



NORTHEAST
BIODEFENSE

C E N T E R

NIAID Region II Center of Excellence for Biodefense and Emerging Infectious Diseases



Desmond Tutu Conference Center
New York, NY
November 1-3, 2011

NBC Seventh Annual Meeting

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NBC Seventh Annual Meeting Agenda

Desmond Tutu Conference Center
New York, New York

TUESDAY NOVEMBER 1

6:00 – 8:00 p.m. **Keynote Event**
Private screening of CONTAGION
Q & A with **W. Ian Lipkin** directly after the film
Tribeca Screening Room
375 Greenwich St., New York, NY

WEDNESDAY, NOVEMBER 2

7:00 – 9:00 a.m. **Continental Breakfast: First floor meeting area**
Meeting Registration: First floor meeting area

8:30 – 8:35 a.m. **W. Ian Lipkin** (Columbia University)
Welcome / Introductory Remarks
Matthews Room

8:35 a.m. – 9:55 a.m. **Plenary Session I: Vaccines**
Matthews Room
Moderator: Rafi Ahmed

8:55 – 9:15 a.m. **Jack Rose** (Yale University)
Vaccine development for Chikungunya and Nipah viruses

9:15 – 9:35 a.m. **Peter Palese** (Mt. Sinai School of Medicine)
Toward a universal Influenza virus vaccine

9:35 – 9:55 p.m. **Charles Rice** (The Rockefeller University)
An overexpression screen to identify antiviral interferon effectors against a broad panel of diverse viruses

9:55 – 10:20 a.m. **Break**

10:20 – 11:20 p.m. **Plenary Session II: Diagnostics and Therapeutics**
Matthews Room
Moderator: Adolfo Garcia-Sastre

10:20 – 10:40 a.m.. **W. Ian Lipkin** (Columbia University)
Microbe hunting, Stardate 2011

- 10:40 – 11:00 a.m **Matteo Porotto** (Weill Medical College)
Antiviral platforms to prevent viral entry
- 11:00 – 11:20 a.m. **Margaret Kielian** (Albert Einstein School of Medicine)
Mechanism and inhibition of alphavirus and flavivirus membrane fusion proteins
- 11:20a.m. – 12:05p.m. **Breakout Session I: Vaccines**
Rusack Room
Discussion Leader: Peter Palese
Panel: Palese, Rose, Rice
- Breakout Session II: Diagnostics and Therapeutics**
Matthews Room
Discussion Leader: W. Ian Lipkin
Panel: Lipkin, Porotto, Kielian
- 12:05 – 2:00 p.m **Lunch / Free Time**
Refectory
- 2:00 a.m. – 3:40 a.m. **Plenary Session III: Passive Immunotherapy**
Matthews Room
Moderator: Arturo Casadevall
- 2:00 – 2:20 p.m. **Thomas Briese** (Columbia University)
Development of fhmAb therapeutics against viral biothreat and emerging disease agents
- 2:20 – 2:40 p.m. **Rafi Ahmed** (Emory University)
Understanding the human B cell response to influenza virus: A first step towards making a universal flu vaccine
- 2:40 – 3:00 p.m. **Bettina Fries** (Albert Einstein School of Medicine)
Staphylococcal enterotoxin B (SEB) specific monoclonal antibody enhances survival in murine models
- 3:00 – 3:20 p.m. **Arturo Casadevall** (Albert Einstein School of Medicine)
Update on passive antibody efforts against *Bacillus anthracis*
- 3:20 a.m. – 3:40 p.m. **James Bliska** (Stony Brook University)
Development of mAb immunotherapy for genetically modified plague
- 3:40 – 4:00 p.m. **Break**

- 4:00 – 4:40 p.m.** **Plenary Session IV: Shared Core Resources**
Matthews Room
Moderator: Thomas Briese
- 4:00 – 4:10 p.m. **David Perlin (UMDNJ)**
Northeast Biodefense Center's small animal core
- 4:10 – 4:20 p.m. **Karen Chave (Wadsworth Center)**
Northeast Biodefense Center protein synthesis core (*E. coli*)
- 4:20 – 4:30 p.m. **Steve Almo (Albert Einstein School of Medicine)**
Gene-to-structure pipeline for biodefense and emerging infectious diseases
- 4:30 – 4:40 p.m. **Erol Gulcicek (Yale University)**
Northeast Biodefense Center proteomics core
- 4:45 – 5:30 p.m.** **Breakout Session III: Passive Immunotherapy**
Matthews Room
Discussion Leader: Arturo Casadevall
Panel: Ahmed, Fries, Bliska, Briese, Casadevall
- Breakout Session IV: Shared Core Resources**
Rusack Room
Discussion Leader: David Perlin
Panel: Perlin, Chave, Almo, Gulcicek
- 5:30 – 6:30 p.m.** **Poster Session and Reception**
Refectory

THURSDAY, NOVEMBER 3

- 7:30 – 8:30 a.m.** **Continental Breakfast: First floor meeting area**
Meeting Registration: First floor meeting area
- 8:30. – 9:30 a.m.** **Plenary Session V: Developmental Projects and Career Development**
Matthews Room
Moderator: Erich Mackow
- 8:30 – 8:50 a.m. **Jorge Benach (Stony Brook University)**
The splenic response to *Francisella tularensis*
- 8:50 – 9:10 a.m. **Irene Jarchum (Memorial Sloan Kettering Institute)**

Critical role of MyD88 and neutrophil recruitment for protection against *C. difficile* colitis

- 9:10 – 9:30 a.m. **Felicia Santa Maria** (NYU School of Medicine)
PKR and mda5 are required for IRF3 nuclear translocation and IFN induction in response to vaccinia virus
- 9:30 a.m. – 11:10 p.m. Plenary Session VI: Microbial Pathogenesis and Innate Immunity**
Matthews Room
Moderator: Sankar Ghosh
- 9:30 – 9:50 a.m. **Craig Roy** (Yale University)
A genetics-squared approach to understanding Coxiella burnetii intracellular replication
- 9:50 – 10:10 a.m. **Sean Brady** (Rockefeller University)
The discovery of new small molecule antibiotics and potential virulence factors relevant to NBC related pathogens
- 10:10 – 10:30 a.m. **David Levy** (NYU School of Medicine)
HDAC-sensitive transcriptional elongation checkpoint during interferon-stimulated gene expression
- 10:30 – 10:50 a.m. **Christopher Basler** (Mt. Sinai School of Medicine)
Modulation of Ebola virus polymerase expression as an antiviral strategy
- 10:50 – 11:10 a.m. **Adolfo Garcia-Sastre** (Mt. Sinai School of Medicine)
The protease of Crimean-Congo hemorrhagic fever virus as a potential antiviral target
- 11:10 – 11:30 a.m. Break**
- 11:30 – 12:15 a.m. **Breakout Session V: Microbial Pathogenesis and Innate Immunity**
Matthews Room
Discussion Leader: Adolfo Garcia-Sastre
Panel: Roy, Brady, Levy, Basler, Garcia-Sastre
- 12:15 p.m. Closing Remarks/Adjournment**
- 12:15 – 2:00 p.m. Lunch**

Refectory

12:10 – 2:00 p.m.

Scientific Advisory Board Meeting (closed session)
Rusack Room

About the NBC

The Northeast Biodefense Center (NBC) is a consortium of academic and governmental biomedical research institutions from New York State, New Jersey, Connecticut, Puerto Rico, and the U.S. Virgin Islands. Along with its strategic partners from other states as well as industry, the NBC, which was established in 2003, is funded by a multi-year federal grant to promote the advancement of scientific research on biodefense and emerging infectious disease.

NBC Participating Institutions

Any institution within the region has access to NBC Cores, Funding Opportunities, and Meetings. Institutions currently participating in the NBC include:

- Albert Einstein College of Medicine of Yeshiva University
- Columbia University
- Cornell University
- Emory University
- Mount Sinai School of Medicine
- New York University
- Memorial Sloan Kettering Cancer Center
- Stony Brook University
- The Rockefeller University
- University of Chicago
- University of Medicine and Dentistry of New Jersey (Public Health Research Institute)
- Wadsworth Center/New York State Department of Health
- Yale University

NBC Steering Committee

W. Ian Lipkin (Columbia), *Ex officio*

Jorge Benach (Stony Brook)

Arturo Casadevall (Einstein)

Anne Moscona (Cornell)

Sankar Ghosh (Yale)

David Levy (NYU)

David Perlin (UMDNJ)

Peter Palese (Sinai)

Charlie Rice (Rockefeller)

Jack Rose (Yale)

Larry Sturman (Wadsworth)

NBC Scientific Advisory Board

Roy Curtiss III

Roy Curtiss III is Professor of Genomics, Evolution and Bioinformatics at The Biodesign Institute and the Director of the Center for Infectious Diseases and Vaccinology at Arizona State University. He was elected to the National Academy of Sciences in 2001. His research has encompassed many aspects of *Salmonella* genetics. Currently, he has been focusing on developing Salmonella as a vehicle to deliver DNA vaccines for *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Clostridium perfringens*, *Yersinia pestis* and many other priority pathogens

Bernard Roizman

Bernard Roizman is the Joseph Regenstein Distinguished Service Professor of virology at the University of Chicago. His research interests are in the molecular biology and pathogenesis of herpes simplex viruses. He also conducts research on the development of targeted oncolytic herpes simplex viruses for treatment of malignant glioblastoma. He serves on numerous editorial boards and also served on study sections and numerous panels of NIH, American Cancer Society, etc. He is an elected member of the National Academy of Sciences, Institute of Medicine, American Academy of Arts and Sciences, American Association for the Advancement of Science, American Academy of Microbiology, Chinese Academy of Engineering (Foreign member) and Hungarian Academy of Science (Honorary Member). He received honorary degrees from US, France, Italy and Spain. He is the recipient of the ICN International Prize in Virology, 1988; J. Allyn Taylor International Prize in Medicine, 1997; Bristol-Myers Squibb Award for Distinguished Achievement in Infectious Disease Research, 1998 and Abbott-ASM Lifetime Achievement Award, 2008. He is the author or co-author of more than 600 articles dealing mainly with the molecular biology of herpes simplex viruses.

Connie Schmaljohn

Connie Schmaljohn is the Chief Scientist at United States Army Medical Research Institute of Infectious Diseases (USAMRIID). She directs the vaccine program for biodefense threat agents, including hantavirus, filovirus, anthrax, VEE and smallpox. She is an expert in hemorrhagic fevers, viral pathogenesis and vaccine development. In 2002, she was awarded the Joel M. Dalrymple award from the Association of Military Surgeons of the United States for her significant contributions to the development of modern biological defense vaccines.

Richard Whitley

Richard Whitley is a University of Alabama Birmingham (UAB) Distinguished Professor, Professor of Pediatrics, Microbiology, Medicine and Neurosurgery, as well as the Loeb Eminent Scholar Chair in Pediatrics. He directs the Division of Pediatric Infectious Diseases and is also the Vice-Chair of the Department of Pediatrics. He co-directs the merged UAB Center for Emerging Infections and Emergency Preparedness and is heavily involved in activities that create awareness of and develop strategies for dealing with pandemic influenza. Dr. Whitley is responsible for the National Institute of Allergy

and Infectious Diseases Collaborative Antiviral Study Group whose role is to perform clinical trials of antiviral therapies directed against medically important viral diseases of children and adults. Dr. Whitley's other research interest is in the translation of molecular biology to clinical application, particularly in the development of human monoclonal antibodies for therapy and engineering of herpes simplex virus for gene therapy.

His research focuses on three areas. First, he directs studies of antiviral agents for unmet medical needs, including herpes simplex encephalitis, neonatal herpes simplex virus infection, congenital cytomegalovirus infection, West Nile Virus encephalitis and influenza in infants, among others. Each clinical trial incorporates natural history of disease, diagnosis and risk factors. Second, he is responsible for the development of antiviral drugs to treat orthopox virus infections. These studies involve coordinating the team efforts of crystallographers, *in vitro* and *in vivo* antiviral testing and iterative medicinal chemistry. Lastly, he is the principal investigator on a program project grant that engineers herpes simplex virus to treat brain tumors.

Keynote Presentation



Biography:

W. Ian Lipkin, MD, is the Director, Center for Infection and Immunity, the John Snow Professor of Epidemiology at the Mailman School of Public Health, and Professor of Neurology and Pathology at the College of Physicians and Surgeons, Columbia University. He is the co-chair of the National Biosurveillance Subcommittee and the director of the World Health Organization Collaborating Centre for Diagnostics in Zoonotic and Emerging Infectious Diseases. He earned his medical degree from Rush University in 1978. Following medical residencies in Internal Medicine and Neurology, he did a research fellowship in neurovirology at The Scripps Research Institute in the laboratory of Michael Oldstone.

Dr. Lipkin is internationally recognized as an authority on the use of molecular methods for pathogen discovery. In the 1980s, Dr. Lipkin identified AIDS-associated immunological abnormalities and inflammatory neuropathy, which he showed could be treated with plasmapheresis, and demonstrated that early life exposure to viral infections affects neurotransmitter function. He was the first to use purely molecular methods to identify infectious agents, implicated West Nile virus as the cause of the encephalitis epidemic in New York in 1999, assisted the WHO and the Peoples Republic of China during the 2003 SARS outbreak, developed MassTag PCR and Greenchip technology and pioneered the use of high throughput sequencing in pathogen discovery. He and his team have discovered or characterized more than 400 infectious agents including Borna disease virus, West Nile virus, LuJo virus, human rhinovirus C, piscine reovirus, canine hepacivirus, and Lloviu virus.

Dr. Lipkin has been featured by the New York Times, the Los Angeles Times, Discover Magazine, Nature Medicine, the History Channel, National Geographic, National Public Radio, Wired, and the Huffington Post.

Dr. Lipkin served as a scientific advisor during the filming of the newly released Steven Soderbergh film CONTAGION. The plot of the movie is centered on an outbreak event of a lethal virus that rapidly progresses to a pandemic. Dr. Lipkin will share his experiences during the pre-, post-, and post-post production of the movie.

Speaker Abstracts

- 9:05 a.m. – 9:55 a.m.** **Plenary Session I: Vaccines**
Matthews Room
Moderator: Rafi Ahmed
- 9:05 – 9:25 a.m.** **Jack Rose (Yale University)**
Vaccine Development for Chikungunya and Nipah Viruses
- 9:25 – 9:55 a.m.** **Peter Palese (Mt. Sinai School of Medicine)**
Toward a Universal Influenza Virus Vaccine
- 9:55 – 10:15 p.m.** **Charles Rice (The Rockefeller University)**
An overexpression screen to identify antiviral interferon effectors against a broad panel of diverse viruses

Vaccine Development for Chikungunya and Nipah Viruses

Anasuya Chattopadhyay and **John K. Rose***

Department of Pathology, Yale University School of Medicine, New Haven, CT

Experimental vaccines based on recombinant vesicular stomatitis virus vectors have been highly effective in animal models for numerous viral and bacterial diseases. These include animal models for HIV, influenza, Ebola, and *Yersinia pestis*. Enrollment for a clinical trial of FDA-approved, live-attenuated VSV/HIV vectors is now underway at multiple sites of the HIV Vaccine Trials Network (HVTN). Last year we reported the development of replication-competent and replication-defective VSV-based vectors expressing the Nipah virus surface glycoproteins, F and G. We showed in a mouse model that these recombinants induced high levels of antibodies capable of neutralizing VSV Δ G/Nipah pseudotypes. These recombinants will be tested in collaboration with the U.S. Centers for Disease Control and Prevention (CDC) in an animal model of lethal Nipah virus infection. The second experimental vaccine being studied is for chikungunya virus (CHIKV). CHIKV is transmitted from mosquitoes to humans and causes a severe crippling arthralgia. In more recent outbreaks it has been reported to also cause encephalopathy and hemorrhagic fever. To develop an experimental vaccine for CHIKV we designed a codon-optimized gene encoding the CHIKV E3-E2-6K-E1 polyprotein. We incorporated this gene initially into a replication-competent VSV-based vaccine vector. We also prepared a Δ G version of this vector. Expression of the E1 and E2 proteins was verified by metabolic labeling of vector-infected cells with [³⁵S]-methionine followed by SDS-PAGE. We also showed, using indirect immunofluorescence microscopy, that CHIKV proteins were expressed on the surface of VSV/CHIKV infected cells. When mice were infected with the VSV/CHIKV vector, they generated neutralizing antibody to CHIKV as measured in a VSV Δ G/CHIKV pseudotype neutralization assay developed in our laboratory. We are testing the efficacy of the VSV/CHIKV vaccine in collaboration with Dr. Scott Weaver's laboratory at the University of Texas Medical Branch, Galveston, Texas.

Toward a universal influenza virus vaccine

Peter Palese*

Department of Microbiology, Mount Sinai School of Medicine, New York, NY

Current influenza virus vaccines are effective when well matched to the circulating strains, but they have clear limitations and improvements are needed. First, specific populations (e.g. the elderly) are not well served by the present vaccine formulations, and vaccines inducing an enhanced protective immune response are needed. Second, yearly administration of vaccines is cumbersome and costly, and could be avoided by the availability of cross-protective (universal) influenza virus vaccines. The identification of cross-protective monoclonal antibodies from patients, which recognize conserved stalk domains of the influenza virus, has suggested that stalk-based vaccines might be possible. Also, we succeeded in specifically inducing in the mouse such broadly cross-protective antibodies by immunizing the animals with hemagglutinins from diverse antigenic variants (that had conserved stalk domains). Finally, based on the concept that seasonal H1 strains are extinguished when a novel H1 (pandemic) strain appears, we propose novel universal influenza vaccine candidates comprising the conserved hemagglutinin stalk domain lacking the globular head (headless hemagglutinin) and peptide immunogens which induce cross-protective responses against the long alpha helix (LAH) in the conserved hemagglutinin stalk.

An overexpression screen to identify antiviral interferon effectors against a broad panel of diverse viruses

John W. Schoggins¹, Mary Y. Murphy¹, Maryline Panis¹, Stephane Pouzol¹, Naoko Imanaka¹, Michael Faelner¹, Balaji Manicassamy², Adolfo Garcia-Sastre², **Charles M. Rice¹**

¹Laboratory of Virology and Infectious Disease, The Rockefeller University, New York, NY; ²Department of Microbiology and Immunology, Mt. Sinai School of Medicine, New York, NY

The cellular type I interferon (IFN) response protects cells from invading viral pathogens by triggering the transcription of interferon-stimulated genes (ISGs). Although hundreds of ISGs have been identified, relatively few have been characterized with respect to antiviral activity. For most, little is known about their antiviral potential, their target specificity, and their mechanisms of action. We have established an overexpression screening platform to evaluate the antiviral potential of more than 380 ISGs against a diverse panel of animal viruses spanning the viral phylogeny. The first round of screening included four category A-C positive-stranded RNA viruses from the *Flaviviridae* and *Togaviridae* families. We identified and confirmed that more than 25 ISGs had antiviral activity against one or more of these viruses. Mechanistic studies revealed that several of these effectors target viral translation as a primary mode of action. We are currently expanding the ISG screens to more than 30 RNA and DNA animal viruses, including several priority pathogens from the *Orthomyxoviridae*, *Bunyaviridae*, *Filoviridae* and *Arenaviridae* families. Functional clustering of ISG specificity indicates that IFN responses may have evolved to use similar mechanisms to inhibit replication of phylogenetically related viruses. Future studies will include genetic and biochemical approaches to pinpoint ISG effector mechanisms of action and systems biology approaches to characterize putative ISG-mediated cellular antiviral networks. Insight into specific and global ISG effector functions may provide a platform for the development of novel antiviral therapies.

- 10:40 – 11:40 p.m.** **Plenary Session II: Diagnostics and Therapeutics**
Matthews Room
Moderator: Adolfo Garcia-Sastre
- 10:40 – 11:00 a.m.** **W. Ian Lipkin (Columbia University)**
Microbe Hunting, Stardate 2011
- 11:00 – 11:20 a.m.** **Matteo Porotto (Weill Medical College)**
Antiviral platforms to prevent viral entry
- 11:20 – 11:40 a.m.** **Margaret Kielian (Albert Einstein School of Medicine)**
**Mechanism and inhibition of alphavirus and flavivirus
membrane fusion proteins**

Microbe hunting, Stardate 2011

W. Ian Lipkin*

Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, New York.

Recent advances in nucleic acid diagnostic methods have revolutionized microbiology by facilitating rapid, sensitive microbial surveillance and differential diagnosis of infectious diseases. Use of these methods in clinical medicine may enable intervention when the prognosis is optimal for limiting replication, dissemination, transmission, morbidity and mortality. This project focuses on development and implementation of a staged strategy for surveillance and discovery in meningoencephalitides, hemorrhagic fevers, respiratory and enteric diseases and unexplained febrile illnesses. Our work is pursued in a global context and surveys not only humans but also domestic animals, wildlife and phlebotomous insects that may serve as reservoirs or vectors for transmission of high consequence pathogens.

Antiviral platforms to prevent viral entry

Matteo Porotto^{1*}, Laura M. Palermo¹, Shohreh Farzan¹, Aparna Talekar¹, Kelly K. Lee², Antonello Pessi³, Branka Horvat⁴ and Anne Moscona¹

¹Departments of Pediatrics and of Microbiology and Immunology, Weill Medical College of Cornell University, New York, NY 10021. ²Department of Medicinal Chemistry and Biomolecular Structure and Design Program, University of Washington, Seattle.

³PeptiPharma, Rome, Italy. ⁴Inserm, Lyon, France.

Nipah (NiV) and Hendra (HeV) viruses are emerging zoonotic paramyxoviruses that cause encephalitis in humans, with fatality rates of up to 75%. Paramyxoviruses fuse at the cell surface, at neutral pH, when the receptor binding protein engages receptor and “triggers” the fusion protein (F) to assume its fusion-ready conformation with the fusion domain exposed. For HeV/NiV, the attachment of G to receptor, and G’s activation of F, is required in order for F to mediate fusion. While activation of F is key for entry, the correct timing is critical; triggering must occur only when F is in proximity to the target cell membrane. We have proposed that this timing of activation represents a target for intervention, and that receptor mimics may induce triggering and inactivate F prematurely, rendering virus noninfectious, and halting spread. The concept was explored using synthetic cells (protocells) bearing NiV receptor molecules; these inactivate HeV/NiV pseudovirions when incubated at 37°C but not at 4°C, implying an energy-requiring step. After screening small compound libraries for molecules that would induce paramyxovirus receptor binding proteins to pre-trigger F, we have identified several compounds, one of which inactivates three paramyxoviruses (NiV, HeV and parainfluenza 3; HPIV3) but not VSV or SV5. Mechanistic studies reveal that this compound inactivates viruses by inducing a conformational change in F consistent with the post-triggered state, like that after receptor engagement by the receptor binding protein. The viruses are intact but rendered non-infectious.

A second antiviral approach complements the pre-triggering strategy by targeting the transitional intermediate stage of F after F’s activation and insertion into the target membrane. Peptides derived from C-terminal heptad repeats (HRC) in F have been shown to inhibit fusion by preventing F’s final folding that drives membrane merger. We recently showed that the addition of a cholesterol group to anti-NiV HRC peptides targets these peptides to the membrane where fusion occurs, dramatically increasing their antiviral effect. Cholesterol tagging renders the peptides active *in vivo*: cholesterol-tagged peptides effectively prevent and treat in an established animal model what would otherwise be fatal NiV encephalitis. This *in vivo* efficacy suggests that these peptides are promising candidates for the prevention or therapy of infection by NiV and other lethal paramyxoviruses. We hypothesized that cholesterol moiety allows the peptides to follow the virus to the site of fusion also for viruses that fuse inside the cell. We present here proof of concept for the applicability of our antiviral strategy to intracellularly-fusing viruses, including known and emerging viral pathogens.

Mechanism and inhibition of alphavirus and flavivirus membrane fusion proteins.

Margaret Kielian^{1*}, Aihua Zheng, and Claudia Sánchez San Martín
Albert Einstein College of Medicine

Flaviviruses and alphaviruses are spread by mosquito and tick vectors and cause severe human and animal illnesses such as encephalitis and hemorrhagic fever. These viruses include many potential bioterrorist agents that are category A-C pathogens, such as the flavivirus dengue virus (DENV) and the alphavirus Chikungunya virus (CV). Our studies aim to discover novel antiviral therapies for DENV (category A) and CV (category C).

The flavivirus and alphavirus membrane fusion proteins are structurally similar elongated three-domain molecules that refold to a homotrimer form to mediate virus fusion and infection. We developed recombinant forms of domain III (DIII) from the alphaviruses Semliki Forest virus (SFV) and CV, and DENV 2. These recombinant DIII proteins act as specific dominant-negative inhibitors of virus fusion and infection. We also developed an expression system in *Drosophila* cells to efficiently produce alphavirus E1 and flavivirus E membrane fusion proteins with all 3 domains or a truncated form containing only DI/II. We used this system to develop core trimers that act as specific targets for DIII binding, thus recapitulating *in vitro* the refolding of the fusion protein that takes place during virus fusion and infection. Our recent studies of CV showed that SFV and CV DIII inhibit SFV or CV fusion and infection, and that CV DIII binds to the core trimer of SFV. This cross-inhibition indicates that the DIII-core trimer interactions are highly conserved, suggesting their potential as broad-specificity inhibitor targets.

We have recently developed a fluorescence polarization-based *in vitro* assay for the binding of DIII to the core trimer of SFV. We have optimized the assay extensively during the past grant period, and are now initiating the use of these assay in high throughput screens of small molecule libraries. Hits will be validated by tests of virus fusion and infection, and ultimately, small molecule inhibitors will be lead compounds for antiviral therapy, and important research tools to understand the virus fusion reaction.

Flaviviruses bud into the endoplasmic reticulum and are transported through the secretory pathway, where the mildly acidic environment triggers particle rearrangement and allows furin processing of the prM protein to pr and M. The peripheral pr peptide remains bound to virus at low pH and inhibits virus-membrane interaction. Upon exocytosis, the release of pr at neutral pH completes virus maturation to an infectious particle. We developed an *in vitro* system to reconstitute the interaction of DENV pr with soluble truncated E proteins, showed that exogenous pr could specifically inhibit virus fusion and infection, and showed that pr is required to silence flavivirus fusion activity during virus secretion. We are currently developing this into a fluorescence polarization-based *in vitro* assay for screening.

- 2:00 a.m. – 3:40 a.m. Plenary Session III: Passive Immunotherapy**
Matthews Room
Moderator: Arturo Casadevall
- 2:00 – 2:20 p.m. **Thomas Briese** (Columbia University)
Development of fhmAb therapeutics against viral biothreat and emerging disease agents
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Understanding the human B cell response to influenza virus: A first step towards making a universal flu vaccine
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Staphylococcal enterotoxin B (SEB) Specific Monoclonal Antibody Enhances Survival in Murine Models
- 3:00 – 3:20 p.m. **Arturo Casadevall** (Albert Einstein School of Medicine)
Update on passive antibody efforts against *Bacillus anthracis*
- 3:20 a.m. – 3:40 p.m. **James Bliska** (Stony Brook University)
Development of mAb immunotherapy for genetically modified plague

Development of fhmAb therapeutics against viral biothreat and emerging disease agents

Thomas Briese^{1*}, Matteo Porotto², Moonsoo Jin³, Gavreel Kalantarov⁴, Ilya Trakht⁴, Anne Moscona², Matthew Scharff⁵

¹*Center for Infection and Immunity, and* ⁴*Dept. of Medicine, Columbia University, New York, NY 10032, USA;* ²*Department of Pediatrics and of Microbiology and Immunology, Weill Medical College of Cornell University, New York, NY;* ³*Biomedical Engineering, Cornell University, Ithaca, NY;* ⁵*Albert Einstein College of Medicine, New York, NY*

Passive immunotherapy has an established track record in the management of viral infections. Reagents include both convalescent serum and monoclonal antibodies (mAb). mAbs have the advantage of defined specificity and enhanced safety profiles, and thus are of value as primary or complementary therapies for emerging infectious diseases. However, in contrast to murine or humanized mAb, only fully human mAb (fhmAb) possess all native effector functions and exclude in patients potential adverse effects to nonhuman components. This project develops therapeutic fhmAbs for specific emerging infections where treatment options are unsatisfactory, limited, or not existing. It also optimizes platforms for rapid development and deployment of therapeutic fhmAbs that will be invaluable as new infections emerge and threaten human health.

We have established methods for creating fhmAb from peripheral blood lymphocytes (PBL) of vaccinees or survivors of natural exposure. We created from donor PBL by fusion to a human partner cell line fhmAb to WNV, RVFV, JUNV and NiV. However, an ideal platform should allow the rapid creation of fhmAb purely from laboratory-generated reagents, without a need of human PBL. Thus, we add a recombinant mouse system (*VelocImmune*®) for generating neutralizing human mAb, to demonstrate how this approach will enable rapid movement from agent identification to therapeutic options.

Using PBL from NiV survivors we identified a potent neutralizing fhmAb, 3B7 that neutralizes both, NiV and HeV. 3B7 interacts obviously with an epitope highly conserved among henipaviruses that promises excellent therapeutic efficacy. In contrast to previously reported mAb commonly targeting the G protein, the neutralization activity of 3B7 appears to act via the F protein, and may directly interfere with the fusion process. This suggests that natural selection during an actual encounter with the pathogen may allow identification of new and broadly reactive neutralizing Ab, which may also inspire more effective vaccine strategies. 3B7 has been produced in quantities sufficient for animal challenge experiments, and in preparation of challenge in non-human primates we analyzed NiV infection of African green monkeys. Surprisingly, infection with a supposedly lethal viral dose resulted in only two animals with fatal disease, while one animal recovered and a fourth showed no signs of disease. Analysis of samples from the three different outcomes of NiV infection is underway.

Understanding the human B cell response to influenza virus: A first step towards making a universal flu vaccine

Rafi Ahmed* and Patrick Wilson

*Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA and
University of Chicago, Chicago, IL*

We have previously shown that, in pandemic (H1N1) 2009 influenza infection, broadly cross-reactive stem-binding antibodies dominated the B cell response. However, the important question remained as to whether similar antibodies could also be induced in humans by vaccination. We therefore analyzed the B cell response in 24 healthy adults immunized with the monovalent subunit pandemic (H1N1) 2009 vaccine. In all cases we found a rapid, predominantly IgG-producing plasmablast response. These were isolated and monoclonal antibodies were generated by single-cell PCR. Over half (45/78) were virus-specific and 62% (28/45) of these bound to the pandemic (H1N1) 2009 hemagglutinin (HA). Strikingly, the majority of these antibodies (25/28) neutralized more than one influenza strain and exhibited high levels of somatic hypermutation, suggesting they were derived from recall of B cell memory. Indeed, memory B cells that recognized the pandemic (H1N1) HA were detectable prior to vaccination not only in this cohort but also in stored samples obtained prior to the emergence of the pandemic strain. Three antibodies demonstrated extremely broad cross-reactivity and were found to bind the HA stem. Furthermore, one of them was found to recognize not only H1 and H5 but also H3 influenza viruses. This exceptional cross-reactivity indicates that antibodies capable of neutralizing all influenza subtypes might indeed be elicited by vaccination. The challenge is now to improve upon this result and design influenza vaccines that can elicit these broadly cross-reactive antibodies at sufficiently high levels to provide heterosubtypic protection.

Staphylococcal enterotoxin B (SEB) specific monoclonal antibody enhances survival in murine models

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¹Department of Medicine, ²Microbiology and Immunology, ³Cell Biology at Albert Einstein College of Medicine, Bronx, NY

Staphylococcus aureus, a major human pathogen that causes a diverse array of infections ranging from minor skin and wound infections to more serious life threatening diseases such as pneumonia, bacteremias, and meningitis. *S. aureus* produces several toxins among them SEB is associated with food poisoning; non-tampon associated TSS, sepsis and has effect on a spectrum of allergic diseases including atopic dermatitis, allergic rhinitis, asthma and food allergies. SEB is excreted by 20% of Methicillin resistant *S. aureus* strains derived from septicemic patients (MRSA). SEB is a superantigen that causes massive T-cell stimulation and shock at very low concentrations and is classified as a class B bioterrorism agent. Our goal is to develop SEB specific mAbs as therapeutics against SEB induced lethal shock and SEB mediated diseases.

Previously, we generated murine monoclonal antibodies (mAbs) to SEB in mice and demonstrated that SEB specific mAbs neutralize the SEB toxin *in vitro* as well as *in vivo*. Now we investigated the protective efficacy of SEB specific mAb *in vivo* in a *S. aureus* septicemia model and a skin/soft tissue infection (SSTI) model using BALB/c mice. In the sepsis model, SEB specific mAb 20B1 (500µg), unrelated mAb or PBS was injected intraperitoneal (*i.p.*) 2h prior to infection. Mice were infected intravenous with a SEB excreting MRSA strain (5×10^7). Mice treated with SEB specific mAb 20B1 survived significantly longer compared to unrelated mAb or PBS treated mice ($p=0.034$). Noteworthy, no difference in *S. aureus* CFU cultured from liver, blood and spleen were documented between treated or untreated mice at early time points of infection (2h, 8h, 24h). To further support the concept that humoral immune response against SEB contributes effectively to protection against a lethal dose of SEB producing MRSA infection, we immunized mice with SEB toxin prior to infection. We found significant survival in SEB immunized mice compared to PBS immunized mice ($p=0.012$). CFU count in liver and spleen of immunized versus sham immunized mice at 19 days post infection was not affected and equal from both groups. Treatment with mAb 20B1 was also beneficial in the murine SSTI model. BALB/c mice (6-8 weeks old) were injected *i.p.* with SEB specific mAb 20B1 (500µg) or unrelated mAb 24h prior to *S. aureus* infection into skin. Results of histological examinations revealed higher inflammation and more invasive infection in skin wounds of mice infected SEB producing MRSA strain and treated with control mAb compared to those treated with SEB specific mAbs. In conclusion, our data supports the hypothesis that SEB is a key virulence factor in *S. aureus* mediated sepsis as well as SSTI. We demonstrate that treatment with SEB specific mAbs improves outcome and thus these mAbs could be used as immunomodulating agents in certain severe *S. aureus* infections.

Update on passive antibody efforts against *Bacillus anthracis*

Arturo Casadevall*

Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY

The presentation will provide an update on the work on *B. anthracis* done in the Casadevall laboratory and collaborating groups. In collaboration with the Ravetch laboratory we have generated mouse-human chimeric antibodies expressing native human Fc domains as well as Fc domains mutated for loss of function and higher affinity to FcR. The mouse-human antibody manifested approximately greater binding to protective antigen (PA) than the parent murine mAb consistent with Fc-mediated effects on V region structure that facilitated binding. The mouse-human antibody with a mutated Fc domain that is unable to bind FcR was not protective confirming the requirement for FcR for a subset of toxin-neutralizing antibodies. We have studied the protective efficacy of combinations of antibodies to PA. One of the curiosities in the antibody response to PA is that most of the mAbs that have been generated are not protective. In fact, highly protective mAbs are relatively rare and several disease-enhancing antibodies have been described. In studying we combination of antibodies we have made some very interesting observations. First, combinations of non-protective mAbs can result in protective outcomes in some concentrations. Second, this effect is dependent on FcR and appears to require the formation of complexes that can crosslink FcR. On the basis of these observations we conclude that combinations of mAbs manifest emergent properties that could not be predicted based on the characteristics of individual antibodies. This result has profound consequences for our understanding of antibody-mediated immunity for it implies that one can define the functional properties of an antibody on the structure of the immunoglobulin alone. Finally the presentation will discuss our findings that platelet activating factor contributes to the lethality of lethal toxin and the discovery of a serum protease that cleaves PA.

Development of mAb immunotherapy for genetically modified plague

Maya Ivanov¹, Stylianos Bournazos² Susan Buhl³, Matthew Scharff³ Jeffrey Ravetch², and James Bliska^{1*}

¹*Stony Brook University*, ²*Rockefeller University* and ³*Albert Einstein College of Medicine*

A type III secretion system (T3SS) essential for virulence in *Y. pestis* exports proteins known as Yops and LcrV. Cytotoxic Yop effectors are delivered across the host cell plasma membrane by the translocators YopD and YopB. The LcrV protein, localized to the tip of the T3SS, is required to insert YopD and YopB into the plasma membrane and is a well-characterized protective antigen. F1 surface fimbriae are a second protective antigen in *Y. pestis*. Murine mAbs specific for LcrV (1) or F1 confer passive protection against plague in mice. However, *Y. pestis* can be genetically modified to be resistant to LcrV- and F1-based immunotherapeutics. F1⁻ mutants remain virulent and protective epitopes within LcrV can be altered. In this project, LcrV and the translocators are being targeted for immunotherapeutics to counteract genetically-modified (F1⁻) plague. Mice can be protected against septicemic plague caused by F1⁻ *Y. pestis* by passive immunization with antiserum to YopD and YopB (2). Antiserum to YopD and YopB increases phagocytosis of *Y. pestis* (2), but does not neutralize T3SS-induced cytotoxicity in macrophages, indicating that these antibodies have a different protective mechanism from that of anti-LcrV.

A panel of murine mAbs recognizing YopD or YopB were developed and functionally characterized. Unlike the LcrV mAb 7.3 (3), the YopB and YopD mAbs did not label the surface of *Y. pestis* or neutralize T3SS-dependent cytotoxicity in macrophages. Several YopB and YopD mAbs tested also failed to passively protect mice against septicemic plague. Alternative strategies being taken to identify protective mAbs include screening for recognition of YopB or YopD epitopes exposed on the surface of *Y. pestis* attached to host cells.

The requirement for FcγR in the neutralizing activity of LcrV mAb 7.3 is unknown. Therefore, we have investigated the role of FcγR-mediated interactions in the neutralization capacity of the 7.3 mAb. A variant of the 7.3 hybridoma that switched from IgG1 to IgG2a was obtained. Functional studies are underway to determine if the IgG2a mAb has increased neutralizing activity. F(ab')₂ fragments and deglycosylated IgG1 mAb 7.3 were produced and tested for in vitro neutralizing activity. As compared to intact 7.3, F(ab')₂ fragments and deglycosylated mAb had reduced neutralizing activity. The sequences of the H and L chain of IgG1 mAb 7.3 were determined and recombinant variants (IgG2a and a D265A mutant in the Fc domain that abrogates binding to FcγR) were purified. The specificity and affinity of the recombinant mAb for LcrV as determined by ELISA and SPR were similar. Recombinant variants of mAb 7.3 are currently being tested for neutralizing activity.

1. J. S. Lin *et al.*, *Vaccine* **29**, 357 (Dec 16, 2010).
2. M. I. Ivanov *et al.*, *Infect Immun*, (Sep 2, 2008).
3. B. L. Noel, S. Lilo, D. Capurso, J. Hill, J. B. Bliska, *Clin Vaccine Immunol* **16**, 1457 (Oct, 2009).

- 4:00 – 4:40 p.m.** **Plenary Session IV: Shared Core Resources**
Matthews Room
Moderator: Thomas Briese
- 4:00 – 4:10 p.m.** **David Perlin (UMDNJ)**
Northeast Biodefense Center's Small Animal Core
- 4:10 – 4:20 p.m.** **Karen Chave (Wadsworth Center)**
Northeast Biodefense Center Protein Synthesis Core
(*E. coli*)
- 4:20 – 4:30 p.m.** **Steve Almo (Albert Einstein School of Medicine)**
Gene-to-structure pipeline for biodefense and emerging
infectious diseases
- 4:30 – 4:40 p.m.** **Erol Gulcicek (Yale University)**
Northeast Biodefense Center Proteomics Core

Northeast Biodefense Center's small animal core

David Perlin*

UMDNJ-New Jersey Medical School, Newark, NJ

Northeast Biodefense Center's (RCE Region II) Small Animal Core is operated out of the Public Health Research Institute (PHRI) in Newark, NJ. The major goal of the core is to support the research objectives of NBC scientists including studies on pathogenesis, host response, vaccine efficacy, novel therapeutics and improved diagnostics.

The PHRI Small Animal Core is a specialized program for select agent and NIAID priority A-C pathogens that serves scientists from more than 15 institutions within RCE Region II. The ICPH and UMDNJ Institutional Animal Care and Use Committee (IACUC) has reviewed/approved over 140 Animal Care and Use Protocols (ACUPs) and has approved 143 amendments since July 2004 to authorize infectious disease studies in the PHRI SAC. The PHRI SAC continues to operate at high capacity and has logged over 1 million animal housing days for ABSL-3 studies. Presently, the Core provides rodent infection models for pneumonic and bubonic plague, anthrax, tularemia, glanders, 2009 pandemic influenza, Vaccinia virus and MDR/XDR/drug sensitive tuberculosis. Studies to examine avian influenza H5N1 in mice and ferrets will be started in January 2012. Furthermore, studies are in development for other emerging pathogens such as Chikungunya virus and multidrug resistant Enterobacteriaceae. Aerosol and upper respiratory tract infections are an area of expertise and include models for *Y. pestis*, *M. tuberculosis*, *B. anthracis*, *F. tularensis*, *B. mallei*, *Influenza* virus and *Aspergillus*. The Core has nose-only aerosol exposure systems for mice and rabbits. Intranasal instillations are routinely performed in mice and hamsters. The UMDNJ Regional Biocontainment Laboratory (RBL) adjoins the existing PHRI RAF opened on July 2010. The RBL ABSL3 provides an additional 7,500 GSF of dedicated animal procedure and housing space for BSL3 studies. The RBL is approved for Select Agent studies from the USDA and CDC. The PHRI SAC also supports animal studies with infected mosquitoes and ticks for vector-borne disease research in the RBL ABSL-3 insectary.

Northeast Biodefense Center protein synthesis core (*E.coli*)

Karen J. Chave*

Wadsworth Center, Albany, NY

Many projects within the Northeast Biodefense Center (NBC) require high-quality purified recombinant proteins. The goal of the Protein Synthesis Core is to facilitate the investigative, developmental, and pilot studies proposed in the various themes Core staff will work with investigators to develop expression systems for the proteins required for their studies either using commercial vectors or customized expression vectors. The facility will provide a complete protein expression and purification service.

We offer well established bacterial expression capabilities at the Wadsworth Center. This will allow the rapid provision of reagents for research projects and provide the NBC with the flexibility to support the wide range of existing needs and to respond rapidly to the changing requirements of principal investigators and changing NIAID mandates. In addition, the Core will maintain stable stocks of proteins and expression strains so that in a time of emergency, production of these materials will be rapid.

An overview of the services provided by the Core will be provided and some specific projects will be used to highlight how the Core can help an investigator's research progress more rapidly by the provision of high quality customized protein reagents.

Gene-to-structure pipeline for biodefense and emerging infectious diseases

Steve Almo*

Depts. of Biochemistry and Physiology & Biophysics and the Einstein Macromolecular Therapeutics Development Facility

We describe a complete Gene-to-Structure Pipeline that is available to all members of the NBC for the expression, purification and/or structure determination of proteins relevant to Biodefense and Emerging Infectious Diseases. The Einstein Macromolecular Therapeutics Development Facility (MTDF) houses both the Eukaryotic Expression Core of the NBC and the protein production core of the New York Structural Genomics Research Consortium (NYSGRC), one of four high-throughput structure discovery centers supported by the NIGMS Protein Structure Initiative (PSI). The NYSGRC infrastructure provides the capacity to clone and perform small-scale expression studies on hundreds of proteins per week and hundreds of liters of large-scale fermentation per week. Over the past several years the members of the NYSGRC have determined the structures of over 300 proteins from Category A-C pathogens and related model organisms. Of particular importance, NYSGRC has continuing efforts focused on the structure determination of enzymes and secreted effector proteins from the Category A-C pathogens, as well as host proteins involved in innate and adaptive immunity. This program provides the members of the NBC full access to the NYSGRC protein production/structure determination pipeline. The synergy between NBC and NYSGRC ensures that accruing structural data will be leveraged for maximal biological insight.

Northeast Biodefense Center (NBC) proteomics core

Erol E. Gulcicek*, Christopher M. Colangelo, Janet Crawford, J. Myron Crawford, Tu Lam, Kathryn Stone, Nancy Williams, Terence Wu, and Ken Williams
Northeast Biodefense Center Proteomics Core, W.M. Keck Foundation Biotechnology Resource Laboratory, Yale University, New Haven, CT

The Northeast Biodefense Center Proteomics Core located within the W.M. Keck Foundation Biotechnology Resource Laboratory at Yale University provides a very broad range of proteomics expertise and analytical capabilities to support basic science, preclinical and clinical research programs for NBC investigators.

The NBC Proteomics Core includes several key Keck Resources (Mass Spectrometry and Proteomics, Amino Acid Analysis, Small Scale Peptide Synthesis, and N-terminal Protein Sequencing) that provide wide number of proteomic services with biostatistics and bioinformatics support that are not often available from many academic core laboratories. Examples include the quantitative protein profiling technologies like differential 2D (fluorescence) gel electrophoresis (DIGE), multiplexed isobaric relative and absolute quantitation (iTRAQ), amino acid analysis using ion-exchange chromatography with post-column ninhydrin detection, and fMoc peptide synthesis. In addition, the MS and Proteomics Resource has recently added state-of-the-art label free quantitation, phosphoprotein profiling, and targeted MS based biomarker quantitation technology tools to its list of existing protein profiling services to offer a “pipeline” approach in implementing technologies for discovering, quantitating, validating, and potentially generating a new diagnostic tool for identification and confirmation of protein biomarkers from clinical samples.

Our presentation at this annual meeting will bring to light the basic technologies provided by the NBC Proteomics Core and will also provide examples of how some of these and other newly developed technologies can be implemented by NBC and RCE investigators.

- 8:30. – 9:30 a.m.** **Plenary Session V: Developmental Projects and Career Development**
Matthews Room
Moderator: Erich Mackow
- 8:30 – 8:50 a.m. **Jorge Benach** (Stony Brook University)
The Splenic Response to *Francisella tularensis*
- 8:50 – 9:10 a.m. **Irene Jarchum** (Memorial Sloan Kettering Institute)
Critical role of MyD88 and neutrophil recruitment for protection against *C. difficile* colitis
- 9:10 – 9:30 a.m. **Felicia Santa Maria** (NYU School of Medicine)
PKR and mda5 are required for IRF3 nuclear translocation and IFN induction in response to vaccinia virus

The splenic response to *Francisella tularensis*

Jorge Benach*

Stony Brook University, Stony Brook, NY

Recent studies have linked the accumulation of the Gr-1+ CD11b+ cell phenotype with functional immunosuppression in diverse pathological conditions, including bacterial and parasitic infections, and cancer. Gr-1+ CD11b+ cells were the largest cell populations in the spleen of mice infected with *Francisella tularensis*. T cells did not increase in the spleen in early infection, and there was a significant delay in the kinetics of accumulation and maintenance of Gr-1+ CD11b+ cells in the spleens of B cell deficient mice. The Gr-1+ CD11b+ in early tularemia were a heterogeneous population that could be further subdivided into granulocytic and monocytes subpopulations using the Ly6G and LyC6 markers, and differentiated into antigen presenting cells in ex vivo culture. Purified CD11b+ Ly6G- cells but not CD11b+ Ly6Gint cells suppressed polyclonal T cell proliferation via a nitric oxide dependent pathway. Although the CD11b+ Ly6Ghi subpopulation of the Gr-1+ CD11b+ cells was able to suppress the T cell responses, the presence of this heterogeneous cell group in surviving mice would also suggest a role in the protective host response.

Critical role of MyD88 and neutrophil recruitment for protection against *C. difficile* colitis

Irene Jarchum*, Mingyu Liu, Chao Shi, Michele Equinda, and Eric G. Pamer
*Immunology Program, Department of Medicine (Division of Infectious Disease),
Memorial Sloan-Kettering Cancer Center, New York, NY*

Clostridium difficile colonizes the large intestine following antibiotic treatment, resulting in mild to severe colitis. *C. difficile* colitis is the most common cause of diarrhea among hospitalized patients. The cellular and molecular requirements for defense against *C. difficile* have not been fully elucidated. Here, we demonstrate that MyD88 signaling is required for recruitment of neutrophils and monocytes to the colonic lamina propria (cLP) during infection. MyD88-deficient mice are markedly susceptible to *C. difficile* colitis, and we set out to investigate whether deficient neutrophil or monocyte recruitment to the cLP may play a role. We demonstrate that neutrophils are critical for protection during *C. difficile* colitis, possibly by preventing systemic dissemination of intestinal bacteria, while monocytes are dispensable. Further, expression of the neutrophil-recruiting chemokine CXCL1 is impaired in the cLP of MyD88^{-/-} mice. Establishing a role for MyD88 signaling and for neutrophil recruitment for defense against *C. difficile* colitis not only contributes to a better understanding of the disease but may also aid in manipulation of immune defenses to better protect against this disease.

PKR and mda5 are required for IRF3 nuclear translocation and IFN induction in response to vaccinia virus

Felicia Santa Maria*, Eugene Friedman and David E. Levy

Department of Pathology, NYU School of Medicine, 550 1st Ave, New York NY 10016 USA

Sensing invading pathogens early in infection is critical for establishing an effective defense. Host proteins responsible for recognizing invading pathogens are collectively referred to as pattern recognition receptors (PRR) and include molecules responsible for detecting single- and double-stranded RNA, DNA, lipids and other pathogen-associated molecular patterns (PAMPs). Two common cytoplasmic sensors are RIG-I and mda5, which recognize specific forms of RNA (5' triphosphate and double-strand, respectively), often in a virus-specific manner. Vaccinia virus (VV) is a model pox virus that encodes multiple mechanisms for evading host innate immunity. However, the major mechanism is encoded by the E3L protein, and VV lacking E3L (VVΔE3L) is capable of inducing IFN in many cell types and is therefore highly attenuated. We have found that VV infection in the absence of E3L is recognized primarily by the sensor mda5 and that E3L protein binds and presumably impairs mda5 function. Cells that lack mda5 fail to induce IFN mRNA following infection with VVΔE3L, while cells that lack RIG-I demonstrate IFN mRNA levels comparable to wild type cells (WT). Interestingly, we also observed an absolute requirement for the double-stranded RNA dependent protein kinase PKR in VVΔE3L-induced IFN production. Mouse embryo fibroblasts (MEFs) lacking PKR showed a near-complete abrogation of IFN production compared to WT MEFs, similar to what was seen with mda5 null MEFs. Furthermore, treatment of WT MEFs or human cells with a PKR inhibitor showed a significant loss in IFN mRNA levels following VVΔE3L infection, compared to untreated cells. Additionally, silencing PKR by RNA interference resulted in an impairment of IFN mRNA levels following infection, mimicking the phenotype seen in WT MEFs.

PKR is best known for its ability to inhibit host protein translation by phosphorylating the translation initiation factor, eIF2 α . However, MEF cells containing a mutation in eIF2 α (S51A) rendering it unable to be phosphorylated by PKR induced IFN mRNA levels in response to VVΔE3L infection similar to those seen in of WT MEFs, indicating that this substrate of PKR is not required for its role in inducing IFN. Impairment in IFN induction in cells lacking PKR appears to derive from an inability of the virus to activate IRF3, since IRF3 nuclear translocation was impaired in PKR KO MEFs and A549 cells lacking PKR. The mechanisms underlying how PKR is involved in mda5 signaling and IFN induction are currently under investigation.

9:30 a.m. – 11:10 p.m. Plenary Session VI: Microbial Pathogenesis and Innate Immunity

Matthews Room

Moderator: Sankar Ghosh

- 9:30 – 9:50 a.m. **Craig Roy (Yale University)**
A genetics-squared approach to understanding Coxiella burnetii intracellular replication
- 9:50 – 10:10 a.m. **Sean Brady (Rockefeller University)**
The discovery of new small molecule antibiotics and potential virulence factors relevant to NBC related pathogens
- 10:10 – 10:30 a.m. **David Levy (NYU School of Medicine)**
HDAC-sensitive transcriptional elongation checkpoint during interferon-stimulated gene expression
- 10:30 – 10:50 a.m. **Christopher Basler (Mt. Sinai School of Medicine)**
Modulation of Ebola virus polymerase expression as an antiviral strategy
- 10:50 – 11:10 a.m. **Adolfo Garcia-Sastre (Mt. Sinai School of Medicine)**
The protease of Crimean-Congo hemorrhagic fever virus as a potential antiviral target

A genetics-squared approach to understanding *Coxiella burnetii* intracellular replication.

Hayley Newton, Justin McDonough and **Craig Roy***

Yale University, Section of Microbial Pathogenesis, New Haven, CT

The intracellular pathogen *Coxiella burnetii* replicates in an acidified vacuole that fuses with host cell lysosomes. How this pathogen creates this specialized organelle and survives inside human cells remains unknown. It is predicted that *Coxiella* has gene products that play a specific role in modulating host cell functions, however, essential virulence factors remain to be determined. To better understand the mechanisms that underlie *Coxiella* infection of cells, we are investigating both host and bacterial products that are needed for successful infection using genetic approaches. Genome-wide siRNA screening has identified host pathways important for *Coxiella* replication intracellularly. Regulators of membrane transport, proteins involved in lipid metabolism, and proteins involved in acidification of endosomes were identified as being important for infection. Using transposon mutagenesis to identify *Coxiella* genes important for infection, it was found that an insertion mutation in the gene encoding *lcmL* abolished intracellular replication. The *lcmL* gene was found to be essential for the ability of the Dot/Icm secretion system to deliver type IV effector proteins. Analysis of the kinetics of effector protein delivery by the type IV system revealed that the Dot/Icm system is not activated until several hours post infection. By silencing host genes important for infection, it was determined that delivery of *Coxiella* to an acidified lysosome is required for the function of this apparatus. Thus, using this genetics-squared approach we show that *Coxiella* remains relatively silent during the early stages of host cell infection, and travels to lysosomes along a canonical endocytic pathway. Upon reaching the lysosome, *Coxiella* begins to deliver type IV effectors into the host cell that are needed to transform the lysosome into a specialized niche that supports replication.

The discovery of new small molecule antibiotics and potential virulence factors relevant to NBC related pathogens.

Sean F. Brady*, John B. Biggins, Zhiyang Feng and Melinda Ternei

Laboratory for Genetically Encoded Molecules, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, NY 10065

One of the key insights to arise from the large-scale sequencing of bacterial genomic DNA is that traditional culture-based strategies used to discover natural products have only provided access to a small fraction of the biosynthetic diversity encoded in bacterial genomes. The complete sequencing of many bacterial genomes indicates that often only a subset of the biosynthetic gene clusters present in a genome is expressed under laboratory fermentation conditions, and the sequencing of DNA extracted directly from environmental samples suggests that the majority of bacteria present in nature have not been cultured in the laboratory. Functionally accessing these previously inaccessible natural product biosynthetic pathways should significantly increase the number and diversity of natural products that are available as probes for understanding bacterial virulence as well the development of new antibiotics. With this in mind, we have focused on the following two independent small molecule discovery efforts 1. the discovery and characterization of molecules encoded by previously inaccessible gene clusters present in the genomes of uncultured bacteria as potential sources of novel NBC pathogen related antibiotics, and 2. the characterization of novel small molecules encoded by cryptic gene clusters found in the genomes of NBC related pathogens. Using *E. coli* and *S. aureus* as model organisms, we have screened molecules produced in environmental DNA heterologous expression experiments for novel antibiotics that might be useful for controlling NBC related pathogens. To date, we have identified two structurally novel families of gram-positive specific antibiotics in this screen. These new antibiotics and their modes of action will be described here. In addition, potential small molecule virulence factors identified through the activation of previously cryptic small molecule biosynthetic gene clusters in the genome of *Burkholderia pseudomallei* will be discussed.

HDAC-sensitive transcriptional elongation checkpoint during interferon-stimulated gene expression

Isabelle Marié, Hao-Ming Chang, Leonid Gnatovskiy, Matthew Tangeman, and **David E. Levy***

Departments of Pathology and Microbiology, New York University School of Medicine, 550 1st Ave, New York NY 10016 USA

IFN-stimulated gene (ISG) expression is mediated by the ISGF3 transcription factor complex, composed of tyrosine phosphorylated STAT1 and STAT2, in conjunction with the DNA binding partner, IRF9. Activation and nuclear translocation of these proteins are common targets by pathogenic viruses for disruption and evasion of innate immunity. We have been investigating the mechanisms of ISGF3 function to better understand how it is targeted by viral virulence factors. We have found that histone deacetylase (HDAC) activity, normally associated with gene repression, is required for induced transcription of ISGs and for establishment of an antiviral state. Inhibition of HDAC activity or reduction of HDAC1, 2, and 3 abrogates ISG transcription without altering the activation or chromatin recruitment of ISGF3. To pinpoint the underlying mechanism of this HDAC requirement, we examined ISG transcription *in vitro* and ISG epigenetic regulation *in vivo*. While transcription of ISGs on nucleosome-free DNA *in vitro* was unaffected by HDAC activity, chromosomal remodeling occurred at ISG promoters *in vivo* in response to IFN stimulation and this remodeling required HDAC activity. To discover factors required for ISG transcription, we purified native ISGF3 complexes and identified STAT2-interacting proteins by mass spectroscopy. The DNA helicases Rvb1 and Rvb2 specifically associated with the transactivation domain of STAT2 and reducing their expression by RNA interference impaired ISG transcription. Neither Rvb1 nor Rvb2 were required for induction of IFN-gamma or TNF-alpha induced transcription. Interestingly, IFN-alpha stimulation recruited RNA Pol II to ISG promoters even in the absence of HDAC activity, and the recruited Pol II became phosphorylated on Ser-5, a hallmark of transcriptional initiation. However, RNA Pol II did not become phosphorylated on Ser-2 and failed to transcribe IFN target genes in the absence of HDAC activity. Pol II Ser-2 phosphorylation is mediated by the P-TEFb complex, and chromatin recruitment of P-TEFb and dismissal of the negative elongation factor NELF occurred in response to IFN stimulation but were blocked in the absence of HDAC activity. Therefore, we conclude that ISG transcription requires Rvb-mediated nucleosomal rearrangement and HDAC-dependent recruitment of P-TEFb to ISG promoters in order to trigger displacement of inhibitory factors and commitment of Pol II for transcriptional elongation. Using chikungunya virus as a model pathogen capable of potently evading IFN-mediated host innate immunity, we are investigating how ISGF3 function is impaired during infection.

Modulation of Ebola virus polymerase expression as an antiviral strategy

Reed Shabman, Ariel Endlich-Frazier and **Christopher F. Basler***

Department of Microbiology, Mount Sinai School of Medicine

The Ebola virus (EBOV) polymerase (Large or L protein), the only EBOV-encoded enzyme, is essential for viral replication and transcription. It is therefore an attractive target for antiviral development. We have identified two mechanisms by which EBOV L expression is regulated; one is at the level of translation, the second is at the level of protein stability. First, a study was performed to characterize the impact of viral 5'-untranslated regions (UTRs) on viral mRNA translation. These experiments were undertaken because EBOV encodes 7 mRNAs which possess unusually long 5'- and 3'-UTRs as compared to most other non-segmented negative-strand RNA viruses. The EBOV 5'-UTRs differentially regulated cap-dependent translation when placed upstream of a GFP reporter gene. Most dramatically, the 5'-UTR of the viral polymerase (L) gene strongly suppressed translation of GFP compared to a β -actin 5'-UTR. The L 5'-UTR is one of four viral genes to possess upstream AUGs (uAUGs). Ablation of each uAUG enhanced translation of the primary ORF (pORF). For the L-5'-UTR, mutating the uAUG dramatically enhanced translation of the pORF. Furthermore, under conditions where eIF2 α was phosphorylated, an intact L uORF enhanced translation of the L pORF. Supporting the biological relevance of these data, modest adjustments in L expression dramatically altered activity of a reconstituted viral polymerase complex. These data suggest a novel mechanism whereby EBOV regulates its polymerase expression and function in response to cell stress levels. Second, L expression is also regulated at the level of protein stability. Expression of epitope tagged, amino-terminal fragments of L consisting of amino acids 1-505 or 1-246 are highly unstable when expressed individually. The instability of L expression requires the first 50 amino acids of L, a region conserved among all filovirus L proteins, and deletion of this region results in significant accumulation of the mutated L protein. Stabilization of L expression requires the EBOV VP35 protein, a viral polymerase co-factor and interferon-antagonist. Stabilization by VP35 does not require intact IFN-antagonist function. However, two VP35 point mutants that are defective for polymerase co-factor function but competent to block IFN- α/β responses, are unable to stabilize L. These observations suggest that VP35 must effectively stabilize L to allow efficient viral polymerase activity. Cumulatively, these studies identify new mechanisms by which the EBOV polymerase is regulated and identify novel functions that may be targeted for antiviral development.

The protease of Crimean-Congo hemorrhagic fever virus as a potential antiviral target

Adolfo García-Sastre*

Mount Sinai School of Medicine, New York, NY

Arthropod-borne viruses constitute important causative agents of emerging hemorrhagic fever diseases. We have been investigating how Crimean-Congo hemorrhagic fever virus (CCHFV), a tick-borne virus of high lethality in humans, antagonize the type I IFN response. We have identified the presence of a protease motif associated with the amino-terminus of the L protein of CCHFV, also known to be the viral RNA-dependent RNA polymerase of this negative strand RNA virus, that removes ubiquitin and the IFN-induced ubiquitin-like molecule ISG15 from conjugated cellular substrates, inhibiting antiviral innate immune responses. The structure of this protease revealed the molecular reasons responsible for substrate specificity. We have also established a high-throughput in vitro assay for the screening of small molecules able to inhibit the protease activity of the amino-terminus of the L protein. Using this assay, we found several compounds that specifically inhibit this protease and do not inhibit closely related mammalian proteases. These molecules represent potential lead compounds for the development of antiviral against CCHFV.

Poster Session Abstracts

POSTER SESSION**Wednesday, November 2, 5:30 p.m. – 6:30 p.m.****Refectory**

POSTER #	PRESENTER	TITLE
1	Wendie Cohick	Expression of ricin A-chain(RTA) mutants in mammalian cells differentially affects ribosome depurination and apoptosis
2	Jesica Levingston Mac Leod	Analysis of compounds to inhibit the OUT Crimean-Congo hemorrhagic fever virus protease activity
3	Aihua Zheng	<i>In vitro</i> and <i>in vivo</i> studies identify important features of Dengue virus pr-E protein interactions
4	Claudia Sanchez San Martin	Search of inhibitors for Alphavirus membrane fusion proteins
5	Oswaldo Martinez	Chemical inhibitors of MAPK p38 block Ebola virus infection of human THP-1 and dendritic cells
6	Samantha Palmer	Development of antiviral treatments for enveloped viruses that fuse inside the cell
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Expression of ricin A-chain (RTA) mutants in mammalian cells differentially affects ribosome depurination and apoptosis

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The plant toxin ricin belongs to the family of ribosome-inactivating proteins (RIPs) that inhibit protein synthesis by removing a highly conserved adenine from the large ribosomal subunit. The ability to develop effective antidotes against ricin or to use it as the active component of an immunotoxin hinges on a thorough understanding of its biological actions in mammalian cells. In addition to its inhibitory effect on protein synthesis, ricin also activates cell signaling cascades that lead to necrosis, apoptosis and inflammation. Previously we conducted a large-scale chemical mutagenesis screen in yeast and identified several mutants that were catalytically active *in vivo* but were not toxic. The objective of the present work was to determine if these mutations would similarly affect biological activity in mammalian cells. The coding region of *Ricinus communis* preRTA (containing the signal sequence) was converted to an optimized codon usage for *Bos taurus* and synthesized. Mature RTA lacking the signal sequence was constructed from preRTA by PCR cloning. Genes were subcloned into the pCAGGS mammalian expression vector and single nucleotide point mutations were generated by site-directed mutagenesis PCR. Constructs were transiently transfected into the bovine mammary epithelial cell line MAC-T and lysates were collected 19 h post transfection. Expression of either the pre or mature active site mutant E177K demonstrated negligible ribosome depurination, caspase activation, nucleosome accumulation and activation of JNK and p38 signaling cascades relative to pre or mature wild-type RTA, which increased these parameters approximately 2- to 3-fold. In cells transfected with either pre or mature E177Q, ribosome depurination was 35% of wild-type RTA. However, pre and mature E177Q had different effects on apoptosis – the ability of pre E177Q to induce apoptosis was reduced relative to wild-type preRTA while mature E177Q induced apoptosis similar to mature wild-type RTA. Other mutations in domains close to or away from the active site cleft including G212E, S215F, and the double mutant P95L/E145K each reduced ribosome depurination between 25 and 40% relative to wild-type RTA controls. However, with the exception of pre S215F, these RTA mutants induced caspase activation, nucleosome accumulation, and JNK and p38 activation similarly to wild-type controls. In summary, we have successfully expressed a variety of RTA mutants that exhibit biological activity in mammalian cells. While depurination activity was reduced in each of these mutants, this parameter can be reduced as much as 40% without affecting the activation of stress-activated signaling cascades and apoptosis relative to wild-type RTA.

Analysis of compounds to inhibit the OTU Crimean-Congo hemorrhagic fever virus protease activity.

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The ubiquitin molecule can be cleaved from target proteins by deubiquitylases (DUBs). Owing to their substrate specificity and the central role that the ubiquitylation plays in cell signaling pathways, DUBs are attractive targets for therapeutic intervention. Characterization of the Crimean-Congo hemorrhagic fever virus (CCHFV) L OTU protease revealed a unique isopeptidase activity, which overcomes the antiviral pathways regulated by ubiquitin (Ub) and by the interferon-inducible ubiquitin-like molecule ISG15. This exceptional dual substrate specificity makes this viral protease an ideal target for antiviral therapy. CCHFV is a *navivirus* that can cause severe hemorrhagic fever in humans, with mortality rates of 30-80%. CCHFV is the most widespread among the tick-born human viral diseases and is endemic in areas of Central Asia, Middle East and North of Africa. As a highly pathogenic virus with no vaccine or effective antivirals available, concerns exist about its potential use as a bioterrorism weapon. Its viral genome consists of three negative-sense RNA segments. The largest segment (L) encodes a 448 kDa viral RNA-polymerase (L) that contains an amino terminal OTU (Ovarian Tumor) protease domain of 18 kDa. Recombinant expressed CCHFV-L OTU protein in bacteria showed protease activity *in vitro*, and thus allowed us to establish a robust fluorometric-enzymatic-assay to screen for small molecule inhibitors. We screened a library of 29,569 structurally diverse compounds and identified 1,118 hits that showed statistically significant inhibition of CCHFV-L OTU. Of those compounds, 82 resulted in a residual activity of <70% and were subjected to a secondary screen that revealed a concentration dependent inhibitory activity and half inhibitory concentrations (IC₅₀) between 2 to 10 μM. These compounds also demonstrated high specificity against the CCHFV OTU, since they had low activity (IC₅₀ values >100 μM) against the mammalian IsoT, A20, OTUB1 and OTUB2 proteases. The isopeptidase activity of CCHFV L-OTU protein expressed in mammalian cells shown previously, allowed us to evaluate the activity of 5 lead inhibitors in culture, which showed no associated cytotoxicity. Expression of the CCHFV polymerase in a baculovirus system allowed us to further assess activity of these inhibitors in the context of the full length L protein. These results provide a promising novel approach to develop lead therapeutics to fight CCHFV.

***In Vitro* and *in vivo* studies identify important features of Dengue virus pr-E protein interactions**

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Flaviviruses bud into the endoplasmic reticulum and are transported through the secretory pathway, where the mildly acidic environment triggers particle rearrangement and allows furin processing of the prM protein to pr and M. The peripheral pr peptide remains bound to virus at low pH and inhibits virus-membrane interaction. Upon exocytosis, the release of pr at neutral pH completes virus maturation to an infectious particle. Together this evidence suggests that pr may shield the flavivirus fusion protein E from the low pH environment of the exocytic pathway. Here we developed an *in vitro* system to reconstitute the interaction of dengue virus (DENV) pr with soluble truncated E proteins. At low pH recombinant pr bound to both monomeric and dimeric forms of E and blocked their membrane insertion. Exogenous pr interacted with mature infectious DENV and specifically inhibited virus fusion and infection. Alanine substitution of E H244, a highly conserved histidine residue in the pr-E interface, blocked pr-E interaction and reduced release of DENV virus-like particles. Folding, membrane insertion and trimerization of the H244A mutant E protein were preserved, and particle release could be partially rescued by neutralization of the low pH of the secretory pathway. Thus, pr acts to silence flavivirus fusion activity during virus secretion, and this function can be separated from the chaperone activity of prM. The sequence conservation of key residues involved in the flavivirus pr-E interaction suggests that this protein-protein interface may be a useful target for broad-spectrum inhibitors.

Search of inhibitors for alphavirus membrane fusion proteins

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The alphaviruses are enveloped viruses that can cause severe human illnesses such as encephalitis and arthritis. Some of these viruses have reemerged to become endemic and they also represent potential bioterrorist agents. An example of this is Chikungunya virus (CV) (category C), which has caused serious outbreaks in the last years with millions of cases reported by the WHO.

Alphaviruses infect cells via endocytic uptake. Viral transmembrane fusion proteins mediate a crucial step for infection, the fusion between virus and endosomes at low pH. The Semliki Forest virus (SFV) E1 glycoprotein is an important model to study the membrane fusion process in alphaviruses. The soluble SFV E1 ectodomain has a central domain I (DI), a domain II (DII), containing the fusion peptide, and the Ig-like domain III (DIII), which is connected to the stem and the transmembrane domain. At low pH E1 ectodomain forms an E1 homotrimer. During trimerization DIII and stem fold back against the trimer core forming a hairpin-like structure. We demonstrated that a recombinant DIII protein can act as a dominant-negative inhibitor of SFV fusion and infection. DIII acts via specifically and stably binding to an E1 trimer intermediate during fusion.

We also developed a *Drosophila* S2 cell-based protein expression system to produce truncated forms of SFV E1 containing domains I and II (E1 DI/II). E1 DI/II is correctly folded and recapitulates the conformational changes occurring during membrane fusion when exposed to low pH in presence of target membranes. The E1 DI/II core trimer also specifically interacts with recombinant DIII, in a reaction that is independent of low pH. Based on this system, we have recently developed and optimized a fluorescence polarization-based *in vitro* assay for the binding of DIII to the core trimer of SFV E1 DI/II. We have initiated the use of this assay for high throughput screening of small molecule libraries in order to find inhibitors of this critical protein-protein interaction. These binding inhibitors are expected to inhibit corresponding virus fusion and infection, and they can also be lead compounds for antiviral therapy against these important viral pathogens.

Chemical inhibitors of MAPK p38 block Ebola virus infection of human THP-1 and dendritic cells

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The *Filoviridae* family member, Ebola virus (EBOV) is a highly virulent negative-stranded RNA virus. Antigen-presenting cells such as dendritic cells (DCs) are early and sustained EBOV targets of infection and infected human DCs are deregulated following EBOV infection. Our previous studies have shown that treatment of DCs with EBOV virus-like particles (VLP) possessing the EBOV glycoprotein (GP) stimulates MAPK signaling. Therefore, we tested whether MAPK p38 inhibitors could inhibit EBOV infection of PMA-differentiated THP-1 human monocytic cells and human monocyte-derived DCs (MDDCs). Using GFP expression as a read-out for DC infection, p38 MAPK inhibitors SB202190, P38KinHIII and SB203580 exhibited IC50s of 5 μ M, 7.5 μ M and 10 μ M. In contrast, control analog SB202474 exhibited less than 10% inhibition at >15 μ M while positive control for inhibition, 3-deazaneplanocin A (DZNep), exhibited an IC50 ~4 μ M. We confirmed that pretreatment with SB202190 and DZNep, but not control analog SB202474 inhibited EBOV infection of MDDCs in a dose dependent manner. P38 MAPK inhibitors exhibit anti-inflammatory activities. Pretreatment with MAPK p38 inhibitors blocked EBOV-stimulated inflammatory cytokine TNFalpha, IL-12, IL-6, IL-1RA, growth factor G-CSF and chemokine IP-10, MIP-1alpha, MIP-1beta and RANTES production. Furthermore, VLP-based entry assays were performed and demonstrated inhibition of EBOV GP but not VSV-G mediated entry. In conclusion, p38 MAPK inhibitors may represent an important focal point for treating EBOV infection.

Development of antiviral treatments for enveloped viruses that fuse inside the cell

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We have previously described fusion-inhibitory peptides that are targeted and anchored to the cell surface membrane by cholesterol-conjugation. These peptides have been found to potently inhibit enveloped viruses that fuse at the cell surface, including HIV, parainfluenza, and henipaviruses. We now examine their effectiveness against viruses that fuse intracellularly. We have previously shown that unconjugated fusion-inhibitory peptides have exhibited low antiviral activity against viruses that fuse inside intracellular compartments. We propose that membrane targeting via cholesterol conjugation may yield potent compounds to inhibit viral entry of intracellularly fusing viruses as the peptide travels with the virus to the site of fusion. Here we compare the activity of fusion-inhibitory peptides derived from the influenza hemagglutinin (HA), and show that the while the unconjugated peptides are inactive, the cholesterol-conjugated compounds are effective inhibitors. The cholesterol moiety localizes the peptides to the target cell membrane, where the peptides can follow the virus to the intracellular site of fusion. The cholesterol conjugated peptides trap HA in a transient intermediate state, after fusion is triggered but before completion of the refolding steps that drive merger of the viral and cellular membranes. These results provide proof of concept for an antiviral strategy that is applicable to intracellularly fusing viruses, including known and emerging viral pathogens.

Natural tissue infection mediated by Nipah envelope proteins: evaluating host target cells for antiviral assessment

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We have previously demonstrated that peptides derived from the heptad repeat regions of paramyxoviruses effectively inhibit Nipah virus fusion and infection *in vitro*. However, poor *in vivo* results led us to evaluate possible reasons for this disconnect between *in vitro* and *in vivo* efficacy. Utilizing a multicycle replication assay employing pseudotyped Nipah virus, we show that antiviral peptides are ~4 fold less effective in primary neurons than previously reported in standard laboratory cells. However, the addition of a cholesterol tag increases efficacy in neurons by 2-3 logs. These results indicate the importance of antiviral evaluation in cells relevant to natural host target tissues.

Premature activation of the paramyxovirus fusion protein before target cell attachment: Corruption of the viral fusion machinery

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Paramyxoviruses, including the deadly emerging zoonotic pathogen Nipah virus (NiV) and the childhood pathogen human parainfluenza virus type 3 (HPIV3), enter host cells by fusion of the viral and target cell membranes. This fusion results from the concerted action of two envelope glycoproteins, the receptor binding protein (hemagglutinin-neuraminidase (HN) for HPIV3, G for NiV) and the fusion protein (F). Receptor binding triggers F to undergo conformational changes that render it competent to mediate fusion of the viral and cellular membranes. We proposed that if the fusion process could be activated prematurely before the virion reaches the target host cell, infection could be prevented. We identified a small molecule that inhibits paramyxovirus entry into target cells, and prevents infection by both pseudotyped NiV and HPIV3. We show that this compound works by an interaction with the receptor binding protein that results in F-activation prior to target cell contact. The fusion process is thereby prematurely activated, preventing fusion of the viral membrane with target cells and precluding viral entry. This first evidence that activation of a paramyxovirus F can be specifically induced before the virus contacts its target cell suggests a new strategy with broad implications for the design of antiviral agents.

Development of broad spectrum antiviral fusion inhibitors

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We have published the *in-vivo* efficacy of peptides derived from Human Parainfluenza Virus type 3 (HPIV3) as treatment for Nipah virus infection. Here we present evidence that the same peptides block infection for a wide range of other paramyxoviruses, i.e. Measles, RSV and PIV5. The broad heterotypic activity of HPIV3 derived peptides prompted us to test whether these peptide are also able to block infection for intra-cellularly fusing viruses, here we present recent data for Ebola virus. The Ebola envelope glycoprotein GP2 contains two HR regions (HRN and HRC), connected by a 25-residue linker containing a CX₆CC motif and the internal fusion loop. The structural determination of the fusion core of Ebola (EBOV) GP2, led to the proposed use of peptides derived from the GP2 HRC as antivirals. However, unlike HIV fusion protein-derived HRC peptides, the EBOV HRC peptides showed low potency (IC₅₀ ~600000 nM), which could be explained by the fact that their target was only accessible in the endosome and not at the cell surface. To test the antiviral activity of our HPIV3 derived peptides for EBOV, we adapted our multicycle replication assay (MCR) to Zaire derived (Z)EBOV GP. We found that HPIV3 derived peptides with a cholesterol tag were able to inhibit the ZEBOV GP MCR with an IC₅₀ of <200nM, while the untagged peptides were inactive. This leads us to believe that the cholesterol moiety allows the inhibitor to 'travel' with the virus into the endosomal compartment, where it can access its target following fusion activation. This supports the hypothesis that cholesterol tagging endows the peptides with the ability to follow and block viruses that fuse inside the cell.

Mechanism of viral entry inhibition by a human monoclonal antibody derived from survivors of Nipah virus infection

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Passive immunotherapy has an established track record in the management of infections with rabies, respiratory syncytial, or variola viruses. Antivirals have been used with success in infections with herpes, lenti-, influenza, and respiratory syncytial virus. However, whereas effects of passive immunotherapy are usually specific and monoclonal antibodies (mAb) promise enhanced safety profiles through defined reactivity and specificity, the toxicity of antiviral agents is a concern and their repertoire is still limited. Although efficacy and safety of antivirals will improve, passive immunotherapy will continue to be attractive as a significant primary and complementary line of defense in emerging infectious diseases. We have established a method to create fully human mAb (fhmAb) through fusion of peripheral blood lymphocytes (PBL) of vaccinees or survivors of natural exposure to pathogens to a fully human fusion partner cell line (MFP-2). Using donor PBL we created fhmAb against Nipah virus (NiV). These antibodies were shown to neutralize NiV and the related Hendra viruses in cell culture. Using neutralizing NiV fhmAb 3B7 preparations we show that the antibody targets the NiV fusion protein (F) and blocks NiV F-mediated fusion in a VSV pseudotype neutralization assay system. While passive immunotherapies developed against NiV virus have targeted mainly the receptor binding protein (G), we show that a potent neutralizing antibody derived from natural infection in humans effectively targets the F protein. The efficacy of a passive immunotherapy approach against NiV G has been validated in animal models, however our results may indicate that the F protein is an alternative and possibly better target. Identifying and understanding the protective antibody epitopes formed during infection of the natural host may help to develop more effective vaccine strategies against NiV and Hendra infection.

Discovery of new antibiotics and their biological targets from the functional analysis of soil derived type II polyketide gene clusters

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The vast majority of bacteria present in nature remain recalcitrant to culturing and as result these bacteria have not yet been explored for the production of novel antibacterial agents. Uncultured bacteria are likely the largest remaining pool of biosynthetic diversity that has not yet been examined for the production of secondary metabolites. Exploiting this genetic diversity should prove to be a useful strategy for uncovering new bioactive metabolites that can serve as novel therapeutics against NBC related pathogens. The inability to culture many of the bacteria present within environmental samples renders these microbes incompatible with the most heavily relied upon techniques for characterizing bioactive natural products. Although it is still not possible to easily culture most bacteria in the environment, it is possible to extract microbial DNA directly from environmental samples and clone this DNA into model cultured bacteria where, for the first time, it can be functionally characterized. Using *E. coli* and *S. aureus* as model assay organisms, we screened molecules produced in environmental DNA heterologous expression experiments for novel antibiotics that might be useful for controlling NBC related pathogens. To date we have identified two structurally novel families of gram-positive specific antibiotics in this screen, and one of them showed high activities against drug-resistant bacteria. These new antibiotics and their modes of action will be described here.

Combinations of antibodies to anthrax protective antigen exhibit emergent properties in protection

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The toxin-neutralizing activity of specific immunoglobulins has been exploited to develop many antibody passive therapies. Antibodies with indifferent or disease-enhancing effects are historically labeled as deleterious by-products of immunization. However, the fundamental questions of why these disease-enhancing antibodies are generated and how they benefit the host remain unresolved.

We generated 24 monoclonal antibodies to protective antigen (PA) of *Bacillus anthracis*, 5 of which are disease-enhancing measured by *in vitro* toxicity assay. When combined with a protective antibody, certain toxin-enhancing antibodies manifested synergic protective efficacy against lethal toxin. In addition, co-administering an enhancing antibody with an indifferent antibody resulted in protection to the macrophage monolayer. Maximum synergic protection occurred at roughly 1:1 molar ratio between protective and enhancing antibodies, suggesting a stoichiometry-mediated toxin neutralization. The use of Alexa Fluor-conjugated antibodies and PA revealed the formation of antibody-antigen supercomplex at the molar ratio which paralleled the peak protection. The large size of the supercomplex did not affect its binding and uptake by the macrophage, indicating that the supercomplex formation itself resulted in the loss of toxicity for the lethal toxin.

These observations shed light on the emergent properties of antibody function against microbial toxin, and for the first time show how individually characterized disease-enhancing antibodies may benefit the host in the context of other antibodies. Furthermore, the results imply the protective efficacy of an antibody molecule cannot be reduced to the individual molecule since the protective efficacy of such antibodies may differ in the presence of other antibodies.

Generation and characterization of murine monoclonal antibodies directed against Lethal factor of anthrax toxin

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Bacillus anthracis, the causative agent of anthrax, is a potential weapon of bioterrorism. The two major virulence factors of *B. anthracis* are the poly-D-glutamic capsule, protecting the bacterium from phagocytosis and anthrax toxin, which causes cell damage and an array of physiological disruptions within the host, ultimately resulting in death. There are three components to anthrax toxin: protective antigen (PA), edema factor (EF) and lethal factor (LF). PA combines with EF to form edema toxin or with LF to form Lethal toxin (LeTx). LeTx is a zinc-dependent metalloprotease which cleaves mitogen-activated protein kinase kinases, in turn disrupting essential cellular signaling pathways. While infection with *B. anthracis* can be treated with antibiotics, there is currently no treatment to neutralize toxin once it spreads systemically. To address this, several studies have sought to raise toxin-neutralizing antibodies as a potential form of passive immunotherapy. The majority of these antibodies are against PA, thus the aim of our project is to generate novel monoclonal antibodies to LF. Six week old C57Bl/6 mice were immunized with purified LF, and titers were measured every two weeks. As expected, the humoral response progressively increased over the course of the experiment. At t=2 weeks the majority of the antibody response was IgG_{2b}, while at 4 and 6 weeks the response was a mix of IgG₁ and IgG_{2b}. Sera were tested for toxin-neutralization capability by a toxin neutralization (MTT) assay using J774 macrophage cells. While the 2 wk sera showed little to no toxin neutralization, the 4 wk and 6 wk sera showed near complete toxin neutralization, suggesting that the mice have generated many anti-LF antibodies that disrupt LeTx function. Future work including cloning and isolation of these antibodies will allow us to further characterize these antibodies and their toxin-neutralizing capabilities alone and in combination with antibodies to PA.

Staphylococcal enterotoxin B (SEB) specific monoclonal antibody enhances survival in murine models

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Staphylococcus aureus, a major human pathogen that causes a diverse array of infections ranging from minor skin and wound infections to more serious life threatening diseases such as pneumonia, bacteremias, and meningitis. *S. aureus* produces several toxins among them SEB is associated with food poisoning; non-tampon associated TSS, sepsis and has effect on a spectrum of allergic diseases including atopic dermatitis, allergic rhinitis, asthma and food allergies. SEB is excreted by 20% of Methicillin resistant *S. aureus* strains derived from septicemic patients (MRSA). SEB is a superantigen that causes massive T-cell stimulation and shock at very low concentrations and is classified as a class B bioterrorism agent. Our goal is to develop SEB specific mAbs as therapeutics against SEB induced lethal shock and SEB mediated diseases.

Previously, we generated murine monoclonal antibodies (mAbs) to SEB in mice and demonstrated that SEB specific mAbs neutralize the SEB toxin *in vitro* as well as *in vivo*. Now we investigated the protective efficacy of SEB specific mAb *in vivo* in a *S. aureus* septicemia model and a skin/soft tissue infection (SSTI) model using BALB/c mice. In the sepsis model, SEB specific mAb 20B1 (500 μ g), unrelated mAb or PBS was injected intraperitoneal (*i.p.*) 2h prior to infection. Mice were infected intravenous with a SEB excreting MRSA strain (5×10^7). Mice treated with SEB specific mAb 20B1 survived significantly longer compared to unrelated mAb or PBS treated mice ($p=0.034$). Noteworthy, no difference in *S. aureus* CFU cultured from liver, blood and spleen were documented between treated or untreated mice at early time points of infection (2h, 8h, 24h). To further support the concept that humoral immune response against SEB contributes effectively to protection against a lethal dose of SEB producing MRSA infection, we immunized mice with SEB toxin prior to infection. We found significant survival in SEB immunized mice compared to PBS immunized mice ($p=0.012$). CFU count in liver and spleen of immunized versus sham immunized mice at 19 days post infection was not affected and equal from both groups. Treatment with mAb 20B1 was also beneficial in the murine SSTI model. BALB/c mice (6-8 weeks old) were injected *i.p.* with SEB specific mAb 20B1 (500 μ g) or unrelated mAb 24h prior to *S. aureus* infection into skin. Results of histological examinations revealed higher inflammation and more invasive infection in skin wounds of mice infected SEB producing MRSA strain and treated with control mAb compared to those treated with SEB specific mAbs. In conclusion, our data supports the hypothesis that SEB is a key virulence factor in *S. aureus* mediated sepsis as well as SSTI. We demonstrate that treatment with SEB specific mAbs improves outcome and thus these mAbs could be used as immunomodulating agents in certain severe *S. aureus* infections.

The effect of *Staphylococcus aureus* infection on human B cell populations

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Staphylococcus aureus (*S. aureus*) is an ectopic bacterium that has been seen to cause a variety of infections, some of which are deadly, in humans. It is currently a growing concern in the medical field due to the rise of antibiotic resistant strains, such as Methicillin Resistant *Staphylococcus aureus* (MRSA); making treatment of virulent *S. aureus* increasingly difficult. Interestingly, infection and clearance of this bacterium from patients does not lead to immunity to future infections. *S. aureus* utilizes a variety of virulence factors in order to evade and actively suppress an effective immune response. One of the virulence factors have been seen to impact the humoral arm of the immune system is *Staphylococcus* Protein A (SpA). SpA is a soluble or membrane-bound protein expressed by *S. aureus* that has the ability to act as a B cell superantigen, selectively targeting and binding to a framework region on VH3-idiotype B cell receptors using one of its five Fab binding portions, and has the ability to prevent Fc-receptor mediated opsonization via its binding to the Fc binding portion of IgG isotype antibodies. In this study, we address the effects of *S. aureus* infection on B cell populations and how the bacterium is able to prevent the induction of long lasting, effective immunity to *S. aureus*; analyzing the effect of infection in humans at the resolution of individual B cells. Looking at human subjects that have experienced infections, we report on our initial efforts to characterize the kinetics and magnitude of B cell activation by flow cytometry and ELISPOT analyses. Further, from individually sorted cells the immunoglobulin genes will be cloned for analysis of both specificity and the antibody repertoires at the sequence level to gain insight into the immune history of the cells. Finally, it is hoped that this will lead to the production of human monoclonal antibodies to a variety of bacterial virulence factors that could be used for therapeutic and prophylactic purposes, or to identify epitopes directing vaccine design. By understanding the B cell biology during the response to *S. aureus* infection it is hoped that it will be possible to determine what is preventing the generation of useful immunity in humans and generate an effective vaccine to *S. aureus*.

Towards a comprehensive B cell epitope map of ricin toxin

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Efforts to develop an effective vaccine against ricin are focused on the engineering of attenuated and stable recombinant forms of the toxin's enzymatic A subunit (RTA). While at least one candidate vaccine is in Phase I clinical trials, vaccine design and efficacy studies are being undertaken in the absence of a fundamental understanding of those regions of RTA that are critical in eliciting protective immunity. In this present study, analysis of representative panels of B-cell hybridomas derived from ricin toxoid or RTA-immunized mice revealed that only a minority (~6%) of the antibodies directed against RTA have neutralizing activity. In an effort to identify the key neutralizing regions on RTA, we produced and characterized a collection of monoclonal antibodies (MAbs) directed against five of the six (I-VI) distinct immunodominant regions on RTA. MAbs directed against regions II and IV, but not regions I, V, and VI, neutralized ricin *in vitro*. MAbs specific to regions II and IV, but not region V, protected mice against a lethal toxin challenge. Neutralizing and non-neutralizing MAbs were similar in their respective affinities for ricin, and in some cases recognized epitopes situated immediately adjacent to one another on the surface of RTA. PyMOL modeling revealed that the neutralizing MAbs identified in this study were primarily directed against epitopes within α -helices located in RTA folding domains 1 and 2, whereas non-neutralizing antibodies recognized random coils and loops that were primarily confined to folding domain 3. These data confer insights into the immunodominant and structural determinants on RTA that give rise to protective immunity, and for the first time provide an immunological rationale for RTA subunit vaccine design. Similar strategies are being employed to identify the key neutralizing epitopes on the ricin B subunit (RTB), as RTB, a galactose-specific lectin that promotes toxin uptake into host cells, has the potential to serve as a ricin vaccine, as well as a carrier of heterologous vaccine antigens.

Integrase-defective lentiviral vector-based vaccine targeting Influenza A nucleoprotein

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A key problem in vaccine development is finding an effective way to induce cellular immunity. DNA vaccines are good inducers of cellular immune responses; however, they require a large amount of DNA and at least three administrations to be effective. Integration-competent lentiviral vectors (IC-LV) are powerful inducers of cell-mediated and humoral immune responses, but they carry an intrinsic risk associated with integration into the host genome. Integration-defective lentiviral vectors (ID-LV) have a mutation in the integrase gene and they neither integrate into the genome, nor replicate. Instead, they are maintained as episomal circular DNA in the nucleus (E-DNA). We have previously shown that E-DNA is stable and can express functional proteins. To define the potential of ID-LV as a vaccine that stimulates cell-mediated responses, we will evaluate the generation of protective immunity in the influenza mouse model using the internal and highly conserved nucleoprotein (NP) as antigen. We chose NP because it is known to elicit CTL responses against heterologous strains of influenza A virus. For this purpose, we generated lentiviral vectors expressing influenza A NP. Protein expression was confirmed by Western blot after vector transfection into 293T cells.

Vectors were prepared by co-transfection of 3 plasmids in the human embryonic kidney 293-T cell line using the calcium phosphate method. Plasmids used for co-transfection include: (i) the packaging vector expressing all viral proteins but Env and either with (ID) or without (IC) a point mutation (D116N) that inactivates the function of the integrase protein, (ii) the Env vector (pMD.G), expressing the envelope G viral protein of VSV to allow broad and efficient transduction and (iii) the transfer vector (pTY2-CMV-NP), expressing NP and containing all the elements necessary for vector packaging into the recombinant virus. By co-transfecting different plasmid combinations, we constructed ID-LV expressing NP, IC-LV expressing NP and empty-LV as a control. Since the packaged vector does not code for Env, ID-LV and IC-LV are replication-defective and will therefore only result in a single round of infection. The NP gene is derived from influenza virus A/PR/8/34 (H1N1). Viral supernatants containing LV were filtered and concentrated by ultracentrifugation on a sucrose gradient. Expression of influenza NP in ID and IC lentiviral vector-transduced 293-T cells was confirmed by FACS analysis and Western blot. Next, vectors were normalized by transduction units and p24 levels, and administered to mice via intramuscular injection. Mice injected with IC-LV or ID-LV were equally able to elicit CTL responses to NP peptides, but not irrelevant peptide controls, as evaluated by ELISPOT. Studies are ongoing to determine whether CTL responses elicited by a single administration of IC-LV or ID-LV will provide protection to mice from influenza A virus challenge. These studies will shed light on the potential use of ID-LV based vaccines for human and animal infections.

Risk factors for Yellow Fever vaccine associated viscerotropic disease (YEL-AVD)

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Although previously considered as the safest of the live virus vaccines, since 2001 live yellow fever virus vaccine has been recognized to cause a severe, often fatal, disease, yellow fever vaccine associated viscerotropic disease (YEL-AVD) that resembles yellow fever. The overall incidence is 3-4 cases per million. Risk factors for YEL-AVD include people ≥ 60 and individuals who have been thymectomized because of thymomas. In marked contrast to the 21% case fatality rate in elderly men and the 50% rate in thymectomized people, a new risk group has recently been recognized, women of child-bearing age in whom the disease has thus far invariably been fatal. A few cases have also been found in individuals with a variety of immunologic defects. Identification of the risk groups stimulates interest in understanding the pathogenesis of the increased susceptibility, encourages measures to decrease the occurrence of the disease, and raises the possibility that similar reactions may rarely occur with live chimeric flaviviral vaccines that contain a yellow fever virus vaccine backbone including vaccines protecting from dengue, Japanese encephalitis and West Nile .viruses.

The role of the human ribosomal stalk proteins in the depurination activity of ricin.

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The plant toxin Ricin is highly toxic for mammalian cells and an attractive tool for bioterrorism. Ricin consists of two subunits: a galactose/N acetylgalactosamine-binding subunit (RTB), which facilitates uptake of the toxin by mammalian cells; and a N-glycosidase subunit (RTA), which specifically cleaves an adenine residue of the sarcin/ricin loop (SRL) of 28S ribosomal RNA and inhibits protein synthesis. In yeast, a component of the large ribosomal subunit known as the stalk, which functions to regulate translation, has been shown by our laboratory to facilitate the interaction of RTA with the SRL. The yeast ribosomal stalk is pentameric protein complex comprised of a single P0 (35 kDa) bound by two dimers of acidic ribosomal proteins (P1 α , P1 β , P2 α and P2 β ; 11-12 kDa). The human ribosomal stalk is also comprised of a pentameric structure except that there are only two types of 11-12 kDa phosphoproteins, P1 and P2. We examined whether the human ribosomal stalk proteins also play a role in facilitating the interaction of ricin with human ribosomes and their subsequent depurination. For these studies we used an inducible P protein-silencing human cell line that enables siRNA-mediated knockdown of P1/P2 expression in the presence of doxycycline. Significantly, ricin depurination activity was markedly reduced in cells depleted of P1 and P2. Biochemical examination of the interaction of RTA with ribosomes isolated from wild-type and P1/P2-depleted cells (by surface plasmon resonance) demonstrated that depleted ribosomes have a reduced ability to bind RTA. Comparable results were also observed when P1/P2 proteins were selectively depleted by NH₄Cl-ethanol treatment. Furthermore, the reduced binding of RTA to depleted ribosomes correlated with reduced depurination activity *in vitro*. These results provide strong evidence that the human ribosomal stalk proteins are critical for the ability of RTA to bind and depurinate human ribosomes.

From genes to structure to function to virulence: a comprehensive genomics based strategy for the discovery of new small-molecule signaling systems and toxins within bacterial pathogenesis.

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Small molecules play important roles in both the establishment and propagation of bacterial infections. Cryptic small molecule biosynthetic gene clusters – gene clusters that do not appear to encode the biosynthesis of any known metabolites – are routinely found in sequenced bacterial genomes. In bacterial pathogens, particularly the NIAID priority pathogens, these cryptic pathways represent the pool of pathways from which additional signaling systems and toxins will be found. Herein, we describe a strategy for selectively activating biosynthetic pathways of priority pathogens, characterizing their molecular structures, and establishing their function as potential virulence factors. Using pathogenic *Burkholderia* spp. as a model, we have developed a chemical biological approach towards assessing putative biosynthetic genes and their subsequent roles in virulence: (a) chemical – whereby we up-regulate “silent” or low-expressing biosynthetic clusters within *Burkholderia* via manipulating the transcriptional regulation of biosynthesis and structurally characterize purified small molecules, and (b) biological – wherein we address the role of small molecule-based virulence within *in vivo* animal models with engineered strains that have the biosynthesis of individual molecules “knocked-out.”

We have purified and structurally characterized multiple unique small molecules encoded within the genomes of *B. mallei*, *B. pseudomallei* and *B. thailandensis*. Based upon molecular structure, the likely pharmacological functions of a subset of these molecules have been further characterized and confirmed by *in vitro* enzyme assays. The possible roles for aiding microbial virulence by these molecules include the disruption of key immunomodulating enzymes within the host proteome. We have additionally identified a putative molecule regulating bacterial motility, which upon disruption of biosynthesis, displays attenuated virulence when assayed against a *Caenorhabditis elegans* animal model.

Gaining access to the small molecules encoded within the genomes of pathogenic bacteria can be a key step towards understanding how pathogens interact with their host and how virulence proceeds. From genes to structure to function to virulence: our protocols have allowed for the production, purification structural characterization, and *in vivo* function of this growing repertoire of small-molecules.

The *Coxiella burnetii* Dot/Icm system acts to facilitate intracellular replication following acidification of the *Coxiella* containing vacuole.

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The intracellular bacterial pathogen *Coxiella burnetii*, the causative agent of Q fever, replicates to high numbers in an acidified vacuole derived from the host lysosomal network. This pathogen encodes a Dot/Icm type IV secretion system that is functionally analogous to the *Legionella pneumophila* Dot/Icm system that delivers bacterial proteins called effectors to the host cytosol to facilitate intracellular replication. The advent of axenic culture media and techniques for genetic manipulation of *C. burnetii* enabled the isolation and identification of a transposon insertion mutation that disrupted the *C. burnetii dot/icm* locus. Importantly, this mutant was found to be defective for intracellular replication and this defect could be overcome by expressing *dot/icm* genes from a plasmid. The Dot/Icm-deficient mutant was used to validate that this apparatus was essential for translocation of novel effectors using a BlaM reporter assay. Translocation of BlaM-effector reporter proteins was also demonstrated to be dependent on bacterial transcription and acidification of the *Coxiella* containing vacuole (CCV). This indicates that, in contrast to *L. pneumophila*, the *C. burnetii* Dot/Icm system is not active upon internalization of the bacteria but once the CCV has matured along the endocytic trafficking pathway. These data highlight key differences between the intracellular lifestyles of *C. burnetii* and *L. pneumophila*. Furthermore, the study verifies that the cumulative activities of the unique cohort of *C. burnetii* Dot/Icm effector proteins enables *C. burnetii* to manipulate an acidified vacuole to support intracellular replication.

Host factors required for vacuole modulation and intracellular growth of *Coxiella burnetii*

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Coxiella burnetii is an intracellular pathogen that establishes a unique lysosome-like vacuole for replication. *C. burnetii* uses a type IV secretion system called Dot/Icm to translocate proteins into the host cell and we predict these effector proteins interact with and modulate host cell factors and processes leading to vacuole formation and maintenance. To gain insight into the molecular mechanisms by which *C. burnetii* modulate the host cell, we used RNA interference (RNAi) to identify host genes required for *C. burnetii* intracellular growth. Using a genome-wide high throughput screening method, we measured *C. burnetii* replication by immunofluorescence microscopy in siRNA-transfected HeLa cells. Here, we describe further characterization of several host factors identified in the screen that show different infection phenotypes following RNAi. *C. burnetii* fluorescence is significantly decreased following siRNA depletion of members of the host retromer complex. In addition, translocation of a Dot/Icm effector protein is impaired after retromer silencing. This complex functions in the retrograde trafficking of membrane receptors from endosomes to the trans golgi, and therefore disruption may affect receptor trafficking events important for *C. burnetii* vacuole formation. In contrast, siRNA depletion of host syntaxin-17 (STX17) resulted in a phenotype in which multiple *C. burnetii*-containing vacuoles were detected in a single cell, compared to a single large vacuole per cell in mock-transfected cells. Since syntaxins function in vesicular fusion, we hypothesize that STX17 may play a role in homotypic fusion events central to *C. burnetii* vacuole expansion. Our results emphasize the host cell specific responses to *C. burnetii* infection, an important step in understanding the mechanisms employed by *C. burnetii* in modulating host cell processes during intracellular growth.

Residue 19 of the late domain motif ${}_{16}\text{PPPX}_{19}$ determines the ability of Marburg virus VP40 to bud in Huh7 cells

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Marburg viruses, members of the *Filoviridae* family, cause severe hemorrhagic fever in humans and non-human primates. The Ravn and Ci67 strain of Marburg virus (RAVV and MARV, respectively) were recently adapted to mice. Although the parental strains are avirulent in mice, the adapted strains are lethal. During adaptation, mutations accumulated throughout the viral genome with several occurring in the gene encoding VP40. VP40, the viral matrix protein, drives Marburg virus budding and also blocks interferon (IFN) signaling. Interestingly, the wild type (WT) RAVV and MARV VP40 inhibit IFN signaling in only human cells whereas the mouse adapted (ma) MARV VP40 inhibits IFN signaling in both human and mouse cells. RAVV and MARV VP40s contain a late domain motif ${}_{16}\text{PPPY}_{19}$, which changed to ${}_{16}\text{PPPH}_{19}$ in the mouse-adapted virus. The late domain mediates interactions with host factors and is required for virus budding. To test the impact of the Y19H mutation on VP40 function, RAVV late domain mutants (RAVV Y19H, maRAVV H19Y) were generated and analyzed *in vitro*. RAVV VP40, maRAVV VP40, RAVV VP40 Y19H, and maRAVV VP40 H19Y all bud from Hepa 1.6 cells, a mouse liver cell line. In contrast, RAVV VP40 and maRAVV VP40 bud efficiently from Huh7 cells, a human liver cell line, but RAVV VP40 Y19H and maRAVV VP40 H19Y lose their ability to bud in these cells. The VP40 late domain mutants, however, retain the ability to inhibit IFN signaling in Huh7 cells. Taken together, the data suggests that 1) the identity of amino acid 19 of late domain motif determines the ability of the RAVV and maRAVV VP40 to bud in Huh7 cells and 2) filovirus VP40 budding can be restricted in a host cell-dependent manner. The identification of cellular proteins interacting with the MARV VP40 late domain in different cell types is in progress along with plans to test the MARV VP40 late domain mutants *in vivo*.

Innate immune responses within Dengue-infected human endothelial cells

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The ability of dengue virus to infect the endothelium provides a means for infection to alter capillary permeability, permit virus replication, and induce a response that activates and recruits immune cells to the endothelium. However, the contribution of infected endothelial cells to vascular permeability is not regarded as a primary factor in dengue pathogenesis. Our recent studies demonstrated that dengue virus productively infects primary human endothelial cells with a rapid increase in viral titers 1 day post infection, suggesting that dengue-infected endothelial cells contribute to viremia and viral dissemination that make them targets of circulating antibodies and immune cells. To further our understanding of endothelial cell responses elicited by dengue virus infection, we compared transcriptional responses in primary human endothelial cells (HUVECs) infected with dengue virus (type 4) with those from mock-infected cells using Affymetrix arrays and quantitative RT-PCR. With both methods we noted a significant induction of interferon β (*IFN β*), IFN-stimulated genes (*ISG20*, *viperin*, *ISG56*, and *MxA*), IFN signaling pathway inducers *RIG-I* and *MDA5*, and IFN-induced chemokines (*RANTES*, *IP-10*, *MIG*, and *ITAC*) 24 and 48 hours post infection (hpi). Transcriptional upregulation followed peak progeny virus production, which occurred between 12 and 24 hpi. These findings suggest that dengue virus regulates early IFN responses. This data is supported by a substantial increase in the number of dengue-infected endothelial cells in the presence of neutralizing IFN β antibody. Collectively, our results suggest that dengue virus regulates early IFN responses within human endothelial cells, resulting in replication and increased viremia, while at later times dengue-infected cells elicit high level IFN and IFN-induced chemokine responses, similar to those observed in dengue patients. Future studies will analyze this biphasic response and define the roles of dengue virus proteins in regulating early IFN responses within infected endothelial cells.

A novel dengue reporter virus to screen for antiviral effectors of type I interferon

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Dengue virus is an emerging disease threat that poses risk to approximately one third of the world's population. The lack of approved antivirals or vaccines targeting dengue virus has prompted interest in developing novel platforms to study dengue virus infection and pathogenesis. While clinical isolates of various dengue virus serotypes exist, these viruses are not easily amenable to discovery of novel antivirals using high-throughput platforms. Molecular clones of dengue virus have also been made, but these constructs are typically unstable and genetically difficult to manipulate. Here, using optimized cloning strategies, we show that a standard infectious molecular clone of serotype 2 dengue virus can be genetically altered to produce viral genomes encoding fluorescent and luciferase-based reporter proteins. Fully infectious reporter viruses can be produced in standard tissue culture cell lines, grown to suitable titers, and are sensitive to anti-dengue compounds and type I interferon. We demonstrate the screening potential of novel dengue reporter viruses by testing more than 350 type I-interferon stimulated genes (ISGs) for antiviral activity. Previously known and novel antiviral effectors were identified in the screen, including *IRF1*, *IRF7*, *RIG-I*, *IFITM3*, *IFITM2*, *STAT2*, *IFI6*, *ADM*, *HPSE*. Collectively, these genes exhibited a range of inhibitory activities that was partially dependent on cellular background. These novel dengue reporter viruses provide a powerful platform for high-throughput screens aimed at uncovering chemical or cellular inhibitors.

The tight regulation of Ebola virus polymerase expression by multiple mechanisms represents a potential antiviral target.

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Ebolaviruses (EBOVs) are highly lethal, emerging negative-sense RNA viruses. Understanding the mechanisms that EBOVs respond to innate antiviral responses may suggest strategies to prevent fatal infection. EBOV genomes encode an RNA-dependent RNA polymerase (L) at its 5' end. When testing the role of the L 5'-UTR on L translation, we show that it strongly suppressed translation compared to a β -actin 5'-UTR in a GFP reporter assay. Suppression by the L 5'-UTR depended upon the presence of an upstream open reading frame (uORF), and ablating its uAUG dramatically enhanced both GFP and L protein translation. Furthermore, the L uORF enhanced L translation initiation under conditions where eIF2 α was phosphorylated (eIF2 α ~P). This demonstrates that the uORF mediated suppression of L is partially overcome in the presence of eIF2 α ~P, identifying a novel mechanism by which EBOV L regulates its translation. Furthermore L expression was strongly enhanced by coexpressing the viral phosphoprotein VP35, representing an additional, post-translational control of L in cells. Supporting the biological relevance of these data, titrating an L expression vector dramatically altered EBOV L-dependent transcription/replication. Since modulating levels of L directly affect the efficiency of EBOV transcription/replication, targeting L translation may provide a platform to screen for EBOV antivirals. Therefore, we are constructing an assay to screen for compounds that stabilize L expression and will test positive compounds in our established EBOV minigenome assay.

Roles of IL-17/22 in West Nile virus pathogenesis

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IL-17 and -22 are Th17 cytokines that have been shown to play a protective role against bacterial infection in the gastrointestinal (GI) tract by enhancing the GI epithelial response to pathogen invasion, such as secretion of anti bacterial peptides. They have also been demonstrated to play a deleterious role in experimental autoimmune encephalomyelitis by enhancing leukocyte infiltration into the central nervous system (CNS). However, their roles in viral infection have not been well defined. Using West Nile virus (WNV), a neurotropic flavivirus, we found that IL-17 and -22 play contrasting roles in WNV pathogenesis. Knockout of *Il17* rendered mice more susceptible to lethal WNV infection, while the *Il22* deficient animals were more resistant, than wild-type. Very interestingly, in the absence of both cytokines, mice responded normally to WNV infection. *Il17*^{-/-} mice had higher viremia and CNS viral loads than wild-type. The transcripts of *Ccl5*, *Ccr5* and *Ifng* in the blood cells of the knockouts were higher than those in the WT; albeit no differences in *Ifna* and *Tnfa* transcript levels were observed. *Il22*^{-/-} showed a quite distinct phenotype from *Il17*^{-/-}. *Il22*^{-/-} mice had viremia and innate immune response comparable to WT. But viral loads, leukocyte infiltrates, proinflammatory cytokines and apoptotic cells in the central nervous system (CNS) of *Il22*^{-/-} mice were strikingly reduced. Further examination showed that *Cxcr2*, a chemokine receptor that plays a non-redundant role in mediating neutrophil migration, was significantly reduced in *Il22*^{-/-} compared to WT leukocytes. Expression of *Cxcr2* ligands, *cxcl1* and *cxcl5*, was lower in *Il22*^{-/-} brains than wild type mice. Correspondingly, neutrophil migration from the blood into the brain was attenuated following lethal WNV infection of *Il22*^{-/-} mice. *In vitro*, recombinant IL-22 induced *CXCL1* and *CXCL5* expression by human brain microvascular endothelial cells and enhanced transmigration of polymorphonuclear leukocytes across endothelial layer. Our results suggest that IL-22 promotes migration of WNV-infected neutrophils into the CNS, thereby exacerbating lethal WNV encephalitis.

Type I interferon activates the interferon antagonist function of yellow fever NS5 protein

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Flaviviruses include important arthropod-transmitted viruses that cause human disease, such as dengue, West Nile, Japanese encephalitis and yellow fever viruses. For most of these viruses, it is known that the viral NS5 protein, in addition to its role in viral genome replication, antagonizes type I IFN signaling, either by preventing STAT phosphorylation (i.e., West Nile virus) or by binding and targeting STAT2 to degradation (i.e., dengue virus). We now show that the NS5 of yellow fever virus also shares the ability with other flavivirus NS5s to inhibit type I IFN signaling. However, it does so by a unique mechanism: by binding and sequestering STAT2 only after exposure to type I IFN. Yellow fever virus NS5, in contrast to dengue virus NS5, does not bind STAT2 in cells that are not treated with IFN. Rather, treatment with IFN, while resulting in normal phosphorylation of STAT2, also promotes STAT2 binding to yellow fever virus NS5. This prevents interaction of the STAT1/STAT2/IRF9 complex with its promoters thus inhibiting transcription of IFN-stimulated genes. Interestingly, the ability of IFN to promote STAT2-NS5 interaction is not due to STAT2 modification, but instead to NS5 modification. We have identified a lysine in NS5 that becomes ubiquitinated in response to IFN treatment. Mutation of this lysine renders NS5 incapable of binding STAT2 after IFN treatment, strongly suggesting that IFN-mediated ubiquitination of NS5 promotes its interaction with STAT2 and its inhibition of the type I IFN signaling pathway. This is a then a unique example of a viral protein that is activated by the very pathway that it inhibits.

Northeast Biodefense Center protein synthesis core (*E.coli*)

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Many projects within the Northeast Biodefense Center (NBC) require high-quality purified recombinant proteins. The goal of the Protein Synthesis Core is to facilitate the investigative, developmental, and pilot studies proposed in the various themes. Core staff will work with investigators to develop expression systems for the proteins required for their studies either using commercial vectors or customized expression vectors. The facility will provide a complete protein expression and purification service.

We offer well established bacterial expression capabilities at the Wadsworth Center. This will allow the rapid provision of reagents for research projects and provide the NBC with the flexibility to support the wide range of existing needs and to respond rapidly to the changing requirements of principal investigators and changing NIAID mandates. In addition, the Core will maintain stable stocks of proteins and expression strains so that in a time of emergency, production of these materials will be rapid.

In the first funding cycle, the NBC Protein Expression Core provided 91 different proteins to 24 NBC investigators including 70 expressed in *E. coli*. A total of over 3.8 g of protein. Since March 1st 2009 the Wadsworth Center has focused on *E.coli* expression and we have delivered to 14 PI's 42 proteins, totaling 3092 mg.

Information about Core services is posted on the NBC website. Investigators funded via theme, developmental or career development mechanisms have priority for service; nonetheless, any investigator in our catchment area pursuing biodefense or emerging infectious disease research is eligible for services.

Northeast Biodefense Center's small animal core

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Northeast Biodefense Center's (RCE Region II) Small Animal Core is operated out of the Public Health Research Institute (PHRI) in Newark, NJ. The major goal of the core is to support the research objectives of NBC scientists including studies on pathogenesis, host response, vaccine efficacy, novel therapeutics and improved diagnostics.

The PHRI Small Animal Core is a specialized program for select agent and NIAID priority A-C pathogens that serves scientists from more than 15 institutions within RCE Region II. The ICPH and UMDNJ Institutional Animal Care and Use Committee (IACUC) has reviewed/approved over 140 Animal Care and Use Protocols (ACUPs) and has approved 143 amendments since July 2004 to authorize infectious disease studies in the PHRI SAC. The PHRI SAC continues to operate at high capacity and has logged over 1 million animal housing days for ABSL-3 studies. Presently, the Core provides rodent infection models for pneumonic and bubonic plague, anthrax, tularemia, glanders, 2009 pandemic influenza, Vaccinia virus and MDR/XDR/drug sensitive tuberculosis. Studies to examine avian influenza H5N1 in mice and ferrets will be started in January 2012. Furthermore, studies are in development for other emerging pathogens such as Chikungunya virus and multidrug resistant Enterobacteriaceae. Aerosol and upper respiratory tract infections are an area of expertise and include models for *Y. pestis*, *M. tuberculosis*, *B. anthracis*, *F. tularensis*, *B. mallei*, *Influenza* virus and *Aspergillus*. The Core has nose-only aerosol exposure systems for mice and rabbits. Intranasal instillations are routinely performed in mice and hamsters. The UMDNJ Regional Biocontainment Laboratory (RBL) adjoins the existing PHRI RAF opened on July 2010. The RBL ABSL3 provides an additional 7,500 GSF of dedicated animal procedure and housing space for BSL3 studies. The RBL is approved for Select Agent studies from the USDA and CDC. The PHRI SAC also supports animal studies with infected mosquitoes and ticks for vector-borne disease research in the RBL ABSL-3 insectary.

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