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PEDIATRIC RESEARCH ALLIANCE

Center for Childhood Infections & Vaccines

Hello from the new CCIV Director!

I was honored to be asked to lead the CCIV this past March. I am stepping into big shoes initially filled by Paul Spearman, then Marty Moore, and most recently by our Interim Director, Bernardo Mainou, who expertly guided the Center through a time of transition. I want to take the time to recognize Bernardo whose excitement about reoviruses is contagious (no pun intended) and who has agreed to spearhead a newly revamped Monday morning CCIV Data Club seminar series starting this Fall (stay tuned). This newsletter will continue to highlight key research contributions from Center members and we are hard at work planning the 3rd Annual CCIV Symposium (save the date: October 17th, 2018). I would also like to share with you an excerpt from the CCIV Progress Report, that describes our mission and long-term goals:

- To serve as the recognized hub in Atlanta for pediatric infectious disease and vaccine research, spanning departments and institutions, to achieve the mission of improving the lives of children. Attainment of this goal will involve linkage of scientists in the fields of immunology, virology, vaccinology, drug discovery, among other areas, to promote identity as a member of the CCIV community.
- To develop and nurture collaborations between clinical and research investigators to support bench-to-bedside research programs that exploit resources and expertise present within the Center and wider community. Synergizing expertise from clinicians and researchers will create an environment that promotes cutting-edge translational research that can directly impact patient outcomes.
- To retain exceptional faculty through endowed chairs and discretionary funds to support shared equipment, personnel, and the development of novel research avenues that will attract new extramural funding. It is essential to provide Center faculty with the resources necessary to develop new research avenues that will establish the Center as a leader in pediatric research.
- To spearhead the recruitment of scientists and clinician-scientists focused on pediatric infectious diseases and/or vaccine development to drive collaborative research within the Center. Achievement of this goal is critical for the long-term sustainability of the research enterprise.
- To identify state-of-the-art research space dedicated to Center faculty to drive collaborative research. Additional research space will enable the Center to recruit and maintain outstanding faculty and foster a research environment that foments collaboration. Identification and acquisition of philanthropic support will promote the growth and profile of the Center. The newly formed Pediatric Institute will provide a framework for fundraising to sustain and grow the mission of the Center.

As you can see, we are aiming high, but I am confident we can realize this vision with support from the Pediatric Institute and our partners at GA Tech and Morehouse as well as several key foundations. I am particularly focused on engaging CCIV members in a meaningful way with other existing programs and institutions on campus (e.g., Emory Vaccine Center, Yerkes National Primate Research Center, Emory Center for AIDS Research, Laboratory of Biochemical Pharmacology, Emory Antibiotic Resistance Center, GA Tech Center for Health and Humanitarian Systems, etc). We have an amazing group of senior and junior faculty, post-doctoral and clinical research fellows, graduate students, and research specialists all contributing to advancing knowledge about childhood infections and vaccine-mediated protection. I know you will continue to drive the mission of the Center and please continue to send news of your accomplishments to myself and Karen Kennedy. Go forth and do great science!

—Ann Chahroudi, MD, PhD
CCIV Director

Ann Chahroudi

Inside this issue:

Recent Publication Highlights

View all recent publications on CCIV's website or click here.

Single HIV-1 Imaging Reveals Progression of Infection through CA-Dependent Steps of Docking at the Nuclear Pore, Uncoating, and Nuclear Transport.

Francis AC, Melikyan GB. Cell Host Microbe. 2018 Apr 11;23(4):536-548.e6. PubMed PMID: 29649444.

The HIV-1 capsid proteins (CA) assembles into a hexagonal cone-shaped lattice surrounding the viral ribonucleoprotein complex. Upon HIV-1 entry into a host cell, the CA dissociates from the cone-shaped core through a process termed uncoating. Timely uncoating is essential for HIV-1 entry into the nucleus where the reverse transcribed viral DNA integrates into the host genome. The extent, timing and cellular location of HIV -1 uncoating remained controversial.

We developed an imaging approach that allows visualization of the single HIV-1 infection process, starting from viral fusion and core trafficking in the cytoplasm that culminates in uncoating, nuclear import and integration. In these experiments, the HIV-1 CA was visualized by incorporating a novel high-avidity CA marker, CypA-DsRed, into virions. Long-term single particle tracking in living cells revealed that CA lattice was required for core docking at the nuclear pore. By contrast, premature uncoating in the cytoplasm promoted proteasomal degradation of HIV-1. We found that productive nuclear import occurred after accelerated loss of CA at the nuclear pore. Imaging nuclear pre-integration complexes labeled with fluorescently tagged integrase revealed a striking correlation between loss of the integrase signal and productive integration manifested in expression of a reporter protein. Using loss of integrase to assess the sites of productive integration, we found that a CA mutation, which has been reported to alter the virus integration site preference, prevented transport of post-uncoating viral complexes into the nucleus.

Together our findings implicate CA in multiple steps of HIV-1 entry. CA lattice protects the viral complexes from cellular degradation machinery, mediates docking at the nuclear pore, and modulates the nuclear penetration depth, following HIV-1 uncoating at the nuclear pore.

-Submitted by the authors, please find the article here: https://www.ncbi.nlm.nih.gov/pubmed/29649444

Melikian Lab

Bacteria and bacterial envelope components enhance mammalian reovirus thermostability.

Berger AK, Yi H, Kearns DB, Mainou BA. PLoS Pathog. 2017 Dec 6;13(12):e1006768. eCollection 2017 Dec. PubMed PMID: 29211815.

Enteric viruses encounter diverse environments and microorganisms as they travel through the gastrointestinal tract to infect their hosts. Antibiotic treatment negatively impacts mammalian orthoreovirus (reovirus) infection, an enteric virus that infects most humans during childhood but seldom causes disease. It is not known how components of the host microbiota affect reovirus infection. In this study, we show that reovirus directly interacts with Gram positive and Gram negative bacteria. The interaction of reovirus with bacteria enhances viral thermostability, allowing efficient infection in the face of an environmental insult. Enhanced viral thermostability is also observed in the presence of bacterial envelope components lipopolysaccharide (LPS) and peptidoglycan (PG). Mucin, a polysaccharide found in the gastrointestinal tract, but not chitin or sucrose, also positively impacts viral thermostability, suggesting the composition of the

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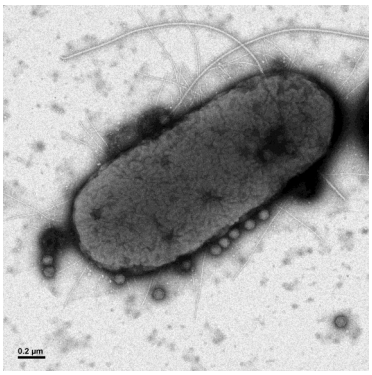
Berger et al. continued

polysaccharide determines its effect on virion stability. Interaction of reovirus with LPS or PG does not alter the ability of the virus to engage its cellular proteinaceous receptor, junctional adhesion molecule-A (JAM-A) and does not alter cell entry kinetics. Interestingly, the neutralization efficacy of reovirus-specific antibodies is not affected by the presence of LPS or PG. These data suggest that reovirus evolved to engage bacterial envelope components on the surface of bacterial cells to enhance its thermostability. The enhanced thermostability likely promotes infection in the face of changing environments encountered by the virus during fecal-oral transmission. These data also show that enteric viruses utilize the microbial rich environment of the gastrointestinal tract to promote infection and dissemination to new hosts.



-Submitted by the authors, please find the article here: https://www.ncbi.nlm.nih.gov/pubmed/29211815

Enhancing the Thermostability and Immunogenicity of a Respiratory Syncytial Virus (RSV) Live-Attenuated Vaccine by Incorporating Unique RSV Line19F Protein Residues.

Rostad CA, Stobart CC, Todd SO, Molina SA, Lee S, Blanco JCG, Moore ML. J Virol. 2018 Feb 26;92(6). pii: e01568-17. PubMed PMID: 29263264.

Christina Rostad, MD

RSV is the leading cause of lower respiratory tract infections in infants and young children, and there is no licensed vaccine available. Live-attenuated vaccines (LAVs) are currently the most advanced RSV vaccine candidates in seronegative children, but challenges associated with LAVs include achieving sufficient attenuation, immunogenicity, and thermostability. Using reverse genetics, we previously generated an RSV LAV named "DB1" that was attenuated by codon-deoptimized non-structural protein genes, deleted short hydrophobic protein gene, and by substitution of a poorly fusogenic subgroup B fusion (F) protein from the Buenos Aires clade (BAF). In this study, we sought to improve upon DB1 and boost its immunogenicity and thermostability by incorporating stabilizing residues from the line19 F protein. Previous data demonstrated that the line19 F protein conferred enhanced thermostability and enhanced stability of the highly immunogenic pre-fusion conformation of the F protein in an LAV candidate. We therefore substituted four unique line19 F residues into the BAF protein of DB1 to generate a new vaccine candidate "DB1-QUAD." We found that DB1-QUAD had significantly enhanced thermostability at 4°C compared to DB1. DB1-QUAD also had higher expression of pre-fusion F as measured by enzyme-linked immunosorbent assays (ELISAs). DB1-QUAD was attenuated in BALB/c mice, in cotton rats, and in normal human bronchial epithelial (NHBE) cells which most closely approximate attenuation levels in seronegative infants. In mice, DB1 was also highly immunogenic and generated significantly higher neutralizing antibody titers to RSV A and B compared to DB1. DB1-QUAD completely protected mice and cotton rats against intranasal challenge with wild-type RSV. Thus, substitution of unique line19F residues into RSV LAV DB1 enhanced viral thermostability, incorporation of prefusion F, and immunogenicity. This strategy could be applied to other RSV vaccine candidates in the future to fundamentally modulate properties of vaccine thermostability and immunogenicity.

-Submitted by the authors, please find the article here: https://www.ncbi.nlm.nih.gov/pubmed/29263264

CCIV Pilot Grants 2018

CCIV is excited to fund two pilots this year. Many thanks to our reviewers who gave NIH-style reviews to our nine applications. Learn about these newly funded projects in the coming pages and look out for updates in the future!

Targeting Novel Inhibition of the Enterovirus 71 RNA-dependent RNA Polymerase
Karen A. Kirby, PhD (PI) & Stefan G. Sarafianos, PhD

Vaccine Induced Serotype-specific Antipneumococcal Antibody Titers and Opsonophagocytic Ability in Hematopoietic Stem Cell Transplant Recipients
Inci Yildirim, MD, PhD, MSc (PI), Evan Anderson, MD, & Lakshmanan Krishnamurti, MD

Targeting Novel Inhibition of the Enterovirus 71 RNA-dependent RNA Polymerase

Enterovirus 71 (EV71) is a picornavirus that is an etiologic agent of Hand, Foot, and Mouth Disease (HFMD), which predominantly affects young children. In some cases, EV71 infection can result in severe neurological or cardiopulmonary effects in children, and even death. There are currently no antivirals approved for the treatment of EV71, however, a vaccine has recently been approved for use in China. While there is extensive variability among picornaviruses in terms of pathogenesis, the type of disease they cause, and the molecular differences among their structural proteins, there is remarkable conservation in some regions of the RNA-dependent RNA polymerase (RdRp), which is absolutely required in viral replication. Specifically, the Sarafianos laboratory has previously identified a highly conserved binding pocket in the RdRp of a related picornavirus, the Foot and Mouth Disease Virus (FMDV), which affects cattle and can cause wide-spread devastation to livestock populations. Preliminary inhibitors of FMDV targeting this pocket were identified through screening of a small subset (~13%) of an in-house compound library. It was also demonstrated that targeting this pocket, which is conserved among multiple picornaviruses, leads to potent antiviral activity in the FMDV picornavirus. Hence, our overarching hypothesis is that compounds that bind at this novel pocket possess broad antiviral activity against multiple picornavirus RdRps. Preliminary experiments in collaboration with the Schinazi laboratory at Emory have recently shown that two of the antivirals targeting FMDV also inhibit EV71 at low micromolar concentrations, albeit with moderate cytotoxicity in human rhabdomyosarcoma cells. This study will focus on validation of the antiviral inhibition mechanism of the preliminary compound hits in EV71, and screening for compounds with improved antiviral and cytotoxicity profiles against EV71. This study is led by PI Dr. Karen Kirby, an Assistant Professor in the Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine. She has 10 years of experience in X-ray crystallography and structural, biochemical, and biophysical studies of viral polymerases. Co-I Dr. Stefan Sarafianos is the Nahmias Schinazi Distinguished Research Chair and Professor and Associate Director of the Laboratory of Biochemical Pharmacology, Department of Pediatrics at the Emory University School of Medicine. He has more than 20 years of experience in virus structural biology, biochemistry, and virology.


Karen Kirby, PhD & Stefan Sarafianos, PhD

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CCIV Pilots continued

Vaccine Induced Serotype-specific Antipneumococcal Antibody Titers and Opsonophagocytic Ability in Hematopoietic Stem Cell Transplant Recipients

Over 13,000 hematopoietic stem cell transplants (HSCT) are performed each year to treat many refractory malignant diseases and various hematologic diseases in the United States. Infections are 1 of the 3 leading causes of mortality after HSCT [with relapse and graft versus host disease (GVHD)]. Hematopoietic stem cell transplant recipients are at a particularly high risk of invasive infections due to *Streptococcus pneumoniae*. Studies have shown a >20-30-fold increase in the rate of invasive pneumococcal disease (IPD) compared with the general population, and 1-10% of HSCT recipients will suffer a life threatening pneumococcal infection following transplantation.

Vaccination is the most important preventive strategy available against pneumococcal infections. However, HSCT recipients go through pre-transplant conditioning regimens that destroy memory T and B lymphocytes which a patient may have accumulated through prior infection or vaccination. Hence, revaccination of HSCT recipients with pneumococcal conjugated vaccine (PCV) is essential to reduce morbidity and mortality secondary to IPD after transplantation. The ability to respond to vaccines that are given in the post-HSCT period depends on the reconstitution of the immune system, and varies by underlying diagnosis, age, type of donor, stem cell source (e.g. double cord blood, conventional or T-cell depleted peripheral blood or bone marrow), onset of GVHD, and degree of immunity present before HSCT. Immunological recovery is a gradual process and may be delayed >1 year from transplantation during which HSCT recipients remain at high risk for IPD with no significant protection.

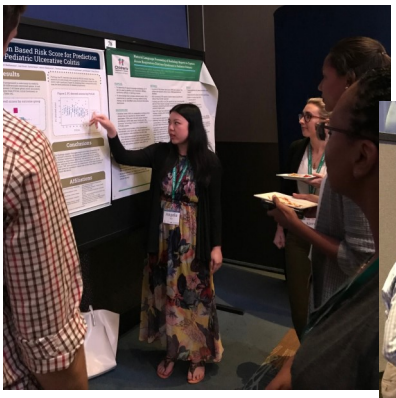
Most circulating T cells in the recipients during the first year after HSCT are graft-derived T cells that have some capacity for reacting to antigens that the donor was exposed to before transplantation. Therefore, HSCT recipients might benefit from pneumococcal serotype-specific donor T-cell responses and the memory-B-cells through adoptive immunity that could provide protection until the recipient's immune system has recovered capacity to fully respond to vaccination. In fact, seven-valent pneumococcal conjugated vaccine (PCV7) given to donors was shown to enhance early antibody responses in adult recipients suggesting that immunity was transferred to the recipient However, data for pediatric HSCT is sparse, and none exists for PCV. In addition, prior studies used antibody levels as the main surrogate of vaccine-induced protection, which may not fully reflect the functional ability of the antibodies to kill *S. pneumoniae in vivo*. Therefore, we propose a prospective cohort study to evaluate how donor antipneumococcal antibody titers and functionality impact the response to PCV13 vaccination in pediatric HSCT recipients. The results of our study will provide preliminary data to support studies of pretransplant vaccination of the donor with PCV13 to reduce pneumococcal disease.

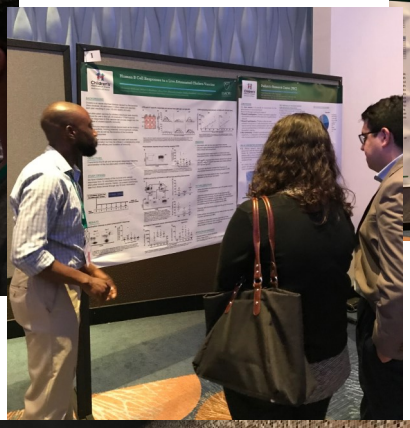


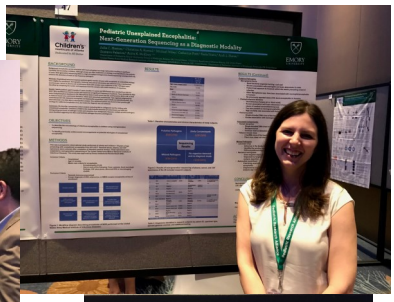
Inci Yildirim, MD, PhD, MSc, Evan Anderson, MD, & Lakshmanan Krishnamurti, MD

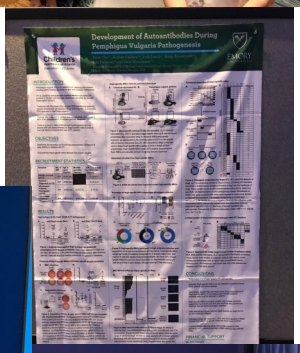
CCIV Labs Represented at Southeastern Pediatric Research Conference June 8, 2018

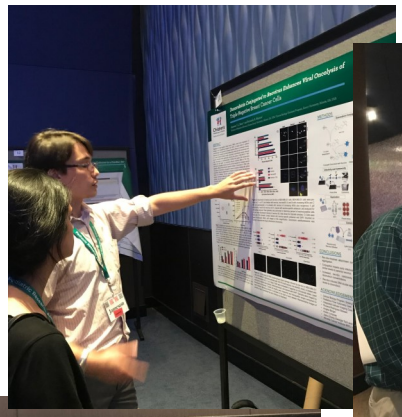
CCIV was well-represented at the 2018 Southeastern Pediatric Research Conference: Precision Medicine, held on June 8 at the Georgia Aquarium. This annual conference started as a chance for CCIV and its sister centers at Children's Healthcare of Atlanta, Emory University, and Georgia Tech to get together to showcase child health-related research. In recent years it has grown to a regional conference. Check out a recap online, including pictures and an evaluation. If you are eligible for CME, you must complete the evaluation. Below are pictures of CCIV research groups enjoying the conference. Thank you to everyone who participated and see you in 2019!

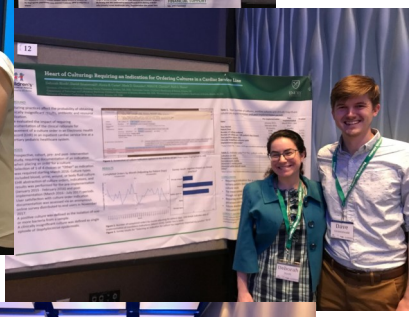


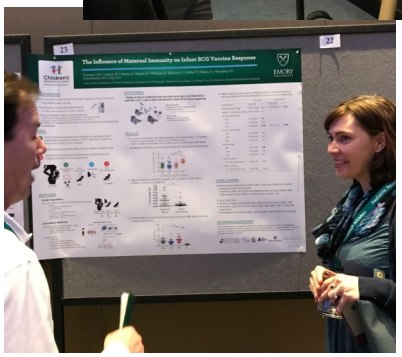


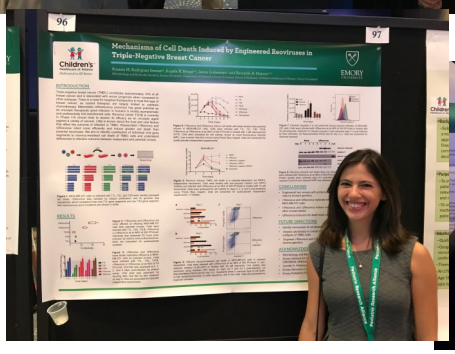


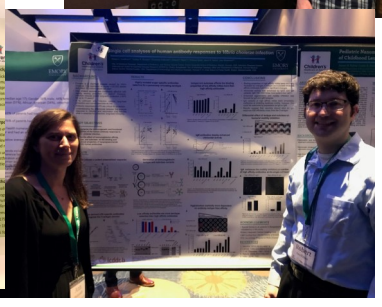












Keep in Touch

Visit our website: www.pedsresearch.org/research/centers/cciv
The CCIV website is part of www.pedsresearch.org!

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Upcoming Events

Pediatric ID Data Club Series
Will resume in Fall 2018, be on the look out for details or contact Bernardo Mainou

Save the Date
The 3rd CCIV Symposium will be on October 17. Keynote speakers will be Latonia Logan, MD & Mathias Lichterfeld, MD, and we will feature local CCIV members, too.

Archive

To see our newsletter archive, check out:
http://www.pedsresearch.org/research/centers/cciv/newsletters/

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