

43rd Annual Symposium on Nonhuman Primate Models for AIDS

October 20-23, 2026
San Antonio, TX



Hosted by
Southwest National Primate Research Center



SOUTHWEST
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2026 NHP Symposium Sponsors

The Organizing Committee would like to express their sincere gratitude to the following sponsors for their participation in the conference and for their generous financial support.

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Welcome to San Antonio!



Dear Colleagues

It is with great appreciation and excitement that we welcome you to San Antonio for the 43rd Annual Symposium on Nonhuman Primate Models for AIDS!

We are so excited to welcome scientists, veterinarians, clinicians, investigators, trainees, and research professionals from around the world to this year's symposium. Our hope is that through friendly conversations, inspiring presentations, and our poster exhibitions, we can truly connect with one another. By sharing our ideas, we can spark fresh perspectives and work together to drive meaningful progress in HIV/AIDS and infectious disease research.

We are honored to welcome our Opening Keynote Speaker, Dr. Beatrice Hahn, Professor of Medicine and Microbiology at the University of Pennsylvania Perelman School of Medicine. Dr. Hahn has fundamentally transformed global health through her research on the origins and evolution of human pathogens. She is globally acclaimed for discovering the primate origins of HIV-1 and HIV-2, engineering the first molecular clone of HIV-1, and tracing the deadliest form of human malaria to West African gorillas. An elected member of the National Academy of Sciences, her revolutionary use of non-invasive molecular tracking continues to drive modern insights into zoonotic diseases and vaccine design.

San Antonio is a vibrant South Texas city celebrated for its rich history, diverse culture, warm hospitality, and distinctive cuisine. Our conference home, the Westin Riverwalk San Antonio, is located in the heart of downtown along the iconic River Walk, just steps from restaurants, attractions, and entertainment.

We encourage you to take some time to explore the River Walk and nearby landmarks, including The Alamo, La Villita Historic Arts Village, and the Briscoe Western Art Museum.

We extend our special thanks to our Session Chairs and Co-Chairs, Scientific Committee, and Organizing Committee for their invaluable time, expertise, and dedication. Their essential work has helped create an outstanding scientific program and has been instrumental in making this year's symposium a success.

Thank you for being part of this important gathering and for contributing your expertise, ideas, and enthusiasm to this year's symposium.

Welcome to San Antonio, Mi casa es su casa!



Corinna (Cory) Ross, PhD

Professor and Director
Southwest National Primate
Research Center (SNPRC)



Riti Sharan, PhD

Assistant Professor
Southwest National Primate
Research Center (SNPRC)

Committees

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Riti Sharan, PhD

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Jacob Estes, PhD

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Timothy Hoang Memorial Travel Award

Elizabeth Wagstaff, The University of Iowa

Andrew Alan Lackner Memorial Travel Fellowship For Early-Stage Investigators

Julien Clain, PhD, Emory National Biomedical Research Center

Bonnie Mathieson Memorial Travel Award

Maggie Hallmets, Tulane National Biomedical Research Center

2026 NHP Symposium Travel Awards

Kali Crofts, PhD, Duke University

Mohammed Daira, University of Pittsburgh

Michael Kuipa, Emory National Biomedical Research Center

Abby LeBlanc, Oregon National Primate Research Center

Abigail Lising, University of California, Davis

Yongbin Liu, PhD, Houston Methodist Research Institute

Ally McCullen, Wisconsin National Primate Research Center

Shashi Prakash Singh, PhD, Southwest National Primate Research Center

Claudia Stroue, Northwestern University

Luanna Vidal, PhD, University of Miami



PROGRAM AGENDA

43rd Annual Symposium on Nonhuman Primate Models for AIDS

All events at The Westin Riverwalk unless otherwise noted.



Tuesday, October 20, 2026

2:00 PM – 7:30 PM	Registration Open - Navarro Foyer
2:00 PM	Sponsor/Exhibitor Setup - Navarro Foyer
4:00 PM	Poster Session Setup - Hidalgo Ballroom
6:00 PM	Welcome Reception & Keynote Address - Navarro Ballroom Beatrice H. Hahn, MD Professor of Medicine and Microbiology, University of Pennsylvania Perelman School of Medicine

Wednesday, October 21, 2026

7:00 AM	Registration Open - Navarro Foyer
7:15 AM	Poster Session Setup - Hidalgo Ballroom
7:15 AM	Continental Breakfast - Navarro Foyer
7:15 AM	Sponsor/Exhibitor Setup - Navarro Foyer
8:00 AM	Welcome and Conference Introduction - Navarro Ballroom Chaired by Dr. Cory Ross & Dr. Riti Sharan
8:15 AM	Travel Award Presentation - Navarro Ballroom

8:30 AM | Acute Infection & Mucosal Immunology

Chair: Dr. Jason Brenchley | Co-chair: Dr. Megan O'Connor | Navarro Ballroom

8:30 AM	Microbiome Alterations Influence SIV Acquisition <i>presented by Dr. Jason Brenchley</i>
9:00 AM	SIV and HIV Proviruses Preferentially Persist in Host Genes Repressed during CD4 T Cell Proliferation <i>presented by Maggie Hallmets</i>
9:15 AM	Charge-Controlled Fc Engineering Redirects Broadly Neutralizing Antibodies to Mucosal Tissues <i>presented by Claudia Stroupe</i>
9:30 AM	Mucosal BCGΔBCG1419c Vaccination Enhances Tuberculosis Control in Juvenile SIV-infected Macaques, with Efficacy Associated with Lower Plasma Viremia <i>presented by Dr. Solomon Jauro</i>
9:45 AM	Heterogeneity at initial infection sites shapes early SIV replication <i>presented by Dr. Brandon Keele</i>
10:00 AM – 10:30 AM	Morning Break Beverage Break & Sponsor/Exhibitor Tables: Navarro Foyer Poster viewing: Hidalgo Ballroom
10:30 AM	Next-Generation Sigh-Deficient Mtb Vaccines Confer Enhanced Protection Against Pulmonary Tuberculosis in Macaques <i>presented by Caden Munson</i>
10:45 AM	Decoupled Evolution of Viral Infectivity and Macrophage Fusogenicity in Early Acute SHIV AD8 Infection <i>presented by Grace Marshall</i>
11:00 AM	Renewal of virally inflamed gut and mucosal immunity through a microbial biotherapeutic lipid metabolite-PPARα-histone crotonylation axis <i>presented by Abigail Lising</i>
11:15 AM	Studying the Role of CD4-Binding Site Proximal Glycans in Mucosal Transmissibility and Pathogenicity of a Subtype C SHIV in Rhesus Macaques <i>presented by Dr. Aimee Pepper</i>
11:30 AM	Lunch (On Your Own)

1:30 PM – 4:30 PM | Prevention: Next-Gen PrEP, bNAbs & Vaccine Platforms

Chair: Dr. Marie-Claire Gauduin | Co-chair: Dr. Mauricio Martins | Navarro Ballroom

1:30 PM	Novel Durable HIV Vaccine Platform Targeting Mucosal Epithelium <i>presented by Dr. Marie-Claire Gauduin</i>
2:00 PM	Administration of an anti-HIV-1 V3 monoclonal antibody with afucosylated Fc glycans controls pathogenic SHIV infection and heterologous SIV superinfection by inducing long-term CD8+ T-cell immunity <i>presented by Dr. Yasuhiro Yasutomi</i>

2:15 PM	The DART Program: Enabling Standardized Ultra-Long-Acting Antiretroviral Delivery for HIV Research in Nonhuman Primate Models <i>presented by Dr. Thi Thao Linh Nguyen</i>
2:30 PM	Adeno-associated virus delivery of Env-specific antibodies affords durable protection against simian immunodeficiency virus challenge <i>presented by Natasha Clark</i>
2:45 PM	Protecting Brain Development from SHIV in Infant Rhesus Macaques through Nanocapsule-Enhanced CNS Delivery of bNAbs <i>presented by Abby LeBlanc</i>
3:00 PM – 3:30 PM	Afternoon Break Beverage Break & Sponsor/Exhibitor Tables: Navarro Foyer Poster viewing: Hidalgo Ballroom
3:30 PM	From Naïve to neutralizing: Tracing the development of HIV broad neutralization in germline-targeting vaccine induced B cell lineages in non-human primates <i>presented by Dr. Patrick Madden</i>
3:45 PM	Limited plasma ADCC activity and Fc receptor engagement following passive infusion of ADCC-mediating monoclonal antibodies in SHIV-challenged rhesus macaques <i>presented by Dr. Kali Crofts</i>
4:00 PM	Enhanced immune protection during the “golden window” for HIV-1 intervention can result in elite control or protection <i>presented by Dr. Sara Sawyer</i>
4:15 PM	Lipid nanoparticle delivery of SIV Gag DNA induces a potent and persistent CD8 T cell response in non-human primates <i>presented by Dr. Tiffany Styles</i>
4:30 PM	Trainee Session: Resilience and Flourishing During Tough Times <i>presented by Dr. Satya Dandekar and Dr. Smita Iyer</i> Madero Room
5:15 PM – 7:15 PM	Poster Reception - Hidalgo Ballroom

Thursday, October 22, 2026

7:00 AM – 5:00 PM	Registration Open - Navarro Foyer
7:15 AM	Poster Viewing - Hidalgo Ballroom
7:15 AM	Sponsor/Exhibitor Tables Open - Navarro Foyer
7:30 AM	Continental Breakfast - Navarro Foyer

8:30 AM – 11:30 AM | Pathogenesis & HIV Co-Infections

Chair: Dr. Smita Iyer | Co-chair: Dr. Riti Sharan | Navarro Ballroom

8:30 AM	Defense, Disease, and Discovery: A Story in Three Acts <i>presented by Dr. Smita Iyer</i>
9:00 AM	Novel Rhesus Macaque Model of JC Polyomavirus Infection Reveals Mechanisms of Early Neurological Infection and Disease <i>presented by Elizabeth Wagstaff</i>
9:15 AM	Administration of a zonulin inhibitor to SIVmac-infected rhesus macaques on antiretroviral therapy improves gut status and alleviates the hypercoagulant status <i>presented by Dr. Mohammed Daira</i>
9:30 AM	Incomplete pulmonary immune recovery despite treatment identifies IL-21 as a host-directed therapeutic target in TB/HIV <i>presented by Dr. Riti Sharan</i>
9:45 AM	Plasmodium fragile malaria in pregnant rhesus macaques induces maternal-fetal immune activation and adverse fetal outcomes <i>presented by Sallie Fell</i>
10:00 AM – 10:30 AM	Morning Break Beverage Break & Sponsor/Exhibitor Tables: Navarro Foyer Poster viewing: Hidalgo Ballroom
10:30 AM	Western Diet and SIV Co-infection Alter Immune Responses to SARS-CoV-2 Infection and Enhance Pathogenesis in Macaques <i>presented by Dr. Kelsie Brooks</i>
10:45 AM	Conserved Sex-Specific Networks in the Aging Rhesus Macaque Hippocampus Reveal Glial and Mitochondrial Mechanisms Relevant to HIV-Associated Neurocognitive Disorder <i>presented by Victoria Harper-Alexander</i>
11:00 AM	Peripheral Cytokine Immunotherapy Reshapes SIV-infected Rhesus Macaque Brain Immune Response <i>presented by Allison Rux</i>
11:15 AM	Deep spatial profiling of granuloma architecture in the tuberculosis lung identifies distinct immunometabolic and cellular niches <i>presented by Dr. Prashant Bajpai</i>
11:30 AM	Lunch (On Your Own)
12:45 PM	Journal of Medical Primatology - Navarro Ballroom

1:00 PM – 4:00 PM | Cure and Substance Abuse Effects on HIV Reservoirs

Chair: Dr. Mirko Paiardini | Co-chair: Dr. Siddappa Byrareddy | Navarro Ballroom

1:00 PM	Combination Immune-Based Strategies for HIV Cure: Insights from Translational Preclinical Models <i>presented by Dr. Mirko Paiardini</i>
1:30 PM	Modeling the Impact of Substance Abuse on SIV Viral Reservoirs <i>presented by Dr. Siddappa Byrareddy</i>
1:45 PM	Modeling Ultra-Small Latent SIV Reservoirs in Rhesus Macaques and Evaluating Viral Rebound <i>presented by Ally McCullen</i>
2:00 PM	Combined SMACm and gp120 targeting Tri-specific killer engager treatment limits the intact SIV reservoir in ART-treated rhesus macaques <i>presented by Dr. Julien Clain</i>
2:15 PM	Targeting the CNS with AAV-Delivered Anti-HIV Antibodies in SHIV-Infected Rhesus Macaques <i>presented by Dr. Luanna Vidal</i>
2:30 PM – 3:00 PM	Afternoon Break – Navarro Foyer
3:00 PM	Building a Kitchen Sink: Iterative Development of Combinatorial HIV Cure Approaches in Nonhuman Primate Models <i>presented by Dr. Christopher Peterson</i>
3:15 PM	Adjunctive cannabinoids remodel the neuroimmune landscape in HIV/ART: A single-cell multi-omic study <i>presented by Dr. Lakmini Premadasa</i>
3:30 PM	CSF1R inhibition reduces myeloid reservoirs and suppresses viral rebound in virally suppressed chronic SIV infection <i>presented by Dr. Hai Duc Nguyen</i>
3:45 PM	Autologous Expanded NK Cell Therapy Delays Viral Rebound and Restricts Reservoir Reactivation Following ART Interruption in a Molecularly Barcoded SIV Model <i>presented by Dr. Tricia Burdo</i>
4:30 PM	Shuttle Transportation: The Ralston at Congo Falls – Pickup Outside Westin
4:45 PM – 8:00 PM	Banquet Dinner - The Ralston at Congo Falls • Gorillas naturally head indoors around 6:00 PM.

Friday, October 23, 2026

7:00 AM – 11:00 AM	Registration Open - Navarro Foyer
7:15 AM	Sponsor/Exhibitor Tables Open - Navarro Foyer
7:30 AM	Continental Breakfast - Navarro Foyer

8:30 AM – 11:30 AM | Translational Innovations & Model Optimization

Chair: Dr. Deepak Kaushal | Co-chair: Dr. Davide Pisu | Navarro Ballroom

8:30 AM	Next-Generation Sigh-Based Double and Triple Knockout Vaccines Targeting TB and TB/HIV <i>presented by Dr. Deepak Kaushal</i>
9:00 AM	Development of Long-Acting Antiretroviral Therapy for Sustained SIV Suppression in Rhesus Macaques <i>presented by Dr. Gabriela Webb</i>
9:15 AM	A Dual-Reservoir Long-Acting Implant for Sustained Combination Antiretroviral Treatment in SHIV-Infected Nonhuman Primates <i>presented by Dr. Yongbin Liu</i>
9:30 AM	PD-L1-based immune shielding confers durable AAV-vectored bNAbs expression and protection against SHIV challenge in rhesus macaques <i>presented by Michael Kuipa</i>
9:45 AM – 10:15 AM	Morning Break - Navarro Foyer
10:15 AM	Optimization of Imaging Cytometry for Cross-Species Translational Tissue Analyses <i>presented by Dr. Harikrishnan Balachandran</i>
10:30 AM	Developing novel CD8 binders for in vivo delivered anti-HIV T cell gene therapies in the macaque model <i>presented by Lidiia Gagarina</i>
10:45 AM	Single-nucleus RNAseq of the Maternal-Fetal Interface in Rhesus Macaques <i>presented by Dr. Orlando Cervantes</i>
11:00 AM	Pharmacokinetics and Protective Efficacy of Fc-Engineered HIV-1 bNAbs 3BNC117-LS in Pregnant Rhesus Macaques <i>presented by Dr. Patricia Hahn</i>
11:15 AM	Closing Remarks & Preview of 2027 Symposium - Navarro Ballroom
11:30 AM	Lunch (On Your Own)
11:30 AM	Remove Sponsor/Exhibitor Tables - Navarro Foyer



Opening Reception Keynote Speaker

Beatrice H. Hahn, MD

Professor of Medicine and Microbiology

University of Pennsylvania Perelman School of Medicine

Dr. Hahn is a Professor of Medicine and Microbiology in the Division of Hematology/Oncology at the University of Pennsylvania Perelman School of Medicine. She received her MD degree from the Technical University of Munich, trained as a postdoctoral fellow in the laboratory of Robert C. Gallo at the National Institutes of Health, and then moved to the University of Alabama in Birmingham where she remained on the faculty in the Department of Medicine for 26 years. Dr. Hahn is recognized for her work deciphering the primate origins of human AIDS viruses and the malaria parasites *Plasmodium falciparum* and *Plasmodium vivax*, including studies of gene functions and disease-causing mechanisms of these pathogens. She is particularly known for developing non-invasive methods to elucidate the prevalence, evolution and zoonotic potential of microbes that infect highly endangered primate species. More recently, Dr. Hahn has studied how type 1 interferon pressures have shaped the biology of HIV-1 both at transmission and after reactivation from latency and has generated novel germline-targeting SHIVs that rapidly and consistently induce broadly neutralizing antibodies in rhesus macaques. Dr. Hahn has authored or co-authored over 500 papers and is a member of many advisory groups and editorial boards. She is an elected member of the American Society for Clinical Investigation, the Association of American Physicians, the American Academy of Microbiology, the National Academy of Medicine, the National Academy of Science, and the American Academy of Arts & Sciences.



SESSION CHAIRS

We extend our sincere gratitude to the session chairs of the 43rd Annual Symposium on Nonhuman Primate Models for AIDS. Their thoughtful leadership, dedication, and expertise were invaluable in shaping the program and guiding its sessions.





Jason Brenchley, PhD

*Chief, Laboratory of Viral Diseases Senior Investigator, Barrier Immunity Section
National Institute of Allergy and Infectious Diseases (NIAID, NIH)*

Dr. Brenchley received a master's degree from Idaho State University in 1999 and received a Ph.D. from the University of Texas Southwestern Medical Center at Dallas in 2003. He joined NIH as a research fellow, studying immunopathogenesis and mucosal immunology in HIV-infected individuals. He became a tenure track investigator in the intramural program of NIAID in 2008, was tenured in 2012, and is now the chief of the Laboratory of Viral Diseases. His work focuses on understanding the mechanisms underlying progressive lentiviral infections. This work explores different species of nonhuman primates, mucosal immunology, and the composition of the GI tract microbiome.



Siddappa Byrareddy, PhD

*Professor & Vice Chair for Research, Pharmacology and Experimental Neuroscience
University of Nebraska Medical Center*

Dr. Siddappa Byrareddy, Ph.D., is the Singer Professor and Vice Chair for Research in the Department of Pharmacology and Experimental Neuroscience at the University of Nebraska Medical Center (UNMC), Omaha. His research focuses on HIV transmission, virology, immunology, substance use disorders, and nonhuman primate models for advancing immunotherapies and understanding disease pathogenesis. Supported by multiple NIH-funded awards and authored >200 peer-reviewed publications. His scientific achievements have earned numerous honors, including recognition as a Stanford Highly Cited Researcher, the Wybran Award for Outstanding Contributions to Neuroimmune Pharmacology, and Indian National Academy Young Scientist. At UNMC, he has received the Scientist Laureate, Distinguished Scientist, Outstanding Mentor of Graduate Students, and Innovator of the Year awards. He is also an elected Fellow of AIMBE.



Marie-Claire Gauduin, PhD

*Professor, Disease Intervention and Prevention
Southwest National Primate Research Center*

Dr. Gauduin has more than 25 years of experience in HIV/AIDS research and medical microbiology. She has been working extensively on HIV and the development of novel vaccine strategies using the non-human primate model for AIDS. In her work, she uses epithelial stem cells and weakened recombinant papillomavirus as vaccine-vectors to protect against multiple low-dose mucosal challenges. Dr. Gauduin is also developing a neonatal model for tuberculosis to study HIV/TB co-infection in pediatric AIDS.



Smita S. Iyer, PhD

*Associate Professor of Pathology, Division of Experimental Pathology
University of Pittsburgh*

Dr. Iyer is an Associate Professor of Pathology at the University of Pittsburgh School of Medicine, where she leads an immunology laboratory focused on nonhuman primate models to understand the cellular basis of immunity to and pathogenesis of HIV infection. Using SIV- and SHIV-infected rhesus macaques, and transgenic mouse models to understand mechanisms, her team investigates T cell trafficking and immune surveillance in the central nervous system, viral persistence during antiretroviral therapy, and mechanisms driving neuroinflammation and cognitive decline. Dr. Iyer serves on the HVTN Early-Stage Investigator Advisory Board. Her research connects viral reservoirs and immunity with brain injury and metabolic disease in people living with treated HIV.



Deepak Kaushal, PhD

*Professor, Host Pathogen Interactions
Southwest National Primate Research Center*

Dr. Kaushal, is a Professor at Texas Biomedical Research Institute (Texas Biomed). He is recognized for having developed the macaque model of TB using the natural inhalation route of infection and a model of Mtb/HIV co-infection. Dr. Kaushal and his research team have made major discoveries about how Mtb interacts with the primate lungs, within the granulomas, and have provided fundamental insights into the biology of the pathogen as well as the host environment. The establishment of a robust macaque model of inhalation TB and TB/AIDS has allowed the platform to be used for testing of novel vaccine candidates as well as drugs against TB.



Mauricio Martins, PhD

*Associate Professor
The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology*

Mauricio Martins was born and raised in Brazil. After studying biology in college, he moved to the United States to pursue a Ph.D. at the University of Wisconsin-Madison, where he was introduced to NHP models of HIV/AIDS. After graduation, he sought warmer climates and landed in South Florida as a postdoc at the University of Miami. Later, as an independent investigator, Dr. Martins's work led to the development of a vaccine modality that protects rhesus macaques against a highly pathogenic SIV clone. Dr. Martins is currently a tenured associate professor at the University of Florida. His lab continues to work on HIV immune interventions, with a focus on gene therapies for promoting persistent in vivo expression of IgG biologics.



Megan O'Connor, PhD

*Assistant Professor, Department of Laboratory Medicine and Pathology
University of Washington*

Dr. O'Connor is an Assistant Professor in Laboratory Medicine and Pathology at the University of Washington and a Core Scientist at the Washington National Biomedical Research Center. Her research focuses on next-generation vaccines and therapeutics for infectious diseases, with emphasis on viral pathogenesis, vaccine responses, host immunity, and the microbiome. The lab studies how infectious diseases and vaccines affect immunosuppressed populations, where immune responses and outcomes can differ markedly from healthy individuals. Research includes evaluating nucleic acid vaccine technologies, developing experimental models of viral pathogenesis and vaccine immunity under immune suppression, and investigating lung microbiome-immune interactions in respiratory disease. Collaborative projects include in vitro lung models and inhaled antibiotic strategies for respiratory infections.



Mirko Paiardini, PhD

Professor, Department of Pathology and Laboratory Medicine, Emory University Division Chief, Microbiology & Immunology, Emory National Biomedical Research Center

Dr. Paiardini research program seeks to unravel the mechanisms that govern viral persistence amid antiretroviral therapy (ART) and adapting this knowledge to devise immunotherapeutic strategies towards a cure for HIV. Based at the Emory National Biomedical Research Center, his laboratory utilizes in vivo simian immunodeficiency virus (SIV) infected NHPs as a comparative model for people with HIV (PWH) to investigate immunopathology, viral persistence, cell-mediated immunity, and as a preclinical platform to test therapeutic interventions to induce long-term viral remission in the absence of ART. In this role, Dr. Paiardini is the contact principal investigator of ERASE HIV, an NIH-funded collaborative for HIV Cure Research.



Davide Pisu, PhD

Assistant Professor, Microbiology and Immunology Tulane National Biomedical Research Center

Dr. Pisu, is an Assistant Professor of Microbiology and Immunology at Tulane National Biomedical Research Center. His research explores how *Mycobacterium tuberculosis* survives in the host and how lung immune cells control or permit infection. His work spans bacterial stress responses and drug tolerance, macrophage biology, and the cellular organization of tuberculosis granulomas. Combining experimental microbiology and immunology with mouse and nonhuman primate models, he investigates how interactions between the bacterium and lung immune cells shape disease outcomes. He is now extending this work to HIV–TB coinfection, examining how HIV changes the lung's ability to control tuberculosis..



Riti Sharan, PhD

Assistant Professor, Host-pathogen interactions Southwest National Primate Research Center

As an NIH-funded Principal Investigator at Texas Biomed and Core Scientist with SNPRC, Dr. Sharan and her team studies host-pathogen interactions and host-directed therapies in TB/HIV co-infection using rhesus macaque model. HIV and TB act synergistically to magnify the burden of disease with deadly consequences worldwide. People living with HIV are at a high risk of reactivating latent TB infection. Sharan Lab seeks to clarify the precise mechanisms of how HIV reactivates latent tuberculosis (TB) and how this causes both diseases to get worse, even when patients are on antiretroviral therapy against HIV. The long-term goal is to explore an intervention targeting the immune system to control chronic immune activation and reduce latent TB reactivation.

Acute Infection & Mucosal Immunology

WEDNESDAY, OCTOBER 21, 2026



SIV and HIV Proviruses Preferentially Persist in Host Genes Repressed during CD4 T Cell Proliferation

Ms. Maggie Hallmets¹, Dr. Joseph Mudd¹, Dr. Charles Goodman², Dr. Chrysostomos Perdios¹, Mrs. Clara Krzykwa¹, Ms. Celeste Coleman¹, Dr. Andrew Rahmberg³, Dr. Jason Brenchley³, Dr. Lori Rowe¹, Dr. Brandon Keele²

1. Tulane National Biomedical Research Center, 2. AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, 3. Barrier Immunity Section, Lab of Viral Diseases, Division of Intramural Research (DIR), NIAID, NIH, Bethesda, MD.

The persistent HIV-1 reservoir consists of proviral clones defined by unique integration sites, many within host genes. Antigen- and homeostatic-driven proliferation of these clones is a major driver of reservoir persistence. Whether the transcriptional behavior of host genes during CD4 T cell proliferation shapes the persistence of genic proviruses remains unknown.

We profiled SIVmac239M integration sites from blood CD4 T cells of ART-suppressed rhesus macaques (n=5) at 3, 10, and 21 months after ART initiation and coupled this to 68,065 publicly-available clones from ART-suppressed persons with HIV-1. To classify host genes by proliferative response, CFSE-labeled rhesus CD4 T cells were stimulated *ex vivo* with anti-cd3/anti-cd28 for 5 days, followed by bulk RNA sequencing of resting and divided cells. Genes containing proviruses were queried for how the gene responded during proliferation (upregulated, downregulated, or unchanged).

We identified 3,465 SIV integration sites, including 49% that were genic, and 130 clonally expanded sites (4.16%). Genic SIV proviruses were enriched in genes downregulated during proliferation (OR = 1.729 [1.285-2.334], $p < 0.001$) and depleted from unchanged genes (OR = 0.737 [0.574-0.946], $p = 0.016$), whereas upregulated genes showed no enrichment. Among proviruses in proliferation-downregulated genes, the majority were highly expressed at rest. This pattern was reproduced in seven of nine independent HIV-1 datasets, including analyses restricted to intact proviruses, and was evident even during untreated viremia.

Proviral integration in genes that become transcriptionally repressed during CD4 T cell proliferation may promote HIV-1 latency during the very cellular process that replenishes the reservoir.

Charge-Controlled Fc Engineering Redirects Broadly Neutralizing Antibodies to Mucosal Tissues

Ms. Claudia Stroupe¹, Dr. Ann Carias¹, Dr. Yanique Thomas¹, Dr. Mariluz Arainga-Ramirez², Mr. Douglas Ferrell², Prof. Francois Villinger³, Prof. Jeremy Smedley², Prof. Thomas Hope¹

1. Northwestern University, 2. University of Louisiana at Lafayette, New Iberia Research Center, 3. University of Texas, MD Anderson Cancer Center

Broadly neutralizing antibodies (bNAbs) offer immediate protection against HIV-1, but their trafficking is poorly understood. VRC07-523LS (523LS), a potent CD4-binding site bNAb, is characterized by low plasma persistence but high tissue accumulation, indicating that 523LS may be selectively pulled from the blood and redirected to tissues. We hypothesize that Fc structural features govern this trafficking and can be engineered to amplify 523LS delivery to mucosal and reproductive tissues, where protection from HIV acquisition is most critical.

We developed five 523LS variants to test this: aglycosylated (negative charge), partial aglycosylation (neutral charge), G0N/G0F, and Fc mutations (LALA, DEL) designed to modulate FcγR binding. We characterized FcγR engagement across multiple allotypes by biolayer interferometry (BLI), then tested each antibody variant in rhesus macaques using ⁶⁴Cu-DOTA labeling and positron emission tomography (PET/CT) imaging paired with ex vivo scintillation quantification over 72 hours.

Fc and N-glycan modifications substantially reshaped FcγR binding profiles and in vivo trafficking patterns, revealing several distinct phenotypes. G0N/G0F and DEL variants sustained low cardiac uptake and preferentially accumulated in the liver. Conversely, WT and LALA antibodies maintained high plasma and cardiac levels with minimal organ localization. Strikingly, complete aglycosylation triggered rapid clearance from blood within 30 minutes, followed by targeted reaccumulation in mucosal tissues at 24 hours. This phenotype was reversed by partial aglycosylation, with strong and persistent signal in the heart, plasma, and liver. These findings demonstrate that Fc charge, determined by glycan composition, is a central determinant of bNAb trafficking, independently controlling whether antibodies remain in circulation or localize to mucosal and lymphoid tissues.

This knowledge reveals that biodistribution can be controlled through rational Fc engineering and provides a strategy for directing antibodies to reproductive and mucosal tissues, where HIV-1 transmission risk is greatest. By transforming antibody biodistribution into a tunable property, Fc engineering opens a new pathway toward tissue-targeted HIV prevention strategies.

Mucosal BCG Δ BCG1419c vaccination enhances tuberculosis control in juvenile SIV-infected macaques, with efficacy associated with lower plasma viremia

Dr. Solomon Jauro¹, Dr. Erica Larson², Mr. Brendon Wahlberg¹, Mr. Mark Rodgers¹, Ms. Alexis Balgeman³, Ms. Persis Sunny¹, Ms. Kara Kracinovsky¹, Mr. Marshall Malin¹, Mrs. Pauline Maiello¹, Dr. Philana Lin⁴, Dr. Mario Alberto Flores-Valdez⁵, Dr. Lishomwa Ndhlovu⁶, Dr. Shelby O'Connor³, Dr. Charles Scanga¹

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Background

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is a global health challenge, particularly among people living with HIV (PLWH) with impaired immunity. Bacillus Calmette-Guérin (BCG), the only licensed TB vaccine, protects against childhood TB but is contraindicated in infants and children with HIV infection and not on antiretroviral therapy. Therefore, safer and effective vaccination strategies are needed.

Methods

Using juvenile Mauritian cynomolgus macaques, we evaluated mucosal vaccination with an attenuated BCG (BCG Δ BCG1419c). Animals were infected with SIV and vaccinated mucosally 3 months later with either BCG Δ BCG1419c or wild-type (WT) BCG. Three months post-vaccination they were challenged intrabronchially with virulent Mtb (Edman strain). Disease progression was monitored by serial PET-CT imaging, and Mtb burden and pathology were assessed at necropsy 8 weeks post-challenge. We measured plasma SIV viral load using qPCR. Animals with sustained plasma viral load $\geq 10^5$ SIV RNA copies/mL were classified as SIV non-controller, whereas those with viral load $< 10^5$ SIV RNA copies/mL are SIV controllers.

Results

Both BCG Δ BCG1419c and WT-BCG were well tolerated and elicited comparable airway and lung immune responses. These included an influx of myelocytic and lymphocytic cells and cytokines produced by CD4⁺ T cells and cytotoxic molecules produced by CD8aa, CD8ab, and NK cells. Despite similar immunogenicity, animals vaccinated with BCG Δ BCG1419c exhibited superior TB control than those vaccinated with WT-BCG, as manifested by lower Mtb load, reduced lung inflammation, fewer granulomas, and less extrapulmonary dissemination. However, the WT-vaccinated group had more SIV non-controllers than the BCG Δ BCG1419c-vaccinated group, and the SIV non-controllers displayed more pathology and higher Mtb burdens.

Conclusions

This study indicates that mucosal BCG Δ BCG1419c vaccination is safe, immunogenic, and protective in SIV+ juvenile macaques. It further highlights that viral control with antiretroviral therapy is important for TB vaccine efficacy.

Heterogeneity at initial infection sites shapes early SIV replication

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Background

There is a brief window between initial HIV exposure and the establishment of systemic infection when the virus is most vulnerable to intervention, yet the events in tissues during this time remain poorly understood.

Methods

Here we combine intravenous infection of 6 purpose-bred Indian-origin rhesus macaques (aged 2.2–8.6 years old; 1 female, 5 male) with a genetically barcoded virus (SIVmac239M2 a virus stock generated from the original Nef-open SIVmac239 clone with a short genetic insertion between the vpx and vpr genes with over 150,000 distinct barcodes). Early necropsy with meticulous tissue sampling was performed to track hundreds of phenotypically identical viral lineages across approximately 200 tissue specimens per animal.

Results

Tissue viral quantification and barcode sequencing across all animals revealed the initial sites of replication for 243 lineages during the first 4-5 days of infection. Initial sites of infection were dominated by spleen and lymph nodes with lymphatic and gastrointestinal (GI) tissues having similar capacity to support viral replication once infection was established. Expansion at initial founder sites was highly variable, and founder-site replication was the dominant predictor of plasma concentration per lineage. In one animal, a single GI lineage achieved extreme amplification, associated with an inflamed duodenal environment enriched for CD4⁺ target cells that drove near-complete systemic dominance.

Conclusions

Identifying local founder-site conditions as a major determinant of early viral replication success revealed that early systemic infection is shaped not simply by anatomical compartment or viral genotype, but by highly localized tissue environments that permits extensive or restrictive viral amplification.

Next-Generation SigH-Deficient *Mtb* Vaccines Confer Enhanced Protection Against Pulmonary Tuberculosis in Macaques

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Background

Mycobacterium tuberculosis (*Mtb*) remains a leading global health threat, responsible for one million deaths and over ten million new infections each year. The only approved anti-TB vaccine, Bacillus Calmette-Guérin (BCG), provides limited efficacy; it prevents disseminated forms of TB in children but consistently fails to prevent pulmonary TB in adults. Consequently, the development of novel, highly effective vaccines is urgently needed to combat the continued spread of *Mtb*. Our work explores the protective efficacy of novel SigH-deficient *Mtb* strains, specifically utilizing attenuated double knockout (DKO) and triple knockout (TKO) strains.

Methods

To rigorously evaluate the protective capacity of these novel vaccine candidates, we utilized the leading pre-clinical non-human primate (NHP) model. Animals were immunized via aerosol vaccination to mimic the natural route of *Mtb* infection and to induce robust localized mucosal immunity. Disease progression and host responses were longitudinally monitored using advanced, non-invasive PET/CT imaging, clinical monitoring, and biweekly sampling. Immunological characterization at the site of infection was evaluated using cutting-edge spatial single-cell technologies allowing for the high-resolution mapping of host-pathogen and cellular interactions within the lung compartment.

Results

Aerosol administration of the SigH-DKO and TKO vaccine strains elicited profound immune responses characterized by robust antigen specificity and marked by an increased frequency of polyfunctional T cells. Following virulent *Mtb* challenge, NHPs vaccinated with the DKO/TKO strains demonstrated a significant reduction in bacterial burden, granuloma formation, systemic inflammation, and overall lung lesions. Additionally, histological assessments revealed marked improvements in disease pathology, specifically reduced cellular inflammation and necrosis. DKO/TKO vaccinated subjects exhibited elite containment of the infection, increased iBALT formation, and altered cellular organization around granulomas all contributing to *Mtb* control.

Conclusion

The robust attenuation, potent immunogenicity, and safety in SIV co-infection models of the SigH-DKO and TKO strains establish them as leading preclinical candidates in the tuberculosis vaccine pipeline. Crucially, these novel vaccine candidates consistently outperform the traditional BCG vaccine in controlling both bacterial replication and subsequent pathological damage in a highly reflective NHP model. Given these promising protective outcomes, the SigH vaccine platforms justify aggressive further evaluation and development as next-generation tools to combat the tuberculosis pandemic.

Decoupled Evolution of Viral Infectivity and Macrophage Fusogenicity in Early Acute SHIV AD8 Infection

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Background

Macrophage-tropic (M-tropic) HIV variants infect myeloid cells expressing low CD4 surface density and are predominantly isolated from tissue-resident reservoirs, complicating cure strategies. During acute infection, standard evolutionary models suggest that adaptation for rapid entry into CD4⁺ T-lymphocytes induces an evolutionary trade-off, causing a rapid loss of macrophage tropism. However, whether this trade-off universally occurs during early acute infection *in vivo* remains unclear. We aimed to characterize the functional evolution of viral infectivity and macrophage tropism during peak acute infection using a pathogenic nonhuman primate model.

Methods

Three Indian-origin rhesus macaques were infected with M-tropic SHIV-AD8. Plasma samples were collected at weeks 2 and 4 post-infection. Single Genome Amplification (SGA) of plasma viral cDNA was performed to isolate intact, single-template envelope (*env*) amplicons, minimizing PCR artifacts. Amplicons were engineered into an infectious, T-tropic pLAI backbone to generate panels of chimeric infectious clones (62–85 functional clones per macaque). Viral entry efficiency and fusogenicity were quantitatively assessed in TZM-bl cells and across 3 primary macrophage donors using a Vpr-β-lactamase (BlaM-Vpr) viral fusion assay.

Results

Chimeric clones derived from all three macaques demonstrated a rapid, uniform, and statistically significant increase in global viral infectivity by week 2 compared to the parental SHIV AD8 stock, which was durably maintained through week 4. Crucially, despite this intense selection for enhanced general entry fitness, macrophage-specific entry efficiency and fusogenicity remained remarkably stable, with the overwhelming majority of acute-phase variants preserving the parental M-tropic phenotype. Clonal resolution revealed only a minor subset of rare outlier variants that lost macrophage fusogenicity, demonstrating that general infectivity and myeloid cell tropism can be molecularly decoupled during early replication.

Conclusions

Acute-phase SHIV AD8 envelopes rapidly optimize overall entry kinetics without compromising low-density CD4 utilization on macrophages. This preservation of macrophage tropism during peak acute viremia indicates that circulating variants retain the capacity to seed long-lived myeloid reservoirs early in infection, carrying important implications for early HIV cure interventions.

Renewal of virally inflamed gut and mucosal immunity through a microbial biotherapeutic lipid metabolite-PPAR α -histone crotonylation axis

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BACKGROUND: Rapid disruption of gut epithelial barrier integrity and mucosal immunity occurs within the first weeks of HIV infection, driving chronic inflammation and disease progression. HIV-induced dysregulation of host lipid metabolism and microbial dysbiosis impair gut mucosal repair and renewal. While antiretroviral therapy (ART) suppresses viral replication, restoration of gut mucosal immunity and gut microbial communities remains incomplete and inconsistent. To build on the success of ART, we developed a novel Accelerated Combination Therapy (ACT) that co-administers ART with a microbiome-derived therapeutic lipid metabolite to promote host mucosal recovery. We previously demonstrated that *Lactiplantibacillus plantarum* induces rapid repair of gut epithelial barriers after direct administration into the chronically inflamed SIV-infected gut lumen in the non-human primate (NHP) model of HIV/AIDS. Untargeted metabolomics identified 10-hydroxystearic acid (10-HSA) as the most enriched metabolite after *L. plantarum* administration, which correlated with increased expression of genes involved in lipid metabolism and gut epithelial renewal.

METHODS: We conducted a 20-week study with 14 rhesus macaques (*Macaca mulatta*) to evaluate ACT by co-administering ART with 10-HSA. Structural characterization of ligand-binding interaction between 10-HSA and PPAR α was performed using X-ray crystallography and functional analyses. *In vitro* experiments employed the Caco2 and HCT-116 PPAR α KO cell lines and *ex vivo* analyses utilized IO-1 human duodenal organoids. Transcriptomic profiling was carried out to assess gene expression changes across experimental conditions.

RESULTS: 10-HSA consistently repaired HIV-disrupted gut epithelial barriers *in vitro*, *ex vivo*, and in the NHP model of HIV/AIDS through direct activation of PPAR α , inducing lipid metabolism and mitochondrial repair. Activation of PPAR α signaling due to 10-HSA treatment was linked to enhanced epigenetic histone crotonylation, which promotes gut epithelial energy reprogramming. Co-administration of 10-HSA and ART in SIV-infected rhesus macaques accelerated viral suppression, resolved systemic inflammation, restored gut epithelial integrity, and recovered the gut microbiota.

CONCLUSIONS: These findings identify a microbiota-derived lipid metabolite-PPAR α -histone crotonylation axis that activates gut epithelial regeneration and enhances the efficacy of ART. Critically, these results demonstrate the restorative impact of harnessing a microbial biotherapeutic lipid metabolite to simultaneously repair host mucosal immunity and gut microbial communities.

Studying the Role of CD4-Binding Site Proximal Glycans in Mucosal Transmissibility and Pathogenicity of a Subtype C SHIV in Rhesus Macaques

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Background: Simian-human immunodeficiency viruses (SHIVs) are challenge viruses that encode the HIV-1 envelope glycoprotein (Env) on an SIVmac backbone. Serial *in vivo* passage of SHIVs enhances mucosal transmissibility, replicative fitness, and CD4⁺ T cell-depleting capacity but also alters the sequence and antigenic properties of the native HIV-1 Env and selects for type I interferon (IFN-I) resistance. Using molecular clones encoding *env* segments from the passaged SHIVC109P3 (SHIV-P3cB) in the background of the parental subtype C SHIVC109F.PB4 (SHIV-109mc), we demonstrated that two passage-associated substitutions, N197D and N276D, which eliminate two glycans proximal to the CD4-binding site, are sufficient to confer IFN α resistance *in vitro*.

Methods: To determine whether loss of N197 and N276 glycans also enhances mucosal transmissibility, replicative fitness, and CD4⁺ T cell depletion *in vivo*, 17 rhesus macaques were challenged intrarectally with repeated low-dose inoculations (up to five exposures) of parental SHIV-109mc (n = 6), passaged SHIV-P3cB (n = 6), or the glycan-deficient mutant SHIV-109mc_{N197D+N276D} (n = 5). Animals were monitored longitudinally for 27 weeks following challenge.

Results: Preliminary analyses indicate that the mucosal transmissibility of SHIV-109mc_{N197D+N276D} (5/5 infected) exceeded that of the parental SHIV-109mc (4/6 infected) and was comparable to that of SHIV-P3cB (5/6 infected). The mean peak plasma viremia of SHIV-109mc_{N197D+N276D} (4.98×10^6 vRNA copies/mL) was similar to that of SHIV-109mc (7.46×10^6 vRNA copies/mL) but ~3.5-fold lower than that of SHIV-P3cB (1.74×10^7 vRNA copies/mL). Persistent plasma viremia was detected in most animals through the 27-week study endpoint across all groups. SHIV-109mc_{N197D+N276D} exhibited an intermediate phenotype with respect to peripheral CD4⁺ T cell depletion, falling between the less pathogenic SHIV-109mc and the more pathogenic SHIV-P3cB.

Conclusions: Preliminary analyses suggest that loss of two CD4-binding site proximal glycans can enhance the mucosal transmissibility and CD4⁺ T cell-depleting capacity, but not the replicative fitness, of an unpassaged subtype C SHIV in rhesus macaques. Ongoing analyses of tissue viral replication, immune responses, and additional virologic parameters will further define the contribution of these glycan changes to SHIV pathogenesis.

Prevention: Next-Gen PrEP, bNAbs & Vaccine Platforms

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Administration of an anti-HIV-1 V3 monoclonal antibody with afucosylated Fc glycans controls pathogenic SHIV infection and heterologous SIV superinfection by inducing long-term CD8⁺ T-cell immunity

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Background: The monoclonal antibody (mAb) 1C10 targets the V3 loop of HIV-1 and neutralizes a broad range of clade B viruses.

Results: We evaluated the therapeutic efficacy of 1C10SW, produced in transgenic silkworms with afucosylated Fc glycans, against pathogenic SHIV89.6P infection in cynomolgus macaques. Plasma viremia declined below the limit of detection in six of seven macaques treated with a high dose of 1C10SW and in one of four macaques treated with a low dose. In contrast, the F(ab')₂ fragment of 1C10SW did not suppress SHIV89.6P infection, suggesting that Fc-mediated immune mechanisms are required for long-term viral control. Six of the seven macaques that suppressed SHIV89.6P infection after 1C10SW administration also controlled challenge with heterologous pathogenic SIVmac239. CD8⁺ cell depletion in controller macaques following infusion of an anti-CD8 mAb led to rapid reappearance of plasma viremia, suggesting that CD8⁺ T cells mediated control of both SHIV89.6P and SIVmac239 infections. Furthermore, 1C10SW administration suppressed viral rebound after combination antiretroviral therapy interruption in three of four macaques.

Conclusion: These results demonstrate that passive immunotherapy with an anti-V3 mAb containing afucosylated Fc glycans facilitates the development of potent CD8⁺ T-cell immunity capable of durably suppressing both SHIV and SIV infections. Induction of robust CD8⁺ T-cell responses through antibody administration may represent a promising strategy for achieving HIV remission.

The DART Program: Enabling Standardized Ultra-Long-Acting Antiretroviral Delivery for HIV Research in Nonhuman Primate Models

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Background

Nonhuman primate (NHP) models play a critical role in evaluating HIV prevention and treatment strategies. However, antiretroviral (ARV) administration often relies on daily oral dosing or repeated injections, requiring frequent animal handling that increases study complexity, cost, and animal burden while introducing variability in drug exposure. Long-acting drug delivery technologies can address these limitations by providing sustained, standardized ARV administration throughout extended studies.

Methods

The Dissemination of Ultra-Long-Acting Antiretroviral Release Technology (DART) Resource Program, funded by NIAID R24, was established to provide the Nanofluidic Drug Delivery Implant (NanoDDI) platform as a shared resource for the HIV NHP research community. NanoDDI is a refillable, drug-agnostic subcutaneous implant that employs a nanofluidic membrane to achieve sustained, near zero-order drug release through passive diffusion. The DART program supports implementation of the technology through standardized deployment workflows, investigator training, and collaborative development of ARV formulations tailored to emerging research needs.

Results

The DART Program provides investigators access to the NanoDDI platform, a technology supported by more than a decade of development and preclinical evaluation. NanoDDI enables autonomous long-term delivery of multiple ARVs, including islatravir, MK-8527, tenofovir alafenamide, emtricitabine, cabotegravir, bictegravir, dolutegravir, and lenacapavir. Sustained drug administration eliminates frequent dosing procedures, reducing handling-associated stress while supporting long-term pharmacokinetic, prevention, and therapeutic studies. Compared with conventional dosing approaches, NanoDDI can reduce ARV administration costs by 3- to 10-fold while maintaining consistent drug exposure. Endorsed by all seven U.S. National Primate Research Centers, the DART Program is facilitating broader adoption of standardized long-acting ARV delivery approaches for HIV prevention, treatment, and pharmacokinetic studies in NHP models.

Conclusions

By enabling sustained, standardized antiretroviral exposure in NHP models, the DART Program addresses key logistical and scientific challenges associated with conventional dosing approaches. This platform has the potential to improve reproducibility, reduce animal burden, lower study costs, and accelerate the evaluation of next-generation

HIV prevention and treatment strategies. Broad adoption of this resource may establish a new paradigm for ARV administration in long-term NHP studies, enhancing the rigor, efficiency, and translational impact of preclinical HIV research.

Adeno-associated virus delivery of Env-specific antibodies affords durable protection against simian immunodeficiency virus challenge

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Conventional vaccines have so far failed to elicit the types of antibodies needed for protection against HIV. As an alternative, we evaluated adeno-associated virus (AAV) delivery of rhesus macaque antibodies to the SIV envelope glycoprotein for protection against SIV challenge. AAV vectors encoding a bnAb or an antibody that only mediates antibody-dependent cellular cytotoxicity (ADCC) were administered individually or together to separate groups of eight rhesus macaques. Sustained antibody expression with minimal anti-drug antibody (ADA) responses was achieved in most animals. After sixteen weeks, animals underwent twelve low-dose, intrarectal (IR) challenges with SIV_{mac}239. All animals that received vectors encoding a control antibody or the ADCC-only antibody became infected after four challenges. However, fourteen of the sixteen animals that received the bnAb, either alone or in combination with the ADCC antibody, resisted all twelve challenges. Of the two animals that became infected despite receiving the bnAb vector, one developed high ADA titers against the bnAb and sequence analysis of the virus population in the other revealed an Env escape mutation that conferred resistance to the bnAb. A year later, serum concentrations of vectored antibodies remained high and unabated, so the protected animals and six naïve control animals were rechallenged by repeated, low-dose IR inoculation with SIV_{mac}239. Whereas all the control animals became infected after six challenges, the fourteen bnAb-expressing animals remain uninfected after all twelve challenges. Thus, sustained expression of a potent bnAb can afford durable protection against a pathogenic SIV strain that is notoriously difficult to protect against by vaccination.

Protecting Brain Development from SHIV in Infant Rhesus Macaques through Nanocapsule-Enhanced CNS Delivery of bNAbs

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Background

HIV-1 infects the central nervous system (CNS) in early infection, establishes persistent viral reservoirs, and contributes to neuropathology. Infants are vulnerable to HIV-associated neurocognitive disorders (HAND), as infection occurs during critical neurodevelopment. Although ART effectively suppresses peripheral viral replication, limited penetration across the blood-brain barrier (BBB) allows CNS reservoirs and neuroinflammation to persist. Broadly neutralizing antibodies (bNAbs) are promising therapeutics to target and deplete persistent reservoirs, but penetration across the BBB similarly restricts efficacy in the CNS. Nanocapsulated bNAbs (n-bNAbs) have been shown to improve CNS delivery, potentially reducing infection-associated neuroinflammation. However, non-invasive assessment of CNS inflammation and its impact on cognitive function remains challenging. Because PET-detected brain inflammation has been associated with impaired executive function in adults, we are integrating TSPO PET neuroimaging with behavioral assessments in an infant SHIV macaque model to determine whether improved CNS delivery of bNAbs reduces neuroinflammation and preserves cognitive function.

Methods

One-month-old rhesus macaques were IV inoculated with barcoded SHIVSF162P3 and divided into SHIV only (n=6), native bNAb treatment (n=3), or n-bNAb treatment (n=3) groups. The treatment groups were given a cocktail of native or encapsulated PGT121+VRCO7-523 (5mg/kg/antibody). n-bNAbs were synthesized by encapsulating individual bNAb molecules within thin, functional polymer shells through *in situ* polymerization. PET imaging will be performed at 13 weeks post-infection using TSPO radiotracer C11-PBR28 with MRI coregistration to quantify uptake across brain regions. Additionally, animals will undergo longitudinal cognitive testing from two weeks pre-infection through 16 weeks post-infection using progressively challenging tasks, including plain reach, object permanence, and recognition to evaluate developmental milestones.

Results

We hypothesize that both native and n-bNAbs will suppress peripheral viral replication, whereas the improved CNS delivery of n-bNAbs will reduce CNS infection. Additionally, we hypothesize a reduction in SHIV-associated neuroinflammation detected by TSPO PET signal in n-bNAb treated animals when compared with controls and animals receiving native bNAbs. Lastly, we hypothesize that both treatment groups will perform better in cognitive testing than untreated SHIV-infected animals, with the greatest benefit observed in the n-bNAb group.

Conclusions

Together, these experiments aim to establish n-bNAb protection against SHIV-associated neuroinflammation and neurocognitive disorders.

From Naïve to neutralizing: Tracing the development of HIV broad neutralization in germline-targeting vaccine induced B cell lineages in non-human primates

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Background

Germline-targeting is a promising avenue for HIV vaccine development that relies on multiple phases, priming of rare precursor B cells followed by maturation of those B cells into bnAbs through sequential immunizations and, finally, conversion of those bnAb-expressing B cells into long-lived responses. We have demonstrated priming of BG18 type I bnAb-precursor B cells in rhesus macaques (Steichen, 2024 Science), despite the precursors being incredibly rare in the naïve B cell repertoire (1 in 53 million in humans and lower in macaques). We recently published a follow-up study using sequential immunization of progressively more native like Env trimers after priming to mature the BG18 type I responses into true bnAbs (Steichen and Madden, 2026 Nature). After each booster BG18 type I memory B cells and germinal center B cells were present and the cells demonstrated progressive SHM and affinity maturation. In $\geq 50\%$ of vaccinated macaques, bnAb-class memory B cells with capacity to neutralize diverse HIV isolates, with 10-67% breadth compared to BG18 were induced. Serum neutralization was also detected in 44% of these animals with breadth from 16 to 84%, at titers predicted to confer protection.

Methods and Results

Tracking vaccine induced lineages across multiple anatomical compartments, blood, lymphoid tissue, and bone marrow has allowed us to dissect the evolution and maturation of neutralization capacity after germline-targeting vaccination regimens. For the most expanded lineages we isolated >1,000 sequences across the experiment and compartments, with one lineage >6,000 sequences. These distinct bnAb producing lineages, from multiple macaques, exhibit broad intra-clonal diversity and multiple maturation pathways in response to the sequential vaccination regimen. Mutational analysis has revealed key mutations in the pathway towards broad neutralization. Strikingly, bnAb lineage B cells are also present as plasma cells in the bone marrow, approaching 2% of sequenced plasma cells in the bone marrow of some animals.

Conclusions

These results demonstrate that germline-targeting vaccines can reproducibly elicit known HIV bnAbs to specific epitopes in primates and represents a major breakthrough in HIV vaccine design. A better understanding of the developmental trajectories of the bnAbs will allow us to optimize the regimen for testing in humans.

Limited plasma ADCC activity and Fc receptor engagement following passive infusion of ADCC-mediating monoclonal antibodies in SHIV-challenged rhesus macaques

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Background: Antibody-dependent cellular cytotoxicity (ADCC) has been identified in both preclinical and clinical trials as an immune function associated with reduced HIV infection risk, acting through recruitment of Fc receptor (FcR)-bearing effector cells to mediate antiviral activity. To determine whether non-neutralizing Abs with Fc-mediated effector functions could protect against infection, adult rhesus macaques (RM) received a cocktail of three ADCC-mediating RM mAbs targeting distinct regions of the HIV-1 envelope, JR4 (C1C2), DH614.2 (V2), and 7B2 (gp41) (n=22) or a negative control anti-DSP mAb (n=10). Animals were challenged intrarectally with low-dose SHIV1157QNEY173H once weekly for eight weeks, beginning seven days following infusion. Despite robust invitro ADCC activity, antibody infusion failed to protect against SHIV infection. This prompted us to investigate plasma ADCC activity in the animals, and engagement of FcR-bearing innate effector cells by infused mAbs longitudinally.

Methods: Plasma ADCC activity was assessed post infusion and post-challenge using both HIV-infected and gp120-coated target cells. FcR engagement was assessed using the Cell-Bound Antibody Assay (CeBAbA), which employs fluorescent gp120 hooks to identify NK cells and monocytes retaining infused antibodies by the FcRs. For CeBAbA analysis, CD4-depleted cryopreserved PBMCs were evaluated pre-infusion, d1 and d7 post-infusion, d3 post-challenge, and at the last negative time point prior to detectable viral load (LN).

Results: Following infusion, plasma ADCC activity was limited, with only 10/22 RMs demonstrating detectable activity against gp120-coated targets and 4/22 RMs against infected cells. Peak ADCC activity against infected cells positively correlated with first detectable viral load in both mAb-infused ($R = 0.69$, $p = 0.00039$) and control animals ($R = 0.77$, $p = 0.0095$). CeBAbA analysis revealed low frequencies of Ab-bound effector cells in vivo. Ab-bound monocytes increased following infusion, peaked at day 7, and declined by LN. Ab-bound NK cells were infrequently detected at all time points measured. In contrast, >80% of monocytes and NK cells retained the ability to bind ex vivo the Ab cocktail used for the infusion.

Conclusions: These findings suggest that limited in vivo FcR engagement, rather than impaired effector functional capacity, may have restricted Fc-mediated antiviral activity and contributed to the lack of protection.

Enhanced immune protection during the “golden window” for HIV-1 intervention can result in elite control or protection

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Background: Developing a vaccine that prevents HIV-1 infection remains one of the most urgent unmet needs in global health. Vaccine-induced immunity must act during the brief interval immediately following HIV-1 exposure, before the latent viral reservoir is established—a “golden window” of opportunity during which infection remains reversible. Studying the effects of immune responses during this narrow period has been difficult because systemic viremia is not even detectable in humans or animal models until weeks after infection. In owl monkeys (*Aotus nancymae*), however, the broadly neutralizing human antibody 3BNC117 has an unusually short serum half-life of approximately seven days, remaining at clinically protective concentrations for only about five days after administration. This unique pharmacokinetic property provides an opportunity to examine the consequences of enhanced immune protection specifically during the golden window.

Methods: Twelve owl monkeys received a single intravenous infusion of either 3BNC117 (n = 6) or an isotype control antibody (n = 6), followed 24 hours later by intravenous challenge with HIV-1 modified for owl monkeys.

Results: Despite the fact that the neutralizing antibody lasted only 5 days at protective levels, animals treated with 3BNC117 exhibited delayed or diminished infection compared with controls, and one animal remained completely uninfected. Remarkably, three of the five treated animals that nevertheless became infected developed long-term elite control of viremia, maintaining viral suppression for months after 3BNC117 concentrations had fallen below clinically active levels.

Conclusions: These findings suggest that immune interventions acting during the golden window can durably alter, and in some cases prevent, HIV-1 infection. More broadly, these findings establish the owl monkey as a promising preclinical model for developing HIV-1 vaccines that prioritize rapid immune recall during the earliest stages of exposure, a parameter that has been difficult to study in other animal models.

Lipid nanoparticle delivery of SIV Gag DNA induces a potent and persistent CD8 T cell response in non-human primates

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Introduction: Lipid nanoparticles (LNPs) have transformed nucleic acid vaccine development, particularly for mRNA vaccines, by enabling potent antibody responses. However, current mRNA-LNP vaccines elicit relatively weak CD8 T cell responses in non-human primates (NHPs) and humans, and the antibody responses they induce decline rapidly after vaccination. In contrast, DNA is more stable than mRNA and supports prolonged antigen expression, which may enhance cellular immunity. Here, we compared the immunogenicity of DNA-LNP and mRNA-LNP vaccines expressing simian immunodeficiency virus (SIV) Gag in rhesus macaques (RMs).

Methods: Two groups of RMs (n=5/group) received three intramuscular immunizations of 100 µg DNA-LNP or mRNA-LNP vaccines expressing SIV Gag. Gag-specific T cell and antibody responses were evaluated after each immunization. Innate immune responses and whole-blood transcriptomic profiles were assessed following the priming immunization.

Results: DNA-LNP vaccination elicited robust and durable Gag-CM9 tetramer⁺ CD8 T cell responses, reaching frequencies of nearly 15% of total CD8 T cells. These responses were **15-fold** higher than those induced by mRNA-LNP vaccination following booster immunization. In addition, DNA-LNP-induced tetramer⁺ CD8 T cells displayed a distinct differentiation profile, remaining in an effector phenotype (CCR7⁻CD45RA⁻) significantly longer, whereas mRNA-LNP-induced cells transitioned to a central memory phenotype (CCR7⁺CD45RA⁻). Although mRNA-LNP vaccination generated higher peak antibody responses, these contracted rapidly after each immunization. In contrast, DNA-LNP-induced antibody responses increased progressively over time with minimal contraction. Mechanistically, DNA-LNP vaccination induced a stronger innate immune response, characterized by increased recruitment of neutrophils and non-classical monocytes. Transcriptomic analyses further revealed significant enrichment of pathways associated with IL-1β production, granulocyte activation, mitochondrial translocation, and phagocytosis following DNA-LNP vaccination, whereas these pathways were not significantly regulated after mRNA-LNP vaccination.

Conclusions: DNA-LNP vaccination induces a distinct immunological program characterized by potent innate immune activation, robust and sustained CD8 T cell responses, and durable antibody responses, supporting its potential as an alternative nucleic acid vaccine platform for applications requiring strong cellular immunity.

One suggestion: the last sentence is stronger if your data support the durability claim statistically. If the antibody durability is only a trend, consider changing “durable antibody responses” to “more sustained antibody responses” to avoid overstating the finding.

Pathogenesis & HIV Co-Infections

THURSDAY, OCTOBER 22, 2026



Novel Rhesus Macaque Model of JC Polyomavirus Infection Reveals Mechanisms of Early Neurological Infection and Disease

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Background

JC Polyomavirus colonizes 60-80% of the adult population in a lifelong, asymptomatic kidney infection. However, in a subset of immunocompromised individuals, it causes a rapidly progressing and often fatal central nervous system (CNS) infection known as Progressive Multifocal Leukoencephalopathy (PML), for which there currently is no cure or prophylactic available. It is unclear when or how JC virus establishes infection within the CNS, and research in this area is severely limited due to the lack of a comprehensive animal model for JC virus.

Methods

In this pilot study, we infected two SIV-infected and CD8-depleted rhesus macaques with a neurotropic strain of JC virus. The first was inoculated intrathecally and the second intravenously; both were euthanized once they reached a humane endpoint, which was at 145- and 85-days post-infection, respectively. Samples of plasma and cerebrospinal fluid (CSF) were collected throughout infection, and structural MRI data, enabling whole-brain assessment of white matter, were acquired before infection and before necropsy. After planned necropsies, frozen and preserved tissues were analyzed for the presence of viral DNA, RNA, and proteins.

Results

JC virus was present in the CNS and peripheral tissues, including bone marrow, kidney, and urine of both animals. The viral load was significantly higher in the intrathecally inoculated macaque brain, with viral nucleic acids and proteins found in astrocytes, oligodendrocytes, and microglia, with predominance in oligodendrocytes, similar to human presentation. Interestingly, infected microglia were often seen overlapping or adjoining perivascular macrophages, which may suggest myeloid cells have a role in trafficking JC virus across the blood-brain barrier. Neuroimaging and Luxol Fast Blue staining were consistent with a decrease in myelin in both macaques, though neither had canonical PML lesions.

Conclusions

This macaque model is a major step in JC virus research as it is the first animal model that shows JC virus infection in the CNS and periphery with trafficking across the blood-brain barrier as well as an early disease state before PML development. These are critical characteristics that will facilitate gaining an understanding of the neuropathogenesis of JC polyomavirus with the goal of improving patient outcomes.

Administration of a zonulin inhibitor to SIVmac-infected rhesus macaques on antiretroviral therapy improves gut status and alleviates the hypercoagulant status

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Background. Intestinal mucosal barrier breaches and endothelial dysfunction are central to HIV/SIV infection, and lead to microbial translocation into the bloodstream, with subsequent macrophage and T-cell activation, and systemic inflammation (INFL), the main predictors of HIV disease progression and death. While numerous studies have been attempted to control chronic INFL as an adjuvant to ART, very few attempted gut healing. Central to HIV-associated mucosal damage are alterations of the tight junctions (TJs), which are critical for barrier homeostasis. The only known physiological regulator of the structure and function of TJs is zonulin, a haptoglobin (Hp) precursor. Therefore, zonulin inhibition could be an effective strategy for controlling gut damage and preventing comorbidities associated with HIV.

Methods. Ten SIVmac239-infected rhesus macaques (RMs) and treated with antiretrovirals starting from 42 days postinfection (dpi) were given a zonulin inhibitor (Larazotide acetate-INN) for 2 weeks, starting at 210 dpi. Five RMs were euthanized on treatment, while in the remaining ones INN was stopped and they were followed for additional three months. Viral loads, major T cell populations and subsets, and their immune activation status were monitored. Soluble biomarkers were tested to monitor systemic INFL (IL-6, IL-12, I-TAC, IP-10 and eotaxin), microbial translocation (sCD163), gut integrity (citrulline) and coagulation status (D-dimer).

Results. INN administration to SIV-infected RMs on ART resulted in an increase of the plasma levels of citrulline (a marker that correlates with the enterocyte mass), a significant improvement of the mucosal continuity (claudin 3), and a significant reduction in the microbial translocation into the lamina propria (LPS), demonstrating INN ability to improve gut health. We also observed a better control of CD4⁺ T cell activation and proliferation, as well as control of systemic INFL, and a major reduction in the plasma levels of D-Dimer, a marker of INFL and hypercoagulation, predictive for HIV/SIV disease progression and associated death. No viral load blips were observed during treatment.

Conclusion. Our pilot demonstrated that INN administration to virally suppressed SIV-infected macaques results in a significant improvement of the gut health which might be beneficial for the outcome of HIV/SIV infection.

Incomplete pulmonary immune recovery despite treatment identifies IL-21 as a host-directed therapeutic target in TB/HIV

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Background: TB remains the leading cause of death among people living with HIV. Our previous work demonstrated that although cART combined with once-weekly isoniazid/rifapentine (3HP) substantially reduces TB risk, pathogen control does not necessarily equate to immune restoration; the extent of pulmonary immune recovery and the mechanisms driving persistent immune dysfunction remain unknown.

Methods: We used scRNAseq and spatial transcriptomics in a rhesus macaque model of latent *Mtb*/SIV coinfection to define pulmonary immune reconstitution following treatment. To uncover mechanisms driving persistent immune dysfunction, we focused on IL-21, a cytokine critical for Th1 immunity, T-cell memory, and antimicrobial host defense.

Results: cART+3HP suppressed viral replication, prevented TB reactivation. Yet, pulmonary immune recovery remained strikingly incomplete. Treated macaques exhibited persistent CD4⁺ T-cell depletion, impaired *Mtb*-specific responses, reduced TNF α production, sustained T-cell activation, and enrichment of Type I interferon transcriptional programs. A key finding was the persistent loss of IL-21-producing CD4⁺ T cells. While abundant during latent TB, these cells declined dramatically following SIV coinfection and were only partially restored by treatment. Integrated single-cell and spatial transcriptomic analyses linked IL-21 deficiency to impaired *STAT1* signaling, persistent *STAT3*-driven inflammatory programs, and chronic immune activation within the lung.

Conclusion/Future Directions: These studies uncover a major limitation of current TB/HIV therapy: pathogen suppression alone is insufficient to restore durable pulmonary immunity. We plan to test IL-21 as a host-directed immunotherapy in the *Mtb*/SIV macaque model, defining how the timing of immune intervention shapes viral reservoir persistence, pulmonary immune reconstitution, and control of TB reactivation.

Plasmodium fragile malaria in pregnant rhesus macaques induces maternal-fetal immune activation and adverse fetal outcomes

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Background

Malaria in pregnancy (MIP) is a major global health burden, adversely impacting maternal and fetal health, but the underlying mechanisms remain incompletely defined. Because rhesus macaques (RMs) recapitulate key features of human pregnancy and immunity, they provide a highly translational model for studying MIP. Here, we sought to define the immunological and pathological outcomes of *Plasmodium fragile* infection in pregnant RMs. We hypothesized that *P. fragile* MIP would result in systemic and maternal-fetal interface immune activation and fetal growth restriction (FGR).

Methods

Pregnant RMs were intravenously inoculated with *P. fragile*-infected erythrocytes (n=9) or saline (n=9) in early, mid, and late gestation. Antimalarial drugs were administered if parasitemia exceeded 0.5%. Necropsy occurred ~4 weeks post-inoculation, and all maternal and fetal tissues were harvested. At necropsy, fetal morphometrics were measured, including weight, head circumference, abdominal circumference, crown-rump length, biparietal diameter, and femur length. Placental parasite burden was measured via *in situ* hybridization and quantified with a machine learning algorithm (HALO). Immune cell frequencies and phenotypes were evaluated in blood and tissues via flow cytometry.

Results

Pregnant *P. fragile*-infected RMs exhibited substantial peripheral parasitemia and anemia, regardless of whether inoculation occurred in early, mid, or late gestation. Flow cytometry revealed elevated CCR5+ CD4+ T cells in peripheral blood (p<0.0001), spleen (p=0.0102), bone marrow (p=0.0221), and decidua (p=0.0549) of *P. fragile*-infected RMs versus control RMs. Increased eosinophil frequencies were observed in spleen (p=0.0515) and decidua (p=0.0041) of pregnant *P. fragile*-infected RMs compared to controls. *P. fragile*-infected erythrocytes were detected in placenta and decidua from pregnant infected RMs, with inter-animal variation in distribution and extent observed. Fetuses from *P. fragile*-infected RMs versus control RMs were smaller when adjusted for gestational age, with some fetuses from *P. fragile*-infected RMs reaching criteria for FGR (z-score<-1.28).

Conclusions

Our data demonstrate that *P. fragile* MIP in RMs mimics hallmarks of human MIP, including parasitemia, anemia, placental parasite burden, and FGR. Immunological assessments highlighted systemically elevated CCR5+ CD4+ T cells, which is highly relevant for susceptibility to malaria/HIV co-infection.

Western Diet and SIV Co-infection Alter Immune Responses to SARS-CoV-2 Infection and Enhance Pathogenesis in Macaques

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Background

Diabetes, obesity, and HIV increase risk for severe COVID-19. Diet can contribute to the development of obesity and diabetes, but its direct impact on SARS-CoV-2 infection is not well-studied. We investigated impacts of a high-fat, high-sugar chow (Western diet, WD) as well as SIV co-infection on SARS-CoV-2 pathogenesis in pigtailed macaques (PTMs).

Methods

Control (n=3) PTMs were infected with SARS-CoV-2 and sampled until necropsy (Nx) at day 30. WD PTMs (n=6) initiated the WD 63 days before SARS-CoV-2 infection and were fed the WD until Nx on days 30-32 SARS-CoV-2. WD+SIV PTMs (n=6) initiated the WD, were infected with SIV 62 days later, and infected with SARS-CoV-2 35 days after SIV; Nxs were days 21-32 SARS-CoV-2. Additionally, inflammatory markers in paired human plasma samples from acute SARS-CoV-2 infection and a follow-up (recovered) time point were investigated for external validation of the NHP infection model.

Results

SARS-CoV-2 subgenomic RNA (sgRNA) shedding was prolonged in stool and nasal swabs (NS) in WD vs. control PTMs. Immune responses to SARS-CoV-2 were significantly reduced: peripheral lymphocytes on day 8 were significantly lower and IL6 was significantly reduced at peak in WD PTMs vs. controls. Lung pathology was modestly increased versus controls. SIV co-infection prolonged SARS-CoV-2 sgRNA shedding in NS and bronchoalveolar lavage fluid, and significantly enhanced lung pathology vs. WD PTMs. SIV-infected PTMs demonstrated significant elevations in inflammatory markers including IL6, CXCL11, and CXCL9 vs. WD PTMs. MX1 expression in lymph nodes also significantly increased with SIV co-infection. IL6 increased modestly from pre-infection to day 4 SARS-CoV-2 in control PTMs, while it decreased modestly from acute infection to recovery in human plasma, suggesting resolution of an acute SARS-CoV-2 infection IL6 inflammatory response.

Conclusions

Both WD and SIV co-infection enhanced SARS-CoV-2 pathogenesis in PTMs, with increased pathology in both conditions and prolonged SARS-CoV-2 RNA shedding suggesting poorer viral control. However, mechanisms of pathogenesis appear distinct, with suppressed immunity in WD PTMs and a heightened inflammatory state with SIV co-infection. PTM SARS-CoV-2 infection also recapitulated an inflammatory response observed in humans. These findings improve our understanding of unique impacts of diet and co-infection on COVID-19.

Conserved Sex-Specific Networks in the Aging Rhesus Macaque Hippocampus Reveal Glial and Mitochondrial Mechanisms Relevant to HIV-Associated Neurocognitive Disorder

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Background: Chronic neuroinflammation is a defining feature of brain aging and is increasingly recognized as a shared hallmark of multiple neurological disorders, including HIV-associated neurocognitive disorders (HAND). Aging and HAND exhibit overlapping inflammatory signatures, including glial activation, mitochondrial dysfunction, and white matter injury, yet the conserved molecular networks underlying these processes remain poorly understood. Because rhesus macaques closely recapitulate human brain aging and are widely used to model HIV neuropathogenesis, they provide a powerful translational system for identifying molecular mechanisms of neuroinflammatory aging. **Methods:** We performed weighted gene co-expression network analysis (WGCNA) on hippocampal RNA sequencing data from young (mean age = 5y) and aged (mean age = 26y) male and female rhesus macaques to identify age- and sex-associated transcriptional networks. Conserved co-expression modules were identified through comparison with human hippocampal single-nucleus RNA sequencing data using ortholog mapping, hypergeometric overlap analysis, and pathway enrichment. Isoform-level WGCNA identified aging-associated alternative splicing networks. **Results:** Aging was associated with extensive sex-specific transcriptional remodeling of pathways implicated in neuroinflammation. Cross-species analysis identified a conserved female-biased neuroinflammatory co-expression module enriched for oligodendrocyte differentiation, myelin maintenance, and axon ensheathment, consistent with disruption of glial homeostasis and white matter integrity, processes that contribute to persistent neuroimmune activation. A second conserved module exhibited reduced expression during female aging and was enriched for oxidative phosphorylation, mitochondrial electron transport, and ATP synthesis, indicating impaired mitochondrial bioenergetics that may promote oxidative stress and inflammatory signaling. Isoform-level network analysis identified six female-specific co-expression modules absent in males, including modules enriched for NF- κ B-associated alternative splicing, microglial activation, and cerebrovascular smooth muscle remodeling, suggesting coordinated regulation of inflammatory, metabolic, and vascular pathways. **Conclusions:** Together, these findings define a conserved female-biased molecular signature of neuroinflammatory aging characterized by glial remodeling, mitochondrial dysfunction, and inflammatory isoform regulation, establishing the rhesus macaque as a valuable translational model for investigating sex-specific mechanisms of chronic neuroimmune activation. The overlap between these aging-associated molecular programs and inflammatory pathways implicated in HAND suggests that healthy brain aging and HIV-associated neuroinflammation engage common biological processes, providing insight into how aging may influence neurocognitive impairment in people living with HIV.

Peripheral Cytokine Immunotherapy Reshapes SIV-infected Rhesus Macaque Brain Immune Response

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Background: HIV-associated neurocognitive disorders (HAND) persist despite anti-retroviral therapy, and peripheral immune interventions may modulate CNS inflammation without direct brain delivery. In SIVmac251-infected rhesus macaques, we tested whether peripheral cytokine administration (IL-21, IL-15) or IL-15 neutralization (anti-IL-15) alters CNS immune responses. Anti-IL-15 reduces proliferation and activation of cytotoxic lymphocytes and was included to test whether limiting IL-15 signaling would dampen, rather than promote, neuroinflammation.

Methods: Four groups of animals received peripheral IL-21, IL-15, anti-IL-15, or PBS during chronic SIV infection. RT-qPCR of plasma from each animal quantified viral loads. Viral loads did not differ across treatment groups. Brain tissue was evaluated by immunohistochemistry and immunofluorescence (IHC/IF) for macrophages and microglia, SIV-infected cells, CD3+ T cells, astrocyte activation, cytokine expression, and blood-brain barrier integrity. Visium HD libraries were aligned to the *Macaca mulatta* genome for section-level bulk RNA sequencing. Curated transcript modules were compared to PBS, and depth-matched downsampling assessed sequencing artifacts.

Results: IHC demonstrated treatment-specific CNS remodeling. Anti-IL-15 increased brain and SIV+ macrophages despite reduced cytotoxic lymphocyte activation. IL-15 increased microglial IL-6 and preserved endothelial CD31. IL-21 increased microglial IL-15, astrocyte activation, macrophage TGF- β , but also worsened BBB. CD3+ T cell abundance was unchanged across treatments. Bulk RNA-seq supported stable T cell abundance, concordant with IHC. The most robust RNA signal was in anti-IL-15 versus PBS, revealing elevated myeloid chemokines, macrophage/disease-associated microglia markers, interferon-stimulated genes, complement, and astrogliosis, indicating that neutralizing IL-15 signaling did not limit, and instead amplified, myeloid-driven neuroinflammation. These patterns were retained after PBS downsampling.

Conclusions: Peripheral infusion of IL-15, IL-21 and anti-IL15 altered CNS immune and inflammatory response without changing SIV viral quantities. Bulk RNA seq identifies peripheral anti-IL-15 with a coherent pro-inflammatory brain transcriptome despite stable T cell abundance, while IHC shows IL-15 affects microglial cytokines and barrier integrity, and IL-21 affects astrocyte activation and BBB. Rather than limiting immune-mediated damage, IL-15 neutralization was associated with heightened myeloid inflammation, suggesting IL-15 signaling may normally constrain, rather than drive, brain myeloid activation. These data support peripheral cytokine therapies with IL-15 and IL-21 to modulate HAND-relevant neuroinflammation.

Deep spatial profiling of granuloma architecture in the tuberculosis lung identifies distinct immunometabolic and cellular niches

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Background

Tuberculosis (TB) is the leading infectious disease killer worldwide and accounts for ~150,000 deaths annually among people living with HIV. Lung granulomas, the hallmark pathological feature of TB, show extensive heterogeneity even within a single lung lobe. Classical necrotic granulomas, which have caseous-necrotic cores surrounded by myeloid cells and lymphocytes, are typically permissive for *M. tuberculosis* (Mtb) growth, while cellular granulomas are non-necrotic and thought to be more restrictive. However, how the geospatial organization of immune cells and their communication networks (granuloma topology) impact the host immune function within granuloma microenvironments remains poorly understood.

Methods

We used tissue cyclic immunofluorescence (t-CyCIF) and spatial transcriptomics (GeoMx) to delineate cellular versus necrotic TB granuloma topology. Rhesus macaques (N=3) were infected with ~200 CFU of Mtb CDC1551 via aerosol. At ~12 weeks post-infection, six lung sections from two lung lobes per animal were obtained at necropsy for histopathological characterization and whole-slide t-CyCIF imaging of phenotypic and functional markers for myeloid, lymphoid, and non-immune cells. Images were processed via MCMICRO; downstream analysis was performed using SCIMAP. Machine learning tools, including KNN, SCIMAP, and QUICHE, were used to analyze granuloma topology; GeoMx data were analyzed with StandR and DESeq2.

Results

A total of 180 individual granulomas were identified and categorized as necrotic (central caseous necrosis) or cellular (dense macrophage/T-lymphocyte aggregates). Spatial machine learning defined 11 distinct cellular zones within granulomas. Within the epithelioid histiocyte layer, necrotic granulomas were enriched in metabolically active (mTOR+COX4+) CD206+HLA-DR+ macrophages expressing immunosuppressive molecules (IDO, IL4I1), found proximal to CTLA4+ T cells. Cellular granulomas instead had significantly higher frequencies of activated Ki-67+IFN γ +GZMB+ CD4/CD8 T cells, HLA-DR+pStat1+ B cells and CD68+ macrophages across multiple zones. Recurrent neighborhood analyses revealed strong neutrophil-macrophage-DC interactions in necrotic granulomas, whereas cellular granulomas showed greater myeloid-T cell crosstalk and robust T/B cell interaction networks.

Conclusions

Using highly multiplexed single-cell spatial profiling, we show striking divergence in granuloma topology and immune networks between cellular vs necrotic TB granulomas. Ongoing work will integrate spatial transcriptomics and extend findings to SIV/Mtb NHP model. These studies provide novel insights that can be leveraged to improve vaccines and immunotherapeutics for TB.

Cure and Substance Abuse Effects on HIV Reservoirs

THURSDAY, OCTOBER 22, 2026



Modeling Ultra-Small Latent SIV Reservoirs in Rhesus Macaques and Evaluating Viral Rebound

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Background

Achieving antiretroviral therapy (ART)-free remission or post-treatment control (PTC) of HIV infection requires the elimination or substantial depletion of the latent reservoir, a population of infected cells that persist under ART suppression and rapidly reactivate upon treatment interruption. Following natural infection, people living with HIV (PLWH) typically rebound within 10-14 days post-ART cessation. It is widely accepted that smaller reservoirs extend time to viral rebound (TTR), although the direct association between reservoir size and TTR is unclear. To address this, we establish defined simian immunodeficiency virus (SIV) reservoirs in rhesus macaques (RMs) to evaluate how reservoir size affects TTR after ART cessation.

Methods

We use a novel method to generate latently infected primary CD4⁺ T cells in vitro using barcoded SIV (SIV-mac239Opt5M). To establish in vivo reservoirs of specific sizes, we autologously infuse defined numbers of these cells into two groups of RMs (n=6 per group) receiving either 100,000 or 10,000 latently infected cells. Based on mathematical models of viral rebound, we hypothesize that TTRs increase as reservoir sizes decrease, predicting average TTRs of 21 and 45 days, respectively. After stopping ART, we monitor viral loads and compare average TTRs across treatment groups.

Results

To date, five RMs have received 100,000 latently infected cells. Four rebounded 11-39 days post-ART (mean 22 days), consistent with the prediction of ~21 days; the fifth remains aviremic more than 6 months after ART cessation. Six RMs have received 10,000 latently infected cells: two rebounded, at 25 days and a remarkable 280 days post-ART, while four remain aviremic at various times off ART. The 280-day rebound is the longest we have observed and shows that a small reservoir can delay rebound for many months. Enrollment, monitoring, and barcode analysis are ongoing.

Conclusions

Our model directly measures how reservoir size affects viral rebound, addressing a key question in HIV cure research that is difficult to resolve with clinical studies alone. By precisely controlling reservoir size at the time of ART cessation, this system enables a direct and quantitative test of the reservoir size–TTR relationship that is not feasible in humans.

Combined SMACm and gp120 targeting Tri-specific killer engager treatment limits the intact SIV reservoir in ART-treated rhesus macaques

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Background:

HIV cure requires strategies capable of both exposing and eliminating persistently infected cells surviving long-term antiretroviral therapy (ART). Specifically, “shock-and-kill” approaches aim to reverse viral latency, induce viral antigen expression, and enhance immune-mediated clearance of infected cells. Here, we evaluated a combination of a novel bivalent second mitochondria-derived activator of caspases mimetic (SMACm) as a latency-reversing agent and a gp120-targeting Tri-specific killer engager (TriKE) to promote NK cell-mediated elimination of infected cells.

Methods:

Ten SIVmac239-infected rhesus macaques (RMs) initiated ART at 4 weeks post-infection. After 13 months of ART, RMs received five intravenous SMACm doses every two weeks using a step-dose escalation (0.125–0.5 mg/kg). Starting with the third dose, TriKE was administered subcutaneously three times during the week following each SMACm infusion. Three additional SMACm doses (0.35–1 mg/kg) were subsequently administered. Plasma viral load, intact proviral DNA, and T/NK cell phenotypes in blood and lymph nodes (LNs) were longitudinally assessed.

Results:

SMACm was bioactive during ART and induced modest viral reactivation in the absence of previously reported adverse effects. TriKE potently and transiently expanded CD16+CD56+ NK cells in blood and LNs, increasing NK activation, proliferation, and cytotoxicity-associated markers. The combined intervention caused transient peripheral CD4 T-cell decreases, increased memory CD4 T-cell proliferation, and central/transitional memory expansion without global activation. Peripheral CD4 T-cells showed reduced BCL-2 expression and decreased CCR5+CXCR3+ transitional memory frequencies. Finally, SMACm/TriKE combination was associated with a significant reduction of the intact viral reservoir in LNs.

Conclusions:

SMACm/TriKE combination therapy is safe, increases antiviral immune responses, and slightly reduces viral reservoirs in ART-suppressed RMs, supporting further development of latency reversal and NK cell-based strategies toward an HIV cure.

Targeting the CNS with AAV-Delivered Anti-HIV Antibodies in SHIV-Infected Rhesus Macaques

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HIV can enter the central nervous system(CNS) early after infection and establish persistent viral reservoirs. While intramuscular administration of recombinant adeno-associated virus(AAV) vectors encoding potent broadly neutralizing antibodies(bNAbs) has the potential to provide durable systemic HIV suppression, antibody penetration into the CNS is limited. In addition, anti-drug antibody(ADA) responses against the expressed bNAbs can reduce antibody levels and compromise therapeutic efficacy. We evaluated whether intrathecal AAV delivery could achieve antibody expression within the CNS while taking advantage of the relative immune privilege of this compartment to limit ADA formation.

Three SHIV-infected rhesus macaques received intrathecal administration of recombinant AAV9 vectors encoding the anti-HIV bNAbs 3BNC117 and 10-1074 at 8 weeks post-infection. Antibody levels and ADA responses were measured in cerebrospinal fluid(CSF) and serum by ELISA. Tissues collected 9 weeks after AAV administration were analyzed by single-molecule RNA in situ hybridization (RNAscope) to assess transgene expression and tissue distribution.

3BNC117 was detected in the CSF of all three animals following AAV administration, while 10-1074 was detected in the CSF of two of the three animals. Both antibodies were also detected in serum. Peak serum antibody levels were associated with a transient reduction in plasma SHIV viral loads. Two animals developed early ADA responses against both antibodies, which correlated with a rapid decline in antibody levels in serum and CSF within 4-6 weeks post-AAV administration. The third animal developed delayed ADA responses and exhibited a more gradual decline in antibody levels. RNAscope analysis confirmed expression of both transgenes in CNS tissues, including the brainstem and cerebellum. Notably, it also revealed transgene expression in peripheral tissues, including the liver, spleen, and lymph nodes.

Intrathecal AAV9 delivery achieved expression of HIV bNAbs in the CNS, generated detectable systemic antibody levels, and was associated with transient antiviral activity. Although this approach did not prevent ADA development, these findings establish the feasibility of CNS-targeted antibody gene transfer and highlight the need for strategies to mitigate ADA responses to achieve durable therapeutic expression. More broadly, intrathecal AAV-delivery may provide a platform for sustained CNS expression of therapeutic antibodies for HIV and other neurological, infectious, and neurodegenerative diseases.

Building a Kitchen Sink: Iterative Development of Combinatorial HIV Cure Approaches in Nonhuman Primate Models

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Background: Potent antiretroviral therapy (ART) regimens for people living with HIV (PLWH) typically integrate small molecules that inhibit multiple stages of the viral life cycle. An increasing body of data suggests that to deplete or eliminate latent HIV reservoirs, multiple orthogonal cure approaches must also be integrated. Over the past decade, we have designed and evaluated multiple so-called “Kitchen Sink” HIV cure approaches in nonhuman primates (NHPs). Here we assess the most and least promising approaches for future NHP and clinical experiments.

Methods: We have studied over 100 rhesus and pigtail macaques with ART-suppressed SIV/SHIV infection. Combinations of two to four candidate cure components included latency reversing agents (LRAs), broadly neutralizing antibodies (bNAbs), therapeutic vaccines, immune-enhancing cytokines, CCR5/provirus gene editing, and/or chimeric antigen receptors (CARs).

Results: Combining Smac Mimetic LRAs (“Kick”) plus eCD4-Ig (“Kill”) failed to reduce reservoir size in SHIV-infected, ART-suppressed rhesus macaques. bNAbs and hematopoietic stem cell gene therapy delayed viral rebound but did not achieve curative endpoints. Our most recent data suggest that analytical treatment interruption (ATI) is essential for antigen-dependent cure strategies, while LRAs may be insufficient.

Conclusions: Our collective experience guides future NHP study designs and informs the best combinations to test in the clinical setting. Frequently, the most promising/potent monotherapies must be carefully balanced to address toxicities that limit feasibility in PLWH. For antigen-dependent therapies, careful alignment relative to ATI and virus recrudescence was critical across our studies. We are currently investigating orthogonal, antigen-independent approaches that could be included in future combinatorial treatments.

Adjunctive cannabinoids remodel the neuroimmune landscape in HIV/ART: A single-cell multi-omic study

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Background: Despite suppressive antiretroviral therapy (ART), chronic immune activation and systemic inflammation persist in people living with HIV, driving comorbidities such as HIV-associated neurocognitive disorders (HAND). HAND pathogenesis involves a complex interplay of persistent neuroinflammation, ART toxicity, metabolic dysfunction, and psychosocial factors. Developing adjunctive therapies to target this unresolved neuroinflammation remains an urgent, unmet clinical need. Having previously demonstrated the anti-inflammatory properties of cannabinoids in the gut and central nervous system during HIV/ART, we sought to elucidate these protective mechanisms at single-cell resolution.

Methods: Accordingly, we investigated the effects of a blood-brain barrier (BBB) permeable, long-term Δ9-tetrahydrocannabinol (THC):Cannabidiol (CBD) (1:3 ratio) formulation on the basal ganglia of SIV-infected, ART-treated rhesus macaques. Using paired single-nuclei RNA-sequencing and ATAC-sequencing, we compared VEH/SIV/ART (n=4) and THC:CBD/SIV/ART (n=4) cohorts, identifying 18 distinct cell populations.

Results: Ingenuity Pathway Analysis (IPA) revealed that oxidative phosphorylation was significantly upregulated across nearly all cell types, except astrocytes, in the cannabinoid-treated group, indicating a metabolic shift toward an anti-inflammatory state. Crucially, THC:CBD treatment significantly downregulated 5 pro-inflammatory interferon stimulated genes, and upregulated 22 anti-inflammatory, M2-associated genes in microglia, including neuropilin-1 (*NRP1*), signaling polarization toward a reparative, immune-regulatory phenotype. Parallel ATAC-seq analysis demonstrated enhanced chromatin accessibility at the *NRP1* locus, confirming that this transcriptional activation is driven by epigenetic remodeling. Finally, immunofluorescence validated a significant increase in NRP1 protein expression. Furthermore, IPA revealed activation of ribosomal quality control signaling in microglia, suggesting improved protein homeostasis and reduced accumulation of damaged proteins that contribute to neurodegeneration. In D1 dopamine receptor-expressing medium spiny neurons, THC:CBD reduced long-term depression signaling, indicating preservation of synaptic integrity and neuronal communication that are typically disrupted during HIV/ART.

Conclusions: Overall, these findings demonstrate that adjunct THC:CBD therapy remodels the neuroimmune landscape by promoting oxidative phosphorylation, anti-inflammatory microglial polarization, and neuroprotection. Given its ability to readily cross the blood-brain barrier, cannabinoid therapy represents a promising adjunctive strategy to alleviate chronic neuroinflammation in HIV/ART, with potential therapeutic applications in other neuroinflammatory and neurodegenerative disorders beyond people living with HIV.

CSF1R inhibition reduces myeloid reservoirs and suppresses viral rebound in virally suppressed chronic SIV infection

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Persistent viral reservoirs in individuals living with HIV-1 remain a major barrier to cure, with myeloid cells such as tissue-resident macrophages contributing to residual inflammation and viral rebound when antiretroviral therapy (ART) stops. Our previous study demonstrated that CSF1R inhibition depleted perivascular macrophages and reduced SIV DNA levels in the brain during acute infection, supporting a role for CSF1R signaling in maintaining macrophage-associated viral reservoirs. However, whether CSF1R inhibition can reduce viral persistence and limit viral rebound during chronic SIV infection remains unknown. Here we show that treatment with the CSF1R inhibitor BLZ945, administered either early (n=8) or late (n=24) during ART, significantly reduced peak plasma viral load following ART interruption in macaques with chronic SIV infection. BLZ945 also suppressed SIV RNA in the cerebrospinal fluid to undetectable levels and reduced viral DNA and RNA across multiple brain regions when compared to ART-only controls (n=10). In the frontal and parietal cortex, BLZ945 treatment reduced the numbers of CD163⁺ and CD68⁺ macrophages and specifically decreased the number of CD163⁺ macrophages harboring SIV RNA and SIV DNA, with a strong positive correlation between macrophage abundance and viral RNA and SIV DNA levels. Transcriptomic analysis revealed downregulation of genes associated with endothelial activation and vascular remodeling, as well as pathways linked to synaptic signaling and immune responses. Immunohistochemical analyses of MAP2, synaptophysin, and CXCL16 were consistent with these transcriptomic changes. Collectively, these findings identify CSF1R inhibition as a potential strategy to reduce CNS myeloid reservoirs and viral persistence and mitigate neuroinflammation in chronic HIV/SIV infection.

Autologous Expanded NK Cell Therapy Delays Viral Rebound and Restricts Reservoir Reactivation Following ART Interruption in a Molecularly Barcoded SIV Model

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Introduction: Despite effective antiretroviral therapy (ART), HIV persistence remains an obstacle to achieving durable ART-free remission. Here, we evaluated the efficacy of autologous ex vivo expanded NK (eNK) cell therapy in the simian immunodeficiency virus (SIV) non-human primate model using a molecularly barcoded SIV to quantify viral rebound dynamics and the complexity of viral lineages following analytical treatment interruption (ATI).

Methods: Fifteen rhesus macaques were infected with molecularly barcoded SIVmac239M2 and initiated ART 14 days post-infection. After 1.5 years of ART, animals were randomized to sham (n=7) or eNK treatment (n=8). NK cells were expanded using feeder cells expressing membrane-bound IL-21. Treated animals received two infusions of autologous eNK cells, one day before and two days after the first ATI (ATI1). Four months after ATI1, all animals resumed ART for 6 months before undergoing a second treatment cycle with two additional autologous eNK infusions followed by a second ATI (ATI2). Following each ATI, plasma viral RNA was quantified to assess time to rebound and post-rebound viral set point and sequenced to determine the number and diversity of rebounding viral barcodes.

Results: Administration of eNK cells delayed plasma viral rebound compared with sham-treated animals following both ATIs, reaching statistical significance after ATI2 using the linear mixed effect model (ATI1, $p = 0.08$; ATI2, $p = 0.03$). However, analysis across both ATIs using the Andersen-Gill proportional hazards model shows that eNK treatment reduced the risk of plasma viral rebound by 83% (95% CI: 64-92%), demonstrating enhanced post-treatment viral control. Barcode analysis further showed that eNK-treated animals exhibited significantly fewer rebounding viral RNA barcodes (eNK median 2, IQR 1-5 vs. sham median 7, IQR 5-16; $p = 0.02$) as well as decreased barcode diversity (eNK median 0.03, IQR 0-0.45 vs. sham median 0.62, IQR 0.53-1.61; $p = 0.001$).

Conclusions: Autologous eNK cell therapy demonstrated efficacy in the rhesus macaque SIV model by significantly delaying plasma viral rebound and reducing the complexity of rebounding viral lineages. These findings support further investigation of NK cell-based immunotherapies as a safe and promising component of HIV cure strategies aimed at achieving durable ART-free remission.

Translational Innovations & Model Optimization

FRIDAY, OCTOBER 23, 2026



Development of Long-Acting Antiretroviral Therapy for Sustained SIV Suppression in Rhesus Macaques

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Background: Long-acting antiretroviral therapy (LA-ART) reduces pill burden and improves adherence, but its effects on viral reservoir establishment and analytical treatment interruptions (ATI) remain poorly understood. We developed LA formulations of lenacapavir (LEN) and cabotegravir (CAB) for SIV suppression in rhesus macaques (RM) and compared this combination to daily ART containing Tenofovir Disoproxil Fumarate, Emtricitabine, and Dolutegravir (QD-ART).

Methods: LEN and CAB formulations were developed based on estimated SIV inhibitory target concentrations. SIVmac239M-infected RMs received 600mg subcutaneous LEN every 8 weeks and 1450mg intramuscular CAB every 4 weeks starting 12 days post-infection and were compared with RMs receiving QD-ART for 13 months (n=5/group). SIV viral loads and reservoirs, drug pharmacokinetics, and tolerability were monitored.

Results: Repeated LEN and CAB injections were well tolerated and maintained target plasma concentrations. At ART initiation, plasma viral loads ranged from 10^7 - 10^8 copies/ml. LA-ART suppressed viremia to the limit of quantification significantly faster than QD-ART. Analysis of cell-associated viral DNA in blood and tissues revealed no differences between groups. However, cell-associated viral RNA was significantly lower in RM receiving LA-ART early after initiation, consistent with faster virologic suppression. Analysis of post-ATI pharmacokinetics, viral rebound kinetics, and rebound virus sequencing are ongoing and will be presented.

Conclusions: LEN and CAB LA-ART achieved durable suppression of SIVmac239M without altering viral reservoir establishment compared with QD-ART. This regimen delivers potent activity, eliminates daily injections, better models clinical LA-ART use, and provides a platform to evaluate the impact of long-acting therapies on ATI outcomes in HIV cure-focused NHP studies.

A Dual-Reservoir Long-Acting Implant for Sustained Combination Antiretroviral Treatment in SHIV-Infected Nonhuman Primates

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Background: Long-acting drug delivery systems represent a promising approach to improve treatment adherence and maintain therapeutic antiretroviral exposure. Here, we evaluated a novel 3D-printed resin implant containing two independent drug reservoirs, each loaded with an antiretroviral agent with a distinct mechanism of action, enabling simultaneous and sustained co-delivery of combination therapy in a SHIV-infected nonhuman primate (NHP) model.

Methods: Chronically SHIVSF162P3-infected NHPs received either islatravir (ISL) + tenofovir alafenamide (TAF) (n=4) or ISL + bictegravir (BIC) (n=3). Uronic acid (UA) was incorporated into the TAF formulation to improve stability, and MCC950 was included to mitigate local inflammation associated with TAF. Plasma viral load (pVL), intracellular ISL-triphosphate (ISL-TP) and tenofovir diphosphate (TFV-DP), and plasma BIC levels were monitored for 90 days.

Results: All four NHPs receiving ISL+TAF achieved complete viral suppression, with pVL declining from $\sim 10^4$ vRNA copies/mL to below the limit of detection within 20 days and remaining undetectable through day 90. Median ISL-TP and TFV-DP concentrations were maintained at 134.5 and 283.5 fmol/ 10^6 peripheral blood mononuclear cells (PBMCs), respectively, exceeding the protective thresholds for ISL-TP (50 fmol/ 10^6 PBMCs) and TFV-DP (40 fmol/ 10^6 PBMCs). In the ISL+BIC group, 2/3 NHPs achieved complete viral suppression, with pVL declining to below the limit of detection around 20 days and remaining undetectable through day 90. The remaining one NHP developed persistent viremia, with subsequent detection of the reverse transcriptase M184V resistance mutation. Across the ISL+BIC group, ISL-TP concentrations remained above the target exposure threshold (median >89.0 fmol/ 10^6 PBMCs), while plasma BIC concentrations remained subtherapeutic at approximately 1 ng/mL. Throughout the study, all NHPs maintained stable body weights with no treatment-related safety concerns observed.

Conclusions: A dual-reservoir implant enabled sustained co-delivery of antiretroviral combinations and durable virologic suppression in SHIV-infected NHPs. The emergence of M184V in an animal with inadequate BIC exposure highlights the importance of maintaining sufficient exposure to both agents for effective combination therapy and resistance prevention. Overall, these findings support the potential of this implant platform as a long-acting strategy for HIV treatment.

PD-L1-based immune shielding confers durable AAV-vectorized bNAb expression and protection against SHIV challenge in rhesus macaques

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Background: A single intramuscular administration of adeno-associated virus (AAV)-vectorized HIV-1 broadly neutralizing antibodies (bNAbs) could confer years of HIV-1 immunity. However, anti-bNAb host immune responses, including anti-drug antibodies (ADA), limit effective bNAb concentrations. Lymphocytic infiltration is commonly observed at sites of intramuscular AAV administration and presents an opportunity to locally inhibit immune responses and preserve transgene expression. To this end, we employed an immune shielding strategy that leverages the PD-1/PD-L1 immune checkpoint pathway to inactivate muscle-infiltrating immune effector cells. Here, we test whether PD-L1 co-delivery enhances the durability of bNAb 3BNC117 expression in rhesus macaques (RMs).

Methods: Three groups of RMs (n=6/group) received AAV9 vectors encoding 3BNC117, 3BNC117 and PD-L1, or PD-L1 only in a 52-week study. Serum 3BNC117 concentrations and ADA titers were determined by ELISA over the study period. Twenty weeks post-AAV administration, RMs were challenged intrarectally with SHIV_{AD8-EO} every 2 weeks for a total of 10 challenges and plasma viremia was determined by RT-qPCR. At necropsy, we assessed inflammation and lymphocytic infiltration at AAV injection sites by immunohistochemistry and performed Visium HD spatial transcriptomics to evaluate immune gene expression and muscle health.

Results: PD-L1 co-delivery resulted in significantly increased 3BNC117 concentrations, reduced ADA endpoint titers, and significantly greater protection from SHIV_{AD8-EO} challenge, whereas 3BNC117-only and PD-L1-only groups were not significantly protected. PD-L1 co-delivery also prevented severe local inflammation, muscle injury, and tertiary lymphoid structure (TLS) formation at the administration site. Importantly, TLSs actively produced immunoglobulins, and greater TLS severity associated with higher ADA titers.

Conclusions: We implicate TLS formation as a driver of ADA responses following intramuscular AAV administration and demonstrate that PD-L1-based immune shielding prevents TLS formation and enables durable bNAb expression.

Optimization of Imaging Cytometry for Cross-Species Translational Tissue Analyses

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Background:

Traditional immunohistochemistry (IHC) techniques used to study disease and biomedical intervention dynamics often require custom antibodies, specialized image analysis pipelines, and destructive tissue processing. Imaging cytometry (ChipCytometry™) overcomes these limitations through iterative cycles of staining, imaging, and photo-bleaching using commercially available fluorescent antibodies, and image analysis post-cell segmentation by flow cytometry-based or spatial analysis pipelines. Although imaging cytometry has been applied to clinical and pre-clinical tissue samples, standardized workflows comparing human and rhesus macaque (RM) model tissues remain limited. Herein, we established and validated cross-species antibody panels and staining protocols for multiple organs and demonstrated quantitative single-cell spatial analysis using standard bioinformatics pipelines.

Methods:

Archived OCT-embedded placenta, liver, kidney, and jejunum from humans and RMs were analyzed. Tissue sections were stained with optimized antibody panels and imaged using the ZellScanner One microscope. Images were processed in QuPath software and analyzed using flow cytometry-style gating and a Python-based Structured Spatial Analysis for CODEX Exploration (SPACEc) pipeline.

Results:

Pan-cytokeratin identified hepatocytes, intestinal epithelium, and trophoblasts in both species, while smooth muscle actin was used to indicate vascular structures. CD45+ immune cells, including macrophages (CD163+), T cells (CD3+CD8+), and NK cells (NKG2A/C+), were detected in liver and jejunum. Placental imaging revealed trophoblast-lined villi, vimentin+ stromal cells, Hofbauer cells (CD163+), and extravillous CD8+ cells. Species- and gestational age-dependent differences in neonatal Fc receptor (FcRn) expression on trophoblasts and Hofbauer cells were identified, which may impact maternal antibody transfer. In the RM kidney, tissue architecture was well preserved despite immune cell infiltration. Although T cells, activated NK cells, leukocytes, and antigen-presenting cells were present, HLA-DR+CD14+ macrophages/monocytes comprised approximately 34% of immune cells, consistent with their recruitment to inflammatory sites. Spatial analysis identified distinct cellular neighborhood zones: epithelium, NK-epithelium, T-APC-epithelium, immune zone, and immune-epithelium regions.

Conclusion:

Imaging cytometry combined with standardized bioinformatics workflows enables high-resolution multiplexed imaging and comprehensive spatial profiling across species. The platform's ability to identify similarities and differences between the species, together with the flexibility to employ complementary analysis pipelines, highlights its potential for investigating tissue homeostasis, pregnancy, transplant rejection, and disease progression relevant to infectious and chronic diseases including HIV/SIV infection.

Developing novel CD8 binders for in vivo delivered anti-HIV T cell gene therapies in the macaque model

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Background: Antiretroviral therapy (ART) fails to reach 23% of people living with HIV (PWH), and is not curative. The persistent viral reservoir is maintained in part by insufficient HIV-specific CD8⁺ T-cell responses. **We aim to accelerate HIV-specific CD8⁺ proliferative responses via *in vivo* CD8⁺ T-cell engineering**, democratizing access globally via a single injection with a modified lentiviral vector (LV) to engineer T-cells within the body. We hypothesize that LVs pseudotyped with mutated vesicular stomatitis virus envelope glycoprotein, engineered to remove native tropism, and co-expressed with engineered anti-CD8 single-chain variable fragments (scFvs) will enable highly specific CD8⁺ T-cell delivery *in vivo* with minimal off-target biodistribution.

Methods: We designed *ex vivo* experiments in primary pigtail macaque cells, chosen for their close genetic, physiological and immunological similarity to humans and unique susceptibility to clinical LVs. We *de novo* engineered 2 anti-CD8 scFvs and compared to 3 anti-CD8 scFvs from the literature. We then generated an initial panel of 14 anti-CD8 scFv-targeted LVs, varying based on scFv sequence, scFv orientation, and linker sequences between the scFv and the transmembrane domain. Each candidate CD8-targeted LV was tested by flow cytometry in vector particle-focused cell delivery assays, evaluating scFv surface display (anti-G₄S-linker staining), binding capacity (soluble fluorescent CD8αβ-heterodimer assay), and transduction potency in primary pigtail macaque cells *ex vivo*.

Results: We demonstrated that 6 LV constructs enable highly specific CD8⁺ T-cell transduction with minimal off-target effects in activated T-cells. Since most LV-targeted T-cells *in vivo* are not activated, ongoing experiments are evaluating LV efficiency in resting T-cells. Top candidates will be tested in primary pigtail macaque PBMCs and in *ex vivo* whole blood assays, and benchmarked against existing delivery systems.

Conclusions: These results confirm that scFv-mediated CD8 targeting is feasible and specific in an NHP primary cell model. Next, we will assess *in vivo* specificity, biodistribution, and safety of a top performing anti-CD8 LV encoding a GFP-reporter in a translationally relevant pigtail macaque model that will inform future clinical studies. Successful development of this platform will pave the way towards our central goal to democratize global access to targeted CD8 T-cell therapies for PWH.

Single-nucleus RNAseq of the Maternal-Fetal Interface in Rhesus Macaques

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Background

Congenital cytomegalovirus (cCMV) infection is the leading infectious cause of birth defects including sensorineural hearing loss. There is as yet no maternal vaccine to prevent cCMV. Our laboratory has established a placental transmission model of congenital rhesus CMV (RhCMV) in rhesus macaques to study immune determinants of cCMV protection in a translational animal model. Macaque pregnancies share key similarities to human pregnancy: long gestational periods, placental architecture, and blood exchange mechanisms, but transcriptional data at single-cell resolution of intact placenta are limited. Single nucleus RNA sequencing (snRNAseq) technologies allow investigators to interrogate biology at high resolution and granularity in frozen tissues.

Methods

To interrogate the maternal-fetal interface, we performed snRNAseq on full-thickness, frozen placenta at near term gestation in four Indian-origin rhesus macaques; two RhCMV-seropositive dams and two RhCMV-seronegative dams experimentally inoculated with RhCMV in early second trimester gestation. Sample libraries were prepared using both Parse Biosciences and 10x Genomics library kits.

Results

After quality control filtering, the Parse dataset contained 113,221 cells and the 10X dataset contained 21,979 cells. The Parse dataset had 1.76-fold greater unique genes/cell and 4.52-fold greater RNA counts/cell relative to the 10X dataset. Out of the total number of cells, immune cells made up 14.4% (16,329 cells) in the Parse dataset and 10.1% (2,222 cells) in the 10X dataset. At a resolution of 0.6, we found that datasets from both kits had comparable frequencies of non-immune cells (trophoblast subtypes, endothelial cells, fibroblasts, pericytes, decidual stromal cells). However, after subsetting the immune cells at a resolution of 0.5 in both datasets, the Parse dataset revealed populations of CD4⁺ T regulatory cells, CD8⁺ T cells, proliferating NK cells, proliferating Hofbauer cells, and monocyte-derived maternal macrophages that could not be distinguished at the same resolution in the 10X dataset.

Conclusions

These findings lead us to support the use of Parse Biosciences kits as a strong alternative to 10X Genomics for snRNAseq library generation. Furthermore, differential gene expression between the two experimental cohorts across all annotated cell types using the Parse dataset is ongoing and will be presented at this conference.

Pharmacokinetics and Protective Efficacy of Fc-Engineered HIV-1 bNAb 3BNC117-LS in Pregnant Rhesus Macaques

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Women bear a disproportionate burden of HIV/AIDS, particularly in sub-Saharan Africa, where they account for >60% of new infections. Pregnancy and the postpartum period are associated with increased HIV-1 susceptibility, creating repeated windows of risk in high-fertility settings. Although broadly neutralizing antibodies (bNAbs) can suppress HIV-1, it remains unclear how pregnancy and placental FcRn activity affect the pharmacokinetics and efficacy of bNAbs containing half-life-extending LS mutations (M428L/N434S). To address this gap, Study A compared the pharmacokinetics of “rhesusized” (rh)-3BNC117-IgG1 with or without LS in pregnant rhesus macaques (RhMs), while Study B evaluated rh-3BNC117-IgG1-LS protection against intravaginal SHIV challenge during pregnancy.

In Study A, third-trimester pregnant RhMs received a single 30 mg/kg infusion of rh-3BNC117-IgG1 (n=7) or rh-3BNC117-IgG1-LS (n=6) and underwent cesarean delivery two weeks later. Maternal serum concentrations were measured during pregnancy and postpartum, and bNAb levels were also quantified in the offspring. In Study B, four second-trimester pregnant RhMs received 30 mg/kg rh-3BNC117-IgG1-LS every two weeks, with intravaginal SHIV-AD8EO challenges administered between infusions. Eight untreated pregnant RhMs and 12 untreated non-pregnant females served as controls. The primary endpoint was the rate of SHIV acquisition across groups.

In Study A, following the first infusion during pregnancy, there was no significant difference in serum half-lives between groups. However, following the second infusion during the postpartum period, rh-3BNC117-IgG1-LS-treated females exhibited a non-significant trend toward longer half-lives compared with 3BNC117-treated females. In infants, neonatal serum concentrations of rh-3BNC117-IgG1-LS at delivery were significantly lower than those of 3BNC117 ($P=0.01$). In Study B, passive bNAb administration significantly delayed SHIV acquisition in treated pregnant macaques ($P=0.001$), whereas nearly all untreated pregnant controls became infected after one or a few exposures. Non-pregnant females exhibited significantly slower SHIV acquisition ($P=0.004$) and lower peak viral loads ($P=0.008$) compared to pregnant controls.

LS-modified 3BNC117 conferred protection against vaginal SHIV acquisition in pregnant macaques. Untreated pregnant females bore significantly higher infection risk than non-pregnant females, mirroring epidemiological observations of heightened HIV susceptibility in pregnant women, though the factors underlying this risk remain unknown. Our model of intravaginal SHIV challenge in pregnant macaques may facilitate investigation of these factors.

POSTERS



Understanding mTOR/AMPK/SIRT immuno-metabolic axis and immune activation during Mtb/SIV co-infection to advance host-directed therapies

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Background: Tuberculosis (TB) and human Immunodeficiency Virus (HIV) remain a deadly co-infection where TB remains the leading cause of mortality in people living with HIV (PWH). Understanding the mechanisms governing TB reactivation can advance the development of improved therapeutic and preventive measures for PWH. HIV harboring activated and proliferative CD4+T cells undergo a metabolic shift facilitating increased glucose uptake necessary for rapid proliferation. This immune metabolism changes are regulated by the mammalian target of rapamycin (mTOR). AMPK is another metabolic sensor that augments autophagy by inhibiting mTOR. The interplay between mTOR and AMPK controls several cellular processes.

Methods: Asymptomatic *Mycobacterium tuberculosis* (Mtb) infected rhesus macaques (~5-10 CFU of Mtb CDC1551) were Co-infected with Simian Immunodeficiency Virus (SIV)(300TCID₅₀ of SIVmac239) followed by Antiretroviral therapy (ART) and anti-TB therapy (3HP) to study immune activation. We also studied the effect of pharmacological inhibition of mTOR in Mtb infected bone-marrow derived macrophages (BMDMs) of rhesus macaque, utilizing mTOR inhibitors or AMPK/SIRT1 agonists to study if mTOR inhibition can reset the immuno-metabolic changes.

Results: HIV infection causes profound immuno-metabolic changes in mTOR/AMPK axis by inducing mTOR, suppressing AMPK and promoting TB reactivation, to regulate immune responses for the benefit of virus. SIV/Mtb co-infected macaques induce TB reactivation characterized by chronic immune activation, including Type I IFN induction and macrophage turnover. ART fails to mitigate immune activation and restore the lung immune environment, despite reducing viral loads and replenishing peripheral and alveolar CD4+T cell counts. We observed that mTOR inhibition in BMDMs upregulated the expression of PRKAA1, SIRT1 AND IDO1 and reduced the intracellular survival of Mtb in BMDMs. This aligns with the ability of Mtb to hijack host mTOR signaling to facilitate its intracellular survival within macrophages.

Conclusions: Concurrent treatment with preventive anti-TB therapy, along with ART, fails to eliminate chronic immune activation. Understanding the mechanisms that modulate mTOR/AMPK axis will establish improved therapeutics and preventive measures for PWH by reducing immune activation and preventing HIV induced TB reactivation. Further validation of using mTOR inhibitors in mouse and non-human primate model of TB will provide better insights into their future translational and therapeutic approaches.

Cryo-EM structure of gp120/gp41 interface region targeting neutralizing antibody from the HTVN-300 clinical trial study

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Introduction. A major goal of HIV-1 vaccine development is to elicit broadly neutralizing antibodies (bnAbs) in humans. Several antigenic hotspots on HIV-1 Env, including the CD4-binding site (CD4bs), V3 glycan region, membrane-proximal external region (MPER), and fusion peptide, are key targets for vaccine design. Induction of bnAbs requires priming immunogens capable of engaging naïve B-cell precursors. The CH505 SOSIP trimer, designed to target CD4bs CDRH3-binding bnAb precursors, has been shown to induce tier 2 autologous neutralizing antibodies that confer protection against homologous SHIV CH505 mucosal challenge. Here, we report findings from the HVTN 300 clinical trial, in which 8 of 11 vaccine recipients developed antibodies targeting the gp120–gp41 interface, as well as antibodies recognizing the N332-independent V3 GDIR motif and the CD4-binding site.

Methods. Single-particle cryo-EM was used to characterize the Env–antibody complex. Cryo-EM data was collected on a Titan Krios 300 kV microscope (Thermo Fisher Scientific) equipped with a K3 detector (Gatan). Data processing was performed in CryoSPARC (Punjani et al., 2017), while model fitting and refinement were carried out using Chimera, Coot, and Phenix. Structural visualization and figure preparation were performed in ChimeraX and PyMOL.

Results. Cryo-EM structure of CH505–Fab complex showed fab to engage in quaternary contacts by recognizing the FPPR of one gp41 protomer using all three heavy-chain complementarity-determining regions (CDRs), while the heavy chain (HC) framework region 3 (FR3) engages the HR2 region of an adjacent gp41 protomer. Additional contacts include HCDR1 interactions with FPPR residues (Leu520, Gly524, and Thr529) and a contact between the HCDR2 residue Ser55 and the gp41 residue Asp624. No light-chain contacts were observed. This HC-only mode of interaction is distinct from the known FPPR-directed bNAb 3BC315, which utilizes both heavy and light chains to engage gp41.

Conclusions. Priming with CH505 SOSIP generated bNAb precursors targeting the FPPR, as well as antibodies against other antigenic hotspots. Although FPPR-targeting antibodies primarily interact through a single Fab heavy-chain domain, their ability to engage two gp41 subunits makes the FPPR an attractive target for bNAb induction. Such antibodies may restrict the conformational transitions in gp41 that are required for membrane fusion and viral entry.

Targeted Rhesus IgG4 Fc Engineering to Generate Primatized Monoclonal Antibodies with Human-Like FcγRIIIA Binding

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Background:

Nonhuman primate (NHP) experiments requiring prolonged human monoclonal antibody (mAb) treatment or serial redosing may be limited by neutralizing anti-drug antibody (ADA) responses. Increasing the sequence identity of the treatment antibody to macaque can help mitigate this immunogenicity risk; however, modification of mAb sequences may also alter its biophysical and functional properties, as rhesus IgG4 has been reported to exhibit higher affinity to Fc gamma receptor (FcγR) than human IgG4. Here, we sought to create rhesus-human chimeric Fc sequences with maximal rhesus sequence identity while preserving a human IgG4-like FcγRIIIA-binding profile, enabling rhesus-compatible IgG4 reagents that narrow the functional gap between human antibody therapeutics and NHP models.

Methods:

To map the IgG4 domains responsible for species differences in FcγR engagement, a panel of rhesus IgG4 anti-CD20 (clone 2B8) antibodies was generated with constant domains swapped with human counterparts, including CH1, hinge, CH2, CH3, and light-chain constant region. Three additional rhesus IgG4 variants carried L234F, A330S/P331S, or L234F/A330S/P331S mutations structurally mapped at the human IgG4 Fc-FcγRIIIA contact site. Wild-type human and rhesus IgG1 and IgG4 antibodies served as controls. Binding to rhesus FcγRIIIA1 and FcγRIIIA3 was measured by surface plasmon resonance with antibodies arrayed in triplicate.

Results:

Human and rhesus IgG1 controls showed no measurable difference. In contrast, wild-type human IgG4 showed approximately two-fold weaker affinity than rhesus IgG4, confirming a species difference in FcγRIIIA binding. Replacing rhesus CH2 with human CH2 increased attenuation toward a human IgG4 binding profile. Human CH1, hinge, CH3, or light-chain constant region swaps produced minimal changes to attenuation. Targeted substitutions L234F, A330S, and P331S within the Fc-FcγR contact site increased attenuation, while the triple mutant produced the greatest reduction in binding and shifted toward a wild-type human IgG4-like profile.

Conclusions:

These data identify CH2 as a determinant of rhesus-human differences in FcγRIIIA binding. Retaining L234F, A330S, and P331S may allow the remaining constant region to be primatized while preserving a human IgG4-like FcγRIIIA profile. These findings support the need for *in vivo* comparison of primatized, wild-type rhesus, and wild-type human IgG4 constructs to assess pharmacokinetics, ADA impact, and effects on target-positive cell populations.

Comparative Long-Read Transcriptomics Reveals Novel Isoforms Across Human and Nonhuman Primates Models

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Background: Nonhuman primates (NHPs) are invaluable models for studying human disease because they share many physiological and immunological features with humans. At the same time, species-specific molecular differences, including patterns of alternative splicing, can provide important biological insight and help explain variation in disease phenotypes across models. With advances in long-read sequencing, it is now possible to capture full-length transcripts and more accurately define isoform diversity across species. Based on our recent results, we hypothesized that long-read sequencing would reveal previously unrecognized isoform diversity across NHP species and identify conserved as well as divergent splicing patterns relative to humans.

Methods: In this comparative transcriptomics study, we mapped the full-length isoform landscape across three NHP species commonly used in human disease research, rhesus macaque (*Macaca mulatta*), olive baboon (*Papio anubis*), and common marmoset (*Callithrix jacchus*), and compared them with human (*Homo sapiens*). We generated long-read transcriptome data from the cerebellum and PBMCs using PacBio Iso-Sequencing. These data alongside publicly available transcriptome datasets were analyzed using IsoQuant to identify isoforms. Quality-control was done using SQANTI3. IsoformSwitchAnalyzeR was used to identify differential isoform usage across species and tissues and to predict the functional consequences of alternative splicing events. Many APOBEC3 isoforms were also identified, and plasmid constructs were generated to assess differences in enzymatic activity and cellular localization by deaminase assay and immunofluorescence, respectively.

Results: We reached isoform discovery saturation in baboon and marmoset transcriptomes, substantially improving poorly annotated reference transcriptomes for these species and potentially enhancing their translational utility. In total, we identified 101,561 isoforms, of which 42,841 were novel (42.2%). At the transcriptome level, exon skipping was the most common alternative splicing event across all four species. Across the three NHP species, multiple APOBEC3 isoforms were identified, with many retaining canonical enzymatic activity and cellular localization relative to human, highlighting conserved antiviral functionality alongside species-specific isoform diversity.

Conclusions: This study reveals extensive, previously unrecognized isoform diversity across human and NHP transcriptomes, underscoring both conserved and species-specific splicing patterns. By improving transcriptome annotation and defining functionally relevant isoform variation, these findings strengthen the use of NHPs as translational models for human disease.

Combining AZD5582 and INI-4001 with early ART and SIV bNAb leads to accelerated viral decay and lower intact provirus in SIV-infect infant rhesus macaques

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Background

Infants who acquire HIV require lifelong adherence to ART, as treatment interruption often leads to viral rebound due to the formation of the viral reservoir. “Surge and purge” pediatric cure strategies aim to disrupt early reservoir establishment by reversing latency of infected cells and enhancing host immune responses. We assessed the impact of a novel liposomal nanoparticle delivering a TLR7/8 agonist, INI-4001, and the latency reversal agent AZD5582 on viral decay and reservoir establishment in conjunction with early ART and bNAbs in SIV-infected infant rhesus macaques (RMs).

Methods

Thirty infant RMs were I.V. infected with SIV_{mac251}. ART was initiated 5-6 days post-infection, and animals were split into three treatment arms. Group 1 (n=10) served as ART-only controls. Group 2 (n=10) received a single dose of an SIV bNAb cocktail (20 mg/kg) at ART initiation and five weekly doses of AZD5582 (0.2 mg/kg). Group 3 (n=10) received five weekly doses of INI-4001 (0.05 mg/kg) in addition to the treatments administered to Group 2. Blood, lymph nodes, and rectal tissues were collected to monitor plasma SIV-RNA and intact proviral DNA.

Results

Plasma SIV-RNA peaked at 10^7 - 10^8 copies/ml one-week post-infection. The median viral load decline rate (log copies/ml/day) post-ART was faster in the two groups that received interventions with ART from weeks 0-2 post-ART (Group 1: 0.214, Group 2: 0.238, Group 3: 0.239) and weeks 2-6 post-ART (Group 1: 0.0385, Group 2: 0.0485, Group 3: 0.0445). Intact SIV DNA in PBMCs trended lower in treatment groups, with significantly less intact provirus in RMs receiving INI-4001 at Week 17 post-infection (Group 1 vs 3, $p = 0.020$). Intact reservoir size was also significantly lower in rectal tissue twelve weeks after viral suppression on ART in Groups 2 and 3 compared to controls (Group 1 vs 2, $p = 0.011$; Group 1 vs 3, $p = 0.036$). Reservoir differences were not found in lymph nodes.

Conclusions

The combination of early ART, bNAbs, AZD5582, and INI-4001 induced accelerated viral decay and smaller intact reservoir sizes in PBMCs and rectal tissue. Evaluation of this combination on viral rebound and virus-specific immune responses is ongoing.

Metformin is well tolerated, but minimally reduces residual inflammation in ARV-treated, SIV-infected NHPs.

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Metformin is well tolerated, but minimally reduces residual inflammation in ARV-treated, SIV-infected NHPs.

Background

Residual inflammation persists in HIV-infected persons and SIV-infected macaques despite effective antiretroviral (ARV) therapy, contributing to long-term comorbidities. Metformin, a widely used antidiabetic drug, has demonstrated anti-inflammatory effects and may be a promising adjunctive therapy to reduce inflammation in people living with HIV receiving suppressive ARVs. Herein, we investigated the effects of metformin on systemic inflammation and immune activation in ARV-treated, SIV-infected rhesus macaques.

Methods

Rhesus macaques were treated for 8 months with ARVs alone or ARVs plus oral metformin (20 mg/kg following a 10 mg/kg induction phase). Safety and tolerability were assessed by clinical monitoring and routine blood parameters. Peripheral blood mononuclear cells (PBMCs) were analyzed by flow cytometry, plasma inflammatory markers (sCD14, IP-10, IL-6, and TNFR1) were quantified by ELISA, plasma samples underwent metabolomic analysis, and monocytes, liver, and pancreas tissues were subjected to transcriptomic analyses.

Results

Metformin was well tolerated, with no adverse effects. Flow cytometry demonstrated no significant differences in CD4⁺ T-cell recovery or Ki-67 frequencies across immune cell populations between treatment groups. Similarly, plasma levels of IP-10, IL-6, sCD14, and TNFR1 were not significantly altered following metformin treatment. Metabolomic and transcriptomic analyses revealed no global shifts in the metabolome or transcriptome. However, pathway analyses identified changes in metabolic and transcriptional pathways despite relatively few changes in individual metabolites or genes.

Conclusions

Collectively, these findings indicate that metformin is safe but has only modest effects on systemic inflammation and immune activation in ARV-treated, SIV-infected macaques.

Evaluation of supplement-containing enemas on intestinal immunity and susceptibility to low dose intrarectal SIV.

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BACKGROUND:

Men who have sex with men (MSM) are disproportionately susceptible to HIV acquisition. Increased susceptibility has been attributed to colorectal epithelial barrier disruption and intestinal dysbiosis, including expansion of *Prevotellaceae*. Although epithelial damage may result from inflammation associated with receptive anal intercourse, rectal douching may also contribute. To improve colorectal epithelial health, we developed an epithelial-enhancing enema containing the prebiotic inulin and the short-chain fatty acid sodium butyrate (EnBF), with or without the antiretrovirals dolutegravir, tenofovir disoproxil fumarate, and emtricitabine (EnBF-ARV). We hypothesized that these formulations would improve colorectal health and reduce susceptibility to intrarectal SIV acquisition.

METHODS:

Rhesus macaques received thrice-weekly iso-osmolar enemas (Normosol-R) alone or supplemented with EnBF or EnBF-ARV (n=6/group). After 28 days of treatment, the fecal microbiome was characterized by 16S rRNA sequencing, and intestinal immunity was assessed by flow cytometry, NanoString transcriptional profiling, and ELISA. Beginning on day 56, animals underwent repeated low-dose intrarectal challenge with 4 TCID₅₀ SIVmac239X. Susceptibility was assessed by time to infection, the number of transmitted/founder (T/F) variants acquired, and post-acquisition viremia.

RESULTS:

All formulations were well tolerated. Supplemented enemas increased circulating monocyte and eosinophil counts without increasing evidence of epithelial injury, microbial translocation, or systemic inflammation, as measured by iFABP2, sCD14, and IP-10. Supplemented enemas also did not alter intestinal T-cell activation. Only 17 rectal genes were differentially expressed following EnBF-ARV treatment, whereas no genes were differentially expressed following EnBF treatment; these changes were not enriched for specific biological pathways. EnBF modestly altered fecal *Bacteroidota*, *Bacillota*, and *Pseudomonadota* amplicon sequence variants and increased *Prevotellaceae* abundance; this *Prevotellaceae* increase was absent with EnBF-ARV. Compared with enema-only controls, supplemented enemas did not alter time to infection, the number of T/F variants acquired, or post-acquisition viremia.

CONCLUSIONS:

Repeated administration of putative epithelial-enhancing enemas was well tolerated but did not measurably improve epithelial integrity, intestinal immune parameters, or reduce susceptibility to intrarectal SIV acquisition. These findings improve our understanding of the biological consequences of repeated enema use in MSM and may inform the development of improved bowel preparations for sexual and clinical use.

A *Tropheryma*-dominant lung microbiome defines respiratory homeostasis in healthy pigtail macaques

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The lung microbiome is an important determinant and indicator of respiratory health; therefore, defining microbial communities in healthy individuals is essential for understanding dysbiosis during respiratory disease. To define the homeostatic lung microbiome in healthy pigtail macaques (PTM), we collected BAL (n = 76) and paired specimens (blood, oral swabs, and stool) across three institutions. Microbial composition was assessed by 16S rRNA sequencing on acellular BAL fluid (BALF), oral swabs, and stool. Peripheral and pulmonary host immune responses were measured by clinical blood tests (serum chemistry, CBC), immunoassays in plasma and BALF (ELISA, Luminex), and bulk RNA-seq of BAL cells. The PTM lung microbiome exhibited low bacterial diversity yet formed a distinct microbial niche separate from the upper respiratory and gastrointestinal tracts. The genus *Tropheryma* was detected in 85% and dominated 55% of PTM lungs. Consistent detection across demographics and institutions, and absence from non-pulmonary samples or controls, support its presence as a recurrent pulmonary resident. Although *Tropheryma*-dominance was strongly associated with housing location, inconsistent cage-pair concordance suggest that environment alone does not fully explain pulmonary colonization patterns. PTM with *Tropheryma*-dominant lungs had less systemic inflammation including lower WBC counts, neutrophil-to-lymphocyte ratios, and CRP concentrations. Ongoing analysis of BAL cytokines and transcriptomic features will further define local lung immune responses associated with microbial communities. Together, these findings describe a *Tropheryma*-dominant homeostatic PTM lung microbiome that could shape host immunity. Furthermore, this study establishes a foundation to improve translational studies of respiratory immunity, dysbiosis, and therapeutic intervention in respiratory disease.

Subcapsular Sinus Macrophage Dynamics During Chronic Viral Infections of Non-Human Primates

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Background: Subcapsular sinus macrophages (SSMs) are lymph node-resident and facilitate antigen presentation of lymph delivered antigens. In specific pathogen free murine models, these cells rapidly turn over following antigen uptake and presentation. The behavior of these cells in non-human primates (NHPs) and during chronic antigenemia have not been comprehensively analyzed.

Methods: We utilized confocal microscopy, flow cytometry, and gene expression analysis to explore SSMs in simian immunodeficiency virus (SIV)-infected and uninfected rhesus macaques. To selectively identify phagocytic cells in LNs, we intradermally injected FITC dextran. Draining lymph nodes were subsequently surgically excised, embedded in paraffin, and harvested into single cell suspensions. FITC-positive cells were isolated using flow cytometry-based cell sorting and analyzed with quantitative PCR to assess the expression levels of CD169, a marker commonly associated with SSMs, and other myeloid cell markers.

Results: We find that in NHPs, myeloid cells expressing CD169 are not uniformly found within the lymph node subcapsular sinus and that antigen drained through the lymph can be captured by macrophages throughout the lymph node anatomy, including the medulla. Cells which had phagocytosed FITC dextran isolated *ex vivo* had increased expression of CD169 compared to T cell negative control. This was true irrespective of SIV infection.

Conclusion: Our data suggest that SSMs frequently turn over in settings of chronic antigenemia and that NHPs are an appropriate model to explore their dynamics to better model outbred populations of humans constantly exposed to antigens.

Evaluation of Oxford Nanopore Sequencing (ONT) for MHC Genotyping in NHP

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Background

Certain MHC class I alleles are associated with exceptional control of chronic SIVmac239 replication in rhesus macaques and can confound the interpretation of SIV studies. Identifying animals carrying these protective alleles is therefore essential. For more than a decade, our group has performed high-throughput MHC genotyping using Illumina MiSeq and PacBio sequencing. Advances in Oxford Nanopore Technologies (ONT) have improved read accuracy while providing long-read sequencing and faster turnaround. Here, we evaluate ONT sequencing as a replacement for MiSeq and PacBio for high-throughput MHC genotyping.

Methods

Genomic DNA from 613 NHPs was amplified using multiplex PCR with primers targeting exon 2 peptide-binding domains of MHC class I and class II loci on a Standard BioTools Biomark X instrument. Amplicons were sequenced on both ONT GridION and MiSeq instruments. Three kb full-length MHC class I and/or MHC-E amplicons were also generated from 406 NHPs and sequenced on the GridION with R10.4 flow cells and super accuracy base calling. The resulting sequence reads from each platform were mapped against species-specific custom allele references.

Results

Validation studies demonstrated that newly developed ONT analytical workflows achieved performance comparable to established MiSeq and PacBio assays, with sufficient accuracy for exact exon 2 genotype matching to reference alleles. To account for ONT indel errors in full-length amplicons, genotype assignments accepted only reads that perfectly matched reference alleles or differed solely by indels, while reads containing SNPs were excluded. Analysis of full-length Mamu-E amplicons from 240 rhesus macaques identified 75 new full-length allelic variants for Mamu-E genes. Likewise, analyses of full-length amplicons of 48 non-Mauritian cynos yielded at least 500 novel Mafa class I allelic variants.

Conclusions

ONT sequencing is a reliable alternative to MiSeq and PacBio for NHP MHC genotyping. Unlike MiSeq which provides lineage-level resolution, ONT long-read sequencing enables high-resolution full-length allele characterization and simultaneous discovery of novel alleles within a single sequencing run. Additionally, ONT sequencing with a MinION is more accessible to laboratories that lack the infrastructure required for Illumina or PacBio systems. The ability to reuse ONT flow cells further enhances scalability and provides a cost-effective solution for MHC genotyping.

Efficacy of estrogen replacement on bone health in SIV-infected ovariectomized female macaques on antiretroviral therapy.

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Current antiretroviral therapy (ART) regimens have rendered HIV a manageable chronic condition rather than a fatal disease, with people who initiate ART soon after infection achieving nearly normal life expectancy. As a consequence of increased survival, more women living with HIV (WLWH) will now undergo menopause and be subject to a disease burden that reflects age along with adverse skeletal consequences of estrogen deficiency. In addition to bone loss and increased fracture risk typical of postmenopausal women, ART also causes decreased bone mineral density (BMD). Therefore ART-suppressed WLWH may experience increased detrimental effects on bone quality and bone turnover due to long-term ART treatment combined with menopause. Our goal was to determine the efficacy of estrogen replacement in preventing bone loss in ART-suppressed SIV-infected ovariectomized (OVX) rhesus macaques, a model of postmenopausal WLWH.

Female rhesus macaques were infected with SIVmac239M and daily ART was initiated two weeks post-infection (PI). All animals were OVX at 35 weeks PI and received either estrogen (n=12) or cholesterol implants (n=11) for 34 weeks. Blood samples were collected and animals monitored longitudinally for bone turnover markers and bone mass/density utilizing dual energy x-ray absorptiometry (DEXA). Femurs were collected at necropsy for micro-computed tomography (μ CT) evaluation of cancellous (metaphysis) and cortical (diaphysis) bone architecture.

Although previous results implicated the estrogen receptor alpha in control of the viral reservoir, there were no differences in plasma or tissue viral reservoirs. The combination of SIV, ART, and OVX negatively impacted pelvic-specific bone mineral content (BMC; -17.5%) and BMD (-9.3%). However, estrogen-replaced animals exhibited increases in BMC (+8.2%) and BMD (+6.4%). Circulating levels of the bone turnover markers osteocalcin and C-terminal telopeptide of type 1 collagen were significantly increased (3x) post-OVX, while estrogen replacement maintained levels similar to baseline. Estrogen replacement resulted in higher femur metaphysis cancellous bone volume/tissue volume (13.9 ± 1.1 vs 10.7 ± 0.8 %), connectivity density (4.9 ± 0.5 vs $3.4 \pm 3.4 \pm 0.4$ mm⁻³), and trabecular number (1.5 ± 0.06 vs 1.3 ± 0.06 mm⁻¹) and lower trabecular separation (695 ± 32 vs 795 ± 35 μ m). Femur cortical bone parameters were unaffected.

Overall, estrogen replacement prevented OVX-induced changes in bone mass and improved cancellous bone architecture in ART-suppressed SIV-infected female macaques.

Intravenous delivery of cationic nanoparticles carrying a RIG-I agonist leads to liver centric innate immunity in rhesus macaques: A preclinical study for HBV therapy

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Background: HBV is a stealth virus that does not induce an interferon response during infection. However, previous studies showed that inducing the Retinoic acid-inducible gene I (RIG-I) pathway with a RIG-I agonist RNA (RAR) suppressed HBV infection and depleted cccDNA in human hepatocytes. Here we tested novel formulations of RAR complexed with proprietary cationic nanoemulsions (LION™) for potency and cytotoxicity.

Methods: After confirming innate immune activation function and anti-HBV efficacy of LION-RAR in primary rhesus hepatocytes and identifying a tolerable dose in rhesus macaques (RMs) we intravenously administered 0.05 mg/kg LION-RAR (N=2) or LION-XRNA (N=2, control) to four RMs. RMs were euthanized after 6h post-drug administration and tissues were harvested. Serum chemistry and CBCs were obtained along with the quantification of innate genes using quantitative PCR and cytokine levels using LEGENDplex™. Flow cytometry was used to identify any changes in immune cell population in the blood.

Results: Our preliminary *in vitro* data showed reduction in HBV RNA, HBV serum antigen (HBeAg) and cccDNA in treated primary hepatocytes. *In vivo* administration of LION-RAR at the optimized dose led to the activation of innate immune genes including IRF3-target genes and ISGs (e.g., ISG15 and MX1) in the liver and elevated cytokine levels (CXCL10 and CCL2) without any signs of toxicity. In contrast, the LION-XRNA that does not activate RIG-I did not induce any changes in these parameters confirming the RAR-specific effects. No significant changes were observed in immune cell populations by either treatment.

Future directions: We are currently evaluating LION:RAR in tenofovir-suppressed, HBV-infected RMs, which will be pivotal in determining the anti-HBV efficacy of LION:RAR. Importantly, these studies will provide critical preclinical data for advancement of LION:RAR to the clinic.

Pyruvate metabolism as a convergent node of methamphetamine-driven immunometabolic dysregulation during treated SIV infection

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Methamphetamine (Meth) use is prevalent among people living with HIV and is associated with worse clinical outcomes, including accelerated disease progression, persistent immune activation, and increased inflammatory complications. However, the biological mechanisms underlying these adverse outcomes remain incompletely defined. Because cellular metabolism governs immune responses and viral persistence, we investigated how Meth alters immune cell metabolism and associated pathways during SIV infection and whether these effects persist after ART initiation. Using an established SIV-infected rhesus macaque model of daily Meth administration, we profiled the metabolic states of peripheral immune cells by high-parameter flow cytometry (MIST), bulk RNA sequencing, and Seahorse T cell metabolic assay, and correlated these measures with plasma viral load and soluble inflammatory biomarkers. *In vivo* Meth exposure induced rapid metabolic remodeling in CD4⁺ T cells and monocytes, characterized by increased expression of glycolysis-associated markers, including higher levels of the pyruvate dehydrogenase 1 (PDK1) enzyme and phospho-(active)-mTOR. While ART initiation normalized most metabolic markers, Meth-exposed animals retained elevated PDK1 expression, reduced mitochondrial spare respiratory capacity in CD4⁺

T cells, and decreased response to TCR stimulation. Our findings suggest that *in vivo* Meth exposure drives dysfunctional pyruvate oxidation, particularly in CD4⁺ T cells and monocytes, that persists despite suppressive ART. Targeting pyruvate metabolism may represent a potential strategy to improve immune function in individuals living with HIV who use Meth

SUPER-ENHANCER LNCRNAs REGULATE HIV TRANSCRIPTION AND LATENCY

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Background

HIV persists in long-lived latent reservoirs within resting CD4⁺ T cells despite effective antiretroviral therapy, enabling viral rebound following treatment interruption. Current latency-reversing agents have limited specificity and potency, underscoring the need for host-directed strategies that precisely regulate HIV latency. Although long non-coding RNAs (lncRNAs) are increasingly recognized as regulators of viral infection and host transcription, the role of super-enhancer-associated lncRNAs (SE-lncRNAs) in HIV transcription and latency remains poorly understood.

Methods

Expression profiles of SE-lncRNAs were analyzed in HIV-infected CD4⁺ T cells. Candidate SE-lncRNAs were functionally interrogated using CRISPR/Cas13d-mediated knockdown in recombinant HIV reporter systems and latent HIV models. Transcript characterization, coding potential prediction, and subcellular localization analyses were performed to define lncRNA identity. Regulation of the neighboring gene INTS1 was assessed by quantitative PCR, immunoblotting, and RNA polymerase II chromatin immunoprecipitation followed by qPCR (ChIP-qPCR).

Results

HIV infection altered the expression of multiple SE-lncRNAs in CD4⁺ T cells. CRISPR/Cas13d-mediated silencing identified lnc-SERIS1 (ENSG00000273230, Super-Enhancer Regulator of INTS1), an SE-lncRNA adjacent to INTS1, as a potent negative regulator of HIV transcription. SERIS1 knockdown enhanced HIV transcription in 293-T-derived Lenti-X and Jurkat T cells infected with a recombinant HIV reporter virus and reactivated latent HIV in J-Lat T-cell models. Transcript characterization and subcellular localization confirmed that SERIS1 is a bona fide nuclear lncRNA lacking protein-coding potential. Mechanistically, SERIS1 depletion significantly reduced INTS1 mRNA and protein levels, and RNA polymerase II ChIP-qPCR showed decreased Pol II occupancy at the INTS1 promoter, supporting a cis-regulatory role in maintaining INTS1 transcription. As INTS1 is a core component of the Integrator complex previously implicated in HIV transcriptional repression, coordinated downregulation of SERIS1 and INTS1 provides a mechanistic explanation for enhanced HIV transcription following SERIS1 perturbation.

Conclusion

These findings identify the SERIS1–INTS1 axis as a previously unrecognized regulator of HIV latency and establish SE-lncRNAs as promising host-directed therapeutic targets for HIV cure strategies.

Quantifying Tissue-Specific KIR mRNA Expression in Naïve and SIV-Infected Rhesus Macaques Using a Branched-DNA Platform

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Background: Killer immunoglobulin-like receptor (KIR) genes are difficult to quantify with conventional methods because high sequence homology and copy-number variation make antibody cross-reactivity unfaithful and qPCR and RNA-seq prone to amplification bias. As a result, KIR expression across tissue compartments remains poorly characterized in rhesus macaques, a key model for HIV/SIV pathogenesis, despite the central role KIRs play in modulating NK and CD8⁺ T cell effector function through MHC class I interactions.

Methods: We used a branched-DNA (bdDNA) amplification-free multiplex assay (QuantiGene Plex, Thermo Fisher) that quantifies mRNA directly from cell lysates without reverse transcription or PCR, offering a reliable alternative to qPCR for this structurally challenging gene family. We measured mRNA expression of six KIR genes (Mamu-KIR1D, -KIR2DL4, -KIR3DH, -KIR3DH5, -KIR3DL0, and -KIR3DL2) in PBMC, lymph node, and spleen mononuclear cells from 16 Indian-origin rhesus macaques, comparing naïve animals with those acutely or chronically infected with SIV or SHIV.

Results: KIR expression in naïve animals was highly compartmentalized: lymph nodes showed higher KIR1D and KIR2DL4 than PBMC ($p=0.0017$ and $p=0.0042$), while spleen showed increased KIR3DL0 and KIR3DH. PBMC consistently showed the lowest expression across all six genes. Chronic SIV/SHIV infection diminished this pattern, particularly in spleen, where KIR3DL0 and KIR3DH decreased by nearly half (fold change $\sim 0.57\times$), along with reductions in KIR1D and KIR2DL4. Acute infection maintained relatively normal expression, indicating that disruption of tissue-specific KIR repertoires is associated with chronic rather than acute infection.

Conclusions: This study establishes a reproducible, scalable bdDNA framework for profiling KIR gene expression in complex tissues from non-human primate models, resolving tissue- and disease-specific KIR signals that are difficult to capture with conventional methods. Chronic lentiviral infection disrupts baseline tissue-specific KIR regulation in a compartment- and stage-dependent manner, with implications for innate immune dysfunction in chronic HIV/SIV disease.

A Computational Bridging Framework to Project Long-Term Antiretroviral Release and Pharmacokinetic Profiles from a Subdermal Delivery Platform

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Long-acting delivery platforms provide sustained antiretroviral exposure, enabling new strategies for HIV prevention and treatment. Their preclinical evaluation often relies on nonhuman primate (NHP) and other large-animal models to characterize drug-release kinetics, antiretroviral pharmacokinetics (PK), and overall platform performance. However, determining the full functional lifespan of these systems may require studies spanning months to years, imposing substantial financial and resource constraints on preclinical research.

To address this challenge, we developed a computational PK-bridging framework that projects long-term release-rate and plasma-concentration profiles from short-duration in vitro or in vivo studies. We applied the framework to our established subdermal nanofluidic drug delivery implant (NanoDDI), which is available to the preclinical research community through the NIAID-funded DART Resource Center (R24AI194877). The framework uses groups of implants with different initial drug loads to represent successive stages of payload depletion. Operating in a drug-remaining-indexed framework, the tool treats each lower-loading group as representing the device at a more depleted state, automatically reconciles overlapping drug-leftover ranges across groups, and applies a guarded tail-extrapolation model to precisely estimate the terminal decline of the release profile. The resulting integrated release-rate and plasma-concentration projections can be directly incorporated into preclinical study design, implant lifetime estimation, and long-term PK analyses.

Application of the framework to in vitro MK-8527 datasets spanning four initial loading levels projected sustained near-zero-order release at approximately 264 µg/day and a platform lifetime exceeding four years. These findings demonstrate that short experimental datasets can be leveraged to estimate long-term release profiles.

By converting brief experimental studies into multi-year release and PK projections, this framework could accelerate the development and evaluation of long-acting HIV prevention and treatment while reducing the time and resources required for platform characterization in NHP models. Both the computational framework and the NanoDDI platform are available to the preclinical research community as shared resources through the NIAID-funded DART Resource Center (<https://dartresourcecenter.org/>).

Improved antibody function after env-mRNA vaccination during cART does not control SHIV post-ATI

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Autologous neutralizing antibodies (aNAbs) may delay viral rebound by targeting the HIV reservoir upon viral reactivation. We evaluated the immunogenicity of an mRNA-based therapeutic vaccine designed to boost and diversify aNAb responses and delay viral rebound following analytical treatment interruption (ATI).

Fifteen rhesus macaques (RMs) were infected with barcoded-SHIV.CH505.TF and treated daily with cART. Following viral suppression, animals were randomized to a vaccine group (n=8), which received HIV-1 CH505 gp160 trimers delivered by mRNA-LNP to elicit neutralizing antibodies targeting the Env CD4-binding site (CD4bs), or to an unvaccinated control group (n=7). Plasma IgG was used to assess neutralization in TZM-bl assays and epitope specificity by EMPER. T-cell responses were measured by ICS. Viral reservoir size and plasma viremia were evaluated by IPDA and qPCR during 10 weeks of ATI, and Env evolution was assessed by single-genome sequencing (SGS). Analyses used exact Wilcoxon tests.

Two weeks after the final immunization, anti-CH505.TF aNAb levels were higher in vaccinated animals than controls (median IC50: 5.64 µg/mL vs. 33.98 µg/mL; p=0.01). CD4bs-specific antibodies were detected only in vaccinated animals. One month after final immunization, CH505 Env-specific CD4 T-cell responses were greater in the vaccine group (median 19.44% vs. 7.63%; p=0.01), with no change in CD8 T-cell responses from baseline or between groups. The viral reservoir was significantly smaller in vaccinated animals 2.5 months after the final immunization and before ATI (median 0 copies/million cells [IQR: 0–1.95] vs. 3.41 copies/million cells [IQR: 3.29–4.17]; p=0.01). All RMs rebounded by 9 weeks after ATI, with no significant difference in time to rebound (p=0.14). However, control animals had higher viral load AUCs during ATI (p=0.01). Env sequence analysis revealed distinct escape patterns: early mutations in CD4bs-associated V5 residues 459–460 occurred in 20% of vaccinated animals but not controls, while V1 loop mutations emerged only in controls at later time points. Functional antibody responses did not correlate with post-rebound viremia.

This mRNA vaccine regimen boosted aNAbs, enhanced Env-specific CD4 T-cell responses, induced CD4bs-targeted antibodies, and reduced reservoir size. Despite these immunologic improvements, it did not delay viral rebound, highlighting the need for more effective therapeutic vaccination strategies.

Advancing Translational immunology research with Non-Human Primates resource reagents

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Background

Non-Human Primates represent an invaluable model for pre-clinical studies of infectious disease, immunology and vaccine research owing to their genetic and immunological similarity to humans. However, research on these models is often limited by the lack of high-quality, species-specific reagents, as many systematically administered human proteins tend to cause ADA.

Methods

Our resource platform addresses this need by engineering, producing and distributing recombinant NHP cytokines, cytokine receptors, and antibody probes, shared over the past 20+ years with NHP investigators and supporting numerous NIH-funded and collaborative research projects spanning infectious diseases, immunizations and vaccine development.

Results

The resource provides anti-SIV and anti-SHIV PET probes for tracking the virus in vivo. In addition to our regular cytokine inventory, newly developed reagents on request include the production of chemokines, e.g. Fractalkine for targeting mRNA-NPs to specific cell lineages in vitro and in vivo. It additionally supplies many immunological cytokines and receptors with extended half-life, such as IL-2Fc, IL-21Fc, IL-7Fc, M-CSF-Fc, SCF-Fc, IL-15R α -Fc, PD-1 and PD-L1 for modulating immune responses in SIV/SHIV studies. Recent additions to the catalog include SIV antigen proteins e.g. p27, p55Gag and Nef for assays and immunization. The platform continues to expand its portfolio through the development of new engineered recombinant reagents based on emerging scientific needs and requests in collaborative relationships whereby specific end use dictates the design and composition of the reagent. These upcoming reagents will further broaden the toolkit available for the research community, supporting new approaches for advances in understanding patho-mechanisms and vaccine development.

Anti-CD4 Chimeric Antigen Receptor T-Cells Potently Deplete Peripheral and Tissue-Resident CD4⁺ T-Cell Reservoirs in a Rhesus Macaque Model of HIV Infection

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Background: Latent HIV-infected cells may be immunologically invisible to the immune system, undermining antigen-specific cure approaches. To address this, we developed CD4-targeting chimeric antigen receptor (anti-CD4 CAR) T-cells. In our rhesus macaque (RM) model of persistent HIV infection, we predicted that anti-CD4 CARs would outperform mAb-based depletion of the CD4-containing reservoir, particularly in tissues.

Methods: Three SHIV.C.CH505.v2-infected, ART-suppressed RMs received anti-CD4 CAR T-cells in a dose-deescalation (high, mid, low) study design to balance toxicity and efficacy. Following CAR infusion, we quantified the kinetics/magnitude of systemic CD4 depletion and depletion of the SHIV reservoir using flow cytometry, tissue-based, and PCR-based assays.

Results: Anti-CD4 CAR T-cells substantially depleted CD4⁺ T-cells in the high- and mid-dose RMs in peripheral blood and tissues. To address CAR-dependent toxicities, we successfully administered cetuximab as a “CAR-OFF” safety switch, which rapidly reduced CAR T-cell counts. CD4 depletion was associated with a dose-dependent reduction in SHIV DNA, including little or no DNA detected in gut, spleen, and most lymph nodes from the high- and mid-dose RMs. Surprisingly, PBMC-associated SHIV DNA rebounded amidst sustained low/undetectable peripheral CD4⁺ T-cell counts.

Conclusions: In our proof-of-principle study, CD4-targeted CAR T-cells expanded *in vivo*, systemically depleted CD4⁺ targets, and dramatically reduced viral reservoirs in an antigen-independent manner. We are currently quantifying the minimum CD4 density that is required for CAR-mediated depletion, and whether CD4^{LO} reservoir cells may escape targeting. Our antibody-mediated “CAR-OFF” safety switch rapidly inactivates CAR function in RMs, a critical first step towards advancing this basic science question to clinical relevance.

IL-10 variant cytokines to enhance therapeutic vaccine responses via modulation of T-cell and dendritic-cell activation

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Though persistent IL-10 signaling in chronic infection can contribute to CD8⁺ T-cell dysfunction or exhaustion, transient IL-10 signaling is an important regulator of T-cell differentiation that limits immunopathology and helps preserve long-term function. In the context of therapeutic HIV vaccines, therefore, controlled IL-10 signaling could be important for sustaining the antiviral response. IL-10 has pleiotropic and cell type-specific effects, including anti-inflammatory responses in myeloid cells and pro-inflammatory responses in T cells. To explore the possibility that IL-10 variants with predominant signaling to T cells will enhance and extend immune responses induced by therapeutic vaccination, we designed novel IL-10 variants based on the macaque wild type cellular IL-10 (cIL-10), including both monomers and dimers with altered binding affinity to the IL-10 receptor. We hypothesized that these variants may change the cell-type specificity and duration of IL-10 signaling. We find that variant IL-10 proteins induce STAT3 and STAT1 phosphorylation and enhance IFN- γ and TNF- α production in CD4⁺ and CD8⁺ T cells stimulated in-vitro. A dimerized variant with lower affinity to the IL-10R β receptor unit induced significantly less pSTAT1 and pSTAT3 signaling even at a saturating dose. IL-10 variants additionally inhibit dendritic cell (DC) subpopulations. These variants were able to limit monocyte-derived (mo)-DC maturation and reduce expression of the co-stimulation molecule OX40L following activation with a TLR-7 agonist. IL-10 variants also significantly lowered TNF- α production in stimulated moDCs and IFN- α production in plasmacytoid DCs. Surprisingly, we found that monomeric IL-10 variants often have comparable EC50s to dimers when comparing IL-10 receptor signaling in an HEK reporter cell line. Thus, IL-10 variants may enhance T-cell responses by direct action on those cells and by reducing IFN- α production. Such activities may be useful for modulating T-cell function in the context of therapeutic vaccination against HIV.

Determining the Zoonotic Transmission Potential of SIVrcm Using Humanized Mice

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Cross-species transmission events gave rise to several human pathogens that resulted in global pandemics/epidemics, including HIV-1 and HIV-2 and more recently SARS-CoV-2. Transmission of simian immunodeficiency viruses (SIVs) from wild non-human primates (NHPs) gave rise to various HIVs infecting the human. Over forty SIVs currently circulate in their native hosts in West and Central Africa. Factors such as deforestation, global warming, and human encroachment into the habitat of wild NHPs continue to accelerate, suggesting that additional cross-species transmissions with potential for the emergence of new human pathogens still exist. Research on the human infection potential of more primitive SIVs is extremely limited. Therefore, a humanized mouse model with a transplanted human immune system which can permit testing of SIV cross-species transmission and evolution is ideal. Here we evaluated SIVrcm from red-capped mangabeys native to West Africa wherein human encroachment into their habitat is not uncommon. Hu-HSC mice were infected with SIVrcm via i/p route and mice were followed for several weeks for evidence of infection. qRT-PCR was used to detect viral RNA from plasma samples collected weekly; flow cytometry was used to evaluate CD4 T cell decline bi-weekly. During the first passage, infected mice displayed chronic viremia with no CD4 T cell decline. In subsequent passages, infected mice displayed higher plasma viral loads and CD4 T cell depletion. Our results from vaginal mucosal transmission experiments also indicated the potential of SIVrcm for sexual transmission. Above findings suggest that hu-mice are a suitable model to evaluate the zoonotic potential of various primitive SIVs.

RANTES-Based Immunoadhesins as a Strategy for Targeting the CCR5+ HIV Reservoir

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C-C Chemokine Receptor Type 5 (CCR5) is a cell surface receptor expressed on lymphocytes and monocytes, which mediates leukocyte trafficking and inflammatory signaling through interactions with endogenous ligands including CCL3, CCL4, and CCL5. In addition to its physiological role, CCR5 is exploited by human immunodeficiency virus (HIV) strains as a coreceptor mediating viral entry. Following the initial binding of the viral envelope glycoprotein, gp120, to CD4, the coreceptor binding site is exposed, permitting attachment to CCR5 and subsequent gp41-mediated membrane fusion. Regulated upon Activation, Normal T Cell Expressed and Secreted (RANTES; CCL5) is a key endogenous C-C chemokine that exhibits high-affinity binding to CCR5 and can inhibit HIV entry via competition. We hypothesized that the early HIV reservoir is marked by CCR5 expression and could be targeted using RANTES or its N-terminal variants. We engineered RANTES-Fc fusions and evaluated their chemotactic activities, effects on CCR5 localization for different cell types, and in-vivo effects on RANTES expressing cells. Some variants were shown to deplete cell-surface CCR5 and others to stabilize the receptor, effectively labeling the targeted cells for potential destruction. Our findings establish a framework for utilizing RANTES immunoadhesins to target the CCR5+ HIV reservoir.

Comparing Threshold- and Machine Learning-Based Segmentation of Sites of Inflammation and Anatomical Regions in *Mycobacterium tuberculosis* – Exposed Non-Human Primates

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Background

Positron emission tomography combined with computer tomography (PET/CT) is a powerful, non-invasive tool for tracking sites of inflammation and lesion development in lungs of non-human primates (NHPs) exposed to *Mycobacterium tuberculosis* (Mtb). Manual labeling of PET/CT scans to define specific regions of interest (ROIs) as, for example, sites of high PET signal, is operator-dependent and laborious, relying on two-dimensional slice-by-slice tracing of standardized uptake values (SUV) and/or Hounsfield units (HU) boundaries for a single time point.

Methods

By using Imaris, a software for imaging data analysis, we have established a reproducible pipeline to 1) align serial scans of the same animal across time points by using anatomical vertebral landmarks, and 2) identify sites of inflammation within the lung boundary by applying threshold-based 3D pixel intensity-based segmentation. Comparison of the generated 3D surfaces to the baseline ROIs demonstrated excellent correlation between maximum pixel intensity and maximum SUV across four animals. Here we expanded this work in four ways. First, we reduced minor positional drift across scans with additional anatomical reference points using drift correction in Imaris. Second, we compared two segmentation approaches – intensity threshold-based and machine learning (ML)-based – against conventional manual segmentation. Third, we developed a ML-based method to define lung boundary using exclusively CT scans, enabling total lung-associated PET intensity visualization. Fourth and finally, we converted Imaris channel intensity into SUV (for PET) and HU (for CT) units (using metadata from DICOM files).

Results

We found that the ML-based approach demonstrated faster segmentation of sites of inflammation, more coincident intensity boundaries, and more robust anatomical segmentation of the lungs. In contrast, threshold-based segmentation generated more artifacts, required more extensive manual cleanup, and occasionally failed to capture relevant lung structures.

Conclusion

Future directions include quantitative statistical comparison of performance between methodologies. Together, these methodological improvements establish a more robust and reproducible framework for the longitudinal analysis of PET/CT data generated in NHPs, offering a scalable foundation for quantitative, multi-site comparisons of infection intensity for NHPs infected with Mtb, SIV, SARS-CoV2, or in settings of coinfection with two or more pathogens.

Differential cysteine utilization is associated with shifts in energy metabolism and SIV disease progression in nonhuman primate models

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Introduction: Determinants of HIV/SIV disease progression remain incompletely understood. Like ART-naïve people with HIV (PWH), Asian-origin cercopithecines, including pig-tailed macaques (PTMs), experience disease progression, with some progressing rapidly (<100 days post-infection). Conversely, SIV natural hosts, like African green monkeys (AGMs), resist immune dysfunction, despite viremia. We leveraged a multi-omics approach to determine mechanisms contributing to SIV progression/non-progression in these species.

Methods: PTMs and AGMs were infected with 300 TCID₅₀ SIVsab. PTMs were retroactively stratified into normal (n=12) and rapid progressors (n=6) and compared at pre-infection, acute infection, and setpoint. Infected AGMs (n=4) were compared to SIV-naïve AGMs (n=6) at setpoint and chronic infection. Untargeted metabolomics was performed by ultra-high-performance liquid chromatography/tandem accurate mass spectrometry. Absolute lipidomic quantitation was performed by Sciex SelexION differential mobility spectrometry. Plasma viral loads, biomarkers (ELISA), and cell counts were measured. Perivisceral (white) adipose tissue was characterized by immunohistochemistry.

Results: Preceding infection, the greatest predictor of rapid progression was higher cysteine sulfinic acid (p=0.002), the first irreversible step in generating taurine, an unrecyclable metabolite. At acute infection, higher taurine was the best predictor (p=0.01). In contrast, AGMs experienced increased cysteine-containing glutathione (re)cycling metabolites by setpoint (all p<0.01). In PTMs, glutathione metabolites negatively associated with traditional progression markers, while taurine correlated with glycolysis-associated cytokines.

Glutathione is required for fatty acid oxidation (FAO), and in AGMs was accompanied by increased acyl carnitines (FAO intermediates; 9/36: p<0.001; 17/36: p<0.05). Lipidomics revealed that at setpoint, free fatty acids (FFAs) were the only changed/increased lipid class in AGMs (p=0.02). Measured by ELISA, reduced adiponectin, which regulates glucose and FAO, predicted progression in PTMs (p=0.03). Immunohistochemistry showed increased UCP1 expression and morphological changes consistent with adipose browning and thermogenic triglyceride catabolism in PTMs but not AGMs.

Conclusions: Basic science has demonstrated that cysteine deficiency induces glutathione depletion, pro-glycolytic metabolic reprogramming, thermogenic adipose browning, and lethal weight loss; mirroring AIDS-associated fever and wasting. Despite antiretroviral therapies, PWH still experience dyslipidemia, co-morbidities, and immunometabolic dysfunction, which remains a barrier in cure strategies. Unraveling inter-relationships between cysteine utilization and systemic energy metabolism will facilitate therapeutic developments to improve immune functionality and reduce co-morbidities in PWH.

Integrated spatial and single-cell transcriptomics reveal mesenchymal stromal cell-driven intestinal mucosal immune recovery and tissue repair during SIV infection

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BACKGROUND: SIV infection disrupts the gut mucosa, driving epithelial barrier disruption, immune activation, and loss of mucosal immune homeostasis. Although antiretroviral therapy (ART) suppresses HIV/SIV replication, it does not fully or consistently repair intestinal damage, highlighting a gap in regenerative therapeutic strategies. Mesenchymal stromal cells (MSCs) possess immunomodulatory and tissue-reparative properties, but their capacity to restore intestinal immune compartments during SIV infection remains incompletely characterized.

METHODS: In the present investigation, we applied spatial transcriptomics to ileal tissue from rhesus macaques to map gut repair and renewal pathways across functionally and cellularly distinct mucosal regions. We then performed single-cell RNA sequencing to characterize these pathways at cell-type-specific resolution across four groups: uninfected controls, chronic SIV infection, SIV infection following analytical treatment interruption (ATI), and SIV treated with MSCs. Over 100,000 cells were profiled and classified into major immune and stromal compartments, followed by differential expression and pathway enrichment analyses.

RESULTS: SIV infection induced significant activation of anti-viral and inflammatory transcriptional programs while reducing epithelial repair pathways associated with cell adhesion, cytoskeletal organization, and barrier integrity. In contrast, MSC treatment led to restoration of extracellular matrix organization, collagen biosynthesis, receptor tyrosine kinase signaling, and gut tissue-repair pathways, particularly within B cells.

CONCLUSIONS: Together, these spatial and single-cell transcriptomic analyses identify MSC-associated restoration of intestinal immune compartments and activation of gut repair pathways in SIV-infected rhesus macaques, supporting MSCs as a potential strategy to promote mucosal immune homeostasis during HIV functional cure interventions.

Impact of vaccine-induced SIV-specific CD8⁺ T cells on early viral dynamics and viral rebound following antiretroviral treatment interruption

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Background: Enhancing immune-mediated clearance of latently infected cells is key to an HIV cure. We evaluated in rhesus macaques (RMs) how vaccine-elicited CD8⁺ T cells impacted SIV dynamics during acute infection, antiretroviral therapy (ART) and analytical treatment interruption (ATI).

Methods: Thirty RMs were infected intravenously with SIVmac239M, started ART at 2 weeks post-infection (wpi), and underwent ATI at 64 wpi. Twenty of these RMs received vaccination prior to SIV infection including 10 RMs that additionally received a second vaccination during ART. The vaccine regimens consisted of three sequential immunizations with heterologous viral vectors expressing SIV-Gag: Vesicular Stomatitis Virus, Vaccinia Virus, and Adenovirus type 5 pre-infection; and DNA-lipid nanoparticles, Modified Vaccinia Ankara, and RNA-lipid nanoparticles during ART. Plasma viral loads (PVL) and SIV-specific CD8⁺ T-cell responses were monitored longitudinally.

Results: RMs vaccinated before SIV infection exhibited reduced viral levels during acute infection and early ART with a 4.9-fold lower median peak PVL at 2 wpi as compared to controls (4.0×10^6 versus 19.5×10^6 copies/mL; $P=0.011$), and a lower PVL AUC up to 8 wpi ($P<0.001$). The pre-infection vaccine group demonstrated higher frequencies of SIV Gag-specific CD8⁺ T cells, during acute infection and maintained on ART until 55 wpi, as compared to controls in peripheral blood, rectal biopsies, lymph nodes, and bone marrow. Vaccination during ART further expanded these responses in the pre- & post-infection vaccine group. Despite sustained SIV-specific CD8⁺ T-cell responses, preliminary data obtained in 5/10 RMs per group that completed ATI indicate a limited impact of vaccination on post-ATI viral dynamics. While post-ATI peak and AUC PVL were comparable across groups, a modest 3-day delay ($P<0.05$) in the time to first detectable viremia was observed in both vaccinated groups compared to controls.

Conclusions: Pre-infection SIV-Gag vaccination generated sustained SIV-specific CD8⁺ T-cell responses on ART that altered acute viral dynamics but only slightly delayed viral rebound post-ATI. These results must be confirmed in the full cohort of RMs included in this study. Further characterization of the viral reservoir and immunological responses is ongoing and will elucidate the impact of vaccine-elicited CD8⁺ T cells on HIV/SIV persistence.

Killing of *Mycobacterium Tuberculosis* by Alveolar Macrophages Prior to Arrival of Adaptive Immunity to the Lung

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Background. Tuberculosis (TB), the disease caused by infection with *Mycobacterium tuberculosis* (Mtb), is responsible for about 1.2 million deaths annually, and is a leading cause of death among people living with HIV. Outcomes of Mtb infection are diverse, with only about 5 to 10 percent of infected individuals ultimately developing TB. The role of early events involving the innate immune response in determining outcomes of Mtb infection is not known. Infection typically begins in the lungs, where alveolar macrophages (AMs) are hijacked by Mtb bacilli that secrete effector molecules to prevent phagosome maturation, subsequently replicating in the resulting protected environment. Recent studies suggest that AMs merely provide a safe haven for Mtb early during infection, doing nothing to limit the growth of infection prior to the arrival of adaptive immunity to the lung. However, while some AMs are disabled by bacteria, it may be that other AMs rapidly kill bacteria and avoid succumbing to the signaling from engulfed bacteria. **Methods.** In order to assess the degree of Mtb death during early infection, CFU must be supplemented with additional indicators of division or death. The rates of killing of Mtb have been estimated during the first three weeks of infection in macaques and during the first two weeks of infection in mice, using chromosomal equivalents (CEQs, macaques) and an Mtb strain carrying a replication clock plasmid (mice); based on the results of these analyses, we performed stochastic simulations of early infection in macaques and in mice. **Results.** We argue that substantial amounts of both Mtb replication and death during early infection are the most reasonable interpretation of existing data, though experiments involving more frequent and/or earlier measurements with CEQs or replication clock plasmid are needed to narrow the range of possible early death rates. **Conclusions.** The best available data suggest that the innate immune response has a possibility of clearing an infection of just one or two bacteria, but that this chance becomes negligibly small (less than 1%) for infection doses greater than about 10 bacteria, as is typically used in animal models.

Prior SIV Infection Dysregulates Pulmonary Immune Responses to SARS-CoV-2, While ART Partially Restores Immune Homeostasis

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Background

Despite durable viral suppression with antiretroviral therapy (ART), people living with HIV (PLWH) remain disproportionately susceptible to severe respiratory viral disease, suggesting that persistent immune dysfunction extends beyond control of HIV replication. Chronic HIV/SIV infection reshapes the pulmonary immune landscape through sustained activation. However, the extent to which this altered immune state influences secondary respiratory viral infections, and whether ART restores effective antiviral immunity, remains poorly understood. Leveraging a translational dual-infection rhesus macaque model, we investigated how chronic SIV infection modulates SARS-CoV-2 pathogenesis and defined the immunologic benefits of ART within the lung.

Methods

Rhesus macaques were assigned to SARS-CoV-2-only, untreated SIV/SARS-CoV-2, or ART-treated SIV/SARS-CoV-2 cohorts. Longitudinal bronchoalveolar lavage (BAL), plasma, and endpoint tissue samples were analyzed for viral burden, cytokine dynamics, and systemic inflammatory markers. Disease progression was evaluated using serial PET/CT imaging, while pulmonary immune remodeling was characterized by flow cytometry, histopathology, multiplex immunofluorescence, and ongoing single-cell RNA sequencing.

Results

Pre-existing SIV infection fundamentally altered the host response to SARS-CoV-2, shifting pulmonary immunity toward a sustained hyperinflammatory state associated with impaired viral control. Untreated SIV-infected macaques displayed amplified and prolonged cytokine responses, persistent IL-8- and Perforin-driven inflammation, and delayed IFN- α 2 resolution, accompanied by prolonged pulmonary SARS-CoV-2 replication and elevated BAL viral loads. Whole-body PET/CT imaging demonstrated enhanced inflammatory activity within the lungs, draining lymph nodes, gastrointestinal tract, and brain, consistent with widespread immune activation that persisted beyond acute infection. Immune profiling revealed remodeling of airway myeloid populations, disrupted T-cell phenotypes, increased cellular activation, and diminished antigen responsiveness. ART largely reversed these pathogenic signatures to levels observed in SARS-CoV-2-only controls. Resolution of inflammatory cytokine networks closely paralleled normalization of clinical biomarkers.

Conclusions

These findings identify chronic SIV-induced immune activation—not immunodeficiency alone—as a central driver of enhanced SARS-CoV-2 pathogenesis. ART confers benefits that extend beyond suppression of lentiviral replication by functionally reprogramming pulmonary antiviral immunity, accelerating inflammatory resolution, and improving control of secondary respiratory viral infection. This dual-infection rhesus macaque model provides a powerful translational platform for defining mechanisms of HIV-associated respiratory disease and for evaluating host-directed therapeutic strategies aimed at reducing respiratory morbidity and post-acute sequelae in PLWH.

Validated Reactivity Database - Evidence-Based Data for NHP Reagent Selection

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1. Nonhuman Primate Reagent Resource (NHPRR)

Background

The NHPRR Reactivity Database has supported the nonhuman primate (NHP) research community for more than 20 years by cataloging reagent reactivity across NHP species and serving as a widely cited resource for experimental planning. However, current reactivity classifications (Yes/No) don't describe an application and are not linked to a supporting evidence. As a result, users either accept classifications without verification or independently locate corroborating publications, reducing transparency, reproducibility, and efficiency in study design.

Methods

The database model was redesigned to link each reactivity claim to supporting evidence and classify the evidence as: Historic database, Patent, Preprint, NHPRR/Collaborator Experiment, or Peer-Reviewed Publication. In the new database, each reagent is linked to species-specific reactivity claims and experimental applications, which means an antibody is linked to many species-applications records. This information is critical because experimental methods can alter target structure and epitope availability, resulting in application-dependent differences in reactivity. The database also accommodates conflicting evidence by allowing multiple studies using different applications to report differing outcomes. After structuring the database, we standardized the target genes and aliases using the Vertebrate Gene Nomenclature Committee (VGNC). We then manually reviewed a subset of reactivity records and annotated them with supporting references and application-specific context to the new database.

Results

We reviewed and updated 516 targets for all 2208 reagent records that are linked to 4600 species-application reactivity records. Of the original reactivity, 35 records were removed because the reagent, target, clone, or vendor could not be confidently identified. The database now have a subset of 145 reagent records linked to a total of 605 species-application reactivity records with at least one peer-reviewed publication supporting it.

Conclusions

This new database transforms the NHPRR Reactivity Database from a repository of reagent classifications into an evidence-based knowledge resource. By linking reactivity claims to supporting publications, capturing application-specific context, and accommodating conflicting evidence, the database promotes more rigorous curation and improves reproducibility. The redesigned database supports formal citation of curated data, and enables access to evidence links, helping researchers make more informed decisions when selecting reagents for NHP studies. The reactivity database can be accessed at www.nhpreagents.org.

Transcriptional and Splicing Dysregulation across PBMC Cell Types during HIV and SIV Infection

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Background

HIV and SIV are retroviruses that induce dysfunctions in major cell types in humans and rhesus macaques, respectively. Prior transcriptomic studies have shown transcriptional alterations in PBMC subsets during HIV/SIV infection, but alternative splicing (AS) signatures remain underexplored. AS is a post-transcriptional process that generates functionally distinct isoforms from a single gene and can modulate immune cell function. How transcriptional and splicing programs of immune cells are reprogrammed during retroviral infection, and how this contributes to immune dysfunction, remains poorly understood. We hypothesized that HIV/SIV infection alters gene expression and isoform usage of regulatory genes across major PBMC cell types, contributing to cellular dysfunction and retroviral pathogenesis.

Methods

We performed single-cell short- and long-read RNA sequencing on banked PBMCs: short-read data enabled cell-type annotation and gene expression analysis, while long-read data enabled isoform detection via full-length transcript resolution. The human cohort comprised PBMCs from three healthy individuals and six people living with HIV-1 on ART. The rhesus macaque cohort comprised PBMCs from three ART-naïve macaques sampled pre-infection, early infection (~14 dpi), and later-stage infection (80-112 dpi) during SIV infection.

Results

We captured 15,639 human and 6,804 rhesus macaque cells across all samples, revealing numerous significantly differentially expressed genes across B cells, CD4+ and CD8+ T cells, monocytes, and NK cells between infection conditions in both species. CPQ was significantly upregulated in CD4+ T cells of PLWH compared to healthy controls, and immune/inflammatory gene sets including TNF- α signaling via NF κ B and interferon- α/γ pathways were upregulated across most cell types during infection in both species. Long-read analysis of these cell types revealed infection-associated isoform expression signatures and identified numerous previously undocumented isoforms, several specific to infection status. For instance, a novel MAMU-E isoform was upregulated in later-stage SIV infection compared to pre-infection, suggesting infection-stage-specific splicing of an immune regulatory ligand.

Conclusions

These findings demonstrate that HIV/SIV infection remodels immune cell transcriptomes and splicing programs in a cell-type-specific manner. Further transcriptomic, splicing modulation, and functional studies are needed to confirm direct links between this modulation and immune dysfunction.

Lack of Antigen-Specific CD8⁺ T-Cell Responses is Associated with Viral Rebound Following ART Interruption

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Background:

Human Immunodeficiency Virus (HIV) infection leads to CD4⁺ T-cell depletion and impaired immune function. Although antiretroviral therapy (ART) suppresses HIV replication, most individuals experience viral rebound following analytical treatment interruption (ATI). In contrast, post-treatment controllers maintain viral suppression after ATI. Because post treatment control (PTC) occurs frequently in Simian Immunodeficiency Virus (SIV)-infected Mauritian cynomolgus macaques (MCMs), they provide a model for studying PTC. CD8⁺ T-cells are critical for PTC, but studies investigating the contribution of antigen-specific (AS) CD8⁺ T-cells versus other CD8⁺ T-cells to PTC are limited. We wanted to test the hypothesis that AS CD8⁺ T-cells are required for PTC in MCMs.

Methods:

To test our hypothesis, we conducted a 32-week comparative study of SIVmac239 and a variant virus, SIV-10x, that contains 10 point mutations in epitopes targeted by M3 MHC alleles, largely eliminating AS CD8⁺ T-cell responses in M3⁺ MCMs. If the hypothesis is correct, M3 homozygous MCMs infected with SIV-10x will experience viral rebound along with an increased percentage of CD8⁺ T-cells expressing exhaustion and a marked decline in CD4⁺ T-cells, unlike their SIVmac239-infected counterparts. To test this, three M3 homozygous MCMs per group were infected with SIV-10x or SIVmac239 and received ART from 2–24 weeks post-infection.

Results:

SIV-10x-infected MCMs exhibited greater viral load fluctuations during ART and higher post-ATI viral loads than SIVmac239-infected MCMs. Additionally, post-ATI SIV-10x-infected macaques exhibited increased numbers of PD1⁺ CD8⁺ T-cells in the lymph node, coinciding with increasing viral load.

Conclusions:

Collectively, these findings demonstrate that loss of antigen-specific CD8⁺ T-cell responses is associated with increased viral rebound and an exhausted CD8⁺ T-cell phenotype, supporting a critical role for these cells in maintaining post-treatment viral control.

CD4 Downregulation Kinetics in African Green Monkey T cells

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Background

African green monkeys (AGMs) are a natural host species for simian immunodeficiency virus and have evolved mechanisms to avoid pathogenicity despite high viral loads. One important mechanism is to downregulate expression of CD4 on the surface of CD4⁺ T cells rendering these cells resistant to infection.

Methods

CD4⁺ T cells from AGMs were stimulated *in vitro* with anti-CD3/CD28 beads to induce CD4 downregulation. CD4⁺ and CD4⁻ cells were then flow cytometrically sorted at multiple timepoints post-stimulation and analyzed by RNAseq for gene expression, ATACseq for chromatin accessibility, and by whole genome methylation analysis.

Results

Surface expression and gene expression of CD4 were both rapidly lost within days of stimulation. Chromatin accessibility at the CD4 promoter region began to be lost by day 3 post-stimulation but even by day 5 did not reach the complete lack of accessibility seen in T cells that have fully lost CD4 expression at steady state. Similarly, DNA methylation increased in two regions near the CD4 transcriptional start site by day 3 post-stimulation but remained lower than in steady state downregulated cells.

Conclusion

Rapid alterations to CD4 mRNA levels suggest control of CD4 surface expression primarily at the transcriptional level while later epigenetic modifications likely serve to preserve the CD4⁻ state. Improved mechanistic understanding of CD4 downregulation could lead to future therapeutic approaches.

Role of IL-6 in T-cell Lineage Restriction of Pre-thymic Progenitors and Impaired T-cell Regeneration Associated with Viremia and Reservoir Burden in the SIV/Macaque Model of AIDS

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Background. T cell reconstitution in people with HIV depends on sustained thymic output, requiring continuous seeding by bone marrow (BM)-derived thymus-seeding progenitors (TSPs). Under inflammatory stress, HSPCs favor myelopoiesis over lymphopoiesis, but whether infection restricts T cell lineage commitment before thymic entry, and whether this associates with SIV burden, is unknown.

Methods. BM CD4⁺CD8⁺ double negative (DN) CD34⁺CD7⁺ TSPs were quantified in SIV-naïve (N=15) and SIV-infected (N=12) rhesus macaques (RMs), and longitudinally in six RMs given 300 TCID₅₀ SIVmac239M intravenously (tracked day 0, weeks 2-to-16). Pre-thymic T cell potential of sorted CD3⁺CD34⁺ HSPCs was assayed in rhesus artificial thymic organoids (RhATO). Plasma cytokines, HSPC RNA-seq/proteomics, and intact provirus (IPDA) were measured. The effect of IL-6 was tested ex vivo using virus-naïve RM and human HSPCs and RhATO/ATO systems.

Results. BM TSPs decreased ~5-fold (P=0.001) against a ~2-fold rise in total DN CD34⁺ HSPCs under SIV infection. TSPs declined >4-fold by week 2, with peak viremia; the CD38⁺ DN1 TSP subset remained reduced at week 16. Concordantly, CD4⁺CD3⁺ immature single-positive thymocyte output decreased ~4-fold (~24% to ~6%, P=0.0001) despite comparable CD45⁺ lymphocyte output, while lymphoid-primed multipotent progenitors expanded >2-fold (P=0.015), locating the block at commitment, not abundance. Plasma IL-6 increased 5-fold at peak viremia and tracked with viremia (r=0.84, P=0.03), TSP attrition (r=0.92, P=0.008), and reduced T cell potential (r=0.83, P=0.04). Viral burden tracked with TSP attrition (r=0.96, P=0.002) and, inversely, with reduced T cell regeneration (r=-0.88, P=0.02); cumulative IL-6 tracked with intact provirus (r=0.87, P=0.024). HSPCs showed IL-6-JAK-STAT and inflammasome activation with suppression of RUNX1, FYN, ZAP70, and RAG2. Ex vivo, IL-6 reduced TSPs dose-dependently (P=0.002) and shifted progenitors toward CD38⁺, reducing DN2 TSPs in rhesus (P=0.0008) and human (P=0.001) organoids; IL-6R blockade restored TSPs from ~18% to ~40%, not significantly different from ~53% control.

Conclusions. IL-6 enforces a CD38⁺CD7⁺ progenitor state in BM HSPCs, blocking the earliest step of T cell lineage commitment before thymic entry. It arises within two weeks of infection, is reversible by IL-6R blockade, and tracks with viremia and reservoir burden, identifying IL-6/IL-6R as a target for restoring T cell regeneration in HIV cure strategies.

Efficacy and Safety of AI-enabled PRS Regimen VI (Clofazimine, Bedaquiline and Pyrazinamide) as Ultra-Short Course Therapy of LTBI in Non-Human Primates in a setting mimicking HIV co-infection

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Background: Latent tuberculosis infection (LTBI) affects nearly two billion people globally and serves as a persistent reservoir for future active TB, particularly in immunocompromised populations. Existing LTBI treatment regimens are lengthy and often poorly tolerated, contributing to low adherence and limited effectiveness. In our previous work, we employed an artificial intelligence-based parabolic response surface (AI-PRS) platform to identify a highly synergistic combination of Bedaquiline (BDQ), Clofazimine (CFZ), and Pyrazinamide (PZA). This regimen demonstrated relapse-free cure in murine TB models within three weeks, marking an approximately 85% reduction in treatment duration compared to conventional therapies.

Methods: In the current study, we aim to assess the efficacy of this optimized three-drug regimen (BCZ) in non-human primates (NHPs) under conditions simulating HIV co-infection via simian immunodeficiency virus (SIV). We have performed a subacute pharmacokinetic (PK) study in Rhesus macaques for 14 days at different dose levels to determine the optimal, clinically relevant doses of each drug in the combination. The plasma drug concentrations were quantified using a validated LC-MS/MS method. Time vs concentration curves were plotted and modeling was done to establish the PK parameters.

Results: PZA at 1x human equivalent dose, and CFZ at half human equivalent dose achieved clinically relevant exposure levels as measured by the Area Under the Curve (AUC). BDQ, at double the human equivalent dose, reach near the therapeutic window levels. However, 2-3 fold higher levels of the M2 metabolite, less potent than the parent molecule, were observed here than in clinical trials. We are currently validating the PK of the optimized BCZ drug combination in NHPs, followed by an efficacy study in an aerosol-induced LTBI model, where animals receive varying durations of the BCZ regimen or the standard isoniazid-rifapentine (3HP) treatment. Subsequent SIV co-infection will be used to evaluate the ability of each regimen to prevent TB reactivation under immune suppression.

Conclusion: Demonstrating robust prevention of TB reactivation with short-course BCZ therapy in this model would support its advancement to clinical trials and may significantly improve LTBI management strategies, accelerating progress toward global TB elimination.

Age-associated alterations in intact SIV genome decay kinetics during suppressive antiretroviral therapy

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Background: Antiretroviral therapy (ART) has extended the lifespan of people with HIV (PWH); however, disparities in health span persist. Over 50% of PWH in the United States are aged >50 years, a proportion expected to exceed 70% by 2030. Immune aging is characterized by clonal expansion of memory T cells and loss of naïve T cells, leading to a naïve-memory imbalance. However, how these changes affect HIV reservoirs persistence remains understudied. We hypothesize that age associated clonal expansion of memory T cells alters HIV reservoirs decay kinetics in older PWH, presenting a barrier to ART-free viral remission.

Methods: Old (19-21yrs) SIVmac251 infected, ART treated rhesus macaques (RMs) were used in this study. Intact SIV proviral genome in peripheral blood CD4⁺ T cells, and CD14⁺ myeloid cells were quantified longitudinally using IPDA to assess reservoir decay kinetics. Additionally, the Primate Cognitive Testing Battery was performed to evaluate cognitive function, and multimodal Magnetic Resonance Imaging was used to assess volumetric and structural alterations in the brain.

Results: Aged RMs exhibited significantly higher plasma and CSF SIV RNA levels during acute infection than young historic controls. SIV reservoirs exhibited cell specific decay kinetics, with biphasic decay in CD4⁺ T cells (phase 1 $t_{1/2}$ =18 days), followed by a steady state plateau during phase 2. In contrast, intact and total proviral DNA in CD14⁺ monocytes exhibited a first-order decay kinetics ($t_{1/2}$ =67 and 51 days). Additionally, intact SIV DNA correlated with activated CD69⁺CD8⁺ T cells. Aged RMs experienced a decline in cognitive function, that did not return to baseline following ART mediated viral suppression. Diffusion Tensor Imaging revealed a significant decline in white matter Fractional Anisotropy post ART mediated viral suppression vs baseline.

Conclusions: In aged RMs, we observed biphasic SIV reservoirs decay in CD4⁺ T cells, distinct from the triphasic decay reported in younger RMs, while monocytes exhibited unique first order decay kinetics. These findings suggest that aging alters viral reservoir dynamics, with important implications for HIV cure strategies in older PWH. Ongoing tissue reservoirs and brain imaging studies will further delineate the relationship between immune aging, viral persistence and cognitive health.

CD7 Identifies Pre-thymic Progenitors with T-cell Regenerative Potential in the Bone Marrow of Rhesus Macaques

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Background. T cell regeneration underpins immune reconstitution and is central to HIV cure, and the rhesus macaque (RM) is an indispensable model for testing T cell-directed interventions. Thymic output depends on continuous seeding by bone marrow (BM)-derived thymus-seeding progenitors (TSPs), yet no phenotypic definition of the RM pre-thymic progenitors in BM carrying T cell regenerative potential exists, a gap limiting interpretation of interventions aimed at restoring thymopoiesis.

Methods. Matched BM and thymus from SIV-naïve adult RMs (N=8) were analyzed by flow cytometry. Using markers established for human lymphoid-committed TSPs, live CD4⁺CD8⁺ mononuclear cells were resolved on CD34 and CD7; CD38 distinguished DN1 (CD38⁺) from DN2 (CD38⁺), and TCF-1 was assessed on thymic progenitors. TSPs were compared by frequency, absolute number per 10⁶ mononuclear cells, and correlation. Sorted CD4⁺CD8⁺CD3⁺CD34⁺CD7⁺ progenitors (1,000-5,000 cells) were seeded per rhesus artificial thymic organoid (RhATO; Wilde et al., *Biomedicine* 2025) and T cell ontogeny assessed at week 4.

Results. DN CD34⁺CD7⁺ TSPs comprised ~3.2% of DN CD34⁺ HSPCs in BM versus ~35% in thymus; though the thymic proportion was >10-fold higher, absolute numbers per 10⁶ mononuclear cells were comparable (~914 vs ~871), indicating a small but continuously supplied BM pool. CD7 was discriminating: CD7-expressing DN CD34⁺ HSPC frequencies correlated between BM and thymus (r=0.80, P=0.01), a correlation abolished when CD7 was excluded (r=-0.30, P=0.47). BM CD34⁺CD38⁺CD7⁺ (DN1) cells associated with thymic DN CD34⁺ (r=0.84, P=0.008) and TCF-1-expressing early progenitors (r=0.74, P=0.03), linking the BM phenotype to the T cell-committed thymic compartment. In RhATO, sorted DN CD34⁺CD7⁺ progenitors recapitulated the full continuum: CD4⁺CD3⁺ ISP and CD4⁺CD8⁺ double-positive thymocytes, then mature CD3⁺ T cells resolving into CD4⁺ and CD8⁺ single-positive subsets. Most naïve T cells expressed CD38, matching the recently reported phenotype of human recent thymic emigrants.

Conclusions. CD4⁺CD8⁺CD34⁺CD38⁺CD7⁺ defines the lymphoid thymus-seeding progenitor in RM bone marrow. CD7 is required for the phenotype to track thymic progenitor content, and the sorted population generates TCR-bearing T cells in a thymus-independent organoid. This provides the reference population needed to test how persistent infection, inflammation, and candidate therapeutics alter pre-thymic T cell lineage commitment in the NHP model.

Anti-Herpes Family Virus Treatment to Reduce Chronic Inflammation in Asian Macaques

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Background

Cytomegalovirus (CMV) is a herpesvirus infecting up to 90% of adults in developing countries and up to 100% in research Asian macaques. While CMV infection is asymptomatic in immunocompetent hosts, it may contribute to chronic inflammation. Indeed, high frequencies of circulating T cells are CMV-specific. Thus, we hypothesized the therapeutic reduction of CMV burden would reduce residual inflammation in SIV+ and SIV- Asian macaques.

Methods

The effective dosing range of oral valganciclovir was tested for rhesus models over 18 weeks (10 mg/kg per day, increasing to 40 mg/kg), followed by a high dose phase for 4 weeks (50 mg/kg per day). Urine and saliva, major dissemination sources during acute and reactive phases of CMV infection, were collected to determine viral shedding concentrations. Inflammation was assessed by measurement of plasma cytokines and biomarkers (IP-10, CD163, and TNFR1) via ELISA. Peripheral blood was analyzed via flow cytometry to assess activation of lymphocytes while on and off treatment.

Results

Viral shedding in both urine and oral samples remained at consistent levels between both control and treatment groups during the dose escalation phase. No significant differences in cytokine levels nor lymphocyte activation (CD4+ and CD8+) were observed during treatment and post-treatment timepoints. Mild neutropenia was observed in the high dose phase at 4 weeks on treatment.

Conclusion

Valganciclovir was incapable of completely reducing rCMV shedding in urine and plasma. Long term valganciclovir does not reduce inflammation in Asian macaques. The influences of valganciclovir on other herpes virus family members was not studied, but our results suggest that these viruses are unlikely to cause significant inflammation in immunocompetent hosts.

Novel breastmilk TF SHIVs to advance development of bnAb strategies to prevent vertical HIV-1 transmission

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Background: In 2025, ~40,000 children acquired HIV via breastmilk transmission, highlighting the need for novel strategies to eliminate vertical transmission. Broadly neutralizing antibodies (bnAbs) are promising candidates for HIV prevention and treatment that warrant evaluation in nonhuman primate (NHP) models.

Methods: Here, we generated a panel of novel breastmilk transmitted/founder Simian-Human Immunodeficiency Viruses (TF SHIVs) for use in NHP bnAb transmission studies. Leveraging two pediatric cohorts (ZEBS and CHAVI 009) (PMID: 18525036, 21191008), we engineered and characterized twelve TF SHIVs (PMID: 27247400) each expressing a unique breastmilk TF Env identified from Malawian and Zambian infants who tested negative at birth and subsequently acquired HIV during breastfeeding.

Results: Pseudovirus neutralization assays revealed that the breastmilk TF Envs display a range of neutralization sensitivities to clinically relevant bnAbs. To enhance replication in NHPs, we generated 6 variants of each TF SHIV with mutations at Env position 375. We tested these variants for preferred entry into modified ZB5 cells expressing rhesus CD4 and CCR5, and for replication in rhesus CD4+ T cells. For each TF SHIV, the most fit variant was identified, barcoded (PMID: 28472156) for *in-vivo* tracking, and sequence confirmed. The neutralization phenotype of each variant will be tested for its fidelity to its parental breastmilk TF Env.

Conclusions: In summary, we have generated a panel of novel, barcoded breastmilk TF SHIVs optimized to advance the clinical development of bnAb strategies to prevent pediatric breastmilk HIV transmission.

Dual tropic HIV-1 infection in owl monkeys (*Aotus nancymae*)

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Background

Recently, we achieved successful CXCR4-tropic HIV-1 infection of immunocompetent owl monkeys (*Aotus nancymae*) that recapitulates key aspects of human HIV-1 infection. However, in humans, HIV-1 infection is nearly always established with CCR5-tropic viruses, making CCR5 usage an essential feature for modeling natural transmission and early infection. To address this limitation, we sought to generate a virus that can utilize CCR5 to infect owl monkeys and give new insight into HIV-1 dynamics in this model.

Methods

We generated a chimeric envelope within our existing HIV-1 backbone modified for owl monkeys (HIVnom3) that contains the variable regions from dual tropic HIV-1 strain 89.6, while maintaining all the features previously determined to be important for replication in owl monkeys. This virus is referred to as HIVnom4. We assessed viral entry into cells expressing owl monkey CD4 alongside either CXCR4 or CCR5. T cells from naive animals were infected with HIVnom4 and progeny virion production was assessed over time via TZMbl reporter cell β -galactosidase assay. Finally, 3 owl monkeys were intravenously inoculated with HIVnom4 and viral load over time was assessed via qPCR.

Results

We show that HIVnom4 can utilize owl monkey CD4 and both co-receptors, CCR5 and CXCR4, for cell entry. This virus replicates efficiently in primary owl monkey T cells *in vitro*. Similarly, following inoculation of immunocompetent owl monkeys, HIVnom4 established productive infection with high levels of viral replication. We further characterize these viral and immune dynamics during infection with this first-in-class dual-tropic virus for the owl monkey model.

Conclusions

In vitro infection of T cells and *in vivo* infection of owl monkeys with HIVnom4 reproducibly generates high levels of viral replication and will enable studies of CCR5-dependent HIV-1 infection in this model. This novel virus expands the utility of the owl monkey model and will enable in-depth comparative studies of pathogenesis, immune responses, and the viral reservoir to evaluate future use as a pre-clinical model of HIV-1 infection.

Plasmodium fragile Infection Induces Innate Inflammatory Responses Within Distinct Brain Regions of Pregnant Rhesus Macaques.

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Background:

Pregnancy alters immune activity in the frontal lobe (FL), hippocampus, basal ganglia (BG), and cerebellum, leaving these areas vulnerable to damage. Neurological injury is a consequence of malaria in pregnancy (MIP). However, the immune factors that drive MIP-associated brain impairment have not been defined. We hypothesized that *P. fragile* MIP would result in upregulation of inflammatory proteins in maternal cerebrospinal fluid (CSF) and microglia inflammation.

Methods:

Pregnant rhesus macaques (RMs) were intravenously inoculated with 20×10^6 *P. fragile*-infected erythrocytes or saline (n=9, each) followed by necropsy ~4 weeks post-inoculation, during which CSF and maternal brain were collected. Anti-malarials were administered when parasitemia exceeded 0.5%. Differentially expressed CSF proteins were identified by data independent acquisition mass spectrometry. Maternal FL, hippocampus, BG, and cerebellum were assayed for tight junctions (ZO-1, GLUT-1) and microglia (Iba1) via fluorescent immunohistochemistry.

Results:

Protein pathways enriched in CSF from RMs with *P. fragile* MIP include the complement system, innate immunity, phagocytosis, interleukin 10 synthesis, and pathways favoring survival of intracellular parasites. Pathways associated with aerobic respiration, translation, biological oxidation, and metabolism of amino acids and lipids were enriched in CSF from uninfected pregnant controls. Tight-junction integrity was significantly reduced in the hippocampus (p=0.0464) but was unchanged in other brain regions assayed in RMs with *P. fragile* MIP compared to controls. In the hippocampus and cerebellar white matter, microglia frequency was significantly increased (p=0.00142 and p=0.0315, respectively) and microglia dendrite length and node number were significantly decreased (hippocampus: p=0.0006 and p<0.0001, respectively; cerebellar white matter: p=0.0275 and p=0.0055, respectively) in RMs with *P. fragile* MIP as compared to controls.

Conclusions:

P. fragile MIP resulted in elevation of protein pathways associated with innate immunity and impaired parasite clearance. Evidence of vascular damage and microglia inflammation suggests that hippocampal and cerebellar white matter are vulnerable to MIP, compared to other regions. These findings demonstrate that brain regions modulated by pregnancy are vulnerable to immune mediated damage due to MIP and that the innate immune system may drive these differences in the brain. Experiments to assess astrocyte activation and morphological changes throughout pregnancy and assessing parasite burden within these brain regions.

Machine learning-based feature selection suggests circulating proteins as best at predicting and diagnosing tuberculosis in *Mycobacterium tuberculosis*-exposed rhesus macaques

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1. Texas Biomedical Research Institute

% background

Mycobacterium tuberculosis (*Mtb*) is a leading cause of morbidity and mortality worldwide and remains the leading cause of death among individuals with concurrent immunosuppressive infections. Although billions carry evidence of past or present *Mtb* infection and over a million develop tuberculosis (*TB*) yearly, most exposed individuals do not progress to active disease and instead control (and perhaps eliminate) the infection. The factors determining progression remain poorly understood, and are of particular concern where coinfection compromises immune control. *Mtb*-exposed non-human primates (*NHPs*) such as rhesus macaques (*RMs*) similarly exhibit variable likelihood of active disease, providing a tractable model for dissecting predictors of progression relevant to both single- and coinfection settings.

% methods

We analyzed over 200 *Mtb*-exposed *RMs*, applying three machine learning (*ML*) techniques—random forest, linear support vector machine, and logistic regression—to quantify the contribution of 20 features (and their derivatives) to predicting development or diagnosis of active disease. Feature discrimination was assessed by ROC/AUC, and feature contributions were attributed using SHAP analysis.

% results

Independent of *ML* technique, C-reactive protein (*CRP*), albumin-to-globulin ratio (*A/G*), and lung CFU were most informative at discriminating animals with active disease from those without. Excluding endpoint (necropsy) measurements, *CRP*.peak and *A/G*.bottom dramatically outperformed all other features at determining progression. The top 5 features (all features) or top 2 (excluding endpoint measurements) achieved nearly the discriminatory power ($\text{AUC} \geq 0.98$) of all 20+ features. Blood leukocyte frequencies (e.g., percent neutrophils or lymphocytes) and their ratios (e.g., neutrophil-to-lymphocyte ratio) had poorer value ($\text{AUC} < 0.8$).

% conclusion

A combination of *CRP* and *A/G* ratio thus has high power for predicting and/or diagnosing *TB* in *Mtb*-exposed *RMs*. Future studies should investigate whether temporal changes in blood proteins and leukocyte frequencies further improve prediction, including in the context of coinfection.

ART Accelerates Viral Clearance and Resolves SIV-Primed SARS-CoV-2 Inflammation

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Background: Persons living with HIV (PLWH) experience heightened respiratory morbidity following secondary viral infections, extending beyond CD4⁺ T-cell depletion to include chronic myeloid and epithelial immune dysregulation¹. HIV tropism is defined by the expression of CD4 and coreceptors (CCR5/CXCR4)^{2,3} rather than cell lineage alone. Alveolar macrophages and epithelial cells are targets for both SIV/HIV^{4,5,6} and SARS-CoV-2⁶, creating a pulmonary microenvironment where prior lentiviral infection may prime secondary coronavirus responses. While antiretroviral therapy (ART) effectively suppresses viral replication, its impact on the kinetics, magnitude, and resolution of pulmonary cytokine responses to secondary viral challenges remains incompletely understood; ART has been reported to modify tissue-specific interferon signaling including types I and III interferons⁷, alters alveolar macrophage infiltration⁸, and reduces systemic immune activation⁹, yet whether these changes improve control of secondary respiratory viruses is unknown. Here, we investigate whether prior SIV infection alters SARS-CoV-2 outcomes and whether ART modulates the kinetics and magnitude of these responses in rhesus macaques.

Results: Untreated SIV infection primed the pulmonary cytokine response to SARS-CoV-2, characterized by exaggerated magnitude and delayed, prolonged kinetics compared to SARS-CoV-2-only infected macaques. ART treatment restored IFN- α 2 kinetics to SARS-CoV-2-only levels while suppressing the magnitude of neutrophil-attracting (IL-8) and cytotoxic cytokines (Perforin). In terms of viral replication, ART treatment effectively controlled SARS-CoV-2 replication in the lungs of SIV-infected macaques, reducing BAL titers to levels comparable to SARS-CoV-2-only controls reaching undetectable levels faster than SARS-CoV-2-only controls (DPI 22 vs DPI 30), whereas non-ART SIV animals exhibited higher viral burdens and prolonged presence. Systemic ART efficacy was confirmed by the expected SIV viral kinetics and subsequent suppression, compared to the sustained high viremia observed in untreated animals.

Conclusion: This current work supports the idea that S/HIV exaggerates secondary coronavirus responses, and treatment with ART ameliorating this heightened inflammatory response while curtailing viral load. These findings provide an early mechanistic framework for understanding respiratory disease in PLWH upon subsequent respiratory virus infection and highlight the importance of maintained antiviral therapy.

Interpreting Clinical Pathology in Infant SHIV Infection: Distinguishing Age-Related Hematologic Changes from Disease

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Background: Clinical pathology is a fundamental translational biomarker used to monitor disease progression and evaluate safety in both nonhuman primate (NHP) studies and human clinical trials. Interpretation of hematologic data in infant rhesus macaques and humans, however, is complicated by rapid physiologic maturation that overlaps with the effects of viral infection and investigational therapeutics. Establishing a framework to distinguish normal developmental variation from disease- or test article-related changes is essential for pediatric HIV research.

Methods: We performed a retrospective analysis of complete blood count (CBC) data from 25 infant rhesus macaques experimentally infected with oral SHIV across multiple pediatric SHIV studies. Longitudinal hematologic findings were evaluated in the context of age at infection, disease progression, and associated clinical and pathologic observations to distinguish expected developmental changes from SHIV-associated abnormalities.

Results: Age-related differences were observed across multiple hematologic parameters, emphasizing the importance of developmental context when interpreting CBC results in infant macaques. Thrombocytopenia was the most consistent clinicopathologic abnormality associated with SHIV infection and ranged from mild to severe among affected animals. Longitudinal assessment demonstrated that platelet abnormalities were more accurately interpreted when considered alongside age-dependent hematologic maturation and concurrent clinical findings rather than by comparison with static reference intervals alone. This integrated approach improved discrimination between normal developmental variation, SHIV-associated disease, and changes that could otherwise be misattributed to investigational interventions.

Conclusions: Clinical pathology represents a highly translatable biomarker for pediatric HIV studies but requires age-appropriate interpretation to maximize its value. Our retrospective analysis provides a practical framework for evaluating hematologic changes in infant SHIV models by integrating longitudinal CBC data with developmental biology and clinical context. These findings support more accurate assessment of disease progression and safety in preclinical studies and strengthen the translational relevance of infant NHP models for pediatric HIV therapeutic development.

Type I Interferon Signatures and Necroptotic Signaling are Associated with CD206⁺CD163⁻ Perivascular Macrophages During Pediatric SIV Infection

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The mechanisms underlying the distinct neuropathogenesis of pediatric and adult HIV/simian immunodeficiency virus (SIV) infection remain poorly understood. We previously identified a novel subpopulation of CD206⁺CD163⁻ perivascular macrophages (PVMs) that is abundant in the healthy infant brain but selectively depleted following SIV infection, suggesting a unique role in pediatric neuroimmune responses. However, the molecular mechanisms regulating the susceptibility of this macrophage subset and the effects of antiretroviral therapy (ART) remain unknown. In the present study, we combined bulk RNA sequencing and multiplex immunofluorescence analyses to characterize CD206⁺CD163⁻ PVMs in uninfected, SIV-infected, and ART-treated infant and adult rhesus macaques. Transcriptomic analyses revealed that untreated SIV infection induced robust activation of type I interferon (IFN) signaling characterized by differentially expressed interferon-stimulated genes IRF7, IFI6, ISG15, MX1, DDX60, and JAK3. Gene enrichment further identified significant activation of innate immune, myeloid cell, macrophage-associated, and autophagy-related pathways, linked to regulated cell death. Successful ART markedly attenuated IFN-associated inflammatory signaling while restoring transcriptional programs involved in neuronal development, synaptic organization, and cellular homeostasis. In contrast, ART non-responders retained persistent inflammatory and neuronal injury signatures despite treatment. Adult transcriptomic analyses similarly demonstrated suppression of chronic antiviral signaling and recovery of neuronal and myelin-associated pathways following long-term ART, whereas ART alone produced minimal transcriptional changes in uninfected brains. Multiplex immunofluorescence was performed using phosphorylated mixed lineage domain-like protein (pMLKL), the execution marker of necroptosis in CD206⁺ macrophages, with the highest frequency observed in untreated SIV-infected adults and lower levels in ART-treated animals, consistent with reduced necroptotic signaling following viral suppression. Phenotypic analyses confirmed that CD206⁺CD163⁻ PVMs persist in ART-treated infant and adult brains and exhibit a distinct immunophenotype characterized by minimal expression of CD68, CD14, CD16, and HLA-DR together with reduced CSF1R levels. Integration of transcriptomic and immunophenotypic analyses supports a model in which type I IFN-driven neuroinflammation promotes susceptibility of CD206⁺ macrophages to necroptotic signaling, while ART attenuates inflammatory responses and reduces activation of this cell death pathway. These findings provide new mechanistic insight into pediatric HIV/SIV neuropathogenesis and identify potential therapeutic targets for limiting persistent neuroimmune dysfunction despite ART.

Comparative approaches to depleting antibodies in SIV-infected pigtail macaques

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Background:

Antibodies play critical roles in adaptive immune responses to a broad range of pathogens through mechanisms including neutralization, opsonization, complement activation, promoting phagocytosis, and recruitment of immune effector cells. Studies in model systems that selectively deplete B cells or specific antibody classes, such as IgG, have been instrumental in defining both protective and pathogenic roles for humoral immune responses. Depleting antibody levels in vivo following Simian Immunodeficiency Virus (SIV) infection and seroconversion or depleting B cells prior to infection to delay antibody generation after infection provide unique opportunities to determine the impact of humoral immune responses on viral pathogenesis.

Methods:

In this pigtail macaque study, we evaluated three methods to deplete total B cells or, specifically, secreted IgG during SIV infection: treatment with 1) rhesusized anti-CD20 (rituximab) to deplete B cells; 2) an anti-neonatal Fc receptor antibody (rozanolixizumab), which blocks IgG recycling following physiologic endocytosis to prevent its return to the systemic circulation; and 3) IdeS (imlifidase), a cysteine protease from *Streptococcus pyogenes* that cleaves IgG into F(ab')₂ and Fc fragments. These treatments were administered to pigtail macaques during or following infection with SIVmac239ΔGY, an SIVmac239 variant that generates high and sustained levels of SIV-specific antibodies and immunity that typically results in virus control. We measured depletion efficacy and rebound kinetics of total and SIV-specific antibodies and assessed their impact on viral control.

Results:

The depletion strategies altered antibody classes, or specifically IgG subclasses, in serum with different efficacies and durability. More efficient depletion of total IgG showed functional consequences with lower binding to Fcγ receptors as well as antibody dependent complement deposition. When antibody levels in serum were allowed to rebound post treatment, they did not always return to baseline with some cases of antibody subclass and antigen spread.

Conclusion:

This study aimed to determine the role of B cells and IgG in lentiviral pathogenesis specifically SIV in nonhuman primates. Overall, this work provides new insights into approaches to modulate humoral immune responses, that could lead to the understanding of antibody responses important for protection against various pathogens or diseases.

A Biodegradable Long-Acting Implant Platform for Maintenance Antiretroviral Therapy in a Rhesus Macaque Model

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Despite advances in antiretroviral therapy (ART), children living with HIV (CLWH) require lifelong daily treatment, creating adherence challenges that increase the risk of virologic failure and drug resistance. Long-acting (LA) implants could address these barriers by providing sustained antiretroviral (ARV) delivery with minimal treatment burden. We developed a biodegradable extended-release implant platform for maintenance ART comprising co-formulated bicitegravir/emtricitabine (BIC/FTC) implants and a separate islatravir (ISL) implant. Preliminary findings show that this implant system maintained virologic suppression in a subset of SIVmac251-infected rhesus macaques following transition from subcutaneous daily tenofovir disoproxil fumarate (TDF), emtricitabine (FTC), and dolutegravir (DTG). Macaques received either two BIC/FTC implants plus one ISL implant (n = 3) or three BIC/FTC implants plus one ISL implant (n = 3), while a control group (n = 3) remained on subcutaneous daily ART. Virologic suppression was maintained in one of three animals receiving two BIC/FTC implants and two of three receiving three BIC/FTC implants; all daily ART controls remained suppressed or exhibited only low-level viremia (<200copies/mL). Explant preliminary observations suggested that, in some animals, adjacent implants were closely co-localized, possibly reducing the effective drug-releasing surface area and potentially limiting systemic drug exposure. Ongoing pharmacokinetic and tissue analyses will examine exposure-response relationships and optimize implant design.

The Pigtail Macaque Lung and Immune Single Cell RNA-Seq Atlas

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1. WaNBRC

Single-cell RNA sequencing (scRNA-seq) allows cell classification using genome-wide transcriptional state. The non-human primate (NHP) provides a powerful translational model to overcome the limitation of studying late development, given its close similarity to human developmental trajectories. We constructed three single-cell RNA-Seq (scRNA-Seq) atlases of peripheral blood mononuclear cells (PBMC), lymph node, and lung from the healthy, uninfected pigtail macaque (*Macaca nemestrina*) aligned to the rhesus macaque genome (Mmul_10). Tissues and blood were dissociated into single cells and processed through the single-cell mRNA-Seq 10X Genomics (Cell Ranger), CellBender (removal of ambient RNA), Scrublet (removal of doublets), and scVI (machine learning for unsupervised clustering) using GPU-accelerated pipelines. Next, aggregation and cluster annotation were performed using a literature-based hand annotation process. Tissues from adult animals were used in all datasets, but the PBMC atlas included samples (N=8) from each of four different demographic groups: adult male, adult non-pregnant female, pregnant female (third trimester), and fetuses. After clustering and annotation, the PBMC, lymph node, and lung atlases had 29, 23, and 30 clusters, respectively. Application of machine learning through CellBender and scVI using GPU acceleration enabled better discrimination of clusters than was possible using standard algorithms. These single-cell RNA-Seq atlases of the PBMC, lymph node, and lung provide a unique reference for healthy and IAV-infected tissues in the adult and during critical windows of nonhuman primate development. A rich diversity of common and rare single-cell populations was identified, which can be compared to respiratory virus-infected datasets.

Vaccination regimen and route differentially program mucosal tissue-resident CD8⁺ T cell immunity in rhesus macaques

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Background: The induction of durable tissue-resident memory CD8⁺ T (T_{RM}) cells at mucosal portals of HIV entry remains a major challenge for vaccine development. Although systemic T cell responses can be readily elicited, the influence of vaccine platform and route of administration on the establishment, persistence, and functional programming of mucosal TRM populations in nonhuman primates remains poorly understood.

Methods: We compared three SIVmac239 Gag-expressing vaccine regimens in rhesus macaques: a DNA-MVA-ChAdC6 regimen administered intramuscularly (DMC-IM) or intravenously (DMC-IV), and a heterologous viral vector regimen consisting of VSV, vaccinia virus, and Ad5 delivered intravenously (HVV-IV). We determined the frequency and function of Gag specific CD4 and CD8 T cells in blood, rectum and vagina and performed single-cell RNA sequencing of vaginal CD8⁺ T cells following intravaginal peptide challenge.

Results: The HVV-IV regimen elicited significantly higher frequencies of Gag-specific CD8⁺ T cells in vaginal and rectal mucosa three months after the final immunization than either DMC regimen, despite comparable systemic responses. In contrast, DMC-IM and DMC-IV induced similar mucosal responses, indicating that vaccine composition rather than route alone determined durable mucosal CD8⁺ T cell accumulation within this platform. HVV-IV also preferentially enriched CD69⁺ tetramer⁺ CD8⁺ T cells in vaginal tissue, consistent with enhanced establishment of mucosal CD69⁺ populations. Functionally, HVV-IV generated more cytotoxic and degranulating CD8⁺ T cells, whereas DMC regimens favored cytokine-biased responses and induced higher frequencies of CCR5⁺ MIP-1β⁻ CD4⁺ T cells, representing a potentially more susceptible HIV target-cell phenotype.

Single-cell transcriptomic profiling identified six vaginal CD8⁺ T cell subsets, with CD137⁺Ki67⁻ and CD137⁺Ki67⁺ populations serving as the principal antigen-responsive subsets following intravaginal peptide challenge. Antigen recall responses were most pronounced in HVV-IV-vaccinated animals and were characterized by activation of cytotoxic, mitochondrial, proteasomal, and MYC-associated transcriptional programs, whereas DMC-induced responses exhibited distinct translational and stress-response signatures.

Conclusions: Together, these results demonstrate that HVV platform elicits more durable and functionally superior CD8 T cell responses than DMC platform. Furthermore, our findings reveal that vaccination regimen and delivery route plays distinct role in mucosal T cell programming. These results are important for designing next-generation vaccines against HIV and other mucosal pathogens.

Infectious Molecular Clones of Human-Simian Immunodeficiency Virus that Evolved In Vivo Establish Replication Competent Reservoirs in Pigtailed Macaques

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We currently do not have a true NHP model of HIV-1 infection for evaluating the efficacy of vaccine and therapeutics targeting HIV-1 immunological targets. We have previously reported the development of Human-Simian Immunodeficiency Virus (HSIV), a minimally modified HIV-1 with a SIV *vif* gene substitution, that replicates persistently in pigtailed macaques (PTMs). We also previously reported the recovery of infectious molecular clones (IMCs) following 3 rounds of serial in vivo passaging of HSIV clones in PTMs. Sequencing showed several nonsynonymous mutations throughout the genome, perhaps suggesting adaptation to PTMs. The goal of this study was to conduct phenotypic characterization of recovered IMCs and to determine their replication potential in PTMs. Results from a virion-cell fusion assay suggested that the observed nonsynonymous mutations in Envelope protein of recovered IMCs do not improve entry of viruses into PTM CD4 T cells. TZM-bl-based neutralization assay results showed that recovered IMCs resist neutralizing antibody response in serum samples collected during serial passages of the parental viruses, and exhibit Tier-2 neutralization resistant phenotype, suggesting that envelope mutations are most likely escape mutations. Since we observed mutations in the Nef-LTR region of recovered IMCs, we also generated derivatives of IMCs that contained identical LTRs. To determine the replication potential of recovered IMCs and its derivatives, 5 PTMs were inoculated via intravenous and intrarectal routes with cell free virus and in once instance through blood transfusion from previously infected macaque. All 5 PTMs exposed to HSIV challenge showed persistence of viral replication for more than 20 weeks, followed by gradual decline in viral loads. Interestingly, one of the infected PTM showed continuously detectable viral loads for more than 100 weeks post-challenge. Furthermore, all 5 HSIV infected PTMs developed autologous Tier 2 neutralizing antibody response. To determine the establishment of replication competent reservoirs, we depleted CD8 T cells in 4 HSIV infected PTMs. All four CD8 depleted PTMs showed rebounds in viral loads suggesting that the PTM-tropic HSIV variant clones persistently replicate, but fail to evade CD8 T cell responses. The data also suggest that PTM/HSIV model could be used to test HIV-1 cure strategies.

An HIV-1 strain modified for owl monkeys is susceptible to most major classes of antiretroviral drugs

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Background: Our laboratory recently developed an immunocompetent nonhuman primate model of HIV-1 which uses a novel, minimally modified virus called HIVnom3 to infect owl monkeys (*Aotus nancymae*). HIVnom3 retains 93% overall sequence identity with HIV-1 strain NL4-3. The pol gene, which encodes the viral enzymes targeted by most HIV-1 antiretroviral therapies (ARTs), remains completely wildtype. Further, although HIVnom3 contains five amino acid substitutions in capsid (CA), these mutations lie outside the binding pocket of the capsid inhibitor lenacapavir. Together, these features suggest that the model could be used to accurately test antiretroviral therapy usage across multiple HIV-1 antiretroviral drug classes.

Methods: To verify this prediction, I compared the *in vitro* susceptibility of HIVnom3 and HIV-1 NL4-3 to representative antiretroviral drugs.

Results: HIVnom3 exhibited drug sensitivities comparable to HIV-1 NL4-3 across multiple drug classes, confirming that the minimal adaptations required for replication in owl monkeys do not substantially alter antiviral susceptibility. Notably, HIVnom3 remained highly susceptible to non-nucleoside reverse transcriptase inhibitors, enabling future evaluation of an entire class of antiretroviral drugs which cannot currently be evaluated in conventional SIV-based nonhuman primate models. Furthermore, HIVnom3 retained full susceptibility to the capsid inhibitor lenacapavir.

Conclusions: These findings validate HIVnom3 as a clinically relevant virus for antiretroviral studies.

Early cellular infiltration is not a feature of anti-drug antibody responses induced by intramuscular gene therapy with AAV vectors expressing HIV-1 bNAbs

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Background: Adeno-associated virus (AAV)-mediated delivery of broadly neutralizing antibodies (bNAbs) is a promising strategy for long-term HIV treatment and prevention following a single intramuscular injection. A major barrier to the clinical application of this approach is the development of anti-drug antibodies (ADAs) against the expressed bNAb, which arise 4-6 weeks after vector administration and can lead to treatment failure. Recent work from Gardner et al. demonstrated that rhesus macaques (RhMs) with ADAs exhibit cellular infiltration at the AAV injection site approximately one year after treatment. Similarly, our group has observed these same infiltrate structures in RhMs with ADA development six months after AAV-bNAb administration, but not in animals that maintained bNAb expression without detectable anti-drug antibodies. While this association suggests that local immune responses may influence treatment outcome, it remains unclear whether these infiltrates contribute to the initiation of the anti-bNAb immune response or instead arise as a consequence of it. Based on the established role of innate immune activation in initiating adaptive immune responses, we hypothesized that animals that develop ADAs would exhibit early cellular infiltration at the injection site.

Methods: To test this, muscle punch biopsies were collected from the injection site of RhMs at 6 hours, 24 hours, or 1 week after AAV-bNAb administration. Tissue sections were evaluated for cellular infiltration and inflammatory changes.

Results: Minimal to no cellular infiltration was observed at any of the early time points examined. These findings indicate that robust cellular recruitment is not an immediate consequence of intramuscular AAV administration and suggest that the infiltrates associated with ADA development arise at later stages following vector delivery.

Conclusions: These results provide insight into the early tissue response to intramuscular AAV gene delivery and suggest that future studies investigating the kinetics of these cellular infiltrates should include time points beyond the first week after vector administration.

Early Targeting of Indoleamine-2,3-dioxygenase 1 exacerbates disease progression in a Non-human Primate model of TB/SIV co-infection and antiretroviral Therapy

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Background

Tuberculosis (TB) remains a leading cause of mortality among people living with HIV (PLWH). HIV infection increases the risk of progression of latent TB to active disease, primarily due to CD4⁺ T-cell depletion and chronic immune activation. Although cART effectively suppresses HIV replication, treatment during chronic infection fails to prevent TB reactivation in macaque model of TB/SIV co-infection. While early initiation of cART controls TB reactivation, it does not resolve chronic immune activation. Therefore, adjunctive therapies that target persistent inflammation are critical. We investigated the immunomodulatory effects of D-1-methyl tryptophan (D1MT), an inhibitor of indoleamine 2,3-dioxygenase 1 (IDO1), administered early after SIV infection in combination with late cART. IDO1 is a potent immunosuppressive enzyme induced within TB granulomas, where it suppresses M.tb-specific immune responses.

Methods

The macaque model of TB/SIV co-infection was used in these studies. The animals were divided into four treatment groups-1-early-cART, 2-late-cART, 3- D1MT+early-cART and 4-D1MT+late-cART. The clinical signs of TB were monitored through bacterial burden, lung pathology and PET-CT imaging. Immune responses were assessed via flow Cytometry. To identify the key immune cells and their spatial organization, Single-cell RNA (scRNA) sequencing of bronchoalveolar lavage (BAL) cells and multiplex imaging of lungs were performed.

Results

We previously demonstrated that the concurrent administration of D1MT with early cART in *M.tb*/SIV co-infected macaques is safe and results in optimal immune responses. However, when D1MT was administered early followed by cART, it reduced the survival while increasing the bacterial burden in the lungs, granulomas, and extrapulmonary tissues, and exacerbated pulmonary inflammation as assessed by PET/CT imaging. D1MT-late cART failed to restore pulmonary CD4⁺ T-cell responses accompanied by reduced B-cell frequencies and increased Th17 cells in the BAL. T-cell proliferative capacity were markedly impaired. scRNA sequencing revealed an expanded population of IFN-responsive macrophages indicating persistent pulmonary inflammation.

Conclusion

Our findings demonstrate that IDO1 inhibition in the absence of effective viral suppression by cART is insufficient to prevent TB reactivation in the *M.tb*/SIV co-infection model. Therefore, we propose that IDO1 inhibition should be considered as an adjunctive host-directed therapy for TB/HIV co-infection only in the context of effective cART-mediated viral suppression.

Integrated Viral Barcoding and Single-Cell Multiomics Reveals Early Tissue Compartmentalization During SHIVAD8EOM Infection in Chinese-Origin Rhesus Macaques

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Background: Simian-Human Immunodeficiency Virus (SHIV) pathogenesis and infection dynamics have been extensively characterized in Indian-origin rhesus macaques. However, comparatively less is known about these important parameters in Chinese-origin rhesus macaques (RMs) despite their increasing use in HIV research. Furthermore, the virologic and immunologic features of SHIV infection in this subspecies of RMs remain incompletely defined, particularly in the central nervous system (CNS). **Methods:** In this study, five Chinese-origin RMs were infected with CCR5-tropic, molecularly barcoded SHIV_{AD8EOM} to enable longitudinal tracking of viral lineages *in vivo*. **Results:** High-dimensional full-spectrum flow cytometry revealed dynamic remodeling of circulating and tissue-resident immune cell populations during acute and chronic infection in blood and peripheral lymph nodes. Viral barcode sequencing demonstrated early compartmentalization of viral lineages across plasma, PBMCs, lymph nodes, and cerebrospinal fluid by approximately 15 weeks post-infection. Brain tissue was analyzed to characterize viral RNA distribution and its colocalization with macrophages, brain-resident microglia, and CD4⁺ T cells. Blood-brain barrier integrity was also assessed to further define CNS involvement during infection. Single-cell RNA sequencing with paired CITE-seq and V(D)J immune repertoire analysis enabled characterization of immune cell composition, transcriptional states, cell-surface protein expression, and adaptive immune receptor diversity throughout infection. **Conclusions:** Overall, this model provides a tractable system for studying HIV persistence, and integration of viral barcoding with multiomic approaches enables resolution of infection dynamics across cell types and anatomical compartments over time. This platform will facilitate investigation of viral persistence mechanisms and evaluation of HIV vaccines, cure strategies, and therapeutic interventions in tissue compartments including the CNS.

Transient CD4 Blockade Does Not Prevent Humoral Responses to AAV-Delivered Anti-HIV Antibodies in Rhesus Macaques

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Background: Persistent delivery of potent, broadly neutralizing antibodies (bNAbs) using recombinant adeno-associated virus (AAV) vectors presents a promising approach for the long-term treatment and prevention of HIV infection. However, anti-drug antibody (ADA) responses against AAV-expressed bNAbs can develop, limiting antibody concentrations and therapeutic efficacy in both non-human primates and humans. Because CD4⁺ T-cell help is critical for humoral immune responses, we evaluated whether transient CD4 blockade could prevent or reduce ADA formation against an AAV-delivered anti-HIV bNAb.

Methods: In a pilot proof-of-concept study, two AAV9-seronegative rhesus macaques received three weekly intravenous administrations of the non-depleting anti-CD4 monoclonal antibody zanolimumab (40 mg/kg). To overlap antibody expression with the tolerization period, animals received intramuscular inoculation of an AAV9 vector encoding the anti-HIV bNAb DH270 during the second zanolimumab infusion. Serum samples and peripheral blood mononuclear cells were collected longitudinally for 20 weeks. DH270 concentrations, ADA responses, and CD4 expression levels were monitored over time.

Results: The anti-CD4 infusions were well tolerated and resulted in effective modulation of CD4 expression. AAV-mediated delivery led to high expression of DH270 in both monkeys, with peak serum concentrations of 102 and 72 µg/ml at weeks 3 and 4 post-AAV administration, respectively. However, despite anti-CD4 treatment, both animals developed ADA responses against DH270, which were associated with a progressive decline in circulating antibody concentrations to near-background levels.

Conclusions: Transient CD4 blockade with the non-depleting monoclonal antibody zanolimumab produced measurable modulation of circulating CD4⁺ T cells but was insufficient to prevent ADA responses against AAV-delivered DH270. These findings suggest that transient disruption of CD4-mediated T-cell help alone is insufficient to establish durable tolerance to HIV bNAbs expressed by AAV vectors. Additional or combination immunomodulatory strategies will likely be required to achieve durable antibody expression following AAV-mediated gene transfer.

Impact of the dose and the number of transmitted/founder viruses on SIV dynamics

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Background

Infection of humans with HIV-1 is typically established by a few transmitted/founder (T/F) viruses, suggesting exposure to relatively low viral doses. In contrast, experimental SIV infection in monkeys is usually performed using high viral doses to ensure infection. The role of the infection dose in shaping SIV dynamics remains poorly understood.

Methods

We analyzed viral load data from 24 rhesus macaques infected intrarectally with a single dose of SIVmac251 ranging from 10^6 to 10^9 viral particles. We fitted the standard target cell-limited (TCL) model to the viral load data from 18 animals that became infected. We also extended the model to incorporate stochastic early infection dynamics of multiple viral strains, enabling us to investigate the effects of vaccination on infection probability and the establishment of T/F viruses.

Results

Lower inoculation doses resulted in fewer detected T/F viruses, and animals infected with two or more T/F viruses exhibited approximately $0.5\log_{10}$ higher set-point viral loads than those infected with a single T/F virus. Model analysis revealed that viral dose, and consequently the number of T/F viruses, significantly influenced the initial viral load and viral replication rate but had no significant effect on target-cell turnover, viral infection rate, or infected-cell death rate. The TCL model failed to accurately capture viral dynamics in several animals, underestimated infected-cell death, predicted excessive target-cell depletion, and produced unrealistically low initial viral loads in the lowest-dose group. A model assuming viral control exclusively by CD8 T-cell responses also failed to explain the observed data. Stochastic simulations of the TCL model showed partially reproduced the observed dose-dependent trends in infection probability and T/F virus numbers and illustrated important role of tissue volume in establishment of infection. The simulations further suggested that infection-blocking and infected cell-killing vaccines have comparable effects on the risk of infection per exposure.

Conclusions

Viral dose strongly influences early SIV infection dynamics, the number of T/F viruses, and subsequent viral load trajectories. Although the TCL model captures several qualitative trends, it is insufficient to fully explain the experimental data, highlighting the need for more realistic models that will combine dynamics of target cells and immunity.

Lineage-Specific APOBEC3G Splicing Defects in Cercopithecinae Alter Viral Mutational Profiles Relative to Humans

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Background: Nonhuman primates (NHPs), especially macaques, are indispensable models for human infectious disease because of their close immunological and physiological similarity. However, molecular differences between NHPs and humans can limit translational relevance. A 2020 study showed that APOBEC3G (A3G) — the most antiviral A3 cytidine deaminase and a major source of HIV hypermutation — undergoes aberrant mRNA splicing in macaques. Because A3 enzymes mediate innate antiviral immunity and restrict cross-species transmission, species-specific splicing differences may affect interpretation of NHP HIV and SIV studies.

Methods: A3-induced viral hypermutation profiles in HIV and SIV sequences were quantified using the Los Alamos National Laboratory (LANL) database. To characterize A3G splicing, publicly available RNA-seq datasets from 15 NHP species were analyzed. A3G protein expression in rhesus macaques was assessed by mass spectrometry of human and rhesus PBMCs, and A3G translation was evaluated using ribosome profiling data from human and rhesus lymphoblastoid cell lines (LCLs). Mechanisms underlying aberrant A3G splicing in rhesus macaques were investigated using splice-site mutagenesis of human and rhesus A3G constructs, and reciprocal intron-swap experiments. Finally, HIV- and SIV-infected human and rhesus PBMCs, respectively, were used in infectivity assays to assess endogenous A3-mediated viral hypermutation and diversification, and PCR-amplified *env*, *gag*, and near-full-length genomes were sequenced using PacBio Revio II.

Results: The A3G mRNA splicing defect was widespread and restricted to the Cercopithecinae subfamily of Old World monkeys, which includes all major NHP species commonly used as models for human disease. As a consequence, rhesus A3G protein was undetectable, consistent with ribosome stalling at two upstream premature termination codons (PTCs) within the novel exon. In line with this defect, compared to HIV, SIV genomes showed highly depleted A3G signature and significantly fewer stop codons.

Conclusions: This human–NHP disparity in A3G expression and activity has important implications for NHP-based studies of viral restriction, diversification, evolution, drug resistance, and immune evasion. Importantly, these differences in viral mutational signatures between humans and NHPs are not limited to HIV/SIV but have also been reported in HTLV/STLV and simian foamy virus (SFV) genomes, underscoring the broad impact of species-specific A3 activity across diverse retroviruses.

Gut dysbiosis during methamphetamine exposure in SIV-infected, ART-treated rhesus macaques impacts neurodysfunction via the microbiota-gut-brain axis

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People with HIV (PWH) experience gut dysbiosis, chronic systemic inflammation, and neuroinflammation. Additionally, a relatively high ratio of PWH suffer from methamphetamine (METH) use disorder (MUD), worsening prognosis despite antiretroviral therapy (ART). We hypothesize that MUD impacts the microbiota-gut-brain axis (MGBA) via bacterial metabolite expression, increasing neuroinflammation and risk of developing HIV-associated neurocognitive disorder (HAND). Using the rhesus macaque model of SIV infection, this study aims to identify how MUD affects host-microbe interactions. The fecal microbiome of SIV-infected, ART-treated animals exposed to METH was compared to SIV-infected, ART-treated animals using shotgun sequencing and bulk RNA-sequencing of the hippocampus. We found significant increases of bacteria *Hallerella porci*, CAG-83 sp900545585, CAG-103 sp000432375, UBA5905 sp900764115, *Oribacterium* sp900772695, *Evtepia* sp004556345, and UBA3818 sp900557155 in the METH-exposed group. These microbes have not been well characterized as members of the microbiome, although we found they positively correlated with measures of bacterial virulence factors. Additionally, significant increases in plasma lipopolysaccharide binding protein during METH-exposure indicate increased damage to the gut epithelium. This, along with significant increases in plasma galectin-3 and IL-8, indicate increased systemic inflammation associated with METH-exposure and is a potential cause for neuroinflammation across the MGBA. Although butyric and hexanoic acid increased with SIV, they normalized during ART. Finally, RNA-sequencing of the hippocampus showed upregulation in genes associated with nervous system signaling, dyskinesia and neurological disorder. In conclusion, we observed systemic inflammation and cytokine signaling across the MGBA, suggesting that gut dysbiosis is associated with increased neuroinflammation and risk of HAND in PWH with MUD.

Fusion of complement fragment C3d strikingly enhances lymph node targeting and persistence of native-like HIV-1 envelope trimers

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Background: Vaccine-induced germinal center (GC) responses are critical for developing an effective HIV-1 vaccine. However, antigen capture on follicular dendritic cells (FDCs) after immunization remains one rate-limiting step for inducing robust and sustained GC reactions. While escalating dosing strategies improve GC responses by increasing antigen retention via complement receptor CD21 or Fc-receptors, they are logistically challenging. To bypass the need for escalating dosing, we hypothesized that targeting antigens to CD21 on FDCs using native-like envelope (Env) trimers conjugated to the complement fragment (C3d) will induce and sustain robust GCs.

Methods: We designed and characterized a native-like, consensus-sequence HIV-1 Env trimer (ConM Env) fused with C3d. Mice were immunized with C3d-tagged or untagged trimers adjuvanted with an AddaS03 emulsion to evaluate GC induction, persistence, and antibody immunogenicity. In vitro clinical translatability was assessed using human tonsil-derived FDCs and HIV-specific B cell lines.

Results: C3d fusion markedly increased ConM Env capture and persistence (up to 56 days) on mouse FDCs compared to untagged trimers. Furthermore, PGT145 antibody staining confirmed a higher frequency of antigen-positive FDCs, demonstrating enhanced in vivo retention of quaternary epitope integrity after a single immunization. Functionally, a three-dose regimen of the C3d-tagged trimer induced significantly higher HIV-1 neutralizing antibody responses without generating autoantibodies against C3d. Notably, female mice exhibited superior antibody responses compared to males. Finally, C3d modification significantly enhanced antigen presentation by human FDCs to HIV-specific B cells.

Conclusion: C3d-modified Env trimers offer distinct structural and immunogenic advantages, presenting a promising strategy for germline targeting of rare B cell precursors in next-generation HIV-1 vaccines.

IL 10 Blockade Enhances Early SIV Transcription and Delays Viral Suppression in ART-Treated, SIV-infected Rhesus Macaques

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IL-10 is an immunoregulatory cytokine that suppresses T cell expansion and differentiation, limiting antiviral immunity. Our previous studies demonstrated that IL-10 promotes survival of infected CD4⁺ T cells in ART-treated SIV-infected rhesus macaques (RMs). We hypothesized transient IL-10 blockade (α IL-10) during acute infection would modulate early viral replication and immune responses, potentially limiting reservoir establishment.

To test this hypothesis, 16 RMs, balanced for age, and weight, were infected with SIVmac239M and received ART starting at day 29 post-infection. Animals were randomized to receive either a de-immunized α IL-10 antibody (10 mg/kg, 3 doses, every 3 weeks) or vehicle beginning one week prior to infection. Blood, lymph nodes (LNs), and cerebrospinal fluid were collected longitudinally. Animals were sacrificed at day 308 (on ART).

α IL-10 treatment resulted in significantly higher peak plasma viremia before ART initiation compared to controls, both at d14 (6.53×10^7 vs. 2.67×10^7 , $p < 0.05$) and d21 (1.27×10^7 vs. 4.73×10^6 , $p < 0.05$). α IL-10 treated RMs also showed a slower viral decay following ART initiation, and greater viral load area under the curve (AUC) across the entire study period, consistent with enhanced viral transcription. Cell-associated viral DNA (CA-vDNA) and intact proviral DNA (IPDA) were comparable between treated and control RMs, suggesting that proviral reservoir size remained unaltered despite sustained higher viremia. Notably, cell-associated viral RNA (CA-vRNA) was increased in LNs and PBMCs, while the CA-vRNA/vDNA ratios were significantly higher in LNs (median 181.85 vs. 117.78 at D14 and 161.96 vs. 81.83 at D29; both $p < 0.05$) and showed a similar trend in PBMCs (median 49.7 vs. 17.9 at D14; $p = 0.05$), indicating a more transcriptionally active viral pool. Longitudinal cytokine profiling revealed early increases in IL-2, IL-6, fractalkine and IFN- γ , followed by dynamic shifts in IL-15, IL-17, TNF- α , and TGF- β , consistent with transient immune activation and inflammatory modulation following IL-10 blockade.

Thus, transient IL-10 blockade increased viral transcriptional activity and immune activation despite unchanged proviral reservoir size. These data provide the first *in vivo* characterization of IL-10 blockade during acute SIV infection. These findings suggest IL-10 contributes to early establishment of a transcriptionally silent viral reservoir and may represent a target for immune-based or latency-modulating strategies.

Visualizing the early immune response to *Mtb* infection in the lungs of IV BCG-immunized NHPs reveals critical functions of polyfunctional T cells in protection

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Background

Tuberculosis (TB) is the leading cause of death from an infectious disease. Intravenous (IV) vaccination with Bacille-Calmette Guérin (BCG), unlike traditional intradermal (ID) administration, induces broad-based immunity and protection against TB in rhesus macaques (RMs). The ability to confer sterilizing immunity and the absence of the development of primary immunity following *Mtb* challenge in most IV BCG-vaccinated RMs suggests rapid bacterial control, but the cellular mechanisms driving protection after IV administration remain unknown.

Methods

To capture the earliest immune events of *Mtb* control in the lung, RMs were immunized with IV-BCG, ID-BCG, or left unvaccinated, then challenged with a high dose of *Mtb* 5 months later. Single-cell spatial proteomics (Pheno-Cycler Fusion) at Day 11 post-infection mapped the cellular composition of early granulomas to identify key cells underlying improved bacterial clearance in IV-BCG vaccinated animals.

To identify early granuloma-like structures, we developed a density-based clustering algorithm that groups IDO1+ cells within 18 µm of one another, defining early granulomas as aggregates of ≥10 IDO1+ cells. Clusters were expanded 30 µm from their outer boundary to capture the surrounding microenvironment.

Results

Healthy lung regions were identified in all animals. Inflamed regions showed complex immune infiltration and foci of early granuloma-like structures marked by *Mtb* and macrophage co-localization, and clustered IDO1 expression. Unvaccinated animals had the highest frequency and largest early granulomas. Bacteria were abundant within IDO1+ clusters in all 3 unvaccinated animals and in 1/3 ID-vaccinated animals, and sparse to absent in 2/3 ID- and 3/3 IV-vaccinated animals.

Early granulomas and their surrounding microenvironment in IV-BCG vaccinated animals were enriched for CD4 T cells (high T cell:macrophage ratio) and B cells and depleted of neutrophils relative to ID-BCG and unvaccinated animals.

Conclusions

Here, we developed an image analysis workflow to identify immune foci during early *Mtb* infection in an unbiased way. Our findings suggest that IV BCG immunization drives spatial enrichment of CD4 T cells around infected macrophages by Day 11 post-infection, contributing to early control of *Mtb*. These findings underscore the potential of IV delivery to enhance the magnitude and quality of pulmonary T cell responses for attenuated TB vaccines.

Enhancement of functional cytotoxic cells in the Lymph node by IL-15/IL-15R α -Fc therapy leads to sustained viral control post-ATI in SIV infected macaques

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Background:

Enhancing the function and effectiveness of CD8⁺ T cells is a key goal in HIV cure strategies. Cytokines such as IL-15 and IL-12 expand CD8 T and NK cells and enhance their cytotoxicity. IL-15 promotes follicular homing of CD8 T cells, while IL-15+IL-12 enhances follicular homing of NK cells during chronic SIV infection. We evaluated the therapeutic effects of IL-15 and IL-12, alone or combined, during chronic SIV infection in rhesus macaques (RMs).

Methods:

Twenty-two RMs infected with SIVmac251 initiated antiretroviral therapy (ART) at 8 weeks post-infection and remained on ART for 9 months. Animals received IL-15/IL-15R α -Fc (n=6), IL-12 (n=5), or IL-15/IL-15R α -Fc+IL-12 (n=6) in two treatment phases (five weekly doses): first at 6 weeks post-infection (2 weeks before ART) and second during ART, beginning 12 weeks before analytical treatment interruption (ATI). ART-only animals (n=5) served as controls. Immunological and virological parameters were monitored longitudinally.

Results:

IL-15/IL-15R α -Fc elicited highly polyfunctional SIV-specific CD8 T cells with proliferative capacity (Ki-67), follicular homing (CXCR5), tissue resident (CD69⁺ CD103⁺), CD16⁺ NK cells in LN. Importantly, IL-15/IL-15R α -Fc significantly expanded degranulating CD107a⁺ CD8 T cells before ATI (P=0.03) in blood. The blood transcriptomic profile confirmed the induction of cytolytic molecules (granzyme-B+perforin), JAK/STAT signaling pathway in the IL-15/IL-15R α -Fc group. In line with LN single-cell RNA sequencing of lymphocytes confirmed the enhanced effector function of CD8 T cells. These immune responses were associated with nearly a 3-log reduction in viral rebound compared with ART-only controls (P=0.004), with 83% of animals maintaining viral loads <500 copies/ml at 20 weeks post-ATI; this was not observed in other groups. SIV-specific CXCR5⁺ CD8 T cells in blood (P=0.04) and lymph nodes (P=0.02), and SIV-specific CD28⁺ CD8 T cells in blood (P=0.002) and lymph nodes (P=0.03), correlated with improved post-ATI viral control.

Conclusions:

These results demonstrate elicitation of functional follicular homing CD8 T cells by IL-15/IL-15R α -Fc therapy at the initiation of ART and during ART markedly enhances the magnitude and function of SIV-specific CXCR5⁺CD8 T cells, enhanced effector function and CD16⁺ NK cells and contributes to profound control of post-ATI viremia. This study defines IL-15/IL-15R α -Fc as a potentially effective immune therapy for HIV cure strategy.

Cell Type-Specific Expression of Minor Intron-Containing Genes Across Human and Nonhuman Primate Immune Cells

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Pre-mRNA splicing is essential for regulating gene expression and transcript diversity across immune cell populations. While most introns are removed by the major spliceosome, a highly conserved subset of approximately 700 human genes containing U12-type introns depends on the minor spliceosome. Although these minor intron-containing genes (MIGs) regulate fundamental cellular processes, their distribution across immune cell types remains poorly characterized.

Here we carried out scRNASeq from PBMCs of humans (healthy controls and plwh) and rhesus macaques (SIV longitudinal infection) to characterize the expression of major and minor spliceosome-dependent transcripts across CD4 T cells, CD8 T cells, natural killer (NK) cells, B cells, and monocytes. Full-length transcript isoforms were integrated with minor intron annotations to identify cell type-specific patterns of MIG expression and associated biological pathways.

Monocytes exhibited the greatest number of expressed MIGs, with gene set enrichment analysis (GSEA) identifying actin cytoskeleton organization and related cellular processes as the most significantly enriched pathways. B cells also displayed increased MIG representation relative to T cells and NK cells and were enriched for pathways involved in cell migration. Despite these differences, few MIGs were differentially expressed between conditions, suggesting that minor spliceosome-dependent transcripts are largely constitutively expressed and may contribute to maintaining immune cell identity rather than mediating dynamic transcriptional responses.

These findings provide a single-cell, long-read atlas of minor spliceosome-associated transcript expression across human and NHP immune populations and establish a framework for investigating the role of minor splicing in immune cell specialization.

Intravaginal mRNA-encoded broadly neutralizing antibodies confer on-demand protection against vaginal SHIV challenge in rhesus macaques

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Background

On-demand HIV prophylaxis that is locally delivered, user-controlled, and clinic-independent remains an unmet need. Oral pre-exposure prophylaxis, long-acting injectables, and parenteral broadly neutralizing antibodies (bnAbs) each face limitations including adherence, clinic-based dosing, and high manufacturing costs. Topical prevention methods have not yielded complete protection and can at time be difficult to negotiate by the female partner.

Methods

mRNA production of HIV-1 bNabs represents a highly flexible approach to generate recombinant bNabs with higher affinity and neutralizing activity. Moreover, intravaginal delivery of mRNA formulated in water encoding multiple versions of HIV-1 bnAbs (PGT121 and VRC07) have shown durable expression, generating a barrier to virus uptake. Based on in vitro neutralization breadth and magnitude, we tested a multimeric IgM-tail and a membrane-anchored IgA like format of PGT121 and VRC07 for their ability to protect female rhesus macaques against vaginal SHIV challenges.

Results

When dosed 24h before each of three weekly clade B SHIV-162p3 challenges, both IgM and anchored IgA formatted PGT121 and VRC07 conferred complete protection. When tested for duration of protection via repeated SHIV162p3 challenges, the IgM-tail format delayed acquisition for several weeks after dosing ceased. Against repeated high-dose clade C SHIV-1157ipd3N4 challenge, the IgM-tail format again conferred complete short-term but not extended protection, whereas the membrane-anchored IgA format delivered only partial protection. Of note the epithelial expression of human recombinant bNabs did not generate ADA in these rhesus macaques, underlining the safety of this topical approach.

Conclusions

These findings provide in vivo proof of concept for intravaginal mRNA-encoded antibodies as a promising on-demand, user controlled and inconspicuous strategy for female HIV prevention.

A self-replicating RNA vaccine induces HIV BG505 Env-specific cellular and humoral responses in systemic and mucosal compartments

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We previously demonstrated that the SARS-CoV-2 repRNA/LION™ vaccine, which consists of a self-replicating RNA (repRNA) formulated with a cationic nanoparticle carrier (LION™), significantly reduced SARS-CoV-2 replication in both the upper and lower respiratory mucosa of nonhuman primates (NHPs). This suggests that repRNA/LION induces mucosal immune responses, which may contribute to protection against HIV by limiting infection in mucosal entry sites. Here, we evaluated the systemic and mucosal immunogenicity of candidate repRNA/LION HIV vaccines. RepRNA/LION vaccines encoding “Env” (engineered membrane-bound BG505 trimer) and “Gag-Env” (co-expressed HIV or SIV Gag and BG505 Env) were administered intramuscularly in BALB/c mice (N=10/group), cynomolgus macaques (N=4/group), and pigtail macaques (N=7/group). Animals received Env, Gag-Env, or “heterologous” (2 doses of Env, followed by 2-4 doses of Gag-Env) regimens, consisting of 3-6 doses spaced 4-12 weeks apart. Cellular and humoral immunogenicity was assessed via IFN γ ELISpot, B-cell ELISpot, and ELISA.

In mice, Env and Gag-Env regimens induced robust BG505 Env-specific binding IgG responses in the blood (4.6 ± 0.14 log₁₀ endpoint titer [EPT]) and T-cell responses in the spleen (119 ± 77 SFU/1e6 cells), lung (433 ± 302 SFU/1e6 cells), and vaginal tract (103 ± 22 SFU/1e6 cells); Gag-Env induced similarly robust HIV Gag-specific T-cell responses in the spleen (1092 ± 826 SFU/1e6 cells) and lung (391 ± 211 SFU/1e6 cells). In cynomolgus and pigtail macaques, the heterologous regimen induced robust BG505 Env-specific binding IgG (5.0 ± 0.14 log₁₀ EPT) and T-cell (787 ± 146 SFU/10⁶ cells) responses in the blood. In the inguinal lymph node, all vaccine regimens induced robust BG505 Env-specific B-cell responses (56.3 ± 34.5 SFU/1e6 cells). In the mesenteric lymph node, which is the draining lymph node for the gastrointestinal tract, Env and heterologous regimens induced modest BG505 Env-specific T-cell (20.7 ± 18.7 SFU/10⁶ cells) and B-cell (9.2 ± 8.9 SFU/10⁶ cells) responses. Lastly, all vaccine regimens induced an increase in BG505 Env-specific binding IgA in nasal secretions ($p=0.001$) and modest increases in BG505 Env-specific binding IgG in nasal secretions ($p=0.067$) and rectal secretions ($p=0.301$) compared to pre-vaccination levels.

RepRNA/LION induced strong systemic, but modest mucosal BG505 Env-specific responses. Future studies will explore the mechanisms of repRNA/LION-induced mucosal immunity and their role in protection against intrarectal SHIV challenge in NHPs.

Expanding MUSA, a Multi-center Allele Database and AIRR Framework for Rhesus Macaque Immunoglobulin Loci

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Accurate immunoglobulin (IG) genotyping in rhesus macaques (RMs) is essential for interpreting antibody repertoire data and improving preclinical vaccine studies, yet the complexity of the RM IG loci has limited population-scale analysis. To address this, we have coordinated a multi-center effort to build integrated genomic and AIRR resources for RM IGH, IGK, and IGL loci. We generated matched targeted long-read IG capture sequencing and IgM/IgK/IgL repertoire data across a large cohort spanning major U.S. primate centers, including completed IG genotyping of more than 500 animals. An initial high-confidence release from 106 animals produced the Macaque Unified Set of Alleles (MUSA), which identified and validated hundreds of novel germline alleles and incorporated coding and regulatory features including leader sequences and recombination signal sequences. Building on this, we have developed improved analytical pipelines for allele inference, validation, and public dissemination through AIRR-compliant resources, and adapted full-length AIRR-seq for RMs to improve chain recovery and constant-region resolution. Together, these advances establish the most comprehensive RM IG resource to date and provide a scalable framework for individualized germline databases, cross-study repertoire analysis, and investigation of how IG variation shapes vaccine-elicited antibody responses.

CD8⁺ T cells have a key role in the sustained reduction of SIV viral loads associated with a short acute-phase course of ART

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Background: The earliest viral and immune events following a mucosal HIV infection are likely to be consequential in determining disease outcome, unfortunately these events are extremely difficult to study. Utilizing an SIV macaque mucosal/oral transmission model, we designed an experiment to assess the viral and immune impact of transiently suppressing viral replication from 3-17 days post-infection. We hypothesized that transient ART administration during the acute phase of infection would result in an improved early immune response that could be evaluated and compared to a control group.

Methods: SIVmac251 (500 TCID₅₀) was administered to juvenile macaques via an intraepithelial oral mucosal injection, an inoculation route we developed and routinely use. The control macaque group received SIV alone (N=8) while a second group received ART (FTC, DTG, TDF) from days 3–17 post-infection (N=12) (early transient ART (et-ART)).

Results: Although ART was stopped at day 17, et-ART macaques experienced a significant (2 log) reduction in plasma viral load observed at 8 weeks post-infection (wpi), and maintained through 22 wpi. Furthermore, lymph nodes of et-ART macaques harbored significantly less SIV RNA in both T cell and B cell zones (RNAscope) while exhibiting intact follicular architecture and actively proliferating germinal centers (in contrast to architectural disruption observed in control macaques). In addition, et-ART macaques exhibited higher anti-SIV-Env IgG endpoint titers (>10-fold) and mediated greater antibody-dependent cell-mediated phagocytosis. At 22 wpi, a subset of et-ART macaques (N=4) were treated with a CD8-depleting antibody resulting in an increase in plasma SIV levels (over next 14 days) that was most evident in et-ART macaques with the lowest pre-depletion viral levels.

Conclusions: These findings demonstrate that fourteen days of ART during acute infection leads to a sustained reduction in viral replication that remains low for up to 20 weeks.

These et-ART SIV⁺ macaques exhibit a preserved lymphoid architecture, improved anti-SIV antibody production, and CD8 T cell responses that contribute to the sustained control. These findings provide evidence that a short-transient administration of ART during acute infection can have a long-lasting vaccinal effect in the SIV/macaque model, with implications for ART administration of people recently infected with HIV.

In Vivo NKG2A Blockade Remodels NK Cell Phenotype in SHIV-Infected Rhesus Macaques

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Background: Natural killer (NK) cells are key mediators of innate antiviral immunity, regulated by a complex balance of activating and inhibitory signals. NKG2A is an inhibitory receptor that suppresses NK cell cytotoxicity upon ligand engagement, making it a promising target for blockade therapies. Here, we evaluated the immunologic and virologic impact of a novel rhesusized anti-NKG2A/C monoclonal antibody administered in combination with the broadly neutralizing antibody VRC07-523LS in SHIV-infected rhesus macaques.

Methods: Twelve SHIV-infected rhesus macaques were enrolled in the study, with six animals receiving a rhesusized anti-NKG2A/C monoclonal antibody in combination with the broadly neutralizing antibody VRC07-523LS and six control animals receiving VRC07-523LS alone. Longitudinal NK cell phenotypes were profiled in peripheral blood mononuclear cells (PBMC) and lymph nodes (LN) by flow cytometry, focusing on major subsets and expression of the activating receptors NKp30, NKp46, and NKp80. Plasma viral load, cell-associated (CA) SHIV DNA and RNA levels in PBMC were evaluated longitudinally as measures of viral replication and frequency of infected cells.

Results: In vivo anti-NKG2A/C treatment induced measurable remodeling of the NK-cell compartment. Treated animals exhibited a decrease in the frequency of NKG2A⁺NKp46⁺ NK cells in PBMC, with a concomitant increase in NKG2A⁺NKp46⁺, NKG2A⁺NKp30⁺ and NKG2A⁺NKp80⁺ NK-cell populations in LN, indicating selective modulation of key activating receptors. Administration of VRC07-523LS was associated with a decline in plasma viral load, CA- SHIV DNA, and CA- SHIV RNA in both groups; however, the addition of anti-NKG2A/C antibody did not confer any further reduction compared with VRC07-523LS alone, indicating that the observed immunologic shifts did not translate into enhanced control of viral replication or elimination of infected cells.

Conclusions: NKG2A/C blockade resulted in a remodeling of the NK-cell phenotype, demonstrating biological activity of this novel immunotherapeutic approach in vivo. Although these changes did not enhance the antiviral effects achieved with VRC07-523LS, they provide evidence that NKG2A blockade can modulate NK-cell composition and receptor expression during chronic SHIV infection. These findings support further evaluation of NKG2A blockade in combination with additional immune-based strategies designed to enhance NK-cell-mediated antiviral activity.

Substance Use Reshapes Cerebrospinal Fluid Monocyte Transcriptomic Signatures in Neuro-HIV

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Background. More than 50% of people living with HIV develop HIV-associated neurocognitive disorders collectively called neuro-HIV, which are further exacerbated by substance use. However, role of different classes of substances like opioids, psychostimulants, and cannabinoids on neuro-HIV remain poorly defined. Using nonhuman primate models, we previously showed that chronic opioid use expands central nervous system viral reservoirs and induces neuroinflammatory transcriptomic signatures in cerebrospinal fluid (CSF) immune cells. Here, we performed a comparative transcriptomic analysis of CSF immune cells across different classes of substances to define their differential effects on neuro-HIV and identify pathways that may inform future HIV cure strategies.

Methods. We conducted scRNA-seq on CSF immune cells from five groups of rhesus macaques (RM): uninfected controls and SIV infected, ART treated RM receiving saline, morphine, cocaine, or THC/CBD. All data analysis was performed using Seurat v5.0.1 or SCANPY pipeline.

Results. We identified 13 CSF immune cell subsets, including B cells, naïve and memory CD4⁺/CD8⁺ T-cell populations, dendritic cells, progenitor and proliferating T-cell, CD14⁺/CD16⁺/intermediate monocytes, and microglia-like cells. Cocaine exposure induced the most profound transcriptional changes, with 2,529 downregulated and 54 upregulated genes, primarily in CD16⁺ monocytes and memory T-cell subsets, involving viral evasion pathways. Morphine dysregulates oxidative phosphorylation, TNF- α , and NF- κ B signaling in CD16⁺ monocytes, whereas THC/CBD induced interferon- α responses and TP53 activation, suggesting enhanced antiviral innate immune activity. Transcription factor activity prediction revealed suppression of NF- κ B, RELA, ETS1, HIF1A, and FOS activity in CD16⁺ monocytes following morphine and cocaine exposure. Metabolite mediated cell-cell communication (MEBOCOST) identified substance-specific metabolic communication changes, including increased ATP-purinergic signaling with morphine, farnesyl pyrophosphate/prostaglandin E2 signaling with cocaine, and GABAergic signaling with THC/CBD. Connectivity mapping using LINCS L1000 identified 1,290 druggable genes, with MG-132 predicted to reverse signatures across all groups and Nrf2 emerging as the top-ranked therapeutic target. Predicted reversal agents included PLK/BTK inhibitors for saline, topoisomerase/protein synthesis inhibitors for morphine, and modafinil/emetine for cocaine groups.

Conclusions. Opioids, psychostimulants, and THC/CBD cause distinct, cell-type specific transcriptional alterations in CSF immune cells, driven primarily by CD16⁺ monocytes. Connectivity mapping identifies targeted adjunctive therapies to reverse substance-induced immune dysfunction and improve neuro-HIV outcomes.

MAIT Cell Activation Drives Antimicrobial Responses Across In Vitro Granuloma-Like Models and Rhesus Macaque Lung Explants

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Background: Mucosal-associated invariant T (MAIT) cells are innate-like T lymphocytes that recognize microbial metabolite antigens presented by MR1 and contribute to early immune responses against *Mycobacterium tuberculosis* (M.tb). Although MAIT cells have been implicated in tuberculosis immunity, their functional role within the lung tissue microenvironment remains incompletely understood. We developed a lung tissue explant model together with an in vitro granuloma-like model to evaluate MAIT-cell activation by bacterial metabolites and its effect on M.tb growth control.

Methods: Metabolite extracts from virulent *M.tb* H37Rv, *Escherichia coli*, and *Listeria monocytogenes* were initially screened by IFN- γ ELISPOT to assess MR1-dependent MAIT-cell activation. The extracts were subsequently evaluated in an in vitro PBMC-based granuloma-like model infected with H37Rv-lux, where bacterial burden was monitored by luminescence at 24, 48, and 72 hours and MAIT-cell activation was assessed by multiparametric flow cytometry. Rhesus macaque lung explants cultured at an air-liquid interface were infected with H37Rv-tdTomato and evaluated by fluorescence imaging and tissue colony-forming unit (CFU) analysis.

Results: Bacterial metabolite stimulation induced measurable IFN- γ responses in the initial ELISPOT screen. In the in vitro PBMC-based granuloma-like model, MAIT stimulation modulated H37Rv-lux luminescence, with the greatest inhibitory effects observed at 48 and 72 hours post-infection. Flow cytometry demonstrated a distinct MAIT-cell activation phenotype characterized by modulation of CD69 and CD26, accompanied by enhanced granzyme B and granulysin production. In rhesus macaque lung explants, H37Rv-tdTomato fluorescence imaging and tissue CFU demonstrated reduced bacterial burden following bacterial metabolite stimulation, consistent with the PBMC model. Histopathological assessment and cytokine profiling are ongoing.

Conclusions: Together, these findings demonstrate that MAIT-directed activation can be evaluated across complementary human PBMC and rhesus macaque lung explant platforms. Concordance among IFN- γ responses, PBMC phenotyping, M.tb luminescence, tissue imaging, and CFU supports an association between MR1-dependent stimulation and improved control of M.tb growth. This integrated approach provides a translational framework for identifying microbial metabolite-based strategies that enhance MAIT-mediated antimicrobial immunity in the lung.

Profiling Maternal Immune Responses to Clade IIb MPXV Infection Across Pregnancy in Rhesus Macaques

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Maternal Monkeypox virus (MPXV) infection during pregnancy is associated with vertical transmission and fetal demise, but the immune responses distinguishing these outcomes remain undefined. Using a pregnant rhesus macaque model of clade IIb MPXV infection, we characterized peripheral and decidual immune responses across three outcomes: fetal demise (FD), MFI infection without fetal demise (MFI+), and no fetal demise (No FD). Peripheral blood was assessed longitudinally through 25 DPI and decidual leukocytes were assessed at necropsy.

In peripheral blood, monocyte frequency increased at 7 DPI in MFI+ and No FD animals but decreased in FD animals. Prior clade I MPXV studies reported robust peripheral NK cell expansion following infection; in this model, NK cells increased only modestly in MFI+ and No FD animals and remained unchanged in FD animals. MPXV-specific IgG to A29L, A35R, and H3L—immunodominant viral proteins—developed in all animals regardless of outcome, indicating antibody development alone did not prevent fetal demise once MFI infection occurred.

In the decidua, MPXV infection shifted macrophage polarization toward an M1-like phenotype relative to mock controls ($p=0.029$), even in animals without detectable decidual viral DNA at necropsy. The animal with the highest decidual viral load but no fetal demise (ID 104) showed an M1:M2 ratio comparable to mock controls, indicating no inflammatory polarization despite substantial viral burden. NK subset composition did not differ significantly across the full cohort; however, animal 104 alone showed an NK profile resembling mock controls. Excluding this animal, MPXV infection was associated with increased cytotoxic NK cells ($p=0.011$) and decreased decidual NK cells ($p=0.038$) relative to mock controls.

These findings suggest that pregnancy outcome following MPXV infection is shaped more by local immune dysregulation at the maternal-fetal interface than by viral burden or systemic humoral immunity alone. Decidual inflammatory changes occurred independent of local viral replication, suggesting that circulating signals from the periphery may drive immune remodeling at the maternal-fetal interface even without local infection. Future studies incorporating peripheral cytokine and chemokine profiling will help determine whether systemic inflammatory signals contribute to this local immune remodeling.

Interrogation of Longitudinal Microbial Communities in Gut Microbiome During SHIV infection of Infant and Adult Rhesus Macaques

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Background: Early-life gut microbial colonization shapes immune development, yet how microbiome composition evolves over time and whether baseline commensals influence viral control remain poorly defined. We evaluated longitudinal gut microbiome composition overtime in Simian-Human Immunodeficiency Virus (SHIV) infected non-human primate Dam-Infant pairs.

Methods: Rectal wecks in Dam-Infant pairs of rhesus macaques (RMs) were obtained at longitudinal timepoints relative to SHIV infection (Infants: month (M) 0 baseline and M24; Dams: prior to M0 [M-0] baseline, M0, M12, and M26). The gut microbiome composition was assessed using 16S rRNA V3-V4 amplicon sequencing and data analysis with DADA2 pipeline.

Results: We studied 11 Dam-infant pairs (N=22) at longitudinal timepoints and found distinct alpha diversity trajectories over time between infants and adults. Specifically, the Shannon diversity metric, which accounts for both evenness and richness of microbial taxa, showed significant differences in the microbial communities over time in both infants (t-test, FDR-adjusted p-value<0.05; M0 vs. M24) and adults (ANOVA, FDR-adjusted p-value<0.01; M0 vs. M26). Beta diversity analyses revealed significant compositional differences between infants and dams at matched timepoints based on Jaccard, Bray-Curtis, UniFrac, and weighted UniFrac metrics (PERMANOVA, FDR-adjusted p-value<0.001 for all). These data suggested that infants had higher inter-individual variability in fecal microbiome composition at genus level compared to dams at M0 based on taxa presence and overall relative abundance profiles. Differential abundance analyses highlighted that early infant microbiomes were dominated by diet-associated and facultative microbes, such as *Bifidobacterium*, *Staphylococcus* and *Escherichia-Shigella*, whose abundances significantly decreased by M24 as infants matured (ANCOM-BC & ANCOM-BC2, FDR-adjusted p<0.05 for both). Additionally, infants had higher plasma viral load over time compared to adults, and higher relative abundance of *Streptococcus* in infants at baseline correlated with an earlier time to peak viral load (Spearman's correlation, rho=-0.83, FDR-adjusted p-value=0.034).

Conclusion: Infant and maternal microbiomes exhibited distinct microbial communities that changed over time. Early-life gut colonizers, specifically *Streptococcus*, are associated with SHIV viral kinetics, supporting a key role for early commensals in shaping host immune function and viral control. Future studies will define the mechanisms of action for gut colonizers in modulating early-life immunity to HIV/SHIV.

Single-Cell Multiomics Reveal Multi-Lineage Immune Modulation and Epigenetic Restructuring of Gut Macrophages by Phytocannabinoids in Chronic HIV/SIV infection

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Background

Chronic HIV/SIV infection causes persistent gastrointestinal dysfunction; while ART partially restores mucosal immunity, residual inflammation drives systemic comorbidities, leaving a critical knowledge gap regarding how to target this refractory inflammation. We previously showed phytocannabinoids reduce intestinal inflammation in SIV-infected rhesus macaques (RMs) via NLRP6 and necroptosis pathways. However, the precise cellular drivers remain unknown. Here, we address this gap using single-cell resolution to identify the specific intestinal immune populations mediating these anti-inflammatory effects and sustaining mucosal recovery.

Methods

Single-nuclei RNA-seq and ATAC-seq were performed on colonic immune cells from SIV-infected, ART-suppressed RMs receiving vehicle (VEH/SIV/ART, n=4) or phytocannabinoids (THC:CBD-1:3; THC:CBD/SIV/ART, n=4). Macrophage, B/plasma and NK cell populations were compared between cohorts by Ingenuity Pathway Analysis.

Results

In ART-suppressed RMs, resident M0 macrophages collapsed, shifting toward a hybrid M1-skewed, fibrogenic phenotype characterized by *CXCL9*, *STAT1*, *ISG15*, *IFI27*, *MMP1*, and *COL5A3*. In contrast, THC:CBD preserved M0 resident identity (*MSR1*, *MRC2*, *MERTK*, *CD36*, *F13A1*) and drove selective M2/reparative programming (*IRF4*, *COLEC12*), while sparing antiviral competence (*RIG-I*, *MX1*, *OAS2*). The most cannabinoid-attributable event was the induction of *HMOX1*, an NRF2-dependent cytoprotective gene that suppressed NF-κB, promoted IL-10, and restrained inflammasome output. ATAC-seq confirmed enhanced chromatin accessibility at the *HMOX1* locus, identifying epigenetic remodeling as the driver of its transcriptional activation. Among other clusters, phytocannabinoids suppressed a senescence/SASP program (*CDKN2A*, *SERPINE1*, *MAPK14*, *IGF1R*) in plasma B-cells, preserving their autophagy-secretory module (*ULK2*, *STX17*, *PLD1/2*). However, *RUNX3*-dependent IgA class-switch transduction remained unrepaired. ART-suppressed RMs also showed reduced *JCHAIN*, *IGHM*, and *IGKC* signatures, indicating impaired secretory IgA assembly; neither deficit was rescued by THC:CBD, though *RELA*, *PPARγ*, *ROR1*, *CD103*, and *IL21R* expression were retained. Finally, NK cells uniquely underwent de-repression under THC:CBD, marked by the releasing of a multi-gene brake (*ADCY9/cAMP*, *PP2A*, *GCN2*, *HDAC9*), *NFATC1* restoration, and *AHR* induction.

Conclusions

Overall, long-term, low-dose THC:CBD beneficially modulates the mucosal immune microenvironment during chronic SIV infection. By epigenetically inducing *HMOX1*, phytocannabinoids switch macrophages from fibrogenic to cytoprotective states, reduce plasma cell senescence, and restore NK cell competence. These multi-lineage, tissue-reparative effects offer a potent strategy to resolve HIV/SIV-driven mucosal inflammation and combat non-AIDS comorbidities in people with HIV.

LRRK2 blockade ameliorates antiviral cytokine expression in microglia

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Immune activation of microglia occurs in people living with HIV even when virally suppressed and in the absence of detectable viral antigen in the brain parenchyma. Chronic immune stimulation due to peripheral infection can contribute to neuroinflammation and neurocognitive dysfunction. Thus, there is a need to identify targetable mechanisms to reduce chronic neuroinflammation that may contribute to or exacerbate neurocognitive decline. Leucine rich repeat kinase 2 (LRRK2) is an immune kinase and potential pharmacologic target for treating Parkinson's disease that has been shown to modulate inflammation. Here we tested the hypothesis that reducing LRRK2 activity would modulate antiviral type I IFN cytokine expression in microglia.

To test how LRRK2 impacts antiviral innate immune responses in microglia, two human microglial cell lines were treated with the LRRK2 inhibitor LRRK2-in-1 overnight and subsequently transfected with the synthetic dsRNA, poly (I:C) for 4 hours. HMC3 cells were treated with 10 μ M LRRK2-in-1, 1 μ g of poly (I:C), or vehicle control. HC69.5 cells, a model for HIV latency, were treated with 2 μ M LRRK2-in-1 and 5 μ g of poly (I:C) to reactivate latent HIV expression. Total RNA was extracted and RTqPCR was used to measure cytokine gene expression.

LRRK2-in-1 treatment alone had no effect on type I interferon gene expression in either microglia model compared with untreated controls. However, exogenous cytosolic RNA resulted in a significant increase in IFN β , the interferon stimulated genes IFIT1 and RSAD2, and the proinflammatory cytokines IL1 β and TNF α , which was partially rescued by inhibiting LRRK2 activity in both microglia cell lines.

These findings show that blocking LRRK2 activity alone does not significantly impact antiviral type I IFN expression in human microglia. Reducing LRRK2 activity partially ameliorated immune gene expression induced by exogenous cytosolic RNA. This suggests LRRK2 may be a potential druggable target for treating neuroinflammation associated with chronic viral infection, however further studies are warranted to determine how these effects impact viral pathogenesis.

Nonhuman Primate Reagent Resource: Specialized Reagents for Your NHP Studies

Dr. Julia de Vasconcellos Castro¹, Dr. Diogo Magnani¹

1. Nonhuman Primate Reagent Resource (NHPRR)

Background

Nonhuman primate models play an important role in preclinical research, including HIV and SIV research, because they allow investigators to study immune responses and evaluate interventions in systems that closely reflect human biology. These studies depend on specialized immunologic reagents, yet many needed tools are not commercially available or are not optimized for NHP use. The Nonhuman Primate Reagent Resource (NHPRR) addresses this need by providing specialized reagents for NHP research. NHPRR has its roots in NHP AIDS research. The Resource was established in 1999 following work using an antibody to selectively deplete CD8⁺ lymphocytes in monkeys, and for more than 27 years has supported investigators developing and using NHP models across biomedical research.

Methods

NHPRR is a resource designed for and shaped by investigators who use NHP models. We identify research needs and develop, characterize, produce, qualify, and distribute reagents for in vitro and in vivo studies. Reagents are improved through primatization to achieve better compatibility and performance in nonhuman primates. The catalog includes tools for immune monitoring, immune modulation, cell depletion, functional assays and flow cytometry, antibody detection, and specialized recombinant proteins. The Resource also provides curated reagent reactivity information across NHP species through the NHPRR Reactivity Database. At www.nhpreagents.org, investigators can explore available reagents, search the Reactivity Database, review publications that have used NHPRR reagents, and submit their own publications.

Results

The Resource has distributed 300 products through more than 10,600 orders, reaching 850 investigators at more than 517 institutions in 27 countries. These resources have been used in research associated with more than 2,000 grants and more than 800 publications — including studies on HIV intervention, prevention, and treatment.

Conclusions

Standardized, well-characterized reagents are essential for reproducible NHP research. NHPRR provides investigators with access to specialized reagents and supporting information that can strengthen the reliability and reproducibility of NHP studies. We welcome questions, ideas, and new reagent needs from the research community.

Stop by the poster to see what is available, ask us about a reagent you need, or tell us what would help move your NHP research forward. Let's advance better science together.

Dynamic shifts in aging under antiretroviral therapy: lessons from pathogenic SIV infection in young and old macaques

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Background: Antiretroviral therapy (ART) has transformed HIV into a stable, long-term condition, but does not fully reverse virus-induced biological aging or prevent comorbidities. Using epigenetic clocks, we previously showed that chronological age influences epigenetic age acceleration (EAA) in untreated pathogenic SIV infection in rhesus macaques. Building on these findings, we hypothesized that ART's impact on biological aging is also age-dependent. **Methods:** We conducted a longitudinal DNA methylation study in PBMCs from rhesus macaques, categorized into young (n=6, mean 4.2 years) and old (n=6, mean 19 years) cohorts. We analyzed DNA methylation across five SIVmac infection phases: baseline (pre-infection), acute, early chronic, early ART, and late ART, using the Rhesus Pan-Tissue epigenetic clock to predict biological age and EAA.

Results: Our results showed that the acute phase did not significantly affect EAA in either cohort. However, young individuals—but not old individuals—exhibited significant EAA during early chronic infection (p-value = 0.031). In contrast to these divergent aging patterns, the initiation of ART induced transient EAA in both young (p-value = 0.045) and old (p-value = 0.0007) macaques, with a more robust p-value in the older cohort, suggesting a more severe maladaptive response to therapy initiation. During late-stage ART, no EAA was detected across individual age cohorts. This suggests that ART-related age acceleration is primarily transient; also, long-term ART reduced the EAA observed in younger individuals.

We also conducted a pooled analysis of both cohorts. This combined approach confirmed that early chronic infection is associated with significant EAA (p-value = 0.02) and reinforced the link between early treatment and transient EAA (p-value = 3.82×10^{-5}). Furthermore, it revealed an EAA during late-stage ART (p-value = 0.02) that was not detectable within the individual age groups.

Conclusions: Together, our findings demonstrate that ART initiation in SIV-infected individuals transiently accelerates biological aging. However, long-term ART is beneficial as it suppresses virus-induced aging in younger individuals; however, as reported in people living with HIV, long-term ART does not fully eliminate EAA. Collectively, these data show that biological aging during SIV infection is a dynamic process shaped by both treatment duration and host age.

Programmable VLP delivery of molecular epitopes drives B cell and T cell immunity against HIV-1

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Background: An effective HIV-1 vaccine likely requires eliciting broadly neutralizing antibodies (bNAbs) and robust CD8⁺ T cells, which synergize to lower the protection threshold. Thus, modular vaccine platforms engaging both adaptive immune arms are highly relevant for clinical development.

Methods: We designed and characterized icosahedral virus-like particles (VLPs) for multivalent display (~200 copies) of peptide antigens (B cell and CD8⁺ T cell targeted epitopes) and CD4⁺ T helper epitopes using the click-chemistry bioconjugation approach. Size, morphology, and peptide loading were verified via dynamic light scattering, transmission electron microscopy, microfluidic electrophoresis and mass spectrometry. C57BL/6 mice received homologous or heterologous VLP-based priming and boosting. Humoral responses were evaluated via ELISA and TZM-bl neutralization assays, while CD8⁺ T-cell responses in blood and tissues were quantified using intracellular cytokine staining and flow cytometry.

Results: All peptides were successfully conjugated to distinct VLPs. Heterologous VLP immunizations elicited significantly higher HIV-1 peptide specific binding and HIV-1 neutralizing antibody (nAb) titers (1:120318 and nAb ID50 = 488) than homologous regimens (1:32891 and nAb ID50 = 244), demonstrating improved immune focusing and overcoming anti-vector immunity. Notably, boosting with BG505.SOSIP.664 or adding exogenous CD4⁺ T-cell help did not further enhance these nAb responses. In contrast, the use of a helper epitope elicited robust effector CD8⁺ T-cell responses (34% IFN- γ ⁺ CD8⁺ T cells) after prime and boost, which persisted in peripheral blood and liver (6% IFN- γ ⁺ CD8⁺ T cells) two months post-immunization. Furthermore, these effects were similarly validated using an unrelated model antigen, demonstrating the modularity of this approach.

Conclusion: A modular VLP platform effectively primes both B- and T-cell anti-HIV immunity, setting the stage for further evaluation in non-human primates for clinical translation.

Metabolic Signatures for Anti-Tuberculosis Immunity in Rhesus Macaque Lung Tissues: a comparative lipidomic study

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Introduction: Lipids play critical roles in physiological, pathogenic, and immune stimulatory processes. We hypothesize that *Mycobacterium tuberculosis* (*M.tb*) infection induces global lipid profile alterations in tissues to regulate anti-microbial immune responses. In this study, we identified lipid metabolites enhanced by BCG vaccination in the *M.tb*-infected lung tissues to suggest candidate metabolites and metabolic pathways associated with anti-tuberculosis immunity. **Methods:** Lung tissues from rhesus macaques in three groups, (i) Naïve (n=4), (ii) BCG-vaccinated/ *M. tb*-infected (BCG-*M.tb*, n=4), and (iii) *M. tb*-infected naïve animals (Naïve-*M.tb*, n=4), were extracted using a modified Matyash method (10:3:2.9 MTBE:MeOH:H₂O). Samples were analyzed on a Vanquish Flex UHPLC coupled to an Orbitrap IQ-X Tribrid mass spectrometer. Heated Electron Spray Ionization (HESI) was used in positive (3500 V) and negative (2500 V) modes, with full MS at 120k resolution. Metabolites were annotated with LipidSearch. **Results/Conclusion:** Results revealed a comprehensive alteration in lipid classes in *M. tb*-infected naïve primate lung tissues, including phospholipids, sphingolipids, and fatty acids. Cholesterol esters increased in *M. tb*-infected lungs, likely associated with lipid storage in foamy macrophages and caseous regions of granuloma upon *M.tb* infection. The increased oxidized lipids likely contribute to an iron-dependent lipid peroxidation, known as ferroptosis, which plays a role in the pathogenesis of tuberculosis. We also observed that ether-linked lipids increased in the *M. tb*-infected lung tissues. Ether-linked lipids are a unique class of lipids formed in the peroxisomes and contribute to cellular structure, membrane trafficking, and cell signaling. They also function as potential endogenous ligands of peroxisome proliferator-activated receptor gamma (PPAR-γ), which promotes lipid droplet formation in macrophages. Together, our data revealed that important lipid metabolites and metabolic pathways were altered in *M.tb*-infected lungs, including cholesterol esters, oxidized lipids, ether-linked lipids, and sphingolipids, which suggest candidate lipid metabolites and metabolic pathways as targets to induce protective anti-tuberculosis immunity. Currently, we are testing the representative lipids identified in this study and their metabolic pathways with small-molecule regulation using an *in vitro* granuloma model to determine the protective effect of candidate lipids against *M.tb* infection.

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BANQUET DINNER

The Ralston at Congo Falls

THURSDAY, OCTOBER 22, 2026

Reception - 4:45pm / Presentations, Dinner & Dancing - 6:00pm

PRESENTERS

Rachael Wolters, DVM, PhD, Research Assistant Professor, Oregon National Primate Research Center

Cory Ross, PhD, Professor & Director, Southwest National Primate Research Center



Let's celebrate the final evening of the symposium with a fiesta to remember! Join us at the beautiful Ralston at Congo Falls for an evening of spectacular views, delicious food, and festive fun. Take in the newly built great ape habitat, enjoy special presentations and dinner, then hit the dance floor with friends and colleagues. Bring your dancing shoes and fiesta spirit as we toast shared discoveries, new friendships, and a wonderful symposium!

Hotel Shuttle Schedule

4:30–6:30 pm: Shuttles will run continuously from the Westin Riverwalk to the Ralston.

7:00–8:30 pm: Return shuttles will run from the Ralston to the Westin Riverwalk.

Westin Riverwalk pickup location: Street outside Westin Riverwalk

Driving or Uber: The Ralston, 950 E Hildebrand, San Antonio, Texas

Suggested Attire: Business, Business Casual or your best Fiesta Flair...





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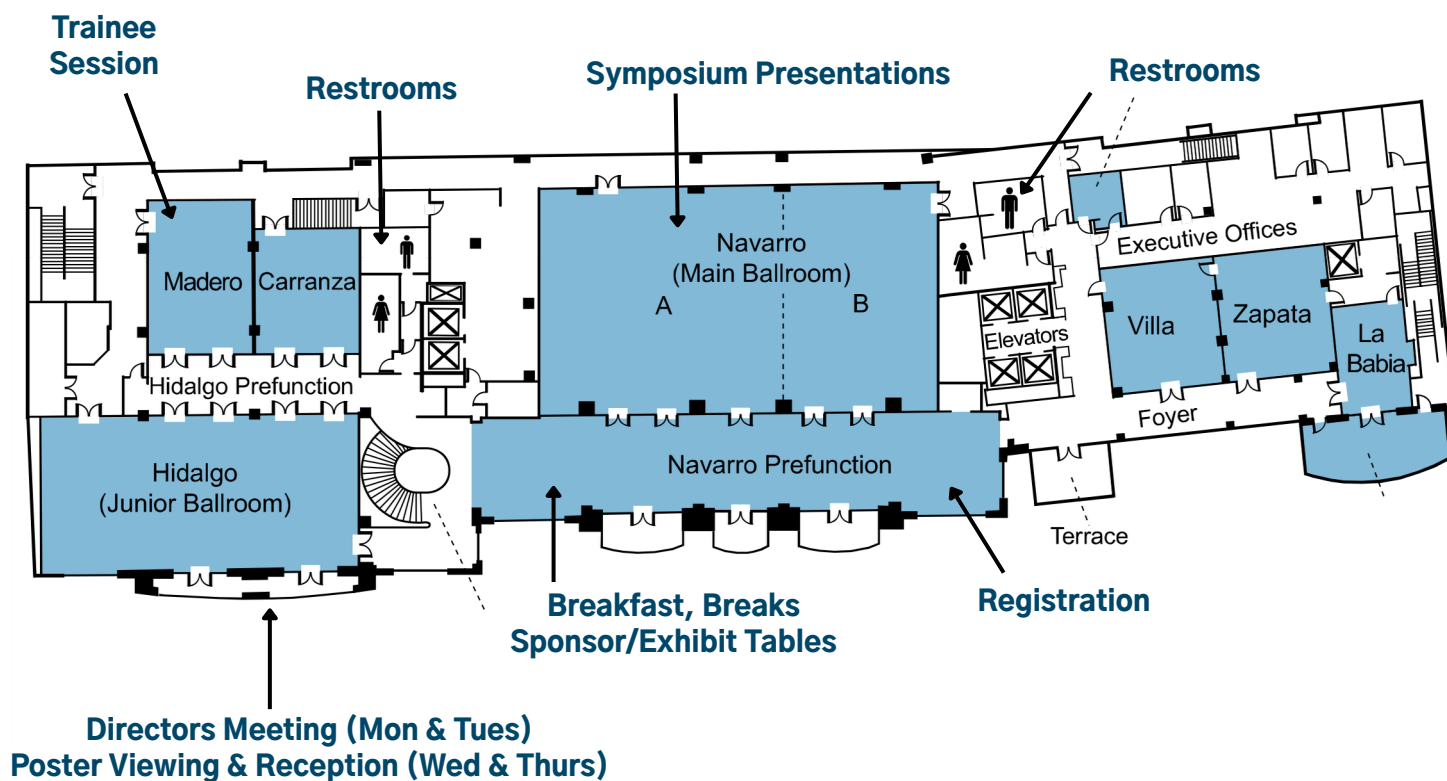
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Seattle, October 19-22



Symposium Map

(Ballroom Level – 2nd Floor)



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