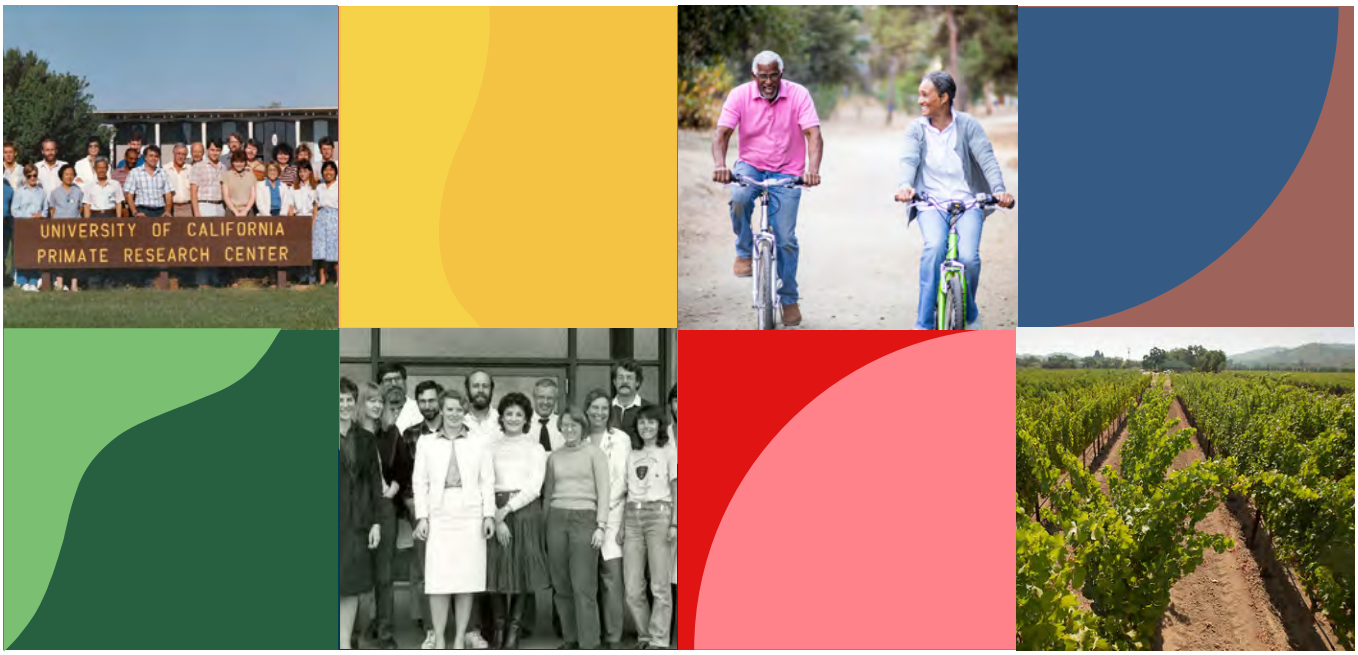
CALIFORNIA NPRC PRESENTS

40TH Annual Symposium on Nonhuman Primate Models for AIDS

DAVIS, CA 2023

October 10 - 13, 2023



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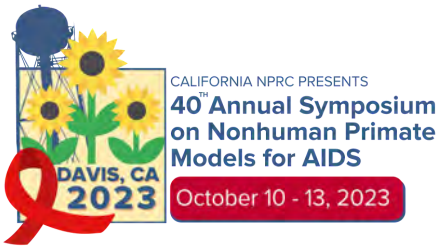

CALIFORNIA NPRC PRESENTS
40th Annual Symposium on Nonhuman Primate Models for AIDS
October 10 - 13, 2023

WELCOME MESSAGE

We welcome you to the 40th Annual Symposium on Nonhuman Primate Models for AIDS, held on October 10-13, 2023, at the University of California Davis Activities and Recreation Center in Davis, California. This symposium is organized by the California National Primate Research Center.

The 40th annual symposium is a unique, world-class opportunity to exchange the latest scientific perspectives, research findings, and emerging technologies for HIV/AIDS and other infectious diseases. The nonhuman primate model is integral to studies that make breakthrough discoveries of causes, preventions, treatments, and cures possible.

We look forward to investigators coming together to present and discuss their work advancing the translation of NHP studies into real-world therapeutic, prophylactic vaccine, and cure strategies. Together we will share the vision to end HIV/AIDS and improve animal and human health.

Vibrant, walkable, bikeable, and fun, Davis always has something going on. Enjoy cycling, culinary delights, shops, breweries, wineries, and an award-winning farmers' market. Davis has something for all ages! Check out visitdavis.org to explore the many amazing things to do in and around Davis, California!

In addition, we welcome you to extend your stay to explore and enjoy the beauty of Northern California. Major tourist attractions such as the Coast with San Francisco, Napa Valley, and the Sierra Nevada with Lake Tahoe are all within a 1 to 2-hour drive from Davis.

Your Host,

UCDAVIS
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Research Center

MESAGE FROM THE ORGANIZING COMMITTEE

Dear colleagues,

A warm welcome to the 40th Annual Symposium on Nonhuman Primate Models for AIDS! We are delighted to see many of you here in person, and happy that others are able to participate online.

This current milestone is a good opportunity to look back on our journey, reflect on our achievements, and share our vision of a world, free of HIV and AIDS, on the horizon. We are grateful to follow the footprints of the early pioneers whose scientific curiosity, dedication, and bold steps, embarked us on this journey with the first nonhuman primate models of HIV/AIDS, which built the foundation for all the achievements we have witnessed over the past 4 decades. Despite many obstacles and hurdles, the perseverance of investigators, innovative use of NHP models and the continuous improvement of resources and technologies, have led to major progress in many areas of HIV research, from basic research - gaining better insights in viral infection, immunology and pathogenesis - to translational "bench-to-bed" research. Many discoveries originally made in nonhuman primate models have led to interventions that have made a tremendous difference for millions of people across the globe. While we currently don't have an effective and affordable HIV vaccine or cure yet, the research data that over the coming days you will present and hear about from others are very promising, and offer an exciting glimpse into further developments that we can expect the coming years. It is also humbling to witness how expertise developed in NHP models of AIDS also benefits many other disease models, and how novel technologies, applied to HIV/AIDS research, unlock doors of new opportunities.

We are especially happy to welcome many young investigators to this symposium. Your fresh energy, creativity, and commitment gives us - the older generation – the confidence that we can gradually pass the baton and know the future of this research is in the right hands. Thank you! We are happy that this year we were able to give 25 Travel Awards to junior investigators.

Over the next few days, we look forward to interacting with many of you, both during the scientific sessions, as well as during the breaks. We encourage you all to connect with other investigators to explore new ideas and collaborations.

A special thanks goes to the Scientific Committee, with representatives from all seven National Primate Research Centers and other academic institutions. The committee spent many hours reviewing and scoring more than 110 abstracts to bring you the exciting and diverse presentations you'll learn about in the coming days in each of the 5 scientific sessions. We are also grateful to our keynote speaker, and chairs and co-chairs of each of the sessions, for sharing your experiences and contributing to the success of this symposium.

This opportunity to gather and share our science would not be possible without the generous 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 more about additional research support services offered by the NPRCs.

We look forward to a great Symposium!

Cheers,

Koen Van Rompay DVM, PhD
Jeffrey Roberts, DVM, DACLAM
Gregory Timmel, DVM, DACLAM
JoAnn Yee, CLS/MLS(ASCP)

Thanks to Simian AIDS Researchers
By Dr. Murray B. Gardner

40 years ago
Infectious disease
Was of little interest
To medical trainees

For non-human primates
Thinking was the same
Genetics and the environment
Were the “name of the game”

Immune suppression
Commonly seen
Unusual infections
Appeared on the scene

Everything changed
Virtually overnight
When AIDS in humans
Came into sight

Veterinary and Medical Schools Pooled
their talent
In a research effort
That was truly valiant

An infectious virus
Was found the cause
Of the disease in humans
And monkeys, without pause

The Simian AIDS model
Became indispensable
It made effective treatment
Comprehensible

Our Primate Centers
And researchers who knew
Were major contributors
We thank all of you!

Simian AIDs Researchers at the California National Primate Research Center

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Samuel Johnson

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Lauren Rust

Thatiane Russo

Yanique Thomas

Arora Garima

Arpan Acharya

Lakmini Premadasa

Muhammad Shoaib Arif

Vidya Vijayan

Arpan Pradhan

Jayme Nordin

Orlando Cervantes

Sonny Elizaldi

Patricia Hahn

Yasmine Issah

NAMED TRAVEL AWARDS

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. This award is sponsored by the Emory National Primate Research Center/Texas Biomedical Research Institute.

Recipient: Brandon Beddingfield (Tulane National Primate Research Center)

Andrew Alan Lackner Memorial Travel Fellowship

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. This award is supported by the Southwest National Primate Research Center.

Recipient: Kyle Rhodehouse (The Johns Hopkins University School of Medicine)

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. This award is supported by the Wisconsin National Primate Research Center.

Recipient: Katherine Turnbull (Tulane National Primate Research Center)

ORAL PRESENTATIONS

Session 1: SIV/SHIV Pathogenesis and Co-Infections

Moderators: Dr. Ivona Pandrea (Univ of Pittsburg)/ Dr. Satya Dandekar (UC-Davis)

Session chair presentation: Dr. Ivona Pandrea: The discrete mechanisms of HIV/SIV disease progression: Role of gut dysfunction.

1.

Targeting Indoleamine-2, 3 dioxygenase (IDO) blockade as a host-directed therapeutic strategy for TB-HIV co-infection

Bindu Singh PhD[1], Ruby Escobedo[1], Erin McCaffrey[2], Michael Angelo[2], Shannan Hall-Ursone[1], Edward J. Dick[1], Xavier Alvarez[1], Deepak Kaushal[1], Smriti Mehra[1]
[1]Southwest National Primate Research Center, San Antonio, Texas, USA. [2]Stanford University, San Francisco, California, USA

Abstract

Background: The TB and HIV co-pandemic continues to be a major healthcare burden. HIV co-infection predisposes the host to TB reactivation, resulting in significant mortality. Granulomas are central to TB pathogenesis; hence, remodeling these lesions can enhance effectiveness of anti-TB responses, reducing HIV-induced reactivation. In macaques, IDO results in inhibition of adaptive immune responses to TB. Blockade of IDO resulted in reorganization of TB granulomas, enhancing effectiveness of anti-Mtb responses. Recently, multiplexed imaging revealed that CD8-T cells mobilization to granuloma core is key to granuloma reorganization and improved Mtb killing. We now seek to study if IDO signaling blockade alone/in adjunct to ART regime could ameliorate immune responses and prevent TB reactivation in latent TB-SIV co-infected macaques.

Methods: In one experiment, macaques with controlled infection were D1MT-treated (or not) and subsequently SIV co-infected, in order to assess residual TB. In another experiment, latent TB macaques were infected with SIV; followed by subsequent ART treatment in presence/absence of D1MT.

Results: IDO inhibition by D1MT in latent TB macaques appears to prevent SIV-induced TB reactivation compared to untreated controls; where former group exhibited improved disease attributes and antigen-specific immune responses. We are assessing if adjunct D1MT and ART treatment could improve Mtb-killing in Mtb-SIV co-infection setting.

Conclusion: Inhibition of IDO-mediated immunosuppression limited Mtb persistence in granulomas, leading to reduced rate of TB reactivation. If D1MT can be used to kill Mtb in Mtb-SIV co-infected macaques adjunctive to ART, this would potentially represent an attractive host-directed therapy in Mtb-HIV co-infected people.

2.

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

Riti Sharan PhD[1], Smriti Mehra PhD[1], Vijay Velu PhD[2], Jyothi Rengarajan PhD[2], Deepak Kaushal PhD[1]
[1]Texas Biomedical Research Institute, San Antonio, Texas, USA. [2]Emory University School of Medicine, Atlanta, Georgia, USA

Abstract

Background: The immune functions to Mtb adversely impacted by HIV remain impaired despite cART. Skewed pulmonary CD4+ TEM responses persist and new TB lesions form despite cART treatment. We hypothesize that coadministration of 3HP with cART would result in superior control of immune activation.

Methods: Six RMs were infected with low dose Mtb via aerosol and co-infected with SIVmac239. They were administered cART+3HP at 2 weeks post-SIV co-infection for 12 weeks. Bacterial burden, PET/CT scans, high parameter flow cytometry, bulk RNAseq, scRNAseq on longitudinally collected BAL samples were performed.

Results: Five out of six macaques treated with cART+3HP were completely devoid of pulmonary bacteria with significantly less lung lesions and pathology relative to macaques treated with cART only. Despite significantly higher Mtb-specific T cell responses, particularly CD4+IFNg+ and CD4+IL-17+ in cART+3HP treated macaques, the concurrent treatment failed to rescue the depleted CD4+ TEM responses in the lungs. Additionally, there was no significant difference in the expression of CXCL8, M1 macrophages, and TH1 transcripts in macaques with concomitant cART+3HP relative to cART only cohort.

Conclusion: Concurrent administration of cART and 3HP improves clinical attributes of the co-infection; inflammation in the lung is however not ameliorated. Our results suggest that Mtb/HIV co-infected individuals may remain at risk for progression due to subsequent infections or reactivation due of persisting defect in pulmonary T cell responses. By identifying lung-specific immune components in this model, it is possible to identify pathways that can be targeted for host-directed adjunctive therapies for TB/HIV co-infection.

3.

Immunopathogenesis and Early Antiretroviral Treatment in SHIV and *Mycobacterium tuberculosis* Coinfected Infant Rhesus Macaques

Katherine Turnbull DVM[1], Huanbin Xu PhD[1], Eunice Vincent[1], Chad Roy PhD[1], Robert Blair DVM, PhD[1], Peter Didier DVM, PhD[1], Smriti Mehra PhD[2], Deepak Kaushal PhD[2], Xiaolei Wang PhD[1]
[1]Tulane University, Tulane National Primate Research Center, Covington, LA, USA. [2]Southwest National Primate Research Center, San Antonio, Texas, USA

Abstract

Background:

Tuberculosis (TB), a disease caused by the bacteria *Mycobacterium tuberculosis* (*Mtb*), still accounts for a huge burden of morbidity and mortality worldwide. Coinfection with Human Immunodeficiency Virus (HIV) further exacerbates and accelerates Mtb infection which is well studied in adult nonhuman primates (NHPs) but limitedly in infants. Currently, a fully optimized clinically relevant pediatric model appropriate for immunopathogenesis studies is unavailable.

Methods:

Eight infant macaques were intravenously infected with Simian-HIV (SHIV) at birth and four of them received early antiretroviral therapy (ART) throughout the study. These SHIV-infected infants along with an additional four naive infants were infected with a low-dose of *Mtb* via aerosol exposure at the same age and monitored for clinical outcomes and disease progression for up to six months before euthanasia. Histological assessment and immunopathological changes were examined in samples collected during the study and at necropsy.

Results:

Untreated SHIV-infected infants progressed to TB pneumonia characterized by the presence of severe pulmonary granulomatous lesions, altered blood chemistries, enhanced immune activation, and cell proliferation within 6 weeks post *Mtb* infection reaching euthanasia endpoints. In contrast, early ART restored most biochemical parameters to normal levels and significantly ameliorated SHIV/*Mtb* coinfection in infant macaques.

Conclusions:

Our study demonstrated that disease progression is accelerated in SHIV/*Mtb*-coinfected infants and early ART could greatly improve host immunity, metabolism, and survival in infants. These findings suggest that SHIV/*Mtb* co-infected infant macaques could recapitulate key properties to understand the immunopathogenesis and test therapeutic strategies for pediatric HIV/*Mtb* coinfection in clinical settings.

4.

Prolonged SARS-CoV-2 Viral Burden and Impaired Immune Response Generation in SIV/SARS-CoV-2 Co-Infected Rhesus Macaques

Megan N Fredericks[1], Hillary Tunggal[1,2], Cecily C Midkiff[3], Jeanna Barrow[2], Anthony Cook[4], Robert V Blair[3], Ankur Sharma[4], Deborah H Fuller[1,2], Megan A O'Connor[1,2]
[1]University of Washington, Seattle, WA, USA. [2]Washington National Primate Research Center, Seattle, WA, USA. [3]Tulane National Primate Research Center, Covington, LA, USA. [4]BIOQUAL, Inc, Rockville, MD, USA

Abstract

Background

People living with HIV (PLWH) have increased risk of morbidity and mortality from COVID-19. SARS-CoV-2 infection in PLWH poses a risk of prolonged infection and viral shedding, and emergence of variants of concern. Using the SIV macaque model for AIDS, we hypothesize that immune dysfunction during HIV infection will impact SARS-CoV-2 viral infection and COVID-19 disease.

Methods

Eight rhesus macaques were intravaginally infected with SIVmac251, then intranasally and intratracheally inoculated with SARS-CoV-2 (WA-1) at 17-34 weeks post-SIV innoculation. Blood, bronchoalveolar lavage, stool, and nasal, oral, and rectal swabs were collected pre-infection through 14 days post-infection (DPI). ELISAs, ELISPOT, qRT-PCR, lung pathology, cytokine multiplex, and virus neutralization assays were performed to measure viral loads, pathogenesis, and immune responses.

Results

We observed no significant changes in SIV or SARS-CoV-2 clinical symptoms, or SIV viral loads. Notably, SARS-CoV-2 RNA levels in nasal swabs were significantly higher at 7-14 DPI in SIV+/SARS-CoV-2 infected rhesus when compared to published data in SIV- rhesus (PMCID: PMC8462335, PMC8829873). Additionally, SARS-CoV-2 replication persisted in the upper, but not the lower respiratory tract. Anti-SARS-CoV-2 binding antibodies in sera were significantly lower in the SIV+ macaques when compared to that from historical SIV-/SARS-CoV-2 infected controls. Furthermore, SARS-CoV-2 spike-specific T-cell responses were dampened at 14 DPI.

Conclusions

Our results suggest that SIV-induced immunosuppression impairs generation of anti-SARS-CoV-2 immunity, that may, in turn, contribute to prolonged SARS-CoV-2 viral shedding, increased transmission windows, and enhanced disease. Studies in progress will determine if SARS-CoV-2 viral evolution is accelerated in SIV-infected macaques.

5.

Immunological and Metabolic Patterns in Ovariectomized SIV-Infected Rhesus Macaques Receiving Estrogen Replacement

Heather Hofmeister, Diana Takahashi, Kristin Sauter, Gabriela Webb, Oleg Varlamov, Charles Roberts, Jonah Sacha, Paul Kievit
Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, Oregon, USA

Abstract

Introduction: Women living with HIV (WLWH) comprise 54% of adults living with HIV globally. Since the introduction of effective antiretroviral therapies (ART), WLWH have near-normal life expectancy and may experience age-related hormonal changes. Increased age, infection, and hormonal changes put WLWH at higher risk for developing metabolic disorders such as obesity, diabetes, and cardiovascular disease. We therefore investigated effects of estrogen replacement on post-menopausal WLWH in a rhesus macaque model.

Methods: Twelve rhesus macaques were infected with SIVmac239M and received ART two weeks post-infection (PI). All animals were ovariectomized (OVX) at 35 weeks PI and received estrogen (n=6) or cholesterol implants (n=6). Weekly blood samples for virus and estrogen levels were collected and longitudinal metabolic assessments and tissue biopsies were collected.

Results: SIV infection resulted in body weight loss (10%) that rebounded as animals achieved ART-associated viral suppression around 25 weeks. Following OVX, estrogen-treated animals demonstrated downward trends in fat mass while retaining bone mineral density. CD4+ T-cells in whole blood and adipose tissue decreased significantly with infection and slightly rebounding post-viral suppression. After 3 months, estrogen replacement reduced the CD4+ cell population rebound in omental adipose tissue.

Conclusion: ART begins remediating negative effects of SIV on metabolic and immunological functions while estrogen replacement post-menopause may attenuate the effects of hormone deficiency and infection. Metabolic changes, as well as additional data for inflammation and immune cell populations will be reported at the conference (8 months post-OVX). Our novel model will continue to study the impact of estrogen replacement in WLWH.

6.

In vivo serial passaging of human-simian immunodeficiency virus clones identifies viral characteristics for persistent viral replication

Rajesh Thippeshappa PhD[1,2], Patricia Polacino[3], Shaswath S Chandrasekar[4], Khanghy Truong[4], Anisha Misra[4], Paula C Aulicino[5], Shiu-Lok Hu[3], Deepak Kaushal[6,7], Jason T Kimata[4]
[1]Texas Biomedical Research Institute, San Antonio, Texas, USA. [2]Southwest National Primate Research Center, San Antonio, Texas, USA. [3]University of Washington, Seattle, WA, USA. [4]Baylor College of Medicine, Houston, TX, USA. [5]Laboratorio de Biología Celular y Retrovirus, Hospital de Pediatría "Juan P. Garrahan"-CONICET, Buenos Aires, N/A, Argentina. [6]Texas Biomedical Research Institute, San Antonio, TX, USA. [7]Southwest National Primate Research Center, San Antonio, TX, USA

Abstract

Inability of HIV-1 to replicate in macaque cells due to the presence of retroviral restriction factors has complicated the development of true nonhuman primate model of HIV-1 infection/AIDS. Since pigtailed macaques (PTMs) lack TRIM5α, and APOBEC3 family of proteins can be counteracted by SIV encoded Vif protein, we constructed Human-Simian Immunodeficiency virus (HSIV) clones by substituting HIV-1 vif with SIVmne027 vif. We constructed both CXCR-4 tropic (HSIV-vif$_{NL4-3}$ based on pNL4-3) and CCR5-tropic (HSIV-vif$_{AD8}$ and HSIV-vif$_{YU2}$ based on pNL-AD8 and Bru-Yu2 respectively) HSIV clones that replicated efficiently in PTM PBMCs. In vivo, HSIV-vif$_{NL4-3}$ replicated persistently for nearly 4 years, suggesting that counteracting APOBEC3 family of proteins enables HIV-1 replication in PTMs. However, infection did not result in high peak viremia and setpoint viral loads as observed during SIV infection of macaques. To further adapt HSIV, we conducted three rounds of serial in vivo passaging in immunocompetent PTMs starting with an initial inoculum containing a mixture of CXCR4- and CCR5-tropic HSIV clones. Interestingly, all the macaques showed peak plasma viremia close to or above 105 copies/ml and persistent viral replication for at least 20 weeks. We have standardized a quick and easy cloning approach to generate infectious molecular clones (IMCs) from proviral DNA. We recovered three IMCs from passage 3 macaque (HSIV-P3 IMCs), which showed nonsynonymous mutations throughout the genome, suggesting adaptation to PTMs. We are currently conducting further in vivo passaging of HSIV-P3 IMCs to generate pathogenic variants, which will be a valuable resource for preclinical vaccine studies.

7.

Epigenetic Age Acceleration In A Progressing SIVmac Infection In The Rhesus Macaque

Anna J Jasinska[1,2], Juozas Gordevicius[3], Robert T Brooke[3], Cuiling Xu[1,4], Sindhuja Sivanandham[1,4], Ranjit Sivanandham[1,4], Cristian Apetrei[1,5], Ivona Pandrea[4,5]
[1]Division of Infectious Diseases, DOM, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA. [2]Department of Molecular Genetics, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Wielkopolska, Poland. [3]Epigenetic Clock Development Foundation, Torrance, CA, USA. [4]Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA. [5]Department of Infectious Diseases and Immunology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA

Abstract

Background: HIV infection increases the risk of early onset of chronic diseases typically developing later in life, leads to a shortened lifespan, and premature DNA methylation (DNAme) changes. We applied pan-mammalian and primate DNAme-based epigenetic clocks, which are objective markers of biological aging, to assess the effect of pathogenic SIVmac infection on the process of aging in the rhesus macaque (RM).

Methods: We analyzed DNAme profiles in 56 tissue samples from the colon, liver, heart, fat, and spleen from the late stage of the SIVmac infection in RMs, and conducted a clock analysis and epigenome-wide association studies (EWAS).

Results: We compared the epigenetic age acceleration in tissues collected from young and old SIV-infected monkeys. Several clocks showed that aging is accelerated in young individuals in the cerebellum, heart, spleen, liver, and colon. In the old adults, colon was the only tissue with a higher age acceleration compared to the young individuals. Duration of infection was associated with massive DNAme changes in the liver, but also involved changes of CpGs in the cerebellum, colon, heart, and spleen, including ubiquitous hypomethylation in the *ACACA*, *ARHGAP23*, *AUTS2*, and *TFAP2A* gene regions, and frequent hypermethylation in the *TNRC6A* and *ANKS1B* regions.

Conclusion: This is the first direct evidence in the RM model that the infection drives age acceleration in tissues, thus providing a mechanistic explanation for the development of multiple comorbidities in the people living with HIV (PWH). Detailed analyses of gene changes may point on geroprotective therapeutics to improve quality of PWH life.

8.

Cocaine addiction affects SIV Reservoirs

Arpan Acharya PhD[1], Urvinder K. Sardarni PhD[1], J J Hudson BS[1], Rajesh Rajaiah Phd[1], Dhananjay Shinde PhD[2], Vinai C Chittezham Thomas[2], Shannon Callen[1], Shilpa J. Buch PhD[1], Siddappa N. Byrareddy PhD[1]
[1]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, USA. [2]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, USA

Abstract

Cocaine has high abuse liability and is associated with high-risk sexual behaviors that increase susceptibility to HIV infection. Herein, we examine the impact of cocaine on HIV pathogenesis and cure using the rhesus macaque (RM) model. Experimental design included one daily injection of cocaine (3mg/kg) to the following: cocaine (n=4); cocaine+SIV (n=6); cocaine+SIV+cART (n=6). RMs were inoculated with SIVmac251 after 8 weeks of cocaine exposure, with daily cART initiated 5 weeks post-infection. Compared to historical controls, a significant elevation in peak plasma ($p=0.0003$) and CSF ($p=0.0465$) viral loads observed in cocaine-treated RMs, with no differences between viral set-points. However, we found significantly lower levels of total SIV DNA in PBMC ($p=0.007$), lymph nodes ($p=0.004$) and rectal tissue ($p=0.017$) vs. controls. Moreover, intact proviral genome size in CD4+ T cells from PBMC ($p=0.008$) and lymph nodes ($p=0.0043$) was significantly reduced in cocaine treated RMs vs. controls. There was no significant difference in other tissue SIV DNA/RNA levels, including several brain regions. In addition, there was a significant depletion of Th1+ (CXCR3+CCR6-,$p<0.001$) and Th17+ (CCR6+CXCR3-,$p<0.001$) CD4+ cells with cocaine exposure. Additionally, cocaine exposure led to a significant increase in classical (CD14+CD16-,$p<0.001$) and decrease in non-classical (CD14dimCD16+,$p<0.001$). Finally, plasma/CSF metabolites were linked to dysregulation of fatty acid biosynthesis, energy metabolism, glutamate/amino acid metabolism pathways, and Warburg effect which suggest that a-bioenergetic imbalance may drive cocaine mediated neuropathogenesis. Our studies warrant a further understanding of cocaine-mediated effects on CNS reservoirs and role of dysregulated metabolites in driving neuropathogenesis.

9.

Evaluating a Novel macrophage-tropic transmitted/founder SHIV model of CNS persistence and pathogenesis in Nonhuman Primates on antiretroviral therapy.

Suvadip Mallick[1], Rachel M Podgorski[2], Ryan J Krause[1], Francesco Elia Marino[1], Gregory D Whitehill[1], Tricia H Burdo[2], Katharine J Bar[1]
[1]University of Pennsylvania, Philadelphia, Pennsylvania, USA. [2]Temple University, Philadelphia, Pennsylvania, USA

Abstract

Characterization of HIV-1 persistence throughout all body compartments, including the brain is a goal of the cure agenda. Emerging evidence suggests that HIV-1 persists in myeloid cells within the CNS. Clade D HIV-1 is described to efficiently infect CD4 T cells and macrophages. We employed SHIV.D.191859, which encodes a clade D TF HIV-1 Env to study persistence in brains of nonhuman primates.

In preliminary studies of 4 SHIV.D.191859-infected rhesus macaques, brain sections necropsied during viremia and on ART suppression showed neuroinflammation by immunohistochemistry. Dual RNAscope and IBA1 revealed RNA-IBA1+ microglia/macrophage co-localization during viremia and on suppressive ART. To further investigate SHIV.D.191859 CNS persistence, 10 RM were IV infected with barcoded SHIV.D.191859. Preliminary data demonstrate peak plasma viremia of 10^5 to 10^7 copies/ml and CSF viral loads of 10^2 to 10^3 copies/ml at 4 weeks of infection, with barcode sequencing revealing shared barcode distribution identity between early infection plasma and CSF clonotypes. Antiretroviral therapy was initiated at 10 weeks of infection with ART suppression of both plasma and CSF levels below assay limit of detection within 4 weeks. Necropsy of 5 RM occurred after 6 months of ART suppression and 5 RM are planned for 24 weeks of ART suppression, with plans for extensive analyses of systemic and CNS reservoirs.

Barcoded SHIV.D.191859-infection demonstrates virus persistence through ART, with overlap between plasma and CSF barcodes suggesting potential compartmentalization of CSF virus populations over time. Ongoing work will further define CSF and systemic reservoirs and extent of CSF virus compartmentalization.

10.

Chronic SIVmac251 in the setting of ART results in CNS Viral integration and Neuroinflammation

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Abstract

Background: Despite antiretroviral therapy (ART), 50% of people living with HIV experience central nervous system (CNS) complications. Consequently, there is a pressing need for modeling virologic and immune activation profiles within the CNS during chronic viral suppression to elucidate underlying mechanisms.

Methods: To investigate early and intermediate stages of CNS-HIV involvement, often understudied, we utilized SIVmac251 with a deferred non-adherent ART regimen. Ten rhesus macaques were infected; four were assessed during the acute phase (week 3 pi), and six during the chronic phase (week 40 pi).

Results: Robust systemic viral replication at week 3, plasma viral(v) RNA levels ($49x10^6$ copies/ml), led to CNS dissemination with cerebrospinal fluid (CSF) levels (44,500 copies/ml). After 4 weeks of Dolutegravir, Tenofovir, and Emtricitabine therapy, viral replication significantly decreased (plasma: 4050 copies/ml; CSF: 65 copies/ml). In the acute cohort, active viral replication was observed in the prefrontal cortex (PFC), indicated by a high vRNA/vDNA ratio [237]. RNA sequencing of CD45+ brain cells identified T cell clusters, with upregulated interferon-induced and anti-viral signatures. During chronic infection, ART effectively suppressed viral replication in the brain (vRNA/vDNA ratio: 0.22), but increased CSF levels of IP-10 and MCP-1 indicated ongoing immune activation in the CNS.

Conclusion: We demonstrate CNS involvement using a R5-T cell tropic SIV model with ART, viral integration, and notably identify establishment of latent CNS reservoirs. These results provide an improved experimental platform for studying mechanisms underlying CNS-HIV complications in the setting of chronic viral suppression with ART.

Session II: Vaccines, Immunology & Prevention

Moderators: Dr. Kristina De Paris (University of North Carolina, Chapel Hill)/ Dr. Gema Mendez-Lagares (UC-Davis)

Session chair presentation: Dr. Kristina De Paris: HIV Vaccines: How Far Have We Come?

11.

Oral Cabotegravir for nondaily HIV pre-exposure prophylaxis (PrEP)

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Abstract

Background

Nondaily oral PrEP is a desirable prevention modality. Cabotegravir (CAB) has a long plasma half-life and high PrEP efficacy as an injectable long-acting regimen. We performed dose-ranging studies in macaques to inform on a weekly oral CAB dose for nondaily HIV PrEP in humans.

Methods

Dose escalation studies were conducted in rhesus macaques (n=6) that received oral CAB doses of 15, 30 or 60mg/kg. Plasma was collected over time (up to 168h) to analyze PK parameters [T_{max}, C_{max}, $AUC_{0\text{-}168h}$, $t_{1/2}$].

Results

For all dosage groups, CAB reached peak concentrations by 4h, demonstrating rapid absorption. The C_{max} and $AUC_{0\text{-}168h}$ values showed dose proportionality for oral doses of 15 and 30mg/kg (C_{max}=7.7 and 18.7µg/ml; $AUC_{0\text{-}168h}$=352.3 and 724.3µg*h/ml, respectively) but saturation was observed at 60mg/kg. Plasma CAB levels with the 30mg/kg dose were >2-fold higher than the protective benchmark (4x-PA-IC_{95}=0.664µg/ml) at day 3 and consistently detected at day 7 (0.042ug/ml). The terminal half-life in macaques was similar across dosage groups and about half the length compared to humans (19h vs 39h). The 30mg/kg dose in macaques translates to a weekly CAB dose of ~120 mgs in humans.

Conclusions

We identified an oral CAB dose that maintains plasma levels above the protective benchmark for at least 3 days in macaques. Given the longer half-life of CAB in humans, achieving similar C_{max} levels would likely sustain protective levels for at least a week. These findings highlight the potential of oral CAB as a once-weekly PrEP option for HIV prevention.

12.

Immunogenicity of germline-targeting BG505 SOSIP trimer immunization in infant and juvenile rhesus macaques

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Abstract

An HIV vaccine that induces protective, broadly-neutralizing antibodies (bnAbs) before sexual debut is critical to eliminate the ~410,000 new infections annually among adolescents worldwide. Recent work has established that children living with HIV develop bnAbs earlier and at a higher frequency than adults. In this study we compared a germline-targeting BG505 SOSIP trimer immunization strategy to induce precursor bnAbs in infant and juvenile rhesus macaques (RM).

Infant (n=5) and juvenile (n=4) RMs received 3 immunizations of the germline-targeting BG505 GT1.1 SOSIP trimer (50mg) with the 3M-052-SE adjuvant 6 weeks apart. All RMs were then boosted 12 weeks later with the BG505.664 WT SOSIP trimer 3 times in 6-month intervals. Vaccine-elicited antibody responses were monitored through 2.5 years.

BG505 GT1.1 SOSIP trimer immunization consistently induced higher magnitude antigen-specific IgG binding responses in infants compared to juvenile RMs. However, plasma autologous virus neutralization responses were similar between the groups, yet the infant response targeted more epitopes. Notably, by week 80, three of five GT1.1 SOSIP-immunized infants exhibited a plasma neutralization signature indicating CD4 binding site-specific (CD4bs) bnAb precursor development, while only one of four juvenile RMs had developed this response. Those same three infants and one juvenile exhibited modest heterologous tier 2 neutralization activity.

Our data indicates that sequential immunization with germline-targeting BG505 SOSIP trimers may induce neutralizing antibodies and CD4bs bnAb precursors more frequently in infants compared to juveniles. Our results support observations in humans suggesting the infant immune environment may be better suited for induction of plasma HIV bnAb responses.

13.

Mucosal modulation of granulocytic effectors in lentiviral infection

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Abstract

Granulocytes are critical innate Fc effector cells, armed with inflammatory and cytotoxic mediators. To study their often-overlooked roles in lentiviral-mediated intestinal pathology and immunoprotection, we characterized granulocytes using non-human primate model of HIV infection.

Immune cells from whole blood and mucosal tissues of naïve, and chronic SHIVsf162p3-infected rhesus macaques (RM), and human blood samples were analyzed by imaging/polychromatic flow cytometry/Luminex for phenotype and functional assays including reactive oxygen species (ROS), intracellular cytokine secretion, extracellular traps generation (NETs), antibody-mediated cell phagocytosis (ADNP) and multiplex signaling.

Flow cytometric and imaging data confirmed granulocyte phenotypes as CD45+CD66abce+CD14+CD49d- neutrophils and CD45+CD66abce+CD14-CD49d+ eosinophils based on their receptor expression, nuclear morphology, and cytoplasmic granularity in blood and tissues. Upon in vitro FcR (CD32 and CD16) crosslinking of granulocytes, generation of ROS and NETs, and expression of important signaling adaptors including p-Syk, p-ZAP70, p-Lck and p-LAT were observed. Interestingly, VRC01-IgA elicited elevated phagocytosis than the VRC01-IgG subtype in human granulocytes, indicating HIV-specific mucosal activity. In SHIV-infected RM, depletion of jejunal eosinophils and vaginal neutrophils and eosinophils were observed, while neutrophils in circulation and colorectal biopsies were elevated indicating tissue-specific modulation of granulocytes in SHIV infection. Further, functional alteration of peripheral eosinophils was evidenced by reduced CD89 expression and intracellular IL-4 secretion upon mitogen stimulation in SHIV-infected RM.

Overall, our study shows lentivirus mediated modulation of granulocytes in mucosal sites of infection. Granulocytic depletion could potentially lead to pathogenic co-morbidities and adversely affect the outcome of antibody-based therapies and mucosal vaccination, thus warranting further studies.

14.

Exogenous Estrogen Increases HIV Target Cell Frequency in the Rectal Mucosa of Male Primates

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Abstract

Transgender women (TGW) are 49-66 times more likely to be infected with HIV than non-transgender individuals over age 15. Considering TGW's high risk of contracting HIV, they stand to benefit greatly from anti-HIV therapeutics, but little is known about the immunomodulatory effects of feminizing hormone therapy (FHT). To relieve gender dysphoria and facilitate physical feminization, many TGW utilize FHT, consisting primarily of 17β-estradiol (E2), which has immune-enhancing effects. To gain a better understanding of the immune consequences of FHT, we set out to model it in primates by treating male rhesus macaques (RMs) subcutaneously with E2 or placebo pellets. The E2 regimen significantly increased serum E2 concentrations and suppressed endogenous testosterone levels, while also inducing physical traits associated with feminization, like enlarged nipples. Importantly, immunophenotyping analysis revealed that CCR5+ CD4+ T-cells, the primary targets of HIV infection, were significantly elevated in both blood and gut from the E2-treated animals. Although the female sex is associated with enhanced immune responses to vaccines, FHT did not significantly alter vaccine-induced anti-Env antibodies in the E2 group following mRNA vaccination. To further define FHT-induced immune perturbations, we used RNA-Seq and plasma Olink proteomics to compare E2- and placebo-treated animals. These analyses revealed profound and sustained differences in gene expression and inflammation markers between the two groups, consistent with the pleiotropic effects of sex hormones. These results demonstrate for the first time the feasibility of modeling gender affirming hormone therapy in RMs and implicate FHT as a potential driver of HIV susceptibility in TGW.

15.

Mucosal HIV Vaccine Targeting Host Epithelial Stem Cells for Long-Term Immunity

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Abstract

Background

We developed a single-cycle vaccine under the involucrin promoter control, which was tested for its ability to drive SIV expression in terminally-differentiated epithelial-cells, induce mucosal immune responses and protection against SIV.

Methods

Twelve female macaques were immunized (1 dose, atraumatic) at week 0 and monitored for specific immune responses in blood, mucosal secretions, various lymphoid/non-lymphoid tissues. Eight animals were challenged with repeated low-doses SIVmac239 at weeks 12 or 24. Eight additional females served as unvaccinated SIV-infected Controls.

Results

Two-weeks post-vaccine, strong mucosal antibody responses (IgG, IgA) and specific CD8+ T-cells were detected. Immunohistofluorescence revealed antigens-expression in epithelia upper-layers. SIVenv was detected via anti-gp120 immunoPET/CT scans in vagina and draining-LN. SIV-challenges showed significant delays and/or lower viremia (2-3 logs-reduction, peak; 4-5 logs-reduction, set-point) to undetectable viremia 15-20 weeks post-SIV in vaccinees. Robust SIV-specific T-cell were also detected in blood, LN and mucosa tissues. Controls had high viremia (log10: 7.2-8.7 viral-RNA copies/ml, peak) and significant gut CD4+ T-cells depletion. We demonstrated a positive correlation between mucosal and systemic T-cell responses and control of viremia, and inverse associations between viremia and post-challenge vaginal antibody responses. All vaccinees manifested durable aviremic SIV-control for 2 years when CD8-depletion was performed. The dramatic fall in viremia coincided with the CD8+ T-cells recovery and significant increase of SIV-specific responses.

Conclusion

The efficacy of epithelial stem cell-based vaccine to serve as antigen-delivery and generate specific mucosal responses led to significant delays in infection, rapid decrease of viremia to durable aviremic CD8α+-cell dependent control.

16.

TCF-1^{+} specific CD8 T cells: Key to Post-Treatment Control of SIV Infection in Rhesus Macaques

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Abstract

Post-treatment controllers (PTCs) are a rare subset of individuals who maintain viral suppression after discontinuation of antiretroviral therapy (ART). Although the mechanisms underlying viral control in PTCs are not fully understood, HIV-specific CD8 T cells have been suggested to play a crucial role. TCF-1, a transcription factor associated with stem cell memory (Tscm), is essential for the development and maintenance of long-lived T-cell responses. In this study, we longitudinally characterized SIV-specific T-cell responses in a cohort of eight therapeutic Ad/Gag recipient Rhesus Macaques, focusing on the expression of transcription factors TCF-1, T-bet, and Eomes. Notably, the magnitude of total CD8+ T cell responses to SIV Gag peptides was similar between SIV controllers and non-controllers, suggesting that successful SIV control is not solely dependent on an exceptionally high frequency of anti-SIV T cells. Instead, successful SIV-specific T cells exhibited higher quality, with increased expression of TCF-1 in controllers, while non-controllers showed higher expression of T-bet within their vaccine-specific CD8^{+} T cells. These findings indicate the potential value of modulating memory differentiation pathways as an adjunct to therapeutic vaccination and emphasize the importance of enhancing the quality, rather than just the quantity, of T-cell responses for achieving successful control of viral infections.

17.

Bispecific 10E8.4/iMab Immunoprophylaxis Against High-dose Intravenous SHIV Challenge in Macaques

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Abstract

Background: In the absence of an efficacious HIV-1 vaccine, pre-exposure prophylaxis with either antiretroviral drugs or HIV-1 broadly neutralizing monoclonal antibodies (bNAb) represent two lead strategies for HIV-1 prevention. The ability of the VRC01 bNAb to prevent transmission of neutralization sensitive but not resistant HIV-1 isolates in the antibody-mediated prevention clinical trial highlights the need for immunoprophylaxis strategies leveraging bNAbs with greater breadth and potency. Methods: To develop a model for evaluation of bNAb peri-exposure immunoprophylaxis in high-risk parenteral exposure settings such as needlesticks, intravenous drug use and unscreened blood transfusions, we evaluated the potent bispecific 10E8.4/iMab targeting the membrane-proximal external region of envelope and the CD4 receptor (Ibalizumab) for prevention of infection following intravenous challenge in rhesus macaques. A single intravenous infusion of 10E8.4/iMab (30 mg/kg) or PBS (N=3/arm) was administered one hour prior to high-dose challenge with SHIV-BG505 (~40 million RNA copies, or ~50,000 TCID50). Transmitted-founder SHIV-BG505 was selected for its well-characterized and stable viremia in macaques, similar neutralization sensitivity profile as HIV-1 strains, and sensitivity to 10E8.4/iMab (IC50<0.01 ug/ml). Results: All control animals developed plasma viremia one-week post-challenge (p.c.), which peaked at 1.1E7-8.7E6 copies/ml two weeks p.c. Evidence of consistent viral replication has yet to be observed in 10E8.4/iMab recipient animals. No adverse events were noted. Conclusion: Single intravenous infusion of a potent bispecific HIV-1 bNAb represents a potential mono or adjunctive peri-exposure immunoprophylaxis which may provide immediate and durable protection against infection transmitted via bloodstream without the need for continued drug adherence.

18.

Evidence of Subclinical SIV Infections in Rhesus Macaques During Neutralizing Antibody Immunoprophylaxis

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Abstract

Broadly neutralizing antibodies (bNAbs) are promising prophylactics to prevent HIV-1 infection. While clinical trials have demonstrated prevention of neutralization-sensitive isolate acquisition, there is limited data on antibody levels at infection. Here, we characterized SIV-specific bNAb levels at SIV infection in rhesus macaques (RMs).

Two groups of 6 RMs were infused with anti-SIV bNAbs ITS103.01 or ITS102.03 that differ 130-fold in their SIVmac239 neutralization potency (IC80s of 22ng/mL, 3µg/mL, respectively). A control group received no antibody. Weekly challenges of barcoded SIVmac239 were initiated 5 days post-infusion. One of 8 uniquely barcoded viruses was sequentially used at each challenge. To determine the exact date of infection, plasma virus barcodes were sequenced.

ITS102.03-treated RMs became infected with median mAb levels 44-fold above the IC80 and viremia onset was slowed, though their time-to-infection did not significantly differ from the controls because of insufficient mAb levels. However, ITS103.01 infusion delayed time-to-infection versus the controls. The median ITS103.01 concentration at the challenge that caused the dominant viremia was 0.25µg/mL. However, 5/6 animals showed earlier transient viremia when mAb was 0.3–3µg/mL. Furthermore, barcode sequencing following sustained viremia revealed subclinical infections occurred while plasma ITS103.01 was 0.47–96µg/mL.

The threshold for ITS102.03 and ITS103.01 preventing infection was similar (44-fold and 11-fold the IC80s, respectively). With surprising frequency, subclinical infections were observed with median ITS103.01 98-fold the IC80. This study demonstrates that antibody immunoprophylaxis can mask subclinical infections, which may affect the interpretation of HIV-1 bNAb preventative therapy and sets a high bar for vaccine-induced, long-lasting antibody responses.

19.

Efficacy of Booster Vaccines Against Omicron BA.5 Variant in Rhesus Macaques

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Abstract

Background: The Omicron variant of SARS-CoV-2 evades vaccine-induced neutralizing antibodies and has dominated recent COVID-19 cases. We assessed the efficacy of mRNA-1273, NVX-CoV2373 (Ancestral spike), and NVX-CoV2515 (Omicron BA.1 spike) booster vaccines against Omicron BA.5 challenge in rhesus macaques (RMs).

Methods: RMs received a primary series of mRNA-1273, were divided into three groups (n=6/group), and given booster vaccines. They were then challenged with SARS-CoV-2 BA.5 variant. We characterized cellular and humoral immune responses to identify correlates of protection against heterologous SARS-CoV-2 BA.5 infection.

Results: All three boosters induced robust cross-reactive binding antibodies against the BA.1 variant and shifted IgG dominance from IgG1 to IgG4 in the serum. They also generated potent neutralizing antibody responses against multiple variants, including BA.5, and resulted in the presence of long-lived plasma cells in the bone marrow. Following BA.5 challenge, the boosters provided significant protection in the lungs and effectively controlled virus replication in the nasopharynx. Both Novavax vaccines exhibited

reduced viral replication in the nasopharynx by day 2. Protection in the upper respiratory airways correlated with activities such as binding, neutralizing, and antibody-dependent neutrophil phagocytosis.

Conclusion: mRNA-1273 and both Novavax boosters, following a primary series of mRNA-1273, generated long-lasting humoral immune responses and effectively protected rhesus macaques against SARS-CoV-2 BA.5 infection. Remarkably, NVX-CoV2373 exhibited superior protection in the airways and enhanced control over viral replication compared to the control group. These findings endorse the utilization of all available boosters to prevent SARS-CoV-2 variant infections and transmission, with adjuvanted protein boosters potentially providing heightened protection.

20.

Intranasal and intramuscular routes of self-amplifying mRNA vaccination against SARS-CoV-2 distinctly modulate immunogenicity, protective efficacy and anamnestic immune responses post-viral challenge in non-human primates

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Abstract

Background: Success with RNA based SARS-CoV-2 vaccines has catalyzed vaccinology research. RNA vaccines, when optimized for delivery intranasally (IN), could offer an unique opportunity in balancing systemic and mucosal immunity. Here, we evaluated immunogenicity and protective efficacy of a clinically relevant nanostructured lipid carrier (NLC)-delivered self-amplifying RNA (saRNA) vaccine encoding the whole Spike (S) via the IN and intramuscular (IM) routes in rhesus macaques (RMs).

Methods: We quantified vaccine-induced serum and mucosal antibodies(Abs) using ELISA and pseudovirus neutralization assays, innate, B cell and T responses using flow cytometry and quantified vaccine-specific plasma cells in blood and bone marrow using ELISpot. Virus in respiratory mucosa was quantified using q-PCR.

Results: IN vaccination with saRNA-(S) was safe, well-tolerated and achieved a significant reduction in respiratory viral load within a week after a heterologous challenge with B.1.617.2 SARS-CoV-2. Virus-specific immunity after IN vs. IM vaccination was most evident as a striking anamnestic S-specific systemic and mucosal response after viral challenge, suggesting establishment of robust mucosal memory. Specifically, 100-300 vs. 5-50 fold increases in S-specific serum and mucosal Abs, T cells in BAL were observed in animals vaccinated with IN vs. IM routes, respectively. Striking frequencies of lung and bone marrow resident S-specific plasma cells after viral challenge highlights why hybrid immunity against SARS-CoV-2 may be superior in durability.

Conclusions: Our data strongly support the use and continued optimization of IN vaccines using the saRNA-NLC platform against SARS-CoV-2, influenza and HIV-1 for which balanced systemic and mucosal immunity may be desirable.

Session III: Therapy & Progress towards HIV Cure

Moderators: Dr. Afam Okoye (Oregon Health and Science University, ONPRC)/Dr. Dennis Hartigan-O'Connor (UC-Davis, CNPRC)

Session chair presentation: Dr. Afam Okoye; Strategies for post-ART viral control: The impact of neutralizing antibody therapy at ART interruption

21.

Adeno-Associated Virus Delivery of Env-Specific Antibodies Delays Viral Rebound Following Treatment Interruption in SIV-Infected Macaques

Vadim A Klenchin[1], Natasha M Clark[1], Kristen Crosno[2], Nell Bekiares[2], Saverio Capuano 3rd[2], Rosmarie Mason[3], Guangping Gao[4], Christine M Fenessey[5], Brandon F Keele[5], Jeffrey D Lifson[5], Mario Roederer[3], Matthew R Gardner[6], David T Evans[1]
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Abstract

High levels of sustained antibody expression with complete protection against immunodeficiency virus challenge have been achieved in animal models following the administration of adeno-associated virus (AAV) vectors expressing Env-specific antibodies. To determine if AAV delivery of Env-specific antibodies can prevent the resurgence of virus replication after the discontinuation of antiretroviral therapy, we infected fourteen rhesus macaques with barcoded SIVmac239M and initiated ART on day 9 post-infection. At week 34 PI, AAV9 vectors encoding natural rhesus macaque antibodies to SIV Env (ITS61.01 and ITS103.01) were administered by intramuscular injection to eight animals and an AAV9 vector encoding a control antibody (17HD9) was administered to six animals. ITS61.01 and ITS103.01 were selected for efficient ADCC against SIVmac239-infected cells. ITS103.01 also potently neutralizes SIVmac239. After discontinuing ART at week 60 PI, SIV replication rebounded within 2 weeks in all six of the control animals, but remained below the limit of detection for at least 34 weeks in four of the eight animals that received Env-specific antibodies. Host antibody responses to the AAV-delivered antibodies were low and generally reflected an inverse relationship with serum concentrations of ITS61.01 and ITS103.01. Sequence analysis of the virus population in plasma revealed only 1-2 barcodes and evidence of antibody escape in animals with viral rebound despite high levels of Env-specific antibodies compared to 5-30 rebounding barcodes in the control group. Overall, these results illustrate the potential for AAV delivery of natural, species-matched antibodies to delay, perhaps indefinitely, the resurgence of immunodeficiency virus replication after ART interruption.

22.

AAV-mediated Delivery of Closer-to-germline Monoclonal Antibodies Mediates Viral Suppression in SHIV-infected Rhesus Macaques

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Abstract

Background: We have three SHIV-infected monkeys which, after receiving recombinant adeno-associated virus (AAVs) encoding a cocktail of broadly neutralizing antibodies (bNAbs) during the chronic phase of infection, have shown suppressed viral loads and appear functionally cured. Our attempts to create more such functional cures have been severely hampered by the generation of anti-drug antibody responses (ADAs) to the AAV-delivered antibodies. Due to prolonged affinity maturation, most bNAbs exhibit unusually high levels of somatic hypermutation and accumulate uncommon features which can trigger unwanted ADA-responses in the recipient, compromising the efficacy of the AAV approach. To overcome this critical issue, we attempted delivery of less mutated antibodies, i.e. antibodies closer to germline.

Methods: Four Indian-origin rhesus macaques were experimentally infected with SHIV-AD8. At week 14 post-infection these monkeys received AAVs expressing bNAbs DH270, PCIN63 and DH511, which were naturally closer to germline than those we had used previously.

Results: High levels (22-327µg/mL) of two AAV-delivered antibodies were obtained in three of the four macaques through the 50 weeks of measurements. Sustained viral load suppression was achieved in two of those three monkeys. The third monkey showed only transient effects on viral load levels and an escape mutant virus was suspected. The fourth monkey had low antibody levels due to ADAs and little or no virologic suppression.

Conclusions: Our data suggest that the use of closer-to-germline bNAbs may be a viable strategy for overcoming ADAs following gene therapy with AAV-bNAb vectors and they also highlight the difficulties associated with achieving long-term suppression.

23.

Viral dynamics of SIV rebound in the CNS following cART cessation

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Abstract

Background

The CNS is a viral reservoir for HIV/SIV which contributes to viral rebound upon cART cessation. However, the resident brain cells that drive rebound from the CNS have not ben established. We aimed to characterize infected cell phenotypes and inflammatory responses that emerge in the CNS during viral rebound.

Methods

We used archived brains from rhesus macaques intravenously infected with SIVmac251 that progressed to AIDS(n=3) or treated with cART(dolutegravir/tenofovir/emtricitabine, n=2) and stopped cART 14 days before necropsy. Combining fluorescence microscopy and viral RNA/DNAscope, we quantified infected brain cell phenotypes including astrocytes(GFAP), macrophages (CD68/163/206), and microglia(Iba1) in the frontal, temporal, and occipital lobes. We quantified CNS cART levels via LC/MS and inflammatory gene pathways via RNAseq.

Results

The majority of infected cells detected following cART cessation were myeloid/microglia cells, which we also detected among the AIDS/no cART animals, albeit at higher levels. We detected astrocytes harboring virus in both groups, although two-fold lower than myeloid cells. The temporal lobe was the only tissue where an association between viral detection and reduced cART levels was evident. TNF- RNA expression was three-fold higher in the temporal lobe post-cART cessation compared to other brain regions.

Conclusion

These findings demonstrate that infected resident brain cells contribute to viral rebound post cART cessation, especially myeloid cells. Thus, novel strategies for HIV cure should not neglect the brain as an HIV sanctuary site and source of re-seeding of HIV from the brain to the periphery.

24.

Investigating the Dynamics of Immunological versus Pharmacological Control of Viral Infection

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Abstract

In the ΔGY model of elite control, pigtail macaques (PTMs) are infected with a variant of SIVmac239 that contains a two amino acid deletion in a conserved Tyr-dependent trafficking motif in the envelope glycoprotein cytoplasmic tail. ΔGY-infected PTMs exhibit high levels of peak viremia, which, upon onset of host immune responses, fall to low or undetectable levels (<15 RNA copies/mL). We studied 9 PTMs infected with barcoded ΔGY. 6 animals controlled the virus as predicted; 3 animals maintained high viral loads and were placed on ART 16 weeks post-infection.

Longitudinal PBMC samples were collected from the animals from weeks 1-66 of infection. The intact proviral DNA assay (IPDA) and hypermutated proviral DNA assay (HPDA) were used to quantify intact and defective proviruses over the course of the experiment. The rates of decay of intact proviruses appear similar between the 2 groups (at week 66: controllers-178 intact proviruses/million CD4+ T cells, noncontrollers-366 intact proviruses/million CD4+ T cells), suggesting that despite different methods of control, populations of infected cells are decaying at similar rates.

Single genome sequencing of envelope revealed a dearth of diversity among proviral sequences in controllers. Mutations associated with a failure to control were observed in the noncontroller animals. These mutations were also observed in the controller animals, suggesting the existence of a critical window early in infection during which control is established.

This study allowed us to observe viral dynamics during the early stages of control that have not been described in human elite controllers.

25.

TGF-b blockade with Galunisertib drives a transitional effector phenotype in T cells reversing SIV latency and decreasing SIV reservoirs in vivo.

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Abstract

We showed that blocking TGF-b in a non-human primate model (NHP) of mucosal SIV infection leads to SIV latency reversal.

Using a different NHP model with barcoded SIVmac239M2 intravenous infection and therapeutic dosing as those adopted during clinical testing of Galunisertib (LY2157299), a small anti-TGFBR1 inhibitor, we confirmed the latency reversal properties of TGF-b blockade. Specifically, 8 SIV infected macaques on suppressive ART for 7 months were treated with 4 2-weeks cycles of 20mg/kg/twice daily of Galunisertib. Galunisertib led to viral reactivation in blood and tissues as indicated by plasma viral load and PET/CT with the 64Cu-DOTA-F(ab')2p7D3 probe. A half to 1 Log decrease in cell-associate (CA-)SIV DNA was detected in lymph nodes, gut and PBMC, while intact pro-virus in PBMC decreased by almost 3 folds. An increase in SIV-specific and non-specific responses was detected in PBMC of all macaques by Elispot. However, no increase in inflammatory cytokines was detected systemically in plasma. Importantly, high-parameter flow cytometry and bulk and single-cell RNAseq of PBMC and lymph node cells revealed a shift toward an effector phenotype in T cells and other immune subsets in absence of an increase in classical activation markers, such as CD69 and Ki67. Particularly, there was a transcriptional increase in the AP1 complex and OXPHOX pathway. In summary, we demonstrated that Galunisertib, a clinical stage TGF-b inhibitor, reverses SIV latency and decreases SIV reservoir by driving T cells toward a transitional effector phenotype, increasing viral reactivation events, and enhancing immune responses in vivo.

26.

Anti-CD3LALA increases SIV RNA in plasma of rhesus macaques on antiretroviral therapy

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Abstract

Background: Identifying latency reversing agents (LRA) that are both effective and well-tolerated is a major challenge for HIV cure strategies. We previously showed that the T cell agonist anti-CD3LALA can increase SIV production rhesus macaques (RM) on ART in the presence of the mTOR inhibitor rapamycin. Here, we assessed whether rapamycin is required for the safety and LRA activity of anti-CD3LALA.

Methods: *In study 1*, 10 SIVmac239+ RM on short term ART +/- rapamycin (n=5 each) received IV infusions of anti-CD3LALA at 0.5, 1.0, 2.0, 5.0 mg/kg to asses saftey. *In study 2*, 10 SIVmac239+ RM on long term ART received two infusions of anti-CD3LALA or control IgG at 1 mg/kg (n=5 each) at 14-day intervals in the absence of rapamycin. SIV RNA in plasma was quantified by RT-qPCR and immune cell dynamics were profiled by flow cytometry.

Results: In the safety study, anti-CD3LALA was well-tolerated at doses up to 5 mg/kg with no adverse events observed +/- rapamycin. Anti-CD3LALA increased frequencies of both CD69+ and Ki67+ CD4 memory T cells in blood but these were lower in rapamycin-treated RM relative to controls (p=0.06 and p=0.03, respectively). In study 2, we observed increases plasma SIV RNA above detection limit of 15 SIV RNA copies/ml in 4 of 5 RM following anti-CD3LALA infusion.

Conclusions: Anti-CD3LALA was safe and could increase SIV RNA in plasma of ART-suppressed RM. While rapamycin was not required for LRA activity, reduced T cell proliferation suggests mTOR inhibition may support virus production without reservoir expansion.

27.

Immunologic and virologic outcomes in pediatric rhesus macaques infected with SIV on early antiretroviral regimens

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Abstract

Background: In our previous study, early initiation of ART in neonatal macaques achieved sustained virologic remission. Multiple factors such as age, antiretrovirals, provirus decay, and immune development remain unclear.

Methods: Newborn, infant, and adult macaques were infected with SIV and received cART starting at 3 days post infection (dpi) for 9 months. Other animal cohorts of neonatal macaques infected with SIV at birth were treated with either DTG alone (n=4, Group 1), Truvada (FTC/TFV, n=4, Group 2), or cART (n=7, Group 3) initiated at 2 dpi for the first 3 months, followed by cART continuation for an additional 6 months in Group 1-3. In some cases of Group 3, treatment was continued for an additional 3 months only (n=2) or discontinued after the first 3 months of therapy (n=2). Blood and tissue biopsies were collected to measure multiple parameters.

Results: Viral rebound occurred after ATI in infants aged 1.5 months or 1 year, as well as in adult macaques. Early treatment initiation at 2dpi followed by discontinuation resulted in viral rebound in Group 1 and 1 of 3 in Group 2, but not in Group 3. Intact proviral DNA was detected in animals with a viral rebound. Plasma drug concentrations declined rapidly in infant macaques. LNMCs of neonates on early cART maintained Ig diversity.

Conclusions: These findings suggest that the outcome of pediatric ART-free remission by early interventions is highly correlated with the age of HIV exposure, timing of treatment initiation, antiretroviral combination, and the decay of intact proviruses.

28.

Modeling ultra-small HIV reservoirs in rhesus macaques

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Abstract

A critical question for HIV cure research is — how low must we go? It is anticipated that the viral reservoir size at the termination of antiretroviral therapy (ART) affects the time to viral rebound (TTR). Yet, the association between reservoir size and TTR is unknown. 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 activate upon stopping ART. This model will be useful for characterizing the impact of viral reservoir size(s) needed for sustained ART-free remission.

29.

No Evidence for Replication on ART in SIV-infected Macaques

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Abstract

Background

The capacity of HIV-1 to replicate during antiretroviral therapy (ART) remains controversial. Limitations of clinical sampling and the high background level of genetic diversity in most persons living with HIV make it challenging to detect additional substitutions on ART. To gain greater sensitivity to detect even small changes to the virus population on ART, we used an NHP model, which allows precise control over the level of pre-ART evolution and subsequent changes to the viral population.

Methods

We infected 21 rhesus macaques with the barcoded virus SIVmac239M to compare near-full-length (nFL) SIV DNA single genome sequences from PBMCs (and in some cases lymph nodes and spleen) obtained near the time of ART initiation and those present after long-term ART. In 4 animals, we also assessed SIV RNA sequences obtained from rebound plasma viremia after ATI. Animals started ART between 10-27 days post infection and were treated for 285-1200 days. We obtained 25-200 intact nFL sequences per animal. We assessed whether the viral populations changed during long-term ART using several tests for molecular evolution.

Results

The genetic divergence, diversity, and composition of the SIV DNA population did not change significantly during ART in any animal. None of the 700 nFL sequences obtained after long-term ART had sufficient mutations to indicate ongoing replication.

Conclusions

The rigorous nature of the RM/SIVmac239M model, which allows more comprehensive analyses than are feasible with clinical samples, reinforces both the emerging consensus of no replication on effective ART, and validates the relevance of the NHP model.

30.

Microbial Postbiotic Repairs SIV/HIV Damaged Gut Epithelium *in vivo* and Gut Organoids *ex vivo* Through PPARa Mediated Histone Crotonylation

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Abstract

HIV causes disruption of gut epithelial barriers early in infection and impairs mucosal immunity promoting chronic inflammation, immune dysfunction and leaky gut syndrome. We previously reported that Lactobacillus plantarum administration in the virally inflamed gut rapidly repaired the gut epithelium through peroxisome proliferator activated receptor-a (PPARa) mediated mitochondrial bioenergetics. High throughput metabolomic analysis of intestinal luminal contents from L. plantarum treated animals with chronic SIV infection identified a highly enriched microbial metabolite with potent gut repair function. We investigated the impact of this microbial product (MLCFA) in mitigating viral pathogenic effects by using SIV infected rhesus macaques in vivo, gut epithelial cell lines in vitro and gut organoid ex vivo. We performed an integrated analysis of immunological, histopathological, genomic and transcriptomic and functional evaluations and utilized several methodologies including flow cytometry immunohistochemistry, RNAseq, ChIPseq, 16S sequencing. MLCFA activated PPARa and induced histone crotonylation which caused activation of gene expression inducing mitochondrial biogenesis and promoting gut epithelial barrier integrity and function both in vivo and ex vivo. MLCFA administration also mitigated SIV induced gut microbiome dysbiosis. Our data for the first time link a specific microbial metabolite to enhanced mitochondrial biogenesis and gut barrier repair through the under-investigated histone crotonylation and PPARa activation in virally inflamed and functionally disrupted gut. Our results link a specific microbial molecule to the symbiotic host gut mucosal immune interface with microbiome in the context of virally inflamed gut and provide novel therapeutic target for HIV vaccine and HIV cure studies.

Session IV: Non-SIV/SHIV NHP Disease Models

Moderators: Dr. Jessica Raper (Emory National Primate Research Center/ Dr. Lark Coffey (UC-Davis)

Session chair presentation: Dr. Jessica Raper

COVID-19 in rhesus macaques produces behavioral, cognitive, and neurological characteristics similar to clinical symptoms of Post-Acute Sequelae of SARS-CoV-2 infection (PASC)

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A significant proportion of COVID-19 patients have lingering symptoms for weeks or even months following initial recovery. These “long COVID-19” symptoms or Post-Acute Sequelae of SARS-CoV-2 infection (PASC) include fatigue, sleep disorders, brain fog, loss of taste or smell, anxiety, and depression. Given the persistence of this viral pandemic, there is a great need to broaden our understanding of the viral pathophysiology to help identify novel targets for further therapeutic development. Animal models are a powerful biomedical research tool for investigating neuropathology, narrowing down novel targets, and testing the efficacy of therapeutics to treat PASC symptoms. We propose that nonhuman primates are an ideal animal model to investigate the neuropathological consequences of SARS-CoV-2 infection. Seven adult rhesus macaques between 6-18 years of age were assessed before and after intranasal infection with SARS-CoV-2 ($1.1x10^6$ PFU of 2019-nCoV/USA-WA1/2020). Despite what seemed to be a rather mild infection with viral clearance similar to human patients (2 weeks), we detected behavioral, cognitive, and neurological changes up to 4 months post-infection. Compared to baseline/pre-infection, SARS-CoV-2 infection led to sleep disturbances as shown by increased activity overnight. Cognitive impairments were detected at 85 days post-infection. SARS-CoV-2 infection altered taste and smell, such that monkeys exhibited decreased preference for their top 5 most highly preferred foods from baseline. Together these data indicate that rhesus macaques can exhibit behavioral, cognitive, and neurological characteristics similar to those reported by PASC patients. As COVID-19 infections and the associated mortality rates continue to recede, the world will be left with a large population of people who will continue to suffer from this debilitating, long-term, post-covid syndrome. We propose that the nonhuman primates provide an ideal translational model to expand our knowledge of SARS-CoV-2 pathophysiology and identify potential targets for much needed therapeutic interventions for PASC COVID-19 patients.

31.

Natural Killer Cells Modulate SARS-CoV-2 Infection And Reduce Lung Damage

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Abstract

The role of Natural Killer (NK) cells in pathogenesis and control of SARS-CoV-2 infection remains unclear, with mixed reports of benefits and pathogenesis associated with NK cell mobilization. To address this deficit, we employed a nonhuman primate infection model with validated experimental NK cell depletion.

Twelve experimentally naïve cynomolgus macaques (CMs) were used, with half receiving anti-IL-15 neutralizing antibody infusions for NK cell depletion and six controls receiving placebo. The CMs were subsequently challenged with SARS-CoV-2 Delta variant at $TCID_{50}$ of $1X10^5$ via intranasal and intratracheal routes. Viral load (VL) measurements, sample collections, and analyses by 23-color flow cytometry, 27-plex inflammatory analyte Luminex, and pathology evaluations were conducted over four weeks of infection.

Circulating NK cells increased in control CMs, reaching a peak at 10 days post-infection (DPI), returning to baseline by 22DPI. The control group also showed activation and proliferation of NK cells. NK cell ablation was highly efficient in blood and tissues of experimental CMs, coinciding with up to 2.5 log increases in VL and a significantly longer duration of viremia compared to controls. Luminex measures in BAL fluid from depleted CMs had universally higher concentrations of inflammatory mediators, most notably a 5-fold higher concentration of interferon-alpha compared to controls. Lung pathology scores were also significantly higher in depleted animals with large increases in overall organ damage.

Overall, we find compelling evidence for NK cell-mediated control of SARS-CoV-2 virus replication and disease pathology, suggesting adjunct therapies for infection could largely benefit from NK cell-targeted or adjunct approaches.

32.

MVA-Based Vaccines Are Protective Against Lethal Eastern Equine Encephalitis Virus Aerosol Challenge in Cynomolgus Macaques

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Abstract

MVA-based monovalent eastern equine encephalitis virus (MVA-BN-EEEV) and multivalent western, eastern, and Venezuelan equine encephalitis virus (MVA-BN-WEV) vaccines were evaluated in the cynomolgus macaque aerosol model of eastern equine encephalitis virus (EEEV) infection. Macaques vaccinated with two doses of 5 x 108 infectious units of the MVA-BN-EEEV or MVA-BN-WEV vaccine by the intramuscular route rapidly developed robust levels of neutralizing antibodies to EEEV that persisted at high levels until challenge at day 84 via small particle aerosol delivery with a target inhaled dose of 107 PFU of EEEV FL93-939. Robust protection was observed, with 7/8 animals receiving MVA-BN-EEEV and 100% (8/8) animals receiving MVA-BN-WEV surviving while only 2/8 mock vaccinated control animals survived lethal challenge. Complete protection from viremia was afforded by both vaccines, with near complete protection from vRNA loads in tissues and any pathologic evidence of central nervous system damage. Overall, the results from this study indicate that both vaccines are highly effective in eliciting an immune response that is consistent with protection from aerosolized EEEV-induced disease.

33.

An owl monkey model of HIV-1 infection

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Abstract

HIV-1 vaccine development has been hindered in part by the lack of an immune competent animal model with which to study the virus. To try and rectify this shortcoming, we are working to develop a nonhuman primate (NHP) model that can be reliably infected with HIV-1 (not SHIV or SIV) without requiring any form of immune suppression. Toward achieving this goal, we first performed genetic analysis on a select group of restriction factors and surface markers from 11 different African, Asian, and South American NHP species. Our results identified a species of owl monkeys (Aotus nancymaae) as the laboratory NHP model with the greatest likelihood of success for infection studies. As part of additional work toward our goal, we created a modified HIV-1 containing eight non-synonymous point mutations and we also replaced the Vif accessory gene. Notably, this virus is still 93% wildtype HIV-1. In subsequent in vivo experiments, we found that owl monkeys are readily infected with our HIV-1 variant when the virus is administered intravenously as a purified virus or through blood transfusions from previously infected animals. These owl monkey infections recapitulate many features of human HIV-1 infections to include: an acute phase of infection with plasma viremia up to 107 copies/mL; subsequent control of the virus; seroconversion; and the establishment of a viable virus reservoir within lymphoid tissues. We believe this immune competent primate model of HIV-1 represents an exciting new platform that will be of benefit to researchers undertaking HIV-1 vaccine and cure development studies.

34.

Single cell RNA-Sequencing reveals neurological perturbations in postnatally ZIKV-infected infant rhesus macaques

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Abstract

Background: Consequences of postnatal ZIKV infection in infants and children are not well understood. We have previously shown abnormal brain structure and function that is predictive of behavior in infant rhesus macaques infected with ZIKV postnatally. Here, we explored the brain regions, cells, and pathways impacted by postnatal ZIKV infection to suggest mechanisms of injury and neuropathogenesis.

Methods: Infant rhesus macaques (RMs) were infected with ZIKV at one month of age and euthanized 14 days after infection for single cell transcriptomic analyses of the hippocampus, amygdala, and striatum. ZIKV-infected infant RMs were compared to age and sex-matched uninfected controls. Bioinformatic approaches using R (V4) and Seurat (V4) were utilized and, after quality control, 105,421 cells from controls and 94,975 cells from ZIKV-infected animals were analyzed.

Results: We identified unique transcriptional phenotypes in the CNS between uninfected and ZIKV-infected RMs, including nervous system development, glial cell differentiation, neuron differentiation, activation of innate immune response and regulation of myelination. As expected, we found a signature of activated microglia along with upregulation of transcription factors involved in interferon signaling and downstream activation of interferon stimulated genes (ISGs) such as IFI6, IRF9 and MX1 in ZIKV-infected RMs. Furthermore, ZIKV infection significantly reduced the expression of several genes involved in myelination processes among mature oligodendrocytes.

Conclusions: Our results show that acute ZIKV infection in infant RMs leads to CNS immune activation and downregulation of critical genes involved in myelination, which may have long lasting neurodevelopmental consequences.

35.

Immune System Dynamics and Transcriptional Perturbations Under BCG Vaccination in Early-Stage *Mycobacterium tuberculosis* Infected Rhesus Macaques

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Abstract

Background

Tuberculosis (TB) is the second most lethal infectious disease globally and the leading cause of infectious disease-related deaths for people living with HIV. While TB is treatable via a 6-12 month antibiotic course, an effective vaccine would be a major boost for TB disease prevention. The most widely deployed vaccine (BCG) has variable efficacy, but newer candidates, including I.V. BCG and CMV-based vectors, show promising results, up to sterilizing protection. In this study, we characterized the early immune response in unvaccinated and BCG-vaccinated macaques, to establish a baseline for comparison against future vaccine candidates.

Methods

We benchmark the BCG vaccine using single-cell RNAseq (scRNAseq) and spatial transcriptomics in intrabronchially challenged Rhesus macaques, collecting longitudinal scRNASeq data from lymph nodes, liver, spleen, bone marrow, lungs, and PBMCs. To link transcriptomic signatures to disease outcome, we use spatial transcriptomics from mature and nascent intra-granulomatous lung tissue substructures coupled with per-granuloma bacterial abundance and tissue-level bacterial burdens.

Results

During the onset of infection, we found the T cell response was heavily skewed toward CD4+ T cells, with little activation in CD8+ T cells. By comparing spatialomics and paired bacterial abundances, we found positive correlations with established TB biomarker TIMP1 and interferon responses and negative correlations with complement subunits of the membrane attack complex and cell proliferation.

Conclusions

These findings establish a baseline of gene signatures that exhibit protection or susceptibility at sub-granuloma resolution and will be used as a guide to evaluate future data on experimental vaccines.

36.

Mayaro virus pathogenesis and immunity in rhesus macaques

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Abstract

Mayaro virus (MAYV) is a mosquito-transmitted alphavirus that causes debilitating arthritogenic disease. While MAYV was reported to infect non-human primates, extensive characterization of MAYV pathogenesis is currently lacking. Therefore, we characterized MAYV infection and immunity in rhesus macaques. To inform the selection of a viral strain for NHP experiments, we evaluated five MAYV strains in mice and showed that $MAYV_{BuenosAires505411}$ induced robust tissue dissemination and disease. RM were subcutaneously infected with 1e5pfu of this strain into the arms. Peak viremia occurred at 2 dpi with evidence of protracted viremia in one animal and rebound viremia at 10 dpi in two RM. The RM were necropsied at 10 dpi to assess viral dissemination, which included the arm and leg muscles and joints, lymphoid tissues, major organs, male reproductive tissues, as well as peripheral and central nervous system tissues. Histological examination demonstrated that MAYV infection associated with appendicular joint and muscle inflammation and the presence of perivascular inflammation in a number of tissues. One animal developed a maculopapular rash and two NHP had vRNA detected in upper torso skin samples, which was associated with perivascular and perifollicular lymphocytic aggregation. The analysis of longitudinal peripheral blood samples indicated robust innate and adaptive immune activation, including the presence of anti-MAYV neutralizing antibodies with activity against related viruses. Inflammatory cytokines and monocyte activation also peaked with viremia. The RM model of MAYV infection recapitulates many of the aspects of human infection and is poised to facilitate the evaluation of novel therapies targeting this re-emerging virus.

37.

Neutralizing Monoclonal Antibody Treatment for Severe Yellow Fever Infection: Evaluation of Dosage and Treatment Timing

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Abstract

Background: With no clinically available treatment, Yellow Fever Virus (YFV) is fatal for 15% of the 200,000 yearly infections. We have shown untreated YFV-infected rhesus macaques (RMs) die within 5 days post-infection (dpi). However, administration of YFV-specific neutralizing monoclonal antibodies (nmAb) at 50mg/kg 2 dpi prevents severe disease and death. To facilitate clinical applicability, we further define protective timepoints and doses of this treatment.

Methods: Ten RMs were infected with pathogenic YFV-DakH1279 (10^3 $TCID_{50}$). Four RMs (Group A) received 50mg/kg nmAb 3.5 dpi, four RMs (Group B) received 10mg/kg nmAb 3.5 dpi, and two RMs (Group C) received 50mg/kg nmAb 4 dpi. Alanine transaminase levels (ALT), serum viral loads (sVL), and clinical evaluations were measured daily.

Results: All RMs in Group A exhibited low ALT (<150 IU/mL) and survived through 21 dpi. Three had low sVL (5×10^5 YFV-RNA copies/mL) that resolved by 15 dpi. In Group B, three RMs survived through 21 dpi. One RM required euthanasia at 6 dpi due to high sVL (2.51×10^8 YFV-RNA copies/mL) and ALT (486 IU/mL). One RM in Group C required euthanasia at 5 dpi due to high sVL (2.6×10^8 YFV-RNA copies/mL) and ALT (8,110 IU/mL).

Conclusions: Eight of ten RMs were able to clear YFV after nmAb treatment. RMs that received a 50mg/kg dose at 3.5 dpi had a 100% survival rate. This study helps inform clinical use of these nmAbs, since many infected individuals will wait until highly symptomatic to seek treatment.

38.

ΔsigH, an attenuated *Mycobacterium tuberculosis* mutant, prevents tuberculosis via efficient antigen presentation and iBALT formation

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Abstract

Background: Novel vaccination strategies are necessary to contain the TB/HIV co-pandemic, as the currently licensed anti-tubercular vaccine BCG, has limited and variable efficacy. Attenuated, live-replicating *Mtb* express the full complement of protective antigens not present in BCG and are most likely to induce long-lived immune responses and generate durable protection. In the absence of SigH, *Mtb* is unable to scavenge host oxidative burst and fails to survive or cause pathology in macaque lungs.

Methods: We performed immunogenicity and efficacy experiments using the resistant cynomolgus macaque species. We used high-throughput flow cytometry to elucidate the dynamics of the host immune response and single-cell RNA sequencing (scRNAseq) to characterize the immune responses in the airways.

Results: Macaques vaccinated with *ΔsigH* were characterized by the presence of robust, antigen-specific, T cell, particularly, CD8 responses in the lung. *ΔsigH* antigens were better presented by APCs relative to Mtb, likely explaining the stronger CD8 T cell responses. scRNAseq and systems serology showed protective T and B cell signatures in only *ΔsigH*-vaccinated and not BCG-vaccinated macaques. *ΔsigH*-vaccinated macaques were significantly protected against high-dose Mtb challenge and devoid of any granulomas indicating that this vaccine may prevent disease prior to the formation of the granulomas. *ΔsigH* is safe in the setting of Mtb/HIV co-infection and therefore its further development may be suitable for high HIV burden settings.

Conclusion: *ΔsigH* is a bon-a-fide lead candidate for development as an antitubercular vaccine. Our results could have transformative implications for the control of the ongoing, global TB pandemic.

39.

Chimeric hemagglutinin influenza nucleoside-modified mRNA vaccine induces protective stalk specific antibody and bone marrow plasma cells in rhesus macaques

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Abstract

Influenza virus infections cause significant morbidity and mortality. There is a great need for the development influenza vaccines that target conserved regions of the virus, like the hemagglutinin (HA) stalk, which would help to circumvent the need for changing the vaccine each year. To this end, we immunized rhesus macaques (RMs) with two mRNA vaccines encoding chimeric HA (cHA) proteins 1) as non-replicating nucleoside-modified mRNA packaged in lipid nanoparticle (LNP) 2) as self-amplifying (sa) mRNA encapsulated in dendron–based nanoparticles. RMs were vaccinated sequentially with cH8/1 followed by cH5/1 mRNA vaccines, which have antigenically distinct heads (H8 or H5) but identical H1 stalks, in the presence of pre-existing influenza virus immunity. To determine the innate immune activation, we monitored the frequency and activation of monocytes and various DC subsets in blood and performed RNAseq analysis of blood. We found that both mRNA vaccine platforms were proficient at activating innate immune cell subsets, particularly intermediate monocytes and CD11c+ mDCs, and RNAseq analysis revealed pathways related to complement, IL-2_STAT5, and IL-6_Jak_STAT3 signaling remained elevated longer in the mRNA-LNP Additionally, the mRNA-LNP immunized RMs induced significantly higher H1 stalk-specific binding antibody, bone marrow plasma cells compared to samRNA immunized RMs. Lastly, adoptive transfer of peak serum (W2 post cH5/1 immunization) from mRNA-LNP but not samRNA immunized RMs protected mice from lethal challenge by cH6/1N5. Collectively, these data demonstrated that cHA mRNA-LNP immunization is superior to samRNA immunization and induces antibody response capable of providing protection against heterologous influenza virus challenge.

40.

Influenza A Virus Disease Severity and Pathogenesis in a Pregnant *Macaca nemestrina* Model

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Abstract

Background: Pregnant women infected with influenza A viruses (IAV) are at higher risk for mortality, severe lung disease and adverse pregnancy outcomes. We hypothesize that an aberrant inflammatory response is responsible for severe disease in pregnant IAV-infected people.

Methods: We inoculated pregnant (N=10) and non-pregnant (N=8) pig-tailed macaques with a 2009 H1N1-pdm IAV strain and euthanized them 5 days post-infection (dpi). To quantify damage to pulmonary physiology, we adapted a human pulmonary function testing protocol to the macaque model, a novel approach, to examine the subjects' lung compliance, oxygenation, and ventilation. We performed qPCR viral load assays, Nanostring nCounter gene expression analysis, and intracellular cytokine staining on adult bronchoalveolar lavage (BAL) and lung.

Results: After infection, most NHPs, regardless of pregnancy status, had an oxygenation measure (PaO2/FiO2) below a threshold indicative of acute respiratory failure in humans. PaO2/FiO2 correlated strongly with viral loads 5 dpi in BAL (R=-0.56, p=0.07). nCounter analysis of adult lungs showed that 3 genes were upregulated and 9 were downregulated in non-pregnant animals when compared to pregnant animals. Of these genes, LILRA5 emerged as significantly and strongly correlated with PaO2/FiO2 (R=-0.65, p=0.03). Th17 frequencies were significantly depressed in blood of pregnant animals relative to non-pregnant ones (p=0.02)

Conclusion: Our data indicates aberrations in the innate and adaptive immune response between pregnant and non-pregnant animals after IAV infection. These results represent a foundation to determine the basis for pregnant people's susceptibility to severe influenza disease.

Session V Innovative Nonhuman Primate Research Tools and Resources (including new technologies, genomics, animal management & experimental procedures)

Moderators: Dr. Jeffrey Stanton Oregon Health and Science University/ONPRC)/ Dr. Rachel Reader(UC-Davis, CNPRC)

Session chair presentation: Dr. Jeffrey Stanton: Managing Graft-vs-Host-Disease in a Nonhuman Primate Model of Hematopoietic Stem Cell Transplantation

41.

Development of Oligonucleotide Barcoded Env Trimers for the High-Throughput Analysis of the Evolution of Neutralizing Antibodies Against HIV Env *in Vivo*

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Abstract

Broadly neutralizing antibodies (bNAbs) exhibit protective efficacy against HIV infection making them an ideal archetype for HIV vaccine design. Presently, no vaccine candidate has induced bNAbs or autologous neutralizing antibodies (NAbs) against neutralization-resistant tier 2 viruses. However, the development of stabilized, native-like env trimers such as BG505.SOSIP.664 has marked a significant advancement in vaccine design, due to their ability to elicit tier 2 NAbs in rhesus macaques (RM). NAb development against tier 2 immunogens in RM remains poorly understood, with hypothesized contributions from genetic variation at the IG loci, naive B cell repertoire, and differential gene expression in B cell lineages. To address these knowledge gaps, we have developed a set of BG505.SOSIP.644 probes capable of recovering paired clonotype identity, antigen specificity, and gene expression of B cells in a high throughput fashion. These probes were constructed by conjugating biotinylated BG505.SOSIP.644 to streptavidin covalently linked to both sc-RNA-Seq compatible DNA oligonucleotides and flow cytometry compatible fluorophores. Using these reagents, we isolated and sequenced BG505.SOSIP.644 specific memory B cells from the PBMCs of RM developing high titers of neutralizing antibodies. To benchmark the accuracy of our technology, we compared our recovered heavy and light chain sequences to those identified from the same animal using conventional methodology, and recovered 100% of previously identified NAbs. Our approach will allow for high-throughput analysis of the evolution of env specific lineages in both RM and humans in response vaccination with HIV env immunogens, including BG505.SOSIP.644.

42.

Pan-respiratory virus screening of a large rhesus macaque breeding colony

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Abstract

Pre-existing immunity (PEI) to seasonal human coronaviruses (hCoV) may provide some protection from disease associated with infection with SARS-CoV-2. The TNPRC houses ~5000 rhesus macaques in both indoor and outdoor areas potentially allowing for exposure to a variety of respiratory viruses. A strikingly high fraction of this colony harbors humoral immune responses both to common cold hCoVs and SARS-CoV-2, presumably due to cross reactive responses induced by infection with seasonal hCoV or unknown coronaviruses. We developed a 2-step approach to screen for and sequence circulating respiratory viruses using both nasal and rectal swabs collected from our colony during semiannual heath assessment as well as from animals that were hospitalized for illness. Samples collected from a single enclosure were pooled, cDNA was made, and a multiplex nested PCR was used for detection of a panel of respiratory viruses. Samples that were positive for any respiratory virus were then sequenced with a respiratory virus panel hybridization assay to determine which virus was present. Preliminary samplings captured low levels of hCoV as well as other viruses, including RSV, polyomavirus, and others. Viruses were detected in both nasal and rectal samples and from multiple enclosures, both indoor and outdoor, potentially suggesting sustained viral spread, similar to what is seen in human populations. Full genome sequencing is currently underway to identify and further characterize the viruses. PEI to respiratory viruses in macaques may impact the utility of these animals for experimental infection studies and allow focused research on the importance of cross-reactive immune responses.

43.

Spatial transcriptomic evaluation of the sites of SIV infection and rebound in NHP models reveals transcriptional patterns of host responses.

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Abstract

BACKGROUND

Despite effective ART, HIV-1 persistence is the major obstacle to cure. Here, we used 10X-Visium spatial transcriptomics to understand the tissue microenvironment harboring viral reservoirs involved in viral persistence.

METHODS

Rhesus macaques (RMs) were intra-vaginally/intra-rectally challenged with SIVmac239, ART initiated at 4 days and maintained for 6 months, followed by PET/CT-guided necropsy either during ART (3-7 weeks) or after ATI (early-ATI: 4-10 days; late-ATI: 18-24 days post-ATI). ^{64}Copper-labelled probe against viral envelope efficiently detected infection sites as early as 4-days post-ATI. In parallel, we optimized a pipeline for immunoPET/CT guided bulk RNASeq and 10X-Visium spatial transcriptomics to characterize the transcriptomic environment of PET/CT+ “hot” areas of the reservoir vs non-reservoir areas in tissues.

RESULTS

RNA-seq analysis showed activation of viral responses and cell migration in late-ATI tissues. Instead, on ART tissues catabolic processes and drug metabolism are upregulated, indicating ART effect on the tissues. ImmunoPET/CT-guided spatial transcriptomics of late-ATI tissues with detectable SIV allowed us to identify foci of viral rebound associated to differential transcriptional patterns compared to non-reservoir areas. Here, we detect the activation of genes associated to metabolic processes, cell localization, and cytokine immune signaling. The combination of both analyses indicates that active SIV rebounding foci might be characterized by immune cell migration and cytokine signaling.

CONCLUSION

Our newly developed techniques combining an immunoPET/CT-guided system with genomics and spatial transcriptomics allow us to identify with unprecedented detail possible markers of tissue reservoirs involved in maintenance and recovery of the SIV reservoir.

44.

Development of a Single-cell Multiomic Assay to Identify and Phenotype SIV/SHIV Infected Rhesus Macaque Cells

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Abstract

Background: SIV infection recapitulates many aspects of HIV-1 infection. However, the SIV reservoir at single-cell resolution remains to be defined. Here, we adapted our single-cell viral Assay for Transposase Accessible Chromatin with Select Antigen Profiling by sequencing (viral ASAPseq) to rhesus macaque SIV infection, to characterize SIV/SHIV reservoir epigenetics and phenotype.

Methods: To adapt viral ASAPseq to the nonhuman primate model, we compiled, titered, and characterized a rhesus cross-reactive antibody panel consisting of 62 immune-related markers using custom and commercially available oligo-tagged antibodies. Using this cocktail, we performed viral ASAPseq on purified lymph node CD4+ T cells at day 13 post-SIVmac251 infection. Finally, we developed a customized bioinformatics analysis pipeline to assess infected cells after viral alignments were made against the SIVmac251 sequence.

Results: From this pilot test, we identified 81 SIV+ cells out of 15,334 cells (0.5%); of these, 42 cells had 2 or more reads aligned to multiple regions of the provirus. Using both the epigenetic and surface antigen profiles, we found that 67% of infected cells had characteristics of T follicular helper, T follicular regulatory, or T regulatory cells. The remaining infected cells appeared to have effector, activated, and/or resident memory characteristics.

Conclusions: These initial findings demonstrate that NHP viral single-cell ASAPseq can be used to identify SIV+ cells and determine the unperturbed epigenetics and phenotype of these cells. Notably, this assay allows an unprecedented view into the SIV/SHIV reservoir to understand the perturbations associated with various cure strategies.

45.

Development of an Automated Digital PCR SIV Viral Load Assay

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Abstract

Background: The Non-Human Primate Core Virology Laboratory (NHPCVL) at the Duke Human Vaccine Institute provides SIV viral load testing to SVEUs nationwide utilizing an optimized RT-qPCR assay. Digital PCR (dPCR) touts higher sensitivity and precision than RT-qPCR by using absolute quantification to detect targets. QIAGEN's QIAcuity dPCR System is a fully automated platform that integrates all steps of dPCR into one instrument. The QIAcuity partitions samples utilizing a microfluidic nanoplate, creating a workflow comparable to qPCR while allowing for reduced run times and sample manipulation. The NHPCVL acquired a QIAcuity Four dPCR System to determine if a dPCR SIV viral load assay would be able demonstrate the same dynamic range and limit of quantification (LOQ) as its existing RT-qPCR assay.

Methods: Historical plasma samples were tested to determine a rate of concordance between PCR methods. Validation panels were created by spiking negative rhesus plasma with an infectious molecular clone of SIVsmmCG7G. Automated QIAGEN platforms were utilized for sample processing and dPCR setup; dPCR assays were run on the QIAGEN QIAcuity Four dPCR System. Validation experiments were performed until a statistically significant N was reached.

Results: Historical testing demonstrated a high correlation between PCR methods across the range of detection. Results from the validation experiments determined that the LOQ for the dPCR assay was 500 RNA cp/mL utilizing 500uL of plasma.

Conclusion: The NHPCVL successfully developed and validated a dPCR SIV viral load assay utilizing QIAGEN's QIAcuity dPCR System. Work is underway to further optimize the assay LOQ.

46.

A SMART method for efficiently isolating monoclonal antibodies from individual rhesus macaque memory B cells

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Abstract

Characterizing antigen-specific B cells is a critical component of vaccine and infectious disease studies in rhesus macaques (RMs). However, it is challenging to capture immunoglobulin variable (IgV) genes from individual RM B cells using 5' multiplex (MTPX) primers in nested PCR reactions. In particular, the diversity within RM IgV gene leader sequences necessitates large 5' MTPX primer sets to amplify IgV genes, decreasing PCR efficiency. To address this problem, we developed a switching mechanism at the 5' ends of the RNA transcript (SMART)-based method for amplifying IgV genes from single RM B cells, providing unbiased capture of Ig heavy and light chain pairs for cloning antibodies. We demonstrate this technique by isolating simian immunodeficiency virus (SIV) envelope-specific antibodies from single-sorted RM memory B cells. This approach has several advantages over existing methods for PCR cloning antibodies from RMs. First, optimized PCR conditions and SMART 5' and 3' rapid amplification of cDNA ends (RACE) reactions generate full-length cDNAs from individual B cells. Second, it appends synthetic primer binding sites to the 5' and 3' ends of cDNA during synthesis, allowing for PCR amplification of low-abundance antibody templates. Third, universal 5' primers are employed to amplify the IgV genes from cDNA, simplifying the primer mixes in the nested PCR reactions and improving the recovery of matched heavy and light chain pairs. We anticipate this method will enhance the isolation of antibodies from individual RM B cells, supporting the genetic and functional characterization of antigen-specific B cells.

47.

Defining genetic diversity of rhesus macaque Fcγ receptors with long-read RNA sequencing

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Abstract

Background: Fcγ receptors (FcγRs) are membrane-bound glycoproteins that bind the Fc constant regions of IgG and mediate important components of the immune response including activation of immune cells for clearance of opsonized pathogens or infected host cells. Many human studies have identified associations between FcγR gene polymorphisms and risk of infection, or progression of disease, suggesting a gene-level impact on FcγR-dependent immune responses. Rhesus macaques are an important translational model, yet little is known about the breadth of rhesus macaque FcγR genetic diversity. This lack of knowledge prevents evaluation of the impact of FcγR polymorphisms on outcomes of preclinical studies performed in rhesus macaques.

Methods: FcγRI, FcγRIIa, FcγRIIb, and FcγRIII genes from 206 Indian-origin Rhesus macaques were amplified by RT-PCR and sequenced using PacBio long-read technology. FcγR sequences were aligned to the rhesus macaque rheMac10 draft genome. Detailed three dimensional models of predicted protein structures of the FcγR genes were made using Alpha Fold.

Results: After sequencing FcγR genes from 206 Indian-origin rhesus macaques we found known and novel single nucleotide polymorphisms (SNPs) in FcγRI, FcγRIIa, and FcγRIII. We also found a frameshift insertion in FcγRI and a non-frame shift insertion in FcγRIIa. In addition to novel SNPs, we identified isoforms of FcγRI, FcgRIIa, FcγRIIb, and FcγRIII.

Conclusions: We identified known and novel FcγR SNPs that result in amino acid changes and that occur in regions that may impact FcgR function and signaling, including SNPs at predicted IgG amino acid contact sites and within the cytoplasmic tail.

48.

New IGRA algorithm for tuberculosis diagnosis and interpretation in cynomolgus macaques (Macaca fascicularis)

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Abstract

Specified pathogen-free cynomolgus macaques (CM; Macaca fascicularis) are required when recruited for biomedical experiments, and tuberculosis (TB) is the first screened out disease. Like humans, CM could develop either latent or active TB after being infected with Mycobacterium tuberculosis complex (MTBC). Apart from intradermal tuberculin test (TST), the gamma-interferon release assay (IGRA) is practically used to detect MTBC infection. Although we recently introduced the monkey IGRA (mIGRA) for TB detection in CM by combining a human QuantiFERON-TB Gold-Plus and monkey IFN-γ ELISApro into the assay, the high numbers of indeterminate results were unexpectedly encountered. We suspected that phytohemagglutinin (PHA) in a positive mitogen control and an algorithm for interpretation might be the cause. Thus, 316 CM naturally infected with MTBC were recruited for this project. PHA or Concanavalin A and pokeweed mitogen (C+P) mixture were used as mitogens and compared. The optical density (OD) from ELISA was used to interpret the results. The results were interpreted as i) positive when ODTB-NIL ≥ 0.05 and ODNIL ≤ 0.18, ii) negative when ODTB-NIL < 0.05, ODNIL ≤ 0.18, and ODMIT-NIL ≥ 0.18, and iii) indeterminate when ODMIT-NIL <0.18 or ODNIL >0.18. As a result, the indeterminate cases were higher (23% for PHA and 3% for C+P), and the negative cases were lower (67% for PHA and 87% for C+P) for the PHA mitogen, while the positive cases (10%) were the same. Combining between the use of C+P mitogen and a new algorithm of mIGRA interpretation, 86% of the indeterminate results were reduced.

49.

The MHC Pangenome of Mauritian Cynomolgus Macaques

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Abstract

Background

Mauritian cynomolgus macaques (MCM) are a valuable population for biomedical research. Founded from a small number of animals, the population exhibits substantially limited genetic diversity today. Only seven major histocompatibility complex (MHC) haplotypes and their recombinants are expressed by the MCM population. MCM have been used in a wide variety of SIV studies to evaluate vaccination protocols, therapeutic treatments, and cure strategies. The limited genetic diversity of MCM makes it possible to easily identify animals with identical MHC genotypes and establish experimental groupings based on MHC haplotypes.

Methods

We used Oxford Nanopore (ONT) ultra-long and Pacific Biosciences (PacBio) high-accuracy technologies for whole genome sequencing of thirteen MCM with known MHC haplotypes. MHC reads for each MCM were extracted from the whole genome dataset, and de novo assembled with Verkko.

Results

We have characterized the totality of genomic diversity of MCM in the MHC region, with seven fully assembled ~5 Mb haplotypes from the thirteen animals. This MHC pangenome of MCM has allowed us to begin characterizing the differences and commonalities between the seven haplotypes. For instance, four of the seven haplotypes contain an extended inverted duplication block containing a single *Mafa-AG* gene and a pair of *Mafa-G* pseudogenes.

Conclusion

The full characterization of MCM MHC diversity provides a unique opportunity to begin unraveling this immune-critical region's complexities. Different MCM MHC haplotypes have been implicated in variable levels of SIV control and viral load setpoint; this study lays the groundwork for exploring potential influencing factors beyond classical MHC antigen-presenting genes.

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In the SNPRC we are imaging from whole animal (PET-CT) to subcellular levels (confocal microscopy) with excellent results. We are using PET-CT scan to follow disease progression and treatment in macaques infected with Mycobacterium tuberculosis (Mtb), Simian Immunodeficiency Virus, Sars-Covid19 and coinfections.

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Abstract

It takes a well-coordinated team to do PET-CT at our primate center. PET-CT is a procedure that combines the pictures from a Positron Emission Tomography scan (PET, uses radioactive isotopes to image) and a Computed Tomography scan (CT, uses X rays to image). The combined scans give more detailed pictures of areas inside the body than either scan gives by itself. We can give you in live animals with CT 3 dimensional radiological images to detect anormal structures. With PET using F18-FDG as tracer we give you: Active glucose uptake. Detect inflammatory process, detect latent or active Mtb granulomas. Follow up disease and treatment without sacrificing the animal. Target tissue collection of the specific tissue of interest by biopsy or at necropsy. We are using PET-CT scan to follow disease progression and treatment in macaques infected with Mycobacterium tuberculosis (Mtb), Simian Immunodeficiency Virus, Sars-Covid19 and coinfections. We also follow Mtb vaccination protocols. We can find out early how well is the treatment working. We also are performing PET-CT of brains of live marmosets. We have a very good team that can perform PET-CT imaging in ABSL3 or ABSL2 for macaques. We have performed 400+ PET-CT scans from Sep21 to Sep22. And then go further to characterize the tissues at cellular and subcellular level, with images from PET-CT, we target collection of tissues to do multicolor immune staining and collect images by confocal microscopy. Including 3D deep imaging, of clarified tissue, up to 300 um.

51.

Real Time Analysis of SARS-CoV-2 Induced Cytolysis Reveals Distinct Variant-Specific Replication Profiles

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Abstract

The ability of each new SARS-CoV-2 variant to evade host humoral immunity is the focus of intense research. Each variant may also harbor unique replication capabilities relevant for disease and transmission. Here we demonstrate a new approach to assessing viral replication kinetics using Real Time Cell Analysis (RTCA). Virus induced cell death is measured in real time as changes in electrical impedance through cell monolayers while images are acquired regularly through an on-board microscope and camera. Using this system, we quantified replication kinetics of five clinically important viral variants; WA1/2020 (ancestral), Delta, and Omicron subvariants BA.1, BA.4, and BA.5. Multiple measures proved useful in variant replication comparisons including the elapsed time to, and the slope at, the maximum rate of cell death. Important findings include significantly weaker replication kinetics of BA.1 by all measures, while BA.5 harbored replication kinetics at or near ancestral levels, suggesting evolution to regain replicative capacity, and both an altered profile of cell killing and enhanced fusogenicity of the Delta variant. More established approaches, such as plaque reduction microneutralization (PRMNT) assay techniques, are also being paired with RTCA methods to provide insight into potential differences of antibody neutralization across SARS-CoV-2 variants. Together, these data show that RTCA is a robust method to assess replicative capacity of, and neutralization against, any given SARS-CoV-2 variant rapidly and quantitatively, which may be useful in assessment of newly emerging variants.

POSTER PRESENTATIONS

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A Rhesus Macaque Model of HIV/HBV Co-infection

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Abstract

HIV/HBV co-infection is common with an estimated 10% of HIV-infected individuals also infected with HBV. Co-infected individuals progress to chronic HBV more frequently with higher probability of extensive liver fibrosis and hepatocellular carcinomas. Thus, a greater understanding of the interplay between HIV and HBV infections is urgently needed to design strategies to prevent accelerated liver disease. Rhesus macaques (RM) are a well-established non-human primate model for HIV research, and we discovered recently that antibody-mediated $CD4^+$ T-cell depletion in RM leads to long-term HBV replication. Thus, we investigated the potential of inducing natural $CD4^+$ T-cell depletion via $SHIV_{DH12\ Clone\ 7}$ and using it to establish HIV/HBV co-infection.

RM were intravenously infected with $SHIV_{DH12\ Clone\ 7}$ ($TCID_{50}$=5000) followed by challenge with $HBV_{genotype\ D}$(10^9 virions) three weeks later.

Preliminary studies showed successful $CD4^+$ T-cell depletion in two RM following SHIV infection. However, one RM controlled SHIV infection (Mamu-B*08^+) and $CD4^+$ T-cells returned concurrent with clearance of HBV. The second RM exhibited SHIV (>10^5 copies/ml) and HBV (>10^4 copies/ml) chronic co-infection (>24 weeks) along with HBsAg in serum and HBV RNA in the liver. Based on the preliminary results, we repeated the study with nine additional animals and found that four exhibited a similar trend of co-infection.

These results indicate that SHIV-mediated $CD4^+$ T-cell depletion helps sustain HBV infection thereby making this the first HIV/HBV co-infection model in RM that can be beneficial for studies investigating pathogenesis associated with co-infection; which will be critical for the further development of this model.

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SRV transmission in Rhesus macaques

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Abstract

Introduction: In the past, to generate SRV-1 positive control rhesus macaques, we performed serial in vivo passage, by inoculating uninfected macaques with whole blood from SRV-1 infected (antibody/DNA positive) macaques. However, recent attempts using this approach have failed. This is contrary to published, historical data. This study investigates why it has become more difficult to transmit SRV.

Methods and results: A naïve macaque was transfused with 30 ml of citrate-anticoagulated blood from an infected macaque. Blood samples were collected from the newly transfused animal on days 3, 7, 10, 14, 21, and 28 for antibody and PCR testing. No infection was detected after 4 weeks. Subsequently, the same macaque was inoculated with supernatant of tissue culture derived SRV-1. SRV infection was detected in blood by PCR on Day 14 and seroconversion followed. To detect whether serial transfusion results in viral evolution, a 400 bp PCR amplified fragment was generated from the neutralization epitope within the env gene. Purified PCR fragments were sequenced using the ABI 3730 Genetic Analyzer. Sequencing of the SRV-1 neutralizing region of env, detected differences from the Genbank sequence in different generations of transfused animals and in the tissue culture inoculum. These nonsynonymous mutations resulted with D265N and C269Y mutants.

Conclusion: The evolution of the env gene upon serial in vivo passage may be a factor in the reduced ability to transmit SRV 1 by transfusion in subsequent generations of monkeys, but further research is needed to confirm this.

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Defining Blood Hematology Reference Values in Pig-tailed Macaques Using An Isolation Forest Algorithm

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Abstract

Background: The antiretroviral drug islatravir induced lymphopenia in humans when administered at high doses during clinical trials. This highlights the importance of thorough preclinical safety evaluation in macaques. Drug toxicities and their associated pathologies are often preceded by changes in blood hematology. To assess the safety of novel pharmaceuticals in macaques the normal ranges of hematological values must be defined.

Methods: Eighteen female pig-tailed macaques with a mean [range] age of 10 [6-17] years and a mean [range] weight of 7.22 [5.65-12.05] kg were evaluated. Blood was collected between 1-25 times per individual for a total of 159 samples. Complete blood counts (CBCs) were performed on the Beckman Coulter AcT diff2 Hematology Analyzer. To remove statistical outliers, an isolation forest method was applied with a contamination proportion of 10%.

Results: The mean [range] of white blood cells (WBCs) was 6.5 [2.6-16.2] 10^3/µl (n=159). To create a reference index, WBC counts were plotted against animal weight and age, as well as CBC hematology parameters (lymphocytes, monocytes, granulocytes, red blood cells, hemoglobin, hematocrit, and platelets), and input into the isolation forest algorithm. After removing all possible outliers, the reference values of WBCs are 6.4 [3.4-11.0] 10^3/µl (n=87).

Conclusions: Here, we defined the normal range of WBCs in female pig-tailed macaques using a relatively large sample size which included longitudinal collections. These methods will be applied to all CBC parameters, and the data generated will be a valuable reference index for studies evaluating drug toxicities in pig-tailed macaques.

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Altered Neutrophil Frequency and Function During *Plasmodium fragile* Co-Infection of ART-treated SIV+ Rhesus Macaques

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Abstract

Background: The mechanisms by which disease progression and pathogenesis is accelerated during HIV/malaria co-infection remain unknown. Neutrophils have been implicated in transmission and pathogenesis of HIV and malaria separately. We hypothesized that *P. fragile* co-infection of antiretroviral therapy (ART)-treated, SIV+ rhesus macaques (RMs) would result in altered neutrophil frequency and function, as measured by neutrophil degranulation.

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. Peripheral blood neutrophil frequencies were assessed via flow cytometry. Neutrophil degranulation was characterized in plasma via ELISA.

Results: Peak *P. fragile* parasitemia occurred at W14 post-SIV (median %parasitemia=25.5% iRBCs) and all animals became anemic (median=19.8% hematocrit). Compared to baseline (median=53.58%) and peak SIV infection at W2 post-SIV (median=56.98%), neutrophil frequencies increased at W14 post-SIV (median=73.25%), then stabilized to baseline levels. Plasma levels of MPO, a component of neutrophil granules, were significantly decreased at W14 post-SIV as compared to W2 post-SIV ($p<0.0001$). In contrast, neutrophil elastase, a marker of neutrophil extracellular trap (NET) formation, was significantly increased at W14 post-SIV as compared to baseline and W2 post-SIV ($p<0.0001$ for both).

Conclusions: Our data indicate that *P. fragile* co-infection resulted in increased peripheral neutrophil frequencies coinciding with peak parasitemia. Neutrophil functional changes, including decreased neutrophil extracellular degranulation and increased production of NET formation markers, also coincided with co-infection and peak parasitemia.

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Non-invasive Buccal Specimen Collection for *Mycobacterium tuberculosis* Detection in Wild Cynomolgus Macaques (*Macaca fascicularis*)

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Abstract

Mycobacterium tuberculosis (Mtb) are pathogenic bacteria that cause tuberculosis in humans and non-human primates. Previously, the prevalence of Mtb in 705 cynomolgus macaques (CM: *Macaca fascicularis*) from 9 populations throughout Thailand was surveyed. Monkeys were captured, anesthetized, collected buccal swabs, determined Mtb using IS*6110*-nested PCR method, and detected 1-12 (0.3-26.7%) positive specimens. Thus, it suggests that buccal swab can be a specimen of choice for Mtb detection in wild CM. The disadvantage of this specimen collection is that the animals must be caught and anesthetized, thus the non-invasive buccal specimen collection, namely rope bait method, was developed. The polyester ropes (10-cm long pieces) were dipped into syrup, and baited to 4 of 9 Mtb positive populations, totally 173 animals. After the animals had taken the rope, chewed it until the sweetness had gone, and discarded the rope onto the ground, the rope was then quickly collected and preserved in sterile lysis buffer, and determined Mtb using IS*6110*-nested PCR technique. The limit of detection of the IS*6110*-nested PCR technique in baited rope solution was 10 fg/mL. All four CM populations were detected Mtb for 4.8% (5/104), 3.6% (1/28), 8.3% (2/24) and 5.8% (1/17) of which the numbers were comparable to those of the invasive specimens. Results of this study denote that the rope bait can be an alternative non-invasive method for buccal specimen collections for Mtb detection in wild macaques.

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Rapamycin Disrupts MHC class 1-restricted Control of SIVmac239 Replication

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Abstract

Background: Previously, we showed mTOR inhibition with rapamycin can limit CD4+ T cell proliferation in SIV-infected rhesus macaques (RM) on ART but it had no durable impact on SIV reservoir dynamics. As SIVmac239 replication was fully suppressed with ART, the impact of rapamycin on SIV-specific CD8+ T cell responses remained undefined. Here we used viremic RM expressing at least 1 protective MHC-1 allele (pMHC) to characterize the effects of rapamycin on the functional activity of CD8+ T cells.

Methods: pMHC+ (Mamu A*01, B*08 or B*17) RM with plasma viral loads (pvl) <10,000 copies/mL received intramuscular injections of rapamycin at 0.02 mg/Kg (n=10) or vehicle control (n=8) twice daily for 6 weeks. Blood was collected before and up to 6 weeks after rapamycin treatment cessation. Pvl were assessed by RT-qPCR, while T cell dynamics and immune responses were assessed with flow cytometry assays.

Results: Rapamycin treatment induced a reduction in CD8+ memory T cell proliferation (%Ki67) and absolute counts, although frequencies of SIV-specific CD8+ T cells remained unchanged. Despite a marked decrease in CCR5 expression on CD4+ memory T cells in blood; there was a significant increase in pvl (p=0.002) in rapamycin-treated RM in contrast to vehicle-treated controls. Immunological and virological effects of rapamycin generally returned to baseline levels within 2-4 weeks of treatment cessation.

Conclusion: The increase in SIVmac239 replication during rapamycin treatment suggests mTOR signaling plays a critical role in the control associated with MHC-1-restricted CD8+ T cell responses.

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

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Abstract

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. Then, ART require lifelong administration, and remission and complete cure that enable drug discontinuation in HIV infection are the current global goal. Development of a vaccine therapy is one of the strategies being pursued for functional cure and seeks to induce immunological control in the absence of ART. In the previous study, we genetically constructed a live attenuated simian human immunodeficiency virus to express the adjuvant molecule Ag85B (SHIV-Ag85B). 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. These results suggest that provide the possibility of eradicating a pathogenic lentivirus from infected cells. In this study, we investigated the therapeutic efficacy of SHIV-Ag85B in pathogenic SHIV89.6P-infected cynomolgus monkeys that began antiretroviral therapy during acute infection. In the cynomolgus monkeys inoculated with SHIV-Ag85B, monkeys did not show viral rebound after discontinuation of ART. In those animals, proviral DNAs in peripheral blood were reduced to low levels and the numbers of peripheral CD4+ T cells were recovered at normal levels. Our findings showed partial therapeutic efficacy of SHIV-Ag85B vaccination following ART discontinuation in pathogenic SHIV-infected cynomolgus macaques.

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High Throughput Tracking of Longitudinal B Cell Dynamics During the Development of Autologous Neutralizing Antibodies in Rhesus Macaques Immunized With A Novel Clade C HIV-1 Envelope

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Abstract

The mechanisms by which B cells engage complex antigens and mature to produce neutralizing antibodies are poorly understood. Here, we used immunological techniques coupled with high throughput immunoglobulin receptor sequencing to characterize the expansion, persistence, recall, and diversity of single antigen specific B cells, as well as the development of neutralizing antibody lineages, following DNA/modified vaccinia Ankara (MVA)/protein vaccination with novel HIV-1 transmitted founder HIV-1 envelope (Env) glycoproteins. In one rhesus macaque (RM), two B cell clonotypes arose from the same immunoglobulin variable domain heavy and light chain germlines and exhibited autologous neutralization against the same Env gp120 epitope. Early antibody variants from both clonotypes present after MVA immunization shared high identity with germline yet neutralized the autologous Env pseudovirus. Later variants acquired somatic hypermutation (SHM) during and after immunization and were abundant at multiple time points post-MVA. In all immunized RM, clonal expansion and SHM occurred throughout immunization with extensive recall of existing B cell clonotypes; however, this was not sufficient to produce neutralizing activity. The presence and pairing of VH/VL germlines capable of neutralization with little to no SHM, early engagement and expansion of the neutralization capable clonotypes, and multiple rounds of affinity maturation and successful competition against many antigen specific B cells during recall likely resulted in neutralization activity. The findings provide new insight into how B cells respond against HIV-1 Env during heterologous prime and boost vaccination in nonhuman primates and why neutralizing antibodies are rare.

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Biodistribution And Pharmacokinetics Of Broadly Neutralizing antibody ePGDM1400v9 In African Green Monkeys

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Abstract

Background

Non-human primates (NHP) provide the closest immunophysiological model to humans in HIV research. We explored the African Green Monkey (AGM) as a model for HIV prophylactic immunogen studies. The broadly neutralizing antibody PGDM1400 binds to the HIV envelope protein, providing potent protection against infection. Consequently, it is imperative to assess the pharmacokinetics of PGDM1400.

Objectives

To determine the tissue distribution and pharmacokinetics of ePGM1400v9 in AGMs.

Methods

PGDM1400v9 was fluorescently tagged using Cy3 or Cy5. Three female AGMs were injected intravenously with 10mg/kg ePGDM1400v9-Cy3 or ePGDM1400v9-Cy5 or PBS at the Institute of Primate Research, Nairobi. Tissue and blood were sampled on alternate days before sacrifice on day7. Cryopreserved tissues were immunofluorescently stained and imaged using a deconvolution microscope. Quantification of antibody in the blood was done using a fluorescence spectrophotometer.

Results

Imaging of the vaginal and rectal mucosa revealed bNAb epithelial saturation at day 7. ePGDM1400v9 presence below the lamina propria in day 4 tissues suggests passive infusion to muscles from peripheral blood as one mode of antibody delivery to the mucosa. The binding of bNAb to macrophages in the liver revealed a second mode of biodistribution via antigen presenting cells. The inverse relationship between bNAb fluorescence intensity in plasma and vaginal mucosa over time demonstrates antibody loading from peripheral blood to tissues.

Conclusion

These findings indicate that the AGM is a viable model for HIV prophylactic immunogen biodistribution and pharmacokinetics research. This study has important implications in HIV preclinical research as it presents an affordable alternative NHP model.

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The Inhibitor of Apoptosis Protein Antagonist Ciapavir Reactivates Latent SIV in Antiretroviral (ART)-suppressed Rhesus Macaques

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Abstract

Background:

Activation of latent HIV may make HIV reservoirs more susceptible to immune-mediated clearance; however, this hypothesis is difficult to test in vivo because few latency reversal agents are well-tolerated at efficacious doses. Recent results indicate that second mitochondria-derived activator of caspases (SMAC) mimetic ciapavir activates the non-canonical nuclear factor kappa-B signaling pathway and induces HIV activation both in vitro and in a humanized mouse model. Here, we tested ciapavir in SIV-infected macaques.

Methods:

Macaques were infected with SIVmac251 and treated with single doses of 0.0, 0.25, 0.5, 1.5, or 4 mg/kg ciapavir. A separate cohort of 22 SIVmac251-infected, ART-suppressed animals were treated with 3 weekly doses of 0.5 mg/kg intravenous infusion of ciapavir or vehicle control. SIV plasma viral load was measured after each dose, along with pharmacokinetic and pharmacodynamic readouts.

Results:

In viremic animals, 0.25, 0.5, and 1.5 mg/kg doses led to significantly increased SIV viral load; the animal dosed with 4 mg/kg died with gastrointestinal hemorrhages. In ART-suppressed, SIV-infected animals, ciapavir induced significant (P=.0008) reactivation of latent SIV and upregulation of genes in the non-canonical pathway. Two (of 10) animals treated with 0.5 mg/kg ciapavir experienced respiratory arrest and died after the third dose. One day after dosing, ciapavir led to significantly increased CD69 on CD4 T-cells but did not affect Ki67 expression.

Conclusion:

Ciapavir robustly reactivated latent SIV in ART-suppressed animals but was associated with toxicity. Future studies should explore strategies for safe dosing of SMAC mimetics to activate latent HIV.

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Utilization of a 30-Color Flow Cytometry Panel for Immunophenotypic Analysis of Innate Immune Subsets at the Maternal/Fetal Interface in Pregnant Rhesus Macaques

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Abstract

Background: Malaria in pregnancy (MIP) is linked with adverse maternal and fetal outcomes. The role of innate immunity in MIP pathogenesis remains incompletely defined. Using *Plasmodium fragile* infection of rhesus macaques (RMs) to model *P. falciparum* in humans, we aim to (1) develop a non-human primate model of MIP and (2) characterize the innate immunophenotype of decidual and placental tissue from RMs with and without malaria.

Methods: Placental and decidual tissue were collected from three RMs in 1st, 2nd or 3rd trimester. Two additional RMs were intravenously inoculated with *P. fragile* (20x10^6 infected erythrocytes) in either 1st or 3rd trimester, with placenta and decidua tissue collection occurring 2-4 weeks post-inoculation. Placental and decidual cells were analyzed via flow cytometry utilizing a 30-color immunophenotype panel targeting innate immune subsets.

Results: In placenta and decidua from *P. fragile*-infected RMs and healthy controls, we identified viable neutrophils (CD45+HLA-DR-CD66abce+CD11b+CD14+CD49d-), basophils (CD45+HLA-DR-CD66abce+CD11b+CD123+), eosinophils (CD45+HLA-DR-CD66abce+CD11b+CD14-CD49d-). Lineage negative (CD3-CD20-CD14-) NK cells (CD45+NKG2A/C+CD8+CD127-), ILC1 (CD45+CD127+c-Kit-ST2-CD56+/-), ILC2 (CD45+CD127+c-Kit-ST2+), and ILC3 (CD45+CD127+c-Kit+NKp44+CD56+) were characterized. Additionally, classical (CD14++CD16-), intermediate (CD14++CD16+) and non-classical monocytes (CD14+CD16+), and dendritic cells (mDCs=CD11c+CD123- and pDCs=CD11c-CD123+) were identified. Markers of activation (CD69), proliferation (Ki-67), and apoptosis (Caspase-3+) were characterized within each subset.

Conclusions: Our 30-color immunophenotyping panel enables characterization of innate immune subsets, which will provide critical insight into the underlying mechanisms of immunopathogenesis during *P. fragile* MIP. Future directions include enrollment of additional animals into MIP and healthy control groups, and expansion to investigate the impact of SIV co-infection on adverse pregnancy outcomes during MIP.

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IL-15 Treatment Rescues Perturbations to NK Cell Mitochondrial Fitness and Effector Function Driven by Acute SIV Infection

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Abstract

NK cells play a central role in limiting SIV transmission and dissemination. However, SIV infection also compromises NK cell functional capacity and lymph node (LN) homing, coinciding with dysregulated mitochondrial fitness and reactive oxygen species (ROS). IL-15 enhances effector function and counteracts metabolic defects in NK cells. Herein, we aimed to elucidate the relationship between mitochondrial fitness, function, and trafficking, and the extent that IL-15 can rescue perturbations driven by acute lentiviral infection.

Peripheral LNs, PBMCs, and spleen samples from naïve and acutely SIV-infected rhesus macaques were used to analyze metabolic and functional profiles of NK cells. Samples were incubated overnight with/without IL-15 prior to multiparameter flow cytometric analysis to assess mitochondrial polarization (ΔΨm), mitochondrial ROS, and intracellular glutathione levels.

SIV infection negatively impacted NK cell metabolic fitness, diminishing ΔΨm and glutathione abundance and increasing mitochondrial ROS. IL-15 treatment reversed these metabolic defects in NK cells from SIV+ samples, and qPCR revealed that IL-15 increased expression of multiple antioxidant enzymes and metabolic transcription factors. SIV infection also decreased NK cell expression of the LN follicle trafficking marker CXCR5 in LNs, a phenotype rescued by IL-15 incubation in PBMC and spleen. Furthermore, NK cell cytokine response, degranulation, and polyfunctionality were also enhanced by IL-15 treatment and correlated with ΔΨm and glutathione abundance.

These data indicate that lentivirus infection systemically induces mitochondrial dysfunction in NK cells and that IL-15 can reverse these effects, highlighting the importance of metabolism in infection and supporting IL-15 as a candidate to augment NK-based HIV therapies.

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Effects of Obesity on Gut Barrier Integrity and Fecal Microbiome Composition in SIV-Infected Rhesus Macaques Treated with Antiretroviral Therapy

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Abstract

Background: Microbial dysbiosis and pathologically leaky gut may contribute to the chronic inflammatory response associated with both obesity and HIV/SIV infection. We sought to determine the impact of SIV infection with antiretroviral therapy (ART) on inflammatory and leaky gut biomarkers and changes in the fecal microbiome between lean and obese rhesus macaques.

Methods: Lean (n=6, weight=10.6 ± 1.41kg) and diet-induced obese (n=5, weight=15.2 ± 1.55kg) adult male macaques were infected intravenously with SIVmac239M, resulting in peak viremia after 2 weeks. ART was initiated at 5 weeks post-infection. Fecal samples were collected at baseline, peak viremia, 4- and 8- months post-ART initiation. Microbiome analysis was performed using 16s rRNA gene amplicon sequencing and Qiime2 software. Blood samples collected at baseline, 4-, 8-, 12-, and 18-months post-ART initiation quantified circulating lipopolysaccharide binding protein (LBP) and C-reactive protein (CRP). Claudin-2, claudin-4, occludin, and tight junction protein 1 gene expression in necropsy colon samples was analyzed by qRT-PCR.

Results: Obese animals exhibited higher initial circulating LBP and CRP levels that did not change significantly throughout the study. Conversely, lean animals exhibited significant increases in circulating LBP and CRP that approached levels of obese animals. No differences in gene expression were observed. Fecal microbiome analysis is ongoing.

Conclusion: Obese macaques exhibited elevated baseline levels of inflammatory/leaky gut biomarkers. After SIV infection and complete ART suppression, both groups exhibited similar LBP and CRP levels, indicating worsening inflammation and gut permeability in the lean cohort. At the time of presentation, fecal microbiome data will be discussed.

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Viral Reservoirs Persist Following B-Cell Follicle Ablation in a Macaque Model of Suppressed HIV-1 Infection

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Abstract

Background: B-cell-targeting CAR T cells, used clinically for B-cell malignancies, present several advantages for depleting HIV reservoirs in B-cell follicles (BCFs). These include influx of HIV-specific $CD8^+$ T cells, augmented antiretroviral therapy (ART) bioavailability, and/or induction of anti-reservoir cytokines that may possess latency reversing properties. Here we quantified the impact of B-cell-targeting CAR T cells in our nonhuman primate (NHP) model of persistent HIV infection.

Methods: Anti-CD20 CAR T cells were manufactured and infused into uninfected and simian/human immunodeficiency virus (SHIV)-infected, ART-suppressed NHPs. We quantified the kinetics and magnitude of CD20 depletion through flow cytometry, immunohistochemistry, and PCR-based methods to determine the ability of CD20-targeted CAR T cells to disrupt BCFs and impact systemic viral reservoir size.

Results: CD20 CAR T cells were well-tolerated and highly functional in NHPs with rapid expansion and trafficking to tissue-associated viral sanctuaries including BCFs and gut-associated lymphoid tissues. CD20 CAR T-cell treatment potently yet transiently ablated BCFs, redistributed SHIV-infected cells in lymphoid tissues, and decreased lymph node-associated T follicular helper (T_{FH}) cells, which were restored following CAR T-cell contraction. Across 27 distinct compartments and via multiple assays, no effective reduction of viral reservoirs was observed.

Conclusion: Our study highlights the unique ability of CD20-targeted CAR T cells to unmask T_{FH} cells within BCF sanctuaries; however, these CAR T cells alone are insufficient for viral eradication in systemic reservoirs. These findings reinforce the need for combination strategies to effectively deplete viral reservoirs, such as incorporating both CD20-directed CAR T cells and directed anti-HIV immunotherapies.

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A novel adjuvant to enhance NK cell responses in HIV preventive vaccines

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Abstract

Background

When considering HIV preventive vaccines, the RV144 clinical trial remains the superior vaccine strategy, utilizing an ALVAC-HIV vector and alum-adjuvanted AIDSVAX B/E gp120 protein, with 31.2% efficacy. This vaccine regimen demonstrated limited yet significant protection and is ideal for further research. Prior studies on rhesus macaques have identified NK cell and ILC subsets as correlates of protection against SIV infection. Using novel adjuvants along with this vaccine regimen may improve vaccine efficacy by enhancing such protective correlates. In vitro studies demonstrate the novel adjuvant LL001's ability to elicit strong mucosal innate immune responses. This study investigates NK cell responses to the RV144 regimen administered along with LL001, in mouse spleen, liver, blood, lung, and bone marrow.

Methods

C57BL/6 mice were categorized into four treatment groups (n=6): PBS, ALVAC, ALVAC + LL001, and LL001 alone. Mice were sacrificed after two weeks of vaccination, and immune signatures and functions were studied using high-dimensional flow cytometry.

Results

LL001 altered receptor expression and the functionality of NK cells. In LL001-treated groups, mature NK cell (KLRG1+) populations were significantly increased in all tissues, while exhausted NK cells (PD1+) were decreased. Furthermore, CXCR5 and CCR2 were expressed more in these NK cells, suggesting lymph-node migration. NK cells in all tissues in LL001-treated mice revealed increased proinflammatory cytokine production (IL6, TNFα, IFNγ, and IL1β). Memory-like NK cells (Ly49H+) demonstrated similar increased functionality ($p<0.05$ for all).

Conclusion

LL001 may serve as an efficacious adjuvant, enhancing NK cell responses of the RV144 HIV vaccine strategy in vivo.

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Optimization Of Resting Periods For Functional T Cell Assays On Cryopreserved Non-Human Primate (NHP) Peripheral Blood Mononuclear Cells (PBMCs)

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Abstract

NHP models are an unrivaled resource for preclinical testing of HIV vaccines and treatments. Human studies are standardized for overnight rest at 37°C of PBMC after thawing, which increases the quality of responses detected by functional assays. Whether resting is similarly beneficial for NHP samples is undetermined. To identify optimal resting periods in NHPs, we tested mitogen and antigen-specific T-cell responses by IFN ELISpot and intracellular cytokine staining (ICS) assays in three SIV-negative rhesus macaque (RM), three SIV-negative pigtail macaque (PTM), and three SIV-infected PTM PBMC. Cryopreserved cells were thawed, and rested for 0, 3, 5, 9, or 16 hours at 37°C before stimulation with PMA/ionomycin, rhesus CMV, or SIVmac239 peptide pools. Resting led to reduced cell recovery with up to 50% cell loss after 16-hrs and increased background of IFN-gamma and TNF-alpha responses, particularly for RM CD8+ T cells. Resting increased the detection sensitivity of antigen-specific T cell responses, although this benefit was variable between animals and stimulants. PMA/ionomycin or CMV peptide stimulation responses from CD4+ T cells were significantly boosted with the 5-9 hour resting times compared to no resting, especially for IL-2 and TNF-alpha secretion. The resting benefit for CD8+ T cell responses in the ICS assay was less striking and confined to increased TNF-alpha secretion. Overall, a window of 3-9 hours resting provided optimal balance of cell recovery with enhanced detection for IFN-gamma ELISpot, CD4 TNF-alpha and IL-2 in the ICS assay, with the rescue of low-frequency responses that would otherwise be undetectable.

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NK Cell Adaptive Functions Driven By The MHC-E/NKG2 Axis Against HIV/SIV And Co-Infections.

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Abstract

Background

Natural killer (NK) cells have been shown to exhibit adaptive immune functions. Our laboratories demonstrated that in non-human primates (NHP) and humans, SIV/HIV infections and vaccinations induce antigen-specific memory NK cell responses largely relying on the MHC-E/NKG2 axis. Understanding the mechanisms regulating memory NK cell function is crucial to harness their potential in future HIV vaccines or cure immunotherapies. Moreover, whether similar mechanisms are involved in NK cell memory against other viruses that disproportionally affect persons living with HIV is unclear.

Methods

To study antigen-specific NK cells in detail, we adapted assays to clonally expand single NK cells (NKCL) from participants with disparate HIV or BK/JC polyomavirus serostatus and from SIV -/+ NHP. NKCL were characterized phenotypically and functionally, including MHC-E-binding peptide-specificity. Models to predict MHC-E-binding epitopes were optimized through computational pipeline.

Results

MHC-E-stabilizing nonamers derived from SIV, HIV and BK/JC polyomaviruses were identified and triggered potent peptide-specific cytotoxic responses by a subset of NKCL generated from participants previously exposed to these viruses. Using blocking antibodies and siRNA knockdown, we confirmed antigen-specific NKCL responses largely depend on NKG2C. Most SIV- and HIV-derived sequences were highly conserved across strains, likely mitigating viral escape.

Conclusion

Our data suggest adaptive NK cell responses to viruses rely on a conserved and epitope-specific targetable mechanism that largely depend on the MHC-E/NKG2C axis and could be harnessed in future

vaccine / therapeutic modalities. Infusion of *ex vivo* expanded antigen-specific NK cells into NHP models will allow evaluation of adaptive NK cell-mediated protection *in vivo*.

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In vivo evolution of env in SHIV-AD8-infected rhesus macaques after AAV-eCD4-Ig therapy

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Abstract

Background

eCD4-Ig is a potent HIV entry inhibitor that mimics the engagement of both CD4 and CCR5 with the HIV Env, a property which imbues it with remarkable neutralization breadth. However, *env* is exceptionally genetically malleable and can evolve to escape a wide variety of entry inhibitors. Here we document the evolution of partial eCD4-Ig resistance in SHIV-AD8-infected rhesus macaques (RMs) treated with AAV encoding eCD4-Ig.

Methods

Six RMs were infected with SHIV-AD8$_{EO}$. At 10 and 14 weeks after infection, five of the six macaques were administered AAV encoding eCD4-Ig. Viral load, serum eCD4-Ig, and anti-drug antibodies (ADA) were monitored, and longitudinal env sequencing was performed for animals that experienced consistent serum levels of eCD4-Ig. Mutations recovered from one animal with a striking phenotype were further characterized.

Results

Two of five treated RMs developed persistent levels of eCD4-Ig in serum but experienced only partial or transient reductions in viremia. In one of the two RMs, setpoint viremia plateaued at 1,000 vRNA copies/ml, despite concomitant serum concentrations of eCD4-Ig in the 70-100 µg/ml range. Three mutations recovered from this animal (R315G, A436T, G471E) were sufficient to confer substantial resistance to eCD4-Ig-mediated neutralization on the parental Env, accompanied by a marked cost to viral fitness.

Conclusions

In vivo selected, eCD4-Ig resistant, SHIV-AD_{EO} is replication deficient in rhesus CD4-T cells. eCD4-Ig resistance appears to arise through: 1) exploitation of a single amino acid difference between rhesus CD4 and eCD4-Ig (I39N), and 2) modulation of CCR5 engagement without altering coreceptor tropism.

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Assessment of pretreatment ART interruption and dual CAR-T cell infusion as a strategy to increase persistence of CAR/CXCR5-T cell therapy in SIV-infected rhesus macaques

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Abstract

During chronic SIV infection, viral replication is concentrated within lymphoid B cell follicles. Infusion of SIV-specific CAR-T cells expressing CXCR5 leads to follicular localization; however, the cells fail to persist in the animal. We hypothesized that a combination of a short interruption of ART, prior to infusion of cells containing dual CD28/4-1BB CARs, would lead to increased persistence.

Dual CD28/4-1BB CAR/CXCR5-T cells, produced by gammaretroviral transduction, were functionally assessed by viral suppression and migration assays. SIV-infected rhesus macaques were released from ART three days prior to infusion of CD28/4-1BB CAR/CXCR5-T cells. The first animal, treated with a dose of 2 x 108 cells/kg and a single dose of Tocilizumab to prevent cytokine release syndrome, died two days post-infusion. Subsequent animals received a reduced dose of 0.5 x 108 cells/kg and three daily treatments of Tocilizumab. Lymph node RNAscope identified CAR/CXCR5-T cells at post-infusion time points.

Use of two separate gammaretroviruses during transduction led to production of functional CAR/CXCR5-T cells, which primarily expressed both CD28 and 4-1BB CARs. Using RNAscope, preliminary post-infusion results suggest that the CAR/CXCR5-T cells were proliferative; however, the number of CAR/CXCR5-T cells peaked at 6 days post-infusion (DPI) and disappeared by 14 DPI.

The results of this study suggest that functional CD28/4-1BB CAR/CXCR5-T cells can be produced by dual gammaretroviral transduction. Addition of a pre-infusion ART interruption and dual 4-1BB/CD28 CARs was not successful in increasing the CAR-T cell persistence in infected macaques. These studies provide guidance for future immunotherapy approaches.

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Production of Improved SIV-specific Engineered Rhesus Macaque T Cells for Adoptive Cellular Immunotherapy using Artificial Thymic Organoids (ATO)

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Abstract

Background

The therapeutic efficacy of adoptive cell transfer (ACT) using ex-vivo engineered lymphocytes is associated with the in-vivo persistence of the infused T cells engineered with either chimeric antigen receptor (CAR) or T cell receptor (TCR). ACT using peripheral blood (PB) lymphocytes as the cell substrate for ex-vivo engineering can be limited by the suboptimal in-vivo persistence of infused engineered cells, especially in situations of limiting amounts of antigen. To address this challenge, we established three-dimensional artificial thymic organoids (ATO) as a consistent cellular source for less differentiated rhesus macaque (RM) CD8+ T cells as a cellular substrate for engineering.

Methods

We co-cultured RM CD34+ hematopoietic stem and progenitor cells (HSPCs) with the murine MS5 stromal cell line expressing human delta-like ligand 4 (MS5-hDLL4) to generate three-dimensional thymus-like structures and tracked recapitulation of “thymic like” T cell development with production of mature, naïve T cells with a thymic emigrant phenotype.

Results

By week 7, CD4+CD8+ double positive, CD4+ single positive, and CD8+ single positive T-cells were observed in both histologic section multiplex immunofluorescence and flow-cytometry assays. Compared to the PB CD8+ T cells, ATO-derived CD8+ T cells engineered for expression of SIV-specific TCR showed higher transduction efficiency and SIV-antigen-specific proliferative responses, and comparable SIV-specific responses by intracellular cytokine staining.

Conclusion

This ATO system may not only provide an alternative cellular source to generate ex-vivo engineered T cells with superior in-vivo persistence, but also provides an ex-vivo platform to study the biology of T cell thymic development in a primate model.

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Development of a Virus Culture Program to Provide SIV and SHIV Challenge Stocks to SVEU Laboratories

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Abstract

Background: The Immunology and Virology Quality Assessment Center at the Duke Human Vaccine Institute holds the NIAID DAIDS contract for the Nonhuman Primate Core Virology Laboratory for AIDS Vaccine Research and Development. In order to facilitate future studies in NHP models for AIDS, our laboratory is developing a program to culture SIV and SHIV to high titer and large volume to provide well characterized challenge stocks to Simian Vaccine Evaluation Unit laboratories.

Methods: PBMCs were isolated from NHP whole blood, viably frozen, and stored at -180°C to create a bank of cells. Cells from multiple animals were thawed, CD4+ T cells isolated, activated, and then allowed to expand. The expanded cells were combined and infected with SIV or SHIV. Additional sets of frozen PBMCs were prepared as above, staggered, to provide "feeder cells" at weekly intervals. Culture supernatant was harvested and viral load measurements were taken at each time point. Culture volumes were increased based on viral load measurements and cell concentrations.

Results: Cryogenic freezing and thawing of NHP PBMCs showed good viable recovery. CD4+ T cell isolations and expansions yielded excess cells for cultures. Infection protocols were effective with SIV and SHIV test viruses. Viral titers of cultures were between 10^8 and 10^9 RNA copies/mL.

Conclusion: Preliminary cultures were successful, demonstrating that the protocols and techniques underlying the procedure are sound. The IVQAC at Duke will continue work on this program, at scale, to produce SIV and SHIV challenge stocks for use in SVEU studies.

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Intracellular Cytokine Assays To Measure Gag-Specific Responses To HIV And SIV by Primary NK Cells

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Abstract

Background

Multiple studies have shown that NK cells are capable of adaptive functions. Our laboratories provided the first evidence of antigen-specific NK cell memory in a primate species, elicited by HIV/SIV infection and vaccination. However, the existence of true HIV-specific memory NK cell responses in humans remains to be clearly demonstrated.

Methods

A modified assay was used to identify CD107a+ and IFN-gamma+ Gag-specific NK cells by intracellular cytokine staining (ICS). Study participants included HIV-negative donors, people living with HIV (PLWH) with untreated infection, virally suppressed on cART, including cART-treated PLWH receiving IFN-α2b. Samples from HIV-naïve participants receiving placebo or either a DNA prime/MVA boost or a DNA prime/rVSV boost regimen that includes Gag were also used to study vaccine-inducible HIV-specific NK cell responses. Naïve and SIV-infected rhesus macaques (RM) were used for comparison.

Results

Up to 10% of NK cells from PLWH reacted against HIV Gag peptides, mirroring responses found in SIV-infected RM, whereas NK cell responses in healthy donors were generally undetectable above background. Vaccine-induced NK cell responses were even more robust, with approximately 60% of vaccinees displaying up to 15% of NK cells that reacted to Gag, while no responses above background were found in placebos. Gag-specific NK cell responses trended to be higher post treatment with IFN-α2b, although differences did not reach statistical significance.

Conclusion

Together these data suggest that HIV-specific responses mediated by NK cells exist in humans and may have the potential to be harnessed for vaccine design or other therapeutic interventions.

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Dynamics of Immune Escape Within Individual Lineages During Acute SIV Infection

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Abstract

Background

HIV-1 replication is fast and error-prone, enabling the virus to generate mutations that evade host immune responses. Investigating the dynamics of immune escape based on population-level measures, such as time to detectable escape mutations and their subsequent trajectories in plasma, has revealed that viral evolution in acute infection is driven by multiple simultaneous selection pressures. Here we employed a barcoded virus model that provides resolution at the viral clonotype level to gain more detailed insight into the generation of and selection for escape mutations within individual lineages during acute SIV infection of rhesus macaques (RM).

Methods

We infected 2 Mamu-A*01 RM with SIVmac239M and used next generation and near-full length single genome sequencing to link the dynamics of individual barcoded lineages to the emergence of distinct mutations across the viral genome. Using targeted next generation sequencing, we further characterized the longitudinal dynamics of the escape mutations in plasma.

Results

We observed three waves of viral genome changes at six putative CTL epitopes in Tat and Nef in each animal. The first escape occurred at the Mamu-A*01-restricted Tat-SL8 epitope by 21 dpi, followed by simultaneous escape at three epitopes by 49 dpi, and at two additional epitopes by 107 dpi. Congruently, the number of distinct barcodes in the plasma of each animal declined over 10-fold, with several minor lineages linked to emerging escape mutations increasing over 100-fold in frequency.

Conclusions

The barcoded virus model was used to delineate simultaneous selection pressures, providing a quantitative measure of the underlying genetic bottleneck.

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Safety and efficacy of the PKC agonist GSK445A with romidepsin in SIV-infected macaques on antiretroviral therapy

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Abstract

Background: The ingenol-based protein kinase C agonist GSK445A is a potent inducer of HIV and SIV RNA transcription, although toxicity was observed in vivo at >15µg/kg. Recent ex vivo experiments suggest the histone deacetylase inhibitor romidepsin may synergize with GSK445A, which may expand its therapeutic window. Here, we assessed the safety and efficacy of combining GSK445A with romidepsin in rhesus macaques (RM) on ART.

Methods: 6 SIVmac239-infected RM started on ART Day 9 for >68 weeks received IV infusions of GSK445A at 10, 15, and 15 µg/kg alone (n=3) or in combination with romidepsin at 0.3 mg/kg (n=3). Plasma viral loads (pvl) at detection limit of 15 RNA copies/mL were assessed via RT-qPCR and lymphocyte dynamics were profiled by flow cytometry.

Results: GSK445A was tolerated at all doses with no severe adverse events observed +/- romidepsin. While transient lymphopenia was observed in both treatment groups, immune reconstitution was delayed in romidepsin-treated RM. Consequently, the rapid increase in CD69+ CD4+ memory T cells ($P<0.0001$) following GSK445A infusion was not observed in RM that received romidepsin. While there was no increase in pvl above threshold in both treatment groups, increased monocyte activation (interferon-inducible CD169) was observed in the combination treatment group.

Conclusions: This study demonstrates that GSK445A combined with romidepsin is safe in SIV+ RM on ART. While no increase in pvl was observed in these RM treated with ART early in acute infection, our

data supports further evaluation of combination latency-reversing agents in NHP models of HIV cure/remission.

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Elevated levels of soluble CD14 in plasma but not in cerebrospinal fluid in ART-treated SIV/*P. fragile* co-infected rhesus macaques

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Abstract

Background: Monocytes have been implicated in central nervous system (CNS) immunopathology in HIV and *Plasmodium falciparum* infection separately, but their role in CNS immunopathology during HIV/*Plasmodium* co-infection has not yet been fully elucidated. We hypothesized that *P. fragile* infection of antiretroviral therapy (ART) treated SIV+ rhesus macaques (RMs) would result in increased levels of circulating and cerebrospinal fluid (CSF) sCD14, a marker of monocyte activation.

Methods: Adult male RMs (n=4) were inoculated intravenously (I.V.) at Week(W) 0 with SIVmac239 (TCID50=50); initiated ART at W8 post-SIV; were I.V. inoculated with *P. fragile* (20x10^6 infected erythrocytes [iRBCs]) at W12 post-SIV; were treated with anti-malarial drugs at W14 post-SIV; and were necropsied at W20 post-SIV. sCD14 concentrations were measured by ELISA in plasma and CSF. Viral load (VL) and parasitemia were measured by qPCR and blood Giemsa staining, respectively.

Results: Peak VL was reached by W2/3 post-SIV (median=9.92x10^6 RNA copies/uL) and peak P. fragile parasitemia occurred at W14 post-SIV (median=23.5% iRBCs). Plasma sCD14 levels were significantly increased at W14 post-SIV (median=2014.41ng/mL) compared to W2 post-SIV (median=756.1ng/mL; p=0.01). CSF sCD14 levels were unchanged at W13 and W16 (median=2.181 and 2.295ng/mL, respectively) compared to W2 post-SIV (median=3.082 ng/mL).

Conclusions: Here, we observed elevated circulating but not CNS levels of sCD14 at peak SIV/*P. fragile* co-infection compared to SIV mono-infection. This study is ongoing and future assessments include characterization of additional markers of inflammation in the CNS at additional timepoints throughout SIV/*P. fragile* co-infection.

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Effect of Delayed Sample Acquisition on Results of a Rhesus Macaque Whole Blood 18-Color Flow Cytometry Panel

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Abstract

To evaluate if the results of a precious flow cytometry experiment is still reliable after delay in sample acquisition due to an unanticipated breakdown in instrumentation or sample storage compromise, we conducted a prospective experiment. We simulated catastrophic failure scenarios due to a cytometer being out of commission, natural disaster, or malfunction of a sample storage refrigerator. To determine the stability of an experiment under such conditions, an 18-color flow cytometry panel was acquired within a day of processing, and then compared to the reacquired data after different storage conditions. These included a 7–21-day delay acquisition, with or without a fixative wash, and either 4ºC or room temperature storage. Both compensation controls and samples were acquired on a BD LSRFortessa. Measured variables analyzed include both distinct populations like CD4+ and CD8+ T lymphocytes and harder to define populations like memory T cells from non-human primate whole blood staining. The aged timepoints were compared with their control timepoints by Mann-Whitney test. The 21-day extreme conditions were altered by frequencies of 3% fewer singlets, 3% more CD4+ T lymphocytes, 4% less natural killer cells, and 10% fewer total memory CD8+ T lymphocytes. The experiment stored at room temperature for 14-days prior to acquisition had non-significant changes in frequencies of the above populations compared to its control of freshly acquired samples. Unexpected robustness of data in 7 days stored at any condition may be attributed to good panel design and titrated reagents. Benefit of washing off fixative is pending deeper analysis.

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Malaria Vaccine-induced Tissue-specific T cell Dynamics in Rhesus macaques: A Time-course Analysis of PBMC and Liver Response to Live-attenuated Vaccination with *Plasmodium knowlesi*.

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Abstract

Background

In 2021, there were approximately 247 million malaria cases globally, leading to 619,000 deaths. Though live attenuated whole-parasite vaccines (WPV) have clinical efficacy in blocking malaria infection, effectiveness in the field is substantially decreased. Studies have shown that T cells are critical to WPV-induced sterile protection, suggesting that prior malaria exposure may lead to decreased efficacy by disrupting the malaria-naïve T cell response. However, human tissue-resident responses are often not detected in the periphery and accessing liver is challenging.

Methods

To investigate the hypothesis that prior malaria exposure disrupts the malaria-naïve T cell response, we have completed a pilot study and are conducting a longitudinal study following attenuated parasite vaccination in malaria-naïve and malaria-experienced Rhesus macaques. We performed single-cell RNA-seq on pilot PBMC and liver samples at baseline, after each of two vaccinations, and at challenge.

Results

We characterize changes in T cell transcriptomic responses during establishment of protective immunity and immediately after challenge. Intracellular cytokine staining flow cytometry of stimulated T cell subpopulations provides evidence for vaccine-induced antigen specific T cell dynamics in liver but not periphery. Investigation of gene expression of clonally expanded T cell populations through TCR analysis showed evidence of distinct immune compartments in blood and liver. We anticipate that later study timepoints will reveal changing T cell dynamics following attenuated whole-parasite vaccination.

Conclusion

This pilot study establishes a baseline characterization of vaccine-induced immunity in malaria-naïve subjects, enabling subsequent contrast with vaccine-induced immunity in previously exposed subjects.

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Exploring CD8+ T Cell Metabolism in SIV-infected Macaques

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Abstract

CD8+ T cells play a critical role in controlling HIV infection but become exhausted due to continuous HIV antigen stimulation. There is growing evidence that a cell's metabolic programming is not only important for survival but critical for carrying out specialized functions, such as cytokine production. However, the role of metabolism in exhausted, antigen-specific CD8+ T cells is not well characterized. Several *in vitro* studies have shown that inhibitors that modulate metabolism to de-emphasize glycolysis improve the functionality of exhausted CD8+ T cells. It has yet to be determined whether this improvement to immunity is achievable *in vivo*. In our study, we infected adult Mauritian cynomolgus macaques (n = 4) intrarectally with SIVmac239. After 3 months, animals were given daily metformin (70 mg/kg, p.o.), an FDA-approved drug used to treat Type 2 diabetes mellitus. Metformin inhibits mitochondrial complex I thereby modulating metabolism. Following SIV infection, SIV-specific CD8+ T cells (MHC Class I tetramer+) exhibited elevated co-expression of markers associated with exhaustion (CD39, PD1 and TIGIT) in blood, peripheral lymph nodes, and airway. Glucose and fatty acid uptake varied among SIV-specific and non-specific CD8+ T cells depending on compartment and co-expression of CD39, PD1, and TIGIT. Flow cytometric and single cell RNA-seq analyses of time points following metformin are underway.

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RANTES Immunotoxins and Immunoadhesions for CCR5 Depletion

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Abstract

Treatment of people with HIV (PWH) with antiretroviral therapy (ART) results in sustained suppression of viremia, but HIV persists indefinitely as integrated provirus in CD4-expressing cells. Intact persistent provirus, the “rebound competent viral reservoir,” is the primary obstacle to achieving a cure. Many in the field have suggested that cure will require reactivation of HIV reservoir cells using a latency-reactivating agent, followed by immune-mediated clearance of the reactivated cells. In cases of cure by transplantation of stem cells that lack CCR5 expression, cure may depend on a combination of reservoir depletion by ablative conditioning and on the graft-versus-reservoir effect. Surprisingly, we recently described long-term SIV remission and apparent cure in SIV-infected infant macaques via targeted depletion of potential reservoir cells that express CCR5. Here we report new immunotoxins and immunoadhesins that can destroy CCR5-expressing cells, and that may be cooperative or synergistic with the bsAb we described. These agents are based on the CCR5-binding chemokine RANTES. Our results demonstrate that both native RANTES and modified versions can guide effector molecules such as Fc and immunotoxins to HIV target cells. We anticipate that these molecules may be future components of HIV cure regimens.

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Interleukin-21 and anti-α4β7 dual administration promotes immune response to SIV and ameliorates gut dysbiosis in ART-treated rhesus macaques.

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Abstract

Despite cART, HIV causes persistent gut barrier dysfunction, immune depletion, and dysbiosis. Further, cART interruption results in reservoir reactivation and rebound viremia. Both IL-21 and anti-α4β7 improve barrier function individually, and we hypothesized combining them would improve outcomes synergistically. Sixteen macaques were inoculated with wildtype SIVmac239, initiated on daily cART (TDF+FTC+DTG) from week 6 until 72 followed by ATI. At week 64, the experimental group was administered seven rounds of IL-21-IgFc weekly and anti-α4β7 antibody every three weeks. Longitudinal VLs, flow cytometry, CD4+ intact proviral DNA assays, and fecal 16S rRNA sequencing were performed. SIV rebounded in all RMs following ATI, with controls reaching pre-cART setpoint by d100 post-ATI. Dual-treated monkeys initially rebounded similarly to controls but showed better viral control replication with a log10 2.2 decrease over pre-cART levels at sacrifice (week 92) ($P<0.0001$) and log10 1.4 copies/mL lower VLs compared with controls. Following ATI, PD-1+ TCMs increased in controls ($P<0.0001$) but not in treated animals. No differences were observed in reservoir size. Microbiota sequencing revealed better recovery from dysbiosis (reduced Spirochaetes ($P=0.02$) and Proteobacteria ($P=0.02$); increased Firmicutes ($P=0001$)) in treated animals compared to controls. The butyrate-producing Firmicute Roseburia predicted PD-1-expressing TCMs ($P=0.04$) and VLs ($P=0.004$) after therapy. Additionally, these PD-1+ TCMs were associated with reservoir size ($P=0.04$), which also predicted VLs ATI ($P=0.007$). Thus, combining IL-21 and anti-α4β7 inhibits PD-1 on CD4+ TCMs, lowers VL setpoints after ATI, and ameliorates dysbiosis, highlighting the importance of targeting PD-1 and microbiome composition during future mucosal immunotherapy efforts..

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Timing of ART initiation, its relation to preservation of mucosal health and its impact on the duration to viral control and replication competence of the SIV reservoir

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Abstract

Background. Gut destruction may delay the control of inflammation/immune activation and thus the SIV suppression with antiretrovirals (ART).

Methods. We compared the efficacy of SIV suppression and reservoir dynamics based on the timing of ART initiation in SIVmac239-infected rhesus macaques (RMs) in which ART was initiated in three clinically relevant situations: at 7 days postinfection (dpi) (before the viral peak, thus prior to gut destruction) (n=16), at 12 dpi (immediately postpeak, prior to massive mucosal CD4+ T cell depletion) (n=8) and at 56 dpi (during early chronic infection, when gut destruction is massive, mucosal CD4+ T cells are at the nadir, yet chronic inflammation is in incipient stages) (n=12).

Results. We found a clear correlation between virus suppression and timing of ART initiation, control occurring earlier and being more robust in monkeys receiving ART earlier: ≈1 month with prepeak ART, ≈2 months with postpeak ART, and >3 months with chronic ART. More frequent postcontrol blips occurred with later ART. Time to viral control correlated with the dynamics of biomarkers of macrophage activation (sCD163), gut damage (I-FABP) and inflammation (IL-12 or eotaxin), which peaked at higher levels and were elevated longer in RMs with later ART. Timing of ART clearly impacted reservoir dynamics, with a higher frequency of intact genomes in the RMs with later ART.

Conclusion. ART should be initiated as early as possible during HIV infection, ideally before the gut damage, to insure gut preservation, rapid viral suppression, control of inflammation and a reduction in the replication-competent reservoir.

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Impact Of IL-15 Neutralization On Neuropathogenesis In Acute HIV/SIV Infection

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Abstract

BACKGROUND:

Immune responses in the CNS during acute HIV/SIV infection are insufficient to prevent viral seeding and reservoir establishment. IL-15 plays a crucial role in anti-viral immune response by stimulating NK and CD8+ T cells. We hypothesized that peripheral neutralization of IL-15 may significantly influence the inflammatory responses in brain. We evaluated the brain immune and inflammatory responses to acute SIV infection after anti-IL-15 infusion in a nonhuman primate model.

METHODS:

Rhesus macaques were administered two doses of rhesusized monoclonal antibodies against IL-15(anti-mRh-IL-15) at days -21 and -7 prior to challenge with SIVmac239X(day 0) and necropsied at 7 and 14 days post-infection(dpi). Peripheral and brain viral load were quantified by qPCR and RNAscope. Sequencing analysis of viral clones in brain regions were compared to those in blood and peripheral lymph nodes. CNS histopathology were analyzed by immunohistochemistry and in situ hybridization.

RESULTS:

IL-15 neutralization did not change SIV quantity in brain between 7 and 14 dpi. Neutralization increased microglial activation; but it decreased GFAP expressing astrocytes in all brain regions. Anti-IL-15 treatment altered an immune and inflammatory response to acute SIV infection in the brain, where fewer microglia cells expressed the proinflammatory cytokine – IL-6, and higher numbers of macrophages expressed the anti-inflammatory cytokine- TGF-β, and decreased M2 macrophage gene pathway activation.

CONCLUSIONS:

IL-15 neutralization altered CNS immune and inflammatory responses to acute SIV infection by decreasing CD8+ T cells and resulting in a tissue environment favoring anti-inflammation, which could overall support the establishment of viral reservoir.

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Development and evaluation of HIV-resistant CAR/CXCR5 T cells

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Abstract

HIV-specific chimeric antigen receptor (CAR) T cells are a potential strategy to eliminate HIV infection. We developed HIV-specific CAR T cells expressing the follicular homing receptor CXCR5 (CAR/CXCR5). Our CAR/CXCR5 T cell product contains both CD4 and CD8 T cells. CAR/CXCR5 CD4+ T cells are susceptible to HIV infection. To address this problem, in this study, we designed and evaluated four different strategies to make CAR/CXCR5 T cells resistant to infection.

We produced four constructs that each encoded CAR/CXCR5 plus a C34 peptide, a 2P23 peptide, an shRNA to CCR5, or an siRNA to CCR5. These constructs were used to engineer T cells. Engineered cells were evaluated for transduction efficiency, resistance to HIV infection, and maintenance of effector function and ability to migrate.

We were successful in producing engineered T cells with each construct showing high transduction efficacies. The C34 and 2P23 constructs showed resistance to HIV infection, suppression of HIV-1 spreading and the cell products migrated specifically to CXCL13. The HIV-specific CAR/CXCR5 expressing the shRNA to CCR5 and siRNA to CCR5 also both showed high transduction efficiencies. The shRNA-engineered cells were not resistant to HIV infection, and studies with the siRNA to CCR5 are ongoing.

These results demonstrate the successful production of CAR/CXCR5 T cells that are resistant to HIV infection. These novel cell products are promising candidates for future preclinical animal studies and take us closer to developing cell products that may lead to the long-term suppression of HIV infections.

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Plasma Lipidomic Alterations During Pathogenic SIV Infection With and Without Antiretroviral Therapy

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Abstract

Background. Lipid profiles change in HIV infection and correlate with inflammation. Lipidomic alterations are impacted by multiple non-HIV-related factors; thus controlled models may inform on the specific lipid changes induced by infection and/or antiretroviral therapy (ART).

Methods. With ultrahigh Performance-Liquid-Chromatography-Tandem Mass Spectroscopy we assessed and compared (ANOVA) longitudinal lipid changes in 25 naïve and 6 ART-treated SIV-infected pigtailed macaques. Key parameters of infection (IL-6, TNFα, D-dimer, CRP and CD4+ T-cell counts) were correlated with lipid concentrations (Spearman).

Results. Sphingomyelins (SM) and lactosylceramides (LCER) increased during acute infection, returning to baseline during chronic infection; Hexosylceramides (HCER) increased throughout infection, being normalized with prolonged ART; Phosphatidylinositols (PI) and lysophosphatidylcholines (LPC) decreased with SIV infection and did not normalize with ART ; Phosphatidylethanolamines (PE), lysophosphatidylethanolamines (LPE) and phosphatidylcholines (PC) were unchanged with infection, yet significantly decreased throughout ART. Specific lipid species (SLS) were also substantially modified by SIV and/or ART in most lipid classes.

Conclusions. Using a metabolically-controlled model, we identified specific lipidomics signatures of SIV infection and/or ART, many of which were similar to HIV-infected individuals. SLS were identical to those involved in development of organ dysfunctions encountered in virally suppressed individuals. Lipid changes also correlated with markers of disease progression, inflammation and coagulation. Our data suggest that lipidomic profile alterations may contribute to residual systemic inflammation and comorbidities seen in HIV/SIV infections, and may be used as biomarkers of SIV/HIV comorbidities. Further exploration into the benefits of interventions targeting dyslipidemia is needed for the prevention HIV-related comorbidities.

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Can voluntary medical male circumcision impact the HIV target cell population of Glans?

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Abstract

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.

Adult male subjects were recruited in Chicago, 21 circumcised and 21 uncircumcised men. A shave-biopsy was obtained from the glans and shaft. A portion of each biopsy was exposed to (PA)GFP-HIV for 4h then frozen in optimal cutting temperature compound (OCT). Cryosectioned tissues were 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 (CD4+, CD3+ and CCR10+) for number of cells per surface area, depth from the epithelial surface, and depth from the basement membrane. Both parameters, virion count and target cells, was compared between circumcised/uncircumcised tissues and tissue locations (glans and shaft).

Our data suggests that the percentage of penetrating virions was higher in both glans and shaft uncircumcised tissues. Also, the Th22 and CD8+CLA+ cells were more present in glans tissues after 4h of HIV challenge. 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.

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Utilizing PET/CT and Multiscale Imaging to Define the Dynamics of the Viral Reservoir and to Analyze the Biodistribution and Function of IV Injected Antibodies.

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Abstract

Positron emission tomography (PET) is a powerful tool for analyzing antibodies as part of a multiscale imaging approach. 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 started on long-term suppressive ART after 4 days, followed by necropsies shortly after ART cessation. PET guided necropsies targeted infected tissues, and PET signals were used to quantify the infected cell population at every stage. Phenotyping the infected cell population in post-ATI tissues by immunofluorescence, revealed a combination of myeloid and mast cells constituting the infected cell population.

Using monoclonal antibodies for therapeutic purposes including HIV treatment, prevention, and cure is also of great interest. We used 89Zr labeled antibodies and PET/CT to study antibody distribution after IV injection. We have recently refined the ability to correlate these multiscale imaging approaches to provide insights into antibody distribution in the rhesus macaque model. Radiolabeling provides the additional ability to monitor antibody levels in bodily fluids and secretions in a sensitive and quantitative manner. These values, combined with imaging data reveal that different antibodies have distinct patterns of distribution and localization that can be influenced by glycoform, Isotype, and optimizing mutations.

PET/CT based approaches have increased the depth and efficiency of our studies and lead to important discoveries that are significant contributions to the Cure field.

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Profiling CD8+CXCR5+ T Follicular Cells in SIV Infection and Vaccination

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Abstract

Background: Virus specific $CD4^+$ T follicular helper (T_{fh}) cells are targets for HIV infection and thus may facilitate viral spread and disease progression. $CXCR5^+CD8^+$ T follicular (T_f) cells, like $CD4^+$ T_{fh}, localize to B cell follicles, where they can kill virus-infected cells and support B cells. Both functions are potentially protective against HIV, so it may be advantageous to generate $CD8^+$ T_f cells by vaccination. However, $CD8^+$ T_f heterogeneity and profiles associated with protection remain undefined. Here, we evaluate phenotypic, functional, and transcriptional characteristics of SIV-specific $CD8^+$ T_f cells from SIV controller and non-controller macaques to identify protective $CD8^+$ T_f features.

Methods: $CD8^+$ T_f cells and autologous B cells, enriched from PBMCs, were co-cultured and stimulated with SIV peptide pools for 9 days. Frequencies of $CD8^+$ T_f cells exhibiting helper (OX40, CD40L, IL-21) or effector (granzyme B, perforin, IFN-γ) phenotypes were assessed by flow cytometry. B cell help was examined via IgG production and expression of genes associated with B-cell differentiation and activation (PRDM1, AICDA, BCL-6). RNA sequencing of $CD8^+$ T_f cells was used to identify transcriptional features of protection.

Results: We predict that $CD8^+$ T_f cells from animals with low viral burdens will be more frequent and exhibit phenotypic and transcriptional profiles spanning B cell help and cytolytic functions.

Conclusions: These studies provide insight into $CD8^+$ T_f biology with respect to HIV/SIV infection and will inform on the possible utility of targeting $CD8^+$ T_f responses with HIV vaccines.

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Pioneer recruitment of CD4 CCR5+ cells from the CSF contributes to viral establishment during acute HIV.

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Abstract

Background: Entry of CCR5+ CD4 T cell effectors/viral targets into the brain parenchyma contributes to viral establishment and the onset of neuroinflammation, emphasizing the critical need to understand the mechanisms governing acute CD4 T cell entry into the brain.

Methods: To investigate the roles of the blood-cerebrospinal fluid barrier (BCSFB), and blood-brain barrier (BBB), in CD4 T cell entry, we used Rh-α4, a primate-specific antibody, to selectively inhibit α4β1 mediated T cell entry via BBB. Macaques received Rh-α4 (25mg/kg, n=4) or IgG before and during acute SIVmac251 infection.

Results: Rh-α4 resulted in complete α4 coverage, leading to significant lymphocytosis (3-fold increase; $p<0.05$), while monocyte counts remained unaffected. α4 blockade increased CSF CD4+ CCR5 frequencies until week 2, which returned to baseline by week 3. This indicated that CCR5+ CD4 T cell migration across the BCSF endothelial and epithelial barriers was independent of α4. BBB integrity assessments using albumin quotient, CSF total protein, and tight junction protein immunofluorescence showed no compromise. However, Rh-α4-treated animals exhibited a 10-fold increase in grey matter viral burden compared to the IgG group. These findings support the hypothesis that the initial recruitment of CCR5+ CD4 T cells through BCSFB triggers neuroinflammation and subsequent CD4 T cell entry into the brain. Additionally, our results highlight the crucial role of anti-viral CD8 T cell influx in controlling grey matter viral replication.

Conclusion: Collectively, these findings suggest that targeting CCR5+ CD4 T cell influx via the BCSF and BBB may be effective for preventing viral establishment and neuroinflammation.

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Displaying HIV Fusion Peptide on Resurfaced Adenovirus Capsids

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Abstract

Induction of neutralizing antibodies is an important goal for prophylactic and therapeutic vaccines against HIV. An epitope on the N-terminus of gp41, the HIV-1 fusion peptide (FP8), is a target of broadly neutralizing antibodies. FP8 has previously been conjugated to the surface of virus-like particles (VLP), and these VLPs together with SOSIP protein elicit anti-FP8 IgG responses in mice that can neutralize HIV. Chemical conjugation of FP8 utilizes lysine groups present on the coat protein of the Qbeta phage VLP. Adenoviral vectors also have abundant surface lysines and tolerate capsid modifications. Due to its larger size and abundance of lysines, adenovirus particles may potentially deliver more copies of FP8.

The goal of this work is to explore FP8 conjugation to wild-type or resurfaced adenovirus type 26 (Ad26) using SMPH. Using wild-type Ad26, this approach will result in conjugation to any lysine groups exposed on the capsid surface; the hexon capsid has 13 lysines in the hypervariable regions which leads to over 9,000 potential FP8 copies displayed. Using Ad26 particles that have been mutated to display more lysines, an even greater density is possible. Here we verify incorporation of FP8 to the adenovirus and report humoral and cellular immunity to fusion peptide after delivery of the FP8-conjugated vectors to mice.

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Generation and testing of human-ready, rationally attenuated and efficacious live *M. tuberculosis* vaccine candidates to protect against tuberculosis and TB/HIV co-infection

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Abstract

Background: In order to effectively reduce the incidence and deaths due to TB by 2030, there is a desperate need for new and highly effective vaccines against this disease. Previous studies have demonstrated the vaccine efficacy of *MtbΔsigH* in TB-SIV coinfected rhesus macaques and in the resistant cynomolgus macaque model. However, in order to satisfy the Geneva Consensus for the development of live attenuated *Mtb* vaccines, additional independent mutation(s) are needed in *ΔsigH* to ensure its complete attenuation.

Methods: We developed ten attenuated double and triple gene knockout (DKO/TKO) vaccine candidates derived from *MtbΔsigH* via homologous recombination using temperature-sensitive mycobacteriophage *phAE159*, developed in the Jacobs lab. Some of these mutants have been reported to generate immune enhancement- or auxotrophy-based attenuation phenotypes, while others render Mtb avirulent in macaques.

Results: Deletion of genes in *Mtb* genome was confirmed by PCR using locus specific primers and sequencing. Studies to assess the safety of these strains in Rag mice and SIV co-infected immunocompromised macaques are ongoing. The strains that consistently provide the best results in terms of safety and immunogenicity will be further evaluated for efficacy via aerosol vaccination in immunocompetent rhesus macaques.

Conclusions: If these D/TKO strains retain the protective efficacy of the parental *MtbΔsigH*, then they would have satisfied the requirement for early human testing. We have already shown that *MtbΔsigH* is nonpathogenic in the SIV co-infection model. We therefore expect that MtbΔsigH-based D/TKO vaccine candidate(s) to be appropriate for vaccinating PLHIV.

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Impaired CNS immunosurveillance by CCR7+ CD4 T cells during HIV-induced chronic neuroinflammation

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Abstract

Background: CD4 T cells critically regulate immune homeostasis in the brain, yet their functions in this context remain poorly understood. Understanding the adaptive immune mechanisms operating under normal, baseline conditions is paramount to effectively develop interventions that can mitigate potential dysfunctions observed during neurodegenerative disorders.

Methods: Utilizing a combination of cutting-edge methodologies such as single-cell (sc) transcriptomic analysis, Assay for Transposase-Accessible Chromatin (ATAC)-seq, and flow cytometry, we conducted an in-depth investigation of chronic HIV-induced neuroinflammation.

Results: Rhesus macaques were infected with SIV CL757 and treated with a deferred non-adherent ART regimen to recapitulate salient features of chronic neuroinflammation. All infected animals showed robust viral replication in the plasma, with peak viral loads of 10^5-10^6 viral RNA copies/mL, and CSF, with peak viral loads of 10^2-10^3 viral RNA copies/mL. Discrete brain regions showed the presence of viral RNA, but viral DNA was below LOD, suggestive of viral replication without viral integration within the brain parenchyma. Evidence of neuroinflammation was evident by microglial activation, upregulation of neurodegeneration-associated genes in myeloid cells, and an increase in CSF IP-10 ($p= 0.03$, 3-fold increase). Additionally, CCR7+ CD4 T cells were significantly depleted from the brain ($p= 0.02$, 3.6-fold decrease), suggesting their presence could be instrumental in controlling chronic neuroinflammation.

Conclusion: Altogether, our findings underscore the critical role of CCR7+ CD4 T cells in immune surveillance within the CNS and highlight their potential as valuable therapeutic targets to mitigate the development or progression of neurodegenerative diseases.

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Longitudinal Surveillance of SIV Dynamics and Immune Responses through scRNASeq of ART-Suppressed Rhesus Macaques

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Abstract

Background

Antiretroviral therapy (ART) is the primary treatment method for HIV management in people living with HIV (PLWH). ART suppresses HIV to undetectable viremic levels in plasma; however, virus is not eliminated, and suppression may be incomplete in all tissues. Understanding the dynamics of HIV/SIV in ART-suppressed subjects is critical for cure research and managing treatment in PLWH.

Methods

To investigate the dynamics of the persistent viral reservoir and antiviral immune response, we conducted longitudinal single-cell RNA-seq (scRNASeq) sampling of Rhesus macaques infected with Simian Immunodeficiency Virus (SIV) on long-term ART. Samples were collected from PBMC, bone marrow, lymph nodes, and spleen, to monitor viral perturbations within the secondary lymphoid system. Additionally, we collected data on plasma viral load (pVL) and cell-associated viral load (CAVL) to establish correlations between nucleic acid evidence of viral activity and immune responses.

Results

We identified systematic differences in the transcriptional profiles of immune cells from late chronic infection, relative to other time points, including pre-infection, indicating there are long-term perturbations in immune cells that do not resolve even after viral suppression. We further demonstrate unique immune environments between mesenteric and peripheral lymph nodes, including different levels of base T cell activation, interferon signaling, and functional differences between regulatory T cells.

Conclusion

Our findings provide insights into ART-dependent heterogeneity within tissues harboring the latent SIV reservoir, which are not accessible outside of animal models. These data demonstrate functional differences between anatomic sites, which may contribute to variable viral persistence or reactivation after ART release.

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LGM1506 Protects Against Ozone-Induced Lung Injury in Rhesus Macaques

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Abstract

Background: Inhaled corticosteroids form the mainstay of current asthma treatment. Previous studies indicate that the toxic air pollutant, ozone (O3) exacerbates airway inflammation in asthma and impairs the anti-inflammatory effects of glucocorticoids. We aimed to evaluate the protective effects of LGM1506, a synthetic single isomer of an antioxidant lignan found in flaxseed, against O3-induced airway changes.

Methods: A cohort of well-characterized rhesus macaques (n=36) from the California National Primate Research Center exhibiting airway hyperreactivity was assessed for lung function and airway inflammation on Day 1. Animals received 7 days of oral placebo or LGM1506 treatment. Macaques were then exposed to O3 or filtered air on Day 7. All measurements were repeated 12 hours post-exposure, on Day 8. A single dose of dexamethasone was injected i.m. for two groups of animals that received either placebo or LGM1506 on Day 8 and underwent necropsy 48 hours later. The left caudal lung lobe was collected and fixed in paraformaldehyde for histopathological evaluation.

Results: O3 inhalation increased the total BAL cell count predominated by eosinophils. LGM1506 (50 mg/kg b.i.d. for 7 days prior O3 exposure) significantly reduced BAL eosinophil counts. In the dexamethasone-treated monkeys, pretreatment with LGM1506 (50 mg/kg b.i.d. for 7 days) prior O3 exposure, significantly reduced lung tissue inflammation, septal thickening, and tissue eosinophilia compared with placebo pre-treatment.

Conclusion: Our data indicate that LGM1506 pretreatment of macaques with pre-existing airway hyperreactivity alleviates eosinophil influx 12 hours after O3 exposure and reduced eosinophilic tissue inflammation in dexamethasone-treated macaques.

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Cannabinoids Modulate the Microbiota-Gut-Brain Axis and Plasma Secondary Bile Acid Profile in SIV Infection Via Reducing Gut Microbiome Dysbiosis and Elevating Neuro/Entero- Protective Metabolite Levels

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Abstract

Despite HIV/SIV suppression by anti-retroviral therapy (ART), people with HIV (PWH) experience dysbiosis, intestinal barrier dysfunction/inflammation and develop co-morbidities such as liver and neurocognitive disorders. Disturbance in bile acid-gut-microbiota cross talk results in accumulation of secondary-bile acids causing liver inflammation. ART is unable to completely reverse HIV-induced dysbiosis or modulate the microbiota-gut-brain (MGB) axis communications.

We hypothesized that low dose phyto-cannabinoids may positively modulate the secondary-bile acid pool and MGB axis. We performed jejunal microbiome and plasma metabolomic profiling in ART treated SIV-infected rhesus macaques (RMs) administered vehicle (VEH/SIV/ART;n=8) or delta-9-tetrahyrodcannabinol (THC)(THC/SIV/ART;n=8).

Plasma metabolome profiling identified significant ($P<0.05$) elevation of secondary-bile acids lithocholic acid and tauro-/glyco-lithocholate in VEH/SIV/ART at 5-month post infection (5MPI). Conversely, THC/SIV/ART RMs showed significant ($P<0.05$) elevation of only non-toxic/excretory glucorinide. Moreover, THC/SIV/ART RMs showed significant ($P<0.05$) elevation of neuro-protective indole-3-propionate (IPA) and entero-protective serotonin (in plasma and jejunum) at 5MPI. Immunostaining confirmed significantly increased protein expressions of IPA binding arylhydrocarbon receptor (AHR) and serotonin receptors; HTR4 and HTR7 in the jejunum epithelium of THC/SIV RMs at 5MPI. Metagenomic profiling detected enrichment of Class *Clostridia* (convert Tryptophan to IPA) and Species *Lactobacillus plantarum* (produce serotonin) in THC/SIV/ART RMs at 5MPI.

Overall, our findings suggest that chronic low-dose THC as an adjunct to ART maintained plasma secondary-bile-acid levels, promotes IPA and serotonin producing gut-bacteria that suppress intestinal inflammation and signal the brain chemically via neuro-protective IPA and mechanically via vagus nerve stimulation by serotonin.

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Advancing NHP Model for Evaluation of Pluripotent Stem Cell Technologies for HIV Immunotherapies

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Abstract

Adoptive T cell therapies with in vitro expanded genetically modified T cells have been considered a valuable strategy to treat and cure HIV. A renewable source of human T cells from induced pluripotent stem cells (iPSCs) would further facilitate and broaden the applicability of these therapies. To enable evaluation of iPSC-based technologies in a preclinical HIV infection model, we explored the feasibility of production of CCR5-edited and anti-HIV chimeric Antigen receptor (CAR) T and NK cells from Mauritian cynomolgus macaque (MCM) iPSCs generated from fibroblasts and peripheral blood T cells. Using CRISPR-Cas9 we successfully introduced deletion within exon 2 of CCR5 including a 24-bp deletion region that was previously found to prevent functional CCR5 expression in NHPs. We demonstrated that T cells and macrophages produced from CCR5-edited iPSCs did not support the replication of the CCR5-T cell-tropic SIVmac239 and macrophage-tropic SIVmac316 simian immunodeficiency viruses, respectively. However, we noted an impaired capacity of iPSCs generated from T cells (T-iPSCs) to re-differentiate into T cells, especially following biallelic CCR5 disruption. In addition, we established MCM iPSC lines with an anti-HIV CD4-MBL CAR inserted into AAVS1 locus under CAG promoter or CCR5 locus under MND promoter. CD4-MBL CAR iPSCs were used succefully to generate T and NK cells which retain CAR expression following differentiation. MCM iPSC-derived CD4-MBL CAR NK cells demonstrated superior cytotoxic activities against HIV1 envelope expressing CHO cells. Overall, these studies provide a platform for further exploration of AIDS therapies based on gene-edited iPSCs in a NHP model.

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Evaluation of innate gene signatures induced by HIV vaccination and their correlation with neutralizing antibody responses in infant and juvenile rhesus macaques

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Abstract

An early-life HIV vaccine could protect infants against HIV infection and has the potential to induce long-lasting immunity in adolescents. The magnitude and quality of vaccine-induced antibody responses are at least partially determined by the early innate immune response.

Here, we identified the early gene signatures induced by immunization with a CH848 gp160 mRNA vaccine formulated in lipid nanoparticles and determined their correlation with antibody responses in infant and juvenile rhesus macaques (RMs). Gene expression was evaluated in whole blood samples collected one day after each immunization at weeks 0, 6, and 12 using the NanoString NHP Immune Panel. Differentially expressed genes(DEG) were subjected to pathway analysis. Vaccine-induced neutralizing antibodies(nAbs) were tested against wildtype CH848, the vaccine N138T DT mutant, and DT N332T KO virus.

The results revealed altered gene expression on day 1 after each immunization in both age groups. However, peak responses in infant RMs were observed at day post prime compared to week 6+day1 in juvenile RMs. Independent of age, DEG were representative of the activation of TLR and interleukin signaling. Although infant RMs trended towards higher nAbs against the DT N138T vaccine virus, infant and juvenile RMs had comparable nAbs against the V3 glycan site. Among the DEG, CCL23, FPR2, IL18RAP, TGFBR2, PLA2G4, and MYC were positively correlated with autologous tier 2 nAbs against the DT N138T vaccine virus .

The study was a first step in identifying specific innate immune pathways that can be modulated to enhance B cell responses

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Antigen-Specific Germinal Center Tfh cells are highly heterogenous in lymph node and Tfh1 and Tfh17 cells are associated with high viral levels during chronic SHIV infection

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Abstract

Background: HIV infection leads to alterations in HIV-specific CD4 T cells including increased expression of inhibitory receptors and skewing toward Tfh cell signature. Tfh cells have been shown to increase during chronic HIV infection. Accumulation of Tfh cells is associated with high viremia and germinal center Tfh (GC-Tfh) in lymphoid tissue may contribute to this process. However, the heterogeneous nature of antigen-specific GC-Tfh cells and the relative contribution of specific GC-Tfh subsets to viral persistence is not understood in HIV/SIV infections.

Methods: Here, we characterized the phenotype and function of antigen-specific GC-Tfh cells based on the expression of chemokine receptors associated with Th1 (CXCR3), Th2 (CCR4), and Th17 (CCR6) along with activation-induced markers (AIM assay) CD25, OX40 and 41BB in the lymph nodes following chronic SHIV1157ipd3N4 infection in rhesus macaques.

Results: In SHIV naïve RM, only a small fraction of total GC-Tfh expressed CXCR3, CCR4 and CCR6. During chronic SHIV infection, there was an increase in GC-Tfh cells. Interestingly the majority of antigen-specific GC-Tfh cells expressed CXCR3 (Tfh1), while a significant proportion of them also expressed CCR4+ (Tfh2) and CCR6+ (Tfh17) cells. These cells expressed OX40, 41BB, and CD25 at higher levels than non-tfh cells. Importantly, the expansion of GC-Tfh1 and GC-Tfh-17 cells is associated with high viremia.

Conclusion: These data demonstrate that chronic SHIV infection promotes the expansion of antigen-specific GC-Tfh cells with heterogeneous populations, which are phenotypically and functionally distinct from conventional GC-Tfh cells and associated with higher viral RNA levels during chronic SHIV infection.

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Early ART plus immune interventions to limit SIV reservoir establishment in infant rhesus macaques.

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Abstract

Background

Persistent reservoirs of HIV-infected CD4+ T-cells remain major obstacles to cure. Broadly neutralizing antibodies control viral replication and may promote infected cell clearance. We tested SIV-Env-specific rhesus IgG1 monoclonal antibodies (RhmAbs), IL-15 superagonist N-803, and early ART in SIV-infected infant rhesus macaques (RMs) to assess impact on viral reservoirs and rebound dynamics upon ART interruption.

Methods

Twenty-two infant RMs were orally-infected with SIVmac251 at 4 weeks-of-life and started on ART 1-2 weeks post-infection, then divided into 3 groups: i) ART only, ii) ART+SIV-RhmAbs (20mg/kg s.c. of ITS09.01-LS, ITS102.01-LS, ITS103.01-LS, ITS113.01-LS, one dose), and iii) ART+SIV-RhmAbs+N-803 (100mg/kg, one dose). Plasma viral loads and total and intact SIV-DNA in CD4+ T-cells were measured longitudinally. ART was discontinued at week 50.

Results

Viral load decay with ART was similar across groups. Levels of CD4+ T-cell-associated SIV-DNA declined on ART with the greatest fold change seen in the ART+SIV-RhmAbs group ($p<0.05$ vs controls; weeks 0-26 of ART). Intact proviral SIV-DNA in CD4+ T-cells from blood or lymph nodes did not differ between groups at week 26; the same was true of total SIV-DNA at week 48. All animals experienced viral rebound 1-4 weeks after ART interruption with no group differences in time to rebound.

Conclusion

Compared to treating with early ART alone, adding RhmAbs +/- one dose of N-803 did not impact the level of persistent SIV once viral loads were suppressed nor did this combination delay viral rebound after ART interruption. Future studies should explore additional doses of RhmAbs and N-803.

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Optimizing Detection Of Functional T-cell Responses In Non-human Primate (NHP) Gut Mucosal Lymphocytes: To Rest Or Not To Rest?

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Abstract

Resting of thawed peripheral blood NHP lymphocytes at 37°C prior to stimulation has shown improved detection of functional T-cell responses in Intracellular Cytokine Staining (ICS) and Enzyme-Linked Immunosorbent Spot (ELISpot) assays. Whether similar conditions are needed for optimal detection of functional mucosal T-cell responses in NHPs is unclear. In this study, we evaluated the effects of resting and stimulation periods on detection of colonic and jejunal T-cell responses in SIV-infected rhesus macaque (RM) and pig-tailed macaques (PTM). T-cell cytokine secretion in response to 6-14 hours of Staphylococcal Enterotoxin B (SEB) or SIVmac239 peptide stimulation was evaluated in the absence or presence of cell resting prior to stimulation. Thawed, cryopreserved colon/jejunal cells from one RM and freshly isolated colon/jejunal cells from two PTM were tested without resting or after overnight resting at 4°C or 37°C. In freshly isolated non-rested cells, the frequencies of SEB-responding cytokine-positive CD4+ and CD8+ T cells were higher after 14-hr compared to 6-hr stimulation. Resting cells at 4°C before stimulation provided no benefit to freshly isolated cells and reduced responses in cryopreserved, thawed cells. Overnight resting at 37°C of thawed or fresh cells prior to a 6-hr stimulation period enhanced jejunal lymphocyte responses but led to a marked decrease in viability (<50%) and cell recovery (<30%), thus limiting the utility of this approach. Based on these results, overnight stimulation of freshly isolated cells without resting appears to be optimal for detection of functional T-cell responses in SIV-infected NHP intestinal lymphocytes.

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Pharmacokinetics of Triple Combination Non-Neutralizing Monoclonal Antibodies in Rhesus Macaques

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Abstract

Passive immunization of HIV-1 broadly neutralizing antibodies (bnAbs) can prevent HIV-1 acquisition. Fc-mediated effector functions contribute to protective effects of bnAbs in animal models. However, whether these functions, in the absence of neutralization, can afford protective immunity is unclear. Moreover, combinations of antibodies, with multiple anti-viral functions and targeting distinct epitopes may be needed for superior in vivo efficacy. We conducted a pilot passive infusion study to evaluate the pharmacokinetics (PK) of non-neutralizing monoclonal antibodies (nnmAbs) in rhesus macaques to inform the design of future immunization and SHIV challenge studies.

Rhesus macaques received intravenous bolus infusions of JR4Rh (C1C2-specific), 7B2Rh (gp41-specific), and DH614.1Rh (V1V2-specific) rhesus mAbs at 25 mg/kg or 10 mg/kg each or DSPR1 control mAb at 25 mg/kg. Plasma and mucosal samples were collected at pre-dose and 16 post-infusion time points, through day 21. Concentrations were measured by binding antibody multiplex assay, with non-compartmental PK analysis performed using PKanalix in MonolixSuite.

mAb concentrations in plasma peaked 1-hour post-second infusion. The 25 mg/kg group exhibited improved peak plasma antibody concentrations (Cmax) and terminal half-life (t1/2) measurements compared to the 10 mg/kg group, with comparable levels and similar rates of decline of each mAb within each animal. Analysis of mAb concentrations in a subset of animals revealed higher levels of 7B2Rh than JR4Rh, with negligible levels of DH614.1Rh in rectal mucosa.

These PK results support the selection of optimal doses of nnmAb cocktail combinations that can potentially afford protection alone or in concert with bnAbs.

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Rhesus macaques as a model for Mayaro virus disease

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Abstract

Background: Mayaro virus (MAYV) is a mosquito-borne alphavirus endemic to Latin America that causes fever, fatigue, aches, rash, and arthritis. MAYV outbreaks are rare and human seropositivity is higher in people living near or in forests compared to cities, likely due to contact with MAYV-infected sylvatic Haemagogus spp. mosquito vectors. Human MAYV viremia kinetics are not defined, which limits biological and epidemiological assessments. The goal of this project is to define MAYV viremia kinetics using rhesus macaques and to evaluate if macaques develop pathology seen in humans, notably joint inflammation.

Methods: We inoculated 12 rhesus macaques that had been previously infected with dengue virus, with either 103 or 107 plaque forming units (pfu) of MAYV subcutaneously or 107pfu intravenously. Animals were monitored via frequent blood collection and were euthanized 10-12 days post inoculation (dpi).

Results: Clinical signs following inoculation were mild or absent. The mean peak MAYV viremia was 1.38 x 105 pfu/ml and occurred 2 dpi in 7 of 12 macaques, the remaining 5 peaking on 1 dpi. All macaques had detectable viremia 1-4 dpi. The inoculation route or dose did not impact the magnitude of peak viremia ($p=0.16$, one-way ANOVA) or area under the viremia curve ($p=0.14$, one-way ANOVA). Pathological analyses showed mild to moderate joint inflammation in all animals, as well as reactive lymphadenopathy and perivascular inflammation.

Conclusions: MAYV infection of rhesus macaques models human MAYV infection. Further optimization and characterization of this animal model can make it useful to explore prophylactic and therapeutic intervention strategies.

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The role of alveolar macrophages in the pathogenesis of chronic lung diseases in aging populations and HIV-infected individuals using a rhesus macaque model

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Abstract

BACKGROUND: Aging and HIV infections lead to chronic inflammation and increased risk of co-morbidities. HIV-infected individuals experience earlier development of chronic lung diseases like COPD. Despite antiretroviral therapy, these complications persist due to dysregulated aging macrophages. Alveolar macrophages (AM) in the lungs can be infected, contributing to viral replication. Persistent viral reservoirs drive inflammation and co-morbidities in HIV/SIV-accelerated aging. To achieve an HIV cure, CD4 T cells and macrophages, which contribute to the virus reservoir, need removal.

MATERIALS and METHODS: Lung tissue from 24 Rhesus macaques infected with SIVmac251 intravenously and 13 uninfected were analyzed by flow cytometry and confocal microscopy to determine frequency of AM (CD163+CD206+) among immune cells. Macaques were treated with dextran intravenously and aerosol, an inert molecule which can be phagocytosed by macrophages and detected at later time points. At necropsy, tissues were collected for digestion and for paraffin-embedded block prior to antigen detection.

RESULTS: AM constituted 76.2% ± 7.1 of immune cells in the alveoli of uninfected macaques, while infected macaques macrophages made up 81.0% ± 3.1 of all immune cells. Dextran positive AM were 89.5%± 9.6 throughout different time points with detection of 99% dextran positive macrophages at 397 days post treatment. CONCLUSION: Alveolar macrophages are a major cell population in the alveolar space highlighting their importance in homeostatic and diseased states. The incorporation of dextran within these macrophages for up to 397 days is indicative of a long-lived tissue-resident macrophage subtype with potential to be a reservoir for HIV/SIV.

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Enhanced antiviral gut mucosal immunity and viral clearance following mesenchymal stem cell therapy in the SIV-infected non-human primate model of HIV/AIDS due to rapid restoration of B lymphoid follicles and NK T cell activation

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Abstract

HIV and SIV cause severe CD4+ T cell depletion, B cell follicle atrophy, and impaired mucosal antiviral immunity in the gut during early viral infection. Antiretroviral therapy (ART) does not fully restore gut mucosal structure and function. We previously reported that mesenchymal stem cell (MSC) treatment of SIV-infected rhesus macaques effectively repaired B lymphoid follicles, restored CD4+ T follicular helper (Tfh) cells and enhanced virus-specific immunity, leading to clearance of SIV-infected cells within the gut effector sites. We sought to spatially map the molecular pathways of mucosal repair and resilience in SIV-inflamed gut using the GeoMx Digital Spatial Profiling platform. Proteomic and transcriptional signatures unique to B lymphoid follicles and villus effector sites were identified. Differential gene expression revealed highly activated BCFs induced by the BCL6 master regulator. Elevated antigen presentation was associated with higher IgG antibody production, and a protective B cell gene signature, previously identified by SIV/HIV vaccine studies, was observed. Robust NKT cell activity (KLRK1, GZMM, CCL5, LAMP1) was detected, suggesting that increased migration to mucosal BCFs of MSC-treated animals led to viral suppression and Tfh cell preservation. In addition, MSC treatment had a positive impact in SIV-infected animals virally suppressed by ART with increased B cells and trafficking of cytolytic T cells in the gut. Our data highlight the novel effects of MSC on mucosal SIV-specific B cell activation through lymphoid follicle restoration and their potential to enhance antiviral immunity, which can be harnessed in future HIV/SIV vaccine and cure studies.

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Profiling T Cell Responses in Nonhuman Primates Immunized with a Self-amplifying RNA Vaccine Encoding HIV-1 Envelope and Formulated with Two Novel LIONs™

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Abstract

Background:

We previously showed that a self-replicating RNA (repRNA) vaccine formulated with LION™, a cationic oil-in-water nanoemulsion formulation, induced robust T cell and antibody responses and provided protection against SARS-CoV-2 in nonhuman primates (NHPs). In this study, we investigated the HIV-specific T cell responses elicited by alternate LION™ formulations.

Methods:

Four groups of pig-tailed macaques (Macaca nemestrina, N=4, 2 males and 2 females) were vaccinated intramuscularly at weeks 0, 8, and 12 with LION™/repRNA vaccine encoding Env (BG505 SOSIP.664). Group 1 received the standard LION™ used in the SARS-CoV-2 vaccine, group 2 received a control lipid nanoparticle (LNP), group 3 received standard LION™ that forms a large complex with RNA (Alt LION™ 1), and group 4 received a large alternate LION™ (Alt LION™ 2). Blood samples were collected pre- and post-vaccination, and HIV-specific T cell responses were analyzed via intracellular staining.

Results:

The standard LION™ induced significant expression of Ki-67 and IFNγ in CD4+ T cells (N=4 and N=1 respectively) and Ki-67, IFNγ, and TNFα in CD8+ T cells (N=2, N=1, and N=1 respectively). In contrast, Alt LION™ 1 induced significant expression of CD107a/granzyme B, IFNγ, and Ki-67 in CD4+ T cells (N=1 for all) and in CD8+ T cells (N=2, N=1, and N=1 respectively). The control LNP and Alt LION™ 2 did not induce significant T cell responses.

Conclusion:

The standard LION™ formulation elicited a stronger, more consistent T cell response than the alternate LION™ formulations. These results show that LION™ complex and particle size can impact immunogenicity.

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Mucosal tissue resident memory T cells induced by different viral vectors and the influence of immunization route in macaques

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Abstract

Background: We recently demonstrated that an intravenous heterologous viral vector (HVV) vaccination consisting of sequential immunization with VSV, vaccinia and Ad5 viral vectors that is designed to induce strong T-RMs against SIV Gag markedly enhances the durability of protection against intravaginal SHIV challenges mediated by a protein vaccination. In this study we compared the T-RMs induced by DMC Gag vaccine (DNA, modified vaccinia Ankara and chimpanzee adenovirus) with HVV Gag vaccine in Mamu A*01 positive macaques. The DNA was delivered via IM and viral vectors were delivered via either IM (DMC-IM) or IV (DMC-IV) routes.

Methods: The Gag-specific CD8 T cells were measured using Gag CM9 tetramer in blood, rectum and vagina longitudinally after each vaccination, and were phenotyped for expression of markers associated with proliferation, cytolytic function, tissue migration and memory differentiation.

Results: All three vaccination regimens induced strong Gag specific CD8 T cells in blood, rectum and vagina. However, the HVV vaccination induced 5-10-fold higher frequency in tissue, and these cells showed better persistence (less than 2-fold decline over 3 months) compared to DMC vaccination. The HVV vaccine induced CD8 T cells also showed higher cytolytic potential. The CD8 T cells induced by IV vaccination showed higher CXCR3 and CD69 expression at mucosal tissues compared to cells induced by IM vaccination.

Conclusions: HVV vaccination induces CD8 T-RM that are distinct from T-RM induced by DMC vaccination, and the route of vaccination significantly influences the functional quality of T-RM.

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Augmented Pro-inflammatory Response of Distinct Liver Macrophage Populations in SIV-infected And cART-treated Rhesus Macaques.

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Abstract

Background

Fatty liver disease is a major source of morbidity and mortality in HIV-infected individuals. Using longitudinal biopsies, we expand upon previous studies to evaluate macrophage phenotype and function throughout SIV infection and cART treatment.

Methods

Liver biopsies were laparoscopically collected from rhesus macaques (SIV-naïve, SIV+, cART-only, and SIV+cART-treated), at baseline and post-infection weeks 2, 6, 16-20, and 32/necropsy. Liver tissue was enzymatically digested, and macrophages assessed by intracellular cytokine flow cytometry. Liver macrophages were identified as cells expressing HLA-DR, CD11b, and CD163 and lacking CD3, CD8, and CD20 in association with markers described below.

Results

In uninfected macaques, two populations of liver macrophages were present: one that was CD11b-high, CD206-negative, and expressed recruitment markers Mac387 and CCR2, and another that was CD11b-intermediate, CD206-positive, without recruitment markers. Following SIV infection, the CD206- subset was significantly associated with liver and plasma viral loads. In contrast, the CD206+ subset did not correlate with the SIV load at either tissue site. TLR stimulation (LPS, Pam3CSK4, heat-killed Listeria monocytogenes) was used to compare macrophages in cART-treated to SIV-infected cART-treated macaques during early chronic infection. CD206- macrophages from cART-treated macaques produced TNF-alpha and iNOS while CD206+ macrophages produced IL-6. In the context of cART-treated SIV infection, IL-6 production was enhanced in the CD206- subset and suppressed in the CD206+ subset.

Conclusion

Liver macrophages demonstrate an altered inflammatory function during SIV infection as well as during cART-treated SIV infection. This differential response provides initial insights into how macrophages promote liver inflammation in these distinct conditions.

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Downregulation of CCR5 on Brain Perivascular Macrophages in Simian Immunodeficiency Virus-Infected Rhesus Macaques

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Abstract

Background

C-C chemokine receptor 5 (CCR5) is a major coreceptor for Human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) cell entry; however, its role in brain pathogenesis is largely understudied. Thus, we sought to examine cell type-specific protein expression of CCR5 during SIV infection of the brain.

Methods

We examined occipital cortical tissue from uninfected rhesus macaques and SIV-infected animals with or without encephalitis using immunohistochemistry and immunofluorescence microscopy to determine the number and distribution of CCR5-positive cells.

Results

An increase in the number of CCR5+ cells in the brain of SIV-infected animals with encephalitis was accounted for by increased CD3+CD8+ cells expressing CCR5, but not by increased CCR5+ microglia or perivascular macrophages (PVMs), and a concurrent decrease in the percentage of CCR5+ PVMs was observed. Levels of CCR5 and SIV Gag p28 protein expression were examined on a per-cell basis, and a significant, negative relationship was established indicating decreased CCR5 expression in productively infected cells. While investigating the endocytosis-mediated CCR5 internalization as a mechanism for CCR5 downregulation, we found that phospho-ERK1/2, an indicator of clathrin-mediated endocytosis, was colocalized with infected PVMs and that macrophages from infected animals showed significantly increased expression of clathrin heavy chain 1.

Conclusions

These findings show a shift in CCR5-positive cell types in the brain during SIV pathogenesis with an increase in the number of CCR5+ CD8 T cells, and downregulated CCR5 expression on infected PVMs, likely through ERK1/2-driven, clathrin-mediated endocytosis.

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Optimization of an in-vitro Blood Brain Barrier (BBB) for testing the passage of antibody based probes to detect SIV reservoirs in brain

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Abstract

Background: Despite effective cART, HIV latent reservoirs persist, preventing a cure. One especially challenging anatomical reservoir for HIV is the CNS due to its selective permeable blood-brain-barrier (BBB) which many ART drugs cannot penetrate. To identify such reservoirs, our team aims to develop HIV/SIV probes capable of penetrating the BBB and locate viral reservoirs using the sensitive immunoPET-CT. To aid in this goal, we are optimizing an in vitro BBB to select the most promising probes.

Methods: Optimized rhesus BBB consist of primary endothelial cells (MBEC) and astrocytes cultured on transwells. Probe passage is tested by incubation of SIV1C target cells in the well bottom after determining the integrity of the BBB using Trans-endothelial electrical resistance (TEER).

Results: While Caco2 epithelial control cells fully blocked the passage of p7D3 F(ab)'2 probes, primary MBEC/astrocytes BBB grown for ~10 days generated markedly lower TEER values and allowed selective passage of various probes tested. Of importance, passage of p7D3 F(ab)'2 fused to Ang2 or odoranalectin peptides appeared more efficient than unfused p7D3 F(ab)'2, This result correlated with the in vivo ability of the same probes to identify SIV signals in the CNS of SIVmac251-infected monkeys with positive SIV mRNA levels in the CSF.

Conclusion: While further optimization is ongoing, we have an in vitro screening tool to evaluate the ability of probes to cross the BBB toward their application to immunoPET-CT visualization of active brain viral reservoirs and the efficacy of CNS targeted antiviral therapies.

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Understanding Enhanced Susceptibility to COVID-19 in People Living with HIV/AIDS

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Abstract

Background: While COVID-19 continues to be a health challenge, very little is known about the mechanisms through which high susceptibility to COVID-19 occurs in PLHIV.

Methods: Our group was amongst few that established the rhesus macaque models of COVID-19 infection early on during the pandemic. We are now leveraging this established NHP model of COVID-19 with the long-standing, highly-validated, pathogenic AIDS NHP model to study a central hypothesis that underlying SIV infection and the resulting immunodeficiency/immune activation promotes severity of COVID-19 presentation by profiling the dynamics of viral titers (measured via molecular virology), induced tissue pathology (measured via live PET-CT imaging and conventional histopathology), and underlying immunological perturbations (measured via flow cytometry and single cell transcriptomics).

Results: Macaques infected with SIV develop acute immunodeficiency within 2-4 weeks and progress to pathogenic AIDS within 6 months, while those infected with SARS-CoV-2 develop mild-to-moderate COVID-19. Macaques co-infected with both viruses demonstrate significantly accelerated progression of diseases caused by both infections including rapid time-to-euthanasia, enhanced lung involvement and novel brain involvement as measured by PET-CT. Investigations of the underlying specific immune mechanisms are ongoing. We are studying changes to airway myeloid cell composition and the phenotype of CD8+ T cell responses to both viruses.

Conclusion: Our studies are outlining the specific mechanisms which result in the enhanced susceptibility of PLHIV to COVID-19. Mechanistic insights developed by this study is imperative for the development of host-directed immunotherapeutic interventions for combating COVID-19 in PLHIV and may have utility for future pandemics caused by coronaviridiae.

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Engineering IOMA for optimal CD4 binding site features informs vaccine design

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Abstract

HIV-1 broadly neutralizing antibodies (bNAbs) that target the CD4 binding site (CD4bs) epitope include rare features that pose challenges in efforts to elicit these bNAbs through vaccination. The IOMA-class of CD4bs bNAbs require fewer rare mutations to achieve broad neutralization, thus presenting a more accessible pathway for vaccine-induced bNAb development. Here, we investigated the role that somatic hypermutations (SHMs) play in conferring IOMA's neutralization potency and breadth by creating a library of IOMA mutants in which each SHM was individually reverted to its inferred germline counterpart. Impacts on neutralization for each mutant were evaluated and this information was used to design IOMAmin mutants that exhibited a reduction of over one-third in SHMs compared to IOMA, while retaining its neutralization capabilities. The binding of this IOMAmin mutant to Env was visualized through a 3.9 Å structure, unveiling distinct CD4bs interactions in comparison to the Env-bound IOMA structure. These results demonstrate IOMA-class bNAbs require even fewer mutations for neutralization and further elucidates how IOMA's structural features correlate with its neutralization mechanism, informing the design of IOMA-targeting immunogens.

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Whole genome and transcriptome profiling of immune cells; a cross-species comparison among nonhuman primate (NHP) models of human infectious diseases.

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Abstract

Nonhuman primates (NHP), specifically macaques, share approximately 93% genome homology with humans, making them the most appropriate animal models to study human infectious diseases such as COVID-19, tuberculosis (TB), and HIV. With the emergence of infectious diseases like COVID-19 and recently monkey pox, there is an increasing need in better understanding primate immunogenomic features that correlates with human disease susceptibility and/or disease transmission. In this study, we perform comparative genomics and comparative single-cell transcriptome immune profiling of peripheral blood mononuclear cells (PBMCs) from four macaque species and humans. Rhesus macaque (macaca mulatta), cynomolgus macaque (macaca fascicularis), pig-tailed macaque (macaca nemestrina), and African green monkeys (chlorocebus sabaeus) are NHP species that are the most commonly used animal models to study human infectious diseases including COVID-19. The comparative genomics and immune cells transcriptomic of these macaque species reveals high homology of several immune cell subtypes of pig-tailed macaque with those of humans. Additionally, the comparative analysis of immune gene orthologous isoforms leveraging long-read sequencing reveals novel species-specific immune orthologs and primate repetitive elements. These novel isoforms and repeat elements play a crucial role in resistance to infectious diseases. The genomics information from our study has broad application in understanding human immune system and development of new therapies leveraging primate comparative immunogenomics.

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Antibody hexamerization to improve HIV bNAb activity in vivo

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Abstract

Background:

Amongst antibody effector functions, complement-mediated activities, which leverage antibody hexamerization and can culminate in lysis of virions or infected cells, have been least well studied. We previously examined the role of complement-associated effector function of broadly neutralizing antibody (bNAb) 10-1074 with ablated and enhanced complement activation profiles in vivo in rhesus macaques. When administered prophylactically against simian-HIV challenge, more bNAb was required to prevent infection when complement activity was eliminated. Conversely, less bNAb was required to protect the animals from infection when complement activity was enhanced.

Methods:

We compared complement cascade activity in humans and macaques across a set of complement-dependent cytotoxicity (CDC) assays in vitro with rhesus, human, and Fc-engineered antibodies. A panel of bNAbs was engineered for enhanced CDC activity by incorporation of the E430G (EG) mutation in IgG1 backbone, which favors Fc domain hexamerization, in turn enhancing C1 complex recruitment and CDC.

Results:

We observed robust CDC activity in both species and reliable increases in CDC activity afforded by EG forms of HIV bNAbs. Preliminary data using binary combinations of bNAbs suggest that hetero-hexamerization also boosts complement activity in vitro, motivating follow up in in vivo models.

Conclusions:

Complement-mediated effector functions contribute to the in vivo antiviral activity of HIV bNAbs. Fc engineering may contribute to the further improvements in the efficacy of antibody-mediated prevention strategies.