Their ongoing studies have shown an association of CRAI with markers of cellular proliferation and neutrophil infiltration of crypt epithelial cells in rectal mucosa, but not with gut microbiome.[26] In MSM taking oral pre-exposure prophylaxis, no shift in microbiota was observed.[27] However, the use of hyperosmolar lubricants (not uncommon for CRAI) resulted in a shift in microbiome from *Bacteroides* to *Prevotella*.[27] Ongoing studies also show that adolescent MSM have distinct rectal immune microenvironment and microbiome compared with adult MSM with samples from young MSM showing higher *Bacteroides* to *Prevotella* ratios and higher HIV replication in an explant challenge model.

Dr. Jason Brenchley from the National Institute of Allergy and Infectious Diseases discussed his findings about the association of gut microbiome and simian immunodeficiency virus (SIV) acquisition in Asian macaques. There is little evidence for dysbiosis induced by SIV infection in macaques.[28] Similarly, many changes in human gut microbiota initially linked to HIV infection appear to instead associate with sexual practices.[29,30] The effects of these externally caused microbial dysbioses on HIV pathogenesis and transmission remain uncertain. The Brenchley laboratory disrupted the microbiome and induced intestinal epithelial damage in macaques using vancomycin then infected them with SIV and found their progression to AIDS was similar to untreated macaques.[31] Vancomycin treatment did appear to induce low-level gastrointestinal tract inflammation and result in an increase in the number of transmitted founder viruses establishing new infections during rectal SIV challenge. To move beyond changes in the bacteria of the gut, the Brenchley laboratory is also working to viably culture translocating bacteria from liver, lymph nodes, and spleen and has identified a methyl transferase potentially shared among many translocating species.

Pathogenesis

MSM have an altered gut microbiome, elevated immune activation, and CCR5 expression even in the absence of HIV infection.[25,29,30,32,33] To investigate this phenomenon, Dr. Eiko Yamada from the University of Colorado presented data showing that fecal microbes, especially *Holdemanella biformis*, a strain enriched in the guts of MSM, can upregulate

CCR5 expression and alter tumor necrosis factor-α/interleukin (IL)-10 ratio, concordant with previous observations of a proinflammatory role for *H. biformis*.[34]

Dr. Yanhui Cai from Gilead Sciences tested fecal microbiome from virologically suppressed HIV-infected individuals and observed no significant differences compared with healthy volunteers. However, the abundance of Proteobacteria in infected individuals correlated with interferon-stimulated gene expression and CD4 T cell activation.

Dr. Shilpa Ray from the Karolinska Institute presented microbiome data on a longitudinal cohort of HIV-infected individuals before and after initiation of several antiretroviral therapy (ART) regimens. They observed relatively little change in bacterial diversity or richness following ART initiation when all ART regimens were pooled but significant decreases in alpha diversity when only patients treated with non-nucleoside reverse transcriptase inhibitor-based therapies were considered. To follow up on this finding, they tested the antibacterial properties of various antiretroviral drugs and found that zidovudine and efavirenz inhibited the growth of several bacterial species, showing the potential for ART to directly affect the microbiome.[35]

James Virga from the National Cancer Institute reported results from the GUTCHEK study, where the effects of oral antibiotic rifaximin[36] on gut inflammation, immune activation, and microbiome were investigated in people living with HIV on long-term ART. Although a significant decrease in total diversity was observed in fecal samples following rifaximin treatment, no significant changes in soluble inflammatory markers, immune cell subsets, or plasma HIV RNA were observed after rifaximin treatment.

Second Keynote Address

In the second keynote presentation, Dr. Cynthia Sears from John Hopkins University discussed her work studying the links between the gut microbiome and colorectal cancer. Rates of colorectal cancer are rising around the world, especially in relatively younger age groups.[37,38] Metagenomic sequencing has revealed ties between cancer and the microbial communities of the gut[39,40] offering the opportunity for characterization of the microbiome to inform prevention and develop therapies. For example, enterotoxigenic *Bacteroides fragilis* express a toxin that can induce DNA damage, trigger inflammation pathways, and lead to carcinogenesis.[41–45] Another sign of a microbial link is that biofilms are often found in association with colorectal tumors.[46–48]

To test the causality of this association, the Sears laboratory and collaborators transferred biofilm-containing microbiota from homogenized biopsies from colorectal cancer patients by gavage to germ-free mouse models of carcinogenesis and observed subsequent biofilm formation in the distal colon, immune cell infiltration into lamina propia, and tumorigenesis.[49] In an unpublished work, the laboratory has cultured bacterial isolates from a patient sample and recapitulated mouse tumorigenesis using a combination of 30 isolates. Further work in patients with familial adenomatous polyposis showed a higher-than-expected frequency of colonic biofilms containing bacteria expressing oncogenic toxins.[50]

The Sears Laboratory has also analyzed databases of clinical and sequencing data to probe links between host and microbiome. Using electronic health records from the United Kingdom, they showed associations between antibiotic use and colorectal cancer diagnosis in a matched case–control study.[51] By careful reprocessing of raw bacterial sequencing data from multiple studies, meta-analyses identified an enrichment of several bacterial taxa in the microbiomes of colorectal cancer patients[48] and decreased diversity associated with HIV infection in woman and men who have sex with women but not in MSM[52] with ongoing analysis to distinguish the effects of HIV infection from those of sexual orientation.

Lessons learned from these analyses included the need for studies to provide sufficient metadata, the potential confounding factor of technical differences between laboratories[53] and the opportunity to integrate data on chemical, social, and environmental exposures.[54]

Prevention

A *Lactobacillus*-dominated vaginal microbiome appears to reduce the risk of acquiring HIV while more diverse communities and bacterial vaginosis associate with an increased risk.[55–57] However, antibiotic treatment of bacterial vaginosis has low cure rates and results in frequent recurrence.[58–60]

Dr. Laurel Lagenaur from Osel, Inc., reported on the results of a phase 2b clinical trial (NCT02766023) of the treatment of bacterial vaginosis aided by the a biotherapeutic formulation of a naturally occurring *Lactobacillus crispatus* strain CTV-05[61,62] called LACTIN-V. The study showed *L. crispatus* colonization in most women treated with LACTIN-V and a marked decrease in bacterial vaginosis recurrence over antibiotics alone.[63,64] Colonization was not affected by condom use or menses and no severe adverse events were observed. A subsequent phase 3 trial is currently being discussed with the FDA.

To better understand the link between lactic acid-producing *Lactobacillus* in the vaginal microbiome and decreased HIV acquisition,[65,66] Brianna Jesaveluk from the Burnet Institute discussed her research testing the effects of lactic acid in a cell model of barrier integrity. Measurements of transepithelial electrical resistance revealed that lactic acid at an acidic pH increased barrier integrity, whereas controls of hydrochloric acid at a similarly acidic pH or lactic acid at a neutral pH did not. To investigate this difference, RNA-Seq was used to characterize gene expression in the cell line with and without lactic acid and confirmed that several tight junction genes appeared upregulated in the presence of lactic acid.

Kaitlin Marquis from the University of Pennsylvania reported on her attempts to discover additional metabolites linking the vaginal microbiome to HIV acquisition risk. Using a meta-analysis of previously published studies of metabolites,[67–70] she found a set of metabolites enriched in high-diversity vaginal communities that have been linked to HIV acquisition. To follow up on these candidates, she developed a high-throughput screen using a reporter cell line to assess the effects of over 500 metabolites on *in vitro* HIV replication and found several candidate metabolites, including 2-hydroxyisovalerate that were both enriched in high-diversity communities and promoted HIV replication.

Male circumcision has been linked to a reduced risk of acquiring HIV.[71–74] Dr. Rupert Kaul from the University of Toronto described his work to study how the foreskin increases HIV risk and whether there are nonsurgical

alternatives to reduce this risk. Circumcision reduces inflammatory cytokines[75] and changes the penile microbiome[76] with a reduction in the abundance of several anaerobic bacterial taxa associated with inflammation and HIV risk.[77] Dr. Kaul reported on ongoing work showing that unlike the vaginal microbiome there do not appear to be clear penile community types or protective bacterial taxa but that several bacterial species were associated with HIV seroconversion. The associations of penile microbiome and HIV acquisition suggest the possibility that antibiotic alteration of the microbiome might provide an alternative to circumcision and a randomized clinical trial to compare the effect of antibiotics to circumcision on penile microbiome and HIV susceptibility in Ugandan men (NCT03412071) was just completed with analysis currently underway.

Comorbidities

Dr. Cara Wilson from the University of Colorado described her studies of host–microbe interactions and chronic immune activation. Dr. Wilson found that primary intestinal CD4+ T cells *in vitro* infected with HIV-1 and cultured in the presence of gut bacterial strain *Prevotella stercorea* displayed marked changes in the expression of genes involved in cellular proliferation and immune activation.[78] These changes included an upregulation of several genes related to the expression of granzymes, serine proteases that can induce apoptosis or promote an inflammatory response.[79,80] To investigate this association, the Wilson laboratory obtained samples from a previous clinical study[81,82] and found that samples from people living with HIV had higher levels of activated CD4 T cells in the blood and colon and higher proportions of granzyme B-positive CD4 T cells in the gut. The levels of granzyme B-positive T cells appeared to correlate with the abundance of several bacterial taxa.

Interactions between diet and the gut microbiome appear to affect cardiovascular risk.[83] For example, trimethylamine, a bacterial metabolite produced after meat, egg, and dairy consumption, is oxidized in the liver to trimethylamine N-oxide (TMAO) and appears to induce atherosclerosis.[84] TMAO can be used as a biomarker of atherosclerosis and heart failure.[85–88]

Dr. Marius Trøseid from the University of Oslo discussed his laboratories' work to study TMAO and metabolic syndromes in people living with HIV. In a cross-sectional study of people living with HIV, he saw little association between TMAO and myocardial infarction perhaps due to an overall increase of TMAO after initiation of protease inhibitor antiviral therapy.[89] Dr. Trøseid and collaborators have recently completed a Copenhagen-Oslo HIV Co-Morbidity and Microbiota Study (COMicS) to assess the links between HIV, sexual practice, and metabolic conditions.[90] They found contrasting effects of sexual practice and HIV infection on gut microbial diversity with MSM tending to have higher diversity, whereas people living with HIV tended to have less diversity resulting in MSM living with HIV having an intermediate diversity. They also identified several taxa previously linked to metabolic syndromes that were associated with HIV.[91] Regulation of gut microbiota by T cells has been proposed to reduce obesity[92] and the COMicS study suggested a link between CD4 count and the amount of visceral adipose tissue and metabolic syndromes.[90] The laboratory has also found soluble biomarkers of IL-1 activation to be predictive of myocardial infarction in people living with HIV,[93] has investigated the association of acylation of gut-derived lipopolysaccharides with inflammation,[94] and is currently exploring the effects of microbial translocation on extracellular vesicles.

Vaccines and Therapeutics

Interactions between the microbiome and the host immune response[95,96] and HIV and the microbiome[91,97,98] suggest the potential for the microbiome to modulate HIV vaccine response. Dr. Roger Paredes from the IrsiCaixa AIDS Research Institute discussed his work to characterize the effects of the microbiome on vaccination response in clinical trials and mouse models. In data collected during the CUTHIVAC 03 (Cutaneous and Mucosal HIV Vaccination) trial, testing a modified vaccinia virus Ankara (MVA) HIV vaccine, Dr. Paredes and colleagues found that the expression of certain genes in whole blood and microbiome abundances in stool and skin swabs were potential predictors of the development of neutralizing antibodies.[99] In a BCN02-Romi trial attempting to reverse viral latency and eradicate infected cells (''kick and kill'') using a combination of MVA vaccination and inducing agent romidepsin, 3 of 13 people living with HIV exhibited control of virus replication (viral load lower than 2000 copies/ml) for the entire duration of the 32-week treatment interruption and potential correlates of control were observed in the microbiome.[100] In a mouse model of multiple vaccinations, potential effects of sex and antibiotics on response to vaccination were observed. Further investigation into these areas is underway in the recently initiated MISTRAL (Microbiome-based stratification of individuals at risk of HIV-1 acquisition, chronic clinical complications, antimicrobial drug resistance, and unresponsiveness to therapeutic HIV-1 vaccination) project.

Dr. Alessandra Borgognone from the IrsiCaixa AIDS Research Institute continued the discussion of the BCN02-Romi trial of MVA vaccination and romidepsin in people living with HIV. She studied the stool bacterial abundances, peripheral blood mononuclear cell gene expression, and plasma protein composition of 3 individuals who controlled viral replication after treatment interruption and 10 individuals who did not control. Controllers appeared to have higher expression of genes related to immune activation and inflammatory response and an enrichment in protein markers of inflammation. Dr. Borgognone further found that controllers seemed to have higher levels of *Bacteroidales* and a depletion of *Clostridiales* bacteria, suggesting a potential role of the microbiome in vaccine response and subsequent control of HIV replication.

In addition to decreasing disease morbidity and mortality, vaccines can be used to probe the immune system.[101] Dr. Bali Pulendran from Stanford University discussed his research to better understand the human immune system utilizing vaccines. Vaccinations offer several benefits, including a defined time of immune perturbation allowing targeted high-throughput analysis, access to large and diverse populations receiving vaccinations, and an array of vaccine and adjuvant technologies providing varied immune stimuli. For example, in a study of the yellow fever vaccine (YF-17D), Dr. Pulendran and colleagues identified signatures of the vaccine-induced

innate immune response that predicted subsequent adaptive immune response.[102] Analysis of the metabolomic, transcriptomic, and cytokine profiles of the response to shingles vaccine Zostavax revealed additional insights.[103] Studies of vaccination also revealed interesting links to the microbiome. For example, the development of an adaptive response to influenza vaccination could be predicted by early molecular signatures, including increased expression of TLR5, a toll-like receptor, which senses flagellin from bacteria and triggers inflammation.[104] This unexpected link to bacteria was clarified by the finding that knockout mice lacking TLR5, along with antibiotic-treated or germ-free mice, exhibited reduced response to influenza vaccination.[105] A clinical trial testing the effects of antibiotic treatment before influenza vaccination (NCT02154061) revealed little effect in humans. However, a second trial focused on individuals with low pre-existing antibody titers showed a strong decrease in antibody titer in antibiotic-treated individuals[106] revealing a critical role of microbiome in immune response.

Anxiety disorders and depression are considerably more prevalent in people living with HIV than among the general population[107,108] and a link between the gut microbiome and brain has been identified in anxiety disorders.[109,110] Dr. Shamsudheen Moidunny from the University of Miami and collaborators observed that both HIV and morphine could induce gut dysbiosis in a humanized mouse model[111] and that anxiety-like behaviors increased with morphine treatment in a Tg26 mouse model expressing HIV proteins. Thus, the Tg26 mouse model may provide an important method to study interactions among HIV, microbiome, and opioid use.[112]

Posters

Several investigators also presented posters summarizing research on HIV, the microbiome or related topics for online discussion of their research. Dr. Matthew Olaniyan from Edo University Iyamho looked at the co-occurrence of mosquito-borne parasites and HIV infection and links to markers of inflammation. Annette Aldous from George Washington University studied the effects of contraceptive use on the vaginal microbiome of adolescent girls. Dr. Alessandro Lazzaro from the University of Turin documented changes in the microbiome of people living with HIV in Zimbabwe before and after initiation of ART. Casey Martin from the University of Colorado Denver reported on the gut microbiomes of children infected at birth with HIV. Dickens Mahwayo from the Given-Secret Foundation discussed the need for an awareness campaign for vaginal ring pre-exposure prophylaxis in Malawi. Dr. Charlotte-Eve Short from the Imperial College London compared the microbiome composition in cervicovaginal secretions collected with menstrual cups with those collected by high vaginal swab.

Conclusion

The research presented at the sixth meeting of the International Workshop on Microbiome in HIV Pathogenesis, Prevention, and Treatment highlighted the broad interdisciplinary nature and promising progress being made in this area. Linkages between the microbiomes of multiple body sites and HIV transmission and pathogenesis were reported. The multiple clinical trials presented show that microbiome research continues to move from observational studies into targeted alterations aimed to directly help patients. Continued collaborations between this broad coalitions of researchers and the communities of people living with HIV promise further improvements in understanding, diagnosing, and treating HIV-1 and its comorbidities.

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