Title

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Journal

AIDS Research and Human Retroviruses, 34(10)

ISSN

0889-2229

Authors

Williams, Brett
Boucher, Charles
Bushman, Frederic
et al.

Publication Date

2018-10-01

DOI

10.1089/aid.2018.0103

Peer reviewed

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 34, Number 10, 2018

DOI: 10.1089/aid.2018.0103

CONFERENCE SUMMARY

A Summary of the Third Annual HIV Microbiome Workshop

Brett Williams,[1] Charles Boucher,[2] Frederic Bushman,[3] Stacy Carrington-Lawrence,[4] Ronald Collman,[5] Satya Dandekar,[6] Que Dang,[7] Angela Malaspina,[7] Roger Paredes,[8] Cara Wilson,[9] Piotr Nowak,[10] Nichole Klatt,[11] Laurel Lagenaur,[12] and Alan Landay[13]

Abstract

Our microbial cotravelers have increasingly apparent roles in both maintaining health and causing disease in several organ systems. Investigators gather annually at the National Institutes of Health to present new discoveries regarding the role of the microbiome in human health and a special focus on persons living with HIV. Here, we summarize the discussions from the third annual Virology Education workshop on the microbiome in HIV, which took place in October of 2017.

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

Introduction

In 2015 Virology Education organized the first HIV microbiome workshop at the National Institutes of Health (NIH), the workshop has continued on a yearly basis to update researchers in the field on the newest developments. The third annual Virology Education HIV Microbiome workshop took place on October 19th and 20th, 2017, at the NIH Fishers Lane Conference Center in Rockville, Maryland. The theme was advances in microbiota/host interactions with a focus on HIV-positive individuals. Speakers were selected by the organizing committee to address the following topics: pathogenesis, treatment and prevention, gastrointestinal (GI) tract and diet, comorbidities, microbiome and vaccines, and metabolomics. Several members of the organizing committee were involved in work that was presented.

Dr. Wendy Henderson, Chief of the Digestive Disorders Unit, Division of Intramural Research, National Institute of Nursing Research, NIH, gave the keynote address entitled ''Brain-Gut-Microbiota interactions and intestinal health.'' She asked, ''why do some patients continue to have gut and or brain clinical symptoms even when HIV replication is controlled and how might the microbiome be involved in this process?'' She noted that up to 20% of the U.S. population has stress-induced GI symptoms and that GI symptoms are a common cause for medical visits with an annual cost of $30 billion.

GI mucosal adherent bacteria are different at baseline in those with irritable bowel syndrome (IBS), particularly overweight individuals with IBS, who were also more likely to report visceral pain.[1,2] They assessed GI permeability in patients with IBS using a four-sugar oral test solution with variable gut permeability based on molar mass. Absorbed sugar is quantified by measuring the fraction that is excreted in the urine. She hypothesized that stress leads to permeability, which leads to microbial translocation and then to abdominal pain (GI symptoms). Participants in an ongoing trial, who had altered gut permeability, were found to be twenty times more likely to have acute GI symptoms during the 5-h urine collection (NIH Clinical Trial No. NCT00824941).

[1]Division of Infectious Diseases, Rush University Medical Center, Chicago, Illinois.
[2]Department of Virosciences, Erasmus Medical Center, Erasmus University, Rotterdam, the Netherlands.
[3]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
[4]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.
[5]Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
[6]Department of Medical Microbiology and Immunology, University of California, Davis, California.
[7]Vaccine Research Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.
[8]Institut de Recerca de la SIDA IrsiCaixa i Unitat VIH, Universitat Autònoma de Barcelona, Universitat de Vic, Catalonia, Spain.
[9]Department of Medicine, University of Colorado at Denver, Denver, Colorado.
[10]Department of Medicine, Unit of Infectious Diseases, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden.
[11]Department of Pharmaceutics, Washington National Primate Research Center, University of Washington, Seattle, Washington.
[12]Osel, Inc., Mountain View, California.
[13]Department of Immunology/Microbiology, Rush University Medical Center, Chicago, Illinois.

Lactobacillaceae were negatively associated with permeability, while Lachnospiraceae were positively associated. Lipopolysaccharide (LPS) binding protein was elevated in diarrhea-predominant IBS, which supports the role of microbial translocation in the development of GI symptoms.

In related models, they assessed the effects of chronic stress on the gut microbiome using water avoidance as a chronic stress model in rats.[3] Stress induced an increase in *Proteobacteria* and genes for fatty acid and sulfur metabolism. Chronic stress decreased the capacity for energy and lipid metabolism. Dr. Henderson's laboratory has also developed a cell culture model to measure membrane permeability under glucocorticoid-induced stress. Butyrate appeared to reduce permeability. Chronic dexamethasone appeared to increase permeability by decreasing the expression of zonula occludens-1 (ZO-1) and occludin (article under review).

Separating the contributions of gut microbiota and chronic stress to GI symptoms and gut translocation in HIV will be difficult as chronic stress is common in many HIV populations. Dr. Henderson's work demonstrates the necessity of concurrently examining these factors and the laboratory methods she has developed will facilitate dynamic analyses.

Pathogenesis

Gut dysbiosis in HIV

The correlations between gut microbial translocation, chronic immune activation, and morbidity/mortality are fairly well established.[4] Controversy remains, however, regarding whether or how HIV infection alters the gut microbial community. Individuals with HIV have consistently been found to have enterotypes distinct from HIV-negative controls, but recently, a study by Noguera-Julian *et al.*[5] suggested that these differences may be due to sexual behavior. Abigail Armstrong, a graduate student in the laboratory of Dr. Catherine Lozupone discussed their study on a cohort of 217 individuals with and without HIV infection. Of the 112 HIV-infected individuals, 45 were on antiretroviral therapy (ART). They found a distinctly different enterotype, which was enriched for *Prevotella*, in men who have sex with men (MSM) compared to women or men who have sex with women. *Prevotella* enrichment is also associated with diets low in animal products, but Ms. Armstrong noted that the MSM-associated *Prevotella*-rich enterotype was different than the diet-associated *Prevotella*-rich enterotype. When plotted on Principal Coordinates Analysis (PCoA), the majority of MSM-associated microbiota did not fit into the *Prevotella*-rich reference space. Further studies will analyze this MSM-associated enterotype in greater depth.

Microbial translocation

Understanding the exact mechanisms by which gut bacteria cause immune activation, including which organisms from the GI microbiome are translocating, remains at an early stage of investigation. Dr. Ivan Vujkovic-Cvijin from the National Institute of Allergy and Infectious Diseases (NIAID) shared some of the data from his work in Dr. Yasmine Belkaid's laboratory. He noted that most previous publications found an increase in *Proteobacteria* and *Erysipelotrichaceae* in those with HIV compared to controls.

Bacteria that are frequently penetrating the gut mucosa and entering the circulation would be anticipated to prompt an immunoglobulin G (IgG) response. Based on this model of the disease process, they decided to search for bacteria that are targeted by high-affinity IgG. HIV-infected and HIV-uninfected individuals were enrolled in a study, their stool sample was incubated with serum to allow IgG to bind to bacteria, and then stool bacteria were sorted into IgG coated and uncoated. *Erysipelotrichaceae* from stool samples of HIV-positive individuals were bound by IgG at a higher level than stool samples from HIV-negative or elite controllers, suggesting a greater degree of microbial translocation. Further supporting the role of *Erysipelotrichaceae* in HIV-associated immune activation was a correlation between the levels of soluble CD14 and both magnitude and affinity of IgG to Erysipelotricheaceae.

Studies using the animal models seem to support the role of *Erysipelotrichaceae* as well. In a gnotobiotic mouse model (the microbiome of the mouse consists only of known microbes), the 7770.E6 *Erysipelotrichaceae* isolate increased the Th17 response in the small intestine. In addition, rhesus and pigtail macaques, which progress to AIDS, had significantly higher quantities of this E7770.E6 isolate in their stool compared to African green monkeys (AGMs), which do not progress to AIDS. Following simian immunodeficiency virus (SIV) infection, the amount of IgG directed at E7770.E6 increased from baseline, but this trend was not seen with IgG directed at another organism *Collinsella* sp.

As noted above, individuals with HIV have been found to have gut microbial communities enriched with *Proteobacteria* and *Prevotella* compared to HIV-negative individuals, some have termed this microbial community state a ''dysbiosis.''[6] *Proteobacteria* appear to be more likely to invade through the gut barrier.[7] It remains unclear as to whether gut dysbiosis is a driver of systemic immune activation. Alexandra Ortiz from Brenchley laboratory at NIH discussed an SIV model for experimentally induced dysbiosis. They used oral vancomycin to deplete bacteroides and clostridia and enrich for *Prevotella* and *Proteobacteria* in SIV-infected macaques. Microbial translocation was quantified by the percentage of tissue that stained positive for *Escherichia coli* in necropsy tissue sections of colon, mesenteric lymph nodes, and liver. Vancomycin-treated macaques were compared to untreated control animals and no differences were seen in viral loads, target cell depletion, microbial translocation, or survival rates. Vancomycin actually appeared to dampen CD8 T effector cell function. It remains unclear whether dysbiosis contributes to HIV-associated morbidity and mortality, but there does not appear to be a clear link in SIV infection.

Microbial interactions with the gut epithelial barrier

The gut milieu is extremely complex, and not all of the effects that microbes have on the mucosa are detrimental. Microbes play a large role in developing mucosal immunity and maintaining barrier integrity.[8] The Wilson laboratory at University of Colorado has pioneered use of an *ex vivo* model to investigate the effects of short chain fatty acids, a microbial metabolic byproduct, on lamina propria mononuclear cells (LPMCs). Jon Kibbie, an MD/PhD candidate in the laboratory discussed some of their recent work. They found that butyrate-producing bacteria (BPB) were reduced in

abundance in untreated HIV-infected individuals and BPB abundance inversely correlated with markers of inflammation and microbial translocation.[9] LPMCs were exposed to HIV *in vitro* and then exposed to varying concentrations of butyrate. The addition of higher levels of butyrate lead to decreased activation and reduced infection of CD4 cells, and decreased production of interleukin (IL)-17 and interferon (IFN)-γ. Lower mucosal butyrate availability may contribute to destruction of gut mucosal $CD4^+$ T cells. Future studies will examine the signaling mechanisms by which butyrate causes these effects.

Knowledge regarding the role of innate lymphoid cells (ILCs), particularly in epithelial barrier immune response, has expanded dramatically over the last few years. Dr. Moriah Castleman from the University of Colorado discussed her work in the Wilson laboratory. ILCs are derived from a lymphoid progenitor, but do not have rearranged antigen receptors. They produce cytokines similar to T cells and are grouped based on that cytokine production. IL-22 production by ILCs appears to improve epithelial barrier function through several mechanisms, while IFN-γ production by ILCs may lead to increased permeability. $NKp44^+CD56^-$ ILCs produce IL-22 in HIV-uninfected subjects, but this same subset produces IFN-γ in those with chronic untreated HIV infection.[10] Dr. Castleman's study sought to define the cytokine responses of human colonic ILCs to luminal bacteria and the role of those cytokines in inflammation associated with HIV infection. She used an *ex vivo* model of human colonic tissue discarded after surgery and isolated ILC3s from the lamina propria. A panel of bacteria known to be altered in HIV was used in the model. All bacteria tested were found to induce IL-22 compared to no bacteria, and this appeared to be dependent on cell wall components lipoteichoic acid from Gram-positive microbes and LPS from Gram-negative microbes. Production of IFN-γ by both ILC1s and ILC3s appeared to only occur in response to Gram-negative bacteria, but not to LPS. The mechanisms by which HIV alters cytokine responses from ILCs and the mechanisms by which bacteria induce these responses remain to be determined.

Microbial metabolites and cardiovascular disease

Gut microbes may contribute to immune activation through mechanisms other than direct invasion and translocation. Bacterial metabolic products have been strongly linked to cardiovascular disease in humans.[11] Tryptophan catabolism by the kynurenine pathway, in particular, appears to be closely related to progression of atherosclerosis in HIV.[12] Kynurenine can be produced by host indoleamine 2,3-dioxygenase-1 (IDO-1) in response to inflammatory cytokines, but some gut microbes, which may be enriched in HIV patients, carry homologous enzyme systems.[13] Dr. Dana Gabuzda from Harvard Medical School discussed her study on serum metabolites in HIV-positive individuals treated with protease inhibitors versus HIV-negative individuals.[14] They found altered tryptophan metabolism in HIV-positive individuals. Those with cardiovascular disease had elevated kynurenine to tryptophan ratio. Levels of several tryptophan metabolites were decreased in individuals taking antibiotics, suggesting that gut microbes are responsible for the production of a significant portion of these metabolites.

The end-organ effects of these tryptophan metabolites remain understudied. Dr. Qibin Qi from Albert Einstein College of Medicine presented his findings from a study in the Women's Interagency HIV study and multicenter AIDS cohort study.[15] About 737 individuals, 520 with HIV and 217 without HIV were monitored for 7 years for the development of carotid artery plaque. Metabolism of tryptophan by the kynurenine pathway was associated with HIV infection and incident development of carotid artery plaque. Preliminary data from another cohort linked levels of host kynurenine metabolites to microbial enzymes assessed by imputed bacterial gene content. He is currently pursuing shotgun metagenomics from stool samples and plasma metabolic profiling to further elucidate the connection between bacterial metabolism and host metabolite levels.

Inhibition of the tryptophan catabolism pathway has potential as an intervention to reduce cardiovascular events. Dr. Zhang Wang from GSK discussed the use of an IDO-1 inhibitor (IDOi) in SIV-infected rhesus macaques. Twelve macaques were infected with SIV, then started on ART, and 10 weeks later, six were started on IDOi and six on placebo. They noted a ''dysbiosis'' following SIV infection, which was reversed by ART and IDOi. Several taxa, particularly Dorea, Slackia, Succinovibrio, and Treponema, were differentially affected by the IDOi and correlated with tryptophan metabolites. These bacterial tryptophan metabolites, which were mostly indoles, appeared to be anti-inflammatory compared to human tryptophan metabolites like L-kynurenine.

Circulating lipid levels have long been known to correlate closely with the development of cardiovascular disease. It has previously been demonstrated that the gut microbiome predicts significant variation in blood lipids and body mass index.[16] Dr. Nicholas Funderburg from The Ohio State University discussed his study on the lipid dynamics of 35 individuals initiating raltegravir-based antiretroviral therapy in ACTG 5248 versus 13 HIV-negative individuals.[17] They found that HIV-positive individuals had lower levels of polyunsaturated fatty acids (PUFAs) at baseline and 48 weeks of ART compared to HIV-negative controls, and that PUFA levels correlated inversely with markers of immune activation. A subset of PUFAs did increase after 48 weeks of ART. He posited that PUFAs may play an important role in immune signaling related to microbial translocation in HIV as they appear to inhibit activation induced by LPS and IL-1b.

Microbial connections to neural development

Data continue to accumulate linking the gut microbiota to neurological outcomes. Mouse studies have suggested that the gut microbiota may play a large role in brain development and behavior. For example, germ-free mice exhibit decreased levels of anxiety and have altered N-methyl-D-aspartate (NMda) receptor expression in the amygdala.[18] Dr. Rebecca Knickmeyer from the University of North Carolina discussed findings from her study of 89 human infants.[19] The infants were followed longitudinally from 1 year of age. Fecal microbiota patterns were broken into three enterostates or clusters. Cognitive performance at 2 years of age differed by cluster and negatively correlated with alpha diversity. At 1 year of age, amygdala functional connectivity differed between clusters. Bacterial gene function also differed by cluster, the high performing cluster had more genes for folate biosynthesis, biotin, and lipolic acid, but less genes for motility and sporulation. Cluster membership differed based on

demographic groups and the higher performing group was more likely to be breast fed, but when demographics were controlled for in the model, the findings were still significant. Interventional studies will be key in determining whether this is a cause-and-effect relationship.

The Relationship Between Diet, the Gut Microbiome, and Health

Diet clearly has a dramatic impact on both the intestinal microbiota and overall health.[20] The immunopathogenesis of HIV appears to be tightly linked with chronic immune activation, a state also seen in humans with obesity, type 2 diabetes, and high-fat diets (HFDs).[21] However, the impact of diet on HIV pathogenesis remains understudied. Dr. Pandrea of the University of Pittsburgh presented her research on the effects of HFD on the progression of SIV in non-human primates (NHP). Pig tailed macaques (PTMs), which develop AIDS, and AGMs, which do not progress to AIDS, were given an HFD or control diet and then infected with SIV. HFD animals had loss of mucosal $CD4^+$ T cells even before SIV infection. Following SIV infection, HFD animals had significantly higher levels of LPS, CD4 activation, and liver fibrosis than those on control diet. There was a trend toward shorter survival in the HFD PTMs and one HFD AGM succumbed to AIDS, an extremely unusual occurrence. HFD animals had changes in several microbial taxa with a notable increase in *Proteobacteria*. Diet may be a critical contributor to persistent microbial translocation and immune activation in people living with HIV. Because diet is modifiable, this may be a highly relevant finding.

Although some diets are clearly healthier than others, it remains uncertain which is the ''healthiest'' diet for adults, but this is not true, however, for infants. Breast milk is the only food shaped by natural selection to improve or facilitate human health. Given the nutritional cost to the mother to produce the milk, all constituents of the milk that are not beneficial to the child should have been selected out by evolution. Human milk oligosaccharides (HMOs) are not digestible by humans, yet comprise the third most abundant class of molecules in human breast milk, suggesting some beneficial role. Dr. Bruce German from UC Davis discussed his work on the interaction of HMOs and the infant gut microbiome. Most bacteria tested were unable to metabolize HMOs, but *Bifidobacterium longum* contains a gene cluster devoted to HMO utilization, resulting in *B. longum* displacing *Enterobacteriaceae* in the breastfed infant gut.[22,23] *Bifidobacteria* appear to have a beneficial effect on the development of T cell responses to infection and enhance response to vaccines.[24] Particular HMOs may also help to protect premature infants from necrotizing enterocolitis.[25] At least in infants, it does appear that one gut microbiome community type is more advantageous than others.

Probiotics are used by millions of people for various indications. Interest has recently developed in probiotics to repair damaged gut epithelium in HIV. Dr. Satya Dandekar from the University of California Davis discussed her work on an *ex vivo* SIV model using intestinal loops. They found rapid (within 5 h) dampening of nuclear factor-κB activation and IL-1b expression following addition of *Lactobacillus plantarum* in the SIV model of AIDS of early viral infection. Both *Bifidobacterium infantis* and *L. plantarum* rapidly restore ZO-1, an epithelial tight junction protein in a model of chronic infection. Significant changes were seen in gut luminal content following addition of *B. infantis* and *L. plantarum*, suggesting a mechanism by which these organisms induce epithelial changes.

Transmission and Prevention

The majority of new HIV infections are transmitted sexually, which means that the initial human-HIV interaction occurs at a mucosal surface.[26] Studies of antiretrovirals for HIV prophylaxis have had dramatically variable results, particularly in women. Dr. Nichole Klatt of the University of Washington discussed her groundbreaking work, which explains much of that variation. She worked with the CAPRISA trial of vaginal tenofovir gel for HIV prophylaxis.[27] She noted that women with a vaginal microbiome dominated by *Lactobacillus* sp. were protected from HIV infection by vaginal tenofovir, whereas women without a *Lactobacillus*-dominant microbiome were not.[28] Cervicovaginal lavage (CVL) specimens were tested for tenofovir concentration and women with a non-*Lactobacillus* dominant microbiome were much more likely to have undetectable tenofovir levels in CVL fluid. Bacteria associated with bacterial vaginosis, such as *Gardnerella*, were found to metabolize tenofovir to adenine *in vitro*. *Lactobacillus* did not metabolize tenofovir. This effect was true both for tenofovir disoproxyl fumarate and tenofovir alefenamide, and for several other drugs with potential for use in HIV prophylaxis, including emtricitabine and dapivirine. Incubation with gut bacteria also resulted in the metabolism of tenofovir, suggesting that the gut microbiome may impact the efficacy of oral preexposure prophylaxis or HIV therapy as well. *In vitro* experiments with gut bacteria are ongoing. Mucosal microbes appear to play a significant role in the metabolism of antiretrovirals and this effect should be considered when assessing their efficacy.

Mucosal microbes also appear to modify the risk of sexual HIV acquisition in the absence of antiretrovirals. In the same CAPRISA trial mentioned above, Masson *et al.* demonstrated that women with elevated levels of inflammation in CVL fluid, as measured by target cell attracting chemokines, were at higher risk of HIV acquisition.[29] Dr. Kwon from the Ragon Institute discussed his work with the FRESH study in South African women.[30] His group divided women into four cervicotypes (CTs) based on their vaginal microbial community type. CT1 (10% of participants) was dominated by *Lactobacillus crispatus*, CT2 (32%) was dominated by *Lactobacillus iners*, and the remaining 58% fell into CT3 and CT4, which were highly diverse and had low *Lactobacillus* prevalence. None of the women in CT1 acquired HIV infection over the course of the study, although 31 participants from the other three groups developed HIV. There was a 17-fold increase in target cells ($CCR\text{-}5^+$ $CD\text{-}38^+$ $HLA\text{-}DR^+$ $CD4^+$) in genital tract specimens from CT4 women compared to CT1 women, suggesting that *L. crispatus* protected against inflammation and thus HIV acquisition. *Lactobacillus*-dominant vaginal communities are much more common in women of European ancestry in the U.S. compared to African American women or South African women, and this discrepancy remains unexplained.[31–33]

Dr. Wessels and her group from McMaster University sought to determine if differences in sexual behavior could be

contributing to the variation in vaginal microbiota noted between different demographic groups.[34] They studied women from the same ethnic group in Nairobi, Kenya, and compared sex workers to nonsex workers. Sex workers were noted to have increased diversity and decreased *Lactobacillus* compared to nonsex workers. Dr. Wessels suggested that these microbiome changes may contribute additional risk of HIV infection to a population already at high risk.

One of the mechanisms by which *Lactobacillus* may blunt inflammatory responses is through production of the lactic acid for which it is named. Professor Gilda Tachedijan of the Burnet Institute discussed her work on the protective effects of *Lactobacillus* and lactic acid. Lactic acid appears to be virucidal to HIV and inactivates *Neisseria gonorrhoeae*.[35,36] Professor Tachedijan's laboratory investigated whether lactic acid also has a direct anti-inflammatory effect on female reproductive tract (FRT) mucosa.[37] They found in an FRT epithelial cell culture model that, lactic acid induced production of the anti-inflammatory mediator IL-1Ra. Production of cytokines, which attract HIV target cells, in response to toll-like receptors (TLR) agonists was blunted by lactic acid, suggesting a mechanism by which *Lactobacilli* reduce the risk of HIV acquisition.

It is also possible to directly study the proteins produced by vaginal bacteria. Dr. Adam Burgener of the University of Manitoba discussed proteomic analysis from the abovementioned CAPRISA study. They found that a more diverse proteome was associated with increased HIV acquisition risk. Several microbial protein families were particularly associated with risk, but we know little about the functions of these proteins. Host proteins associated with epithelium were most predictive of HIV acquisition risk, consistent with mucosal inflammation as a risk factor.

Modulation of the vaginal microbiome, in combination with antiretroviral prophylaxis, has the potential to be a potent weapon in the prevention of HIV. However, maintaining a *Lactobacillus*-dominant vaginal microbiome and adequate lactic acid levels remains a difficult task. Recurrence of bacterial vaginosis is high, despite antibiotics aimed at anaerobes, while attempts to replete *Lactobacilli* with probiotics or suppositories have yielded mixed results.[38,39]

Microbiome Effect on Vaccine Responses

The gut microbiota interacts with the host to develop and regulate not only local but also systemic immune responses and homeostasis.[40] Multiple studies using the mouse models have demonstrated that the host immune response to vaccines is dependent on the gut microbiome.[41,42] The effect of gut microbiota on host immune response is apparent in humans as well. The abundance of *Bifidobacterium* has a positive impact on the immune response to oral polio vaccine, and Bacteroidete abundance positively impacts oral rotavirus vaccine-specific immune response.[24,43] Furthermore, a growing body of evidence suggests that responses to immunomodulating drugs like the PD-1 inhibitors may be modulated by manipulation of the gut microbiome.[44]

At the first Virology Education HIV microbiome meeting held at the NIH in Bethesda, Maryland, on April 7th and 8th of 2015, Dr. Wilton Williams presented data from several HIV Vaccine Trials Network (HVTN) studies, which suggested that commensal gut microbes induced non-neutralizing HIV gp-41 antibodies.[45] There has been an increasing interest in understanding the association of gut microbiome composition with quality and magnitude of host immune responses to vaccines. Dr. James Kublin, executive director of the HVTN, discussed findings from the HVTN 096 study, which investigated gut microbiome associations with HIV vaccine responses. The study was performed in Lausanne and utilized rectal secretions for microbiome analysis. It was found that several taxa, including Clostridiales family XI, Peptoniphilus, and Finegoldia, were associated with baseline anti-HIV gp41 antibody response. However, the microbiota composition did not predict vaccine response to other antigens. Different microbial taxa were associated with anti-gp-120 antibody response. There may be a defined composition of gut microbiome that is conducive for the optimal HIV vaccine response, but this has not yet been elucidated.

NHP models

Dr. Daria Hazuda from Merck Research Laboratories presented findings on the age-related effects on the gut microbiota composition and potential impact on vaccine responses. In previously reported human clinical study of aged individuals, prevaccination gene expression signatures consisting of inflammation and B cell signaling in peripheral blood correlated with age-related hyporesponsiveness to hepatitis B virus (HBV) vaccination.[46] Dr. Hazuda presented comparable data in the rhesus macaque model, showing that aged rhesus macaques with different gut microbiota had reduced HBV vaccine responsiveness compared to the young counterparts. Current investigations are evaluating both host and microbiome-based factors that may correlate with this age-dependent vaccine hyporesponsiveness. Dr. Satya Dandekar and her research group at the University of California, Davis, examined gut microbiota and host immunity in the preclinical rhesus macaque model and found that there were striking differences in the immune cell subsets and gut microbiota composition of animals housed in the outdoor environment compared to those in the controlled indoor environment.[47] These differences were driven by the presence of subclinical viral infections, including cytomegalovirus (CMV). Immune response to Flu vaccine was influenced by the presence of CMV. These findings suggest that altered gut microbiota and activated immune cells due to subclinical CMV infection induce immune heterogeneity and influence vaccine responses.

Conclusion

The study of host–microbe interactions remains in its infancy, but dramatic advances have been made regarding the effect of diet and gut microbiota on immune function, identifying translocating bacteria and the protective effects of vaginal *Lactobacilli*. The field appears to be moving past descriptive studies and into more mechanistic and systems-based approaches. Industry interest in microbiome-based therapeutics has increased significantly as well, a development that will hopefully lead to more clinical trials in the short term.

Acknowledgments

We would like to acknowledge virology education for organizing the annual HIV Microbiome Workshop. We would also like to acknowledge the presentors.

Author Disclsosure Statement

No competing financial interests exist.

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Address correspondence to:
Brett Williams
Division of Infectious Diseases
Rush University Medical Center
600 South Paulina #143
Chicago, IL 60612

E-mail: brett_williams@rush.edu