39th Annual Symposium on Nonhuman Primate Models for AIDS

SEPTEMBER 13 – 16, 2022

HOSTED BY
Emory National Primate Research Center

IN COLLABORATION WITH
Oregon National Primate Research Center

The Nines Hotel
525 SW Morrison St, Portland OR 97204

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Dear colleagues,

Welcome to the 39th Annual Symposium on Nonhuman Primate Models for AIDS! We are pleased to have so many of you with us in person and are thankful technology allows virtual participation by even more of our colleagues.

Please join us in thanking our Scientific Committee, which includes representatives from all seven National Primate Research Centers, other academic research centers and the pharmaceutical industry. The committee reviewed more than 100 abstracts to bring you the exciting and diverse presentations you'll hear across these scientific sessions in the coming days: SIV Pathogenesis and Co-Infections; Vaccines, Immunology, and Prevention; Progress Toward HIV Cure; COVID and Other Infectious Diseases; and Genomics and Emerging Technologies. In addition, we added an Animal Management category this year. Leaders in each of these areas will chair and co-chair the sessions, and the chairs will start each session with the latest information about their research.

We also ask you to thank our session chairs and co-chairs, and to join us in thanking Nancy Haigwood, PhD, Director of the Oregon National Primate Research Center. Nancy, who is retiring as director before the end of the year, will give the keynote talk during the Opening Reception. She is an esteemed colleague and dedicated advocate for science education and public outreach.

During the daily breaks and evening events, we encourage you to discuss the science, forge collaborations and connect with our early career investigators. We selected 22 of them for travel awards this year. Three of these awards are named in memory of our colleagues Drs. Tim Hoang, Andrew Lackner and Bonnie Mathieson.

The opportunity to gather and share our science would not be possible without grant support from the Office of Research Infrastructure Programs at the National Institutes of Health as well as the generous support of our sponsors. Please be sure to visit the exhibit tables to thank our sponsors and learn about the services they offer. We also have an NPRC Core table where you can learn about additional research support services the NPRCs offer.

If you have not done so yet, be sure to download the Whova app and complete your profile, which will facilitate connection among meeting participants and provide you with easy access to the agenda and more information about this year's meeting.

We look forward to a great 39th NHP AIDS Symposium!

Sincerely,
Joyce Cohen, VMD, DACLAM
R. Paul Johnson, MD
Mirko Paiardini, PhD
Meeting Co-Chairs

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ATTENDEE LIST

The attendee list for the 39th Symposium may be found online in the conference app at whova.com. Simply login at https://whova.com/portal/webapp/snma_202209/Attendees/All with the same email you used to register, and click on "Attendees" in the left-hand sidebar. This will allow you to connect with all attendees at this event.

Note: You must activate permission to be contacted through the event portal if you wish to view others' contact information.

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We thank the following for their generous financial support, which makes this symposium possible:

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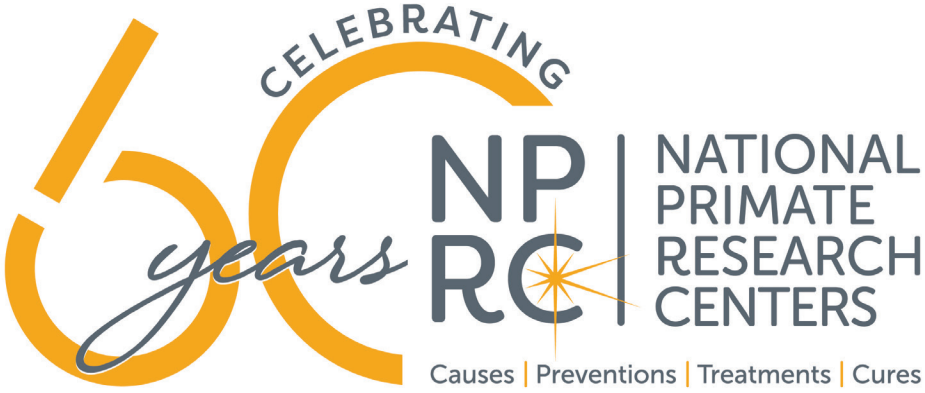

NAMED TRAVEL AWARD RECIPIENTS

Sho Sugawara, Andrew Lackner Travel Award

The Andrew Alan Lackner Memorial Travel Fellowship celebrates excellence in research in any area of infectious diseases using nonhuman primate models. It is awarded to a top ranked early stage investigator at the Annual Symposium on Nonhuman Primate Models for AIDS. The award celebrates the life of former Tulane National Primate Research Center Director Andrew A. Lackner, DVM, PhD, DACVP, and his countless contributions to veterinary medicine, pathology and research in the area of infectious diseases. The Southwest National Primate Research Center supports this award.

Elise Viox, Timothy Hoang Memorial Travel Award

The Timothy Hoang Memorial Travel Award celebrates the life of Dr. Timothy Hoang, a graduate student at the Emory National Primate Research Center whose dedication to scientific discovery and advancement inspired all the lives he touched. Dr. Hoang made critical contributions to HIV cure research and pioneered SARS-CoV-2 studies in nonhuman primates during his short but impactful career, and will be remembered for his intelligence, drive and love for science. The Emory National Primate Research Center supports this award.

Michelle Lee, Bonnie Mathieson NHP Symposium Travel Award

The Bonnie Mathieson NHP Symposium Travel Award was established to honor Dr. Mathieson who always attended the Annual Symposium and was a champion for early stage scientists, serving as a mentor and advocate. She had a specific interest in helping early stage researchers develop their ideas and initiate new areas of HIV vaccine research. The Wisconsin National Primate Research Center supports this award.

EARLY STAGE INVESTIGATOR TRAVEL AWARD RECIPIENTS

Michelle Ash
Fredrik Barrenäs
Sreya Biswas
Ning Chin
Amir Dashti
Hiroshi Ishii
Hannah King
Antonio Solis Leal
Matilda Mostrum
Mukta Nag
Sydney Nemphos
Megan O'Connor
Caroline Subra
Katherine Turnbull
Amit Upadhyay
Anil Verma
Zoey Wallis

EMORY CENTER FOR AIDS RESEARCH TRAVEL AWARD RECIPIENTS

Sakthivel Govindaraj
Bhrugu Yagnik

KEYNOTE SPEAKER

Nancy Haigwood, PhD
Director, Oregon National Primate Research Center

Antibodies as Therapies for HIV: A Forty Year Perspective

Nancy L. Haigwood, PhD, is a professor of Pathobiology & Immunology and Director of the Oregon National Primate Research Center (ONPRC) at Oregon Health & Science University. She received her Ph.D. from UNC Chapel Hill in microbiology and immunology and completed postdoctoral training in virology at The Johns Hopkins University. She has over 40 years of research experience in in the area of global health, both in the biotech and non-profit sectors, and since the mid-1980s, she has been actively engaged in vaccine discovery and immunotherapy research in nonhuman primate (NHP) models for HIV/AIDS.

Her group has shown that passive immunotherapy using potent human neutralizing monoclonal antibodies and other early intervention strategies can change the course of SHIV infection in infant macaques, preventing the establishment of a permanent viral reservoir. These findings are helping to guide the application of antibody-based therapies to prevent vertical transmission in the clinic. Dr. Haigwood was inducted into the American Academy of Microbiology in 2014. She is an advocate for education and openness to increase understanding of science for the public.

AGENDA

Please note: All times Pacific Time

TUESDAY, SEPTEMBER 13, 2022

Opening Reception 6 PM – 8 PM
Keynote Speaker:
Nancy Haigwood, PhD, Director, Oregon National Primate Research Center

WEDNESDAY, SEPTEMBER 14, 2022

Breakfast and Sponsor/Exhibitor Tables Open 7:30 AM – 8 AM

Welcome and Named Travel Award Presentation 8 AM – 8:15 AM
Andrew Lackner Travel Award
2022 Recipient: Sho Sugawara, Duke University

Scientific Session 1: 8:15 AM – 8:45 AM
SIV Pathogenesis and Co-Infections
Chair: Michaela Muller-Trutwin, PhD, Institut Pasteur
Co Chair: Keith Reeves, PhD, Duke University
Dr. Muller-Trutwin's presentation: NK cells responses in tissues during SIVagm and SIVmac infections

A novel multiplex analysis reveals global downregulation of CD16 signaling pathways via an IL18/ADAM17-dependent mechanism during lentivirus infection 8:45 AM – 9 AM
Speaker: Sho Sugawara

The Effects of P.fragile Co-Infection on Antiretroviral Treatment and Immunological Responses in SIV-Infected Rhesus Macaques 9 AM – 9:15 AM
Speaker: Sydney Nemphos

Natural killer cells regulate acute SIV replication, dissemination, and inflammation, but do not impact independent transmission events — 9:15 AM – 9:30 AM
Speaker: Sabrina Tan

Origin of viral rebound in SIV-infected Chinese rhesus macaques during early antiretroviral therapy interruption — 9:30 AM – 9:45 AM
Speaker: Antonio Solis Leal

Epigenetic Modifications in CD8+ T cells in Simian Immunodeficiency Virus Infection of Rhesus Macaques. — 9:45 AM – 10 AM
Speaker: Mukta Nag

Break and Sponsor/Exhibitor Tables Open — 10 AM – 10:30 AM

Antiretroviral therapy reduces but does not eliminate the traffic of SIV-infected perivascular and meningeal macrophages into and out of the central nervous system. — 10:30 AM – 10:45 AM
Speaker: Zoey Wallis

The Role of CD4+ Naive T Cells in SIV Persistence during Antiretroviral Therapy — 10:45 AM – 11 AM
Speaker: Ben Varco-Merth

Characterization of early cellular targets of infection after an intra-vaginal inoculation of simian immunodeficiency virus into rhesus macaques — 11 AM – 11:15 AM
Speaker: Muhammad Shoaib Arif

Bridging anti-lentivirus granulocyte biology across humans and rhesus macaques — 11:15 AM – 11:30 AM
Speaker: Cordelia Manickam

Lunch (on your own) — 11:30 AM – 1 PM

Scientific Session 2: Vaccines, Immunology, and Prevention 1 PM – 1:30 PM

Chair: Michael Betts, PhD, University of Pennsylvania
Co Chair: Smith Iyer, UC Davis
Dr. Betts' Presentation: Manipulation of T Cell Trafficking in SIV-Infected Macaques to Understand SIV Immunopathogenesis

Cytomegalovirus vaccine vector-induced, unconventionally MHC-restricted effector memory T cells protect cynomolgus macaques from lethal aerosolized H5N1 influenza challenge 1:30 PM – 1:45 PM
Speaker: Joseph Hwang

Tailoring helper profile of HIV-1 vaccine induced CD4 T follicular helper cell responses 1:45 PM – 2 PM
Speaker: Anil Verma

Adjuvanted pox-protein vaccination elicits exceptional effector antibody and T cell responses, but does not protect against heterologous SHIV rectal challenge in rhesus macaques 2 PM – 2:15 PM
Speaker: Caroline Subra

Deep Integration Network Analysis of RhCMV/SIV vaccines links gene expression with IL-15 response and non-conventional antigen presentation for SIV control 2:15 PM – 2:30 PM
Speaker: Fredrik Barrenas

Break and Sponsor/Exhibitor Tables Open 2:30 PM – 3 PM

Single-cell Blood Leukocyte Signature of the Rhesus Cytomegalovirus-based Vector Protection Signature of SIV Vaccination 3 PM – 3:15 PM
Speaker: Leanne Whitmore

Preclinical development of Modified Vaccinia virus Ankara vaccines against SARS-CoV-2 infections — 3:15 PM – 3:30 PM
Speaker: Nanda Kishore Routhu, PhD

Single cell repertoire analyses of blood and bone marrow antibody secreting cells reveals superior ability of 3M-052-alum adjuvant in comparison with alum to promote HIV-1 envelope specific long-lived immunity — 3:30 PM – 3:45 PM
Speaker: Sudhir Kasturi, PhD

Cytomegalovirus infection alters the transcriptomic response to RhCMV/SIV vaccination — 3:45 PM – 4 PM
Speaker: Ning Chin

Special Session: Animal Management — 4 PM – 4:15 PM
Chair: Joyce Cohen, VMD, Emory National Primate Research Center
Dr. Cohen's Presentation:
The Value of Animal Management in Supporting Research

Refinements to Nursery Practices for Infant Rhesus Macaques — 4:15 PM – 4:30 PM
Speaker: Lisa Houser

Survey of Macaque and Baboon Samples Shows No Human Coronavirus Infections and Low Prevalence of Other Common Respiratory Pathogens — 4:30 PM – 4:45 PM
Speaker: Richard Grant

Sponsor/Exhibitor Tables Open — 4:45 PM – 6 PM

Poster Reception — 6 PM – 8 PM
Approx. 65 researchers will be available to discuss their science during the poster reception.

THURSDAY, SEPTEMBER 15, 2022

Breakfast and Sponsor/Exhibitor Tables Open 7:30 AM – 8 AM

Scientific Session 3: Progress Toward HIV Cure 8 AM – 8:30 AM

Chair: Katharine Bar, MD, University of Pennsylvania

Dr. Bar's presentation: Advances in HIV-1 Cure via NHP models: Transmitted Founder SHIVs for Persistence

Precursor Effector TCF1+ CD39+ Tox+ CD8 T Cells Emerge in Lymph Node After SIV Infection and Are Associated with Better Viral Control 8:30 AM – 8:45 AM

Speaker: Zachary Strongin

Massive viral RNA burden in SIV-infected CD4+ T-cells coincides with latency transcription factor downregulation 8:45 AM – 9 AM

Speaker: Diane Bolton

RNAscope Characterization of the CNS SIV Reservoir Reveals Elevated Infected Astrocytes Following cART Cessation 9 AM – 9:15 AM

Speaker: Michelle Ash

LAIR-1 is a negative regulator of NK cells during chronic SIV infection 9:15 AM – 9:30 AM

Speaker: Sakthivel Govindaraj

Blockade of TGF-β signaling reactivates HIV-1/SIV reservoirs and immune responses in vivo 9:30 AM – 9:45 AM

Speaker: Sadia Samer

Combination Of PD-1 Blockade And AZD5582 Therapy In SIV+ Monkeys 9:45 AM – 10 AM

Speaker: Bhrugu Yagnik

Break and Sponsor/Exhibitor Tables Open 10 AM – 10:30AM

Impact of the IL-15 Superagonist N-803 on Latency Reversal Induced by AZD5582 and on Immune Cells in ART-Suppressed Rhesus Macaques 10:30 AM – 10:45 AM
Speaker: Amir Dashti

Passive transfer of SIV Env-specific rhesus mAbs to rhesus macaques: a robust model for ATI/functional cure studies 10:45 AM – 11 AM
Speaker: Hannah King

Targeting the SIV Reservoir with Alemtuzumab (anti-CD52) 11 AM – 11:15 AM
Speaker: Morgan Chaunzwa

Constitutive NKG2A levels and timing of antiretroviral therapy initiation impact the potential role of NK cells after treatment interruption—the pVISCONTI study 11:15 AM – 11:30 AM
Speaker: Anais Chapel

Lunch (on your own) 11:30 AM – 1 PM

Special Presentation 1 PM – 1:10 PM
Journal of Medical Primatology
Speaker: Ron Veazey, PhD

Named Travel Award Presentation 1:10 PM – 1:15 PM
Timothy Hoang Memorial Travel Award
2022 Recipient: Elise Viox, Emory National Primate Research Center

Scientific Session 4: 1:15 PM – 1:45 PM
COVID and Other Infectious Diseases
Chair: Robert Seder, PhD, NIH Vaccine Research Center
Co Chair: Mirko Paiardini, PhD, Emory National Primate Research Center
Dr. Seder's presentation: Mechanisms of mRNA Vaccine Immunity and Protection in NHP: The scientific rationale for boosting against variants and do we need intranasal vaccines to prevent infection and transmission

Modulation of Type I Interferon Responses Inhibits SARS-CoV-2 Replication and Inflammation in Rhesus Macaques 1:45 PM – 2 PM
Speaker: Elise Viox

Induction of adaptive MHC-E restricted lung tissue-resident NK cells associated with persistent low antigen load in alveolar macrophages after SARS-CoV-2 infection 2 PM – 2:15 PM
Speaker: Nicolas Huot

A self-amplifying Replicon RNA COVID-19 vaccine induces durable protection from SARS-CoV-2 in pigtail macaques and protects even after neutralizing antibodies have waned to undetectable levels 2:15 PM – 2:30 PM
Speaker: Megan O'Connor

Reduced COVID vaccine responses in CMV seropositive rhesus macaques associated with decreased ribosomal protein gene transcription of memory B cells 2:30 PM– 2:45 PM
Speaker: William Chang

TREM2+ and interstitial-like macrophages orchestrate airway inflammation in SARS-CoV-2 infection in rhesus macaques 2:45 PM – 3 PM
Speaker: Amit Upadhyay

Break and Sponsor/Exhibitor Tables Open 3 PM – 3:30 PM

Pulmonary RhCMV Reactivation Following SARS-CoV-2 In Aged Rhesus Macaques: Implications for Immunopathology — 3:30 PM – 3:45 PM
Speaker: Jamin Roh

The presence of indoleamine 2,3-dioxygenase in an aerosol-mediated infant rhesus macaque tuberculosis model — 3:45 PM – 4 PM
Speaker: Katherine Turnbull

Congenital cytomegalovirus infection drives metabolic rewiring of amniotic fluid in a nonhuman primate model — 4 PM – 4:15 PM
Speaker: Matilda Mostrom, PhD

Protective efficacy of a vaccine inducing anti-Env antibodies against HTLV-1 challenge in cynomolgus macaques — 4:15 PM – 4:30 PM
Speaker: Hiroshi Ishii

Banquet — 6 PM – 10 PM
(First bus leaves at 5:30 pm)

Friday, September 16, 2022

Breakfast and Sponsor/Exhibitor Tables Open — 7:30 AM – 8 AM

Welcome and Named Travel Award Presentation
Bonnie Mathieson NHP Symposium Travel Award
2022 Recipient: Michelle Lee, Emory Vaccine Center and Emory National Primate Research Center

Scientific Session 5: — 8 AM – 8:30 AM
Genomics and Emerging Technologies
Chair: Steven E. Bosinger, PhD, Emory National Primate Research Center
Co Chair: Claire Deleage, Frederick National Laboratory
Dr. Bosinger's presentation: Advances in AIDS and COVID-19 research via NHP genomics

Multi-omics analysis at single-cell level of SIV-specific CD8+ T cells in ART-suppressed SIV infected rhesus macaques — 8:30 AM – 8:45 AM
Speaker: Michelle Lee

Increased Chemokine Production is A Hallmark Of Rhesus Macaque Natural Killer Cells Mediating Robust Antibody-Dependent Cellular Cytotoxicity — 8:45 AM – 9 AM
Speaker: Junsuka Nohara

Defining the dynamics of SIV infection and the viral reservoir from early ART initiation to Rebound using PET/CT analysis and a multi-scale imaging approach — 9 AM – 9:15 AM
Speaker: Yanique Thomas

SHIV Reservoirs: from In Vivo Labeling and PET/CT Imaging to Ex Vivo Identification of Infected cells — 9:15 AM – 9:30 AM
Speaker: Kenneth Rogers

Transgenic Rhesus Macaques Expressing Human Na+ Taurocholate Cotransporting Polypeptide: A Promising Model to Study Chronic Hepatitis B Infection — 9:30 AM – 9:45 AM
Speaker: Sreya Biswas

Break and Sponsor/Exhibitor Tables Open — 9:45 AM – 10:15 AM

Integration of Spatial and Single Cell Transcriptomics Identifies Novel Pathologically Relevant Markers in SIV- and Mycobacterium tuberculosis-infected Rhesus Macaques — 10:15 AM – 10:30 AM
Speaker: GW McElfresh

Alternative splicing and genetic variation of MHC-E: Implications for rhesus cytomegalovirusbased vaccines — 10:30 AM – 10:45 AM
Speaker: Xinxia Peng

Tailoring Gene Space Facilitates Phenotyping of T cell Subpopulations from Single-cell RNA-seq Data Obtained from SIV-infected Rhesus macaques — 10:45 AM – 11 AM

Speaker: Gregory Boggy

Nimble: A Novel Tool to Maximize and Augment Information From Bulk and Single-Cell RNA-Seq Data — 11 AM – 11:15 AM

Speaker: Sebastian Benjamin

The Rhesus IgSeq project: Population genotyping of the germline immunoglobulin repertoire in AIDS-designated Rhesus macaque breeding colonies — 11:15 AM – 11:30 AM

Speaker: Corey Watson, PhD

Closing Remarks and Preview of 2023 NHP AIDS Symposium — 11:30 AM – Noon

R. Paul Johnson, MD

Koen Van Rompay, DVM, PhD

Smita Iyer, PhD

ABSTRACTS
ORAL PRESENTATIONS

SCIENTIFIC SESSION 1:
SIV Pathogenesis and Co-Infections

Chair: Michaela Muller-Trutwin, Institut Pasteur
Co Chair: Keith Reeves, PhD, Duke University

NK cells responses in tissues during SIVagm and SIVmac infections

Presentation by Michaela Muller-Trutwin (Speaker)
Institut Pasteur, Professor

ABSTRACT 1

A novel multiplex analysis reveals global downregulation of CD16 signaling pathways via an IL-18/ADAM17-dependent mechanism during lentivirus infection

Sho Sugawara[1], Brady Hueber[1], Griffin Woolley[1], Kyle Kroll[1], Cordelia Manickam[1], Daniel R Ram[2], Stephanie Jost[1], R. Keith Reeves[1]

1 Duke University, Durham, NC, USA.

2 Beth Israel Deaconess Medical Center, Brookline, MA, USA.

Natural killer (NK) cells elicit critical innate effector responses to control human immunodeficiency Virus (HIV)-1 and simian immunodeficiency virus (SIV) infections. However, dysregulated NK cell responses are frequently exhibited in both, yet the underlying mechanisms remain unclear. Whereas altered surface receptor expression and NK cell functions have been widely characterized during lentiviral infection, its impact on subsequent signaling activation remains understudied due to a previous lack of complex NK cell signaling analyses. In order to fill this knowledge gap, we aimed to characterize the humans and rhesus macaque (RM) NK cell signalomes using our newly-established multiplex NK cell signaling assay in greater detail during HIV-1/SIV infections. Human NK cells and NK cells from control and chronically SIVmac251-infected RMs were stimulated through multiple NK cell receptors including CD16, NKp46, NKG2D, and CD2, and their signaling activation was comprehensively assessed by monitoring the phosphorylation (p) events of 10 major signaling molecules critical for NK cell functions: p-Syk, p-lck, p-LAT, p-ZAP70, p-JNK, p-NFkB, p-p70S6K, p-Akt, p-STAT3, and p-STAT5. Compared to controls, NK cells from SIV-infected animals demonstrated dampened immunotyrosine activation motif-based signaling from CD16 stimulation in accordance with their reduced surface CD16 expression. Moreover, we elucidated CD16 surface expression and signaling downregulation was mediated by IL-18-induced ADAM17 elevation during SIV infection. Taken together, we were able to establish a multiplex platform to analyze complex NK cell signaling, and delineated global suppression of CD16 signaling during SIV infection, which could be targeted for improving current NK cell-based HIV-1 immunotherapeutics.

ABSTRACT 2

The Effects of *P.fragile* Co-Infection on Antiretroviral Treatment and Immunological Responses in SIV-Infected Rhesus Macaques

Sydney Nemphos[1], Hannah Green[1], Kelly Goff[1], Megan Varnado[1], Kaitlin Didier[1], Natalie Guy[1], Matilda Moström[1], Coty Tatum[1], Chad Massey[1], Robert Blair[1], Mary Barnes[1], Carolina Allers[1], Monica Embers[1], Nicholas Maness[1], Preston Marx1,[2], Brooke Grasperge[1], Amitinder Kaur[1], Jennifer A Manuzak[1]

1 Tulane National Primate Research Center, Covington, Louisiana, USA.

2 Tulane School of Public Health and Tropical Medicine, New Orleans, Louisiana, USA.

Background: HIV and malaria are co-endemic. The mechanisms by which co-infection accelerate disease pathogenesis are unknown. We hypothesized that *P. fragile* co-infection of ART-treated, SIV+ rhesus macaques (RMs) would result in elevated viral load (VL) and decreased CD4+ T-cell counts.

Methods: Male RMs (n=4) were intravenously (i.v.) inoculated with SIVmac239 (TCID50=50); initiated daily ART at Week (W)8 post-SIV infection (post-SIV); were i.v. inoculated with *P. fragile* (20x10^6 infected erythrocytes [iRBCs]) at W12 post-SIV; and were treated with anti-malarials at W14 post-SIV. Plasma VL and peripheral parasitemia were monitored via qPCR and Giemsa-stained blood smears, respectively. CD4+ T-cell absolute counts were assessed using flow cytometry.

Results: Peak VL (median=9.92x10^06 RNA copies/ul) was reached by W3 post-SIV. Upon ART initiation, VLs decreased, with 2/4 RMs becoming undetectable by W12 post-SIV. Within two weeks of *P. fragile* inoculation, parasitemia reached peak levels (W14 post-SIV median %parasitemia=25.5% iRBCs), which declined after anti-malarial treatment. Beginning one week post-*P. fragile* inoculation (W13 post-SIV), all RMs exhibited detectable VLs through W17 post-SIV (median=9.34x10^02 RNA copies/ul). Compared to baseline (median=306.905 cells/ul), CD4+ T-cell counts declined by W8 post-SIV (median=140.5 cells/ul), increased after ART (median=193.5 cells/ul), and declined after *P. fragile* inoculation (median=173.59 cells/ul).

Conclusions: In this pilot study, our observations suggest that *P. fragile* co-infection may lower ART efficacy, characterized by lesser SIV viral suppression and poorer CD4+ T-cell reconstitution. More work is needed to characterize the immune mechanisms contributing to disease progression and pathogenesis during SIV/malaria co-infection.

ABSTRACT 3

Natural killer cells regulate acute SIV replication, dissemination, and inflammation, but do not impact independent transmission events

Matthew Mosher[1], Griffin Woolley[1], Sabrina Tan MD[2], Kyle Kroll[1], Rhianna Jones[1], Brady Hueber[1], Sho Sugawara PhD[1], Cordelia Manickam DVM, PhD[1], Valerie Varner[2], Michelle Lifton[2], **Brandon Keele**[3], R. Keith Reeves PhD[1,2]

1 Duke University, Durham, NC, USA.

2 Center for Virology and Vaccine Research, Boston, MA, USA.

3 AIDS and Cancer Virus Program, Frederick, MD, USA.

Natural killer (NK) cells are potent effectors of the innate immune system possessing both cytotoxic and immunoregulatory capabilities; which contribute to their crucial role in controlling HIV and SIV infections. However, despite significant evidence for NK cell modulation of HIV disease, their specific contribution to transmission and control of acute infection remains unclear. In this acute necropsy study, rhesus macaques (RM) were subjected to pre-infection depletion of systemic NK cells using established methods of IL-15 neutralization, followed by challenge with barcoded SIVmac239X. Our study showed that depletion was highly effective resulting in near total ablation of all NK cell subsets in blood, liver, oral and rectal mucosae, and lymph nodes that persisted through the duration of the study. Meanwhile, frequencies and phenotypes of T cells remained virtually unchanged, indicating that our method of NK depletion had minimal off-target effects. Importantly, NK cell-depleted RM demonstrated early and sustained 1-to-2 log increase in viremia over controls, but single genome analysis suggested no difference in independent transmission events. Acute bulk, central memory, and CCR5+ CD4+ T cell depletion was similar between experimental and control groups, while CD8+ T cell activation was higher in NK cell-depleted RM as measured by Ki67 and PD-1 expression. Using 27-plex Luminex analyses, we found modestly increased inflammatory cytokine profiles in NK cell-depleted RM compared to control animals. Collectively, these data suggest NK cells are important modulators of lentivirus dissemination and disease but may not independently impact individual transmission events.

ABSTRACT 4

Origin of viral rebound in SIV-infected Chinese rhesus macaques during early antiretroviral therapy interruption

Antonio Solis Leal Ph.D[1], Binhua (Julie) Ling Ph.D., M.D.[1], Boby Nongthombam Ph.D.[1], Xabier Alvarez Hernandez Ph.D.[1], Suvadip Mallick Ph.D.[1], Yilun Cheng Ph.D.[2], Fei Wu Ph.D.[1], Jason Dufour Ph.D.[3], Grey De La Torre M.S.[1], Vinay Shivanna Ph.D.[1], Jeffrey Lifson Ph.D.[4], Qingsheng Li Ph.D.[2], Brandon F Keele Ph.D.[4]

1 Host Pathogen Interaction Program & Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, Texas, USA.

2 Nebraska Center for Virology & School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska, USA.

3 Tulane National Primate Research Center, Covington, Louisiana, USA. |

4 AIDS and Cancer Virus Program, Frederick National Laboratory, Frederick, Maryland, USA.

Background: Viral persistence in tissue reservoirs is the major obstacle in eradicating HIV infection. These reservoirs are responsible for viral rebound following antiretroviral therapy (ART) interruption (ATI). A better understanding of the origin of the early rebound virus could help to develop effective strategies to prevent systemic spread.

Methods: Seven Chinese rhesus macaques were infected with the barcoded virus SIVmac239M and treated with ART starting at 12 weeks post-infection for 42 weeks, followed by a 3-week ATI1, ART resumed for 13-18 weeks, and a second ATI (ATI2) was initiated one week before each necropsy. Deep sequencing, single genome amplification, qRT-PCR, intact proviral DNA assay, immunofluorescence analysis, and combined CODEX/RNAscope in situ hybridization (Comb-CODEX-RNAscope) analysis were used to evaluate the reservoir size and the rebounding source.

Results: Plasma viral load (PVL) was maintained at <15 copies/mL while on ART and at necropsy. Despite "undetectable" PVL, a barcode clonotype signal was detected in plasma of 4/7 animals, with a total number of 11 clonotypes detected among 4 animals at ATI2. In these animals, spleen, mesenteric, and inguinal lymph nodes showed high viral barcode clonotypic diversity, a larger intact proviral reservoir size, and the same barcodes than plasma clonotypes. Viral RNA was detected in these tissues by Comb- CODEX-RNAscope.

Conclusion: Spleen, mesenteric, and inguinal lymph nodes may contribute to the rebounding virus population during the first week after ATI. While novel strategies have to target these anatomical sites for potential viral eradication, it may be challenging considering the complexity of these reservoirs.

ABSTRACT 5

Epigenetic Modifications in CD8+ T cells in Simian Immunodeficiency Virus Infection of Rhesus Macaques

Mukta Nag[1], Jonathan E. Fogle[2], Sathoshan Pillay[3], Ellen Bak[1], Jeffrey D. Lifson[1], Gregory Q. Del Prete[1], Kristina De Paris[4]

1 AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA.

2 Boehringer Ingelheim, Raleigh, North Carolina, USA.

3 Enlabeler, Cape Town, Western Cape, South Africa.

4 University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA.

During chronic HIV/SIV infection CD8+ T cells become functionally impaired due to persistent antigen stimulation. To assess associated epigenetic changes, specifically DNA methylation, we studied cryopreserved CD8+ T cells from PBMCs, lymph nodes and intestinal tissues of SIVmac239/SIVmac251- infected (5-31 wpi) or uninfected infant and adult rhesus macaques. We performed promoter-wide and site specific CpG methylation quantitation by targeted bisulfite sequencing and chromatin immunoprecipitation- qPCR (ChIP-qPCR) for Foxp3 binding at cytokine loci. Methylation signatures at the cytokine promoters of PBMC and intestinal CD8+T cells were similar but distinct from those in lymph nodes irrespective of the animals' infection group. In CD8+ T cells of SIV-infected compared to uninfected animals, the % CpG methylation was lower at the IL2 and IFNG promoters and higher at the TNFA promoters for PBMC and intestinal specimens, whereas methylation was unchanged at the IL2 but higher at the IFNG and TNFA promoters of lymph node samples. By determining methylation of individual CpG sites within the region 1000bp upstream of the transcription start site of each the IL2, IFNG and TNFA promoters, we identified the specific CpG residues that were differentially methylated based on the infection status. We also observed a trend towards increased Foxp3 binding to the cytokine promoters in SIV-infected compared to uninfected animals. Tissue-specific DNA methylation differences at cytokine promoters during chronic SIV infection are associated with altered binding of transcription factors, including the repressive transcription factor Foxp3, suggesting a potential role for epigenetic modifications in contributing to CD8+ T cell dysfunction.

ABSTRACT 6

Antiretroviral therapy reduces but does not eliminate the traffic of SIVinfected perivascular and meningeal macrophages into and out of the central nervous system

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Antiretroviral therapy (ART) reduces central nervous system (CNS) virus but its effect on myeloid turnover is understudied. We use two different colored, fluorescent superparamagnetic iron oxide nanoparticles (SPIONs) administered into the cerebrospinal fluid of SIV+ rhesus macaques during early (12 dpi) and late infection (30 days prior to sacrifice). SPIONs are selectively taken up by CD163+ perivascular (PV) and meningeal macrophages and used to monitor turnover. SPION+ cells were more concentrated in the meninges vs parenchyma in SIV encephalitis (SIVE, 40x) and ART animals (150x). SIVE animals had a higher density of SPION+ cells in the parenchyma compared to ART and ART interruption animals [SIVE 0.63, ART 0.16, ART Interruption 0.23 SPION+ cells/mm2]. SIVE monkeys have 40x more SPION+ cells in the meninges than the parenchyma and 150x more following ART. The majority of SPIONs in the CNS were from early SPIONs. SPION-labeled cells were differentially distributed and most concentrated in the dorsal root ganglia (DRG)> deep cervical lymph node (cLN)> spleen. The majority of SPION+ cells outside the CNS were labeled during early infection. ART decreased the number of SIV-RNA+ cells in the parenchyma [0.06 RNA+ cells/mm2], meninges [0.03 RNA+ cells/mm2], cervical lymph node [0.3 RNA+ cells/mm2], spleen [2.1 RNA+ cells/mm2], and DRG [0.2 RNA+ cells/mm2]. With ART, no SPION+SIV-RNA+ or gp41+ cells were detected inside or outside the CNS; however with ART interruption, SPION+SIV-RNA+ cells were in the meninges [0.05 cells/mm2], deep cLN [0.003 cells/mm2], DRG [0.002 cells/mm2], and spleen [0.05 cells/mm2], but not the parenchyma.

ABSTRACT 7

The Role of CD4+ Naive T Cells in SIV Persistence during Antiretroviral Therapy

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Background: The contribution of CD4+ naïve T cells (TN) to HIV persistence during ART remains unclear. Here we used a rhesus macaques (RM) model of experimental CD4+ TN-deficiency to elucidate the role of the CD4+ TN compartment to SIV rebound dynamics.

Methods: To deplete CD4+ TN, RM received thymectomy (n=9) or sham surgery (n=5) prior to anti-CD4 antibody depletion. After CD4+ T cell reconstitution in blood (~400 days post-depletion), all RM were IV inoculated with 2 infectious units of SIVmac239 before starting ART at 7 days post-infection (dpi). ART was interrupted at 475 dpi to characterize SIV rebound kinetics.

Results: Despite a substantial loss in CD4+ TN in blood and tissues, we observed no difference in plasma viral loads (pvl) or cell-associated viral loads in blood, lymph nodes or bone marrow between CD4+ TNdeficient RM and sham controls during ART. Following ART cessation, 11 of 14 RM rebounded within 60 days, with no difference in time to viral rebound or post-ART pvl set points between CD4+ TN-deficient RM and controls. However, time to SIV rebound did correlate with pre-ART peak pvl. Indeed, 2 CD4+ TNdeficient RM and 1 control RM with no rebound after > 660 days of ART release, had the lowest pvl at time of ART.

Conclusions: These data suggest that CD4+ TN may not play a primary role in driving SIV reservoir persistence during ART or substantially contribute to SIV rebound kinetics following ART cessation. The non-rebounders also confirm previous observations that early reservoirs can be labile.

ABSTRACT 8

Characterization of early cellular targets of infection after an intra-vaginal inoculation of simian immunodeficiency virus into rhesus macaques

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Prevention of HIV acquisition in women requires a substantial increase in our knowledge about early HIV targets and is critical to design effective prevention strategies. Using a rhesus macaque (RM) vaginal challenge model, we identify potential SIV infection sites 48hr after challenge and Th17s as preferred viral targets. Here, we expanded our analysis into later timepoints (72- and 96hr) and analyzed the phenotype and distribution of SIV-infected cells.

8 female RMs were challenged intravaginally with a non-replicative luciferase reporter, LiCH, and SIVmac239 mixture and sacrificed 72-, or 96-hrs post-challenge. Monitoring luciferase signal allowed us to identify female reproductive tract (FRT) regions likely containing infected cells which were further confirmed by immunofluorescence staining of infected cells.

Phenotyping of >10,000 SIV-infected cells demonstrate that Th17s and immature DCs (iDCs) are the primary SIV targets. Comparing the two timepoints main differences were observed in the infection of Th17s and iDCs. A decrease in infection of Th17s were observed from 72- to 96hr (84%;SD$\pm$12% to 62%;SD$\pm$21%), followed by an increase in iDCs infection (10%;SD$\pm$4% to 23%;SD$\pm$10%). We also studied the distribution of infected cells within the tissue using average nearest neighbor distance and Ripley's L. The emerging results suggest that clustering is increased at 96hr, relative to 72hr that might be due to infiltration of susceptible cells into these sites.

Our findings shed light on the earliest steps of mucosal transmission and support previous data demonstrating that entire FRT is susceptible to infection and Th17s are the predominant early targets.

ABSTRACT 9

Bridging anti-lentivirus granulocyte biology across humans and rhesus macaques

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Granulocytes are important innate cytotoxic and regulatory cell subpopulations armed with preformed pools of inflammatory mediators and localized at mucosal portals of entry including the gastrointestinal tract. To better understand their often-overlooked roles in lentiviral-mediated intestinal pathology/immunoprotection, we characterized NHP granulocytes in naïve and SIV/SHIV-infected rhesus macaques. Flow cytometric and imaging data from Imagestream and Chipcytometry analyses confirmed granulocyte phenotypes as CD45+CD66abce+CD14+CD49d- neutrophils, CD45+CD66abce+CD14-CD49d+ eosinophils and CD45+CD66abce-CD123+HLA-DR- basophils based on their surface marker expression, nuclear morphology, and cytoplasmic granularity. Although NHP granulocyte subsets differed from their human counterparts by the absence of CD16 (Fc receptor; FcRgIII) expression, they expressed similar levels of other FcRs including CD32 (FcRgII), CD64 (FcRgI), CD89 (FcRa) and FcRe. Functionally, NHP granulocytes generated reactive oxygen species upon CD32 crosslinking similarly to human granulocytes and formed extracellular DNA traps (NETosis) upon mitogen activation in vitro. Multiplex signaling assays using the Luminex platform showed phosphorylation of important signaling adaptors including Syk, ZAP70, Lck and LAT when human neutrophils were cross-linked with CD32 but not with same concentrations of CD16. These data suggested disparate FcR-mediated functional and signaling modulation. Interestingly, eosinophils which were enriched in naïve jejunum but were depleted in SHIVinfected samples. Furthermore, elevated colorectal and reduced vaginal frequencies of neutrophil and eosinophil subsets were evident in SHIV-infected macaques indicating tissue dependent modulations due to infiltration of granulocytes into the inflamed mucosae. Overall, our study underscores the mucosal significance and potential therapeutic implications of NHP granulocytes, thus warranting further studies utilizing NHP models of AIDS.

ABSTRACTS
ORAL PRESENTATIONS

SCIENTIFIC SESSION 2:
Vaccines, Immunology, and Prevention

Chair: Michael Betts, PhD, University of Pennsylvania
Co Chair: Smita Iyer, UC Davis

Manipulation of T Cell Trafficking in SIV-Infected Macaques to Understand SIV Immunopathogenesis

Presentation by Michael Betts (Speaker)
University of Pennsylvania, Professor of Microbiology

ABSTRACT 10

Cytomegalovirus vaccine vector-induced, unconventionally MHC-restricted effector memory T cells protect cynomolgus macaques from lethal aerosolized H5N1 influenza challenge

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Background: A vaccine for influenza that overcomes viral sequence diversity and provides long-lived heterosubtypic protection is needed to protect against seasonal and pandemic influenza viruses. To circumvent HA and NA sequence variability, we hypothesized lung-resident effector memory T-cells induced by cytomegalovirus (CMV)-vectored vaccines expressing conserved internal influenza antigens would protect against lethal heterologous influenza challenge.

Methods: We constructed a vaccine set of double deleted cynomolgus CMV (ddCyCMV) vectors that are devoid of all identified inhibitors of unconventional T cell priming, and that also express 1918 influenza M, NP, and PB1 antigens (ddCyCMV/influenza), thus creating a vaccine that primes unconventionally MHC-restricted CD8+ T cells targeting influenza internal proteins. Six Mauritian cynomolgus-macaques (MCM) received two subcutaneous doses three months apart. A group of six MCM received no vaccine serving as the control group. Sixteen weeks after final vaccination, macaques were challenged in blinded groups with 5.5 log10 PFU of aerosolized H5N1 (A/Vietnam/1203/2004), then monitored via telemetry, plethysmography, and bronchoalveolar lavage (BAL).

Results: The ddCyCMV/influenza vaccine set induced lung-resident, effector memory influenza-specific CD8+ T-cells that were either MHC-E or MHC-II restricted. Following challenge with aerosolized H5N1 influenza, all six unvaccinated MCM died by seven days post-infection. Vaccination with dd- CyCMV/influenza protected 4/6 MCM from death following aerosolized H5N1 challenge (p=0.04).

Conclusions: These data demonstrate that CMV-induced effector memory T-cells targeting conserved internal influenza proteins can provide protection against highly pathogenic heterologous influenza challenge and establish proof-of-concept for effector memory T-cell-based vaccines in the development of universal influenza vaccines.

ABSTRACT 11

Tailoring helper profile of HIV-1 vaccine induced CD4 T follicular helper cell responses

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Generation of durable antibodies against the HIV-Envelope (Env) glycoprotein hinges on optimal cytokine and co-stimulatory help from CD4 T follicular helper cells (Tfh). We demonstrated that adjuvant-induced stimulation of T helper (h) 1- Tfh cells (Tfh1) enhances magnitude and quality of anti-Env antibody to a Clade C DNA-prime/protein-boost platform relative to one designed to induce Tfh2 responses. Based on the significance of Th17 cells in mucosal immunity, here we asked whether adjuvant driven modulation of cytokines is an effective strategy to elicit Tfh1/Tfh17 germinal center (GC) response. To simultaneously prime and boost Tfh1/Tfh17 cells, we immunized rhesus macaques with a Clade C DNA plasmid molecularly adjuvanted with interferon protein 10 (IP-10) and interleukin 6 (IL-6). Ex vivo characterization of the DNAIP-10/IL-6 plasmid demonstrated expression of trimeric gp160 with production of IP-10 and IL-6 in supernatants. Animals were then boosted with a Clade C gp140 Env protein (Pro) adjuvanted with cationic liposome-based formulation (CAF01) resulting in induction of IP-10 and IL-17 in sera of most animals. We observed robust GC responses with significant induction of GC Tfh cells co-expressing chemokine receptors, CXCR3, and CCR6 exemplifying Th1/Th17 skewing of the GC with the DNAIP-10/IL-6 /ProCAF01 platform. Notably, strong Env-specific Tfh responses were elicited within lymph nodes. These data demonstrate the ability to tailor GC Tfh responses; ongoing studies are underway to determine whether induction of Th1/Th17 GC Tfh cells enhances rectal anti-Env IgA antibodies, and augments functional quality and durability of anti-Env IgG compared to a Th1 vaccine platform.

ABSTRACT 12

Adjuvanted pox-protein vaccination elicits exceptional effector antibody and T cell responses, but does not protect against heterologous SHIV rectal challenge in rhesus macaques

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An effective HIV-1 vaccine remains elusive, though the modestly efficacious RV144 clinical trial highlighted non-neutralizing effector antibody and polyfunctional T cell responses as immune correlates of vaccine-mediated protection. Optimizing vaccine adjuvants to induce desirable immune responses against HIV-1 is an active area of investigation. We previously reported 90% vaccine efficacy against heterologous mucosal SHIV-1157ipd3N4 challenge in macaques using recombinant modified vaccinia virus Ankara encoding HIV-1 gag/pol and env, co-administered with multimeric gp145 protein and next generation liposome-based adjuvant, Army Liposomal Formulation (ALF) adsorbed to aluminum hydroxide (ALFA). To assess protection against SHIV encoding transmitted-founder Env, rhesus macaques were vaccinated with the identical regimen, adjuvanted with either ALFA or the more potent, related ALF with QS-21 (ALFQ) and challenged intrarectally with low-dose SHIV-CH505. Vaccination elicited high-titer Env-specific binding IgG and antibodies with Fc-mediated effector functions (e.g. antibody-dependent cell-mediated phagocytosis (ADCP) and neutrophil phagocytosis (ADNP)), with greater responses in ALFQ-adjuvanted animals. No neutralizing antibody responses were observed. ALFQ adjuvanting also generated remarkable Env-specific memory CD4 Th1 polyfunctional T cells (0.9-5.9%) expressing B cell helper molecules CD40L and IL21, and Env-specific CD8 T cell responses (0.4-3.4%). Despite high magnitude ADCP, ADNP, and T cell responses, vaccination with either adjuvant did not protect against SHIV-CH505 acquisition. Improved early viral control post-infection was associated with ADCP, ADNP and Env-specific CD4 T cell responses, but not CD8 T cell responses. These results support further development and optimization of viral vector and adjuvanted protein vaccine regimens for prophylactic and therapeutic applications targeting robust adaptive immune responses.

ABSTRACT 13

Deep Integration Network Analysis of RhCMV/SIV vaccines links gene expression with IL-15 response and non-conventional antigen presentation for SIV control

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Rhesus cytomegalovirus (RhCMV) vectors derived from the 68-1 CMV strain expressing SIV antigens, serve as potent vaccine vectors for inducing protective immunity against SIV infection in rhesus macaques (RM). Animals that manifest vaccine protection mount a persistent vaccine-induced transcriptomic response in peripheral blood, that displays an IL-15 response signature. Here, we investigate the mechanisms of protection using 6 additional versions of the RhCMV/SIV vaccine.

We performed longitudinal blood transcriptomic profiling of 138 RMs in total. 64 were given the RhCMV/SIV 68-1 vector vaccines, 46 were given 68-1 derived vaccines modified for spread deficiency, and 28 female RMs were given vaccines exclusively using MHC-E antigen presentation. We evaluated the time course of vaccine response to define persistent gene correlates of protection (GCOPs).

68-1 vaccine GCOPs featured an immune effector and chemotaxis signature of T cell and myeloid cells while cell-restricted vaccine GCOPs were associated with tissue inflammation and immune effector functions. MHC-E exclusive vaccines exhibited partial association with both 68-1 and cell-restricted vaccines. We developed Deep Integration Network Analysis (DINA) approach that integrates a large compendium of transcriptomic datasets to define core network genes of vaccine function. A core network of GCOPs from all vaccines included genes encoding BTN3A1 and BTN3A3, each of which can activate non-conventional MHC-I antigen presentation and is expressed in several immune cell types linking with 68-1 based vaccine protection.

BTN3 genes were connected in DINA to IL-15 activated genes, this defining a GCOP network possibly linked with MHC-E restriction and immune programming.

ABSTRACT 14

Single-cell Blood Leukocyte Signature of the Rhesus Cytomegalovirus-based Vector Protection Signature of SIV Vaccination

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Nonhuman primate models of SIV infection are used to evaluate HIV-vaccine candidates. Approximately 50% of rhesus macaques (RM) receiving strain 68-1 rhesus cytomegalovirus (RhCMV)-based vaccine vector expressing SIV antigens can arrest and clear SIV infection in a manner linked with antigen presentation by MHC-E. An interleukin (IL)-15 and immune activation bulk mRNA sequencing transcriptome signature identified in whole blood was linked with RhCMV/SIV vaccine protection. To identify the cells that harbor this protective signature, we performed single-cell sequencing (scRNAseq) on PBMC isolated at baseline and pre-challenge (88 weeks post-prime immunization). Two cohorts of RMs were administered the 68-1 RhCMV/SIV vaccine orally and subcutaneously, groups O and S respectively. A validation cohort (group X) had previously received a modified non-protecting version of the vaccine (68-1.2 vector) received the 68-1 vaccine subcutaneously which also resulted in 50% of animals being protected. Genes in the protective IL-15 signature were primarily expressed in monocytes and NK cells. Differential gene expression (DE) analysis between pre-challenge and baseline time points for protected and non-protected for all cohorts revealed that blood monocytes were the most transcriptionally responsive cell-type to the vaccine, with a distinct set of significant DE genes present in protected animals across the three vaccine groups. These genes were enriched in pathways involved in cell activation and innate immunity within an overall response to IL-15. The 68-1 vaccine platform links MHCE restriction and vaccine immunity with blood monocytes. Monocytes then elicit an IL-15 response that imparts immune programming for vaccine protection.

ABSTRACT 15

Preclinical development of Modified Vaccinia virus Ankara vaccines against SARS-CoV-2 infections

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Background: SARS-CoV-2 vaccines should induce broadly cross-reactive humoral and T cell responses to protect against emerging variants of concern (VOCs). Here, we created a modified vaccinia Ankara (MVA) virus vector containing spike with an inactivated furin cleavage site and nucleocapsid (MVA/SdFCS-N) and investigated immunogenicity and protective efficacy of MVA/SdFCS-N vaccination in rhesus macaques against the Delta SARS-CoV-2 VOC.

Methods: Three groups (n=5/group) of RMs were immunized with MVA/SdFCS-N vaccine on weeks 0 and 4, via intramuscular (IM), needle-free oral (buccal (BU), or Sublingual (SL)) routes. An additional group (control) received non-recombinant MVA (MVA/Empty) via IM. Following vaccination, all RMs were challenged with B.1.617.2 strain (Delta) of SARS-CoV-2 at week 8 via intratracheal and intranasal routes simultaneously.

Results: IM vaccination induced spike-specific IgG in serum and mucosae (nose, throat, lung, and rectum) that neutralized the homologous (WA-1/2020) and heterologous VOCs, including Delta, with minimal loss (<2-fold) of activity. IM vaccination also induced both spike- and nucleocapsid-specific CD4 and CD8 T cell responses in the blood. In contrast, the SL and BU vaccinations induced less spike-specific IgG in secretions and lower levels of polyfunctional IgG in serum compared with IM vaccination. After challenge, the IM route induced robust protection, the BU route induced moderate protection, and the SL route induced no protection.

Conclusion: Vaccine-induced neutralizing and non-neutralizing antibody effector functions positively correlated with protection, but only the effector functions correlated with early protection. Together, we have created a SARS-CoV-2 vaccine vector that can protect against VOCs.

ABSTRACT 16

Single cell repertoire analyses of blood and bone marrow antibody secreting cells reveals superior ability of 3M-052-alum adjuvant in comparison with alum to promote HIV-1 envelope specific long-lived immunity

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Background: A significant challenge in vaccinology is to promote long-lived vaccine induced immune responses. Live vaccines such as against small pox and measles highlight that the induction of long-lived plasma cells (LLPCs) is critical in maintaining a durable antibody (Ab) response. In contrast, potent adjuvants are needed to improve the magnitude and durability of Ab responses against protein vaccines. Here, we investigated and compared innate, T and B cellular as well as Ab responses induced by 3M-052- alum, a TLR-7/8 targeted and alum alone adjuvanted HIV-1 derived Env native trimer-based vaccine in rhesus macaques.

Methods: We used a combination of techniques such as multi-parameter flow cytometry, bulk and single RNA-sequencing as well as Ab repertoire analyses, ELISpot, EMPEM (epitope mapping) and pseudo-virus neutralizing activity against HIV-1.

Results: 3M-052-alum in comparison with alum induces; a) qualitatively distinct vaccine enriched blood antibody secreting cells (ASCs), b) significantly higher clonal overlap at early (4-6 weeks) and late (up to 2 years) time points between blood and bone marrow ASCs, c) enhanced epitope recognition on Env antigens (by EMPEM) and, e) striking durability (up to 2 years) of HIV-1 neutralizing activity and LLPCs in the bone marrow.

Conclusions: We highlight that the 3M-052-alum adjuvant offers significant promise in enhancing the magnitude, breadth and durability of HIV-1 env specific immunity in comparison with alum. We also conclude that transcriptional and Ab repertoire analyses of vaccine induced B cell responses can further support the identification

ABSTRACT 17

Cytomegalovirus infection alters the transcriptomic response to RhCMV/SIV vaccination

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Rhesus cytomegalovirus (RhCMV) -vectored vaccines against simian immunodeficiency virus (SIV) have been shown to provide impressive protection against repeated low-dose challenges. Because 50-90% of adult humans are CMV infected, the protective mechanisms used by the vaccines may be complicated by the immunologic impact of wild-type CMV. We and others previously showed that RhCMV infections cause transformative immune changes. Such changes, including expansion of a subset of innatememory CD8+ T cells, may cooperate with adaptive immune responses to protect against HIV/SIV. On the other hand, wild-type CMV infection has previously been associated with weaker antibody responses against influenza A vaccination.

To uncover effects of pre-existing CMV infection on vaccine efficacy, we performed transcriptomic analysis of PBMC and gut biopsy samples from RhCMV-seronegative and -seropositive animals before and after RhCMV/SIV vaccination. We found that wildtype RhCMV infection was associated with downregulated translational pathways and upregulated cell-cycle and T-cell-receptor pathways. Vaccination with RhCMV/SIV induced similar changes to wildtype RhCMV infection, while additionally upregulating interferon signaling pathways. Interestingly, pathways related to antigen processing were upregulated only in RhCMV-naïve animals after vaccination, while innate-immune pathways were downregulated only in RhCMV+ animals after vaccination. Thus, prior RhCMV infection alters the host's immune response to RhCMV-based vaccination.

ABSTRACTS
ORAL PRESENTATIONS

SPECIAL SESSION
Animal Management

Chair: Joyce Cohen, VMD, DACLAM
Emory National Primate Research Center

The Value of Animal Management in Supporting Research

ABSTRACT 18

Refinements to Nursery Practices for Infant Rhesus Macaques

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Background: The past decade has seen an increase in the number of SIV/SHIV studies utilizing infant macaques to model HIV vertical transmission and cure research. Such studies often rely on nurseryrearing subjects, and require daily administration of ART. We have refined several nursery procedures in an effort to provide a more ethologically relevant environment, thereby promoting welfare and scientific rigor. A primary focus of these refinements is increasing socialization and positive reinforcement training for the infants, known to be critical for psychological health.

Methods: We designed specialized infant cages that allow infant rhesus macaques to be housed in small groups (e.g., 4-6 individuals) while still allowing temporary separations for feedings or research-related procedures. These provide both vertical and horizontal access for the infants, and allow the use of swings and manipulatable objects. Another important refinement is increased acclimation and use of an "infant procedure cage." This cage was designed to gently hold infants undergoing daily ART injections without sedation. Infants are trained to enter the procedure cage, which is temporarily attached to the front of the cage.

Results: We have used the specialized caging with over 40 rhesus macaque infants, and have not seen any evidence of viral transmission. Once inside the procedure cage, infants hold onto a fleece-covered bar while remaining prone. Most infants readily adapt to this procedure cage and remain relatively calm for the injections without sedation or additional restraint.

Conclusions: These refinements have allowed improvements in animal welfare while supporting scientific outcomes.

ABSTRACT 19

Survey of Macaque and Baboon Samples Shows No Human Coronavirus Infections and Low Prevalence of Other Common Respiratory Pathogens

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Introduction: Surveys for respiratory pathogens were conducted using samples from macaques and baboons from several US facilities, including some from recent international sources, chimp health screening samples and human blood bank samples. Evidence of exposure to these viruses might be expected to be seen in nonhuman primate (NHP) populations due to high prevalence in humans (>90% for some pathogens), the high transmissibility of respiratory pathogens and close interactions between humans and NHPs in their natural settings and in animal facilities over many years.

Methods: Serological and/or PCR surveillance testing of over 1,500 macaques and 60 baboons housed in US primate facilities from 2018 – 2022 were conducted using multiple assay platforms including Luminex, MSD, ELISA and immunoblot and direct virus tests using traditional PCR and Biofire PCR.

Results: There is a lack of antibody and PCR positivity for common-cold coronavirus pathogens in macaques and baboons, yet some common human pathogens such as adenovirus and rhinovirus are commonly present (42% and 63%, respectively) in macaques year-round. In opposition to macaques, chimps in contact with humans show high prevalence of exposure to seasonal HCoV (not SARS).

Conclusions: NHPs in contact with humans show evidence of infection from common human respiratory pathogens such as adenovirus and rhinovirus/enterovirus. However, some common human respiratory pathogens such as seasonal coronaviruses, do not show evidence of exposure in macaques or baboons but do show evidence of exposure in chimps. These results point out species specificity of some pathogens and broader host susceptibility for other pathogens.

ABSTRACTS
ORAL PRESENTATIONS

SCIENTIFIC SESSION 3:
Progress Toward HIV Cure

Chair: Katherine Bar, MD, University of Pennsylvania
Co Chair: Afam A. Okoye, PhD,
Oregon National Primate Research Center

Advances in HIV-1 Cure via NHP models: Transmitted Founder SHIVs for Persistence

Presentation by Katharine Bar (Speaker)
University of Pennsylvania, Assistant Professor of Medicine

ABSTRACT 20

Precursor Effector TCF1+ CD39+ Tox+ CD8 T Cells Emerge in Lymph Node After SIV Infection and Are Associated with Better Viral Control

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CD8 T-cell responses to HIV/SIV infection critically contribute to control of viremia, but lymph node (LN) CD8 have been shown to be phenotypically and functionally distinct from those in blood. To further understand the unique populations in LN, we explored during early chronic SIV infection (D42 postinfection) dynamics of LN CD8 T expressing Tox, TCF1 and CD39, 3 well-described markers delineating exhausted, stem-like and terminally-differentiated populations. Tox is upregulated after SIV infection ($p=0.002$) and primarily expressed in PD-1+ and TIGIT+ cells. Notably, we observed high levels of Tox on a previously undescribed TCF1+CD39+ population that significantly expands after infection, distinct from the known TCF1+CD39- stem-like and TCF1-CD39+ effector/exhausted CD8. These TCF1+CD39+ cells expressed inhibitory receptors, intermediate levels of Ki-67, and are uniquely high in GzmK but low in GzmB, a profile consistent with a precursor effector cell. After SIV peptide stimulation, TCF1+CD39+ cells degranulated at similar levels to TCF1-CD39+ cells but did not produce as much IFNy. scRNA-seq revealed an intermediate profile of TCF1+CD39+ cells between the stem-like TCF1-CD39+ cells and the differentiated TCF1+CD39- cells. Importantly, a higher frequency of both Tox+ ($p<0.0001$) and TCF1+CD39+ ($p=0.0003$) CD8 in LN at d42 p.i. was strongly associated with lower plasma viremia, lower levels of cell-associated SIV DNA (Tox $p=0.0007$; TCF1+CD39+ $p=0.01$) and better CD4 preservation (Tox $p=0.02$; TCF1+CD39+ $p=0.065$). Overall, these data are consistent with a unique precursor effector CD8 T-cell population that expands in LN after SIV infection and is associated with increased viral control and reduced disease progression.

ABSTRACT 21

Massive viral RNA burden in SIV-infected CD4+ T-cells coincides with latency transcription factor downregulation

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Persistent HIV-1/SIV reservoirs of infected CD4+ T-cells able to support viral replication upon antiretroviral therapy interruption are the primary barrier to HIV-1 cure and yet remain poorly characterized due to their rarity and lack of unique biomarkers. To study properties of cells infected in vivo and identify factors influencing viral replication fate, we isolated memory CD4+ T-cells from either lymph node (N=727 cells) or PBMC (N=923 cells) of two acutely SIV-infected rhesus macaques by flow cytometry followed by single-cell RNA sequencing. Infected cells, defined as harboring SIV reads, were classified with respect to viral life cycle stage as follows: 1) replication-active: expressing viral RNA (vRNA) encoding multiply- and singly-spliced genes; and 2) replication-inactive: lacking spliced vRNA and <1% SIV reads, suggesting a quiescent state. The median vRNA content for replication-active cells was 10.3% (IQR 6.3-15.4%) and 7.5% (IQR 3.8-10.6%) of the transcriptome in the animals, respectively. Cell surface CD3 and CD4 were also diminished, consistent with viral protein expression. Host genes differentially expressed in replication-active cells relative to vRNA-negative and replication-inactive cells included downregulation of FOS, a transcription factor previously identified as mediating HIV-1 latency in vitro. Three abundant T cell clones shared across vRNA-negative and -positive CD4+ T-cells suggests that expanded clones present during acute infection are independent of cellular infection status. These findings highlight remarkable viral transcription among infected cells, clonal proliferation during the earliest stages of host infection, and potential mechanisms regulating viral latency in vivo that may be harnessed for viral reactivation in HIV-1 therapeutic strategies.

ABSTRACT 22

RNAscope Characterization of the CNS SIV Reservoir Reveals Elevated Infected Astrocytes Following cART Cessation

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Background

While advances in combined antiretroviral therapy (cART) have prolonged the lives of people living with HIV (PLWH), HIV reservoirs lead to viral rebound following cART cessation, posing a challenge for developing a functional cure. The central nervous system (CNS) has been identified as a reservoir site and compartmentalizes HIV within the brain. We hypothesize that following cART cessation, there will be a distinct cell type that emerges in the CNS harboring rebounding virus.

Methods

Utilizing rhesus macaques intravenously infected with SIVmac239, we devised a method to identify SIV infected cells in AIDS and two-weeks post-cART cessation tissues, utilizing uninfected tissues as controls. Through a combination of traditional fluorescence microscopy and RNAscope, we have quantified cell phenotypes including astrocytes (GFAP), macrophages (CD68/163/206), and microglia (Iba1) in brain regions including frontal lobe, midbrain, and hindbrain, harboring viral RNA.

Results

We found that fluorescent RNAscope detection of infected cells can be quantified by a halo of RNA signal surrounding the nucleus. While microglia and macrophages exhibited the highest levels of infection in AIDS as expected from the literature, astrocytes exhibited elevated levels of infection during cART cessation, and expressed increased GFAP when closely associated with infected macrophages/microglia.

Conclusion

These findings, especially the discovery of differential cell types containing rebounding virus, have implications for therapies that can block viral egress from the CNS. We are currently measuring cART penetrance in these tissues to correlate with the amounts of RNAscope

ABSTRACT 23

LAIR-1 is a negative regulator of NK cells during chronic SIV infection

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Leukocyte-associated Ig-like receptor-1 (LAIR-1) is a surface molecule expressed on human peripheral blood mononuclear leukocytes that function as an inhibitory receptor on human NK cells. However, the role of LAIR-1 on NK cells during SIV/HIV infection remains to be fully elucidated.

A total of 13 macaques were infected with SIVmac251 and studied for the dynamics of LAIR-1+ NK cells during acute and chronic infection using flow cytometry. We performed immunohistochemistry to stain for LAIR-1 in Lymph nodes and used RNA sequencing to study the transcriptomic profile.

Following acute SIV, most (>80%) of blood NK cells expressed LAIR-1, and the level of LAIR-1 per cell increased during chronic infection. The distribution of LAIR-1 during SIV infection displays higher expression on CD16+ NK cells than on CD56+ NK cells. Using IHC, we found that the level of LAIR-1 expression per cell in LN increased during chronic SIV infection. Transcriptionally, the blood LAIR-1+ NK cells exhibited a reduced expression of IL-2, STAT-6, and NFKB signaling genes during the chronic phase. Furthermore, the accumulation of LAIR-1+ NK cells during SIV infection showed a strong positive association with plasma viral RNA levels. Indeed, In vitro blockade of LAIR-1 using LAIR-1-Fc/anti-LAIR-1 antibody resulted in enhanced proliferation of NK cells with cytotoxic capacity (CD107a, cytokine production). These results serve as a foundation for future in vivo trials of the use of rMamu-LAIR-1 to enhance and/or restore antiviral immune responses in vivo, especially in secondary lymphoid tissues, which may be important for the HIV cure strategy.

ABSTRACT 24

Blockade of TGF-ß signaling reactivates HIV-1/SIV reservoirs and immune responses *in vivo*

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Elevated levels of TGF-ß, a potent immunosuppressive factor, are present in HIV-1 infected individuals even after years of antiretroviral therapy (ART). TGF-ß plays a critical role in maintaining immune cells in a resting state by inhibiting cell activation and proliferation. Resting HIV-1 target cells represent one of the main cellular reservoirs after long term ART. We hypothesized that releasing cells from TGF-ß-driven signaling would promote latency reversal. To test our hypothesis, we compared ex-vivo models of HIV-1 latency reactivation with and without TGF-ß and a TGF-ß type 1 receptor (TGFßR1) inhibitor, galunisertib. We tested the effect of galunisertib in SIV-infected, ART-treated macaques by monitoring SIV envelope (env) expression via PET/CT using the Cu64-anti-gp120 Fab (7D3) probe, along with plasma and tissue viral loads (VL). Exogenous TGF-1ß reduced HIV-1 reactivation in U1 and ACH2 latency models. Galunisertib increased HIV-1 latency reversal both ex vivo and in PBMC from HIV-1 infected, cART treated aviremic donors. In vivo, oral galunisertib promoted increased SIV env protein total standardized uptake values (SUVtot) in PET/CT images of tissues (gut and lymph nodes) of 5 out of 7 aviremic, long-term ARTtreated, SIV-infected, macaques. This increase correlated with an increase in SIV RNA in gut tissue. Two out of 7 animals also exhibited increases in plasma viral load. Higher anti-SIV T cell responses and anti-SIV env antibody titers were detected after galunisertib treatment in most animals. In summary, our data suggest that blocking TGF-ß signaling simultaneously increases retroviral reactivation events and enhances anti-SIV immune responses.

ABSTRACT 25

Combination Of PD-1 Blockade And AZD5582 Therapy In SIV+ Monkeys

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Latency reversal and restoring T cell function are the two major barriers to HIV cure. PD-1 blockade has not only been shown to potentiate HIV/SIV latency reversal but also to reverse immune exhaustion, improve antiviral immunity and reduce viral reservoirs. The objective of this study was to combine PD-1 blockade with AZD5582, a potent latency reversal agent, as a "shock and kill" approach and study safety, virological and immunological effects in SIV infected and ART suppressed rhesus macaques (RMs).

A total of 9 RMs, chronically infected with SIVmac239, received five weekly infusions of AZD5582 under ART. Of these, four RMs received two primatized anti-PD-1 antibody infusions at 1st and 4th cycle of AZD5582 infusion.

Strong latency reversal and instances of sustained viremia were observed in both the groups with no significant difference in events of viral rebound between the groups. Interestingly, transcriptomics and phenotypic data revealed that combination of PD-1 blockade and AZD5582 but not AZD5582 alone, showed a robust increase in proliferating total and memory CD4 and CD8 T cells with each AZD5582 administration in PBMCs. Surprisingly, there was no significant change in the antigen specific T cell response. AZD5582 treatment decreased cell associated RNA copies in PBMCs and addition of PD-1 blockade didn't reduce it further.

In summary, combined treatment is safe and effective in inducing latency reversal. Despite no additive advantage on reservoir size, the combined treatment changed the landscape of T cell responses in blood and tissues.

ABSTRACT 26

Impact of the IL-15 Superagonist N-803 on Latency Reversal Induced by AZD5582 and on Immune Cells in ART-Suppressed Rhesus Macaques

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Background
We have shown systemic SIV latency reversal in rhesus macaques (RMs) using the SMACm/cIAP inhibitor AZD5582. Here, we evaluated the impact of the IL-15 superagonist N-803 on latency reversal activity of AZD5582 and immune cells in SIV-infected, ART-suppressed RMs treated with SIV Env-specific rhesus monoclonal antibodies (RhmAbs) to enhance immune-mediated clearance of infected, reactivated cells.

Methods
9 SIV-infected RMs received 0.1mg/kg AZD5582 i.v. wkly for 10wks. Another group of 9 RMs also received 100mg/kg N-803 s.c. twice (pre-dose1 and pre-dose6 AZD5582). Latency reversal was assessed by plasma SIV-RNA-PCR. Immune cells were monitored longitudinally by CBC and flow cytometry. Antibody-dependent neutrophil phagocytosis (ADNP) was measured ±N-803 in vitro.

Results
78% AZD5582-treated and 100% AZD5582+N-803-treated RMs experienced on-ART viremia. Absolute lymphocyte counts increased after each N-803 dose, with pronounced expansion of CD8+ T-cells and NKcells after N-803 dose1. By contrast, absolute lymphocyte, CD4+ and CD8+ T-cell counts declined after 1-2 doses of AZD5582 without N-803. Ki67 in CD4+ and CD8+ T-cells increased after 1-2 doses of AZD5582 in both groups, but the increase was less marked in CD4+ T-cells in RMs exposed to N-803. A sharp decline in circulating neutrophils was found following both N-803 doses, although neutrophil viability was unaffected by culture with N-803. Interestingly, N-803 enhanced ADNP mediated by SIV Env-RhmAbs (p=0.01).

Conclusions
Our findings support a role for N-803 in enhancing latency reversal induced by AZD5582. The complex interactions between N-803 and AZD5582 in vivo were revealed by longitudinal tracking of immune cell numbers and function.

ABSTRACT 27

Passive transfer of SIV Env-specific rhesus mAbs to rhesus macaques: a robust model for ATI/functional cure studies

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4 AIDS and Cancer Virus Program, Frederick National Laboratory, Frederick, MD, USA.

Antiretroviral therapy (ART) limits seeding of viral reservoirs when administered shortly after infection but fails to eradicate the virus as viral rebound is typically observed upon analytical treatment interruption (ATI). SIV-infected rhesus macaques are a robust preclinical model with consistent viral replication that is suppressible with ART, enabling evaluation of HIV-1 cure strategies in a manner potentially more stringent than SHIV-based models. We tested anti-SIV neutralizing mAbs in 21 rhesus macaques infected with SIVmac251 with ART initiated 7 days post infection. Animals received 8 doses of anti-SIV mAbs administered biweekly (weeks 15-29) spanning ATI (week 19) in 3 treatment groups: i) no mAb; ii) 4-mAb combination; iii) 2-mAb combination; where both mAb groups included at least 1 CD4bs bNAb. Compared to controls, mAb recipients had delayed viral rebound, lower setpoint viremia, fewer viral lineages at rebound, and extended lifespan. Rebound virus in all mAb-treated animals exhibited escape mutations, equivalent to VRC01 escape mutations in humans, at one of two glycan sites known to reduce neutralization by CD4bs mAbs, underscoring the relevance of this model for HIV. Intriguingly, three NHPs in the 2-mAb group displayed delayed rebound at 14 to 22 weeks post-ATI, while all NHPs in the 4-mAb group rebounded by 6 weeks post-ATI. 2 mAb recipients had significantly greater antigenspecific Tfh cells than control animals 20 weeks post-rebound. Our data in a robust preclinical model demonstrate the clear anti-viral effect of potent anti-SIV mAbs in the context of ATI and highlight their potential use in adjunctive therapeutic studies.

ABSTRACT 28

Targeting the SIV Reservoir with Alemtuzumab (anti-CD52)

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Background
Alemtuzumab (AZM) is a licensed pan-lymphocyte-depleting monoclonal antibody (mAb) that targets CD52+ cells. Here we administered AZM to SIV-infected rhesus macaques (RM) at the time of ART initiation (early) or during full ART suppression (late) to assess its ability to deplete latent, SIVinfected cells.

Methods
RM were initially screened for CD52 expression on erythrocytes and then IV inoculated with 500 infectious units of the barcoded SIVmac239M. ART was initiated 7 days post-infection (dpi). RM also received 4 weekly doses of AZM or control mAb at 5mg/kg starting 7 dpi (early; n=8 AZM, n=6 control) or 294 dpi (late; n=8 AZM, n=4 control). ART was discontinued at 533 dpi to assess SIV rebound dynamics.

Results
AZM induced massive lymphocyte depletion, including >95% of CD4+ T cells in blood. CD4+ T cell depletion was also observed in the lymph nodes. Depletion was followed by CD4+ memory T cell proliferation and the reconstitution of cells in blood. Following ART cessation, 24 of 26 RM rebounded, with no difference in time to rebound or rate of reactivation of SIVmac239 barcodes between AZMtreated RM and controls. However, time to rebound did correlate with pre-ART peak pvl. Interestingly, 2 early AZM-treated RM with lowest pvl at time of ART showed no rebound >400 days post-ART release.

Conclusions
Alemtuzumab depletion was not sufficient to delay or prevent SIV rebound, in most RM. However, the lack of rebound in 2 of 8 early AZM-treated RM suggests the reservoir may be more liable at time of ART initiation.RM. However, the lack of rebound in 2 of 8 early AZM-treated RM suggests the reservoir may be more liable at time of ART initiation.

ABSTRACT 29

Constitutive NKG2A levels and timing of antiretroviral therapy initiation impact the potential role of NK cells after treatment interruption -the pVISCONTI study

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The pVISCONTI study aims to assess the mechanisms leading to post antiretroviral treatment (ART) control in a cynomolgus macaque (CyM) model of SIVmac251 infection. We found that 24-months ART initiated at day 28 post infection (p.i.) favored a delayed viral rebound after treatment interruption (TI) and a high frequency of post-treatment controllers when compared to ART initiated at 6 months p.i. We evaluated the impact of time to ART initiation on NK cell dynamics, and their potential role on post treatment SIV control, in 12 early-treated and 12 late-treated CyMs. We analyzed phenotypically (flow cytometry) NK cells from blood and tissues (Lymph Nodes (LN), Bone Marrow (BM) and Broncho Alveolar Lavage (BAL)) at various timepoints before ART and following TI. We found that NK cells from CyMs constitutively expressed differential levels of NKG2A. The presence of NK cells with high expression of NKG2A before infection was significantly correlated with a lower viral load during primary-infection and at d3 and d7 following TI. In addition, the timing of the treatment initiation had a strong impact on the phenotype of the NK cells following TI. In particular, late treated animals showed a higher frequency of NK cells expressing the activating markers NKp46 and NKp30 from d3 after TI in the blood and lymph nodes. We propose that the constitutive presence of NKG2Ahi NK cells may impact SIV viremia during primary-infection and prepare for better SIV control, whereas the timing of ART impact the distribution of the NK cells subsets after TI.

ABSTRACTS
ORAL PRESENTATIONS

SCIENTIFIC SESSION 4:
COVID and Other Infectious Diseases

Chair: Robert Seder, PhD, National Institutes of Health
Co Chair: Mirko Paiardini, PhD, Emory
National Primate Research Center

Mechanisms of mRNA Vaccine Immunity and Protection in NHP: The scientific rationale for boosting against variants and do we need intranasal vaccines to prevent infection and transmission

Presentation by Dr. Robert Seder (Speaker)
National Institutes of Health,
Chief, Cellular Immunology Section

ABSTRACT 30

Modulation of Type I Interferon Responses Inhibits SARS-CoV-2 Replication and Inflammation in Rhesus Macaques

Elise G Viox[1], Timothy N Hoang[1], Amit A Upadhyay[1], Zachary Strongin[1], Gregory K Tharp[1], Maria Pino[1], Rayhane Nchioua[2], Maximilian Hirschenberger[2], Matthew Gagne[3], Kevin Nguyen[1], Justin L Harper[1], Jacob Estes[4], Thomas H Vanderford[1], Monica Vaccari[5], Daniel C Douek[3], Konstantin Sparrer[2], Frank Kirchhoff[2], R. Paul Johnson[1], Gideon Schreiber[6], Steven E Bosinger[1], Mirko Paiardini[1]

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The mechanisms by which SARS-CoV-2 drives inflammation have been linked to type I interferons (IFN-I), which, although essential for defense against viral infections, can increase disease severity if left unchecked. Here, we used IFN modulator (IFNmod), a mutated IFNα2 previously demonstrated to block binding of endogenous IFN-I, to characterize the role of IFN-I in SARS-CoV-2 infection.

The impact of IFNmod on interferon stimulated genes (ISGs) in uninfected rhesus macaques (RMs) and SARS-CoV-2 replication in Calu-3 cells was first examined. To determine the effect of IFNmod on SARSCoV- 2 replication and pathogenesis in vivo, 18 RMs (9 untreated and 9 IFNmod-treated from -1 to 2 days post infection (dpi)) were inoculated with SARS-CoV-2 WA1/2020. Blood, nasal and throat swabs, and BAL were collected longitudinally for viral load, Mesoscale immunoassay, flow cytometry, and RNAseq analysis. RMs were euthanized at 2, 4, or 7dpi.

IFNmod resulted in low-level stimulation of antiviral genes without induction of inflammatory genes in uninfected RMs and inhibited SARS-CoV-2 replication in Calu-3 cells. Administration of IFNmod to SARSCoV- 2-infected RMs reduced: i) viral loads in BAL (>3 log reduction) and swabs during treatment, ii) lung pathology, iii) inflammatory cytokines and chemokines in BAL, iv) expansion of inflammatory monocytes and v) antiviral and inflammatory ISGs.

IFNmod administration in SARS-CoV-2-infected RMs resulted in early control of viremia while also dampening endogenous IFN-I signaling and limiting immunopathology. These data are consistent with a model where an early and controlled IFN-I response is beneficial following SARS-CoV-2 infection, whereas a sustained response leads to detrimental effects.

ABSTRACT 31

Induction of adaptive MHC-E restricted lung tissue-resident NK cells associated with persistent low antigen load in alveolar macrophages after SARS-CoV-2 infection

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Natural killer (NK) cells are innate lymphocytes with potent activity against a wide range of viruses. In SARS-CoV-2 infection, NK cell activity might be of particular importance within lung tissues. Here, we investigated whether NK cells with activity against Spike+ cells are induced during SARS-CoV-2 infection and have a role in modulating viral persistence beyond primary clearance from nasopharyngeal and tracheal tissues. We performed an integrated analysis of NK cells and macrophages in blood and bronchoalveolar lavage fluids (BALF) of COVID-19 convalescent non-human primates in comparison to uninfected control animals. SARS-CoV-2 protein expression was detected for at least 9-18 months postinfection in alveolar macrophages. Convalescent animals segregated into two groups based on cellular phenotypes and viral persistence profiles in BALF. The animals with lower persistent antigen displayed macrophages with a regulatory phenotype and enhanced MHC-E restricted NK cell activity toward cells presenting peptides derived from the SARS-CoV-2 Spike protein leader sequence, while NK cell activity from the other convalescent animals, control animals and healthy humans were strongly inhibited by these Spike peptides. The adaptive NK cell activity was not detected in blood but in tissue-resident NK cells, and cross-reacted against MERS-CoV and SARS-CoV Spike-derived peptides.

ABSTRACT 32

A self-amplifying Replicon RNA COVID-19 vaccine induces durable protection from SARS-CoV-2 in pigtail macaques and protects even after neutralizing antibodies have waned to undetectable levels

Megan A O'Connor[1,2], David Hawman[3], Kimberly Meade-White[3], Shanna Leventha[3], Wenjun Song[4], Samantha Randall[1], Jacob Archer[1,5], Thomas B Lewis[1,2], Brieann M Brown[1,2], Naoto Iwayama[2], Chul Ahrens[2], William Garrison[2], Solomon Wangari[2], Kathryn A Guerriero[2], Patrick W Hanley[3], Jamie Lovaglio[3], Greg Saturday[3], Paul T Edlefsen[4], Amit P Khandhar[5], Heinrich Feldmann[3], Deborah H Fuller[1,2], Jesse H Erasmus[5,1]

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5 HDT Bio, Seattle, WA, USA.

Background
SARS-CoV-2 variants of concern, waning immunity, evidence that vaccines may not prevent transmission and inequitable vaccine distribution have driven continued development of vaccines against SARS-CoV-2 to address these public health needs.

Methods
Eight pigtail macaques were vaccinated with two doses (5-50µg), spaced 4-12 weeks apart, of a novel self-amplifying SARS-CoV-2 replicon RNA vaccine (RepRNA-CoV2S) encoding the Spike protein. Animals were challenged with SARS-CoV-2 5-30 weeks following the final immunization and disease and viral outcomes were compared to six unvaccinated controls

Results
All vaccine doses/intervals induced robust immunity but were the strongest in doses >25µg. At the time of challenge, serum neutralizing antibody (nAb) responses were detected in the groups boosted <11 weeks prior but were undetectable in animals boosted 30 weeks prior. Compared to unvaccinated animals, all vaccinated animals were protected from SARS-CoV-2, as evident by reduced viral replication and disease in the lower respiratory tract, reduced viral shedding in the nasal cavity and lower concentrations of pro-inflammatory cytokines in the lung. In animals with undetectable nAb at the time of challenge, a rapid recall antibody response corresponded with suppression of viral replication and protection from disease. Among all animals, binding antibody significantly correlated with lower viremia in the lung.

Conclusion
Cumulatively, our data in pigtail macaques demonstrate that a self-amplifying RepRNA vaccine can elicit durable and protective immunity to SARS-CoV-2 infection. Furthermore, these data provide evidence that self-amplifying RepRNA vaccines can provide durable protective efficacy and reduce viral shedding even after nAb responses wane to undetectable levels.

ABSTRACT 32

A self-amplifying Replicon RNA COVID-19 vaccine induces durable protection from SARS-CoV-2 in pigtail macaques and protects even after neutralizing antibodies have waned to undetectable levels

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Conclusion
Cumulatively, our data in pigtail macaques demonstrate that a self-amplifying RepRNA vaccine can elicit durable and protective immunity to SARS-CoV-2 infection. Furthermore, these data provide evidence that self-amplifying RepRNA vaccines can provide durable protective efficacy and reduce viral shedding even after nAb responses wane to undetectable levels.

ABSTRACT 33

Reduced COVID vaccine responses in CMV seropositive rhesus macaques associated with decreased ribosomal protein gene transcription of memory B cells

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CMV is a highly prevalent herpesvirus that establishes life-long persistent infections in ~50% of young adults and >90% of elderly people in developed countries. In developing countries, CMV seroprevalence is even higher among young adults (80-90%). Increasing evidence suggests that CMV plays a significant role in immunosenescence and reduced vaccine responses in elderly populations via memory inflation, a gradual accumulation of highly differentiated effector memory T cells. CMV infection also mobilizes a large pool of memory B cells with an effector phenotype and a higher level of intrinsic inflammation. Using the nonhuman primate model, we have previously shown that CMV+ animals manifest lower antibody responses to influenza vaccination. In this study, we observed eight-fold higher RBD-binding antibody titers (both peak and longitudinal) in CMV-naïve macaques boosted with an adenovirus-26 based COVID vaccine, in comparison to CMV+ macaques. To further investigate the impact of subclinical CMV infection on B-cell biology and vaccine efficacy, we performed transcriptomic analysis of FACSsorted class-switched memory B cells from four naïve and seven experimentally infected (10-11 weeks post seroconversion) animals. While primary CMV infection triggered sustained expansion of activated memory B cells, we found evidence that the protein synthesis machinery of these B cells was severely impacted. Specifically, ribosomal protein genes were significantly downregulated and genes involved in spliceosome formation were upregulated. The expression of transcription factors remained unchanged. This association between CMV-induced changes to the B-cell repertoire and suppressed COVID vaccine responses could have implications for vaccine strategies in CMV-seropositive individuals, including most older adults.

ABSTRACT 34

TREM2+ and interstitial-like macrophages orchestrate airway inflammation in SARS-CoV-2 infection in rhesus macaques

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The continued surges of COVID-19 due to mutating variants highlight the importance of understanding the immunopathology of SARS-CoV2 infection. Here, we utilize a rhesus macaque (RM) model of SARSCoV- 2 infection to delineate perturbations in the innate immune system during acute infection.

We used high dimensional flow cytometry, multi-analyte cytokine detection, and bulk and single-cell RNA-Seq (scRNA-Seq) to investigate the early events of SARS-CoV-2 USA-WA1/2020 infection in the blood and lower airway of RMs. We used two different strategies employing single and bulk RNA-Seq references, to classify the macrophage/monocyte populations and determined analogous populations in SARS-CoV2 infected human airway samples.

At two days post-infection, we observed a rapid infiltration of plasmacytoid dendritic cells into the lower airway, natural killer cell activation, induction of interferon-stimulated genes, and a significant increase of blood CD14-CD16+ monocytes. There was rapid recruitment of CD163+MRC1+TREM2+ and CD163+MRC1- macrophages that resemble the monocyte-derived and interstitial macrophages at four days post-infection and these were the predominant source of inflammatory cytokine expression. These populations were analogous to the SPP1hi and FCN1hi macrophages that were described in SARS-CoV2 infected human BAL where they were found to increase with disease severity. Treatment of SARS-CoV-2 infected RMs with baricitinib (Olumiant®), a novel JAK1/2 inhibitor, was effective in eliminating the influx of infiltrating macrophages.

Collectively, this study defines the early kinetics of pDC recruitment and Type I IFN responses and identifies discrete subsets of infiltrating macrophages as the predominant source of pro-inflammatory cytokine production in SARS-CoV-2 infection.

ABSTRACT 35

Pulmonary RhCMV Reactivation Following SARS-CoV-2 In Aged Rhesus Macaques: Implications for Immunopathology

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Background
Aged individuals with co-morbidities are most vulnerable to SARS-CoV-2 immunopathology, however the mechanisms driving aberrant immune responses in this high-risk population are not fully elucidated. Cytomegalovirus (CMV) is highly prevalent in humans and CMV seropositivity was reported to be associated with increased hospitalization among people with SARS-CoV-2 infection. We hypothesized that CMV reactivation exacerbates SARS-CoV-2 pathogenesis since reactivation is both a cause and consequence of inflammation. To address this hypothesis, we utilized the naturally infected rhesus macaque model of RhCMV to investigate reactivation in SARS-CoV-2 infection in sites of SARS-CoV-2 pathology.

Methods
To assess RhCMV reactivation, eight aged, type 2 diabetic RhCMVseropositive rhesus macaques were infected with SARS-CoV-2 and monitored for 7 days then euthanatized. Tissues were collected to assess viral and inflammatory dynamics. Results: SARS-CoV-2 replication was observed throughout the respiratory tract, which associated with local and systemic inflammation. Lung histopathological assessments revealed development of interstitial pneumonia with colocalization of SARS nucleocapsid protein within pneumocytes. Assays targeting RhCMV gB and IE1 showed CMV DNA within the caudal lung lobe, ileum, and brain. The animal with the highest CMV DNA presented with the most profound clinical symptoms, without any detectable spillover into plasma. CMV DNA copies strongly correlated with T cell activation indices in blood and spleen.

Conclusion
SARS-CoV-2 infection of RhCMV-seropositive macaques results in CMV reactivation in the anatomic sites where SARS-CoV-2 causes pathology below detection in plasma. Future experimental studies should address

ABSTRACT 36

The presence of indoleamine 2,3-dioxygenase in an aerosol-mediated infant rhesus macaque tuberculosis model

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Background
Tuberculosis (TB) is a disease caused by the bacteria Mycobacterium tuberculosis (Mtb) and remains an important cause of morbidity and mortality worldwide. Unlike adults, little is known about the immune landscape of the lungs, disease progression or latency, and expression of IDO in infant rhesus macaques. We established an aerosol-mediated infant NHP model to study immunopathogenesis of pediatric TB and expression of IDO in Mtb-exposed infant macaques.

Methods
Eight 10-week-old infant rhesus macaques (Macaca mulatta) were infected with aerosolized Mtb CDC1551 with two different doses and monitored for disease progression. All animals were euthanized at the end of the study to evaluate pathological and immunological changes. Immunohistochemistry and microscopy were performed to examine the expression of IDO in the pulmonary tissues collected from necropsy.

Results
TB symptoms in all 4 high-dose Mtb-exposed infants were more acute in onset and resulted in concurrent pulmonary and extra-pulmonary TB (EPTB), which is distinct from the 4 infants with reduced infectious dosing that had a chronic disease course with pathology limited to primary infectious sites. Acute infant TB models also presented with high levels of IDO expression in pulmonary granulomas co-localizing within the band of epithelioid macrophages, suggesting inhibition of tryptophan metabolism via IDO blockade may also enhance immune-mediated control of TB in the infants.

Conclusions
We have experimentally demonstrated increased susceptibility to TB disease by using Mtb aerosol inoculation in infant rhesus macaques, and this model can be used to understand immunopathogenesis of TB in young children and evaluate promising pediatric treatments.

ABSTRACT 37

Congenital cytomegalovirus infection drives metabolic rewiring of amniotic fluid in a nonhuman primate model

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Congenital cytomegalovirus (CMV) infection complicates 40,000 births in the U.S. annually and remains the most common viral cause of congenital infections. There is currently no treatment for congenital CMV and the mechanistic factors contributing to pathology remain poorly defined. Healthy pregnancies are dependent on tightly regulated metabolic pathways. Increases or decreases in available metabolites or type of metabolite may adversely affect the fetus and/or the pregnancy. CMV depends on metabolic rewiring for replication. In this study, we hypothesize that congenital CMV alters metabolic and lipidomic profiles that contribute to placental and fetal pathology. Using untargeted LC-MS/MS, we measured metabolite and lipid levels in amniotic fluid comparing non-transmitters (n=3) and CMV-transmitters (n=3) from our published CD4+ T lymphocyte depletion congenital rhesus CMV transmission model. We identified 5663 metabolites in the positive and negative ion modes. Using ANOVA analysis 569 metabolites were found to be significantly different ($p < 0.05$) between transmitters and nontransmitters. Lipidomics detected 229 different lipids, 62 being significantly different. Of note, we observed significantly reduced levels of triglycerides in CMV-transmitters compared to non-transmitters. Low triglycerides are associated with reduced fetal size, a feature observed in human congenital CMV infection. This analysis presents possible therapeutic targets for novel interventions to minimize congenital CMV-associated pathology in utero.

ABSTRACT 38

Protective efficacy of a vaccine inducing anti-Env antibodies against HTLV-1 challenge in cynomolgus macaques

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Human T-cell leukemia virus type 1 (HTLV-1) causes severe diseases such as adult T-cell leukemia (ATL) and HTLV-1 associated myelopathy in some individuals after long-term asymptomatic phase of latent infection. Control of horizontal HTLV-1 transmission is a globally important issue, but there is no available vaccine. We have recently developed a chimeric antigen consisting of the HTLV-1 gp63 ectodomain and the Sendai virus (SeV) F transmembrane-cytoplasmic domain (HtlvEnvF). A vaccine using a SeV vector expressing HtlvEnvF (SeV-HtlvEnvF) and a non-infectious SeV particle carrying HtlvEnvF (NVP-HtlvEnvF) has been shown to induce anti-HTLV-1 neutralizing antibodies in mice (Vaccine, 40:2420-2431, 2022). In the present study, we examined the protective efficacy of the vaccine using SeV-HtlvEnvF and NVPHtlvEnvF against HTLV-1 challenge in cynomolgus macaques. The vaccinated macaques (n = 5) showed significantly lower proviral loads compared to the unvaccinated (n = 6) after an intravenous challenge with 108 HTLV-1-producing cells (an ATL cell line provided by Dr. Yuetsu Tanaka). In the three out of five vaccinated macaques that showed substantial anti-HTLV-1 neutralizing antibodies, proviruses were undetectable after the challenge. These results indicate that neutralizing antibody induction by vaccination can result in protection from HTLV-1 transmission.

ABSTRACTS
ORAL PRESENTATIONS

SCIENTIFIC SESSION 5:
Genomics and Emerging Technologies

Chair: Steve E. Bosinger, PhD, Emory
National Primate Research Center
Co Chair: Claire Deleage, PhD,
Frederick National Laboratory

Advances in AIDS and COVID-19 research via NHP genomics

Presentation by Steven E. Bosinger (Speaker)
Emory University, Assistant Professor

ABSTRACT 39

Multi-omics analysis at single-cell level of SIV-specific CD8+ T cells in ARTsuppressed SIV-infected rhesus macaques

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The mechanisms contributing to SIV latency remain incompletely elucidated. A potential strategy is to harness the CD8+ T cell response to target the latent reservoir by reversing the dysfunction and exhaustion of these cells. Here, we developed a novel multi-omic approach to study SIV-specific CD8+ T lymphocytes, CD4+ target cells and innate cells at single-cell resolution in the lymph node during suppressive ART in nonhuman primates. We developed a workflow that utilizes barcoded Gag-CM9 tetramers and custom reagents for NHP cell surface markers to conduct simultaneous detection of epitope specificity, transcriptome, surface protein expression, TCR- and BCR-profiling at the single cell level. We applied our method to SIV-specific CD8+ T cells sorted from lymph nodes of three Mamu-A*01+ SIVmac251 infected rhesus macaques that had been maintained on ART for 18 months. We compared these cells to total LNMCs and CD3- CD20- innate cells, and to samples obtained from ARTnaïve animals. We observed that Gag-specific CD8+ T cells in the LN were comprised of heterogeneous populations that formed distinct clusters, including a cluster with high shared expression of PD1 and TIGIT that was enriched for gene signatures representative of exhaustion. Gene expression from LNresident Gag-specific CD8+ T cells differed significantly from pre-ART samples, with divergent expression of interferon-stimulated genes and inflammation signatures. In sum, this novel tool provides greater insight into immunological processes in lymphoid tissue during ART suppression and characterize CD8+ T cell exhaustion during HIV latency at greater resolution.

ABSTRACT 40

Increased Chemokine Production Is A Hallmark Of Rhesus Macaque Natural Killer Cells Mediating Robust Antibody-Dependent Cellular Cytotoxicity

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Background
In Rhesus Macaques (RM), Natural killer (NK) cells have been demonstrated to play a crucial role in the protection conferred by some HIV vaccine candidates by mediating antibody-dependent cellular cytotoxicity (ADCC). We intended to fill a gap in our knowledge of the molecular pathways of RM NK cells responsible for ADCC.

Methods
We collected PBMCs from six RM and measured ADCC mediated by a pool of antibodies with different epitope specificities. NK cells were subsequently sorted into degranulating (CD107a+) and nondegranulating (CD107a-) populations and analyzed via single-cell RNA sequencing (scRNAseq) to identify differences between and within the two populations.

Results
Seven NK cell clusters with distinct gene expression profiles were identified in every RM. Degranulating NK cells displayed activation-associated signatures, and 55.38% of these cells segregated into two clusters with high expression of chemokine transcripts such as MIP-1α, MIP-1β, and XCL1. In contrast, non-degranulating NK cells displayed a higher proportion of cells with a deficiency in ribosomal proteins, suggesting a dysfunctional profile. Importantly, trajectory analysis suggested that degranulating cells originated from a population of non-activated NK cells that could commit to activation and cytokine production.

Conclusion
Our study provides for the first time insights into the cellular transcriptome diversity at single-cell resolution among and within the RM NK cell. We observed a unique presence of a chemokine producer population. Since MIP-1α/β can recruit other immune cells and prevent virus entry by binding to CCR5, this suggests an anti-viral role for NK cells broader than just mediating ADCC.

ABSTRACT 41

Defining the dynamics of SIV infection and the viral reservoir from early ART initiation to rebound using PET/CT analysis and a multi-scale imaging approach

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Regular long-term adherence to antiretroviral therapy (ART) is effective but not curative. Human immunodeficiency virus (HIV) persistence during ART has been attributed to long-lived, latently infected memory T-cells. Here we utilize a PET/CT 64Cu-FAB2 probe(7D3) for iterative whole-body imaging to quantitatively localize SIV envelope at various timepoints, allowing us to explore the dynamics of infection in the rhesus macaque(RM) model.

RMs were challenged with a single high-dose of SIVmac239 and suppressive ART was initiated 4 days post-challenge. Following at least 6 months of ART, treatment was discontinued and RMs were necropsied as early as 4 days post-ART cessation. During ART and ATI, PET/CT scans using 64copperlabelled antibodies against viral envelope proteins revealed the sites of SIV gene expression. We used the PET signal to guide the isolation of small pieces of tissues at the time of necropsy. Infection sites identified with PET/CT were correlated with fluorescent imaging which revealed the presence of SIV infected cells.

SIV infections sites were efficiently detected by PET/CT as early as four days post-ART cessation in multiple tissues and infected cells were identified in these tissues by immunofluorescence. Rebound PET signals were isolated and quantified in the anatomical sites where discrete signals are initially established. Phenotyping the infected cells in post-ATI tissues revealed a mixture of myeloid and mast cells constituting the infected cell population. This finding is important to understand the dynamics of viral persistence and rebound, and to provide more avenues for the Cure field to explore.

ABSTRACT 42

SHIV Reservoirs: from *In Vivo* Labeling and PET/CT Imaging to Ex Vivo Identification of Infected cells

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Background
Upon infection, SIV/HIV viral reservoirs are rapidly established, evading ART and immune based elimination. Upon ART interruption, the virus uniformly and rapidly rebounds in spite of decades of efficient ART mediated control, suggesting sanctuaries of residual replication instead of true latency. Using a SHIV rhesus macaque model, we identified viral reservoirs with immunoPET/CT, and defined target tissues and cells, early upon ART interruption.

Methods
Seven rhesus macaques infected with SHIV162p3 were given anti-ENV PGT121-Cu64 and 3BNC117- Alexaflour467 i.v., scanned by PET/CT and sacrificed. Tissues identified as SHIV+ by PET/CT were collected for cell isolation, phenotypic analysis, sorting and analysis by SHIV RNAscope.

Results
PET/CT identified secondary lymphoid tissue as the main reservoirs of SHIV+. Flow cytometry analysis confirmed that the anti-ENV marked cells in vivo were indeed productively infected cells via confirmatory intracellular SIVgag staining. The majority of splenocytes positive for Env and Gag were T cells, but infected T cells were found to down regulate CD3 and CD4 from the surface (surface CD3lo but intracellular CD3+). Env+/Gag+ sorted cells were confirmed SHIV+ by RNAscope.

Conclusion
ImmunoPET/CT provides for a highly sensitive method to identify viral signals in real time in vivo even after several months of ART. More importantly, the technique readily identifies rebounding virus 3-4 days post ART interruption. Such monitoring tool will be critical in support of strategies aimed at HIV cures.

ABSTRACT 43

Transgenic Rhesus Macaques Expressing Human Na+ Taurocholate Cotransporting Polypeptide: A Promising Model to Study Chronic Hepatitis B Infection

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Chronic hepatitis B (CHB) is a major global health concern, with an estimated 300 million individuals affected worldwide and causing 820,000 deaths annually. Curative treatments remain elusive due to the lack of physiologically relevant HBV models for therapeutic testing. Previously our group demonstrated that rhesus macaque (RM) hepatocytes transduced with adenoviral (Ad) vectors expressing the HBV receptor, human Na+ taurocholate cotransporting polypeptide (hNTCP), support HBV infection both in vitro and in vivo. Unfortunately, Ad transduction results in a limited number of hNTCP-expressing hepatocytes and expression wanes over time since Ad does not integrate into the genome. Furthermore, Ad is immunogenic and may alter HBV dynamics in vivo. Hence, a transgenic model would ensure permanent hNTCP expression on all RM hepatocytes, making them susceptible to HBV infection. Here, we present the first transgenic RM expressing hNTCP from the germline. We injected RM zygotes with piggyBac mRNA and a transposon plasmid expressing hNTCP via a liver-specific transthyretin (TTR) promoter. Developing embryos were transferred into surrogate females, leading to successful pregnancies and six healthy infants. We confirmed germline editing in one male RM, consisting of a single transposon integrant on chromosome 5 in genomic DNA from liver, skin, muscle, lymph nodes, and rectum. Moreover, this infant exhibited hNTCP expression (RNA) exclusively in the liver that was comparable to levels found in human liver. Our findings indicate that this methodology, along with breeding, can be used to generate further transgenic animals to study CHB and test pre-clinical HBV therapies.

ABSTRACT 44

Integration of Spatial and Single Cell Transcriptomics Identifies Novel Pathologically Relevant Markers in SIV- and Mycobacterium tuberculosis-infected Rhesus Macaques

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Background
Novel spatial RNA-Seq technologies are capable of measuring gene expression from local cellular neighborhoods or even individual cells, providing essential spatial context to interrogate disease pathogenesis and the immune response. Nonetheless, even the most innovative spatial platforms currently compromise on either spatial resolution or transcriptomic fidelity. Integrating spatial RNA-Seq and single cell RNA-Seq (scRNA-Seq) of phenotypically similar tissues can mitigate some of the weaknesses of each platform.

Methods
These approaches broadly focus on determining highly expressed, cell-type defining marker genes used to deconvolve a spatial sample into its constituent cell-types. These methods are foundational, but subtle pathologically relevant signals can be obscured due to either weak signature gene expression or rare phenotypic occurrence within a spatial neighborhood. Our work focuses on leveraging the strengths of both scRNA-Seq and spatial RNA-Seq to co-annotate the data and gleam unique insights from each assay. In these analyses, we use paired tissue data from two distinct studies, SIV-infected lymph nodes and Mycobacterium tuberculosis (Mtb) infected lung tissue, to demonstrate discovery of marker sets at variable resolutions.

Results
Within Mtb-infected lung, we identify a novel marker that defines intra-granuloma macrophages in NHPs, and in SIV-infected lymph nodes, we identify cytotoxic lymphocyte signatures within SIV+ cellular neighborhoods that allow for more specific targeting of lymphocytes proximal to infected cells.

Conclusions
These methods and findings provide valuable insight into understanding the complicated interplay between spatial in situ hybridization and droplet-based scRNA-Seq in infected tissues.

ABSTRACT 45

Alternative splicing and genetic variation of MHC-E: Implications for rhesus cytomegalovirus-based vaccines

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Rhesus cytomegalovirus (RhCMV)-based vaccination against Simian Immunodeficiency virus (SIV) elicits MHC-E-restricted CD8 + T cells that stringently control SIV infection in ~ 55% of vaccinated rhesus macaques (RM). Here, we focused on determining how accurately the RM model reflects HLA-E immunobiology in humans. Using long-read sequencing, we identified 16 Mamu-E isoforms and all Mamu-E splicing junctions were detected among HLA-E isoforms in humans. We also obtained the complete Mamu-E genomic sequences covering the full coding regions of 59 RM from a RhCMV/SIV vaccine study. We found that the Mamu-E gene was duplicated in 32 (54%) of 59 RM. Among four groups of Mamu-E alleles: three ~ 5% divergent full-length allele groups (G1, G2, G2_LTR) and a fourth monomorphic group (G3) with a deletion encompassing the canonical Mamu-E exon 6, the presence of G2_LTR alleles was significantly ($p = 0.02$) associated with the lack of RhCMV/SIV vaccine protection. These genomic resources will facilitate additional MHC-E targeted translational research.

ABSTRACT 46

Tailoring Gene Space Facilitates Phenotyping of T cell Subpopulations from Single-cell RNA-seq Data Obtained from SIV-infected Rhesus macaques

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Background
Single-cell sequencing technologies provide unparalleled resolution into the transcriptional state of individual cells, thereby enabling high-resolution characterization of disease pathogenesis and the immune response. Spatial transcriptomic platforms generate similar high-dimensional gene expression data, while retaining spatial context. A common downstream analysis for these data is unsupervised clustering, whereby cells are clustered based on transcriptional similarity in high-dimensional space. This is a powerful method to identify novel cell populations or states, yet it can be a challenge to connect these data-defined clusters to established cell phenotypes and lineages, grounded in immunology knowledge.

Methods
The conventional approach to clustering involves discovery of cell neighborhoods in the gene space defined by the 2,000-3,000 most variable genes. While capturing the theoretically most informative features, this can both exclude key marker genes and bias toward genes with high variation due to technical artifacts. Using a combination of literature-derived markers and empirically identified genes with high subject-to-subject variation, we validated semi-supervised gene spaces, which we used for analysis of scRNA-seq data.

Results
Here we demonstrate that tailoring the gene space prior to clustering, by including key marker genes and excluding genes empirically shown to have high subject-to-subject variation, is an effective approach to both eliminate batch effects, and facilitates phenotyping of T cell subpopulations obtained from tissues collected during studies of SIV progression.

Conclusion
Performing dimensionality reduction using a semi-supervised gene space improves scRNA-seq clustering analyses from immune cells.

ABSTRACT 47

Nimble: A Novel Tool to Maximize and Augment Information From Bulk and Single-Cell RNA-Seq Data

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Background
RNA sequencing (RNA-seq) technology can measure whole transcriptome gene expression from tissues or even individual cells, providing a powerful tool to study the immune response. Analysis of RNA-seq data involves mapping relatively short sequence reads to a reference genome, and quantifying genes based on the position of alignments relative to annotated genes. While this is usually robust, genetic polymorphism or genome/annotation inaccuracies result in genes with systematically missing or inaccurate data. These issues are frequently hidden or ignored, yet are highly relevant to infectious disease, where minor sequence differences in gene isoforms result in critical functional consequences, and balancing selection often generates polygenic gene families not represented in a 'one-size-fits-all' reference genome.

Methods
Here we present nimble, a tool to supplement standard RNA-seq pipelines. Nimble uses a pseudoaligner to process either bulk- or single-cell RNA-seq data using custom gene spaces. Importantly, nimble can apply customizable scoring criteria to each gene set, tailored to the biology of those genes.

Results
Using single-cell RNA-seq data from SIVmac239-infected rhesus macaques, we demonstrate that nimble is able to generate counts for non-genomic features, such as SIVmac239, discriminate highly similar gene isoforms, and quantify genes missing or incorrectly annotated in the reference genome. We demonstrate that nimble can even genotype complex families including MHC-I and KIR.

Conclusion
Combining nimble-derived counts with standard pipelines enhances the fidelity and accuracy of the data, maximizing the value of these expensive datasets, and identifying cellular subsets not possible with standard tools alone.

ABSTRACT 48

The Rhesus IgSeq project: Population genotyping of the germline immunoglobulin repertoire in AIDS-designated Rhesus macaque breeding colonies

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Background
An urgent priority of contemporary HIV vaccine research is the development of strategies to elicit broadly neutralizing antibodies (bNAbs) capable of providing protection against diverse strains. Currently, some of the most promising vaccine strategies are those in which specific classes of germline immunoglobulin (IG) genes are targeted by immunogens and Ab evolution is directed by sequential immunization with a changing set of epitopes. For these pre-clinical studies to progress, a key technical roadblock is a lack of information on the composition, population diversity and extent of humanorthology in the IG loci of Indian-origin rhesus macaques (RMs). The RM IG loci are poorly characterized at the genomic level, in part due to technical challenges assessing these structurally complex loci.

Methods
To overcome this barrier, we have developed novel approaches for re-constructing full-locus IG haplotypes utilizing long-read sequencing and pairing this data with expressed repertoire sequencing (RepSeq) analyses.

Results
We completed the first long-read assembly of the Indian rhesus macaque genome, from which we compiled a unique dataset of RM germline IG alleles which we used to create a preliminary database of Ig alleles and generate a rhesus-specific capture reagent. We have completed paired genomic and mRNA sequencing for 86 rhesus from centers across the US.

Conclusions
In the RhIG-Seq project, we have greatly extended the availability of sequence information for the IgH/IgL/IgK loci by generating full-locus reference assemblies and germline variant catalogues for the IG loci across multiple RM centers in the USA.

ABSTRACTS
POSTER PRESENTATIONS

SCIENTIFIC SESSION 1
SIV Pathogenesis and Co-Infections

Poster 2

Viral evolution of SIV chimpanzee towards HIV-1 using humanized mice

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How chimpanzee-derived simian immunodeficiency viruses (SIVcpz) evolved into pathogenic HIV-1 group M viruses and the less widespread HIV-1 group N viruses following repeated exposure to a human immune environment has been the subject of previous studies including our own. Mice engrafted with human hematopoetic stem cells (hu-HSC) permit the de novo production multiple human immune cell lineages, providing an ideal system to recapitulate adaptive changes required for SIV evolution. Previous studies analyzed singly passaged SIVcpz and limited regions of the viral genomes identifying two notable mutations in the env gene. To improve upon these earlier studies and better understand the long-term evolution of these viruses, we serially passaged SIVcpzMB897 (Group M) and SIVcpzEK505 (Group N) over four, six-month passages in hu-mice to model serial viral transmission and subsequent spread. Viral fitness was assessed through plasma viral loads and CD4+ T cell decline. Additionally, we used Illumina-based deep sequencing of the entire viral genome to identify genetic changes related to viral adaptation. SIVcpzMB897 showed increased viral loads and CD4+ T cell decline, while SIVcpzEK505 showed fluctuating plasma viral loads and CD4+ T cell loss across all four passages. Surprisingly, the historical Gag M30K/R mutation was not detected but the Gag V35I mutation was maintained at high frequencies in both viruses. Mutations affecting viral pathogenicity identified by us and others were still maintained by the end of the fourth passage. While the functional impact of these mutations is still being investigated, these data provide insight into the evolution of SIVcpz into HIV-1.

Poster 5

Effect of concurrent ART and 3HP therapy on LTBI reactivation in nonhuman primate model of Mtb/SIV co-infection

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We have shown that timing of ART is critical in mitigating chronic immune activation. We have also modelled 3HP treatment of LTBI in our co-infection model with significant sterilization of macaque lungs. Here, we administered ART+3HP at 2 weeks post-SIV co-infection for 12 weeks to study i) if restoration of CD4+ T cell immunity occurred more broadly, ii) led to better control of bacterial replication and iii) if this prevented reactivation of LTBI compared controls. Bacterial burden, PET/CT scans, T-cell responses, cytokine levels, pathological analysis will be performed.

In this ongoing study, we have observed no significant increase in bacterial burden in BAL, inflammatory marker CRP, weight loss or body temperature in macaques treated with ART+3HP up to 6 weeks post treatment initiation. We expect to observe a significant decrease in bacterial burden within alveolar macrophages, better reversal of dysfunctional IL-22 and IL-17 production as well as skewed CD4+ T effector memory responses in ART+3HP treatment group compared to ART and 3HP only groups upon study completion. Finally, we expect to observe a significant decrease in chronic immune activation in the gut, reduced microbial translocation into systemic circulation and LTBI reactivation in the ART+3HP treatment group compared to control groups. Using concurrent ART and 3HP to treat rhesus macaques with LTBI and SIV coinfection is an innovative approach to tease out components of TB immunity that are impaired by SIV versus those that are partially restored after ART and those that are better restored by concurrent ART and 3HP.

Poster 10

Tissue ILC precursor differentiation and cytokine profiles in tissues in acute phase differ between nonpathogenic and pathogenic SIV infection

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Natural killer cells (NK) and innate lymphoid cells (ILCs) specialize in the rapid secretion of polarized sets of cytokines and chemokines to fight viral infections and promote tissue repair, particularly at mucosal barriers. Both cells differentiate from committed ILC precursors (ILCPs), but how ILCPs give rise to fully mature tissue resident NK and ILCs with antiviral activity remains unclear. Here we investigated the differentiation, functional specialization and lineage relationships of ILCPs and downstream differentiated cells in tissues during acute SIVmac and SIVagm infection.

Seven cynomolgus macaques infected IV with SIVmac251 and 14 AGMs infected with SIVagm.sab92018 were evaluated. Monkeys were sacrificed at either 4 or 28 dpi. Blood was collected before infection and at days 1, 2, 3, 4, 7, 9, 14 and 28 pi; bone marrow before infection and at days 4, 9, 28 pi; and lymph nodes before infection and at days 1, 2, 3, 4, 9, 14, and 28 p.i. A combination flow cytometry analysis using 60 markers for defining the distinct differentiation stages of ILC, NK cells and of cytokine production profiles (i.e., IFN-g, IL-17, IL-21) was optimized specifically for this study. ILC differentiation profiles differed between non-pathogenic and pathogenic SIV infection, leading to a profound difference in cytokine profiles in early and late acute phase of SIV infection in tissues.

This unbiased analysis contributes to better understand biological processes promoting or blocking the generation of ILC with efficient antiviral activity, and on the long term, might contribute providing novel targets for immunotherapies.

Poster 17

Isolation and Proteome Profiling of Translocating Bacteria in SIV Infection Identifies Unique Drug Targets to Inhibit Function

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Introduction: Microbial translocation contributes to inflammation in HIV-infected humans and has been associated with increased mortality and morbidity in individuals treated with antiretrovirals. Thus, there is interest in understanding the mechanisms underlying microbial translocation. To characterize translocating bacterial populations, we isolated and identified translocating bacteria from chronically SIV-infected macaques and characterized their genomes, transcriptomes, and proteomes.

Materials & Methods: Liver, mesenteric lymph node, and spleen samples were taken during necropsy from one uninfected and twenty chronically SIV- or SHIV-infected RM. Tissue samples were homogenized and plated under aerobic and anaerobic conditions. Isolates were identified using MALDI-TOF and/or 16S rDNA sequencing. Bacterial proteomes were analyzed by tandem mass spectrometry and genomics by next generation sequencing. Decitabine, a cytosine-specific DNA methyltransferase inhibitor, mediated growth inhibition was determined in vitro.

Results: Thirty-six translocating bacterial taxa were identified from 4 bacterial phyla. Translocating bacteria showed different proteome features from non-translocating bacteria with 47.21% of proteins identified as being unique. Top hits included cytosine-specific DNA methyltransferases and copper homeostasis protein CutC, which were found in five of the eight translocating bacterial species. Decitabine preferentially inhibited the growth of translocating bacterial species compared to non-translocating species.

Conclusions: Microbial translocation does not seem to be stochastic and unique taxa of translocating bacteria commonly express DNA methylation enzymes. Inhibition of these enzymes in vitro results in significant reduction of growth in these taxa. Blocking activity of these enzymes in vivo may offer unique treatment modalities to reduce microbial translocation and improve the prognosis of HIV-infected individuals.

Poster 24

Boosting Vγ9Vδ2 γδ T cells early in SIV/Mtb coinfection does not reduce TB disease or bacterial burden

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HIV infection in children increases their risk of Mycobacterium tuberculosis (Mtb) coinfection and worsens TB outcome. Vγ9Vδ2 γδ T cells can control Mtb through direct killing or by enhancing conventional T cell responses. We investigated whether boosting Vγ9Vδ2 γδ T cells could improve TB outcome using a nonhuman primate model of pediatric HIV/Mtb coinfection. Juvenile Mauritian cynomolgus macaques (MCM), equivalent to 4-8 year old children, were infected with SIVmac239M (i.v.). After 6 months, they were coinfected with low dose, intrabronchial Mtb. MCM were randomized to receive zoledronate (ZOL, n=5; 0.2 mg/kg, i.v.) at days 3 and 17 followed by recombinant human IL-2 (800,000 IU, s.c.) daily for 5 days to boost Vγ9Vδ2 γδ T cells or saline (n=5) at days 3 and 17. In the saline group, Vγ9Vδ2 γδ T cell levels did not change in the blood or airways following Mtb coinfection. A spike in Vγ9Vδ2 γδ T cells occurred in ZOL+IL-2-treated MCM in the blood, but not airways, between day 7 and 10 following the first dose. Vγ9Vδ2 γδ T cells were refractory to the second dose of ZOL+IL-2. Eight weeks after Mtb coinfection, animals were necropsied and TB pathology, Mtb bacterial burden, and immunology were assessed. ZOL+IL-2 did not reduce pathology or bacterial burden. Moreover, the frequency of γδ T cell subsets in granulomas did not differ between treatment groups. These data show transiently boosting γδ T cells with ZOL+IL-2 early in SIV/Mtb coinfection does not improve Mtb pathology and bacterial burden.

Poster 49

SIV and SARS-CoV-2 Coinfection in a Nonhuman Primate Model

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Persistent and uncontrolled SARS-CoV-2 replication in immunocompromised individuals may be an important source of novel viral variants that continue to drive the pandemic. Importantly, immunodeficiency associated with chronic HIV infection may lead to enhanced COVID-19 disease or persistence and evolution of SARS-CoV-2 but has not been directly addressed in a controlled setting.

We conducted a small pilot study wherein two pigtail macaques (PTM) infected with SIVmac239 for one year were then infected with SARS-CoV-2 and monitored for six weeks for clinical disease, viral replication and evolution as compared to a published cohort of SIV-naïve SARS-CoV-2 infected PTM.

We show that one PTM exhibited profound immunodeficiency at the time of SARS-CoV-2 infection, with almost no CD4+ T cells detectable in gut, blood, or bronchoalveolar lavage (BAL). The other PTM harbored a small population of CD4+ T cells in all compartments. Although neither PTM showed signs of enhanced COVID-19 disease, the more immunocompromised PTM developed a progressive increase in pulmonary infiltrating monocytes throughout the first month of SARS-CoV-2 infection. Viral kinetics and evolution in all sampled mucosal sites were indistinguishable from control animals, suggesting that underlying SIV infection is insufficient to drive uncontrolled SARS-CoV-2 replication. Neither PTM mounted anti-SARS-CoV-2 T cell responses in blood or BAL, and the PTM with the most profound SIV induced immunodeficiency also failed to mount anti-SARS-CoV-2 neutralizing antibodies.

Together, our data suggest that underlying SIV induced immunodeficiency likely impacts immune responses to SARS-CoV-2 but may be insufficient to drive the emergence of novel viral variants.

Poster 53

Evaluation of 2-hydroxypropyl-β-cyclodextran treatment on systemic immune parameters in healthy rhesus macaques

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Background: SIV infection of Asian macaques is an excellent model for HIV infection. Recent coformulations of nucleoside analogs and an integrase inhibitor have been used for parenteral administration, resulting in undetectable SIV RNA in plasma of infected non-human primates (NHPs). In a cohort of SIVmac239-infected macaques, we observed that this ARV therapy resulted in an unexpected increase in soluble CD14 (sCD14), associated myeloid cell stimulation. We hypothesized that the solubilizing agent Kleptose™ (2-hydroxypropyl-δ-cyclodextran) may have induced systemic myeloid cell activation and the release of sCD14.

Methods: We treated 5 healthy rhesus macaques with 15% Kleptose™ at 1 ml/kg/day and evaluated immune responses in these and 5 control animals by flow cytometry, Nanostring, and ELISA. We further stimulated PBMCs from untreated RMs with 2-hydroxypropyl-δ-cyclodextran from different sources and evaluated inflammatory cytokine production in response to stimulation in vitro.

Results: 2-hydroxypropyl-δ-cyclodextran induced immune responses in vitro and in vivo. In vitro, treatment of PBMCs resulted in increased myeloid cell IL-1δ production and destabilized lymphocyte CCR5 surface expression. In vivo, we observed modestly increased myeloid cell frequencies and numbers in treated animals. Nanostring assessment of intestinal homogenates revealed a shift in the expression of several antigen processing and antimicrobial peptide genes.

Conclusions: 2-hydroxypropyl-beta-cyclodextran is sufficient to induce myeloid cell cytokine production in vitro and low levels of inflammation in vivo. Further studies are needed to determine the effect of 2-hydroxypropyl-δ-cyclodextran containing ARV regimens on viral suppression, microbial translocation, inflammation, and the development of co-morbidities in NHP models of HIV infection.

Poster 66

Maintained mycobacterial growth inhibition potential of lipid-reactive T cells during SIV infection in Bacillus Calmette-Guérin (BCG) inoculated macaques

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Background: As BCG vaccine fails to protect against pulmonary TB in adults and HIV coinfection significantly increases the risk of TB, it is essential to explore novel therapeutic options beyond targeting of conventional adaptive immune responses. We have earlier demonstrated in NHPs that unconventional CD1-restricted T cells, including gamma delta (gd) T cells and NKT cells, innately respond to mycobacterial lipid antigens in BCG/Mtb-naïve setting. Here, we evaluated mycobacterial lipid-specific T cell responses following BCG exposure and then SIV infection in cynomolgus macaques.

Methods: Immune responses to total cellular lipids of M. bovis and MTb H37Rv were examined ex vivo in blood and bronchioalveolar lavage (BAL) fluid by IFN-γ/Perforin ELISPOT and Flow cytometry.

Results: BCG exposure induced significant increase in IL-7R expression on circulating gdT cells, greater CXCR3 and CD161 expression in BAL gdT cells, and greater IFN-γ responses in gdT and NKT cells at 1-3 weeks, suggesting induction of Th1/Th17-type responses in the lungs. SIV infection resulted in a significant loss of IL-7R expression on T cells including gdT cells, confirming the hallmark disruption of IL-7/IL-7R pathway during HIV/SIV infections; along with impaired IFN-α responses in lipid-reactive T cells. Interestingly, however, their ability to inhibit in vitro BCG growth was maintained during SIV infection.

Conclusion: Despite SIV-induced decline in IFN-γ production and IL-7R that disrupts T cell homeostasis, lipid-reactive T cells maintain their ability to inhibit mycobacterial growth. Stimulating these functions in vivo has the potential to enhance TB control in HIV-infected persons.

Poster 71

Peripheral IL-15 Neutralization Decreases Inflammation in Brains of Rhesus Macaques during Acute SIV Infection

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Background: Acute SIV infection activates inflammatory responses in both resident and migrated immune cells in brain. We hypothesized that neutralization of the pleiotropic cytokine IL-15 and consequently the associated cytotoxic effector cells, may significantly influence the inflammatory responses in the brain. Here, we comprehensively evaluated brain immune and inflammatory responses to acute SIV infection after IL15 neutralization.

Methods: Eight rhesus macaques were administered with two doses of anti-mRh-IL-15 antibody at days -21 and -7 prior to challenge with SIVmac239 (day 0) and were necropsied at 7 (n=3) and 14 (n=5) days post-infection (dpi). Peripheral and brain viral load were quantified by qPCR and RNAscope; immune cells dynamics and inflammation profiles assessed by IHC and RNASeq. SIV-infected macaques without anti-IL-15 treatment (non-depleted) and depleted without infection served as controls.

Results: IL-15 neutralization did not change SIV quantity in brain between 7 and 14 dpi, Neutralization did not change microglial activation, but it decreased GFAP expressing astrocytes in all brain regions. Fewer transmigrated CD163+/CD68+ and CD8+ cells were detected at 14 dpi in those treated with anti-IL-15 treatment. IL-15 neutralization significantly decreased percentages of IL-6 expressing Iba-1+ microglia, while increasing CD4+ T-cells, and TGF-α expressing CD163+/CD68+ cells.

Conclusion: While IL-15 neutralization did not influence quantitative and anatomical distribution of SIV in the brain, infiltration of immune cells and the inflammatory milieu in brain were significantly altered. This provides new insight that peripheral immune conditions, rather than brain viral load, may influence inflammatory conditions in the brain during acute SIV infection.

Poster 73

Effect of Obesity on Response to Antiretroviral Therapy in SIV-infected Rhesus Macaques

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Background: Modern antiretroviral therapy (ART) regimens including integrase inhibitors are associated with increased risk of metabolic disease. We employed the macaque SIV model to determine if a modern ART regimen comprised of TDF, FTC, and DTG would elicit metabolic dysfunction and if this effect was exacerbated by pre-existing obesity.

Methods: Lean, metabolically healthy (n=6) and western-style diet-induced obese (n=5) adult male macaques were infected iv with SIVmac239 and ART was initiated at 5 weeks post-infection and continued for 16 months. Baseline and longitudinal assessments of plasma and cell-associated viral load, circulating and white adipose tissue (WAT) immune cell profiles, WAT morphology, and systemic measures of metabolism were obtained.

Results: Plasma viral load peaked in both groups at 2 weeks and declined to the limit of quantitation (30 copies/ml) after ~27 weeks of ART, with the obese group exhibiting a slightly slower initial rate of decay. Cell-associated viral loads were reduced by ART in both groups. SIV infection decreased CD4 and increased CD8 T cell proportions in both blood and WAT, with greater changes in the latter. Systemic metabolic parameters in the lean and obese groups were not significantly affected by SIV infection or subsequent ART.

Conclusion: Tissue-specific responses to SIV infection and ART were observed in both experimental groups, but significant metabolic effects were not seen, contrary to expectations. This may reflect the recently described NLRP3 inflammasome inhibitory activity of the FTC class of NRTIs, which would potentially mitigate the adverse metabolic effects of integrase inhibitors such as DTG.

Poster 83

Development of qPCR Assays for the Discrete Detection of SIVmac251 and SIVsmE660 in Plasma from Co-Infected Animals

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Background: The Non-Human Primate Core Virology Laboratory (NHPCVL) at the Duke Human Vaccine Institute provides viral load testing to SVEUs nationwide. The NHPCVL typically performs SIV viral load testing utilizing an optimized, high-throughput SIV qPCR assay with a robust primer/probe set. To address the need for differential quantification of SIVmac251 and SIVsmE660 in co-infected animals, our laboratory developed two new qPCR assays utilizing our existing assay workflow that are able to discretely detect SIVmac251 and SIVsmE660 in the same plasma sample.

Methods: Primer/probe sets were developed and optimized for each qPCR assay. Infectious molecular clones were prepared and quantified for use in the creation of validation panels. Automated QIAGEN platforms were utilized for sample processing and PCR setup; qPCR assays were run on ThermoFisher QuantStudio3 Real-Time PCR Systems. The reliability and reproducibility of the assays was determined through the analysis of performance parameters such as accuracy, precision, specificity, and limit of quantitation.

Results: Preliminary testing of the discrete assays has been successful and showed no cross-reactivity between SIVmac251 and SIVsmE660. Since both qPCR assays are based on our existing SIV qPCR assay workflow, we can conjecture that the LOQ for each discrete assay will also be 62 RNA cp/mL utilizing 500uL of plasma. Similarly, the dynamic range for the assays will be 5 RNA cp/rxn up to 5E10 RNA cp/rxn.

Conclusion: The NHPCVL has successfully developed two qPCR assays with the ability to discern between SIVmac251 and SIVsmE660 viral loads in plasma from co-infected animals.

Poster 89

SIV co-infection during LTBI diminishes tissue and lymph node homing receptors on circulating NK cells in non-human primate model of Mtb/SIV co-infection

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Immune correlates of protection against Mycobacterium tuberculosis (Mtb) infection and TB disease have not been well-defined. Natural killer (NK) cells are increasingly recognized as a critical component of the innate immune response to Mtb. We used Mtb/Simian immunodeficiency virus-coinfected macaques to study the impact of Mtb and Mtb/SIV co-infection on NK cells distribution and function.

A total of 18 macaques were infected with low dose aerosol infection with Mtb CDC1551. Nine weeks post Mtb (LTBI), animals were infected with SIVmac239. We evaluated the dynamics of circulating NK cells for their phenotype and function during LTBI and following SIV co-infection using multicolor flow cytometry.

First, we observed a significant increase in the proliferation (Ki-67) and activation (CD69) of NK cells within a week post Mtb infection. The proliferation and activation were coupled with increased tissue homing (CCR6, CXCR3), lymph node homing (CCR7), and follicular homing (CXCR5) receptors, which were maintained seven weeks post Mtb. Following SIV co-infection (week two post-SIV), NK cell proliferation increased significantly with higher activation. However, they lose the capacity to express lymph node, tissue, and follicular homing receptors. In addition, SIV-coinfection reduces the functional CD27+ NK cells.

Altogether, our data demonstrate that NK cells are highly activated during LTBI and display increased tissue homing receptors. However, following Mtb/SIV co-infection, the phenotypic and functional profiles of NK cells were altered. This will inform future studies aimed at defining NK cell-mediated immune correlates that may be associated with Mtb disease progression in Mtb/SIV co-infection settings.

Poster 92

SHIV C109 NHP Induces Rapid Disease Progression in Elderly Macaques with Extensive GI Viral Replication

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Background: Chimeric Simian/ Human immunodeficiency viruses (SHIV) with clade C, R5 tropic transmitter/founder (T/F) Env provide NHP based models critical for studies of HIV immunopathogenesis, efficacy of vaccine and mucosal microbicides. SHIV C109 was developed with these objectives in mind.

Methods: SHIV C109 virus was serially passaged in two rhesus macaques (RM) to generate a high titer virus stock. The co-receptor usage was confirmed before infecting 5 aged RMs (4 female and 1 male) IV. Plasma viral load were monitored by qRT-PCR. Multiplex analysis of 23 cytokine/chemokine were conducted as well as biomarkers associated with GI tract damage. Antibody and cell mediated responses were also measured and viral dissemination into tissue.

Results: SHIV C109 infection of aged RMs lead to high plasma viremia and rapid disease progression necessitating euthanasia from 3 to 12 weeks post infection. Four out of 5 monkeys showed elevated I-FABP at the peak of infection while REG3α showed marked increases in the 3 out of 5 animals surviving the longest, at necropsy suggesting antimicrobial peptides produced by the GI epithelium in response to microbial translocation though only after acute infection. Luminex analysis showed elevated plasma levels of MCP-1, IL15, IL12/23. Gag specific TNFα+CD8+ and MIP1α CD4+ and Env specific IFN-γ+CD4+ and CD107a responses were observed though only in the monkeys that survived 4 weeks post infection. Viral RNA detection in gut and secondary lymphoid tissues was extensive via RNAscope.

Conclusion: SHIV C109 is a highly pathogenic R5 tropic SHIV causing rapid disease progression in aged RM.

Poster 95

ALPHA 4 INTEGRIN+ CCR5+ CD4 T cells mediate acute SIV CNS seeding in rhesus macaques

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CCR5+ CD4 T cells survey the CNS during homeostasis and the majority of their counterparts in circulation express the integrin α4 receptor, important for CNS entry. These data implicate CCR5+ α4+ cells in CNS viral seeding during HIV infection. To determine whether blocking α4 reduces early CNS viral seeding, we treated rhesus macaques with Rh-α4 (25mg/kg, n=4) or IgG (n=4) before and after SIV infection. Rh-α4 resulted in complete receptor coverage leading to profound lymphocytosis prior to and during acute SIVmac251 infection (3-fold; $p<0.05$). Within the CSF, a trend for elevated lymphocyte counts was noted with a surprising increase in frequencies of CCR5+ CD4 T cells prior to infection. Following infection, CSF CD4+ CCR5 frequencies increased in all Rh-α4-treated animals, while only two IgG treated animals displayed a similar trend. Based on a negative correlation between CSF vRNA and CCR5+ CD4 T cells in a previous study ($r = 0.5$, $p< 0.01$), we predicted that increased CCR5+ CD4 T cells at week 1 post SIV was consequent to decreased CNS viral seeding in Rh-α4 treated animals. Consistently, Rh-α4 resulted in significantly lower CSF viral loads at week 1 (median vRNA (copies/ml CSF): Rh-α4, 305; IgG, 12,650). Thus, CCR5+ α4+ CD4 T cells mediate early viral seeding within the CNS. Ongoing studies assessing whether Rh-α4 treatment decreases viral seeding and attenuates SIV induced microglial and T cell activation within the brain parenchyma will provide insights into the role of CD4 T cells in acute CNS viral seeding and subsequent neuroinflammation.

Poster 103

Anti-α4ß7 antibody reduces intestinal myeloid cell turnover in SIV-infected macaques in a microbiome-dependent manner

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Background: People living with HIV (PLWH) experience gastrointestinal symptoms and barrier dysfunction. Emerging evidence suggests vedolizumab (anti-α4ß7 mAb) efficacy during inflammatory bowel diseases depends on microbiome composition and myeloid cell differentiation. Since gut macrophage turnover is associated with accelerated SIV pathogenesis and CD103+ DCs orchestrate gut trafficking, inhibiting these with anti-α4ß7 may improve disease outcomes.

Methods: Nine CD8-depleted rhesus macaques were infected with SIVmac251. At week 2, daily cART and infusions (anti-α4ß7: n=5; IgG: n=4) every three weeks were initiated. The cART was discontinued at week 14, but infusions continued until week 23. At week 28, necropsies were performed. Duodenum cells were isolated, and Bujko's (2018) gating strategy was utilized to determine macrophage maturity from recently differentiated (Mf1) to mature lamina propria macrophages (Mf3). Fecal DNA was isolated for 16S rRNA sequencing. Duodenum viral loads (VL) were quantified.

Results: Mf1s were lower (p=0.01) and Mf3s higher (p=0.02) in the anti-α4ß7 group. Independently, CD103+ expression was lower on CD11c+ cells (p=0.03). VLs correlated with myeloid cells: (Mf1: r=-0.8758, p=0.0098; Mf3: r=0.9332, p=0.0021, CD103+CD11c+: r=-0.8298, p=0.0209). This association of macrophage turnover with VLs suggests timing of infusions may influence viral reservoir establishment when their turnover is reduced. At week 14 dysbiosis marker Prevotella: Roseburia ratio (P:R) was lower with anti-α4ß7 (p=0.03). Week 23 P:R correlated with Mf1s (r=0.7833, p=0.0372) and CD103+CD11c+ (r=0.8119, p=0.0266).

Conclusions: These findings implicate microbiome-immune crosstalk as a novel factor in anti-α4ß7 efficacy offering new avenues for targeting pathogenic mucosal immune response during HIV/SIV infection.

Poster 104

Replication, Phenotype and Interferon Stimulated Gene Expression Within Liver T cells Assessed Throughout the SIV Disease Course

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Background: Non-alcoholic fatty liver disease (NAFLD) contributes significantly to morbidity and mortality in HIV-infected individuals, even under combination antiretroviral therapy (cART). Utilizing previous murine studies as a guide, we evaluated changes in hepatic T cell replication and interferon stimulated gene (ISG) expression throughout the SIV infection of macaques.

Methods: Liver biopsies from naïve and SIV-infected rhesus macaques were laparoscopically collected at multiple time points (weeks -4, 2, 6, 16-20, and 32/necropsy). T cells were evaluated by immunofluorescence microscopy (CD3, Ki67, Mx1 (interferon stimulated gene)) and flow cytometry (CCR5, Ki67, CD69, HLA-DR, PD1 on central memory (CD28+) and effector memory (CD28-) populations).

Results: Microscopy and flow cytometry assessment during acute infection (week 2) identified increased levels of T cell replication (Ki67) ($P< 0.0001$). Additional markers of activation (CD69 and HLA-DR) and exhaustion (PD1) remained elevated on CD8+ T cells (CD69 increasing on EM and PD1 on CM CD8 T cells). T cell Mx1 expression increased in SIV-infected macaques at necropsy, though it remained a minimal contributor to overall Mx1 expression in the liver (T cells were often associated with Mx1-highly expressing macrophages). Finally, assessment of liver tissue identified altered T cell zonation, with elevated T cells in centrilobular regions of SIV-infected macaques.

Conclusion: T cell replication and activation increased at numerous timepoints following SIV infection, including evidence of altered liver zonation. However, T cells only contribute minimally to the IFN-1 response when compared to other cell types, providing insights regarding the role of T cells in SIV/HIV associated liver dysfunction.

Poster 111

Long non-coding RNA profiling identifies a role for MMP25-MMP25-AS1 interactions in intestinal epithelial barrier dysfunction and trans endothelial neutrophil migration in ART naïve and experienced chronically SIV-infected rhesus macaques

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Background: Disruption of intestinal epithelial barrier/chronic intestinal inflammation is a hallmark of HIV/SIV infection that persists despite ART. Although underlying mechanisms remain unclear, emerging evidence suggests a critical role for long-noncoding RNAs (lncRNAs) in maintaining epithelial homeostasis. Since long-term Δ9-tetrahydrocannabinol (Δ9-THC) administration reduced intestinal inflammation in SIV-infected rhesus macaques (RMs), we hypothesized that modulation of lncRNA expression may represent an epigenetic mechanism underlying its protective effects. Methods: Using microarray, we profiled lncRNA and mRNA expression in colonic epithelium (CE) of uninfected (n=6) and SIV-infected RMs administered either vehicle (VEH/SIV; n=5) or Δ9-THC (THC/SIV; n=6). Results: Relative to controls, fewer lncRNAs were up/downregulated in CE of THC/SIV compared to VEH/SIV RMs. Importantly, reciprocal expression of natural antisense lncRNA MMP25-AS1 (up-2.3-fold) and its associated protein-coding gene MMP25 (attracts neutrophils by inactivating alpha-1-anti-trypsin) (down-2.2-fold) was detected in the CE of THC/SIV. LncTAR confirmed two significant homology regions and an energetically stable (nDG=0.2626) mRNA-lncRNA duplex structure between MMP25-MMP25-AS1. Overexpression of MMP25-AS1 blocked interferon-gamma induced MMP25 mRNA and protein expression in-vitro. Elevated MMP25 protein expression in CE of VEH/SIV but not THC/SIV RMs was associated with increased infiltration of lamina propria by myeloperoxidase/CD11b++ neutrophils. Interestingly, similar reciprocal expression of MMP25-AS1 and MMP25 mRNA and protein expression was confirmed in jejunal epithelium of THC/SIV/ART RMs. Conclusion: Data suggests that MMP25-AS1 is an epigenetic negative regulator of MMP25 and low-dose Δ9-THC can inhibit neutrophil infiltration by reducing MMP25 mRNA/protein expression through upregulation of MMP25-AS1.

ABSTRACTS
POSTER PRESENTATIONS

SCIENTIFIC SESSION 2
Vaccines, Immunology and Prevention

Poster 4

Preventive efficacy of an adjuvant-containing live-attenuated AIDS vaccine in pathogenic SHIV-infected cynomolgus macaques

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The combination antiretroviral treatment (ART) has led to a dramatic reduction in HIV-related morbidity and mortality; however, it cannot eradicate HIV from viral reservoirs established before the initiation of therapy. As such, developing an effective preventive vaccine against HIV are essential to prevent or limit the continuous spread of the virus as well as to control the disease progression and to eradicate the virus from HIV infected patients, respectively. Antigen 85B (Ag85B) can induce strong Th1-type immune responses in mice model as an adjuvant. We genetically constructed a live attenuated simian human immunodeficiency virus to express the adjuvant molecule Ag85B (SHIV-Ag85B). In this study, we investigated the preventive efficacy of SHIV-Ag85B to pathogenic virus infection. Most of macaques inoculated with SHIV-Ag85B showed protective effects against the intravenous challenge of pathogenic SHIV89.6P. Also, eradication of SHIV89.6P was confirmed by an adoptive transfer experiment and CD8+ cell depletion study. In these macaques, SHIV antigen-specific CD8+ T cell responses with polyfunctionality were rapidly induced in the acute phase of SHIV89.6P challenge. The SHIV antigen-specific T cell responses inversely correlated with virologic control. These results suggest that SHIV-Ag85B elicited viral antigen-specific CD8+ T cell responses against pathogenic SHIV and provide the possibility of eradicating a pathogenic lentivirus from infected cells.

Poster 14

Depletion of SIV-specific CD8+ T cells does not alter viral load kinetics during SIV infection

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The emergence of SIV-specific CD8+ T cells coincides with declining viremia in monkeys acutely infected with SIV. Antiviral CD8+ T cells may also contribute to immune control in chronically infected animals treated with antiretrovirals. We explored these relationships using toxin-conjugated MHC class I tetramers to deplete Gag CM9-specific CD8+ T cells in rhesus macaques infected with a pathogenic strain of SIV. Mamu-A*01+ rhesus macaques were infected with SIVmac239. Gag CM9 (CTPYDINQM) tetramers conjugated to saporin were administered to animals with progressive infection and one animal with spontaneous control of viremia to <10,000 copies/mL. Lymphocyte populations in blood, bronchoalveolar lavage fluid, lymph nodes, colon, and jejunum were enumerated via flow cytometry. Plasma viremia was measured via quantitative RT-PCR. Immunotoxin-conjugated CM9/Mamu-A*01 tetramers induced a transient and significant depletion of CM9-specific CD8+ T cells in blood with lesser effects in tissues. This manipulation resulted in viral recrudescence in the animal with spontaneous control of viremia but did not alter the kinetics or magnitude of viremia in other animals during acute or chronic infection, irrespective of treatment with antiretrovirals. Our results suggest that immunotoxin-conjugated MHC class I tetramers effectively induce transient depletion of circulating SIV-specific CD8+ T cells. Furthermore, CM9-specific CD8+ T cells were important for elite control of viral replication but played a minimal role in the transition from acute to set-point viremia in animals with progressive infection. No evidence was found to support the notion that SIV-specific CD8+ T cells contributed to the reduced viremia observed in animals treated with antiretrovirals.

Poster 16

Optimal resting times for detection of antigen-specific T lymphocyte responses in cryopreserved lymphocytes from SIV-infected macaques

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Within research for HIV treatment and prevention, non-human primates (NHP) are irreplaceable as an animal model for preclinical testing of HIV vaccine candidates. Optimization of immunological assays in NHP models is of the utmost importance to achieve best possible translational applications of data. Clinical research has long employed an overnight rest at 37 degrees for thawed human lymphocyte samples used in functional assays. In contrast, peripheral blood mononuclear cells (PBMCs) stocked from non-human primate (NHP) models have been known historically to suffer viability and functional issues with overnight resting. We sought to identify optimal resting conditions for NHP PBMC prior to assessment of antigen-specific CD4+ and CD8+ T-cell responses. Archived cryopreserved PBMC from one SIV-infected pig-tailed macaque were tested in three separate experiments to assess reproducibility and inter-assay variation. Cells were thawed, rested for 0, 3, 5, and 9 hours at 37°C, and then stimulated with SIV peptide pools. Viability and recovery were determined by automated cell counting, and SIV-specific T-cell responses measured by IFN-gamma ELISpot and intracellular cytokine staining (ICS). Our results show that resting improved the detection sensitivity of cytokine responses, with maximal benefit observed by 5 hours. The benefit was particularly apparent in IFN-gamma (ELISpot) assays and for IL-2-positive responses in the ICS assay. Although recovery and viability of thawed PBMC decreased after resting, the reduction was <20%. Ongoing experiments are planned to expand characterization and reproducibility of rested responses in PBMC from additional SIV-infected and uninfected pigtail and rhesus macaques.

Poster 47

Characterization of Conserved Nonhuman Primate Cell Specific-Transcriptomic Response to Interleukin-15

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Interleukin-15 (IL-15) is an immunomodulatory cytokine that directs immune cell activation and supports T cell effector functions but is overall poorly understood in the context of viral infection. We applied a systems biology approach on bulk and single-cell (sc) RNA sequencing (RNAseq) datasets to define a whole blood transcriptomics response signature linked with IL-15 signaling in RM and Mauritian-origin cynomolgus macaques (MCM). We analyzed whole blood collected over a 21-day course from RMs and MCMs treated with increasing doses of recombinant purified heterodimeric macaque IL15/IL15Rα (rRh-Het-IL-15) and scRNA-seq was performed on corresponding RM PBMCs post IL-15 administration. Additionally, bulk mRNAseq and scRNAseq were performed on cultured RM PBMCs treated with a single dose of either 4 or 40 ng/ml of rRh-Het-IL-15 and collected at 5 time-points post-treatment. We applied co-expression analyses and integrated bulk mRNAseq and scRNAseq cell-specific transcriptome analyses to further define and refine IL-15 response gene expression across these model systems. We show that IL-15 imparts both innate and adaptive immune response activation across myeloid cells, NK cells and T cells. Single cell data revealed myeloid cells were the first to elicit an innate immune response from IL-15 followed by NK and T cells. Further, we found that the IL-15-induced innate immune genes are specifically expressed in T cell subsets (CD4 and CD8 T cells) and remain up-regulated over time. These data identify a conserved transcriptomic IL-15 response across two primate species furthering our understanding the function of IL-15 in viral infections in NHP models.

Poster 55

Development of a fully optimized 28-color panel for immunophenotypic analysis of decidual leukocytes and peripheral blood mononuclear cells (PBMC) in rhesus macaques

Matilda Moström, Amitinder Kaur, Marissa Fahlberg

To date, most of the maternal-fetal interface immunology in humans has focused on reproductive research. Few attempts have been made to determine the role of the maternal-fetal interface during viral congenital infections. Acquiring human samples to address this gap is challenging as placental studies are not routine and mothers with congenital infections are often undiagnosed and therefore samples are not collected. Thus, using the rhesus macaques model to characterize the immunological environment of the maternal-fetal interface is a powerful approach to improving our knowledge of immune correlates of protection against congenital infections. To address this gap, a 28-color flow cytometry panel was developed and optimized as a tool to identify conventional and non-classical T lymphocytes, B lymphocytes, NK cells, and myeloid antigen presenting cells at the maternal-fetal interface and in PBMCs of pregnant rhesus macaques. This panel further delineates memory T lymphocyte populations along with characterization of their tissue residency, activation, proliferation, cytotoxicity, trafficking, and exhaustion status. It was aimed for cryopreserved decidual leukocytes and PBMCs but also works comparably on freshly isolated tissue lymphocytes and other cryopreserved samples such as splenic lymphocytes. We believe this panel would improve the toolbox available for non-human primate researchers working in infectious disease, reproductive sciences, and inflammation research. In addition, commonly used fluorochromes such as FITC, APC, and PE could be exchanged in our panel to identify rare or unique antigens of choice without altering the panel backbone.

Poster 69

Designing a Bispecific Antibody to Cross the Blood Brain Barrier to Target HIV

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Background:
PGT121, a broadly neutralizing antibody (bnAb), is in clinical trials to suppress HIV progression. Due to low penetrance of PGT121 into the CNS, we engineered a bispecific antibody, PGT121/anti-HTfR, where one of the arms of the antibody binds to the human transferrin receptor (HTfR) to cross the blood brain barrier through transcytosis.

Methods:
Viral neutralization efficiency, binding properties, and in vivo toxicity of bispecific antibody were tested. Organ distributions of both parent (PGT121) and bispecific (PGT121/anti-HtfR) antibodies were also studied though fluorophore tagging. Efficiency against virus was studied in two group of macaques infected with SHIV-YU2 and administered with PGT121 for 1st dose, and the experimental group received PGT121/anti-HTfR for the 2nd dose, whereas control group received another dose of PGT121. One week later, animals were necropsied and viral load (peripheral, tissue) determined using qPCR and RNAscope.

Results:
Bispecific antibody PGT121/HTfR neutralized most of the SIV and SHIV strains in vitro and was safely administered in vivo. Peripherally administered bispecific antibody was found to be present in CNS and peripheral tissues more than the parent antibody. Although no significant difference in plasma and CSF SHIV viral load was found between parent and bispecific antibody groups, bispecific antibody administered animals had low viral load in brain and peripheral tissues.

Conclusion:
The bispecific antibody preparation is a safe and potential alternative in controlling plasma and CSF viral replication, relatively better in controlling brain and tissue viral load. On further investigation, the bispecific antibody might have potential implications in neuroHIV treatment.

Poster 74

Delay of Post-cART Viral Rebound After Infusion of SIV-specific Engineered T Cells

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Background: To assess the potential for engineered virus-specific T cells to prevent or control viral rebound after cART withdrawal, two SIVmac239M-infected Mamu B*08+A*01- rhesus macaques received autologous T cells transduced with SIV specific TCRs, along with IL-15, three days after discontinuation of long-term cART (TDF/FTC/DTG).

Methods: One animal received MHC matched TCRs (B*08: Nef RL10, Rev KL9, Env KL9; experimental animal), the other received cells with an MHC mismatched TCR (A*01: CM9; control animal), to control for non-specific effects of infusions. Approximately half of all transduced cells in both animals were engineered to co-express HuCXCR5 to promote infused cell trafficking to B cell follicles of lymphoid tissues, an immune privileged viral sanctuary site.

Results: Plasma viremia (>15 vRNA copies/mL) was detected in the control animal 1 week after cART release, but not until 23 weeks post cART release in the experimental animal. Cell Trace Violet (CTV) labeled infused cells were detectable in blood through 26 weeks after infusion in both animals but had waned considerably. At the time of viral rebound, CTV+ cells were ~6% of CD3+ PBMC in the control animal and <0.5% in the experimental animal. Additional tissue analyses are in progress.

Conclusion: We hypothesize that infused cells in the experimental animal suppressed viral replication in tissues well enough to allow endogenous responses to mature and control further viral replication until ~6 months after cART release. Evaluation of potential immune escape mutations is underway to understand the late loss of control in the experimental animal.

Poster 79

Autologous Tier 2 Serum IgA Neutralizing Antibodies in Macaques Vaccinated with BG505.664 SOSIP

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Background: One goal of human immunodeficiency virus type 1 (HIV) vaccines is to generate mucosal IgA antibodies that neutralize HIV at sites of mucosal entry. However, no HIV envelope (Env) vaccine immunogen has yet been shown capable of eliciting IgA neutralizing antibodies (nAbs), even in serum.

Methods: We used Peptide M+SSL7 and Protein G to purify total IgA and IgG, respectively, from serum of 8 rhesus macaques that developed serum nAbs after receiving subcutaneous immunizations with BG505.664 SOSIP. Controls were IgA and IgG from vaccine naive animals. The purified IgA and IgG preparations were adjusted to 10mg/ml and assessed for ability to neutralize autologous tier 2 BG505 T332N pseudovirus using the TZM-bl assay and for levels of anti-SOSIP binding antibodies (bAbs) by ELISA.

Results: nAbs were detected in all IgA preparations, except those from vaccine naive macaques, including BG505 SHIV-infected animals. However, titers of nAbs (ID50) and bAbs (ED50) in IgG preparations were roughly 20- and 40-fold greater than those in the IgA preparations. Nonetheless, after adjusting titers of nAbs relative to titers of bAbs, neutralization by anti-SOSIP IgA was found just as effective and sometimes better than anti-SOSIP IgG from the same animal.

Conclusion: BG505.664 SOSIP or other stable native-like trimeric HIV Env immunogens could potentially generate tier 2 HIV neutralizing IgA antibodies in mucosal tissues and secretions if administered by a mucosal route. Funded by NIH grant UM1 AI24436 (Emory Consortium for Innovative AIDS Research in Nonhuman Primates, E.H. and R.R.A).

Poster 84

HIV clade C gp140, combined with SIV-Gag/Nef protein vaccine adjuvanted with NE/AS01B generated antibody-mediated effector functions and reduced viral load in SHIV-infected macaques

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Background: HIV is primarily transmitted through the mucosal route, and advancements in antiretroviral therapy have rendered the infection manageable, though not without co-morbidities and accelerated aging. Here, we tested the hypothesis that combining cell-mediated immunity gut homing with the nanoemulsion (NE) adjuvant and humoral immunity with AS01B will elicit robust immune responses and protect against SHIV.

Methods: Rhesus macaques (RMs) (n=4) were initially immunized with HIV gp140 envelope glycoprotein and SIVmac239 P55 Gag and Nef antigens delivered in 3x Pure Soybean oil-nano emulsion and AS01B mucosal adjuvants intranasally and subsequently boosted IM and subQ. Both vaccinated and naïve (n=3) macaques were challenged intrarectally with repeated low-dose SHIV.

Results: Vaccinated macaques developed potent gag-specific lymph node CD107a+ responses in CD4+ (all $P>0.05$ but <0.07) and PBMC CD8+ T cells ($P=0.057$), as well as robust CD8+ gag IFNα responses within axillary and inguinal lymph nodes ($P<0.05$), and CD8+ gag TNF α+ responses within inguinal lymph nodes ($P<0.05$). We did not observe robust neutralizing antibodies, but significant antibody-dependent complement deposition (ADCD) and phagocytosis (ADCP) were observed ($P<0.01$) in vaccinated animals. Next, increased mucosal but not plasma retinoic acid signaling in vaccinated animals. Control monkeys experienced acute SHIV infection-associated dysbiosis not seen in vaccinated monkeys. Immunization did not completely protect against SHIV infection; however, vaccinated animals had significantly reduced viral load in the plasma/CSF ($P<0.003$), and reduced levels SHIV DNA in the gut.

Conclusions: Gut-homing properties of the NE adjuvant may serve as a valuable mucosal adjuvant strategy for future HIV vaccine design.

Poster 93

HIV gp140 Vaccine Induces Distinct Outcomes in Lymph Nodes that Drain the Site of Vaccination When Compared to Adjacent Non-draining Lymph Nodes

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Background: Activation of germinal centers (GCs) in draining lymph nodes (LNs) is a crucial event in the establishment of immunity. Here, we utilize immunofluorescence microscopy to evaluate T and B cell populations within draining and non-draining/ adjacent LNs at three different vaccination sites as identified by near infrared imaging.

Methods: The SOSIP F8 gp140 immunogen adjuvanted with liposomal QS21+MPLA was administered to rhesus macaques via intramuscular (IM, n=3), intraepithelial oral mucosal (IEPO, n=3), and intraepithelial vaginal mucosal (IEPV, n=3) routes, then boosted at 4 weeks. At 2 weeks post-boost, indocyanine green (ICG) dye was administered at the vaccination site and LNs were obtained via biopsy to identify draining (ICG+) and nondraining (ICG-) LNs.

Results: Immunofluorescence microscopy was undertaken to assess proliferating B cells (CD20+, Ki67+) and T follicular helper (Tfh) cells (PD-1+ and CD3+) within GCs. GCs of axillary (IM vaccine route), submandibular (IEPO), and mesenteric (IEPV) LNs revealed a significant increase in the number of proliferating B cells per GC ($p<0.001$) when compared to adjacent ICG- LNs. Tfh cell numbers per GC were also significantly elevated in the ICG+ LNs ($p=0.005$). This increase in two key cell populations was likely due in part to the significantly increase in GC size in the ICG+ LNs ($p<0.0001$).

Conclusion: This study provides evidence for the utility of ICG dye to locate draining LNs following vaccination (increased Tfh levels, proliferating B cells and GC size). Identification of draining LNs may provide clues regarding elicitation of an optimum vaccine response.

Poster 107

Immunogenicity of a Self-Amplifying Replicon RNA (repRNA) Vaccine for HIV, Formulated in a Novel LIONTM Nanoparticle

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Background:
We previously showed that a self-amplifying replicon RNA (repRNA) SARS-CoV-2 vaccine formulated with a novel LIONTM emulsion generated robust binding and neutralizing antibody responses in nonhuman primates. Here, we seek to adapt the repRNA/LION platform to develop an HIV-1 vaccine that induces broadly neutralizing antibodies and to determine if this vaccine induces mucosal responses.

Materials and Methods:
Four groups of two male and two female pig-tailed macaques (Macaca nemestrina, N=4), will be vaccinated at week 0, 8, and 12, with a repRNA/LION vaccine encoding a full-length, disulfide-stabilized HIV-1 Env antigen. Group 1 received the clinical trial version of LION, delivered via intramuscular injection. Group 2 received a control lipid nanoparticle saRNA vaccine delivered intramuscularly, while Groups 3 and 4 received a repRNA/LION vaccine with alternate complex structures. In both the periphery and gut mucosa, binding antibody titers will be quantified via ELISA, while neutralizing antibody titers will be determined with a pseudovirus neutralization assay. Peripheral and mucosal SIV-specific T-cellular responses will be assessed by flow cytometry.

Results:
Preliminary data shows the first vaccinations were well-tolerated in all groups, with no overt adverse events. A booster immunization was recently administered, and analysis of binding and neutralizing antibody in the blood and gut, as well as peripheral and mucosal T-cell responses, are in progress.

Conclusions:
Preliminary results indicate the repRNA/LION platform can be developed for HIV-1, and merits further experiments to determine whether our study supports advancement of an repRNA/LION SIV vaccine to NHP challenge studies.

ABSTRACTS
POSTER PRESENTATIONS

SPECIAL SESSION
Animal Management

ABSTRACT 12

2018 and 2020 Surveys of Social Housing Status of Nonhuman Primates on SIV Studies

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Social housing status is frequently unreported in infectious disease study publications, more specifically SIV (simian immunodeficiency virus) studies. To gauge the prevalence and spectrum of housing configurations utilized on nonhuman primate (NHP) SIV studies, a preliminary survey was distributed in 2018 to six facilities known to conduct them. Survey findings revealed that 48% of studies were utilizing pair housing during at least some phase of studies, including those during which animals were SIV infected. Social housing was implemented via full contact housing or protected contact housing in which animals can interact through barriers preventing entry into others' cages. To determine if this sample population was reflective of the larger U.S. based NHP SIV population, a second more extensive survey was sent to the same six facilities and four additional institutions, providing information on over 3000 NHPs assigned to over 250 SIV research protocols. The 2020 survey confirmed the 2018 preliminary findings and further clarified the types of studies, demographic of animals, and phases of the study that monkeys are allowed various forms of social housing. Eight out of the 10 respondents allow pair housing throughout infection for at least some of their protocols. IACUCs were identified as the most common driving force for advocating and advancing this type of housing. At institutions where pair housing was used, superinfection was not considered to be an adequate scientific justification for single housing. This presentation will discuss the survey questions, responses, and next steps in this area research and animal welfare.

ABSTRACT 68

Maintenance of MHC-defined social groups for HIV/AIDS work

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Monkeys of defined MHC type enhance the predictability and reproducibility of SIV/AIDS progression in studies of rhesus macaques. The Oregon National Primate Research Center maintains a breeding colony of approximately 3,500 Indian-origin rhesus macaques. MHC-defined breeding groups result in predictable phenotypes in our colony, allowing assignment of monkeys of any age to HIV/AIDS projects. We utilize a comprehensive genetic and behavioral management plan to ensure that physically and psychologically healthy monkeys are produced in social groups, while ensuring that overall colony genetic heterogeneity is maintained. This process includes a projection of the number of monkeys needed for infant and adult projects over time, allowing proactive formation of social groups. Each breeding group is maintained to ensure that all males of breeding age (3+ years old) are negative for the Mamu-B*08 and Mamu-B*17 alleles. Additionally, a high proportion of females negative for the Mamu-B*08 and Mamu-B*17 alleles are maintained in each MHC-defined social group. This ensures predictable offspring phenotypes and easy access to animals for subsequent studies without the need for MHC typing to determine suitability for project work. Offspring are harvested in a collaborative, iterative process between colony management, behaviorial, and operations staff in an effort to minimally impact the social stability and genetic merit of any given group. This colony management paradigm directly supports production of monkeys for HIV/AIDS-related work while improving behavioral outcomes for these animals.

ABSTRACT 77

Positive Reinforcement Training to Enhance an HIV Sexual Transmission Model in Rhesus Macaques (Macaca mulatta)

Caroline Widmaier, Kristin Killoran, Claire Deleage

Voluntary cooperation of non-human primates through positive reinforcement training reduces potential injury to both the human and NHP involved, and can reduce stressful events, which can have a negative impact on animal welfare and research outcomes. SIV, SHIV, and stHIV-infected animals pose an additional bloodborne pathogen risk to personnel and, therefore, leveraging voluntary cooperation in such NHPs is even more beneficial. Our goal was to both administer oral treatments (experimental group) and collect voluntary vaginal swabs daily from female rhesus macaques to track menstrual cycling as part of a model of HIV sexual transmission. Positive reinforcement training with hand to hand offered treats was used to shape the behavior required for daily swabbing. The investigator identified 12 animals for the project without prior formal temperament assessment and with limited duration for training. Four animals were deemed poor candidates due to highly inhibited temperaments incompatible with study end goals. Three animals were used as controls because desensitization to accepting treats was unachievable within the allotted timeframe. One animal was treated orally, despite failure to complete swabbing training. Four animals successfully completed swabbing training (defined as voluntary rump presentation and cooperation with daily swabbing). Duration to successful training was four weeks to more than four months, consistent with previously documented natural discrepancies in animal temperament and fitness for training. Given these results, we recommend temperament testing a pool of animals prior to enrollment in such studies and disqualification of highly inhibited animals for consideration for this procedure.

ABSTRACT 98

Gastrointestinal pathogen free (GPF) status in a colony of rhesus macaques (Macaca mulatta) substantially reduced diarrhea cases and eliminated the need for diarrhea treatment across a 2.5-year period

Rachele Bochart, Hugh Crank, Kimberly Armantrout, Samantha Uttke, Danika Whitcomb, Chris Schriver-Munsch, Tonya Swanson, Miranda Fischer, Cassandra Moats, Joseph Sciurba, George Lawrence, Michael Axthelm, Jeremy Smedley

Background
Bacterial and parasitic endemic pathogens induce profound immunologic/physiologic changes that negatively impact SIV models, and are significant zoonotic risks associated with Rhesus macaques (RM). The annual incidence of diarrhea can exceed 10% and frequently necessitates antibiotics and other drugs that can result in confounding variables.

Methods
RM treated with a multimodal treatment regimen and confirmed free of detectable GI pathogens and maintained with barrier practices were considered GI pathogen free (GPF). We compared GPF animals to untreated (nonGPF) animals housed at the ONPRC across the same 2.5-year period for number of cases of diarrhea (#DC), number of diarrhea days (#DD), number of days of treatment (#Tx), and number of days of antibiotic treatment (#Abx).

Results
Only one GPF animal had a diarrhea case which required no treatment and lasted 3 days during the entire 2.5-year span with a #DC 2.86x10-5 of and # DD of 8.58x10-5. NonGPF RM had significantly more diarrhea with a #DC 2.4x10-4 ($p=0.0115$; odds ratio (OR) 8.4) and a #DD of 9.0x10-3 ($p<0.0001$; OR 106.0). No GPF animal required treatment compared to a #Tx for non-GPF 8.8x10-3 ($p<0.0001$; OR 621.6) and a #Abx for non-GPF of 4.0x10-3 ($p<0.0001$; OR 283.4).

Conclusion
GPF animals were maintained for extended periods, had >100 fold less diarrhea days and required no treatment resulting in odds ratio of >600. Combined with published data demonstrating improved consistency of GI mucosa and background inflammation GPF animals represent significant improvements in animal welfare, research readiness, and personnel safety compared to standard SPF4 macaques.

ABSTRACT 99

Effect of Psychosocial Stress on Innate Immune Activation in an SIV-Infected Pigtail Macaque (Macaca nemestrina) Model of Acute HIV Infection

Natalie Castell, Selena Guerrero-Martin, Leah Rubin, Erin Shirk, Jacqueline Brockhusrt, Claire Lyons, Kevin Najarro, Suzanne Queen, Ming Li, Brandon Bullock, Bess Carlson, Robert Adams, Craig Morrell, Lucio Gama, David Graham, Christine Zink, Joseph Mankowski, Janice Clements, Kelly Metcalf Pate

Simian immunodeficiency virus (SIV) infection of macaques recapitulates HIV pathogenesis and is similarly affected by both genetic and environmental factors. Chronic stress is known to have deleterious effects on the immune response, and psychosocial stress is associated with worse clinical outcomes in people with HIV (PWH). This study assessed the impact of psychosocial stress on the innate immune responses of pigtailed macaques (Macaca nemestrina) during acute SIV infection.

A retrospective analysis of acute SIV infection of juvenile male pigtailed macaques was performed to compare the innate immune responses of socially and singly housed animals. Absolute monocyte (n= 76, n= 58 for monocyte subsets) and platelet counts (n= 18), and flow cytometry data were analyzed using a mixed effects model.

Innate immune parameters were affected by SIV infection, with a modulating effect from social housing. Monocyte count increased post-infection for both groups, driven by classical monocytes (CD14+CD16-) with a greater increase in socially housed animals ($P< 0.001$ compared to pre-inoculation timepoints). Platelet numbers of socially housed animals recovered more quickly from the acute decrease observed in both groups. Platelet activation, as assessed by P-selectin ($P= 0.004$ and $P <0.001$) and MHC-I ($P= 0.009, 0.044$) surface expression, was observed only in socially housed animals, while there was no change in singly housed animals.

Psychosocial stress may play an immunomodulatory role on the innate immune response to acute retroviral infection and this could contribute to immune suppression and increased disease severity in PWH through changes in innate immunity.

ABSTRACTS
POSTER PRESENTATIONS

SPECIAL SESSION 3
Progress Toward HIV Cure

Poster 1

A Brain-Penetrant CSF1R Inhibitor Reduces Brain Virus Burden in SIV-Infected Macaques

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Perivascular macrophages (PVMs) are targets and reservoirs of HIV and SIV in the brain, hindering viral clearance despite antiretroviral therapy. We previously demonstrated that colony-stimulating factor-1 receptor (CSF1R) in PVMs was upregulated and activated in simian immunodeficiency virus (SIV)-infected rhesus macaques with encephalitis, correlating with SIV infection of PVMs. Herein, we investigated the role of CSF1R in the brain during acute infection using BLZ945, a brain-penetrant CSF1R kinase inhibitor. Among a total of 9 SIV-infected macaques, 6 animals received a daily oral dose of either 10 or 30mg/kg of BLZ945 (n=3 each), starting on days 10 post infection, for 20-30 days until euthanasia. In necropsied brain tissue, we counted immunohistochemistry-stained macrophages (CD206+ or CD163+) and microglia (P2RY12+) by microscopy and also measured tissue viral DNA (vDNA) load by qPCR. In 9 out of 11 tested brain regions, the vDNA load was significantly reduced with at least one of the two doses by 95 to 99%, and in some instances, even to undetectable levels. With the high-dose BLZ945 treatment, there was a significant reduction in CD206+ or CD163+ cells across all 3 brain areas examined, compared to low-dose treatment and control groups. Decreased numbers of CD163+ and CD206+ cells significantly correlated with lower vDNA levels in all corresponding brain areas. Our results indicate that doses as low as 10 mg/kg of BLZ945 are sufficient to reduce brain vDNA load. This study provides evidence that infected PVM are highly sensitive to CSF1R inhibition, opening new possibilities to achieve viral clearance.

Poster 6

Beneficial impact of Glatiramer Acetate treatment in SIVmac-infected macaques in combination or not with early antiretroviral treatment on immunological parameters

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In people living with HIV, residual chronic inflammation can persist during suppressive ART and likely is multifactorial, with a major contribution of microbial translocation through the damaged intestinal barrier. It leads to an increased risk of non-AIDS comorbidities and mortality, with an unmet need for novel therapeutics targeting HIV-induced inflammation. Glatiramer acetate (GA), an FDA-approved drug, used for its immunomodulatory effects in the treatment of relapsing forms of multiple sclerosis, has shown regulatory effects in other inflammatory diseases, particularly in the gut. To investigate whether GA treatment could reduce inflammation and intestinal tissue damage in SIV infection, we conducted a study on 24 SIV-infected cynomolgus macaques. GA was administered on day 28 or 84 post-infection for 4 months. One group of animals received GA in combination with ART initiated on day 28 pi. Control groups were untreated SIV-infected animals or treated with ART alone. Levels of viremia, cell-associated viral DNA, inflammation, gut barrier, immune cell activation, and effector phenotype of NK cells, T cells, and unconventional NKG2A+CD8+ T cells were analyzed. In the animals treated with GA, we observed: (i) a trend towards lower viral load (ii) higher ratios of CD4 T lymphocytes in blood and gut (iii) lower activation (CD8 HLA-DR+ T cells) and inflammation (plasma IP-10) levels. These improved profiles were not durably maintained for long after treatment interruption. GA treatment could play a role in repairing gut homeostasis and might represent an interesting strategy in HIV cure research to be further investigated.

Poster 11

Infusion of autologous in vitro generated SIV latently infected cells into SIV-naïve rhesus macaques results in viral rebound after ART withdrawals

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An enduring question for HIV cure research is how small must viral reservoirs be to attain clinically relevant periods of antiretroviral therapy (ART)-free remission. The viral reservoir size at ART termination is anticipated to affect the time to viral rebound (TTR). Yet, the association between reservoir size and TTR is unclear. To address this question, we developed a simian immunodeficiency virus (SIV)/rhesus macaque model to precisely set the size of latent reservoirs in vivo. To do so, we infuse defined numbers of autologous in vitro generated SIV latently infected cells into SIV-naive rhesus macaques treated with ART. We piloted this approach in two rhesus macaques. For each animal, we infected resting CD4+ T cells with barcoded SIVmac239M and cultured the cells with immunosuppressive cytokines and antiretroviral drugs to block replication and induce quiescence. At the end of the culture period, we enumerated the latently infected cells and infused ~50,000 SIV DNA-containing CD4+ T cells into the macaques, which were started on ART one week prior. We maintained ART for one-month with no detectable SIV in the plasma. However, upon ART withdrawal, we detected emergent viremia after 18- and 21-days, with one and two distinct SIVmac239M clonotypes present, suggesting that one or two latently infected cells reactivated to seed virus replication. These results show that transferred cells can persist in vivo for at least one-month post-infusion and can activate upon stopping ART. This model may help evaluate the impact of ultra-small viral reservoirs on ART-free remission.

Poster 19

Pharmacological modulation of Wnt and Notch pathways to inhibit the proliferation of the HIV reservoir

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Latently HIV-infected memory CD4+ T cells persist indefinitely through proliferation. We previously showed that inhibition of proliferation and induction of differentiation of central and stem cell memory CD4+ T cells can be achieved in ART-treated SIV-infected rhesus macaques (RMs) through modulation of Wnt pathway. Here, we evaluate a combined approach targeting both Wnt and Notch pathways during SIV acute infection of RMs to disrupt viral reservoir establishment.

A dose-finding study was performed in two uninfected RMs that received 8-week treatment cycles of Wnt inhibitor PRI-724 at the previously defined dose of 20mg/kg daily and Notch/gamma-secretase inhibitor LY3039478 at escalating doses of 1.5 and 2.5mg/kg three times a week. Plasma drug concentrations were measured by LC-MS. Five RMs were infected i.v. with SIVmac239 before receiving an 8-week treatment with PRI-724+LY3039478. ART was initiated 8wpi and maintained for one year during which PBMC were collected to sort memory CD4+ T cell subpopulations by FACS for assessment of viral reservoir distribution as compared to controls.

Plasma concentrations of LY3039478 increased > dose-proportionality with pharmacokinetic parameters at the increased dose of 2.5mg/kg achieving those observed in clinical trials. The pharmacodynamic activity of LY3039478 was demonstrated by a transient decrease in plasma levels of amyloid-beta peptide. The combined treatment PRI-724+LY3039478 demonstrated an acceptable safety profile in uninfected and SIV-infected RMs with no adverse events and only transient elevations in liver enzymes. Virological analyses in sorted cells are ongoing.

Combination treatments targeting pathways regulating T cell proliferation represent a novel strategy to reduce SIV/HIV persistence.

Poster 22

Modeling SIV latency in vitro by directly infecting primary rhesus macaque CD4+ T cells

Lea Matschke[1], Jason Weinfurter[1], Matthew Reynolds PhD[1,2]

A major hurdle to curing HIV is the rapid establishment of long-lived viral reservoirs after infection, promoting research into compounds that reactivate latently infected cells to deplete viral reservoirs. The activity of candidate latency reversal agents (LRAs) is initially screened with in vitro latency models, yet these models have not been adapted to the SIV/macaque system. To address this deficiency, we developed a novel in vitro latency model using primary rhesus macaque CD4+ T cells and SIVmac239. Our goal was to generate latently infected cells in vitro that recapitulated the resting CD4+ T cell reservoir in vivo. Resting T cells, however, are inherently resistant to retroviral infection. Therefore, we sought to increase cell permissiveness by releasing cell cycle-associated blocks to infection. Thus, we designed our culture conditions around two objectives: (1) inducing the G0 to G1 phase transition, and (2) reverting infected cells to G0 phase without cell division. After iterative testing, we increased the frequency of SIV-infected cells while maintaining ≥98.9% of cells in G0/G1 phase. To quantify latently infected cells, we measured intracellular viral protein expression with and without stimulation in conjunction with an intact proviral DNA assay. To further characterize these populations, we assessed functional phenotypes, activation marker expression, and responses to benchmark LRAs. In sum, our model generated homogeneous populations of resting memory CD4+ T cells that produced viral protein upon stimulation by well-characterized LRAs. This model may aid in identifying effective LRAs for preclinical macaque studies and translating research to a clinical setting.

Poster 36

Impact of CD4 Binding-site bNAb Therapy on Virus-specific T Cell Immunity in Barcoded TF-SHIV-infected Rhesus Macaques During Antiretroviral Treatment Interruption

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Broadly neutralizing antibody (bNAb) administration can suppress viremia after analytical treatment interruption (ATI) in SHIV-infected rhesus macaques (RM). It has also been proposed that bNAb administration can improve T cell function after ATI. Here, we evaluated this bNAb-mediated effect on SHIV-specific T cell function during ATI in two NHP studies using SHIV-infected RMs treated with CD4 binding-site (CD4bs) bNAbs. In study#1, 18 RM infected i.v. with barcoded TF-SHIV-D were treated with antiretrovirals (ART) at day 120p.i. for 6 months, followed by ATI during which 9RM received a single 30 mg/kg i.v. dose of VRC07.523.LS. In study#2, 12 RM infected i.v. with barcoded TF-SHIV-C were treated with ART at day 10p.i. for 4 months, followed by ATI during which 6 RM received a single 30mg/kg i.v. dose of VRC07.523.LS. Peripheral blood was collected for viral quantification and evaluation of SIV-GAG-specific T cell function (cytokine production). Virus rebounded in all RM in both studies, with time to viral rebound significantly delayed in VRC07.523.LS-treated versus control RM (study#1:median values=41d and 28d, respectively; study#2:median values=50d and 12d, respectively;$p<0.05$). We found a trend towards a higher proportion of cytokine-producing GAG-specific CD8+T cells in treated RM in study#2, though the difference was not significant as compared to control RMs ($p=0.065$). In conclusion, CD4bs-bNAb monotherapy in TF-SHIV-C-or-D-infected RM at ATI recapitulates key features of bNAb monotherapy in humans, including a delay in time to viral rebound. However, we found no significant effect on the level or function of SHIV-specific CD8+T cells.

Poster 40

Pilot study of a cIAP inhibitor (AZD5582) + Bcl-2 inhibitor (ABT199) in SIV-infected, ART-suppressed rhesus macaques

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Background: Combining the cIAP inhibitor AZD5582 with the Bcl-2 inhibitor ABT199 (venetoclax) to reverse latency and enhance clearance of infected cells via apoptosis is a novel approach to cure HIV. In this pilot study, we evaluated the safety, pharmacokinetics, and pharmacodynamics of ABT199 with AZD5582 in a nonhuman primate (NHP) model.

Methods: Two juvenile rhesus macaques (RMs) were orally infected with SIVmac251 and ART was initiated 4 weeks post infection (wpi). At 112 wpi, RMs received escalating single doses of ABT199 at 2.5mg/kg, 10mg/kg and 20mg/kg intramuscularly (i.m.). Next, AZD5582 was administered once intravenously at 0.1mg/kg with 4 daily doses of ABT199 (at 15mg/kg i.m.). Peak plasma concentrations (Cmax) of ABT199 were evaluated and changes in absolute CD4+ and CD20+ cell counts were assessed by flow cytometry.

Results: ABT199 was safely administered with average Cmax of 0.42ug/ml, 1.69ug/ml and 3.45ug/ml at the 2.5mg/kg, 10mg/kg and 20mg/kg doses, respectively. The 20mg/kg dose was associated with mild adverse effects. When the combination of ABT199 and AZD5582 was administered, average ABT199 Cmax was 3.30ug/ml after the first dose and 2.25ug/ml after the fourth dose. A decline in absolute CD4+ T-cells was seen at 24hrs post dose and ranged from 22%-97%. CD20+ B-cell counts were reduced 62%-91%. CD4+/CD20+ cell counts recovered by 7 days post-ABT199 dose.

Conclusions: Concentrations of ABT199 dosed at 15mg/kg approximated the steady state Cmax observed in humans after 400mg oral dosing. The observed reduction in CD4+ T-cells demonstrates the potential for this therapeutic approach to reduce viral reservoirs in vivo.

Poster 41

Study of MHC-E restricted suppressive activities in macaques infected with an ENV-signal peptide recombinant SIVmac

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In African green monkeys (AGM), natural killer (NK) cells mediate strong control of SIVagm infection in secondary lymphoid tissues. SIVagm infection induces the expansion of terminally differentiated NKG2Alow NK cells displaying an adaptive transcriptional profile with increased MHC-E restricted cytotoxicity in response to nonamer peptides derived from the Env leader sequence of SIV. In contrast, such NK cell differentiation was found to be lacking in chronically SIVmac-infected macaques (MAC). To better understand the impact of MHC-E-Env peptide interactions on NK cell activity and viral control, we infected 6 rhesus MACs with a recombinant SIVmac239 clone coding for a SIVagm nonamer Env leader sequence peptide. This SIVagm Env peptide is known to bind to MHC-E and to inhibit simian NK cells less than the corresponding SIVmac peptide. As a control, we infected 6 MACs with wild-type SIVmac239. The recombinant virus replicated well in vitro in human cell lines and rhesus MAC PBMC. However, following in vivo infection, we observed delayed replication kinetics and lower viremia in blood and lymph nodes in the group of MACs infected with the recombinant virus. Quantification of viral load, phenotypical analyses of immune cells (NK, NKG2A/C+CD8+T, CD4+T, monocytes, dendritic cells, including CXCR5 and HLA-E expression levels), anti-SIV Env antibody titers, functional assays and quantification of inflammation markers in blood and tissues are performed. This study provides novel insights into the roles of these host factors with potential implications for NK and CD8 cell-based immunotherapies toward HIV cure.

Poster 42

Delivery and long-term expression of CCR5-blocking monoclonal antibody Leronlimab with AAV

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Background
CCR5 blockade represents a scalable non-transplantation approach for long-term ART-free HIV remission. Here, we tested if AAV vectors could induce long-term expression of CCR5-blocking monoclonal antibody Leronlimab in SIV- and SHIV-infected Mauritian cynomolgus macaques (MCM).

Methods
Two MCM received AAV9 encoding human Fc Leronlimab (AAV9-HuLeron), and two MCM received AAV9 encoding macaque Fc Leronlimab with stabilizing, silencing, and half-life extending mutations (AAV9-MacLSLeron). To limit immune activation, MCM received dexamethasone (-12, -1, and 5 hours post-AAV) and daily tacrolimus (days -8 to 28 post-AAV).

Results
One AAV9-HuLeron and one AAV9-MacLSLeron MCM exhibited transient CCR5 receptor occupancy (RO) on blood CD4+ T-cells, but subsequently developed anti-drug antibodies (ADA) and lost RO. One AAV9-MacLSLeron MCM did not express due to pre-existing ADA. The final AAV9-HuLeron MCM achieved 100% CCR5 RO on blood CD4+ T-cells within 2 weeks and possessed detectable plasma Leronlimab >1ug/mL within 3 weeks without ADA, with CCR5 RO and plasma Leronlimab maintained through 33 weeks post-AAV. Mesenteric lymph node and spleen CD4+ T-cells from week 13 post-AAV exhibited >98% RO. SHIVsf162p3 viremia became undetectable within 4 weeks post-AAV and remained undetectable through 33 weeks post-AAV with the exception of 3 blips of plasma viremia ~103 copies/mL which coincided with small dips in blood CD4+ T-cell CCR5 RO.

Conclusions
While further investigation is needed to develop AAV vectors and/or regimens that reduce the incidence of ADA, these data demonstrate the potential of AAV vectors for sustained antibody delivery and gene therapy approaches for long-term ART-free HIV remission.

Poster 60

Circumcision Increases the Barrier Function of Glans Epithelium

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Background
Male circumcision has been shown to reduce the risk of heterosexual penile acquisition of HIV by approximately 60% and is recommended by the WHO as part of a comprehensive protection strategy. The foreskin has been extensively studied and shown to be rich in HIV target cells. But little is known about the effects of circumcision on the glans as it transitions from being in a moist environment to being constantly exposed
to air.

Methods
Adult male subjects were recruited in Chicago, 21 circumcised and 21 uncircumcised men, for evaluation of skin barrier function with noninvasive hydration and water loss meters in glans and shaft tissues. A shave-biopsy was obtained from the glans and shaft to be exposed to PA-GFP-HIV for 4h then imaged by deconvolution microscopy for virion count, proportion of penetrators, and depth of penetration into the tissues. Additionally, we analyzed by immunofluorescence images of potential HIV-1 target cells in these tissues, as CD4+, CD3+ and CCR10+ for number of cells per surface area of tissue imaged, depth from the epithelial surface, and depth from the basement membrane. Both parameters was compared between locations and circumcised/ uncircumcised tissues.

Results
Our data suggests that circumcised glans exhibited reduced total water content and TEWL. Also, the percentage of penetrating virions that are deep penetrators is halved in circumcised glans with respect to uncircumcised tissue.

Conclusion
Our results will help define how HIV enters the glans and establish a method to follow changes with future interventions aimed at altering HIV susceptibility.

Poster 62

Assessment of anti-CD20 antibody pretreatment as a strategy to augment CAR/CXCR5-T cell therapy in SIV-infected rhesus macaques

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Background

During chronic SIV infection, the majority of viral replication occurs within B cell lymphoid follicles. Infusion of SIV-specific CAR-T cells expressing the follicular homing receptor, CXCR5, leads to follicular localization of the cells, but the CAR/CXCR5-T cells fail to persist long-term. We hypothesized that a temporary disruption of follicles, with anti-CD20, would create space for CAR/CXCR5-T cell engraftment leading to increased abundance and persistence.

Methods

SIV-infected, ART-suppressed rhesus macaques were treated with 7 mg/kg anti-CD20. Seven days later, CAR/CXCR5-T cells were infused, and the animals were released from ART. Paired animals, not receiving CAR/CXCR5-T cells, served as controls. The first animal, treated with 2 x 108 CAR/CXCR5-T cells/kg, experienced apparent cytokine release syndrome (CRS). Subsequent CD20-depleted animals were pre-treated with Siltuximab to prevent CRS and were used in a CAR/CXCR5-T cell dose escalation study (1 to 8.3 x 107 cells/kg).

Results

CAR/CXCR5 T cells underwent a robust expansion in lymph nodes, with expanding cells clustered in and around small follicles, and showed increasing quantities of cells with dose. There was a sharp decline of cells at day 14, all occurring prior to the detection of recrudescing vRNA in lymph nodes. An anti-CAR antibody response was detected. There was no apparent impact on viral loads. CD20+ cells returned to normal levels by 60 days post-depletion.

Conclusion

The results of this study suggest that pre-treatment with anti-CD20 antibodies led to a robust expansion of therapeutic cells, and for safety, may require prevention of a CRS response.

Poster 72

The In Vivo Effects of TIGIT Blockade in Chronic SIV Infection

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TIGIT is a negative checkpoint receptor associated with T-cell exhaustion in cancer and HIV. TIGIT is upregulated in virus-specific CD8+ T cells and NK cells during HIV/SIV infection resulting in dysfunctional effector capabilities. In vitro studies targeting TIGIT on CD8+ T-cells suggested TIGIT blockade as a strategy to restore SIV-specific T-cell responses. Here we extend these studies in vivo, using TIGIT blockade in cynomolgus macaques in an effort to reverse T-cell and NK cell exhaustion in the setting of SIV infection.

SIV-infected cynomolgus macaques were treated with 5 biweekly doses of 10 mg/kg anti-TIGIT antibody and control animals were left untreated. All animals were monitored for changes in Ki67, perforin, and granzymeB in CD4+ and CD8+ T-cells. Plasma concentrations of anti-TIGIT were monitored as well as anti-drug antibodies against anti-TIGIT.

Anti-TIGIT blockade was well-tolerated in cynomolgus macaques. Despite high plasma concentrations of anti-TIGIT antibody and minimal anti-drug antibodies, we observed no consistent improvement in T-cell proliferative capacity or functionality. Plasma viral loads in both groups remained stable indicating the anti-TIGIT antibody treatment failed to increase the antiviral function of CD8+ T-cells.

The enhancement of virus-specific T-cell proliferative responses observed in vitro with single/dual blockade of TIGIT and/or PD-1 highlighted TIGIT as a potential target to reverse T-cell dysfunction. Our studies, however, show that targeting the TIGIT pathway alone was insufficient is ineffectual and that combining checkpoint blockade for mediating a functional cure for HIV is may be the a future path forward.

Poster 80

IL-15/IL-15Ra Synergizes with IL-12 to Enhance NK Cell Function In Vivo in Chronically Infected ART-treated Macaques

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Natural killer (NK) cells are known to limit viremia very early upon HIV/SIV infection. However, during chronic infection, NK function appears decreased. Thus, elicitation of functional NK cells is important for HIV cure strategy. IL-15 and IL-12 individually can activate NK cells during SIV infection. However, the synergistic effect of these cytokines in inducing functional NK cells has not been studied during chronic SIV and following ART.

We performed an in vivo experiment in macaques to evaluate the effect of cytokines IL-15 (20µg/Kg)/IL-15Rα-(100µg/kg) and IL-12 (10µg/Kg) and its combination for the induction of functional NK cells during chronic SIV (week 6 post SIV, 2 weeks prior ART) and following ART (30 weeks of ART). Lymphocytes from blood and lymph nodes were analyzed for functional NK cells using flow cytometry and immunohistochemistry.

Our data demonstrate that administration of IL-15/IL-15Rα plus IL-12 during chronic SIV rapidly enhanced NK cell proliferation (Ki-67) and cytotoxicity (Granzyme-B+) of NK cells in blood and LN. Similarly, the CD16+ (FCgRIII) NK cells and the degranulation capacity of NK cells (CD107a+) were enhanced substantially in the combination group. Interestingly, combined cytokine group during ART enhanced NK cell proliferation with a follicular homing (CXCR5) phenotype compared to other groups.

Altogether, these data suggest that combining IL-15/IL15Rα plus IL-12 treatment increases NK cell function with follicular homing capacity during chronic SIV and following ART, which may significantly contribute to HIV cure strategies, especially in directing functional NK cells to immune-privileged sites (B cell follicles) during ART therapy.

Poster 86

TLR7 agonist administration to SIV-infected macaques receiving cART initiated during chronic infection does not induce plasma viremia

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Lim and coworkers previously showed transient plasma viral load (PVL) increases, viral DNA (vDNA) declines in PBMC and tissues, and, in a subset of animals, prolonged off-cART virologic remission following TLR7 agonist (Vesatolimod (VES) or GS-986) administration in cART-suppressed SIVmac251-infected macaques (Lim SY et al, Sci Transl Med, 2018). However, in several subsequent studies, measurable viral induction was not observed following TLR7 agonist administration in SIV- or SHIV-infected macaques on cART. These subsequent studies utilized earlier cART initiation, lengthier cART treatment before TLR7 agonist administration, and/or less sensitive virologic assays compared to Lim et al, raising the possibility that quantitative or qualitative differences in the established reservoirs, and/or a reduced capacity to detect reactivating virus, may underlie these discrepant findings. To better match the conditions of the Lim study, we treated ten SIVmac239M-infected rhesus macaques with cART starting 65 days post-infection. Beginning 17–18 weeks after cART initiation, six animals received six doses of VES (0.15 mg/kg, oral, once every 2 weeks); the remaining four received vehicle. Despite clear, transient immunomodulatory effects following VES treatment, including IFN-stimulated gene upregulation in blood and tissues, and characteristic modulation of immune cell population phenotypes, VES treatment did not result in measurable increases in peripheral blood or hepatic portal vein PVL, increases in cell-associated vRNA or vRNA:vDNA ratio, nor decreases in vDNA in blood or tissues. Our findings are consistent with a recent clinical VES evaluation and confirm that TLR7 stimulation does not consistently induce robust viral reactivation in vivo.

Poster 94

CCR7+ CD4 TCM Cells Actively Survey Rhesus Central Nervous System During Homeostasis

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Antigen-experienced CD4 and CD8 T lymphocytes are the foundation for immune surveillance but our understanding of their roles in the central nervous system (CNS) is incomplete. Here, we defined the division of memory T cell subsets within the non-inflamed CNS to ascertain if this balance was dysregulated during chronic HIV infection. Examining samples of the brain parenchyma and CNS-associated border tissues (cerebrospinal fluid (CSF) and dura mater) from healthy rhesus macaques, we found that CD4 and CD8 T cells patrolling the CNS possessed distinctive differentiation states. The majority of CD4 T cells expressed high levels of CD28 and exhibited features of either resting/central memory cells or tissue-resident cells. Within CD4 T cells, CD69 and CCR7 expression delineated distinct CD4 subsets in CNS, with CCR7+ CD4 T cells presenting features of central memory T-cells (TCM). Indeed, preventing egress of TCM cells from lymph nodes using Fingolimod significantly decreased frequencies of CCR7+ CD4 T cells in CSF and resulted in increased monocyte influx. In SIVCL757 infected macaques (115 weeks post-infection) on deferred non-adherent anti-retroviral therapy, CCR7+ CD4 TCM frequencies were decreased (2.2 fold, $p<0.05$) with a corresponding increase in frequencies of CD69+CD4 (1.4 fold, $p=0.11$) T cells in the brain parenchyma, CSF & dura mater. Our findings show that CCR7+ TCM-like CD4 T cells constitute an important subset in the CNS during homeostasis and suggest that decreased frequencies of TCM-like cells may drive immune dysfunction in neurodegenerative diseases.

Poster 96

SIV/HIV infection of primary tissue resident mast-cell is modulated by cytokines and soluble factors in the microenvironment

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Mast cells (MCs) are known for their role in allergy and anaphylaxis. They are immune cells of myeloid lineage and are abundantly present in connective tissue throughout the body. Precursors of MCs traffic from bone marrow to the tissue where they mature under the influence of stem cell factor and remain as long-lived resident cells. Their phenotype is modulated by cytokines, provided by the tissue microenvironment

We found that primary skin/gut MCs and MC-derived cell lines express CD4 and CCR5 and they are susceptible to R5-tropic viruses. Considering their long life and high frequency at mucosal sites, which is the primary site of HIV invasion, we investigated primary skin/Gut MCs and MC derived cell lines ex-vivo susceptibility to various R5 Tropic-replication competent-reporter viruses.

Viral replication was assessed by (1) mCherry/GFP expression and (2) tracked by p24 quantification in the supernatant. Adding IL33 and TGFβ to cultures synergistically increased p24 production, when compared to control samples. When p24 production decreased, LPS stimulation could reverse it. (3) Productive infection was confirmed by quantification of pro-viral DNA (gag). Immuno-fluorescent staining of SIV-Envelope and Gag protein in various tissues (female reproductive tract and gut) of Rhesus macaques also confirmed infection of MCs.

Taken together this data suggests that (1) MCs may serve as a viral reservoir and could contribute to viral rebound during ATI. (2) considering their role in inflammation and LPS driven re-activation, they could also contribute to residual viremia during ART and could reseed and recrudesce infection during ATI.

Poster 105

Enhancing HIV-Specific Immunity with Virus-Specific CAR T Cells

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Background
We previously reprogrammed rhesus macaque T-cells with chimeric antigen receptor (CAR) molecules containing the Env-binding CD4 ectodomain (CD4CAR) to target HIV- and SIV-infected cells. Notably, the impact of this approach on post-ART viral rebound was promising but variable. The goal of the current study was to quantify the function of an augmented CD4CAR molecule and compare to key virological correlates in vivo.

Methods
Donor T-cells collected pre-infection (Pre-I) and in SIV/SHIV-infected, ART-suppressed animals (Post-I) were CRISPR-edited, transduced with two CD4CAR vectors containing distinct rhesusized domain structures, and infused into the autologous host. To determine the infused cells' virus-specific function, we analyzed molecular and immunological characteristics of CAR-expressing and SIV TCR-specific T-cells within the infusion product.

Results
Intracellular cytokine expression in response to SIVmac239 gag, pol, and env peptide pools was evaluated in Pre-I and Post-I T-cells within each CD4CAR infusion product. Post-I cells, which should be enriched for virus-specific TCR molecules, exhibited greater polyfunctionality in response to SIVmac239 Env, with the CAR+ fraction contributing to an increase in TNFα and IFNγ.

Conclusion
Our study provides critical preclinical insights on the activity of CD4CAR T-cells in an NHP model of HIV persistence. A clearer understanding of the cellular phenotypes and functions contributing to virus control in vivo will directly aid in the design of analogous approaches in ongoing clinical trials. We are continuing to evaluate the trafficking and distribution of CAR-expressing cells into key tissue sites of HIV persistence, including the gut, brain, and B cell follicles.

ABSTRACTS
POSTER PRESENTATIONS

SCIENTIFIC SESSION 4
COVID and Other Infectious Diseases

POSTER 13

Inhibition of Indoleamine dioxygenase leads to better control of tuberculosis adjunctive to chemotherapy

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The expression of indoleamine 2, 3, dioxygenase (IDO), a robust immunosuppressant, is significantly induced in macaque TB granulomas, where it is expressed on interferon-responsive macrophages and myeloid derived suppressor cells. IDO expression is also highly induced in the human TB granuloma, and products of its activity are detected in TB patients. In-vivo blockade of IDO activity resulted in the reorganization of the granuloma with significantly more T cells being recruited to the core of the lesions. This correlated with better immune control of TB and reduced lung Mtb burdens. In order to study if the IDO blockade strategy can be translated to a bon-a-fide host-directed therapy in the clinical setting of TB, we studied the effect of IDO inhibitor D1MT adjunctive to suboptimal anti-TB chemotherapy. While 66% of controls and 33% of chemotherapy- treated animals progressed to active TB, inhibition of IDO adjunctive to the same therapy protected macaques from TB, as measured by clinical, radiological and microbiological attributes. While chemotherapy improved proliferative T cell responses, adjunctive inhibition of IDO further enhanced the recruitment of effector T cells to the lung. These results strongly suggest the possibility that IDO inhibition can be attempted adjunctive to anti-TB chemotherapy in clinical trials. Shortly, we will do an experiment to assess the effect of IDO inhibition in M.tb-SIV co-infected Rhesus macaques. Here, we will study if IDO inhibition in adjunct to ART/ anti-TB chemotherapy regimens or alone, could serve as better strategy to control TB reactivation in M.tb-SIV infected macaques.

POSTER 18

Therapeutic Neutralizing Monoclonal Antibody Administration Protects Against Lethal Yellow Fever Infection

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Despite ongoing vaccination campaigns, infections caused by Yellow Fever Virus (YFV) result in 200,000 cases and 60,000 deaths annually, with no clinical treatments available. We show that treatment of YFV-infected rhesus macaques (RMs) with two YFV-specific neutralizing monoclonal antibodies (nmAb) prevents severe disease and death. We screened 489 YFV-specific monoclonal antibodies isolated from memory B-cells of YFV-17D vaccinated humans. Of these 489 antibodies, 38 had IC50 values < 200 ng/mL. Two antibodies (MBL-YFV-01 and MBL-YFV-02) were found to neutralize primary Brazilian strains of YFV in vitro with IC50 < 100 ng/mL. These two nmAbs were selected as candidates for in vivo studies. RMs (N=10) were infected subcutaneously with the pathogenic YFV-DakH1279 strain (103 TCID50), and 8 of these animals received an infusion (50 mg/kg) of either MBL-YFV-01 (N=4) or MBL-YFV-02 (N=4) two days post-infection. Both control animals exhibited markers of severe disease including elevated alanine transaminase (ALT) levels and high serum viral loads (sVL, >1011 RNA copies/mL), and met clinical endpoints requiring humane euthanasia by day 5 post-infection. In contrast, all 8 nmAb treated RMs showed no clinical signs of severe disease, maintained low ALT levels and sVL (<5,000 RNA copies/mL), and survived through day 21 post-infection. RNAscope staining of livers from the two control RMs showed widespread YFV RNA expression, while treated RMs had minimal YFV RNA expression. These data show that administration of YFV-specific nmAbs can prevent severe disease in YFV-infected rhesus macaques, providing a strong rationale for further clinical development.

POSTER 43

A model of lymphocryptovirus-associated lymphoproliferative disease in Mauritian cynomolgus macaques

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Background
Immunosuppressed individuals are at risk for developing Epstein-Barr virus-associated lymphoproliferative diseases, such as post-transplant lymphoproliferative disease (PTLD) and AIDS-related lymphomas. Here, we developed a nonhuman primate model of lymphocryptovirus-associated lymphoproliferative disease in SIV-infected Mauritian cynomolgus macaques (MCM) using cynomolgus lymphocryptovirus (CyLCV).

Methods
Five CyLCV-seropositive, SIV-viremic MCM received anti-CD8a depleting antibody and an infusion of autologous B cells infected with CyLCV cultured from a hematopoietic stem cell transplant recipient MCM that developed PTLD. After infusion, MCM received varying degrees of immunosuppression including tacrolimus, belatacept, prednisone, dexamethasone, and/or anti-CD8b depleting antibody. Two MCM underwent fluorodeoxyglucose (FDG)-positron emission tomography (PET) to monitor disease development.

Results
Four of five macaques developed CyLCV plasma viremia and masses detected by ultrasound. Upon necropsy, all five macaques exhibited signs of CyLCV-associated lymphoproliferative disease, ranging from plasmacytic hyperplasia to multicentric lymphomas often involving the stomach, liver, and adrenal glands. Masses contained lymphocytes positive for both CD20 and LCV EBNA2 antigen, high levels of cell-associated LCV DNA, and large frequencies of proliferating (Ki67+) B-cells. Longitudinal FDG-PET of one MCM revealed increasing FDG uptake, particularly in the adrenal glands, during disease development.

Conclusions
These data suggest that MCM represent a promising preclinical NHP model of lymphocryptovirus-associated lymphoproliferative diseases that could be employed to test novel diagnostic and therapeutic modalities.

POSTER 46

Detection and analysis of Zika virus in tissues from the pigtail macaque model of acute Zika virus

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Zika virus (ZIKV) disseminates in a host during acute infection. We isolated ZIKV from NHP plasma and mucosal compartments for viral sequence analysis to assess changes in the ZIKV genome that may contribute to viral tropism. NHP (n=4 females and n=4 males) were inoculated subcutaneously with 5 x 105 PFU of 2015 Brazil ZIKV. Longitudinal plasma, cervico-vaginal, and rectal cytobrush samples were collected for RNA analysis and virus isolation. Samples confirmed ZIKV+ by qPCR were added to C6/36 cells to isolate virus. ZIKV stocks were tittered on Vero cells and subjected to immunofluorescence assay to detect ZIKV E antigen. A total of 7 ZIKV stocks confirmed positive were subjected to total RNA sequencing for viral sequence analysis. Multi-sequence alignment was performed by generating consensus sequences from aligned reads and then aligning those to the ZIKV genome (GenBank: KX811222.1). Alignment to the ZIKV genome exhibited 8% diversity across tissue types. Variant calling was performed to discover intrahost tissue-specific viral genome mutations. Multi-sequence analysis identified 6 amino acid mutations found across tissue types compared to the 2015 Brazil ZIKV challenge virus genome. Unique mutations were found in both the cervico-vaginal and rectal samples. These observations show that ZIKV can be cultured directly from several NHP tissue types following acute infection and within those sample types the ZIKV genome undergoes diversification from the virus inoculum. Thus, these changes in the viral genome may contribute toward viral dissemination from the periphery and into the mucosal tissues enhancing the incidence of sexual transmission of ZIKV.

POSTER 48

Changes in Maternal-Fetal Interface Immunity in a Primary Infection Model of Congenital Cytomegalovirus Infection in Immunocompetent Rhesus Macaques

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Congenital CMV (cCMV) is the leading infectious cause of neurological defects in the newborn affecting roughly 1 in 200 births annually. Yet, a maternal vaccine to prevent in utero transmission remains elusive. cCMV vaccine development is hindered by our gap in knowledge of maternal immune determinants of protection. In this study, we used the placental transmission model of rhesus CMV (RhCMV) to investigate maternal-fetal interface immunity during primary RhCMV infection in immunocompetent pregnant rhesus macaques. Six CMV-seronegative rhesus macaques were inoculated intravenously with two RhCMV strains, FL-RhCMV (a fully repaired molecular clone based on 68-1) and the primary isolate UCD52, using 1x106 pfu each in early 2nd trimester and studied until elective C-section at near term. Transmission of RhCMV to the amniotic fluid was found in 2 of 6 dams. Flow cytometric evaluation at C-section revealed enrichment of granzyme B+ memory CD4+ and CD8+ T lymphocytes in the decidua relative to peripheral blood suggestive of accumulation of cytotoxic CMV-specific decidual T lymphocytes. Compared to gestation-matched healthy CMV-seropositive controls, decidual leukocytes in the primary CMV infection dams showed an increase in effector memory CD8+ T lymphocytes, increase in activated HLA-DR+, granzyme B+ memory CD4+ and CD8+ T lymphocytes, with a decline in CXCR3+ T lymphocytes. Our data demonstrate that RhCMV impacts the immune system at the tissue level of the maternal-fetal interface, irrespective of detectable congenital transmission. Ongoing further studies are likely to provide novel insights into tissue-based immune correlates of protection against cCMV transmission in this model.

POSTER 50

Non-invasive Specimen Collection from Wild Cynomolgus (Macaca fascicularis) and Rhesus (M.mulatta) Macaques for Tuberculosis Detection

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Since the climate changes have led to the interface between humans and wildlife, the zoonotic transmission across species has become a concern. Mycobacterium tuberculosis (MTB) are pathogenic bacteria that cause tuberculosis in both humans and non-human primates. Thailand is listed as one of the 30 high tuberculosis burden countries according to WHO global list. Cynomolgus (Macaca fascicularis; MF) and rhesus (M. mulatta; MM) macaques are wildlife that commonly encounter with humans in Thailand. Previously, the prevalence of MTB in 1,836 wild MF and MM from 32 locations throughout Thailand was surveyed using IS6110-nested-PCR method. Monkeys were captured, anesthetized, collected throat and buccal swabs, and released back to their natal habitats. The positive results were detected in 128 throat swabs (7.0%) and 41 buccal swabs (2.3%) with a significant correlation between these two types of specimens (r2= 0.19, p value = 0.01). Thus, it suggests that buccal swab can be an alternative method of specimen collection for tuberculosis test. Here, we modified a non-capturing, non-invasive rope bait method for buccal specimen collection (Toyoda et al., 2021). The polyester ropes were dipped into syrup and provided to 3 of 17 macaque populations that showed high prevalence of MTB (10–23%). Sixty-nine baited ropes were collected and determined MTB using IS6110-nested-PCR method. The positive results were detected in all three populations; 5.9% (1/17), 3.6% (1/28), and 8.3% (2/24). Results of this study denote that the rope bait can be an alternative non-invasive method to collect buccal specimens for MTB detection in wild macaques.

POSTER 51

Chemokines temporal landscape and their role in myeloid trafficking and lung inflammation in SARS-CoV-2 infection

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Chemokines are crucial mediators of immune responses following pathogenic infections however, their excessive release can lead to hyperinflammation. Several clinical investigations suggest a direct role of chemokines in lung symptoms of COVID-19, but their role has not been fully elucidated. Here, we investigated the early dynamics and role of chemokines in mediating the trafficking of myeloid phagocytes to the lung during SARS-CoV-2 infection in macaques. We used in vivo 5-bromo-2'-deoxyuridine (BrdU) and serial collections of blood and bronchoalveolar lavage to capture the marrow egress of myeloid cells. We measured an array of chemokines involved in lung trafficking by mesoscale. Viral replication was measured in the nasal cavity, and lungs.We observed a rapid increase of myeloid cell egress from the bone marrow that peaked at five days following exposure to SARS-CoV-2. In addition, we identified a group of three chemokines in plasma, CCL2, IP-10, and CCL17, that were strongly associated with the levels of viral replication in the nasal cavity and the lungs. CCL2, a key mediator of monocyte migration into the lung, was also highly correlated with monocytic egress and worse disease scores. We corroborated the possible relevance of these chemokines in humans; the levels of CCL17 and IP-10 were elevated in the plasma of 20 adults following acute SARS-CoV-2 infection compared to non-infected adults that received three doses of SARS-CoV-2 vaccine (n = 20). These data indicate chemokines' critical and early role in regulating COVID-19 progression and emphasize the importance of understanding their dynamics for developing therapeutic strategies.

POSTER 52

Role of Type I Interferons in COVID-19

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Background

Amidst the COVID-19 pandemic, unprecedented efforts culminated in the rapid development of nonhuman primate models of COVID-19 which helped in accelerating vaccine testing and therapeutics development. Now, the emergence of mutant SARS-CoV-2 strains necessitates a better understanding of the early viral dynamics, host responses and immunopathology.

Methods

Single-cell RNA sequencing (scRNAseq) allows for deep immunophenotyping of individual cells, uncovering heterogeneous and variable responses to environment, infection and inflammation. Combining the rhesus macaque model of COVID-19 with 10x scRNAseq platform we characterized the immune responses triggered in the airways of young macaques during acute and post-acute phases of SARS-CoV-2 infection.

Results

Our scRNAseq analyses of longitudinal bronchoalveolar lavage (BAL) cells from young rhesus macaques demonstrated distinct dynamics of immune cells. We observed the accumulation of distinct populations of both macrophages and T-lymphocytes expressing interferon-driven inflammatory gene signature as well as induction of a myeloid cell-mediated Type I IFN response.

Conclusion

Type I interferons (IFN) possess critical anti-viral functions. Our results point to the importance of early innate immune responses and cytokine signaling, particularly Type I IFN signaling, in protecting against COVID-19. Further GSEA analysis of upregulated genes in these macrophages revealed interferon-driven innate antiviral defense and negative regulation of viral genome replication suggesting a prominent role of macrophages-driven innate immunity in the resolution of SARS-CoV-2 infection. Paradoxically, late- and end-stages of COVID-19 disease are characterized by Type I IFN-driven cytokine storm. We are now performing mechanistic studies to dissect the specific role of IFN-signaling in SARS-CoV-2 infected macaques.

POSTER 58

Atypical Astrocytes as a Marker of Exposure to an Acute Maternal Zika Virus or Influenza A Virus Infection in Fetal White Matter Near the Putamen

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Background

Infections in pregnancy can cause neurodevelopmental or neuropsychiatric disorders in the offspring. We investigated commonalities in fetal brain pathology in pregnant nonhuman primate models of Zika virus (ZIKV) and influenza A virus (IAV) infections.

Methods

Second- or third-trimester pregnant pigtail macaques were inoculated with ZIKV (n=16), IAV (n=7) or saline/media (sham; n=7) and necropsied in the third trimester. Fetal brain tissues were examined histologically, immunohistochemically, and ultrastructurally. Digital spatial mRNA profiling was performed using the Nanostring GeoMx.

Results

In ZIKV and IAV models, fetal brains necropsied 4-22 days after third-trimester maternal inoculation contained abundant atypical astrocytes primarily in a discrete region of white matter ventral to the putamen. The cytoplasm of these "inclusion cells" was packed with eosinophilic and periodic-acid-Schiff-positive granules shown by immunohistochemistry (LAMP1-positive) and electron microscopy to be multivesicular bodies. Similar cells were absent or rare in other parts of the brain, sham controls, or fetuses from dams infected earlier in gestation with a longer time interval between infection and delivery. Differential gene expression indicated an increase in gene sets associated with neurological disease and organismal injury in the inclusion cell-rich white matter. Neither electron microscopy, immunohistochemistry nor PCR demonstrated evidence for active ZIKV infection within the inclusion cells.

Conclusions

Atypical astrocytes, packed with multivesicular bodies, within a discrete region of fetal white matter is a previously unrecognized response to acute maternal ZIKV or IAV infection, independent of active brain infection. The mechanism underlying this striking cellular phenotype and its clinical significance need further investigation.

POSTER 61

Tyrosine Kinase Inhibitor Masitinib Does Not Reduce Viral Replication in SARS-CoV-2 Infected Rhesus Macaques

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Background

As SARS-CoV-2 continues to infect and re-infect individuals despite high community seropositivity, treatments to reduce morbidity are critical, particularly those which might reduce viral shedding and infectiousness. The tyrosine kinase inhibitor masitinib has been evaluated for cancer treatment in clinical trials, and has been shown to directly bind the SARS-CoV-2 protease. Masitinib inhibits viral replication in vitro and significantly reduced viral titers in the lungs of ACE2 transgenic mice.

Methods

We dosed rhesus macaques with approximately 10 mg/kg daily oral masitinib from day -1 to day 8 of intranasal/intratracheal infection with 2x10^6 TCID50 units US/WA-1/2020 SARS-CoV-2. Untreated control animals were similarly infected or inoculated with inactivated virus to assess decay of viral RNA (vRNA) from inoculum. Animals were monitored for fever, weight loss, respiratory distress, inflammation, antiviral immune responses, and vRNA levels in acute infection; necropsies were conducted at day 10 post-infection.

Results

Infection was achieved with viable virus recovered from respiratory tract samples, and SARS-CoV-2 specific immune responses were induced. Masitinib treated animals did not demonstrate significantly different vRNA levels in nose or throat swabs over controls, and did not have significantly different symptomology, inflammation, or antiviral responses. Furthermore, treated macaques did not demonstrate significantly different indicators of gastrointestinal pathology.

Conclusions

Although masitinib demonstrated considerable antiviral activityin vitroand in a mouse model of COVID-19, we did not detect similar antiviral activity in a rhesus macaque model of SARS-CoV-2 infection. These results indicate the importance of NHP models in comprehensively evaluating drug treatments for human efficacy.

POSTER 76

Sequential Immunization of Updated SARS-CoV-2 DNA and RNA Vaccines Expressing Novel RBD Immunogens From Different Variants of Concern Induce Broadly-Specific Antibody Responses in Nonhuman Primates

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SARS-CoV-2 vaccines that expand immunity against emerging variants of concern (VOCs) are needed. Current mRNA vaccines express Spike from the original strain, but waning antibodies and VOCs are diminishing efficacy, prompting development of updated vaccines matching VOCs. We investigated the effects of sequentially boosting with updated DNA or RNA vaccines.

Cynomolgus macaques were immunized by Gene Gun (GG) or intramuscularly with lipid nanoparticle (LNP) formulation at weeks 0 and 6 with an IL-12 adjuvanted DNA vaccine (GG/DNA N=5) or with a self-amplifying replicon RNA (repRNA) vaccine (GG/repRNA N=5, LNP/repRNA N=4) encoding SARS-CoV-2 A.1 prefusion Spike. Macaques were boosted at week 17 with DNA or repRNA vaccines expressing SHARP, a novel chimeric immunogen comprising B.1.351 and B.1.617 Spike receptor binding domains (RBD) fused to influenza HA2 stem domain (designed by AIR/JP). Binding antibody responses specific for full length Spikes and RBDs of VOCs were measured.

Two doses of the A.1 Spike vaccines induced broad antibody responses against all VOCs. The LNP/repRNA vaccine induced the highest titers. Updated VOC SHARP immunogens significantly boosted antibody responses against all VOCs and by week 25, all 3 groups had comparable antibody titers against each VOC. Binding and neutralizing antibody determinations against a broad range of coronaviruses are in progress.

These results demonstrate that updated vaccines can boost for broadly specific antibodies against VOCs including Omicron (which was not included in the boosting vaccine) and show that Gene Gun delivered DNA and RNA vaccines induce comparable responses to LNP-delivered RNA vaccines.

POSTER 91

Interaction of SARS-CoV-2 spike protein with Amyloid beta

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The long-term consequences of SARS-CoV-2 infection on accelerated aging and age-related neurodegenerative disorders are unknown. SARS-CoV-2 might induce a worsening cognitive decline in Alzheimer's disease (AD) patients. In this study, we have investigated the interactions of SARS-CoV-2 spike (S) protein using HADDOCK protein docking software, and by amyloid aggregation assay in which monomeric form of Aß1-42 was incubated with S protein. Spike protein induced higher rates of Aß1-42 aggregation compared to control. Similar increase in Aß1-42 aggregation was observed in the presence of SARS-CoV-2 pseudovirus. Aß melting temperature reflects intrinsic protein stability, which arises from non-covalent forces therefore we assessed the effect of spike protein on Aß1-42 melting temperature. Consistent with our hypothesis, we observed significant increase in Aß1-42 melting temperature in the presence of spike. To provide direct physical evidence of enhanced Aß aggregation, we performed atomic force microscopy (AFM). The results indicate significantly increased Aß1-42 aggregation and fibril length in the presence of spike protein. We then tested the effects of Aß with or without spike on mouse hippocampal cell line. Cell viability was significantly reduced in the presence of Aß alone, and Aß and Spike, but not with Spike alone, suggesting that spike protein exerts pathogenic effect through interaction with Aß. To the best of our knowledge, our studies identify, for the first time, evidence of interaction between SARS-CoV-2 spike protein and Aß. Our data suggest toxicity of spike-Aß interactions on neurons and identify a novel mechanism of SARS-CoV-2 induced neuropathology.

ABSTRACTS
POSTER PRESENTATIONS

SCIENTIFIC SESSION 5
Genomics and Emerging Technologies

POSTER 3

Characterizing the phenotypic and genetic changes of pre-epidemic HIV-2 Group F virus following serial passage in humanized mice

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HIV-2 emerged from the cross-species transmission of SIV native to sooty mangabeys into humans nine independent times giving rise to genetically diverse viral lineages (A-I). HIV-2 groups A and B are responsible for the ongoing epidemic, while the remaining groups were from individual cases except for HIV-2 Group F, which was isolated from two separate individuals, one of whom was symptomatic. The lack of human-human spread and milder symptoms of these atypical HIV-2 groups suggests that they are not fully adapted to humans but may represent transitionary intermediate viruses between non-adapted SIVsm and virulent HIV-2. Humanized (hu)-mice provide an effective model for characterizing HIV-2 evolution that goes beyond what is possible in nonhuman primates. Following engraftment, hu-mice become reconstituted with a wide range of human immune cells. Previously, we characterized the necessary evolutionary genetic changes that arose from serial passaging SIVsm into HIV-2. Here, we serially passaged HIV-2 Group F in hu-mice to identify the evolutionary genetic changes in comparison to those that arose after serially passaging SIVsm. Viral adaptation and pathogenicity were assessed through plasma viral loads and CD4+ T cell decline. HIV-2 Group F plasma viral loads increased over two serial passages, while CD4+ T cell decline was moderate, indicating that HIV-2 Group F is still adapting to human immune cells. Additionally, Illumina-based deep sequencing identified multiple nonsynonymous mutations that increased in frequency over time, primarily in gag, env, and nef. Further analysis of these changes may shed light onto the nature of HIV-2 adaptation.

POSTER 70

Increased Chemokine Production Is A Hallmark Of Rhesus Macaque Natural Killer Cells Mediating Robust Antibody-Dependent Cellular Cytotoxicity

Junsuke Nohara[1], Tyler Evangelous[1], Madison Berry M.S.[1], Shalini Jha M.S.[1], Whitney Beck[1], Sarah Mudrak Ph.D.[1], Keith R Reeves Ph.D.[1], Justin Pollara Ph.D.[1], Bradley C Todd Ph.D.[2], Georgia Tomaras Ph.D.[1], Guido Ferrari M.D.[1]

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Background
In Rhesus Macaques (RM), Natural killer (NK) cells have been demonstrated to play a crucial role in the protection conferred by some HIV vaccine candidates by mediating antibody-dependent cellular cytotoxicity (ADCC). We intended to fill a gap in our knowledge of the molecular pathways of RM NK cells responsible for ADCC.

Methods
We collected PBMCs from six RM and measured ADCC mediated by a pool of antibodies with different epitope specificities. NK cells were subsequently sorted into degranulating (CD107a+) and non-degranulating (CD107a-) populations and analyzed via single-cell RNA sequencing (scRNAseq) to identify differences between and within the two populations.

Results
Seven NK cell clusters with distinct gene expression profiles were identified in every RM. Degranulating NK cells displayed activation-associated signatures, and 55.38% of these cells segregated into two clusters with high expression of chemokine transcripts such as MIP-1α, MIP-1β, and XCL1. In contrast, non-degranulating NK cells displayed a higher proportion of cells with a deficiency in ribosomal proteins, suggesting a dysfunctional profile. Importantly, trajectory analysis suggested that degranulating cells originated from a population of non-activated NK cells that could commit to activation and cytokine production.

Conclusion
Our study provides for the first time insights into the cellular transcriptome diversity at single-cell resolution among and within the RM NK cell. We observed a unique presence of a chemokine producer population. Since MIP-1α/β can recruit other immune cells and prevent virus entry by binding to CCR5, this suggests an anti-viral role for NK cells broader than just mediating ADCC.

POSTER 70

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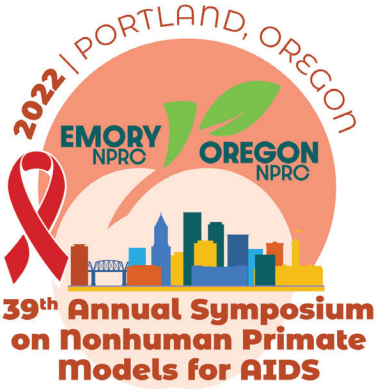
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