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EMORY+CHILDREN'S PEDIATRIC RESEARCH CENTER

December 2015

Center for Childhood Infections and Vaccines

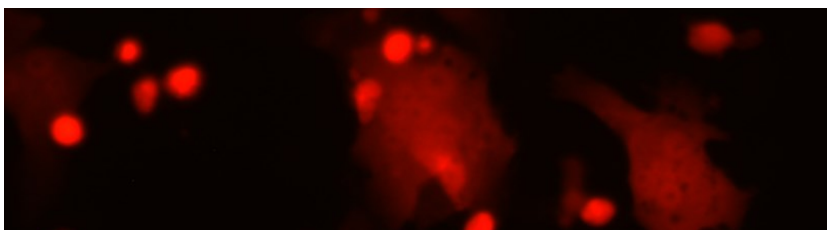
Clinical Fellow Research Highlight: Christina Rostad, MD

Christina Rostad, MD
Emory University

Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infections in infants. Although RSV causes substantial morbidity and mortality worldwide, a safe and effective vaccine is not yet available. Live attenuated vaccines (LAVs) are the safest and most advanced RSV vaccine candidates in pediatric clinical trials; however, achieving an optimal balance of vaccine attenuation and immunogenicity has proven challenging. As a fellow in Pediatric Infectious Diseases at Emory, I have worked in Dr. Martin Moore's lab to generate a novel RSV LAV candidate by reverse genetics. This vaccine candidate "DB1" balances attenuation and immunogenicity by incorporating a poorly fusogenic subgroup B fusion (F) protein. I characterized DB1 in vitro and in vivo in BALB/c mice and found that it was attenuated, highly immunogenic, broadly neutralizing, and completely protective against RSV challenge. My next step in the development of DB1 will be to introduce stabilizing mutations into the F protein to boost immunogenicity and confer thermostability to facilitate vaccine storage and distribution. This project is the subject of a Child Health Research Career Development (K12) award funded by the NICHD (Scholar = Christina Rostad / Co-mentors = Martin Moore and Paul Spearman). Our goal is to generate a successful RSV LAV which combines the features of safety, attenuation, immunogenicity, efficacy, and thermostability.

I am grateful for the opportunities I have had throughout my training to participate in research which I believe has meaningful implications for the future of child health. These opportunities date back to my undergraduate research experience at Georgia Tech with the Petit Undergraduate Research Scholars Program in Dr. Mark Prausnitz' lab, which was foundational for my pursuit of a career in science and medicine. I hope that students interested in research, regardless of their level of training, will take advantage of the unique opportunities available through our collaboration between Emory, Georgia Tech, Morehouse, and CHOA to develop new solutions to address problems affecting the health of children.

-Submitted by Christina Rostad, MD



RSV live attenuated vaccine tagged with red fluorescent protein mKate2 forming syncytia in Vero cells.

Inside this issue:

New CCIV Faculty Profile: Bernardo Mainou, PhD

Bernardo Mainou, PhD
Emory University

I have been a microbiologist at heart since I was a kid. A Santa Claus-delivered toy microscope allowed me to appreciate the complexity of small organisms like brine shrimp and insects. My interest in viruses didn't begin until I joined the laboratory of Dr. Bertram Jacobs while pursuing an undergraduate degree in microbiology at Arizona State University. Starting as a dishwasher my sophomore year and then as a member of an undergraduate research program that gave me the opportunity to have my own project in Dr. Jacobs' lab, I was smitten by how viruses, vaccinia virus in this case, hijack the cellular machinery for their own benefit. I continued my training in Dr. Nancy Raab-Traub's laboratory at the University of North Carolina at Chapel Hill where I obtained my Ph.D. based on studies aimed at figuring out how a viral oncogene of the herpesvirus Epstein-Barr virus commands cells to undergo unregulated growth. My post-doctoral training in Dr. Terry Dermody's laboratory at Vanderbilt University focused on understanding how reovirus uses cellular uptake mechanisms to enter cells. My training exposed me to different ways to approach scientific problems and gave me the opportunity to work with amazing people. My laboratory will focus on two aspects of reovirus biology: understanding how cellular factors and the microbial environment regulate the outcome of infection and identifying how small molecules that target specific cellular molecules enhance the virus-induced killing of cancer cells. We will establish 3-dimensional cell cultures using a rotating wall vessel system to create a tractable microenvironment that more closely recapitulates the setting the virus encounters in vivo. This 3D culture system will be used to identify viral and host factors that regulate the outcome of virus infection. We are interested in determining how lipopolysaccharide and peptidoglycan, components of bacterial outer membranes, and bacteria the virus would encounter in the gut affect reovirus attachment to cells and virion stability. We will use our experience with high-throughput screens to find small molecules that potentiate reovirus cell killing of cancer, but not healthy, cells. This research program will enhance our understanding of the interplay between host, the host microbiome, and pathogen and yield better tools for our fight against cancer.

Outside of the lab, I can be found hanging out with my wife Natalie Thornburg, who works at the Hope Clinic in Adult Infectious Diseases, and our son Tyler. I also like to run, play hockey, watch college basketball, hockey, and football.

-Submitted by Bernardo Mainou, PhD

Natalie Thornburg and Bernardo Mainou

CCIV Faculty Highlight: Elizabeth Wright, PhD

Elizabeth Wright, PhD
Emory University

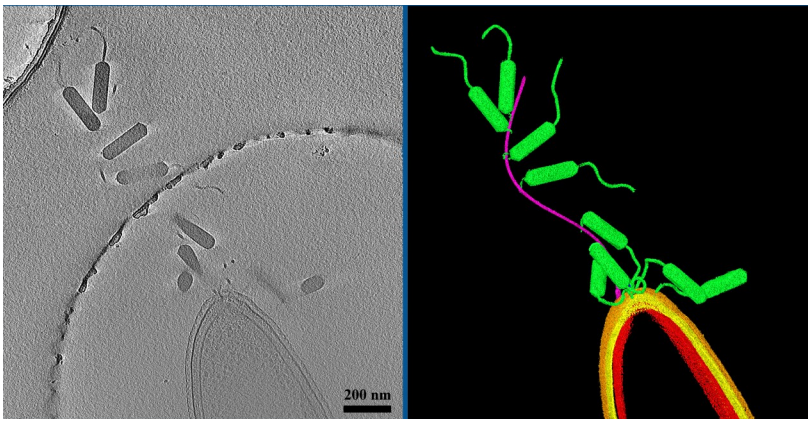
I joined the Department of Pediatrics in 2008. I am a faculty member in the Division of Infectious Diseases and am the Director of the Robert P. Apkarian Integrated Electron Microscopy Core. I have an adjunct appointment in the School of Biology at Georgia Tech and I have a secondary appointment in the Emory Department of Microbiology and Immunology. My research focuses on the use of cryo-electron microscopy (cryo-EM), cryo-electron tomography (cryo-ET), and molecular biology approaches to explore the three-dimensional (3D) structures of isolated viruses and cells and specific host-pathogen interactions. I have pioneered work on determining the 3D structure of HIV-1, measles virus, respiratory syncytial virus (RSV), and bacteria-bacteriophage interactions. I am also a major developer of cryo-EM technologies that are used to improve our capacity to address difficult biological questions, including: enhanced phase contrast through the use of Zernike and hole-free phase plates; affinity capture methods; sample preservation approaches; and cryo-correlative light electron microscopy (cryo-CLEM).

One of my long-term goals is to define the progression and spatial regulation of viral infection in a host that has a well-orchestrated developmental cycle. Caulobacter crescentus is our preferred platform because it is genetic tractability and has a well-defined, asymmetric life cycle. These characteristics allow us to monitor the relationship between the cell's regulatory pathways and viral infection. We have identified contacts made between bacteriophage ϕCbK and C. crescentus during the initial stages of infection by determining their 3D structures using cryo-ET. We are moving our cryo-ET investigations into the cell volume to examine how processes associated with bacteriophage replication are either in synchrony with the cell-cycle or exert forces that modulate the cell-cycle. Our data will be used to develop predictive C. crescentus cell-cycle models in response to bacteriophage infection to further our quantitative understanding of host-pathogen interactions.

I also have significant interest in determining the viral and cellular factors governing the replication cycle and structure of pleomorphic enveloped viruses. I have targeted two paramyxoviruses, respiratory syncytial virus (RSV) and measles virus (MeV), as model systems because both viruses severely impact the pediatric population worldwide and insufficient vaccines and antivirals are available to reduce or eliminate the global burden of infection. Publications from my group and our collaborators have reported on the 3D structure of measles virus and its glycoproteins, RNA-sensitive probe delivery for imaging of RSV in cells, the 3D structure of purified RSV particles, and the native 3D structure of RSV at sites of assembly on cells cultured on EM grids. Current and future studies target additional questions regarding fusion and entry or assembly and egress.

Outside of the laboratory, I enjoy spending time with my family, hiking, and drawing.

-Submitted by Elizabeth Wright, PhD



This is a slice from a three-dimensional (3D) tomographic reconstruction of a Caulobacter crescentus cell infected with bacteriophage phi-CbK. The bacteriophage utilize a head-filament to adsorb along the length of the bacterial flagellum. The bacteriophage then use the flagellar motility of the bacterial cell to be translocated up to the cell pole and sites of irreversible attachment. Courtesy of Rebecca Dillard in the Wright lab.

Recent Publication Highlights

Fluorescent protein-tagged Vpr dissociates from HIV-1 core after viral fusion and rapidly enters the cell nucleus

Desai TM, Marin M, Sood C, Shi J, Nawaz F, Aiken C, Melikyan GB.
Retrovirology. 2015 Oct 29;12(1):88. PMID: 26511606

Tanay Desai, PhD

HIV-1 Viral protein R (Vpr) is a multi-functional accessory protein packaged with the viral core. It is implicated in nuclear import of viral DNA, induction of cell cycle arrest, and is important for efficient viral replication in macrophages. Vpr also bears a nuclear localization signal that results in its translocation to and retention in cellular nuclei. Fluorescently-tagged Vpr incorporated into viral particles has been used to follow the fate of post-fusion HIV-1 cores as they traverse the cytoplasm. In this study we employed fluorescence confocal and deconvolution microscopy to extensively track fluorescent-Vpr labeled viruses in living cells prior to and well beyond their fusion step, and found that Vpr is rapidly shed from cytosolic viral cores within minutes after fusion. Vpr shedding was found to be independent of the viral envelope glycoprotein, capsid protein stability or target cell-type, and did not occur as a result of viral degradation within the cell. We observed that Vpr shed from fused viral cores rapidly trafficked to the cell nucleus, where it existed in a monomeric state and also as part of very large complexes. These results imply that the bulk of Vpr shedding may precede capsid uncoating, which is known to occur on a longer time-scale. Although further studies are needed to delineate the possible role(s) of Vpr shedding, the present study raises the possibility that this protein can modulate the early steps of HIV-1 entry.

-Submitted by the authors, please find the article here: http://www.ncbi.nlm.nih.gov/pubmed/26511606

Association between mixed rotavirus vaccination types of infants and rotavirus acute gastroenteritis

Mohammed A, Immergluck L, Parker TC, Jain S, Leong T, Anderson EJ, Jerris RC.
Vaccine. 2015 Oct 13;33(42):5670-7. PMID: 26322843

Anaam Mohammed

Rotavirus is the leading cause of severe diarrhea in children under five years old worldwide. There are currently two rotavirus vaccines available in the US, Rotarix® (RV1) and RotaTeq® (RV5). Studies have evaluated the impact of RV1 or RV5 on rotavirus disease, but little is known about the impact of incomplete or mixed vaccination upon vaccine effectiveness. We conducted a case-control study to examine the association of combined RV1 and RV5 and rotavirus acute gastroenteritis (AGE), factoring severity of diarrheal disease. 1,127 children with AGE from all three Children's Healthcare of Atlanta hospitals were approached for enrollment over the course of three rotavirus seasons. Parents of the 708 enrolled children completed a questionnaire, a stool specimen was collected, and patients' vaccination records were obtained. We found that children >12 months of age were more likely to have rotavirus than younger children. Complete rotavirus vaccination with a single vaccine type resulted in protection against rotavirus diarrhea and a decrease in severity of rotavirus gastroenteritis. Our findings also suggest that incomplete rotavirus vaccination, either with a single vaccine or mixed vaccination types, provide some protection against rotavirus disease.

-Submitted by the authors, please find the article here: http://www.ncbi.nlm.nih.gov/pubmed/26322843

Recent Publications by CCIV Members

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Continued on the next page

Recent Publications continued

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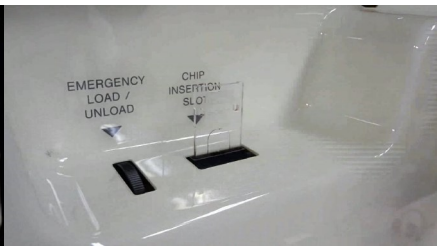
Note: If you have a publication that you would like included in the next CCIV newsletter, please contact kmurra5@emory.edu

Coming soon to the Pediatric Flow Cytometry Core: Sony SH800 Cell Sorter

The Emory+Children's Flow Cytometry Core is pleased to announce the arrival of the new Sony SH800 Cell Sorter in early December. This 6-color, 4-laser (405 nm, 488 nm, 561 nm and 638 nm) instrument will provide users with state of the art microfluidic chip sorting into tubes or 96/384 well plates. It has fully automated adjustment of the laser beam and drop delay. The 6 freeform PMTs can be adjusted to detect fluorescence signals from any laser based on the filter selection. Importantly, once trained, users can setup the instrument easily without the need of a specialist operator or core staff and the instrument will be available 24/7.

The flow cytometry core would like to thank the VTEU and GRA for their generous support in purchasing this instrument.



Images from: http://www.sonybiotechnology.com/sh800_overview.php
Left to right: Chip nozzle package, insertion slot, front view of machine

Recent Funding Awards to CCIV Members

Investigator	Title	Sponsor
Evan Anderson	R2222-RSV-1332: A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study	REGENERON PHARMACEUTICALS
Larry Anderson	Herpes Simplex Virus Type 1 and Type 2 Antibody Testing for NHANES 2012-2017	CENTERS FOR DISEASE CONTROL
Larry Anderson	IPA for Larry J. Anderson	CENTERS FOR DISEASE CONTROL
Anita McElroy	Defining the roles of CD4+ T cells in generating a protective immune response against Rift Valley fever virus	BURROUGHS WELLCOME FUND
Martin Moore	Host and viral determinants of infant and childhood allergy and asthma	VANDERBILT UNIVERSITY
Mark Mulligan	VTEU 15-0038 Chickungunya (MV-CHIK) Task Areas B-C	NIH NATL INST OF ALLERGY AND INFECTIOUS
Mehul Suthar	RIG-I-like receptor regulation of T cell immunity against flavivirus infection	UNIVERSITY OF WASHINGTON SEATTLE

Note: If you have been awarded a grant that you would like included in the next CCIV newsletter, email kmurra5@emory.edu

Keep in Touch

Visit our website: www.pedsresearch.org/centers/detail/immunology-vaccines

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Emory+Children's Pediatric Research Center
An Atlanta-based research alliance
Children's Healthcare of Atlanta | Emory University | Georgia Institute of Technology | Morehouse School of Medicine

Upcoming Events

Pediatric ID Seminar Series

Meets each Thursday at 1 pm in the Emory-Children's Center Room 202

December 10: Elizabeth Wright
December 17: Rania Chirkova (L. Anderson Lab) and Junghwa Choi (Spearman Lab)
December 24 and 31: No meeting, happy holidays!
January 7: Sarah Takushi (Spearman Lab) and Thayer King (Lamb Lab)
January 14: TBA
January 21: Zhengde Xie, visiting from Beijing
January 28: TBA
February 4: Wah Chiu, visiting from Baylor College of Medicine
HSRB Auditorium