INTERNATIONAL WORKSHOP ON

MICROBIOME & HIV

PATHOGENESIS, PREVENTION, AND TREATMENT 2021

WORKSHOP | 19-21 OCTOBER 2021

Program & Abstract Book

virology education

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Welcome

Dear Delegate,

We are pleased to welcome you to the 7th edition of the **International Workshop on Microbiome and HIV Pathogenesis, Prevention, and Treatment 2021**.

The interplay between human gut microbiota and the immune response is an integral component of many physiological processes, but also pathological conditions. Therefore, understanding the microbiome-immunity crosstalk is central in order to illuminate molecular mechanisms that maintain hemostasis in health, but also to decipher what goes wrong in a disease.

This rapidly evolving research area may offer a comprehensive understanding on the interplay between the HIV infection and the host immunity, and how it contributes to an altered immune status and disease pathogenesis. This ultimately may lead to the identification of novel drug targets with a distinct mode of action compared to currently available therapies. Whereas research in this area may be presented at major conferences, there is often limited time for in-depth discussion and debate among cross-disciplinary experts on the new data and their implications.

This 3-day workshop will have invited lectures by key opinion leaders, oral abstract presentations, and poster presentations. This data will contribute to translating research achievements into opportunities for the development of preventive approaches to reduce virus transmission and to provide a better understanding of the impact of the microbiome on host immunity.

Clinicians and researchers with an interest in Microbiome and HIV Pathogenesis, Prevention, and Treatment are most welcome to attend this workshop.

We look forward to a productive and inspiring program!

The Microbiome 2021 Chairs

Workshop Chairs

Grace Aldrovandi MD, CM

University of California, United States

Ronald Collman MD

University of Pennsylvania, Perelman School of Medicine, United States

Alan Landay PhD

Rush University Medical Center, United States

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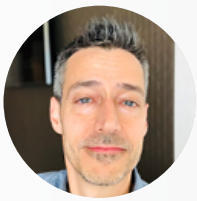

James Cummins
PhD
NIH, NIAID, DAIDS,
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Scientific Committee

Shaun Barnabas, MD	University of Cape Town, South Africa
Stacy Carrington-Lawrence, PhD	NIH/OAR, United States
Que Dang, PhD	NIAID/NIH, United States
Wendy Henderson, PhD, MSN, CRNP, FAAN	NINR, NIH, DHHS, United States
Steve Innes, MBChB, MRCPCH, MPhil, PhD	Stellenbosch University, South Africa
Douglas Kwon, MD, PhD	Ragon Institute of MGH, MIT, Harvard, United States
Angela Malaspina, PhD	NIAID/NIH, United States
John McGowan, PhD	NIAID, United States
Piotr Nowak, MD, PhD	Karolinska University Hospital, Sweden
Sandra Pinto Cardoso, PhD	Centre for Research in Infectious Diseases, National, Institute of Respiratory Diseases, Mexico City, Mexico
Angela Wahl, PhD	University of North Carolina at Chapel Hill, United States
Cara Wilson, MD	University of Colorado, United States

Practical Information

Abstracts
Accepted abstracts are published in this Program & Abstract Book. The e-book can be downloaded from the official Microbiome & HIV 2021 website.

Microbiome 2021 Virtual Platform
All registered participants are provided with personal credentials consisting of a login account email and a PIN code to access the workshop portal during the event dates and 3 weeks following the closure of the event. If you do not receive the information please contact the Conference Secretariat at eva.vamvounaki@amededu.com

Meeting Secretariat
The workshop organizers can be contacted for all questions concerning the logistics of the event. You can submit technical inquiries via the Live Support icon in the upper-right of your scree on the vitual platform (see below) or contact us at eva.vamvounaki@amededu.com and/ or at info@amededu.com for program-related questions during the event hours.

Presenters & Session Chairs
Presenters and session chairs will receive a separate set of login details for the session(s) they are involved in. Please log on at least 15 minutes before the session time.

Certificate of attendance
A Certificate of attendance will be sent by e-mail once you complete the post-event survey.

Post-Event Survey
Your feedback is very valuable to us and enables us to further improve this meeting. An electronic session survey form will be available on your screen at the end of each session. A post-event survey will be sent by email at the end of the conference. A Certificate of Attendance will be sent by e-mail once you complete the post-event survey.

Posters
Posters are accessible in the Poster Gallery on the workshop platform. Participants can connect via Meeting Hub with posters authors to ask questions, send messages or schedule a call. Posters can be saved and downloaded from the Handouts widget, on the right side of the Poster.

Presentations and webcasts
Recordings of all sessions and e-posters, will remain accessible to all registrants on the Microbiome & HIV 2021 virtual platform until **12 November**. Materials provided with the author's permission will be moved to AcademicMedicalEducation.com after **12 November**.

Speakers

Catherine Lozupone
PhD
University of Colorado,
United States
View Bio >

Catriona Bradshaw
MD, FAChSHM, PhD
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Alfred Hospital, Central Clinical
School, Monash University, Australia
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Jo-Ann Passmore
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Leopoldo Segal
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Tuesday, 19 October 2021

Eastern Daylight Time (EDT)

11:00 AM	**Opening of the Workshop** *Chair: Grace Aldrovandi*
11:05 AM	**Introduction to the Microbiome for HIV Clinicians and Researchers** **Catherine Lozupone, PhD** University of Colorado, United States
11:25 AM	**Discussion - Live Q&A**
	Session 1: Prevention *Co-Chairs: Gilda Tachedjian & Nichole Klatt*
11:30 AM	**Evidence for Sexual Transmission of Bacterial Vaginosis** **Catriona Bradshaw, MD, FAChSHM, PhD** Melbourne Sexual Health Centre, Alfred Hospital and Central Clinical School, Monash University, Australia
11:45 AM	**Geo-adapted Live Biotherapeutic Strategies for HIV Prevention** **Jo-Ann Passmore, PhD** University of Cape Town, South Africa
	Abstract - Driven presentations:
12:00 PM	**Immunomodulatory Properties of Cervicovaginal Lactobacillus Isolates Are Associated with Lactic Acid Production and Bacterial Proteome Profiles (#1)** **Monalisa Manhanzva** South Africa
12:07 PM	**Transkingdom Connections in the Female Reproductive Tract in Health and Bacterial Vaginosis (#2)** **Ferralita Madere** United States
12:15 PM	**Live Q&A**
12:25 PM	Break
	Session 2: Transmission *Co-Chairs: Catherine Lozupone & Laurel Lagenaur*
12:40 PM	**Vaginal Lactobacillus-Derived Extracellular Vesicles in Protecting from HIV-1 Transmission** **Rogers Alberto Nahui-Palomino, PhD** NIH / NICHD, United States
12:55 PM	**Penile Anaerobic Bacteria as a Risk Factor for HIV Infection** **Cindy Liu, MD, MPH, PhD** George Washington University, United States
	Abstract - Driven presentations:
01:10 PM	**HIV Replication, Transmission, and the Metabolome of the Female Reproductive Tract (#4)** **Kaitlin Marquis** United States
01:20 PM	**Live Q&A**
01:30 PM	Break

Tuesday, 19 October 2021

	Session 3: Vaccines *Co-Chairs: Satya Dandekar & James Cummins*
01:45 PM	**Gut Microbiota During Early Life Modulates Vaccine Immunogenicity in Infants Exposed to HIV** **Heather Jaspan, MD, PhD** Seattle Children's Research Institute, United States University of Cape Town, South Africa
02:00 PM	**Exploring the Role of the Microbiome on HIV Infection in Humanized Mice** **Angela Wahl, PhD** University of North Carolina at Chapel Hill, United States
	Abstract - Driven presentations:
02:15 PM	**Rational Donor Fecal Microbiota Transplantation in HIV (Refresh Study): Preliminary Results of Shotgun Sequencing Analysis (#5)** **Alba Talavera** Spain
02:20 PM	**Gut Microbiome and Immune Phenotype Response to Art/Co-trimoxazole Treatment Differs among Plwh of Rural versus Urban Zimbabwe: A Multicenter Longitudinal Interventional Study (#6)** **Alessandro Lazzaro** Italy
02:30 PM	**Live Q&A**
02:40 PM	End Of Day 1

Wednesday, 20 October 2021

Eastern Daylight Time (EDT)

11:00 AM	**Opening of the Workshop** *Chair: Ronald Collman*
11:05 AM	**Microbiome and the Aging Brain: Moving Toward Mechanism** **John F. Cryan, B.Sc. (Hons), PhD** University College Cork, Ireland
11:25 AM	**Live Q&A**
	Session 4: Treatment *Co-Chairs: Jason Branchley & Grace Aldrovandi*
11:30 AM	**Metabolome-Microbiome Crosstalk Towards Natural Immune Control in HIV** **Ujjwal Neogi, MSc, PhD** Karolinska Institute, Sweden
	Abstract - Driven presentations:
11:45 AM	**Isolation and Proteomic Profiling of Translocating Bacteria in Progressive SIV Infection of Rhesus Macaques (#7)** **Jacob Flynn** United States
11:52 AM	**Butyrate Administration Does Not Improve Immune Reconstitution in Antiretroviral-Treated SIV-Infected Macaques (#8)** **Alexandra Ortiz** United States
12:00 PM	**Live Q&A**
12:10 PM	Break
	Session 5: Comorbidities *Co-Chairs: Ronald Collman & Rick Bushman*
12:25 PM	**Microbiome, Diabetes, and HIV Inflammation** **Jean-Pierre Routy, MD, FRCPC** McGill University Health Centre, Canada
12:40 PM	**HIV, HPV and the Microbiome: Partners in Crime?** **Sergio Serrano-Villar, MD, PhD** University Hospital Ramón y Cajal, Spain
	Abstract - Driven presentations:
12:55 PM	**Age-Associated Gut Dysbiosis, Marked by Loss of Butyrogenic Potential, Correlates with Altered Plasma Neuroactive Tryptophan Metabolites in Older People Living with HIV (#9)** **Smitha Ghare** United States
01:00 PM	**Interactions among Mycobiome, Bacteriome, Inflammation and Diet in HIV Infection (#10)** **Maria Jose Gosalbes** Spain
01:15 PM	**Live Q&A**
01:30 PM	Break

Wednesday, 20 October 2021

	Guided Posters Tour *Moderator: Ronald Collman*
01:45 PM	**HIV-1 infection and Gut Microbiome (#1)** **Jyotsna Jaiswal** India
01:48 PM	**Longitudinal Analysis of the Lung Microbiome in an Immune-Compromised Patient Population (#2)** **Samantha Whiteside** United States
01:51 PM	**Peptidoglycans from Enteric Bacteria Differentially Induce Epithelial Cell Activation (#3)** **Charles Neff** United States
01:54 PM	**Factors Related to Composition of a More Western versus Agrarian Diet in HIV-Infected Individuals in Zimbabwe (#4)** **Nichole Nusbacher** United States
02:00 PM	**Live Q&A**
02:10 PM	End Of Day 1

Thursday, 21 October 2021

Eastern Daylight Time (EDT)

11:00 AM	**Opening of the Workshop** *Chair: Alan Landay*
11:05 AM	**The Gut Microbiome in Health and Disease States: Challenges and Opportunities for Developing Microbiome-based Diagnostic and Interventions in the Era of Precision Medicine** **Eugene B. Chang, MD** University of Chicago, United States
11:25 AM	**Live Q&A**
	Session 6: Pathogenesis *Co-Chairs: Ronald Collman & Roger Paredes*
11:30 AM	**High Resolution Metagenomic Analysis of the Human Microbiome** **Nicola Segata, PhD** University of Trento, Italy
11:45 AM	**Microbiome and HIV: A Clinical Perspective** **Irini Sereti, MD** NIAID/NIH, United States
	Abstract - Driven presentations:
12:00 PM	**Lung Microbiome in Highly Immunosuppressed Lung Transplant Recipients Drives Inflammation and Primary Graft Dysfunction (#11)** **John McGinnes** United States
12:07 PM	**Gut Microbiome Signatures Linked to HIV-1 Reservoir Size and Viremia Control (#12)** **Alessandra Borgognone** Spain
12:15 PM	**Live Q&A**
12:25 PM	Break
	Session 7: COVID-19 and Microbiome *Co-Chairs: Aland Landay & Sergio Serrano-Villar*
12:40 PM	**Lower Airway Microbiome in Critically Ill COVID-19 Patients** **Leopoldo Segal, MD** NYU Grossman School of Medicine, United States
12:55 PM	**Signatures of COVID-19 Severity and Immune Response in the Respiratory Tract Microbiome** **Ronald Collman, MD** University of Pennsylvania, Perelman School of Medicine, United States
	Abstract - Driven presentations:
01:10 PM	**SARS-CoV-2 Infection Associated with Microbial Translocation Across Intestinal Mucosa (#13)** **Kelsie Brooks** United States
01:17 PM	**Inflammation and Pulmonary Dysfunction Associated with Elevated Sars-Cov-2-Specific T Cells in Post-acute Sequelae COVID-19 (#14)** **Katherine Littlefield** United States
01:25 PM	**Live Q&A**
01:35 PM	Closing Remarks & End of the Workshop

Abstract Book

Oral Abstract Presentations

O_1

Properties of cervicovaginal Lactobacillus isolates are associated with lactic acid production and bacterial proteome profiles Immunomodulatory

Manhanzva M[1], *Alisoltanidehkordi A[1], Abrahams A[1], Bell L[2], du Plessis M[2], Calder B[1], Gamieldien H[1], Froissart R[3], Jaspan H[1,4], Jaumdally S[1], Barnabas S[1], Dabee S[1], Blackburn J[1], Bekker L[5], Gray G[6,7], Passmore J[1,8], Masson L[1,91]Institute of Infectious Disease and Molecular Medicine (IDM), University of Cape Town, Cape Town, South Africa, [2]Centre for Proteomic and Genomic Research, Cape Town, South Africa, [3]UMR 5290 MIVEGEC, French National Centre for Scientific Research (CNRS), Montpellier, France, [4]Seattle Children's Research Institute, University of Washington, Seattle, USA, [5]Desmond Tutu HIV Centre, University of Cape Town, Cape Town, South Africa, [6]Perinatal HIV Research Unit, University of the Witwatersrand, Johannesburg, South Africa, [7]South African Medical Research Council, Cape Town, South Africa, [8]National Health Laboratory Service (NHLS), Cape Town, South Africa, [9]Disease Elimination Program, Life Sciences Discipline, Burnet Institute, Melbourne, Australia*

Background: Bacterial vaginosis (BV) and non-optimal microbiota are associated with female genital tract (FGT) inflammation which increases HIV acquisition risk. Lactobacilli are thought to protect against pathogens by modulating immune responses in the FGT. We aimed to characterize the immunomodulatory properties of vaginal Lactobacillus isolates and determine the mechanisms underlying these relationships.

Methods: We isolated 64 non-iners vaginal Lactobacillus species from South African women. Growth rates, adhesion to vaginal epithelial (VK2) cells, culture acidification and D/L-lactate production were evaluated. The production of cytokines (IL-6, IL-1α, IL-1β IL-8, IP-10, MIP-3α, MIP-1α, MIP-1β and IL-1RA) by VK2 cells in response to lactobacilli was measured using Luminex. Proteome profiles of 22 Lactobacillus isolates that induced higher inflammatory cytokine production and 22 isolates that induced low levels of cytokine production were analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS) to investigate underlying mechanisms leading to different inflammatory profiles.

Results: Lactobacilli isolated from women with non-optimal microbiota produced less lactic acid and induced greater inflammatory cytokine production by VK2 cells than those from women with optimal microbiota. A total of 5087 Lactobacillus proteins were identified by LC-MS/MS, with 164 proteins differentially abundant between the non-inflammatory and relatively inflammatory isolates. The majority of the protein molecular function gene ontologies that were underabundant in inflammatory isolates were enzymatic pathways, suggesting that more inflammatory isolates had less metabolic activity. D-lactate production by the isolates correlated positively with D-lactate dehydrogenase relative abundance and D-lactate dehydrogenase was inversely associated with IL-6, IL-8, IP-10 and MIP-1α production by VK2 cells in response to the isolates (adjusted p=0.001, 0.001, 0.029 and 0.014, respectively).

Conclusion: Lactobacillus isolates from women with optimal versus non-optimal microbiota differed functionally, producing more lactic acid and inducing lower cytokine responses. The immunomodulatory properties of lactobacilli are likely multifactorial and may be associated with metabolic activity and lactic acid production.

Oral Abstract Presentations

O_2

Transkingdom Connections in the Female Reproductive Tract in Health and Bacterial Vaginosis

***Madere F[1]**, Sohn M[2], Winbush A[3], Barr B[10], Grier A[5], Java J[5], Meiring T[6], Williamson A[6,7], Bekker L[8], Adler D[9], Monaco C[1,41]Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester , United States, [2]Department of Biostatistics and Computational Biology, University of Rochester Medical Center, Rochester, United States, [3]University of Rochester School of Medicine & Dentistry, Rochester, United States, [4]Department of Internal Medicine, Division of Infectious Diseases, University of Rochester Medical Center, Rochester, United States, [5]UR Genomics Research Center, University of Rochester Medical Center, Rochester, United States, [6]Institute of Infectious Diseases & Molecular Medicine and Division of Medical Virology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa, [7]National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa, [8]Desmond Tutu HIV Centre, Institute of Infectious Diseases & Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa, [9]Department of Emergency Medicine, University of Rochester Medical Center, Rochester, United States, [10]Department of Rural Family Medicine, West Virginia University , Morgantown, United States*

Background: The female reproductive tract (FRT) microbiome plays a vital role in maintaining vaginal health. Low diversity, Lactobacillus-dominant bacterial communities, known as bacteriomes, help to prevent urogenital diseases like bacterial vaginosis (BV) and sexually transmitted infections (STIs) such as HIV. Conversely, diverse FRT bacteriomes have been linked to increased inflammation and STI transmission. Viruses play a key role in regulating other microbial ecosystems, yet little is known about how FRT viruses (virome), particularly bacteriophages, influence FRT health and dysbiosis. An improved understanding of transkingdom interactions between bacteria and bacteriophage in the FRT is crucial to discerning and treating diseases in this central environment. We hypothesize that urogenital diseases such as BV are associated with alterations in the FRT virome, and these changes significantly correlate with shifts in the FRT bacteriome. Materials and Methods: We conducted a retrospective, longitudinal analysis of vaginal swabs collected twice yearly between October 2012 and October 2014 from 50 HIV-positive and 50 HIV-negative, sexually active young women ages 16-21 in Cape Town, South Africa. 16S rRNA gene amplicon sequencing was performed with V3-V4 region-specific and bacteriome characterization and diversity analysis was conducted using QIIME 2. A subset of 38 baseline samples, 14 of which were BV-negative and 24 were BV-positive, were used to characterize the FRT DNA virome through virus-like particles (VLPs) enrichment and Illumina NovaSeq sequencing resulting in an average of 29 million reads per sample. Resulting viral sequences were assigned to known viral taxa using VirusSeeker, a BLAST-based virome analysis pipeline.

Results: Bacteriome analysis identified five major bacterial community groups distinguished by Lactobacillus-dominant or higher diversity microbiomes, with increased bacterial alpha diversity significantly associating with clinical BV diagnosis. Investigation of the FRT virome on a baseline subset of samples revealed that FRT bacteriophages clustered into novel viral state types (VSTs), a viral community clustering system based on virome composition and abundance that are linked to bacteriome composition. Distinct bacteriophage signatures, including increased alpha diversity along with Bacillus, Burkholderia and Escherichia bacteriophages were identified in BV. Discriminate transkingdom associations between bacteria and bacteriophage were further identified between Bacillus and Burkholderia viruses and BV-associated bacteria such as Gardnerella, Sneathia, A. vaginae and Prevotella providing key insight for future mechanistic studies elucidating transkingdom interactions inducing BV-associated microbiome perturbations.

Conclusions: Herein, we are the first to describe bacteriophage communities within the FRT and their association with bacterial composition and clinical BV in this South African cohort. Our

Oral Abstract Presentations

findings provide insight into putative interactions between bacteriophage and bacteria that may contribute to development and maintenance of FRT dysbiosis.

Oral Abstract Presentations

O_3

Abstract number 3 has been withdrawn.

Oral Abstract Presentations

O_4

HIV Replication, Transmission, and the Metabolome of the Female Reproductive Tract

Marquis K[1], *Hwang Y[1], Petucci C[2], Schultz D[1,3], Cherry S[1,3], Bushman F[11]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States, [2]Metabolomics Core, Cardiovascular Institute, Smilow Translational Research Center, University of Pennsylvania, Philadelphia, United States, [3]High Throughput Screening Core, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States*

Background: HIV disproportionately affects young women, yet we have a poor understanding of the factors contributing to a successful transmission event in the female genital tract. Colonization of the vaginal tract with highly diverse, Lactobacillus-deficient microbial communities (HDCs) increase a woman's risk of acquiring HIV-1 compared to colonization with Lactobacillus-dominant communities (LDCs). The inherent polymicrobial nature of these communities has made it exceedingly challenging to elucidate the microbial mechanisms responsible for modulating HIV transmission. Despite this phenomenon, several studies have identified conserved changes in the small molecule metabolome of HDC and LDC lavage fluid. Since bacterial production of metabolites can profoundly reprogram cell physiology, we interrogated whether any of these metabolites can modulate susceptibility to HIV-1.

Material and Methods: We developed a TZM-bl cell-based reporter assay to screen a library containing over 500 microbial and host derived metabolites. TZM-bl cells were incubated with metabolites for 24 hours prior to infection with the dual-tropic HIV-1 89.6. Forty-eight hours post infection, a luciferase assay was conducted to quantify HIV replication. Screening was conducted in duplicate and the results were interpreted in light of four published metabolomics studies comparing the metabolome of HDC vs LDC lavage fluid. The effect of metabolites on HIV-1 replication was validated using a combination of p24 ELISA assays in antiCD3 activated donor CD4+ T cells and/or flow cytometry staining for HIV-1 core antigen in unstimulated donor CD4+ T cells. Flow cytometry staining for CCR5+CD38+HLA-DR+ triple positive cells was conducted to measure the metabolite's effect on T cell activation status in HIV target cells. Lastly, we interrogated the genomes of representative HIV-associated HDC and LDC stains for differences in microbial metabolic machinery and used liquid chromatography-mass spectrometry on bacterial culture supernatants to quantify metabolites secreted by individual bacterial strains.

Results: Comparison of our screening results to the cervicovaginal metabolome of HDC and LDC colonized women yielded several metabolites of interest that might play roles in the interaction between the vaginal microbiome and HIV-1 infection. We have particularly focused on 2-hydroxyisovalerate (2-HV), a branched chain amino acid catabolite, that is enriched in HDC lavage fluid and boosted viral replication 3-6x in primary CD4+ T cells. Preliminary data suggests that this metabolite acts at an early stage of the viral life cycle without significantly influencing T cell activation status. Additionally, we have confirmed that at least one HIV-associated HDC strain, Veillonella montpellierensis, can secrete 2-HV and are currently characterizing the ability of other HDC strains to produce this metabolite.

Conclusions: We have identified a number of conserved changes in the small molecule metabolome of HDC and LDC colonized

Oral Abstract Presentations

women, mainly in amino acid catabolism, that could potentially influence HIV replication in the vaginal tract. These changes may be driven in part by differences in HDC and LDC microbial metabolic machinery. Ultimately, we hope this study will guide future efforts to modulate the environment of the female genital tract to diminish HIV transmission.

Oral Abstract Presentations

O_5

Rational Donor Fecal Microbiota Transplantation in HIV (Refresh Study): Preliminary Results of Shotgun Sequencing Analysis

Talavera T[1], Gosalbes M[2], Madrid N[1], Gutiérrez C[1], Fernández V[1], Dronda F[1], Pérez-Molina J[1], Gutiérrez C[1], Moreno E[1], Jiménez D[1], Martínez-Sanz J[1], Ron R[1], Vivancos M[1], Moreno S[1], Serrano-Villar S[1] [1]Hospital Universitario Ramon Y Cajal, Madrid, Spain, [2]Area of Genomics and Health, FISABIO-Salud Pública, , Spain

Background: An altered interplay between mucosal immunology and the microbiota contributes to chronic immune dysfunction during treated HIV infection. It is unknown whether oral fecal microbiota transplants (FMT) can affect the gut microbiota and systemic immunity of HIV-infected individuals.

Methods: Thirty ART-treated HIV-infected subjects with a CD4/CD8 ratio <1 were allocated to receive either weekly oral fecal microbiota capsules or placebo for 8 weeks (10 capsules at week 0; 5 capsules/week from weeks 1-7). Three stool donors were selected from a universal donor stool bank based on bacterial abundance of Fecalibacterium and Bacteroides (high) and Prevotella (low) together with high fecal butyrate concentrations. We previously published the Illumina 16S rRNA sequencing analysis. We now report the preliminary shotgun sequencing results to explore the species level.

Results: Twenty-nine participants, with a mean CD4 count of 641±286 cells/µL and CD4/CD8 ratio of 0.63±0.26 completed the 48-week follow-up. FMT was well tolerated, with no grade 3-4 related adverse events. The donors' microbiota was dominated by species belonging to the Bacteroides, Faecalibacterium, Intestinimonas, Lachnospiraceae, Roseburia, and Clostridiales genus, with a marked butyrogenic profile. Within the FMT group we found numerous species insertions absent at baseline in patients but present in donors. The microbiota composition showed greater variability in the FMT group than with placebo over time. FMT was associated with an increase in Shannon diversity in the FMT group (p=0.021) than in the placebo group (p=0.109). This effect was attenuated in the 4 subjects in the FMT group that had received antibiotics weeks before the first FMT. Beta-diversity analysis using Bray-Curtis dissimilarity trajectories indicated mild engraftment of donors' microbiota in the FMT arm, which appeared to be donor-dependent and enhanced by previous antibiotics. LEfSe results show that the number of species inserted over time in the FMT group was significantly higher than in the placebo group. After identifying the species most likely to explain the longitudinal differences between the two FMT and placebo groups, we found 37 differentially abundant species at the follow-up visit in the placebo arm, and 99 species at week 7 and 170 at week 8 in the FMT arm. The species Lachnospiraceae bacterium, Anaerostipes hadrus, Methylobacterium gnaphalii, Christensenella minuta, Mageeibacillus indolicus, Clostridium hylemonae, Eubacterium cellulosolves were the most robustly engrafted over time. Participants in the FMT arm experienced a significant decline in intestinal fatty acid-binding protein (IFABP).

Conclusions: Repeated oral capsular FMT from rationally selected donors was safe in HIV-infected subjects on ART and introduced incremental compositional changes in the fecal microbiota at the species level. Our results indicate that manipulating the gut microbiota using a non-invasive and safe strategy of FMT delivery is feasible. We plan to address changes in fungal communities, impacted pathways, and engraftments at the strain level.

Oral Abstract Presentations

O_6

Gut Microbiome and Immune Phenotype Response to Art/Co-trimoxazole Treatment Differs among Plwh of Rural versus Urban Zimbabwe: A Multicenter Longitudinal Interventional Study

Lazzaro A[1], *Fiorillo S[2], Nusbacher N[3], Neff C[4], Schneider J[3], Martin C[3], McDonald B[4], Darcy J[3], Borok M[5], Campbell T[2], Palmer B[4], Lozupone C[3] [1]Department Of Public Health And Infectious Diseases, Sapienza University Of Rome, Policlinico Umberto I of Rome, Roma, Italy, [2]Division of Infectious Diseases, Department of Medicine, University of Colorado Anschutz, Aurora, Colorado, United States of America, , , [3]Division of Biomedical Informatics and Personalized Medicine, , Department of Medicine, University of Colorado Anschutz, Aurora, Colorado, United States of America, , , [4]Division of Allergy and Clinical Immunology, Department of Medicine, University of Colorado Anschutz, Aurora, Colorado, United States of America, , , [5]Department of Medicine, University of Zimbabwe, Harare, Zimbawe, ,*

Background: People living with HIV-1 (PLWH) have gut microbiome characteristics that differ from healthy controls despite successful treatment with antiretroviral therapy (ART). However, studies conducted to date have largely focused on PLWH in Western Countries, and not highly impacted areas in sub-Saharan Africa (SSA). Also, little attention has been given in the developing world to whether HIV pathogenesis, response to treatment, and gut microbiome associations may differ in urban and rural areas, even though differences such as diet, environmental exposures, and socio-economic status may influence these parameters.

Methods: We conducted a multicenter longitudinal interventional study in Zimbabwe, recruiting in an urban and a rural area. Blood and stool samples were collected from PLWH enrolled in three cohorts: A) treatment naïve PLWH (TN-PLWH) (n=68); B) treated for >12 months PLWH (TE-PLWH) (n=39); C) healthy controls (HC) (n=41). All three cohorts were sampled at 2 timepoints 6 months apart, with the TN-PLWH being sampled before and after treatment. Treatment consisted of co-trimoxazole and ART composed by efavirenz/lamivudine/tenofovir disoproxil fumarate. We assessed fecal microbiome composition (through Illumina sequencing of the V4 region of 16S rRNA gene) and systemic immunity (through flow cytometry of blood samples), focusing on immune activation (HLA-DR+CD38+ T lymphocytes) and immune exhaustion (PD1+ T lymphocytes). Statistical tests were performed using Kruskal-Wallis, paired Wilcoxon signed-rank tests for immune data, and ANCOM-BC for microbiome composition analysis. All results reported below had p-values less than 0.05 with FDR corrections applied where appropriate.

Results: PLWH with untreated and treated infection had median CD4+ T cells count and CD4+/CD8+ ratio below healthy ranges, with TN-PLWH showing the lowest levels. PLWH also had higher levels of immune activation and immune exhaustion compared to HC, with TN-PLWH showing the highest levels. Individuals living in the urban but not the rural area had significant improvements in levels of T cell activation and exhaustion following 6 months of treatment, even though urban and rural cohorts had comparable levels of viral suppression. ART/co-trimoxozole treatment and not HIV-1 infection itself nor CD4+ T cell count was associated with intestinal microbiome disturbances. Indeed, treatment had an overall negative impact on the gut microbiome, associating with lower diversity (Shannon, Faith PD and Simpsons) and higher gut microbiome dysbiosis (assessed as average UniFrac distance from HC). The intestinal microbiome of people living in rural areas showed higher alpha diversity and lower dysbiosis with long term treatment. However, longitudinally assessed

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microbiome disturbances with treatment were evident in rural and not urban Zimbabwe and overall were influenced by levels of baseline dysbiosis. Cross-sectional compositional analysis allowed us to identify unique taxa found significantly more prevalent in specific cohorts and areas.

Conclusions: The intestinal microbiome of individuals living in rural areas had higher diversity than people from urban areas at baseline; yet, was more prone to disturbance due to short term treatment with ART/co-trimoxazole. Further work is needed to explore whether this may explain the higher improvement with treatment in immune phenotype observed solely among individuals from the urban area.

Oral Abstract Presentations

O_7

Isolation and Proteomic Profiling of Translocating Bacteria in Progressive SIV Infection of Rhesus Macaques

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Introduction: Microbial translocation is a significant contributor to chronic inflammation in HIV-infected humans and has been associated with increased mortality and morbidity in individuals treated for long periods of time with antiretroviral medicines. Thus, there is interest in understanding the mechanisms underlying microbial translocation. Translocating bacterial taxa are not representative of the gut microbiota, with Proteobacteria appearing to preferentially translocate. To fully characterize translocating bacterial populations, we isolated translocating bacteria from chronically SIV-infected macaques and characterized their proteome.

Materials & Methods: Liver, mesenteric lymph node, and spleen samples were taken during necropsy from one uninfected and twenty chronically SIV- or SHIV-infected RM, including treated with Vancomycin. Tissue samples were homogenized and plated on: a) Brain Heart Infusion, b) TSA+Tween 80, and c) TSA+5% Sheep's Blood media under aerobic conditions, and d) Brucella Blood and e) CDC Blood media under anaerobic conditions. Isolates were grown for 1-7 days, colonies re-streaked for purity, and identified using MALDI-TOF and/or 16S rDNA sequencing. Eight translocating species and five non-translocating species, isolated from stool, were analyzed via mass spectrometry.

Results: Thirty-six species were identified, 5 Proteobacteria (Enterobacteriaceae), 4 Actinobacteria (50% Actinomycetaceae, 25% Corynebacteriaceae, 25% Coriobacteriaceae), 2 Bacteroidetes (50% Odoribacteraceae, 50% Prevotellaceae) and 25 Firmicutes (32% Lactobacillaceae, 16% Streptococcaceae, 12% Enterococcaceae, 8% Aerococcaceae, 8% Eubacteriaceae, 8% Leuconostocaceae, 4% Bacillaceae, 4% Planococcaceae, 4% Staphylococcaceae, 4% Veillonellaceae). Unique proteome signatures were identified in translocating bacteria, with 47.21% of proteins identified in translocating bacteria being unique. Top hits included cytosine-specific methyltransferases and copper homeostasis protein CutC, which were found in five of the eight translocating bacterial species.

Conclusions: Unique taxa of translocating bacteria are frequently present in tissues and commonly express DNA methylation enzymes. Blocking activity of these enzymes may offer unique treatment modalities to reduce microbial translocation and improve the prognosis of HIV-infected individuals.

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O_8

Butyrate administration does not improve immune reconstitution in antiretroviral-treated SIV-infected macaques.

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Background: Defective gastrointestinal barrier function and in turn, microbial translocation have been identified as significant contributors to persistent inflammation in antiretroviral-treated people living with HIV. Although it remains unclear whether bacterial dysbiosis is a contributing factor to incomplete immune reconstitution and inflammation in treated PLWH, metabolic supplementation of short-chained fatty acids otherwise produced by the commensal microbiome may improve these outcomes.

Methods: Herein we assessed whether supplementation with the dietary supplement sodium butyrate would improve immune reconstitution and inflammation in antiretroviral treated, SIV-infected rhesus macaques. Naturally produced by the commensal microbiome, butyrate is essential for the development and maintenance of intestinal immunity, with butyrate having a known role in supporting epithelial integrity, Treg development, T-cell memory function, and antagonism of many pathobionts.

Results: We demonstrate the butyrate supplementation does not significantly improve immune reconstitution, with no differences observed in systemic CD4+ T-cell frequencies, T-cell functionality or immune activation, intestinal integrity, or microbial translocation. Conclusions: Our findings demonstrate that sodium butyrate is not sufficient to reduce persistent inflammation and microbial translocation in treated, SIV-infected macaques, suggesting that this therapeutic may not reduce co-morbidities and co-mortalities in antiretroviral-treated people living with HIV.

Oral Abstract Presentations

O_9

Age-associated gut dysbiosis, marked by loss of butyrogenic potential, correlates with altered plasma neuroactive tryptophan metabolites in older people living with HIV

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Background: Imbalance in tryptophan metabolism and its neuroactive metabolites, serotonin, and kynurenine is a known pathogenic mechanism underlying neurocognitive impairment. Gut microbiota plays an important role in tryptophan metabolism and the production of these neuroactive molecules affects neurocognitive function. Although, both HIV infection and normal aging independently induce gut dysbiosis and influence tryptophan metabolism, their interactive effects on compositional/functional changes in gut microbiota and consequent alterations in tryptophan metabolites remain largely undetermined.

Methods: Older people living with HIV infection (PLWH, 50-70 years of age, n=22) were enrolled in this cross-sectional pilot study. Metagenomic analysis of fecal microbiome employing 16S rRNA gene sequencing and metabolomics analysis of plasma employing mass spectrometry with a reverse-phase LC-MS/MS was performed. Statistical analyses included the univariate linear regression and Spearman correlation analysis.

Results: Age-associated changes in plasma levels of key neuroactive tryptophan metabolites serotonin and kynurenine were seen in PLWH. Specifically, we observed age-dependent decreases in serotonin and increases in kynurenine and kynurenine-to-tryptophan ratio (KYN/TRP), indicative of dysfunctional tryptophan metabolism. Furthermore, the gut dysbiosis seen in older PLWH is characterized by a reduction of Firmicutes/Bacteroidetes (F/B) ratio and butyrate-producing microbial families Lachnospiraceae and Lactobacillaceae. Importantly, correspondent with gut dysbiosis, increasing age was significantly associated with decreased plasma butyrate levels, which in turn correlated positively with serotonin and negatively with KYN/TRP ratio.

Conclusions: Age-dependent gut microbial dysbiosis distinguished by a decrease in butyrogenic potential is a key pathogenic feature associated with the shift in tryptophan metabolism from serotonin to kynurenine in older PLWH.

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O_10

Interactions among Mycobiome, Bacteriome, Inflammation and Diet in HIV Infection

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Background: Few studies have addressed the characterization of the mycobiome. We focused on the gut fungal community in patients with HIV on ART. We sought to assess the inter-kingdom interactions with the bacteriome, the impact of diet, and the correlations with chronic immune activation.

Material and Methods: Fungal DNA extraction, ITS2 amplification (ITS3-F and ITS4-R primers) and MiSeq sequencing from fecal samples of 24 ART-treated HIV-infected subjects (HIV+ART+) and 12 healthy controls (HIV-) were performed. The sequence analysis was performed with the ITS version of the DADA2 workflow. The taxonomic information of the ASVs was obtained by BLAST comparison against the UNITE ITS database (v8.0). The relative abundance values were smoothed and arcsine square root-transformed to better approximate normality. We used mixOmics package in R to select fungal discriminant ASVs (sPLS-DA) and to compute pairwise associations between compositional data, clinical or diet features (sPLS), adjusting for multiple comparison using the Benjamini–Hochberg correction. We used Adonis test to assess the effect of external factors on microbial composition.

Results: At the alpha diversity level, Chao1 was significantly higher in the HIV+ART+ than in the HIV- group (p=0.029). The bacteriome was richer and more diverse than fungal community in both HIV+ART+ and HIV- participants. Principal coordinate analysis showed the two fungal communities, HIV+ and HIV-, clustered separately at ASV level on the base of Bray-Curtis dissimilarity index and the Adonis test (pvalue= 0.0017). The sPLS-DA model indicated that Debaryomyces hansenii (adjusted p=3.29e-05), Candida albicans (adjusted p =3.29e-05) and Candida parapsilosis (adjusted p =3.29e-05) were enriched in the HIV+ART+ group, although they presented a low discriminant power. The sPLS analysis indicated that vegetable and fibre intake were strongly associated with Candida genus abundance, while Prevotella genus correlated with carbohydrates and energy intake. Fats and oils, monounsaturated fatty acid and total lipids intake correlated with Faecalibacterium and Lachnospiraceae NK4A136 group abundance, as well as with different species of Candida. In the mycobiome of the HIV+ART+ participants, Candida is highly correlated, and showed a positive and strong association with Faecalibacterium. In a sPLS analysis including systemic markers of immune dysfunction and the microbiome (mycobiome and bacteriome), bacterial translocation (LTA, LBP), and monocyte activation (sCD14) positively correlated with 4 fungal and 7 bacterial ASVs.

Conclusions: Subjects with HIV on ART showed a distinct mycobiome fingerprint mycobiome with high abundance of Candida, specifically C. albicans. Different inter-kingdom interactions were identified with potential effect on the host physiology. The identified inter-kingdom consortia could be involved in vegetable fibre degradation and SCFA production as well as lipid metabolism. Given the correlations with systemic predictors of clinical progression (LTA, LBP and sCD14), a deeper understanding of the functional consequences of the mycobiome in HIV infection is needed.

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O_11

Lung microbiome in highly immunosuppressed lung transplant recipients drives inflammation and primary graft dysfunction

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Background: Primary graft dysfunction (PGD) is the principal cause of early morbidity and mortality after lung transplantation. It is thought to be an ischemia-reperfusion type of acute lung injury, however the factors that prime an allograft for PGD early after transplant are not well understood. Recent advances have identified the lung microbiome as a potential mediator or modulator of host response. We sought to understand the relationship between the microbiome and inflammatory host response at the time of lung transplantation and risk for subsequent PGD.

Methods: We performed a single-center prospective cohort study of individuals undergoing lung transplantation. Airway lavage samples were obtained from organ donors immediately prior to organ procurement (pre-transplant), and from recipients immediately after implantation and re-perfusion (post-transplant). The lung microbiome was defined by bacterial 16S rRNA gene sequencing. Post-transplant samples were analyzed for cytokines and chemokines by 41-plex Luminex array and for pepsin by sandwich ELISA.

Results: We enrolled 149 subjects and analyzed pre-transplant (n=109) and post-transplant (n=136) lung microbiomes. Pre-transplant lungs were significantly different from historical healthy control lung (n=12) in alpha (Shannon index, p = 0.000019) and beta diversity (weighted UniFrac, PERMANOVA, p=0.014). Compared to pre-transplantation, immediate post-transplantation allografts had increased microbial biomass, reduced diversity, and enrichment of oral-type taxa (all p<0.05). Among enrolled subjects, 13 developed severe PGD (persistent grade 3) while 40 were without PGD (persistent grade 0). Individuals with severe PGD had distinct post-transplantation microbiomes, with significantly greater enrichment of Prevotella and a decrease in Streptococcus (both p < 0.05) and distinct compositions on weighted UniFrac analysis (PERMANOVA, p < 0.05). To assess for biochemical evidence of aspiration we measured pepsin levels and found that they were greater in PGD-3 than PGD-0 subjects (median 1.23 ng/dL vs 0.35 ng/dL, p<0.05). Pepsin also correlated with beta diversity of post-transplant samples. Next, to assess the host response our multiplex cytokine/chemokine analysis found significant differences in an unbiased principal components analysis and elevations in persistent PGD-3 compared to PGD-0 subjects in: CC-chemokines Eotaxin, MCP-1, MIP-1a, RANTES (CCL5); colony stimulating factors G-CSF, GM-CSF; CXC-chemokines: IL-8, IP-10; the growth factor Flt-3L; and the inflammatory cytokines IL-6, and TNF-alpha (p<0.05; Kruskal-Wallis). We found that a composite model of Prevotella/Strepotoccus ratio, pepsin, and RANTES, TNFa had a high discriminatory power to predict severe PGD (AUC 0.83).

Conclusions: Immediately post-transplantation, lung transplant recipients' microbiome differs between those who develop severe PGD and those who do not. In addition, the subjects with PGD exhibited biochemical evidence of aspiration with an associated hyper-inflammatory phenotype. Combining microbiome, pepsin, and inflammatory measurements holds potential for prediction of PGD and these findings point to a potentially modifiable factors that prime allografts for post-transplantation acute lung injury.

Oral Abstract Presentations

O_12

Gut Microbiome Signatures Linked to HIV-1 Reservoir Size and Viremia Control

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Background: The potential role of the gut microbiome as a predictor of immune-mediated HIV-1 control in the absence of antiretroviral therapy (ART) is still unknown. In the BCN02 "kick and kill" clinical trial, which combined the MVA.HIVconsv immunogen with the latency-reversing agent romidepsin in early-ART treated HIV-1 infected individuals, 23% (3/13) of participants showed sustained low-levels of plasma viremia during 32 weeks of a monitored ART pause (MAP). We previously reported that viremic controllers during MAP exhibited higher Bacteroidales/Clostridiales ratio, lower microbial gene richness and depletion in butyrate-producing bacteria and methanogenic archaea before vaccination and throughout the study intervention when compared to non-controllers. Also, baseline transcriptome profiling showed that upregulated genes in viremic controllers were functionally enriched in immune activation and inflammatory response. Here, we present a multi-omics correlation and integrative analysis aimed at identifying salient signatures associated with HIV-1 control in the BCN02 trial.

Materials and Methods: Fecal samples from 13 participants in the BCN02 trial before vaccination were collected for metaproteome profiling. Microbial proteins were measured by mass spectrometry and protein identification performed using Mascot search engine (Matrix Science) and Scaffold Q+ software (Proteome Software). To estimate the viral reservoir size , longitudinal cell-associated (CA) HIV-1 RNA and HIV-1 DNA were measured in peripheral CD4+ T cells by ddPCR. Correlations were computed based on Spearman's rank coefficients and integrative multi-omics analyses inferred using the mixOmics R package and Cytoscape for network visualization. Intra and inter-group comparisons were calculated using paired and non-paired Wilcoxon test, respectively. Benjamini-Hochberg method was used for multiple comparison correction (FDR=5%).

Results: Baseline fecal metaproteome analyses showed that functional differences between viremic controllers and non-controllers were mainly driven by Clostridiales, which were actively producing bacterial proteins in both groups albeit in distinct functional contexts. Specifically, viremic controllers were enriched in proteins from Blautia and Ruminococcus mainly involved in starch/sucrose and glyoxylate/dicarboxylate metabolic pathways, and depleted in proteins derived from other Clostridiales genera. Before vaccination and over the intervention, the Bacteroidales/Clostridiales ratio negatively and significantly correlated with the reservoir size in terms of both HIV-1 DNA and CA-HIV-1 RNA, whereas an opposite trend was observed for microbial gene richness. Integration analysis showed that the baseline Bacteroidales/Clostridiales ratio positively correlated with pre-existing immune activation transcripts and proteins from Blautia, Ruminococcus and Prevotella. Furthermore,

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pro-inflammatory Bacteroidales species as well as pre-existing host immune activation signatures, both increased in viremic controllers, inversely associated with the viral reservoir size, while butyrate producing Clostridiales species showed an opposite trend.

Conclusions: This proof-of-concept study suggests pre-existing gut microbial and immune activation signatures as potential predictors of HIV-1 reservoir size and sustained viral control in the absence of ART, providing a potential target for future microbiome-based treatment strategies and opening up new avenues for a functional HIV cure.

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O_13

SARS-CoV-2 Infection Associated with Microbial Translocation Across Intestinal Mucosa

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Background: Gastrointestinal symptoms such as diarrhea have been described in SARS-CoV-2 infection of humans, and viral RNA has also been detected in human stool samples. However, intestinal pathology has not been well described in humans or animal models. Here we investigate the effect of SARS-CoV-2 infection on the gut mucosa in rhesus macaques.

Materials and Methods: Six adult rhesus macaques were infected with 2x10^6 TCID50 units of US/WA-1/2020 strain of SARS-CoV-2, with 1x10^6 units instilled intranasally and 1x10^6 units instilled intratracheally. Animal temperature and weight were monitored during the course of infection. Nasal and throat swabs were conducted prior to infection and periodically throughout infection along with blood draws and stool collection up to 10 days post-infection, at which point animals were euthanized. RNA was extracted from swab and stool samples and SARS-CoV-2 RNA measured by quantitative reverse-transcriptase PCR. Plasma samples were assessed for inflammatory markers by ELISA. Tissues collected at necropsy were formalin fixed and assessed for microbial translocation through immunohistochemical (IHC) staining of E. coli, while hematoxylin and eosin staining was also performed. Tissues were additionally collected from uninfected rhesus macaques and processed in the same manner.

Results: SARS-CoV-2 infection did not induce fever nor weight loss over 5% of body mass. Viral RNA was detected in all animals in nasal and throat swabs, with peak viral burden at day two post-infection in nasal swabs and day one in throat swabs. Viral RNA was additionally detected in stool samples. Clinical scores for translocating bacteria in colon sections stained by IHC for E. coli we higher for SARS-CoV-2 infected animals than uninfected control animals. Additionally, follicles made up a higher percentage of total mesenteric lymph node area in SARS-CoV-2 infected animals than healthy controls. Furthermore, soluble CD14 in plasma increased significantly from baseline to day 10 of SARS-CoV-2 infection (p=0.03).

Conclusions: SARS-CoV-2 infections span a wide range of disease severity in humans, from asymptomatic to fatal. Adult rhesus macaques experienced a mild to moderate clinical disease, yet demonstrated evidence of microbial translocation, as indicated by clinical scores of translocating bacteria in the colon and corresponding increased follicle area in gut draining lymph nodes and increased plasma sCD14 levels. These data suggest gut involvement in SARS-CoV-2 infection that is not exclusive to severe disease.

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O_14

Inflammation and Pulmonary Dysfunction Associated with Elevated Sars-Cov-2-Specific T Cells in Post-acute Sequelae COVID-19

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Background: After infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a significant number of individuals develop post-acute sequelae of COVID-19 (PASC) marked by prolonged symptoms, including persistent pulmonary dysfunction. T cells and inflammation contribute significantly to severe COVID-19; however, little is known about the role of persistent inflammation and SARS-CoV-2-specific immunity in PASC. The aim of this study is to compare inflammatory markers, frequencies of SARS-CoV-2-specific T cells, microbiome composition and pulmonary function in subjects who recovered from acute COVID infection (AC) and PASC.

Material and Methods: We collected blood samples and stool samples from individuals after recovery from SARS-CoV-2 infection and divided the cohort into AC (n=17) or PASC (n=18) based on symptom duration. Peripheral blood mononuclear cells (PBMC) and plasma were then isolated. PBMC were stimulated with SARS-CoV-2 surface proteins and the frequencies and phenotypes of SARS-CoV-2-specific T cells were measured by flow cytometry and identified by production of tumor necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ) or interleukin-2 (IL-2). Inflammatory markers levels in the plasma assessed by ELISA and pulmonary function measured by spirometry during the standard of care were both compared to T cell responses. The Mann-Whitney U test or Wilcoxon's matched pairs test were utilized to determine significance of differences between groups. Correlations were calculated using the nonparametric Spearman test. P values of <0.05 were considered statistically significant.

Results: Compared to AC, subjects with PASC had significantly elevated plasma CRP and IL-6 and up to a hundred-fold increase in the frequency of IFN-γ- and TNF-α-producing SARS-CoV-2-specific CD4+ and CD8+ T cells in blood. Importantly, the frequency of SARS-CoV-2-specific, TNF-α-producing CD4+ and CD8+ T cells in PASC positively correlated with plasma IL-6 (P=0.002, R=0.64; P=0.05, R=0.41 respectively). SARS-CoV-2-specific IFN-γ-producing CD4+ and CD8+ T cells negatively correlated with measures of lung function, specifically FEV1 (P=0.01, R=-0.81; P=0.007, R=-0.90 respectively), while increased frequencies of positively associated with the duration of respiratory symptoms during the post-acute period.

Conclusions: Significant immunological differences exist between subjects with PASC and AC that associate with increased inflammation and pulmonary dysfunction. Various studies have demonstrated prolonged viral RNA presence in the distal digestive and respiratory systems after SARS-CoV-2 infection and the elevated presence of SARS-CoV-2 specific T cells in PASC we find suggests that persistent viral presence may drive ongoing symptoms. Studies of the effect of the gut microbiome on systemic inflammation, T cell immunity and lung function in PASC are ongoing.

Poster Presentations

P_01

HIV-1 infection and Gut Microbiome

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Background: The prevalence of Human Immunodeficiency Virus type 1 (HIV) infection globally was recorded to be 29.8 million in 2001 and 37.9 million in 2018 showing a trend of gradual increase in over the time. HIV 1 infection weakens the immune system and increases the risk of infection by other pathogens (opportunistic infections) in an individual. According to an estimate in year 2019 about 23.49 lakh people were living in India with the increase in prevalence of HIV. The gut microbiota playing a key role in maintaining health and immune system. Any imbalance in the level of gut microbiome due to viral or bacterial infections may adversely influence the human health. HIV-infected adults have a gut microbiome associated with decreased bacterial richness and diversity, and associated with systemic inflammation and immune activation.

Materials and Methods: The information about Transmission and Prevention of HIV infection was collected from published sources using different search engines.

Results: HIV infections are reported to cause changes in the gut microbiome which have been shown to lead adverse consequences on the vital organs of humans especially the liver and brain. The microbial translocation into the blood stream have been found to cause increased indolamine 2,3-dioxygenase (IDO) enzyme expression and activity, HIV disease progression and increase in inflammation and immune activation. In addition, an understanding about the mechanisms of HIV-1 infection mediated alterations in microbiome and subsequent physiological / biochemical alterations in an individual could help to achieve a "functional cure" from HIV infection.

Conclusion: The knowledge about the gut microbiota of an individual and its fallout after HIV infection may provide leads about new and effective diagnosis and novel therapy of the disease. Some strategies are required to be used to prevent HIV transmission both at the individual and community levels keeping in view the gut microbiota. It includes screening of all blood and blood products, followed by taking precautions, educating people for safer sex practices, identification and treatment of sexually transmitted infections (STIs) or other infections.

Poster Presentations

P_02

Longitudinal Analysis of the Lung Microbiome in an Immune-Compromised Patient Population

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Background: HIV infection is fundamentally a disease of immune dysfunction, but as a result of effective antiretroviral therapy lung diseases in HIV-infected individuals have transitioned from primarily being acute opportunistic infections to being dominated by chronic pulmonary diseases. By studying lung transplant patients, we can investigate alterations in microbiome adaptations under chronic inflammatory and immunocompromised states. Cystic fibrosis (CF) is one of the most common indications for lung transplant. We sought to understand the microbiome of the upper and lower airways of CF lung transplant recipients in the first-year post-transplant, in an effort to identify predictive markers and mechanisms of lung dysfunction.

Materials and Methods: We collected longitudinal oropharyngeal wash (OW) samples as a representation of the upper respiratory tract, bronchoalveolar lavage fluid (BALF) sampling of the lung, and bronchoscope pre-wash (PW) as an environmental control from 26 CF lung transplant recipients followed over the first-year post-transplant. DNA was extracted and 16S rRNA gene sequences amplified using V1V2 primers, sequenced on the Illumina platform, and processed using QIIME2. Bacterial taxonomy was assigned using a naïve Bayesian classifier and the SILVA database. Diversity metrics were calculated using QIIME2. Statistical analyses were performed using R.

Results: Shannon diversity was significantly higher in OW relative to BAL ($p < 0.001$; paired t-test). There were large variations in the longitudinal Shannon diversity indices of both OW and BALF in most patients. Principal coordinate analysis (weighted UniFrac) revealed that OW (upper respiratory tract) and PW (environmental controls) comprised distinct clusters, while the BALF (lung) spanned the space between these two sample types. Outlier analysis comparing paired BAL and OW samples identified significant enrichment (outgrowth) in lung of known respiratory pathogens, including Pseudomonas, Stenotrophomonas, Staphylococcus, and Burkholderia. Pseudomonas was the most frequent dominant taxa (defined as >30% of reads) in BALF, seen in 32.9% of samples; in the OW, Streptococcus was the most frequent dominant taxa, seen in 53.1% of samples. Sequencing results closely corresponded with the clinical culture for most samples, including subjects chronically colonized by Burkholderia cepacia complex and the emerging pathogen, Segniliparus rugosus. Unexpectedly, we found striking differences between analyses carried out using Amplicon Sequence Variants (ASVs) that reflect 100% sequence identity, and Operational Taxonomic Units (OTUs) clustered at 97% sequence identity, indicating the profound impact of methodological approaches to taxonomic identification.

Conclusions: This study provides a unique, longitudinal analysis of the respiratory microbiome of CF lung transplant recipients. The reduced Shannon diversity of BALF likely reflects bacterial outgrowth, which was demonstrated in the outlier analysis. Importantly, taxonomic outgrowth in the BALF implies that lung and upper respiratory tract are distinct communities and that the lung microbiome is independently replicating in this population. Further, it

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suggests that lung microbiota are metabolically active, with implications for host homeostasis and therapeutic management of chronic lung disease. Finally, different analytic approaches to taxonomic identification can substantially alter community profiles. As long-term outcomes accrue, these data will provide a basis for identifying predictors and potential mechanisms of pulmonary complications in this immunocompromised microbially-colonized cohort.

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P_03

Peptidoglycans from Enteric Bacteria Differentially Induce Epithelial Cell Activation

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Background: HIV-associated enteric microbiota has been shown to induce immune activation; however, the underlying mechanisms are poorly understood. When properly controlling for sexual behavior, differential inflammatory properties are observed between microbiota from HIV-infected compared to uninfected men-who have sex with me (MSM) despite a lack of taxonomic differences. Having these compositionally similar microbiomes with distinct inflammatory properties presents a unique opportunity to identify causal bacteria and their components that drive cell activation. Bacterial peptidoglycan (PGN) has strain specific structures with varying capacities to induce or evade cell activation. It is well known that epithelial cells can facilitate innate immune activation and recent studies have highlighted the involvement of epithelial cells in microbiome interactions.

Material and Methods: To identify PGNs responsible for epithelial cell activation, we cultured PGN isolated by HPLC from commensal and pathogenic bacteria with T84 immortalized epithelial cells and epithelial cells isolated directly from resected gut tissue. Epithelial cell activation was evaluated by conventional flow cytometry, cytokine production and ImageStream and membrane integrity was measured by transepithelial electrical resistance. To characterize the interactions of epithelial cells and immune cells, autologous epithelial cells were first stimulated with PGN and then were either directly cultured with lamina propria mononuclear cells (LPMC), or supernatant was collected and used to stimulate LPMC. Innate immune cell activation was evaluated by flow cytometry and cytokine production.

Results: We found PGN from different gut bacteria induce differential levels of epithelial cell activation markers, including ALCAM and E cadherin, and IL-15 and GMCSF cytokine production. Using ImageStream, we also found that epithelial cell activation effects the surface expression of TLR2, TLR4 and TLR9. When cultured with PGN from inflammatory bacteria, there was a greater loss in membrane resistance. Lastly, we found that epithelial cells can induce the activation of innate immune cells, particularly monocytes in the blood and macrophages in the gut, both directly and through signaling molecules.

Conclusions: Our data shows that various PGNs differentially induce epithelial cell activation which subsequently activate innate immune cells. These findings implicate PGN and these cells as being essential for HIV-associated enteric microbiota induced immune activation.

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P_04

Factors Related to Composition of a More Western versus Agrarian Diet in HIV-Infected Individuals in Zimbabwe

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Background: The prognosis of people living with HIV (PLWH) in sub-Saharan Africa (SSA) is influenced by their diet. Consumption of a more Western versus Agrarian type diet has been shown to increase inflammatory phenotypes in a manner related to differences in gut microbiome composition, whose composition has been linked with HIV disease pathogenesis. Furthermore, consumption of a high-fat, low-fiber "Western" type diet has been shown to promote inflammation, dysbiosis of the intestinal microbiome, and disease progression in SIV-infected macaques. A traditional diet in Zimbabwe is high in fiber and low in fat and sugar and processed foods, but consumption of more "Western" type foods in Zimbabwe is also common and has been associated with increased prevalence of diseases that are disproportionately present in developed countries, such as obesity.

Methods: We developed a novel, regionally specific food frequency questionnaire (FFQ) focusing on western and agrarian food types to describe average consumption in Zimbabwe. The FFQ was administered to 168 PLWH and healthy controls from urban and rural communities in Zimbabwe in 2018-2019. Foods were separated into 34 categories and scored based on self-reported average consumption.

Results: Significant differences were found both by community and by HIV-infection status overall and within communities. Based on a principal component analysis, PC1 values represented variation in consumption of a diet dominated by Sadza, a traditional staple food of corn maize, versus a diet with more diverse food items. PC2 correlated with consumption of more agrarian type foods high in fiber and low in fat versus more western items such as burgers and sugary drinks. Diet significantly varied between urban and rural communities and cohorts (i.e. HIV-positive:ART-naïve, HIV-positive:ART-treated, and healthy controls). The urban community and HIV-positive:ART-naïve trended together along PC1, while the rural and HIV-positive:ART-treated trended together. The urban community reported a higher overall food diversity as well as higher consumption of several food types including grain, dairy, protein, and fast food. The period over which these FFQs were gathered was a time of high turmoil in Zimbabwe with government instability and high rates of inflation. Therefore, we looked at food consumption longitudinally and found that consumption of all foods in the urban community and grain products, in particular, decreased between visits, but no change in consumption was seen in the rural participants.

Conclusions: We characterized patterns in consumption of food items in rural and urban Zimbabwe that have previously been shown to influence inflammation and HIV

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pathogenesis in animal models. We are currently using this knowledge to account for diet as a potential driver of differences in ART treatment effectiveness and microbiome differences that we have observed in PLWH in rural versus urban Zimbabwe.

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