Center for Childhood Infections & Vaccines

8th Annual Symposium

November 6th, 2024

12:00 PM – 5:00 PM

Health Sciences Research Building (HSRB-II)

EMORY UNIVERSITY · Children's Healthcare of Atlanta · MOREHOUSE SCHOOL OF MEDICINE · Georgia Institute of Technology

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Welcome

Dear Colleagues,

Welcome to the 8th Annual Center for Childhood Infections and Vaccines (CCIV) Symposium! We are thrilled to have you join us for what promises to be an informative and engaging event.

This year's symposium features 30 abstracts showcasing the outstanding work of our faculty, staff, trainees, and students. Our agenda is packed with presentations highlighting cutting-edge infectious disease and vaccine research taking place at Emory University, Children's Healthcare of Atlanta, and our partner institutions.

We are honored to have Dr. Grace John-Stewart as our keynote speaker this year. Dr. John-Stewart is a distinguished Professor of Global Health, Medicine, and Epidemiology at the University of Washington. Her pioneering research focuses on infectious diseases and global maternal-child health, and we are excited to hear her insights.

In addition, we are pleased to welcome our internal guest speakers, Dr. Vineet Menachery and Dr. Una O'Doherty, who will share their expertise and contributions to the field.

Thank you for taking the time to attend the symposium. We look forward to a day of stimulating discussions and collaboration.

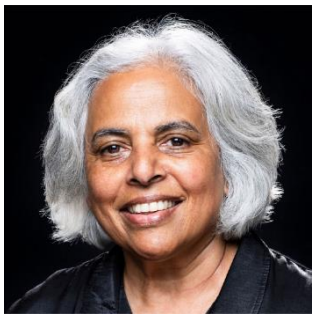
Sincerely,

Gregory Melikian, PhD
Symposium Co-Chair
Professor
Division of Infectious Disease
Department of Pediatrics
Emory University School of Medicine
Children's Healthcare of Atlanta

Christina "Kristy" Rostad, MD
Symposium Co-Chair
Assistant Professor
Division of Infectious Diseases
Department of Pediatrics
Emory University School of Medicine
Co-investigator in the Emory Vaccine and Treatment Evaluation Unit (VTEU)

Mehul Suthar, PhD
Symposium Co-Chair
Director, Center for Childhood Infections and Vaccines (CCIV)
Associate Professor, Emory Vaccine Center
Emory University

Our Keynote Speaker



Grace John-Stewart, MD, PhD
Professor
Departments of Global Health, Medicine, Pediatrics, and Epidemiology
University of Washington

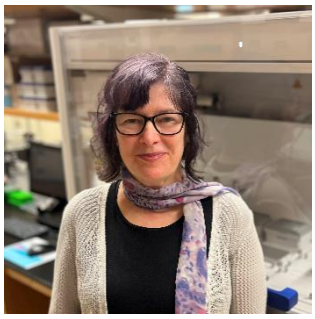
Dr Grace John-Stewart is a Professor in the Departments of Global Health, Medicine, Pediatrics, and Epidemiology at the University of Washington. Dr. John-Stewart is an internist/pediatrician infectious diseases epidemiologist who has led clinical research focused on HIV and the interconnected health of women, adolescents, and children as part of a collaborative research team in Kenya. Her research group has conducted research across a methodologic spectrum that has included clinical trials, molecular epidemiology, implementation science, and large-scale evaluations.

Dr. John-Stewart enjoys mentoring the next generation of research scientists and has mentored over 100 pre-doctoral and post-doctoral trainees. Through mentorship she has sought to catalyze new research with new investigators to advance studies of growth, TB, PrEP in pregnancy, and mental health in mothers, adolescents, and children. Overall, her research has been disseminated in >500 peer-reviewed publications. She is Director of the University of Washington Center for Global Health of Women, Adolescents and Children (Global WACh) and is an Associate Director of the UW-Fred Hutch Center for AIDS Research (CFAR).

Our Internal Guest Speakers

Vineet Menachery, PhD
Associate Professor
Department of Pediatrics
Emory University School of Medicine
Emory Vaccine Center
Emory University

" Using reverse genetics to probe SARS-CoV-2 infection and pathogenesis."



Una O'Doherty, MD, PhD
Professor
Department of Pathology and Laboratory Medicine
Emory University School of Medicine
Pathologist, Anatomic Pathology
Emory Healthcare

" The Central Role of Naïve T cells for HIV Reservoirs"

Schedule

Time/Room	Presentation
12:00 – 1:00 PM	**Keynote Presentation**
N600	*"Pediatric HIV: Challenges and Opportunities"* **Grace John-Stewart, MD, PhD** Professor Departments of Global Health, Medicine, Pediatrics, and Epidemiology University of Washington
1:00 – 1:30 PM	**Internal Speaker**
N600	*" Using reverse genetics to probe SARS-CoV-2 infection and pathogenesis."* **Vineet Menachery, PhD** Associate Professor Department of Pediatrics Emory University School of Medicine Emory Vaccine Center Emory University
1:30 – 1:45 PM	**Oral Abstract Presentation #1**
N600	*"eMASH: Exploring Feasibility and Acceptability of mHealth Visits for Adolescents Living with HIV in South Africa"* **Lily Acheampomaa-Piasare, BS** Research Assistant. Department of Pediatric Infectious Disease
1:45 – 2:00 PM	**Oral Abstract Presentation #2**
N600	*" Breast milk is enriched with functional anti-mycobacterial antibodies that decline over time"* **Lisa Marie Cranmer, MD, MPH** Associate Professor of Pediatric Infectious Diseases and Epidemiology
2:00 – 2:15 PM	**Oral Abstract Presentation #3**
N600	*" A Shock and Kill Cure Strategy for SIV-Infected Infant Macaques: Utilizing a Combination of a Broadly Neutralizing Protein Delivered Through AAV9 and a Latency Reversal Agent "* **Jairo Fonseca, MD** NIH Office of AIDS Research Scholar, Research Fellow, PGY-6 Clinical Fellow. Division of Infectious Diseases
2:15 – 2:30 PM	**Break**

2:30 – 3:00 PM	Internal Speaker
N600	*" The Central Role of Naïve T cells for HIV Reservoirs"*
	Una O'Doherty, MD, PhD Professor Department of Pathology and Laboratory Medicine Emory University School of Medicine Pathologist, Anatomic Pathology Emory Healthcare
3:00 – 3:15 PM	**Oral Abstract Presentation #4**
N600	*" CD4+ and CD8+ T cells are required to prevent SARS-CoV-2 persistence in the nasal compartment"*
	Meenakshi Kar, PhD Post-doctoral Fellow, Suthar Lab
3:15 – 3:30 PM	**Oral Abstract Presentation #5**
N600	*"Unraveling the mechanism of potent DENV and ZIKV neutralization by a quaternary epitope targeting antibody"*
	Sanjeev Kumar, PhD Post-doctoral Fellow, Wrammert Lab
3:30 – 3:45 PM	**Oral Abstract Presentation #6**
N600	*"Postnatal Zika Virus Infection Alters Temperament and Social Attention in Infant Rhesus Macaques"*
	Kaitlyn Love, BS Research Specialist, Raper Lab
3:45 – 4:00 PM	**Break (Proceed to the Atrium for the poster session and reception)**

4:00 – 5:00 PM	Poster Session / Reception

Atrium

2. *CRISPR/Cas9-Mediated Gene Editing of Cultured Primary Human Hepatocytes for HBV Studies*
Antonios Athanasiadis, BS

3. *Environmental Surfaces in Healthy Households as Reservoirs of Extended-Spectrum Cephalosporin-Resistant Enterobacterales in the Community*
Barret Breeze, BS

4. *Cytokine profiling analysis for HIV+ and SARS-CoV-2+ pregnant women and in vitro investigation of HIV-1 antiretroviral drug exposure on pro-inflammatory gene expression in human placental cells*
Dara Brena, MS

5. *Development of Novel Functional Assays of Fc Effector Antibody Responses to SARS-CoV-2*
Xuemin Chen, MD

6. *Infectious Etiologies of Acute Respiratory Illness in Older Adults and of CHF and COPD Exacerbations Requiring Hospitalization During Two Pre-Pandemic Respiratory Seasons*
Jong-Ha Choi

7. *Multiplex Th1/Th17 cytokine detection identifies a high proportion of South African and Indian infants with Mycobacterium tuberculosis (Mtb) sensitization*
Lisa Marie Cranmer, MD, MPH

8. *Sex-based Differences in Macaque Models of HIV Pathogenesis and Persistence*
Kedan Endrias, MS

9. *Clinical Features and Outcomes of Pediatric and Adult Patients Hospitalized for COVID-19: A Comparison Across Age Strata*
Fnu Gopchandani

10. *Sex-specific Immune Responses to ALVAC-HIV and Bivalent Subtype C gp120/MF59 in the HVTN 100 Clinical Trial in South Africa*
Cassie Grimsley Ackerley, MD, MSc

11. *Targeting Wnt/β-catenin Signaling Pathway During Latency Reversal in ART-suppressed SIV-infected Rhesus Macaques*
Riri Hamid, MS

12. *Persistence of Plasma TGF-β in CLWH is associated with Lower Inducible HIV Despite High Latent Reservoirs*
Mojahidul Islam, PhD

13. Infants' Humoral Immune Responses to Primary Influenza Vaccination and H3-Infection
Devyani Joshi, PhD

14. *Safety Profile of AZD5582 in Infant Macaques on ART*
Alexis King, B.S

15. *Opt-Out HIV Screening Highlights Syphilis Trends in Pediatric Emergency Departments*
Lauren Middlebrooks, MD

16. *Eosinophils protect against SARS-CoV-2 following a vaccine breakthrough infection*
Kathryn Moore, PhD

17. *Profiling Calcium and cAMP Responses in Human Monocytes Toward Development of Improved Vaccine Adjuvants*
Maria Parilla, BS

18. *Regulation of IFITM Proteins' Antiviral Activity Through Sequestration into Intraluminal Vesicles of Late Endosomes*
David Prikryl, PhD

19. *Inflammatory and Immune Dysfunction-Targeting Therapeutics for Unmet Clinical Needs in Children with Kawasaki Disease and Multisystem-Inflammatory Syndrome (MIS-C)*
Guadalupe Quinones, BS, BA

20. Elucidating changes in CPSF6 mobility and interactions in HIV-1 infected cells
Sara Erlan Sagadiev, BS

21. *Evaluation of IgA enzyme immunoassays to detect primary Respiratory Syncytial Virus infection in infants and young children*
Ranjini Sankaranarayanan, PhD

22. *Characterizing Contact Patterns of Children Under 5 Years Old: A Comparative Study across Four Low- and Middle-Income Countries*
Machi Shiiba, MPH

23. *RSV pneumonia among adults hospitalized with acute respiratory infections, CHF, or COPD exacerbations*
Janelle Spencer-Ramirez, BS

24. *Influence of Maternal Immune Activation and HIV Exposure on Monocyte Cytokine Profiles to Mycobacterium tuberculosis in Early Childhood*
Arijita Subuddhi, PhD

25. *Combination cIAP and BCL-2 Inhibition Reduces Intact Reservoirs in ART-Suppressed SIV-Infected Rhesus Macaques*
Benedicth Ukhueduan, PhD

eMASH: Exploring Feasibility and Acceptability of mHealth Visits for Adolescents Living with HIV in South Africa

Authors: Acheampomaa-Piasare, L; Goldstein, M; Bayeni, M; Sibaya, T; Archary, M; and Zanoni, B.

Presenting Author: Lily Acheampomaa-Piasare

Background: South Africa has one of the highest HIV prevalence rates among youth ages 15 to 24. Youth with HIV (YWH) face many barriers to engagement in care and non-adherence to treatment, including access to care, conflict with school hours, transportation costs, and stigma. The COVID-19 pandemic exacerbated these barriers and highlighted a need for innovative solutions to improve engagement in care. Conducting virtual healthcare visits through mobile health (mHealth) may address such barriers.

Methods: We recruited 10 healthcare providers and 20 YWH from healthcare facilities in Durban, South Africa. After healthcare providers and YWH participated in mock mHealth visits through a WhatsApp video or audio call, we conducted in-depth interviews to evaluate the acceptability and feasibility of these virtual healthcare visits. We used thematic analysis to code interview transcripts in Dedoose using deductive and inductive codes until saturation was met. Codes were then organized into themes.

Results: Virtual healthcare visits were highly acceptable among youth and providers, who noted that mHealth visits provided a more convenient and cost friendly alternative to in-person visits. Benefits included continuity of care in stable patients, more openness from the youth in discussing care, and the ability to discuss the same information as in an in-person visit. Most participants preferred a video call over audio alone since video reassured them of confidentiality and allowed for identification of acute physical changes in the participant. Barriers reported by providers and youth included the novelty of mHealth visits, distractions, and phone, network, and data access. Youth voiced concerns about not being in a private setting during the call and providers noted difficulty in building rapport with unfamiliar patients and the inability to directly refer patients to additional services, if needed.

Conclusion: This study demonstrated potential of utilizing mHealth visits for HIV care in South Africa to address barriers to care such as delays in receiving care, transportation costs, and the stigma attached to visiting clinic in person. While mHealth visits were viewed favorably, we identified important, yet addressable barriers that demonstrated their utility as a supplementation to in-person health visits for YWH to increase engagement in care.

Breast milk is enriched with functional anti-mycobacterial antibodies that decline over time

Authors Lisa Marie Cranmer, Vandana Kulkarni, Adrianna Westbrook , Prasanthi Chappa, Prasad Deshpande, Mallika Alexander, Patricia Grace, Medrine Kihanga, Riya Goel, Aarti Kinikar, Cheryl L. Day, Jens Wrammert, Galit Alter, Amita Gupta, Jyoti S.Mathad, Ramesh Bhosale

Presenting Author: Lisa Marie Cranmer, MD, MPH

Background: Breast milk plays a significant role in shaping infant respiratory tract mucosal immunity, but little is known about Mycobacterium tuberculosis (Mtb)-specific humoral immunity in breast milk. We aimed to characterize the longitudinal antibody profile of anti-mycobacterial antibodies in plasma and breast milk of lactating people living in a TB-endemic setting.

Methods: People of known HIV and Mtb infection status were enrolled during pregnancy in the PRACHITi observational cohort study at Byramjee Jeejeebhoy Government Medical College (BJGMC)/Sasson Hospital and followed postpartum. We measured anti-PPD IgA and IgG levels by ELISA, and PPD-specific antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent neutrophil phagocytosis (ADNP) from cryopreserved plasma and breast milk supernatant at 6 weeks and 12 weeks postpartum. Median antibody levels (OD) and phagocytosis scores in plasma and breast milk were compared using paired Wilcoxon ranksum tests.

Results: Of 56 study participants with available paired plasma and breast milk samples, 28 (50%) were HIV positive and 25 (44%) had Mtb infection as detected by Quantiferon Gold-Plus. Overall, anti-PPD antibody levels and function were similar by HIV and IGRA status in plasma and breast milk. Anti-PPD IgA levels and antibody-dependent neutrophil phagocytosis (ADNP) were higher in breast milk compared to plasma at 6 weeks ($p<0.001$). Anti-PPD antibody-dependent cellular phagocytosis (ADCP) and ADNP function decreased in breast milk from 6 to 12 weeks postpartum ($p<0.001$) (Figure).

Conclusions: Breast milk was enriched with functional anti-mycobacterial antibodies, which declined over time. Future studies to evaluate the role of breast milk antibodies in infant mucosal Mtb immunity are warranted and may inform future TB vaccination strategies prior to or during pregnancy.

A Shock and Kill Cure Strategy for SIV-Infected Infant Macaques: Utilizing a Combination of a Broadly Neutralizing Protein Delivered Through AAV9 and a Latency Reversal Agent

Authors: Fonseca, Jairo Andres; King, Alexis C; Farinre, Omotayo; Liang Shan; Da Costa, Lucas; Enhert, Stephanie; Wood, Jennifer; Gardner Matthew; Van Rompay, Koen; Cottrell, Mackenzie; Martins, Mauricio; Chahroudi, Ann

Presenting Author: Jairo Fonseca, MD

Background: Latency reversal and clearance is an HIV cure strategy focused on reactivating and eliminating latently infected CD4+ T cells. eCD4-Ig, a fusion protein comprising the ectodomain of CD4, an IgG1 Fc portion, and the tyrosine-sulfated regions of CCR5, enhances antibody-mediated cellular cytotoxicity, making it a promising clearance agent. To induce latency reversal AZD5582, a non-canonical NFκB stimulator with efficacy demonstrated in adult rhesus macaques (RM), was selected.

Methods: 72 infant RM were screened for neutralizing antibodies against AAV9, resulting in the selection of 20: 17 with < 25% and 3 with ≤ 35% neutralization. These macaques were orally infected with SIVmac251 at 4 weeks of age and initiated ART at 21 days post-infection. Half received an intramuscular injection of AAV9-eCD4-IgG1. Previous studies indicated that the latency reversal effect of AZD5582 is less pronounced in infants due to pharmacokinetic differences. Pharmacodynamic modeling was conducted to determine the appropriate infant dose of AZD5582, which was administered at 40 weeks post-infection.

Results: eCD4-IgG1 expression exceeded 10 mg/mL in all treated macaques, with sustained levels for at least 59 weeks. Only one animal developed transient anti-drug antibodies, which did not affect long-term eCD4-IgG1 expression. Pharmacokinetic modeling indicated that higher doses of AZD5582 were needed to replicate adult pharmacokinetics. During AZD5582 treatment, ultrasensitive viral load measurements (LOD: 3 copies/mL) showed all 10 treated RM had on-ART viremia exceeding the pre-intervention baseline, peaking at 5600 copies/mL. Of 67 viral load measurements taken, 47 (65.7%) were above baseline, with significantly higher on-ART viremia in the intervention group versus controls ($p = 0.0147$). Six animals from each group underwent Analytical Treatment Interruption. All treatment animals rebounded within 8 days, similar to controls; however, 2/6 in the treatment group experienced a ~4.5 log decline in viremia post-rebound, which was not observed in controls.

Conclusions: Due to its long-lasting transgene expression, AAV-9 demonstrates promise as a delivery platform for HIV-neutralizing molecules, including bNAbs. Higher doses of AZD5582 induced on-ART viremia in infant macaques, with peak levels surpassing those observed in previous studies. Further research is needed to elucidate the underlying mechanisms responsible for viremia control in the treatment group.

CD4+ and CD8+ T cells are required to prevent SARS-CoV-2 persistence in the nasal compartment

Authors: Meenakshi Kar, Katherine E.E. Johnson, Abigail Vanderheiden, Elizabeth J. Elrod, Katharine Floyd, Elizabeth Geerling, E. Taylor Stone, Eduardo Salinas, Stephanie Banakis, Wei Wang, Shruti Sathish ,Swathi Shrihari, Meredith E. Davis-Gardner, Jacob Kohlmeier, Amelia Pinto, Robyn Klein, Arash Grakoui, Elodie Ghedin, Mehul S. Suthar

Presenting Author: Meenakshi Kar, PhD

In SARS-CoV-2 infection, circulating virus-specific CD4+ and CD8+ T cells have been shown to target multiple viral proteins, displaying polyfunctionality and long-lasting effects, crucial for antibody response promotion and mitigating disease severity. However, their role within the respiratory tract remains largely unexplored. We used wild-type C57BL/6 mice, infected with a naturally occurring Beta variant to evaluate the role of T cells in the respiratory tract and found that following SARS-CoV-2 infection, while both CD4+ and CD8+ T cells are recruited to the nasal airways and lungs, displaying cytotoxic activity and antigen-specific effector responses. We see a higher infiltration of CD8+ T cells in the nasal airways as compared to the lungs. Depletion of both CD4+ and CD8+ T cells leads to persistent virus replication in the nasal compartment but not the lungs, independent of neutralizing antibody levels, indicating a crucial role for T cells in controlling viral spread in the upper respiratory tract and innate responses in the lower respiratory tract. CD4+ and CD8+ T cells play overlapping roles in controlling SARS-CoV-2 in the nasal airways as depletion of only CD4+ or CD8+ T cells does not lead to this persistent virus phenotype. In situ hybridization revealed this persistent virus to be located predominantly in the nasal epithelial layer. Deep sequencing of the viral isolates from persistently infected mice revealed mutations across the genome, including a large deletion in ORF6 implicating evolutionary selection in CD4+/CD8+ tandem depleted mice. Our findings underscore the critical role of T cells in controlling SARS-CoV-2 infection within the respiratory tract and highlight potential differences in immune responses between the upper and lower respiratory compartments.

Unraveling the mechanism of potent DENV and ZIKV neutralization by a quaternary epitope targeting antibody

Authors: Kumar Sanjeev; Xu Lingling; Moore Kathryn; Vander Velden Jake; Suthar Mehul; Wrammert Jens

Presenting Author: Sanjeev Kumar, PhD

Background: Envelope (E) glycoprotein is a major target of broadly neutralizing antibodies (bnAbs) to Dengue virus (DENV). Few DENV bnAbs have shown cross-neutralizing potential to Zika virus (ZIKV). Understanding the mechanism of virus neutralization of such potent bnAbs can guide rational vaccine design aimed to elicit such potent bnAbs upon vaccination.

Methods: In this study, we characterized an antibody 33.3A06 having a potent cross-neutralizing potential to DENV and ZIKV. We generated IgG1-4 isotype variants of 33.3A06. We evaluated the binding reactivity of 33.3A06 in IgG1-4 forms to whole DENV and ZIKV virus, viral lysates, EDIII receptor binding domain, native-like E-protein dimers and monomers. Octet/BLI was performed to determine the affinity of 33.3A06 to E-protein dimers. Neutralizing potential was tested by live virus based high throughput focus reduction neutralization test (FRNT) assays.

Results: ELISA binding data revealed that 33.3A06 doesn't bind to E-protein monomers and EDIII domain whereas it targets a conformational / quaternary epitope present on whole virus and E-protein dimers. 33.3A06 IgG1 neutralized DENV1-3 and cross-neutralized ZIKV in IgG1 format but failed to neutralize DENV4, however IgG3 version potently neutralized DENV4. Deletion of somatic hypermutations (SHMs) of the light chain gene lead to reduction of neutralization potential of 33.3A06, but no major impact observed due to deletion of heavy chain SHMs.

Conclusion: Our findings suggests that potent neutralization activity of 33.3A06 is mediated by its light chain in majority. Our results further implicate developing 33.3A06 or its isotype variants as useful clinical reagents for DENV and ZIKV diagnostics, prophylactic and therapeutic reagents in future.

Postnatal Zika Virus Infection Alters Temperament and Social Attention in Infant Rhesus Macaques

Authors: Love, Kaitlyn; Matsuoka, Joy; Van Schoor, Alex; Richardson, Rebecca; Suthar, Mehul; Chahroudi, Ann; and Raper, Jessica

Presenting Author: Kaitlyn Love, BS

Zika virus (ZIKV) is now endemic in mosquito populations in many countries, presenting a continued risk for human health. Fetal ZIKV exposure can cause congenital defects, including microcephaly, brain structural abnormalities, visual impairments, cognitive deficits, and changes in socioemotional behavior. However, the potential consequence of ZIKV infection during infancy remains largely unexplored. Considering the rapid postnatal brain development that occurs during the first years of life, it is crucial to understand the potential impact of ZIKV neurotropism during infancy.

This study investigates the effects of postnatal ZIKV infection using a rhesus macaque (RM) model with twelve infant RMs infected with the Puerto Rican ZIKV strain (105 pfu PRVABC59) at one month of age (ZIKV-1), six uninfected controls (UIC), and six immune stimulation controls (Poly-IC, PIC). During the first two months of life, infants undergo weekly standardized neurodevelopmental assessments, similar to the Brazelton Test for human infants. Subsequently, at four and six months of age, their attention is tracked while viewing videos focusing on social interactions, non-social stimuli, and visual acuity videos.

ZIKV-1 infants displayed a decline in orientation scores post-infection, corresponding with elevated temperament scores during neurodevelopmental testing compared to UIC and PIC controls. Groups did not differ in their performance on visual acuity test at four or six months of age. Interestingly, compared to UIC and PIC, ZIKV-1 infants directed more visual attention to the mouth and less visual attention to the eyes of social stimuli at 4 months of life, whereas visual attention did not differ for nonsocial videos.

Despite a decline in orientation scores after ZIKV infection, there is no evidence of visual impairment as indicated by the scores for visual acuity. Therefore, differences in orientation during early infancy are more plausibly linked to heightened emotional reactivity (elevated temperament scores) resulting in a lack of attention span during the neurodevelopmental assessment. Considering the rapid neural plasticity happening during the first years of life, altered attention to social cues could improve or worsen with age. Further characterization of these animals will help shed light on the potential impacts of ZIKV infection on the developing brain.

Poster Number: 2

CRISPR/Cas9-Mediated Gene Editing of Cultured Primary Human Hepatocytes for HBV Studies

Authors: ATHANASIADIS, ANTONIS; Stenzel, Ansgar F.; Dangas,Georgios; Barbosa,Luana;Zhou, Luana; Levenson,Kenneth C.; Zou, Chenhui; Rice,Charles M.; de Jong,Ype P.; and Michailidis,Lefteris

Presenting Author: Antonis Athanasiadis, BS

Background & Aims: Hepatoma cell lines with NTCP overexpression (HepG2-NTCP) support HBV infections and can efficiently be gene-edited with CRISPR. However, the proliferative and transformed nature of cancer cells do not recapitulate primary human hepatocyte (PHH) biology. A PHH cell culture system that is based on the isolation and culture of PHH from human liver chimeric mice, which we termed mouse-passaged PHH (mpPHH), overcomes several of these limitations. Yet, because mpPHH cultures do not proliferate and technical challenges transfecting PHH, efficient gene editing remains a significant problem for host-pathogen studies. We therefore aimed to establish methods to efficiently CRISPR edit PHH cultures.

Methods & Results: Here, we report the development and application of two novel methods for efficient gene editing in mpPHH: 1) a lipofection-based Cas9-RNP system and 2) a CRISPR-based lentiviral delivery system. Unlike widely used electroporation methods that can cause irreversible damage to isolated hepatocytes, these methods can yield high editing efficiency while minimizing cell disturbance. We have also optimized both methods for arrayed screens, enabling high-throughput applications using mpPHH.

The use of huFNRG mice models also provides a practical advantage of re-transplanting and expanding edited mpPHH cells, creating a unique in vivo model to validate edited phenotypes. This capability addresses the laborious nature and reproducibility issues of gene-edited PHH experiments. Post-engraftment, these mice can be exposed to HBV to assess the impact of specific gene knockouts, such as NTCP KO, by comparing infection levels with non-targeting controls.

Conclusion: We can perform gene edits in PHH without affecting their viability and with a significant success rate. The ability to efficiently perform CRISPR-based gene editing in cultured PHH enables both targeted and high-throughput studies of host and viral factors in the context of HBV infection.

Poster Number: 3

Environmental Surfaces in Healthy Households as Reservoirs of Extended-Spectrum Cephalosporin-Resistant Enterobacterales in the Community

Authors: BREEZE, BARRETT; Babbs, Catherine; Shen, Katherine; Boyle, Mary; Babiker, Ahmed, Heidbreder, Ainsley; Green, Stefan; Fritz, Stephanie and Logan, Latania K.

Presenting Author: Barrett Breeze, BS

Background: Community-acquired extended-spectrum cephalosporin resistant (ESCr)-Enterobacterales (E) are increasing in healthy populations; the sources of acquisition are unknown. We assessed household (HH) environmental surface samples (HS) in St. Louis, MO, to identify the HS most frequently contaminated with ESCr-E and associated antibiotic resistance (AR) mechanisms.

Methods: From 150 HHs, samples from 21 HS and inguinal fold (IF) samples of healthy adults and children were cultured for bacteria. Families were recruited from outpatient pediatric settings. Bacterial identification and antibiotic susceptibility testing were conducted (Vitek®2, bioMérieux). Molecular characterization of ESCr-E was performed by PCR detection of beta-lactamase (bla) and plasmid-mediated quinolone resistance (PMQR) genes. Whole genome sequencing (WGS) was performed on select HH with >1 colonized HS to evaluate for genetic relatedness, plasmids, and AR genes. A cluster was defined as <15 single nucleotide polymorphism differences between 2 isolates.

Results: Of 627 IF and 3018 HS in 150 HHs, the majority harbored bacteria (IF, 95-100% and HS, 79-100%) and gram-negative bacteria, (IF, 19% and HS, 4-50%). IF colonization with Ent was ~23% and ranged from 7-58% on HS; The most common ESCr-E genera were Enterobacter (73%), Pantoea (10%), and Klebsiella (7%). ESCr-E were found on IFs in 5% of HHs. HS most notably colonized with ESCr-E were the kitchen sink faucet (11%), oven door handle (5%), sofa (5%), bathroom sink faucet (3%) and bed sheets (3%). ESBL-E were recovered from ~5% of HHs. MDR-E (>3 classes) were found in 5% of HHs.

WGS of 20 E. cloacae from 4 HHs revealed within HH clusters. Of interest, we found that among E. cloacae, 10/20 (50%) contained AR plasmids (ex. IncFI, IncFII, IncX5, IncHI2, Col440I); bla, other AR (ex. PMQR, aminoglycoside, sulfa, fosfomycin) and virulence genes were detected in E. cloacae in all HHs. We also found related E. cloacae strains in 2 HHs (ST108), indicating local strains circulating potentially related to a common exposure.

Conclusions: ESCr-E are present in healthy household environments. Future directions will be to identify candidate clinical and epidemiological features linked with ESCr-E HH colonization.

Poster Number: 4

Cytokine profiling analysis for HIV+ and SARS-CoV-2+ pregnant women and in vitro investigation of HIV-1 antiretroviral drug exposure on pro-inflammatory gene expression in human placental cells

Authors: BRENA, DARA; Schuch, Viviane; Huang, Ming-Bo, Badell, Martina; Floyd, Riaun; Hossack, Daniel; Wilson, Cristina; Bond, Vincent; and Johnson, Erica.

Presenting Author: Dara Brena, MS

Background: In the combination antiretroviral therapy (cART) era, the prognosis for people living with HIV (PLWH) has radically shifted from a progressively lethal disease to a chronic infectious disease. However, there is no cure for HIV and cART suppressed PLWH face a disproportionate risk for chronic non-AIDS-related morbidities including adverse pregnancy outcomes. The etiology of this clinical burden is unknown. This study's purpose is to examine cytokine profiles of pregnant cART suppressed PLWH with SARS-CoV-2 as compared to healthy pregnant controls and to evaluate the in vitro effects of ART exposure on placental cell pro-inflammatory gene expression.

Methods: HIV+ and SARS-CoV-2+ maternal peripheral plasma and umbilical cord plasma were acquired through the Study of Pregnancy Outcomes in women with Respiratory illness due to suspected or confirmed coronavirus infection (SPORE) biorepository. With written informed consent, corresponding controls were obtained from uninfected pregnant women recruited from Grady Memorial and Emory University Midtown Hospital, GA. Multiplex cytokine analysis was conducted through Eve Technologies on 15 analytes. Placental cells (JEG-3) were treated with four different dosages of HIV-1 antiretroviral drugs dolutegravir, efavirenz, tenofovir disoproxil fumarate, tenofovir alafenamide, and emtricitabine. Total cellular RNA was extracted, cDNA generated, and RT-qPCR conducted targeting NLRP3, IL-6, NFKB, and TNF-alpha.

Results: Interestingly, maternal and cord blood plasma cytokine profiles of each group (infected and uninfected) did not always exhibit a positive correlation, but rather had differential cytokine profiles between the mother and fetus. This could indicate a possible protective immunological role. For example, IL-12p40, an anti-inflammatory cytokine was higher in the cord plasma for both HIV+ & SARS-CoV-2+ and the healthy control groups. For the in vitro experiments, DTG decreased NLRP3 expression by approximately 10-fold at the highest dosage. TAF increased NLRP3 expression. DTG decreased NFKB expression by approximately 2-fold.

Conclusions: This study seeks to address the knowledge gap for non-AIDS-related morbidities specific to pregnancy through exploring placental immunology. The chronic immune activation with accelerated immunosenescence experienced by cART suppressed PLWH could similarly be occurring within the in utero/placental environment.

Poster Number: 5

Development of Novel Functional Assays of Fc Effector Antibody Responses to SARS-CoV-2

Authors: Chen, Xuemin; Li, Grace; Ciric, Caroline, Gibson, Theda; Anderson, Larry J and Rostad, Christina A

Presenting Author: Xuemin Chen, MD

Background: The Fc regions of antibodies mediate important immune responses to infections, including antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), antibody-dependent neutrophil phagocytosis (ADNP), and complement-dependent cytotoxicity (CDC). These functions play a significant role in the immune response. This study described that different vaccine dosing regimens impact Fc-mediated immunity and the role of Fc-effector functions in long-term vaccine efficacy.

Method: We developed four assays to evaluate the Fc effector functions of SARS-CoV-2 antibodies. The first two assays assess antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) using stably transfected cells expressing luciferase and SARS-CoV-2 variant spike proteins (Ancestral, Omicron XBB.1.5, and EG.5). Target cell lysis, mediated by Fc engagement through complement (CDC) or ADCC antibody binding to Fc receptors, is measured via luciferase activity. The other two assays measure phagocytosis mediated by macrophages (ADCP) and neutrophils (ADNP) using fluorescently labeled virus-like particles that display SARS-CoV-2 variant spike proteins as target antigens, with phagocytosis quantified by flow cytometry.

Results: We developed and characterized assays for the determination of functional Fc-effector antibodies. Prior to administration of the COVID-19 XBB.1.5 booster vaccination, cross-neutralizing antibodies against XBB.1.5 and EG.5 variants were minimally detectable, while cross-functional Fc-effector antibodies were present at higher baseline levels. The COVID-19 XBB.1.5 booster vaccination significantly boosted both neutralizing and Fc-effector antibodies in magnitude and breadth. The greatest increase in neutralizing antibodies was against the XBB.1.5 strain, while Fc-effector functional antibodies had similar fold-increases in antibody titers against the breadth of SARS-CoV-2 variants tested.

Conclusion: Our study demonstrated that the XBB.1.5 COVID-19 booster vaccination significantly enhanced the magnitude, quality, and breadth of antibody responses against SARS-CoV-2. These novel Fc-mediated functional assays combined with neutralizing antibody assays provide a more comprehensive model of the in vivo virus-antibody interaction leading to effective pathogen control.

Poster Number: 6

Infectious Etiologies of Acute Respiratory Illness in Older Adults and of CHF and COPD Exacerbations Requiring Hospitalization During Two Pre-Pandemic Respiratory Seasons

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Presenting Author: Jong-Ha Choi

Introduction: In adults, the infectious etiologies of acute respiratory illnesses (ARIs) and of exacerbations of cardiopulmonary diseases requiring hospitalization are incompletely understood.

Methods: We conducted a prospective surveillance study of older adults hospitalized with acute respiratory illnesses (ARI) and adults of any age hospitalized with exacerbations of Congestive Heart Failure (CHF) or Chronic Obstructive Pulmonary Disease (COPD) at two urban hospitals in Atlanta, Georgia during the 2018-2019 and 2019-2020 respiratory seasons. All participants had nasopharyngeal and oropharyngeal swabs collected and tested for common viral and bacterial etiologies using a BioFire multiplexed panel. A subset of patients had paired serological testing for respiratory syncytial virus (RSV), and standard-of-care (SOC) microbiologic testing results were collected when available. Participant demographics, clinical characteristics, and outcomes were determined through a combination of participant interview and medical record abstraction. Statistical comparisons between groups were made using Fisher's exact test, and analyses were performed using SAS v9.4.

Results: Of 3142 potentially eligible participants, 1584 were enrolled, of which 1558 were included in the analysis. 757 participants (48.9%) were hospitalized for ARI, 490 (31.5%) for CHF exacerbation, and 311 (19.3%) for COPD exacerbation. 476 (30.6%) participants had at least one pathogen detected, most commonly rhinovirus/enteroviruses (127/1558, 8.2%), influenza (119/1558, 7.6%), and RSV (92/1558, 5.9%). Although co-infections and bacterial and fungal infections were uncommon, these were associated with severe clinical outcomes.

Conclusions: Respiratory viruses contributed substantially to hospitalization for ARI and cardiopulmonary disease exacerbation among our cohort of high-risk adults, underscoring the need for effective therapeutic and preventive interventions.

Poster Number: 7

Multiplex Th1/Th17 cytokine detection identifies a high proportion of South African and Indian infants with Mycobacterium tuberculosis (Mtb) sensitization

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Presenting Author: Lisa Marie Cranmer, MD, MPH

Background: Detection of Mycobacterium tuberculosis (Mtb) infection among infants has been hampered by low sensitivity of interferon-gamma (IFN-) release assays (IGRAs). Multiplex detection of additional Mtb-specific Th1/Th17 cytokines from IGRA supernatants is a means to more broadly evaluate for adaptive Mtb cellular immunity, or "Mtb sensitization," but infant cohorts in TB-endemic settings have not been well-characterized.

Methods: Infants born to mothers enrolled in the PRACHITI cohort in Pune, India and the iNFANT cohort in Cape Town, South Africa were followed from birth to age 12 months. IGRAs were performed between 6 and 12 months of life. Multiplex detection of Th1/Th17 cytokines were performed on cryopreserved IGRA supernatants using Human Th9/Th17/Th22 Fixed Panel Luminex assay (PRACHITi) and a customized MiliporeSigma Luminex panel (iNFANT). Mtb sensitization was defined as detection of ≥2 Mtb-specific cytokines (IFN-, IL-2, TNF, GMCSF and IL-17) per published cutoff criteria. We describe the frequencies of composite and individual cytokine responses and evaluated clinical cofactors using univariable regression, t-tests or Fisher's exact test, as appropriate.

Results: Of 351 infants evaluated by IGRA, 19 (5.4%) infants were IGRA-positive, and multiplex Th1/Th17 cytokine testing detected an additional 63 infants with Mtb sensitization (69/348, 18%) who were IGRA-negative. IL-2 was the most frequently detected cytokine overall (56/328, 17%). Higher maternal education was associated with lower odds of Mtb sensitization (OR 0.53 [95%CI 0.34, 0.84]. TNF expression was associated with maternal HIV exposure (OR 0.44 [95%CI 0.22, 0.85]) and older gestational age (OR 1.33 [95%CI 1.06, 1.71]). Longer duration of breastfeeding was associated with a trend for lower odds of positive IGRA (OR 0.90 [95%CI 0.81, 1.01]), but higher odds of Mtb sensitization (OR 1.06 [95% CI 1.0, 1.13]).

Conclusion: Mutliplex detection of infant Th1/Th17 Mtb-specific cytokines identified a greater proportion of infants with evidence of Mtb sensitization compared to IGRA, which could indicate early Mtb infection after household or community TB exposure or maternal breast milk immune priming. Maternal HIV exposure and gestational age may affect Mtb-specific immune profiles. Future studies are needed to evaluate the prognostic value of Mtb-specific Th1/Th17 cytokines for TB disease progression.

Poster Number: 8

Sex-based Differences in Macaque Models of HIV Pathogenesis and Persistence

Authors: Kedan Endrias, Soma Sonawane, Vidisha Singh, Rama Rao Amara , Steven E. Bosinger, Mirko Paiardini, Vijayakumar Velu, Guido Silvestri, Ann Chahroudi

Presenting Author: Kedan Endrias, MS

Background: Despite women representing >50% of people living with HIV globally, they are underrepresented in HIV cure research. Current literature suggests biological sex influences HIV pathogenesis, with women tending to have lower set point viral loads and increased immune activation. Studies of HIV reservoir size on antiretroviral therapy (ART) have been contradictory, underscoring a need to interrogate potential sex differences in HIV curative studies.

Methods: We conducted a meta-analysis to explore if viral persistence differed by biological sex among animal studies using the rhesus macaque SIV/SHIV infection model. Inclusion criteria included infant or adult studies of SIV or SHIV infection and daily ART treatment with available data on viral dynamics and reservoir size. Data were extracted from 17 studies containing 191 rhesus macaques, of which 117 (61.2%) were male, and 142 (74.3%) were adults. Demographic data, viral strain, viral dynamics, ART duration, and viral reservoir measurements in peripheral blood and tissues were included. We assessed peak plasma viral load (VL), pre-ART VL, and cell-associated SIV/SHIV DNA levels by sex. Descriptive statistics and Mann Whitney U tests were conducted in GraphPad Prism.

Results: Female adult macaques had significantly higher peak VL compared to males (median 1.8×107 versus 8.2×106 copies/ml plasma, respectively, p=0.0011). There was not a significant difference in pre-ART VL by sex nor in peak VL or pre-ART VL when comparing infants. Among SIV-infected animals, females exhibited significantly higher viral DNA levels in peripheral blood CD4+ T-cells (1.3×104 versus 6.2×102 copies/million cells, respectively, p<0.001). Considering both SIV- and SHIV-infected adults, females also displayed significantly higher levels of viral DNA in CD4+ T-cells isolated from lymph nodes (p=0.0028) and gastrointestinal tract (p=0.0002).

Conclusion: These data suggest that female macaques infected with SIV/SHIV have larger reservoirs than males, potentially related to higher peak viremia, and that biological sex may indeed play a critical role in modulating immune responses to HIV infection. Our data support greater inclusion of females in HIV cure research to more accurately capture population level distributions and adequately address the distinct cure potential of females compared to males.

Poster Number: 9

Clinical Features and Outcomes of Pediatric and Adult Patients Hospitalized for COVID-19: A Comparison Across Age Strata

Authors: Li, Grace; GOPCHANDANI, KOMAL; Brazer, Noah; Tippet, Ashley; Choi, Chris; Hsiao, Hui-Mien; Oseguera, Miriam; Foresythe, Abiodun; Bhattacharya, Sanchita; Servellita, Venice; Gonzalez, Alicia Sotomayor; Spinler, Jennifer; Gonzalez, Mark; Gulick, Dalia; Kraft, Colleen; Kasinathan, Vyjayanti; Wang, Yun; Bard, Jennifer Dien; Chen, Pei Ying; Flores-Vazquez, Jessica; John, Audrey Odom; Planet, Paul; Devaraj, Sridevi; Annapragada, Ananth; Luna, Ruth Ann; Chiu, Charles; and Rostad, Christina A.

Presenting Author: Komal Gopchandani, MBBS, MPH

Background: COVID-19 continues to cause hospitalizations and severe disease in children and adults.

Methods:This study compared the risk factors, symptoms, and outcomes of children and adults hospitalized for COVID-19 from March 2020 to May 2023 across age strata at five US sites participating in the PreVAIL consortium. Eligible patients had an upper respiratory swab that tested positive for SARS-CoV-2 by nucleic acid amplification. Adjusted odds ratios of clinical outcomes were determined for children versus adults, for pediatric age strata compared to adolescents (12-17 years), and for adult age strata compared to young adults (22-49 years).

Results: Of 9101 patients in the PreVAIL cohort, 1560 were hospitalized for COVID-19 as the primary reason. Compared to adults (22-105 years, n=675), children (0-21 years, n=885) were less commonly vaccinated (14.3% vs. 34.5%), more commonly infected

with the Omicron variant (49.5% vs. 26.1%) and had fewer comorbidities ($p<0.001$ for most comparisons), except for lung disease ($p = 0.24$). After adjusting for confounding variables, children had significantly lower odds of receiving supplemental oxygen (aOR 0.57, 95%CI 0.35, 0.92) and death (aOR 0.011, 95%CI <0.01, 0.58) compared to adults. Among pediatric age strata, adolescents 12-17 years had the highest odds of receiving supplemental oxygen, high-flow oxygen, and ICU admission. Among adults, those 50-64 years had the highest odds of mechanical ventilation and ICU admission.

Conclusions: Clinical outcomes of COVID-19 differed across pediatric and adult age strata. Adolescents experienced the most severe disease among children, while adults 50-64 years experienced the most severe disease among adults.

Poster Number: 10

Sex-specific Immune Responses to ALVAC-HIV and Bivalent Subtype C gp120/MF59 in the HVTN 100 Clinical Trial in South Africa

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Presenting Author: Cassie Grimsley Ackerley, MD, MSc

Background: Innate and adaptive immune responses differ between individuals assigned male and female at birth, and these differences may impact vaccine-induced immunity. Little is known about sex-specific immunogenicity to HIV-1 vaccine candidates.

Methods: HVTN 100 was a phase 1-2 randomized controlled, double-blind trial conducted in South Africa. Participants aged 18-40 years without HIV received either vaccine regimen (intramuscular injection of a canarypox vector (ALVAC) at 0, 1, 3, 6, and 12 months plus bivalent subtype C envelope gp120 and MF59 adjuvant at 3, 6, and 12 months) or placebo. Immuneresponses at month 6.5, including IgG binding antibodies (bAb), neutralizing antibodies (nAbs), antibody-dependent cell-mediated cytotoxicity (ADCC), and CD4+ IFNg and/or IL2 intracellular cytokine staining (ICS) responses, were compared between per-protocol vaccine-recipients assigned male at birth (AMAB) and assigned female at birth (AFAB). T-cell ICS responses to cytomegalovirus (CMV) pp65 and staphylococcal enterotoxin B (SEB) at month 6.5 were also compared between AMAB and AFAB vaccine- and placebo-recipients. Response rate comparisons were conducted using Barnard's test and response magnitude comparisons using Wilcoxon Rank Sum test. P-values were Holm-adjusted for multiple comparisons.

Results: Of the 185 vaccine-recipients, 73 were AFAB, 112 AMAB. AFAB had a higher ADCC response rate (57.5% vs 29.5%; padj = 0.0003) and a trend toward greater area under the curve granzyme B activity (16.1 vs 11.2; padj = 0.05) to TV1.C gp120 compared to AMAB. In contrast, AMAB vaccine recipients had a higher CD4+ T cell ICS response rate to ZM96.C gp120 (54.1% vs 36.8%; padj = 0.04) and a trend toward a greater response rate to 1086.C gp120 (44.1% vs 29.4%; padj = 0.05) compared to AFAB. There were no differences in response rate or magnitude of IgG bAb to vaccine-matched antigens (1086.C, ZM96, TV1.C) or in nAbs to TV1C8.2 or MW965.26. Among 185 vaccine-recipients and 37 placebo-recipients (18 AFAB, 19 AMAB) no sex differences were observed in CD4+ or CD8+ responses to CMV or SEB.

Conclusions: We found selected differences in immune responses by sex assigned at birth for the HVTN 100 vaccine regimen; however, these varied by immune response type. Considering the variation in HIV-1 candidate vaccine platforms and dosing regimens, future HIV vaccine trials should investigate whether there are sex-specific differences in immunogenicity and efficacy.

Poster Number: 11

Targeting Wnt/β-catenin Signaling Pathway During Latency Reversal in ART-suppressed SIV-infected Rhesus Macaques

Authors: HAMID, RIRI; Ruiz-Salinas, Inna; Schoof, Nils; Colvin, Alora; Keele, Brandon; Silvestri, Guido; Chahroudi, Ann; and Mavigner, Maud

Presenting Author: Riri Hamid, MS

Background: The key obstacle to cure HIV infection is a reservoir of latently-infected CD4+ T cells that cause recrudescence of viremia if ART is stopped. Among these reservoir cells, CD4+ central memory and memory stem cells continually maintain their own pool size through homeostatic proliferation. We showed that inhibition of proliferation and induction of differentiation of these memory CD4+ T cells can be achieved in ART-treated rhesus macaques (RM) through pharmacological modulation of the Wnt/β-catenin signaling pathway. Here we evaluated whether the transient induction of long-lived memory CD4+ T cell differentiation potentiated AZD5582-induced latency reversal in absence or presence of CD8+ cells in ART suppressed SIV-infected RMs.

Methods: Sixteen RMs were infected i.v. with 5,000 IU of barcoded SIVmac239M and initiated on ART 4 weeks post-infection. After 76 weeks of ART all RMs were administered five weekly i.v. doses of AZD5582 at 100 μg/kg. Eleven RMs also received the Wnt inhibitor PRI-724 administered subcutaneously daily at 10 mg/kg starting a week before the first dose of AZD5582. Six of these RMs additionally received subcutaneously a single dose of the CD8α-depleting antibody MT807R1 at 50 mg/kg 24h prior to AZD5582 treatment. On-ART plasma viral loads (PVL) were monitored to assess for latency reversal.

Results: On-ART viremia above 60 copies/ml was experienced by 60% of RMs treated with AZD5582 only, 80% of RMs treated with AZD5582 + PRI-724 and in 83.3% of RMs when CD8+ cell depletion was additionally performed. We also found a higher frequency of latency reversal events in the combined treatment groups with 88% of PVL measurements above baseline in the AZD5582 +PRI-724 group and 90% in the AZD5582+PRI-724+MT807R1 group versus 58% in the AZD5582 only group. These results suggest that PRI-724 enhanced the latency reversal activity of AZD5582. Furthermore, the area under the curve of the PVL was significantly higher in the triple intervention group than in the AZD5582+PRI-724 group, consistent with a role for CD8+ T cells in the maintenance of HIV/SIV latency on ART.

Conclusions: These studies support the latency reversal effect of AZD5582 that can be potentiated by CD8+ cell depletion and Wnt/β-catenin modulation.

Poster Number: 12

Persistence of Plasma TGF-β in CLWH is associated with Lower Inducible HIV Despite High Latent Reservoirs

Authors: Islam, Mojahidul; Johnson, Hadiya; Kakkar, Fatima; Bitnun, Ari; Brophy, Jason; Samson, Lindy; Read, Stanley; Hawkes, Michael T; Chahroudi, Ann; Sékaly, Rafick; Soudeyns, Hugo; and Sharma, Ashish.

Presenting Author: Mojahidul Islam, PhD

Background: Globally, approximately 3,000 new perinatal HIV infections occur each week. Early antiretroviral therapy (ART) reduces HIV reservoir size in children living with HIV (CLWH), but the latent HIV reservoir in CD4+ T cells persists, leading to viral rebound after ART interruption. In adults, latent HIV-harboring CD4+ T cells are linked to cytokines like TGF-β and IL-10, which drive T cell dysfunction. Notably, these cytokines are elevated in early life, suggesting they may influence the HIV reservoir in CLWH. We hypothesize that the size of the HIV reservoir in childhood is shaped by a cytokine environment (e.g., IL-10 and TGF-β) that sustains the persistence of latent HIV-harboring CD4+ T cells.

Methods: To address this hypothesis, we obtained plasma samples from the EPIC4 cohort—a group of children and adolescents (53.5% female) with perinatal HIV infection enrolled from 9 clinical centers across Canada. Three longitudinal samples from 64 virally suppressed CLWH, aged 5-18 years, were collected. The HIV reservoir in these participants spanned a 3-4 log range and showed no decay over three years. Plasma levels of 33 chemokines and cytokines were measured using Mesoscale Discovery's S-plex and U-plex assays.

Results: As expected, an unbiased principal component analysis (PCA) of the data showed that global variability in the plasma cytokine profile on the first PC (explaining 27.4% of the variability) was significantly ($p<0.05$) associated with age at sample collection, while the second PC (15.04% variability) was linked to HIV reservoir cell frequencies. Plasma levels of TGF-β and T-helper 2 cytokines (e.g., IL-4) were significantly elevated in children under 10. A subset of older CLWH (>10 years) with high TGF-β levels did not exhibit high frequencies of cells with inducible HIV RNA, despite maintaining latent HIV. Conversely, older CLWH with reduced TGF-β showed higher effector T-helper cytokines (IL-17A, IFN-γ) and increased cells with inducible HIV RNA and latent HIV DNA.

Conclusions: These data demonstrate an abundance of plasma TGF-β in early childhood CLWH, suggesting its role in shaping the HIV integration landscape. More importantly, persistent TGF-β in later childhood is associated with reduced effector T cell cytokines and poor HIV inducibility from CD4 T cells. These findings emphasize the need for interventions to purge latent HIV in CLWH, particularly in cases of elevated TGF-β.

Poster Number: 13

Infants' Humoral Immune Responses to Primary Influenza Vaccination and H3-Infection

Authors: JOSHI, DEVYANI; Kumar, Sanjeev; Burrell, Allison; Bedoya, Shamika; White, Brendon; Nyhoff, Lindsay; West, Richard; Lowen, Anice; Staat, Mary; and Wrammert, Jens

Presenting Author: Devyani Joshi, PhD

Background: A major obstacle in the development of the universal vaccine against influenza is a rapidly shifting nature of the viral immune dominant epitopes. The further confounding obstacle is the antigenic imprinting, where an individual's first exposure to influenza virus can shape the humoral immune response to subsequent infections and vaccinations. Here we studied the effects of imprinting in infants. The infants were followed with weekly nasal swabs and timely blood collections beginning soon after birth, allowing us to identify symptomatic and asymptomatic respiratory infections and evaluate the immune response both prior to and longitudinally after each influenza vaccination and infection. The blood collections starting at birth also allowed us to study the effects of maternal antibodies on influenza specific response in the infants.

Methods: The IgG binding antibody responses to the HA proteins of various influenza viruses, including H1N1, H3N2 and B, were measured by ELISA analysis. The antibody responses were evaluated to study the magnitude and durability of maternal antibodies, impact of number of vaccinations, infection, and hybrid immune response to influenza vaccination and infection. We compared the infants' responses to a cohort of adult donors who received 2021-2022 influenza vaccination.

Results: The infants had high magnitude of influenza-specific maternal antibodies at birth. These antibodies showed a fast decline and persisted for close to 9 months from birth. The infants showed an increasing antibody response with increasing numbers of yearly influenza vaccinations. However, the response showed a fast decline post vaccination, comparable to the adults' response to influenza vaccine. As opposed to vaccination, infants' antibody response to was much more durable. For the infants who received an influenza vaccination post infection, the antibody response post vaccination was strongly skewed towards the infecting strain of the virus.

Conclusion: Infants produced increasing response to influenza vaccine with increasing numbers of yearly vaccinations. The antibody response to influenza infection is higher in magnitude and more durable than the response to vaccination. In case of prior influenza infection, the antibody response to influenza vaccination is skewed towards the infecting strain of the virus, underscoring the original antigenic sin.

Poster Number: 14

Safety Profile of AZD5582 in Infant Macaques on ART

Authors: King, Alexis; Fonseca, Jairo; Farinre, Omotayo; Wood, Jennifer; Davis, Kaleaha; Cottrell, Mackenzie; and Chahroudi, Ann.

Presenting Author: Alexis King, BS

Background: AZD5582, a mimetic of the second mitochondrial activator of caspases (SMACm), is known for its role in inducing latency reversal in adult rhesus macaques (RMs) through the ncNF-κB pathway, thereby mitigating harmful generalized immune activation. However, its efficacy appears reduced in infants, a phenomenon potentially linked to the inherent resistance to latency reversal of vertically acquired HIV reservoirs compared to horizontally acquired. This study aimed to address this disparity by evaluating the safety and potential efficacy of AZD5582 in an infant RM model.

Methods: We administered AZD5582 to simian immunodeficiency virus (SIV) infected infant RMs on antiretroviral therapy (ART) to assess the safety profile of higher doses not previously tested. Our study utilizes a "shock and kill" strategy, where AZD5582 was used to reactivate latent SIV reservoirs, complemented by an AAV9-delivered eCD4-IgG1 molecule to enhance viral clearance. The AZD5582 dosing regimen included prolonged intravenous infusion times based on pharmacokinetic modeling, aiming to align the efficacy in infants with that observed in adults.

Results: AZD5582 doses of 0.2-0.3 mg/kg were evaluated in infants, as compared to the standard 0.1 mg/kg dose used in adults. Following the third through fifth doses of AZD5582 administered at 0.3 mg/kg over 1.5 hours in 2/2 RMs, there were several adverse events and reactions observed. After reducing the AZD5582 dose to 0.25 mg/kg infused over 0.75 hours we saw similar adverse events and reactions in 2/5 RMs. Notable symptoms observed in the four affected RMs included fever, respiratory distress, and gastrointestinal problems. In contrast, AZD5582 was well-tolerated in 3/3 infant RMs that received a single 0.25 mg/kg loading dose followed by five weekly 0.2 mg/kg maintenance doses, with each infusion lasting 0.75 hours. Complete blood count and serum chemistry profiles also remained within normal limits in these infants.

Conclusions: These results suggest that AZD5582 can be administered at higher doses to infant macaques, although careful attention to adverse effects is warranted. Optimizing dosing strategies of cure-directed interventions using pediatric models is crucial to achieve a balance between efficacy and safety.

Poster Number: 15

Opt-Out HIV Screening Highlights Syphilis Trends in Pediatric Emergency Departments

Authors Middlebrooks, Lauren; François, Sandy; Cameron, Melissa N.; Daniel, Jordan E.; Wynn, Bridget A.; Brown, Sara P.; Thompson, Sarah; Carter, Rebekah G.; DeNaples, Kelly; Kandaswamy, Swaminathan; Orenstein, Evan; Camacho-González, Andrés; Griffiths, Mark A. and Morris, Claudia R.

Presenting Author: Lauren Middlebrooks, MD

The Centers for Disease Control and Prevention (CDC) recommends universal HIV screening for all patients ≥13 years. There is an important association between HIV and other sexually transmitted infections (STIs), specifically syphilis. HIV and syphilis co-infection are linked to increased HIV transmission, viral shedding, and viral loads. Children's Healthcare of Atlanta (Children's) started utilizing a best practice alert (BPA) in their medical record EPIC to capture HIV and STI testing in 2016. The pediatric emergency department (ED) is often used as a main source of care for adolescents which fostered our aim to identify HIV and syphilis testing patterns over the last decade.

Children's population discovery tool-Grandpop was used to compare Children's ED HIV and syphilis testing volumes of patients 13–24-year-olds, for the past decade (January 1, 2013, to December 31, 2023). The data was categorized by sex, positive results, average age of patients screened, and overall positivity rate. Descriptive statistics, a two tailed T-Test, and a Pearson Correlation Coefficient were utilized.

In the past ten years, there were 9283 patients tested for syphilis, 7056(76%) females and 2227 (24%) males with a mean age of 16.0± 0.3. There were 203 positives, 138(68%) females and 65(32%) males, and a positivity rate of 2.2%. For HIV 11,055 patients were tested, 8037(73%) females and 3018(27%) males with a mean age of 16.0± 0.2. There were 61 positives, 8(13%) females and 53(87%) males with a positivity rate of 0.6%. Testing from 2013 to 2023 increased >7-fold for both HIV and syphilis with a strong correlation ($r = .98$, $P<0.001$); screening increase was also seen after the BPA alert launch. For positive results, HIV cases more than doubled while syphilis cases increased 8-fold when comparing 2013 to 2023 results.

This reveals an unexpectedly high prevalence of syphilis positive adolescents in our community. Screening in the ED can play a pivotal role in identifying this public health crisis. Earlier detection, linkage to care and amplified preventative resources are key components that can target syphilis and HIV rates. The CDC should consider recommending opt-out syphilis testing along with HIV given the growing disease burden in adolescents.

Poster Number: 16

Eosinophils protect against SARS-CoV-2 following a vaccine breakthrough infection

Authors Moore, Kathryn M.; Foster, Stephanie L.; Kar, Meenakshi; Floyd, Katharine A.; Elrod, Elizabeth J.; Williams, Elliott M.; Vander Velden, Jacob; Ellis, Madison; Malik, Ansa; Wali, Bushra; Lapp, Stacey; Metz, Amanda; Bosinger, Stephen E.; Menachery, Vineet D.; Seder, Robert A.; Amara, Rama Rao; Kohlmeier, Jacob E.; Grakoui, Arash; and Suthar, Mehul S.

Presenting Author: Kathyrn Moore, PhD

Waning immunity and the emergence of immune evasive SARS-CoV-2 variants jeopardize vaccine efficacy leading to breakthrough infections. We have previously shown that innate immune cells play a critical role in controlling SARS-CoV-2. To investigate the innate immune response during breakthrough infections, we modeled breakthrough infections by challenging low-dose vaccinated mice with a vaccine-mismatched SARS-CoV-2 Beta variant. We found that low-dose vaccinated infected mice had a 2-log reduction in lung viral burden, but increased immune cell infiltration in the lung parenchyma, characterized by monocytes, monocyte-derived macrophages, and eosinophils. Single cell RNA-seq revealed viral RNA was highly associated with eosinophils that corresponded to a unique IFN-γ biased signature. Antibody-mediated depletion of eosinophils in vaccinated mice resulted in increased virus replication and dissemination in the lungs, demonstrating that eosinophils in the lungs are protective during SARS-CoV-2 breakthrough infections. These results highlight the critical role for the innate immune response in vaccine mediated protection against SARS-CoV-2.

Poster Number: 17

Regulation Of IFITM Proteins' Antiviral Activity Through Sequestration Into Intraluminal Vesicles Of Late Endosomes

Authors: Parrilla, Maria; Luthra, Deepali; and Tirouvanziam, Rabindra.

Presenting Author: Maria Parrilla, BS

Background: Adjuvants are critical determinants of the potency of vaccines. Key to their function is the capacity to induce acute stress responses in myeloid cells, including scavenger and antigen-presenting cell (APC) activities. Calcium (Ca^{2+}) and cyclic adenosine monophosphate (cAMP) are key signaling intermediates in adjuvant-exposed myeloid cells. Thus, understanding upstream and downstream signaling events associated with Ca^{2+} and cAMP signaling is essential for optimizing adjuvant-induced responses. Here, we present early findings in a human monocytic cell line engineered with Ca^{2+} and cAMP reporters as a model to study adjuvant responses.

Methods: The human THP-1 monocyte line engineered to express luciferase (Luc) under control of Ca^{2+}-driven NFAT (nuclear factor of activated T cells) or cAMP-driven CREB (cyclic AMP response element binding protein) were used. Cells were seeded in a white flat-bottom 96-well plate in a staggered pattern to prevent signal bleed-through and treated with Ca^{2+} agonist ionomycin and cAMP agonist forskolin, respectively, at various concentrations (2.5-20 μM). Luc activity (Promega Bright-Glo™ substrate) was detected using a multi-mode microplate reader (SpectraMax® iD3) and used as a quantitative readout of pathway activation at various timepoints (2-12 hrs.). Recombinant Luc (QuantiLum®) was used as a positive control, and media and cell-only controls were used for background subtraction.

Results: In NFAT-Luc and CREB-Luc expressing monocytes, signal strength, measured in relative luminescence units, showed a range of 5-6 Log10. As expected, signal strength increased with higher agonist concentrations and longer incubation periods eventually plateaueing. CREB-Luc, but not NFAT-Luc, monocytes displayed substantial RLUs at baseline, suggesting tonic activation of cAMP signaling at rest.

Conclusions and Next Steps: Our initial findings support the use of NFAT-Luc and CREB-Luc monocytes in further exploration of Ca^{2+} and cAMP signaling in tissue adaptation and stress responses. The broad signal range and ability to measure dose-responsive activation with chemical standards open broad avenues for future studies. In ongoing studies, we are migrating these cells through an airway monolayer to emulate lung APCs and testing established adjuvants and bacterial outer membrane vesicles for their Ca^{2+} and cAMP activation potential in addition to using primary patient samples.

Poster Number: 18

Regulation Of IFITM Proteins' Antiviral Activity Through Sequestration Into Intraluminal Vesicles Of Late Endosomes

Authors: Prikryl, David; and Melikian, Gregory B.

Presenting Author: David Prikryl, PhD

Background: Expression of interferon-induced transmembrane proteins (IFITMs) in cells inhibits the entry of numerous enveloped viruses. Humans encode three antiviral IFITMs - IFITM1, IFITM2, and IFITM3. The range of restricted viruses is mainly determined by the IFITMs' subcellular localization. IFITM1, which is primarily found at the cells' surface, tends to inhibit viruses that fuse with the plasma membrane. On the other hand, IFITM2 and IFITM3 concentrate in late endosomes, preventing the fusion of viruses entering from intracellular compartments. We have previously found that treatment of the cells with cyclosporine A (CsA) relieves the fusion block for the Influenza A virus, apparently by inducing rapid relocalization of IFITM1 and IFITM3 from their respective locations to the Golgi apparatus. However, challenges persist, as other groups have observed drug-induced degradation of IFITM3 protein without evident relocalization. In this study, we aim to deepen our understanding of the effect of CsA on IFITM proteins.

Methods: The majority of experiments were conducted on the adenocarcinomic human alveolar basal epithelial cell line, A549, with ectopic expression of IFITM3 or IFITM1. We utilized modified versions of the standard indirect immunostaining protocol for confocal and super-resolution microscopy.

Results: We report the existence of at least two distinct pools of IFITM3 in CsA-treated cells – one that is associated with the Golgi, and one that is getting trapped in late endosomes. The existence of these pools is apparent from distinct differences in IFITM's accessibility to antibodies against the N-terminus of this protein, depending on cell fixation and permeabilization conditions. Whereas the Golgi-localized pool is readily accessible to antibodies from the cytosol, IFITM3 in late endosomes appears to relocalize to intraluminal vesicles (ILVs). Control experiments show that such differential accessibility of the antibody epitope is not due to a CsA-mediated change in IFITM's membrane topology.
Conclusions: We conclude that loss of IFITM3's antiviral activity after CsA pretreatment is caused by its relocalization from the limiting membrane of multivesicular bodies to ILVs. A corollary to this model is that ILV-localized IFITM3 does not effectively interfere with productive viral fusion culminating in infection. This work was supported by the NIH R01 AI135806 grant to GBM.

Poster Number: 19

Inflammatory and Immune Dysfunction-Targeting Therapeutics for Unmet Clinical Needs in Children with Kawasaki Disease and Multisystem-Inflammatory Syndrome (MIS-C)

Authors: Quinones, Guadalupe; Roa, Sebastian; Reece, D. Monica; and Gavegnano, Christina

Presenting Author: Guadalupe Quinones, BS, BA

Background: Kawasaki disease (KD), an acute, self-limited vasculitis primarily affects coronary arteries in children. Multisystem Inflammatory Syndrome in Children (MIS-C) is a serious complication of SARS-CoV-2 infection. KD and MIS-C induce inflammatory signaling resulting in chronic immune activation and immune dysfunction, conferring susceptibility to opportunistic infection and coronary and circulatory comorbidities. The existing standard of care, IVIG does not alleviate all complications of these diseases, including heart failure and myocardial infarctions, underscoring the unmet clinical need for safe and potent therapies.

Methods: We developed an in vitro model of KD exposing Human Umbilical Vein Endothelial Cells (HUVEC) to a proinflammatory cytokine cocktail at physiologic concentrations: CD40-L (1ng/mL), TNF-α (2.5 ng/mL), IL-1-α (10 ng/mL), IL-1-β (10 ng/mL), and IL-18 (50 ng/mL). The following conditions were evaluated: Negative control (HUVEC media only), cocktail+0.1 uM novel therapeutic, positive control (cocktail alone). Flow cytometry quantified vascular dysfunction (CD31), a key marker of HUVEC immune dysregulation. Cytokine levels in patient samples from MIS-C serum were measured using a cytokine multiplex assay, versus aged matched healthy controls. Medians were compared with a non-parametric Mann-Whitney U test.

Results: Flow cytometry data demonstrated a statistically significant ($p<0.05$) reduction in percent positive cells in treated HUVECs versus untreated cell, with a statistically significant decrease in percent positive activated cells and an increase in inactivated cells in the treatment group. The following concentrations were significantly different across control and MISC groups for patient serum samples ($p<0.05$): IL-10 (2.73 pg/mL), IL-1-β (0.299 pg/mL), IL-6 (3.816 pg/mL), IL-8 (10.37 pg/mL), and TNF-α (1.032 pg/mL). While the TNF-α was not significant, the results were trending ($P=0.19$).

Discussion and Clinical Relevance: This novel therapeutic significantly reduces cellular immune activation in an in vitro model of KD at physiological concentrations. Human serum samples demonstrate significant immune dysregulation signatures versus healthy controls, highlighting the need for agents that can block these cytokines and restore vascular and cellular immune dysregulation. These data provide a foundation for further examination of our novel agent for restoration of immune function in KD patients.

Poster Number: 20

Elucidating changes in CPSF6 mobility and interactions in HIV-1 infected cells

Authors: Sagadiev, Sara; Raghunath, Gokul; Engelman, Alan N; and Melikian, Gregory B

Presenting Author: Sara Sagadiev, BS

Background: CPSF6 (cleavage and polyadenylation factor 6) is a well characterized nuclear host factor that is crucial for proper integration of HIV-1. CPSF6 binds HIV-1 capsid directly and mediates transport of capsid cores from nuclear envelope/pore to nuclear speckles. Nuclear speckles are membraneless organelles within the nucleoplasm that behave as biomolecular condensates and are important hubs for mRNA transcription and processing. We and others have shown that, upon HIV-1 infection, viral replication complexes and CPSF6 accumulate at nuclear speckles. However, several details about the speckle microenvironment and how it affects protein mobility/diffusion in the context of HIV-1 infection remain poorly understood. The aim of this investigation is to characterize the changes in the speckle environment upon infection and define how those changes affect mobility of CPSF6 and CPSF6-interacting proteins.

Methods: To address these gaps in the literature, we use fluorescence correlation spectroscopy (FCS), which is a powerful single-molecule technique, to determine the concentration and diffusion coefficient of CPSF6 in nuclear speckles and elsewhere in the nucleoplasm.

Results and conclusions: Our results so far indicate that CPSF6 relocated to nuclear speckles upon HIV-1 infection exhibits restricted mobility compared to diffusion in the nucleoplasm. We also found that the nuclear speckle environment does not slow down protein diffusion.

Poster Number: 21

Evaluation of IgA enzyme immunoassays to detect primary Respiratory Syncytial Virus infection in infants and young children

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Presenting Author: Ranjini Sankaranarayanan, PhD

Respiratory syncytial virus (RSV) a leading cause of acute lower respiratory infections in children under 2 years of age. Prior infection in a child is usually determined by RSV antibodies; however, in young children, persisting maternal IgG and/or exogenously administered antibodies can incorrectly indicate past RSV infection. In this study, we explored the sensitivity and specificity of four IgA antibody enzyme immunoassays (EIAs) based on RSV F, Ga, Gb proteins, or RSV lysate antigens to distinguish infection induced from persisting maternal RSV antibodies. We tested the EIAs against 62 cord blood specimens (group A), 39 plasma specimens from infants not exposed to an RSV season (group B), 102 plasma specimens from infants with a documented RSV infection (group C), and 124 plasma specimens from infants exposed to their first RSV season but without a documented RSV infection (group D). Among the two negative control groups, no group A specimens and one of the group B specimens were positive in all four IgA EIAs, giving a specificity of 100% and 97% respectively. The sensitivity of the F, Ga, Gb and Lysate IgA EIAs were 88%, 31%, 26% and 61% respectively for groups C specimens. Among the group C specimens, the RSV IgA antibody levels were significantly higher in children who were older at the time of their infection. As expected, the level of RSV IgA antibodies were lower with increased time since infection, though this difference was not statistically significant. Forty four percent of the 124 specimens in group D were positive in the RSV F IgA EIA. In conclusion, the RSV F protein IgA EIA exhibited a high level of sensitivity and specificity for detecting previous RSV infections in the presence of maternal antibodies and can help in RSV clinical trials and epidemiologic studies in young children. However, the relative rate of decline in RSV IgA antibody and detection up to 300 days after infection suggests that the presence of RSV IgA antibody in children exposed to a second RSV season will indicate previous infection but not in which season it occurred.

Poster Number: 22

Characterizing Contact Patterns of Children Under 5 Years Old: A Comparative Study across Four Low- and Middle-Income Countries

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Presenting Author: Machi Shiiba, MPH

Background: Social contact plays an important role in the transmission of infectious diseases. Young children have a recognized higher risk of severe respiratory and gastrointestinal infections. Therefore, analyzing the contact patterns of young children is critical to understanding the epidemiology of infectious diseases in this vulnerable population.

Methods: The GlobalMix study is a population-based study, aimed to characterize contact patterns across all ages in four low- and middle-income countries; in this analysis we focus on contact patterns of children under 5 years old. Contact diaries gathered characteristics of contacts of children in Guatemala (333 participants, 4162 contacts), India (348 participants, 5047 contacts), Mozambique (409 participants, 4603 contacts), and Pakistan (396 participants, 7653 contacts).

Results:The proportion of non-household contacts increased among 1- to 4-year-olds compared to those under 1 year in all countries. Children in Mozambique had a relatively higher proportion of non-household contacts. In India, most young children's contacts were with individuals aged 20 years or older, while Guatemala and Pakistan had a more even distribution across age groups. In Mozambique, over half of the contacts were with those under 20 years old. Additionally, we examined 'second-order' contacts, defined as the individuals with whom the participants' direct contacts interact. The largest proportion of second-order contacts was with those aged 20-39 years in Guatemala, India, and Pakistan, and with those aged 10-19 years in Mozambique. In a regression model examining factors influencing the number of contacts, we found that the number of non-household contacts significantly increased among 1- to 4-year-olds compared to children under 6 months old, controlling for participants' sex, study site, and household size. Additionally, being in an urban site significantly reduced the number of non-household contacts in Mozambique and Pakistan.

Conclusions: Overall, children's contact patterns vary across countries, with differing exposure to household and non-household contacts. These variations may have implications for the transmission risks of respiratory and enteric infections, shaping how these diseases spread and impact differently among young children in different countries.

Poster Number: 23

RSV pneumonia among adults hospitalized with acute respiratory infections, CHF, or COPD exacerbations

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Presenting Author: Janelle Spencer-Ramirez, BS

Background: Respiratory syncytial virus (RSV) is a common respiratory virus that can lead to severe disease in older adults and those with underlying comorbidities. The objective of this study was to describe RSV pneumonia (PNA) in hospitalized adults.

Methods: Adults ≥50 years of age hospitalized for acute respiratory illness and adults of any age hospitalized for CHF or COPD exacerbations were enrolled at two hospitals in Atlanta, GA during the 2018-2019 and 2019-2020 respiratory seasons. Nasopharyngeal and oropharyngeal swabs were collected and tested with BioFire FilmArray respiratory panels and standard-of-care results were recorded. Acute and convalescent sera were collected when possible and analyzed for seroconversion (≥four-fold increase in RSV-A/B binding antibodies). Demographic and clinical data were collected through patient questionnaire and electronic medical record abstraction. PNA was categorized as definite or probable based on chest imaging reports. Baseline characteristics were summarized using descriptive statistics, and study groups were compared using t-test, chi-square, or Fisher's exact in SAS v9.4.

Results: Of 3,142 eligible patients, 1,584 were enrolled, and 1,538 had chest imaging performed and NP/OP specimens available for analysis. Overall, 83 (5.4%) were RSV-positive, of whom 80 (96.4%) were ≥50 years of age and 25 (30.1%) had radiographic PNA. Among participants with RSV, those who had PNA had similar sociodemographic characteristics as those who did not develop PNA. In terms of comorbidities, participants with RSV PNA less commonly had baseline CHF (20.0% vs. 55.2%, p=0.004) or COPD (20.0% vs. 43.1%, p=0.05), but tended to be immunocompromised (44.0% vs. 24.1%, p=0.07) compared to RSV-positive participants without PNA. Clinical features did not differ significantly between RSV-positive participants with and without PNA, with the exception of myalgias. The most common clinical features in both groups were cough, dyspnea, and fatigue. Clinical outcomes were similar between RSV-positive participants with and without pneumonia in terms of ICU admission (20.0% vs. 17.2%, p=0.76), requirement for mechanical ventilation (8.0% vs. 15.2%, p=0.49), and death (8.0% vs. 0.0%, p=0.09).

Conclusion: Approximately one-third of adults hospitalized with RSV in our cohort developed radiographic PNA, and severe clinical outcomes were observed.

Poster Number: 24

Influence of Maternal Immune Activation and HIV Exposure on Monocyte Cytokine Profiles to Mycobacterium tuberculosis in Early Childhood

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Presenting Author: Arijita Subuddhi, PhD

Background: HIV-exposed uninfected (HEU) children are exposed in utero to HIV and antiretroviral therapy, which may impact the development of both innate and adaptive immune responses in infancy and early childhood. Monocytes are critical in the initial innate immune response to Mycobacterium tuberculosis (Mtb) and we hypothesized that maternal HIV exposure may modify monocyte cytokine production profiles in HEU children, compared with children who are not exposed to HIV.

Methods: Pregnant women with and without HIV were enrolled in Kisumu, Kenya during their 2nd or 3rd trimester of pregnancy. Plasma was collected at enrollment and evaluated for soluble markers of immune activation and inflammation using Luminex or ELISA. PBMCs from children born to enrolled women were collected at 6 weeks, 12 months, and 24 months of age. Adherent monocytes were stimulated overnight with Mtb antigens and E. coli lipopolysaccharide (LPS, positive control). Levels of IL-1β, IL-6, IL-10, IL-12p40, IL-12p70, and TNF-α were measured in supernatants by Luminex.

Results: Women with HIV had higher plasma levels of IP-10, CD14, CRP, and neopterin, compared with women without HIV, indicating residual maternal immune activation and inflammation despite ART. For both HEU and HUU children, IL-6 was the predominant cytokine produced, followed by TNF-α, upon stimulation of monocytes with Mtb Ag and LPS at each time point. There were no significant differences in cytokine levels between HEU and HUU infants to any Ag at 6 weeks and 12 months. However, at 24 months, monocytes from HEU children produced higher levels of IL-1β, IL-6, IL-10, and IL-12p40 following stimulation with LPS, and higher levels of all 6 cytokines following stimulation with Mtb Ag, compared with HUU children.

Conclusions: While monocyte cytokine profiles were similar between HEU and HUU children in early infancy, monocytes from HEU children exhibited increased cytokine production capacity at 2 years of age. These data suggest that maternal HIV exposure may be associated with differential monocyte maturation in HEU children.

Poster Number: 25

Combination cIAP and BCL-2 Inhibition Reduces Intact Reservoirs in ART-Suppressed SIV-Infected Rhesus Macaques

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Presenting Author: Benedicth Ukhuedua, PhD

Background: Despite the efficacy of antiretroviral therapy (ART) in controlling HIV replication, the persistent reservoir of latently infected CD4+ T cells remains a significant barrier to cure. Here we investigated a synergistic strategy using the cIAP-inhibitor AZD5582 and the BCL-2 inhibitor Venetoclax (VTX) to reverse latency and enhance the apoptosis-mediated clearance of reactivated infected CD4+ T cells.

Methods: Thirty SIVmac239M-infected rhesus macaques (RMs) were initiated on ART at 4 weeks post-infection (wpi). At 68 wpi, RMs were divided into three treatment groups: A) ART only, B) ART + VTX, C) ART + VTX + AZD5582. VTX was dosed at either 15 mg/kg intramuscular or 300 mg oral, with 3 cycles of 4 daily doses in groups B and C. Group C received 10 weekly intravenous infusions of AZD5582 at 0.1 mg/kg. Analytical treatment interruption (ATI) commenced 4 week post the last dose of AZD5582 infusion.

Results: Treatment with VTX caused a transient decrease in CD4+, CD8+, and CD20+ lymphocytes in Groups B and C. AZD5582 induced latency reversal with on-ART viremia >60 copies/ml in 6 of 10 RMs in Group C. Comparing pre- to post-intervention, a significant decline in intact proviral SIV DNA was observed in CD4+ T cells from peripheral blood in Group C ($p=0.0001$) and from bone marrow in Groups B ($p=0.0007$) and C ($p=0.0001$), but not in Group A control RMs. The fold change in the peripheral blood intact reservoir was significantly greater in Group C compared to Group A ($p=0.0034$). A similar reduction in intact proviral SIV DNA was not found in CD4+ T cells from lymph nodes or rectum, however. Despite the impact on reservoir size, there were no significant differences between the groups in time to rebound or viral load area under the curve post rebound after ATI.

Conclusions: The combination of AZD5582 and VTX had a favorable effect in reducing the level of blood and bone marrow CD4+ T cells with intact SIV DNA in ART-suppressed RMs. This reduction was insufficient to modulate viral rebound dynamics during ART interruption, underscoring the challenge of eliminating rebound-competent reservoirs in established SIV/HIV infections.