5th Southeastern Mycobacteria Meeting

January 24-26, 2014

University of Alabama at Birmingham

Heritage Hall & Edge of Chaos,
Lister Hill Library
University Blvd, Birmingham, AL

Keynote Speaker: Lalita Ramakrishnan, M.B.B.S, Ph.D.
University of Washington, Seattle, WA

Organizing Committee:

Michael Niederweis, Ph.D.
University of Alabama at Birmingham

Miriam Braunstein, Ph.D.
University of North Carolina, Chapel Hill

Jyothi Rengarajan, Ph.D.
Emory University, Atlanta

Frank Wolschendorf, Ph.D.
University of Alabama at Birmingham

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Infectious Diseases, Global Health and Vaccines Strategic Planning Initiative

CFAR CENTER FOR AIDS RESEARCH

Meeting Program

Friday, January 24, 2014

6:30 – 9:30 p.m. **Welcome reception and meeting registration**
Edge of Chaos, 4th floor of Lister Hill Library
1700 University Blvd, Birmingham, AL

Saturday, January 25, 2014

8:00 a.m. – 12:00 p.m. **Oral presentations**
Heritage Hall, 1401 University Blvd

12:00 p.m. – 1:45 p.m. **Lunch and poster session I**
Edge of Chaos, 4th floor of Lister Hill Library

2:00 p.m. – 3:35 p.m. **Oral presentations**
Heritage Hall

3:35 p.m. – 4:25 p.m. **Keynote Address: Lalita Ramakrishnan**
Heritage Hall

4:30 p.m. – 6:15 p.m. **Poster session II**
Edge of Chaos, 4th floor of Lister Hill Library

7:00 p.m. – 11:00 p.m. **Dinner and entertainment**
Iron City Grill, 513 22nd St. S, Birmingham, AL

Sunday, January 26, 2014

9:00 a.m. – 11:25 a.m. **Oral presentations**
Heritage Hall

11:30 a.m. – 12:00 p.m. **Awards and prizes**
Heritage Hall

12:00 p.m. – 1:00 p.m. **Lunch**
Heritage Hall

Detailed Meeting Schedule

Friday, January 24, 2014

6:30 – 9:30 p.m. **Welcome reception and meeting registration**
Edge of Chaos, 4th floor of Lister Hill Library
1700 University Blvd, Birmingham, AL

Saturday, January 25, 2014

Please enjoy breakfast at your hotel

8:00 – 8:30 a.m. **Registration** Heritage Hall

Powerpoint presentations should be uploaded at this time.

8:30 – 8:45 a.m. **Opening remarks** Heritage Hall
Michael Niederweis, UAB
Mike Saag, CFAR Director, UAB

Oral presentations session I – Moderator: Miriam Braunstein Heritage Hall

8:45 – 9:05 a.m. **Adrie Steyn**, **K-RITH**
Energy and redox homeostasis during Mycobacterium tuberculosis infection

9:05 – 9:25 a.m. **Hillary Bengtson, University of Central Florida**
Nanosensors for the Detection and Differentiation of Mycobacteria Species

9:25 – 9:45 a.m. **Rahul Sharma, National Hansen's Disease Programs**
Leprosy: an emerging zoonosis in the United States

9:45 – 10:05 a.m. **Alasdair Leslie, K-RITH**
Interaction of Mycobacterium tuberculosis with innate immune cells

10:05 – 10:30 a.m. **Coffee Break**

Oral presentations session II – Moderator: Frank Wolschendorf Heritage Hall

10:30 – 10:50 a.m. **Gail Cassell, Harvard Medical School**
The Global Challenge of Drug Resistant TB

10:50 – 11:10 a.m. **Sylvie Garneau-Tsodikova, University of Kentucky**
Towards understanding and combating resistance in tuberculosis

11:10 – 11:30 a.m. **Mehri Haeili, University of Alabama at Birmingham**
Copper Ions in Disguise – A Novel Strategy to Attack Copper Homeostasis of Mycobacterium tuberculosis

11:30 – 11:50 a.m. **Daniel Kalman, Emory University**
Host-directed tyrosine kinase inhibitors as therapeutics for antibiotic-resistant TB

Lunch and Poster session I – Posters 1-13 Edge of Chaos

12:00 – 1:45 p.m. All posters should be set up at this time. Posters will be presented from 12:30-1:45.

Oral presentations session III – Moderator: Jyothi Rengarajan Heritage Hall

2:00 – 2:20 p.m. **Karen Lacourciere, NIH**
NIAID TB Research Program: Priorities, Funding and Collaborations in Global TB Research

2:20 – 2:40 p.m. **Ed Khan**, **UAB**
Lessons from a Recent TB Case in Birmingham

2:40 – 3:00 p.m. **Vikram Saini**, **University of Alabama at Birmingham**
Cigarette smoke contributes to the development of drug resistance in Mycobacterium tuberculosis

3:00 – 3:20 p.m. **Martin Cheramie, University of Louisiana-Lafayette**
Investigation into Protective Mucosal Immunity in Fish against Mycobacteriosis

3:20 – 3:35 p.m. **Coffee Break**

3:35 – 4:25 p.m. **Keynote Address**
Lalita Ramakrishnan, University of Washington, Seattle Heritage Hall
A zebrafish's guide to tuberculosis pathogenesis and treatment

Poster session II – Posters 14-37 Edge of Chaos

4:30 – 6:15 p.m. All posters should be set up at this time.

Dinner and entertainment Iron City Grill

7:00 – 11:00 p.m. 513 22nd Street South, Birmingham, AL

Sunday, January 26, 2014

Please enjoy breakfast at your hotel

Oral presentations session IV – Moderator: Michael Niederweis Heritage Hall

9:00 – 9:20 a.m. **Jenny Hayden, University of North Carolina**
Mycobacterial metabolism is regulated by lysine acetylation

9:20 – 9:40 a.m. **Maria Georgieva, Emory University**
Mycobacterium tuberculosis Hip1 is a serine protease that cleaves GroEL2 and modulates macrophage responses

9:40 – 10:00 a.m. **Konstantin Korotkov, University of Kentucky**
Structure-based approach to identify inhibitors of mycosin protease from ESX-1 secretion system

10:00 – 10:20 a.m. **Rebecca Beerman, Duke University**
Visualization and Manipulation of Calcium Dynamics in Inflammation and Infection

10:20 – 10:35 a.m. **Coffee Break**

10:35 – 11:05 a.m. **Hardy Kornfeld, University of Massachusetts**
Infection-Induced Apoptosis and Necrosis in tuberculosis

11:05 – 11:25 a.m. **Jim Sun, University of Alabama at Birmingham**
CpnT: A novel protein toxin of Mycobacterium tuberculosis

11:30 – 12:00 p.m. **Awards and prizes**

12:00 – 1:00 p.m. **Lunch**

Other Information

Arrival & Registration

Upon your arrival to the reception at The Edge of Chaos (1700 University Blvd, 4th floor of Lister Hill Library) on Friday January 24th, please visit the registration table to pick up your meeting materials. Attendees may also pick up their registration packets on the day of the meeting, Saturday January 25th from 8:00 – 8:30 a.m. at the Heritage Hall (1401 University Blvd).

Oral presentations

All Powerpoint presentations should be uploaded on January 25th, 2014 between 8:00 – 8:30 a.m. Presentations should not exceed 20 minutes including 5 minutes for questions unless otherwise indicated.

Poster session

Each poster will be assigned a number. Posters that are assigned numbers1-13 will be presented during Poster session I (12:30 – 1:45 p.m.). Posters that are assigned numbers 14-37 will be presented during Poster session II (4:30 – 6:15p.m.).

Posters should be set up on January 25, 2014 between 11:50 a.m. and 12:15 p.m.
Poster dimensions should not exceed 48 inches by 72 inches in size.

Prizes will be awarded for the best oral presentation and poster presentation by a student or a post-doctoral scientist.

Directions and Lodging

For details on travel and lodging and campus maps, please visit the 2014 SEMM webpage at:
http://www.uab.edu/medicine/microbiology/welcome

Talks

Talk #1

Energy and redox homeostasis during *Mycobacterium tuberculosis* infection

Adrie J.C. Steyn

KwaZulu-Natal Research Institute for Tuberculosis and HIV (K-RITH), Durban, South Africa
Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, USA. Centers for AIDS Research and Free Radical Biology, University of Alabama at Birmingham, Birmingham, AL, USA.

A major obstacle to the development of successful therapeutic intervention strategies for tuberculosis (TB) is the lack of a mechanistic understanding of how *M. tuberculosis (Mtb),* maintains a persistent, non-replicating state in humans for years, insensitive to antimycobacterial drugs, to then unexpectedly resume growth and cause disease. Since numerous host factors can profoundly affect *Mtb* physiology and almost certainly redox balance, it is likely that the mechanisms used by the bacilli to maintain redox balance during active disease, are critical towards the outcome of disease. What are the redox and bioenergetics states of infected host cells, and *Mtb*? How do we define and measure it? Studies have shown that changes in bioenergetic metabolism accompany a wide range of human diseases, and targeting shifts in bioenergetic metabolism may hold strong therapeutic potential. My laboratory have used a combination of quantitative metabolomic, transcriptomic, bioenergetic and mass spectrometric methods to examine mechanisms whereby *Mtb* balances the cytoplasmic redox state and maintains bioenergetic homeostasis. I will discuss approaches for characterizing the intracellular redox status of *Mtb* and the implications this knowledge have for studying the mode of action of antimycobacterial drugs. I will provide evidence of a redox hierarchy in *Mtb* that is facilitated by the *Mtb* “redoxome”. I will also discuss how *Mtb* infection of macrophages redirect metabolism from oxidative phosphorylation to glycolysis. Lastly, I will discuss how metabolic flux analysis can be exploited for the non-invasive study of real-time *Mtb* bioenergetics.

Talk #2

Nanosensors for the Detection and Differentiation of Mycobacteria Species

Hillary N. Bengtson, Yulia V. Gerasimova, Kyle H. Rohde, Dmitry M. Kolpashchikov

Chemistry Department and Burnett School of Biomedical Sciences, College of Medicine. University of Central Florida. 4000 Central Florida Blvd, Orlando, FL 32816

In this study we use the recent developments in DNA nanotechnology to design new efficient point-of-care (POC) diagnostic tools for the detection and differentiation of *Mycobacterium tuberculosis* (*Mtb*). Specifically, we have developed two classes of sensors with fluorescent readout: the molecular beacon-based differential receptor and deoxyribozyme-based sensors. Conventional methods of *Mtb* diagnostics assays (culture-based assays, acid-fast bacilli smear microscopy, PCR) have several drawbacks including lack of rapidness, high cost, and either false positive or false negative responses. The assays developed in this study are quick and straightforward and will enable clinicians to rapidly detect and diagnose *Mtb*. We demonstrate a multicomponent-differential receptor that allows for the identification of rifampin resistant *Mtb* strains that differ by a single mutation in the 81 bp hot spot region of the rpoB gene. This differential receptor is able to produce a specific pattern of responses, the 'fingerprint' of the analyte. Our second class of sensors - deoxyribozyme sensors - target the high copy number 16S and 23S rRNA, and offer an advantage of low detection limits without the need of target amplification. Due to the abundance of RNA in the cell and the catalytic power of deoxyribozymes we are able to develop a very sensitive assay for the detection of *Mtb* and differentiation of RNA from closely related mycobacterial species. We are applying these sensors for the detection of *Mtb* in whole-cell and sputum formats. Our technology provides the foundation for future development of cost-effective and sensitive alternatives for molecular POC diagnostics.

Talk #3

Leprosy: an emerging zoonosis in the United States

Rahul Sharma[1,2], Pushpendra Singh[3], David M Scollard[2], Maria Pena[1,2], Ramesh Subramanian[2]

Vladimir N Chouljenko[2], Steward T Cole[3] and Richard W Truman[2]

[1]HHS/HRSA/HSB/National Hansen's Disease Program, LSU School of Veterinary Medicine Baton Rouge, Louisiana 70803. [2]Louisiana State University School of Veterinary Medicine, Department of Pathobiological Sciences, Baton Rouge, Louisiana 70803. [3]Global Health Institute, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Leprosy is an infectious disease caused by *Mycobacterium leprae.* Other than humans, nine-banded armadillos (*Dasypus novemcinctus*) are the only known natural host for *M. leprae* and these animals range from Northern Argentina to Central United States. To better understand the role of armadillos in the ecology of leprosy in southern United States, we re-sequenced genomes of *M. leprae* isolated from 3 human patients and 1 infected armadillo, and developed a single nucleotide polymorphism (SNP) based algorithm and a set of 10 variable number tandem repeat (VNTR) to define the geographical affiliation of *M. leprae* strains. Using this combination, we found that 88% of the armadillos and 64% of the autochthonous human cases from southern United States are sharing a single unique strain (3I-2-v1) of *M. leprae*, probably through zoonotic transmission. Although armadillos in Georgia and Florida were thought to be free of the infection some 20 years ago, we recently examined serum and tissue samples from 645 armadillos obtained in that region since 2004. 16.43 % (106/645) armadillos were found serological positive for the infection and confirmed with two sites PCR. In addition to the previously described strain, another zoonotic *M. leprae* strain strain (3I-2-v15) was found among patients and armadillos from central Florida with 94 unique SNPs. Leprosy appears to be an emerging zoonosis in the US and may be other parts of Americas. Detailed studies are required throughout the range of armadillo's natural habitat to identify any emerging infection and potential risk factors for inter–species transmission.

Talk #4

Interaction of *Mycobacterium tuberculosis* with innate immune cells

Alasdair Leslie

KwaZulu Natal Reseach Institute for TB and HIV (K-ITH), Durban, South Africa

The innate immune response is crucial to the control of human TB. Alveolar macrophages, neutrophils and dendritic cells are rapidly infected with mTB in the lung and the outcome of this interaction is likely to determine the subsequent course of infection. We use high-resolution label free quantitative mass spectrometry to study the effect of mTB infection in primary innate immune cells both in-vitro and ex-vivo. In vitro infection of primary dendritic cells with H37Rv and/or BCG followed by enrichment of phospho-peptides identifies novel reciprocal phosphorylation changes in sets of proteins involved in cell autonomous immunity to intracellular pathogens, including RAB7a and RAB7-GAP, which are involved in phagosome maturation; STAT1 and IRF9, which are crucial for the interferon response; GBP1 and Sequestosome1, involved in autophagy and the cytochromes p67pHOX, p91pHOX and p22pHOX (NOX2), which are central to the generation of reactive oxygen species. Furthermore, we detected significant regulation in several interesting proteins with no known role in mTB control and/or pathogenesis. In addition, we have used mass spectrometry to evaluate the neutrophil response to HIV/TB co-infection directly ex-vivo, as an alternative approach to studying this notoriously challenging cell type. This reveals striking changes in important neutrophil proteins, including decreased levels of MPO, SERPINB1 and NOX2 and increased levels of Cystatin A and GMFG. The potential of these as biomarkers for disease is under investigation.

Talk #5

The global challenge of drug resistant TB

Gail Cassell

Harvard Medical School

Gail H. Cassell is a Senior Lecturer, Department of Global Health and Social Medicine at Harvard Medical School and a Senior Research Scientist at Brigham and Women's Hospital. She is also currently Vice President of TB Drug Development at the Infectious Disease Research Institute (IDRI). Gail recently retired as Vice President, Scientific Affairs and Distinguished Lilly Research Scholar for Infectious Diseases at Eli Lilly and Company in Indianapolis where she was responsible for launching the Lilly MDR-TB philanthropic effort and establishing and leading the Lilly TB Drug Discovery Initiative, a nonprofit launched in 2007. Prior to Lilly, Gail served as the Charles H. McCauley Professor and Chairman of the Department of Microbiology at the University of Alabama Schools of Medicine and Dentistry at Birmingham.

Gail has served on a number of boards and committees, and is currently a member of the NIH Science Management Board, the newly appointed NIH Board of Trustees, and the Advisory Council of the Fogarty International Center of NIH. A member of the Institute of Medicine of the National Academy of Sciences, Gail has been widely recognized for her research accomplishments including two honorary degrees.

Gail received her BS from the University of Alabama in Tuscaloosa and obtained her PhD in Microbiology from the University of Alabama at Birmingham.

Talk #6

Towards understanding and combating resistance in tuberculosis

Sylvie Garneau-Tsodikova

Department of Pharmaceutical Sciences, University of Kentucky, BioPharm Complex, room 423, 789 South Limestone Street, 40536-0596, KY, USA.

The emergence and global spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) is an increasingly alarming health threat. We recently established the unprecedented mutli-acetyltransferase activity of Eis, the enzyme responsible for resistance to kanamycin A in a large fraction of *Mycobacterium tuberculosis* clinical isolates. Mechanistic studies of this unique resistance enzyme along with novel strategies and molecules that alleviate its activity in XDR-*Mycobacterium tuberculosis* strains will be presented.

Talk #7

Copper Ions in Disguise – A Novel Strategy to Attack Copper Homeostasis of *Mycobacterium tuberculosis*

Mehri Haeili[1,2], James B. Cochran[1] and Frank Wolschendorf[1]

[1]Department of Medicine, University of Alabama at Birmingham, Birmingham, USA
[2]Department of Microbiology, University of Tehran, Tehran, Iran

Macrophages contain invading bacteria in their phagosomes where they are killed by a variety of mechanisms including acidic pH, proteolytic enzymes and exposure to high concentrations of copper ions. *Mycobacterium tuberculosis (Mtb)* has evolved to evade these innate immune functions by stalling the phagosome maturation process rendering the otherwise bactericidal phagosome highly ineffective against the intruder. Therefore, drugs which replicate specific antimicrobial features of matured phagosomes may provide a novel opportunity to antagonize *Mtb*-corrupted phagosome functions. We developed a novel drug screening technology to identify compounds which potentiate the anti-mycobacterial activity of copper ions. A small compound library was screened in copper limiting and copper supplemented conditions. We identified several compounds and FDA approved drugs that act against growing, as well as non-growing *Mtb* bacilli in a strictly copper-dependent and copper-specific manner. These copper-boosting compounds (CBC) are membrane permeable. For that reason, the outer membrane, which reconstitutes the most important barrier for extracellular copper ions, has no protective function towards copper-loaded CBCs. Invisible to copper resistance proteins, CBCs enable and conceal the passage of copper ions across the mycobacterial cell envelop thereby facilitating their accumulation in the cytoplasm. We conclude that *Mtb* succumbs to the destructive action of free roaming intracellular copper ions which attack crucial metabolic functions.

Talk #8

Host-directed tyrosine kinase inhibitors as therapeutics for antibiotic-resistant TB

Daniel Kalman

Emory University, Atlanta GA

The lengthy course of treatment with currently used anti-mycobacterial drugs and the resulting emergence of drug-resistant strains have intensified the need for alternative therapies against Mycobacterium tuberculosis (Mtb), the etiologic agent of tuberculosis. The Abelson (ABL) tyrosine kinases (TKs) are essential for growth and differentiation of human cells and tissues, and ABL1 is dysregulated in human cancers such as chronic myelogenous leukemia (CML). We found that ABL and related host TKs are co-opted by a variety of viral and bacterial pathogen. We show that Mtb likewise use ABL and related tyrosine kinases for entry and intracellular survival within macrophages. In mice, the ABL-family tyrosine kinase inhibitor Gleevec, a frontline FDA-approved therapy for CML, reduced bacterial load. Gleevec was also effective against antibiotic-resistant strains, and acted synergistically when co-administered with current first-line antibiotics. Finally, Gleevec had potent immunostimulatory effects, which mimic a physiological innate immune response to infection. Together, these data implicate host ABL-family tyrosine kinases in mycobacteria pathogenesis, and suggest that Gleevec may have therapeutic efficacy against Mtb, including antibiotic resistant strains. Because Gleevec targets the host, it is less likely to engender resistance compared to conventional antibiotics. Moreover, Gleevec may decrease the development of resistance against co-administered antibiotics, and render ostensibly antibiotic-resistant strains now sensitive to antibiotics. Progress on the development of a human clinical trial will be discussed. This work was supported by a grant from Institute Merieux.

Talk #9

NIAID TB Research Program: Priorities, Funding and Collaborations in Global TB Research

Karen LaCourciere

NIH

Global control of tuberculosis (TB) requires the participation of multiple stakeholders that cross the spectrum of biomedical research, product development, and implementation and operational research. The National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), plays a critical role in TB biomedical research and product development by directly supporting and leveraging other funding support strategies and providing research resources to facilitate the translation of knowledge about TB into strategies and tools to more effectively combat disease. The primary mission of NIAID is to support high quality, peer reviewed, investigator initiated research that contributes to innovation in infectious disease research. NIAID offers a variety of funding mechanisms to support research from basic fundamental science, to translation research, preclinical research and clinical research. Funding supports research conducted by U.S. and international academic institutions, pharmaceutical companies, public private partnerships, research organizations and U.S. small businesses. It is also within the mission of NIAID to assure that research findings are translated into vaccines, diagnostics, and drugs to better prevent, diagnose, and treat this devastating disease. NIAID supports resources available to researchers to provide services including bioinformatics resources and databases, genome sequencing, research reagents, in vitro drug screening, animal model testing for drugs and vaccines, evaluation of diagnostic assays, preclinical services and clinical evaluation.

Talk #10

Lessons from a Tuberculosis Case in Alabama

Ed Khan

Jefferson County Department of Health

We will discuss a recent challenging case of tuberculosis diagnosed and treated in Alabama, which presented several unique challenges with regards to quarantining and treatment, and how modern molecular techniques have greatly assisted clinicians treating TB in making critical therapeutic decisions. The case is presented of a nineteen year old college freshman from West Africa who was beginning school at a local university. Shortly after her arrival in the U.S. she developed symptoms of TB and she was soon diagnosed by acid fast bacilli (AFB) smear and a rapid PCR based test (TB GeneXpert) to have active pulmonary tuberculosis. Additionally, the PCR test detected rifampin resistance, which is a herald sign for multi-drug resistance (MDR-TB) or other polyresistant profiles. An empiric MDR-TB regimen was designed for her, which was further modified after both rapid molecular testing of drug resistance from the CDC as well as phenotypic drug susceptibility results from the Alabama state lab as well as two other reference laboratories were reported. The challenges of managing such resistant cases are discussed, including monitoring for toxicities, and several of the newer agents being used which hold considerable promise for the future of MDR and XDR-TB treatment.

Talk #11

Cigarette smoke contributes to the development of drug resistance in *Mycobacterium tuberculosis*

Vikram Saini[1,2], Aisha Farhana[1], Ranjit Kumar[1], John Osborn[1], Krishna Chaytanya Chinta[1], Jason Freeman[1], Joel N. Glasgow[1], Eliot J Lefkowitz[1] and Adrie JC Steyn[1,2,3]

[1]Department of Microbiology, [2]UAB Centers for Free Radical Biology, AIDS Research and Emerging Infections and Emergency Preparedness, University of Alabama at Birmingham, AL 35294, USA, [3]KwaZulu-Natal Research Institute for Tuberculosis and HIV, Congella, Durban-4013, South Africa

Rationale: Cigarette smoke (CS) is considered one of the most important risk factors for tuberculosis (TB). However, the genetic mechanisms of how CS exacerbates TB are not clearly understood. In this study, we sought to identify mechanisms by which CS exposure alters *M. tuberculosis* (*Mtb*) pathogenesis.
Methods and Results: Using a novel *in vivo* assay, we have established that exposure to CS causes *Mtb* DNA damage. Next-generation genomic sequencing analysis of seven representative CS-exposed MDR and XDR *Mtb* strains revealed mutations in known target genes for anti-TB drugs. In addition to SNPs, we also scored for synonymous and non-synonymous mutations as well as indels, and observed that the largest insertion is 6 bp present in *rpoB*, the target for rifampicin. Whole genome microarray profiling of CS-exposed *Mtb* enabled us to identify an 'Mtb smoke regulon', which is comprised primarily of genes encoding membrane transporters, PE/PPE proteins, the *mce* operon and lipid metabolism machinery. Following chronic exposure of *Mtb*-infected mice to CS, we observed increased numbers of drug resistant bacilli from CS-exposed animals compared to unexposed animals.
Conclusions: These findings, for the first time, show that exposure to CS leads to the development of drug resistance in *Mtb*. The establishment of a role for CS in the emergence of drug-resistant TB has major public health and socio-political implications.

Talk #12

Investigation into Protective Mucosal Immunity in Fish against Mycobacteriosis

Martin Cheramie, Kristy Frady, Hunter McClendon, April Savoy, Meagan Bahlinger, Michael Kim, and Don G. Ennis

Department of Biology, University of Louisiana-Lafayette, Lafayette, LA.

Our lab investigates mycobacteriosis in an aquatic animal model, using *Mycobacterium marinum* (Mm) as the pathogen and Japanese medaka (*Oryzias latipes*) as the host. Mm shares at least 80% amino acid sequence identity with over 3,000 orthologous genes of *Mycobacterium tuberculosis*. Mm disease presentation in medaka parallels many aspects of human TB, including growth in macrophages, granulomatous lesions and life-long chronic disease. We have also shown that a major route of infection in fish is oral, such as ingestion of small invertebrate prey carrying Mm, including mosquito larvae. We are currently investigating how Mm is able to efficiently traverse the gut epithelia and then populate target organs, such as kidney, liver and spleen. We are also investigating if mucosal immunity against Mm can be induced in medaka. As a candidate fish vaccine strain, we are employing an attenuated Mm strain carrying the analogous “Region of Difference-1,” ten-gene deletion that was uncovered in the anti-TB vaccine strain, BCG. In two separate studies, fish that were first orally exposed to the Mm vaccine strain. The fish were found to be largely protected from a subsequent challenge by a virulent wild-type Mm. To test for even greater protection against virulent Mm, we are again orally vaccinating fish as before but adding a booster exposure. I anticipate that my studies will not only lead to insights into future live vaccines against mycobacteriosis, but also document how mycobacteria are able to breach the epithelia and initiate infections of target viscera.

Talk #13 – Keynote address

A zebrafish's guide to tuberculosis pathogenesis and treatment

Lalita Ramakrishnan

University of Washington, Seattle

We have developed as a model for tuberculosis the zebrafish infected with its natural pathogen *Mycobacterium marinum*, the closest genetic relative of the *Mycobacterum tuberculosis* complex. This model has enabled surprising discoveries that change our thinking about tuberculosis pathogenesis and suggest immediate therapeutic applications. The most current aspects of our work will be discussed.

Talk #14

Mycobacterial metabolism is regulated by lysine acetylation

Jenny Hayden*, Lanisha Brown*, Ellen Perkowski*, Harsha Gunawardena†, Xian Chen†, and Miriam Braunstein*

*Department of Microbiology and Immunology, †Department of Biochemistry and Biophysics.
University of North Carolina at Chapel Hill

Mycobacterium tuberculosis metabolism is critical for pathogenesis. While our understanding of the metabolic pathways *M. tuberculosis* uses during infection is increasing, very little is known about how those pathways are regulated. Because lysine acetylation is an important regulator of metabolism in other bacteria, we hypothesized it may regulate mycobacterial metabolism as well. Lysine acetylation is a protein modification in which an acetyl group is covalently attached to lysine residues. The modification can affect protein function, stability, and localization. Lysine acetylation is mediated by acetyltransferases, which add acetyl groups to lysine residues, and deacetylases, which remove the acetyl groups. In order to investigate the role of lysine acetylation in mycobacteria, we used acetyl-CoA synthetase (Acs) as a model acetylated protein. We confirmed that Acs is acetylated in *Mycobacterium smegmatis* cells and showed that Acs is required for acetate metabolism. By measuring acetate metabolism in various mutants, we identified that the lysine acetylation/deacetylation system in *M. smegmatis* cells is comprised of one acetyltransferase, PatA, and one deacetylase, SrtN. Now that we have defined the lysine acetylation regulatory circuit in mycobacterial cells, we can determine what other proteins are lysine acetylated and examine how various cellular signals are integrated into the lysine acetylation pathway.
Since there are obvious *M. tuberculosis* orthologs of the *M. smegmatis* acetyltransferase and deacetylase, we expect that in *M. tuberculosis* cells, PatA and SrtN also carry out lysine acetylation and deacetylation. If lysine acetylation regulates *M. tuberculosis* metabolism, this post-translational modification is likely to be crucial to pathogenicity

Talk #15

Mycobacterium tuberculosis Hip1 is a serine protease that cleaves GroEL2 and modulates macrophage responses

Maria Georgieva[1,*], Jacqueline L. Naffin-Olivos[2,*], Nathan Goldfarb[3,*], Ranjna Madan-Lala[1], Dagmar Ringe[2], Ben M. Dunn[3], Gregory A. Petsko[2, #] and Jyothi Rengarajan[1, 4, #]

[1]Emory Vaccine Center, Emory University, Atlanta, GA, USA, [2]Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA, USA, [3]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL, USA, [4]Division of Infectious Diseases, Department of Medicine, Emory University, Atlanta, GA, USA

[*]These authors contributed equally.

Mycobacterium tuberculosis (*Mtb)* employs multiple strategies to evade host immune responses and persist within macrophages. We have previously shown that the cell envelope-associated *Mtb* serine hydrolase, Hip1, prevents robust macrophage activation and dampens host pro-inflammatory responses, allowing *Mtb* to delay immune detection and accelerate disease progression. We now provide key mechanistic insights into the molecular and biochemical basis of Hip1 function. We establish that Hip1 is a serine protease with activity against protein and peptide substrates. Further, we show that the *Mtb* GroEL2 protein is a direct substrate of Hip1 protease activity. Cleavage of GroEL2 is specifically inhibited by serine protease inhibitors and is optimal at intraphagosomal pH conditions. We mapped the cleavage site within the N-terminus of GroEL2 and confirmed that this site is required for proteolysis of GroEL2 during *Mtb* growth. Interestingly, we discovered that Hip1-mediated cleavage of GroEL2 converts the protein from a multimeric to a monomeric form. Moreover, ectopic expression of cleaved GroEL2 monomers into the *hip1* mutant complemented the hyperinflammatory phenotype of the *hip1* mutant and restored wild type levels of cytokine responses in infected macrophages. Our studies point to Hip1-dependent proteolysis as a novel regulatory mechanism that helps *Mtb* respond rapidly to changing host immune environments during infection. These findings position Hip1 as an attractive target for inhibition for developing immunomodulatory therapeutics against *Mtb*.

Talk #16

Structure-based approach to identify inhibitors of mycosin protease from ESX-1 secretion system

Konstantin V. Korotkov

Department of Molecular & Cellular Biochemistry and Center for Structural Biology, University of Kentucky, Lexington, KY 40536

The ESX secretion system, also called type VII secretion system, is a unique multicomponent protein transport machinery that plays an important role in the virulence of pathogenic mycobacteria. In *Mycobacterium tuberculosis*, three paralogous systems — ESX-1, ESX-3 and ESX-5 — secrete their specific set of substrates during defined stages of infection process. Each system includes a substilisin-like protease (mycosin or MycP) as a core component essential for secretion. We solved the crystal structure of *M. thermoresistibile* $MycP_1$, the mycosin from the ESX-1 system. We also systematically characterized the specificity of $MycP_1$ using peptide libraries, and showed that it has evolved a narrow specificity relative to other subtilisins. In addition, to identify small molecules $MycP_1$ inhibitors, we developed an efficient 3D shape-based similarity algorithm including an effective 3D shape fitting procedure and a robust scoring function. Using this approach, we identified a number of compounds that show promising antagonistic activity in the $MycP_1$ inhibition assay. Although the potency of these small organic inhibitors is only moderate (IC_{50} 50-100 μM), this study clearly validates the efficiency of our virtual screening method and shows that it is possible to design a non-peptide, small-molecule inhibitor of $MycP_1$. These compounds thus represent a promising starting point for the development of potent small-molecule antagonists of $MycP_1$, with potential for development of novel anti-tuberculosis treatment.

Talk #17

Visualization and Manipulation of Calcium Dynamics in Inflammation and Infection

Rebecca W. Beerman[1], Molly A. Matty[1], Kingshuk Roy Choudury[2], Loren Looger[3], Philipp Keller[3], and David M. Tobin[1]

[1] Molecular Genetics and Microbiology, Duke University, Durham, NC. [2] Duke Radiology, Duke University, Durham, NC. [3] Howard Hughes Medical Institute, Janelia Farm Research Campus, Ashburn, Virginia.

Innate immune cells utilize calcium signaling during chemotaxis, phagocytosis and phagolysosome maturation. Pathogens can manipulate each of these processes to promote their own survival. Studies of immune cell calcium dynamics have largely been restricted to *ex vivo* and cell culture systems. Here we combine light sheet microscopy with genetically encoded calcium indicators to analyze whole-animal calcium dynamics in live animals during inflammation and infection.

We have developed multiple transgenic zebrafish lines to provide an unprecedented window into calcium signaling in neutrophils and macrophages as they migrate toward a wound site during the first steps of a sterile inflammatory response and in macrophages in the context of infection with diverse bacterial pathogens. We find that migrating neutrophils display unique calcium flux depending on the distance to inflammatory cues at a wound site. To assess the role of these patterns *in vivo*, we developed a broadly applicable approach to pharmacologically reprogram calcium levels in individual cell types. We demonstrate a functional requirement for calcium signaling in neutrophils *in vivo* in the context of wound healing and bacterial infection. Finally, we observe differential calcium signaling in macrophages infected with *Mycobacterium marinum* compared with other bacterial species. These findings represent the first *in vivo* analysis of immune cell calcium dynamics in whole animals during mycobacterial infection.

Talk #18

Host Cell Apoptosis and Necrosis in TB

Hardy Kornfeld

Department of Medicine, University of Massachusetts Medical School. Worcester, MA, USA

Mycobacterium tuberculosis (Mtb) is a facultative intracellular pathogen of macrophages. Following inhalation, the bacillus requires alveolar macrophages as a niche for initial replication in order to establish infection in a new host. In later stages of TB disease bacilli also populate the extracellular environment in the lung, which is a requirement for transmission. Alveolar macrophages lack innate effector mechanisms to restrict Mtb growth. They may respond to infection by undergoing TNF-mediated apoptosis which eliminate the replication niche and packages bacilli in apoptotic bodies that facilitate killing and/or antigen presentation but might also facilitate dissemination. Virulent Mtb strains suppress the apoptotic response to preserve their sanctuary that for optimal growth. After reaching a threshold intracellular burden in macrophages analogous to the burst size of some viruses, virulent Mtb triggers a primarily necrotic form of cell death. This death mode is independent of caspase-3, caspase-1, TNF, TLR4, MyD88, cathepsins B or L, free radicals of oxygen or nitrogen, and calcium flux. It involves widespread damage to lipid bilayers through depletion of host phospholipid species. That results in mitochondrial injury independent of Bax/Bak and disruption of plasma membranes that permits spreading infection to naïve alveolar and inflammatory macrophages, dendritic cells and neutrophils. The spectrum of secondary host cells shifts over the course of TB disease, reflecting both pathogen-specific and host-specific factors that will be discussed.

Talk #19

CpnT: A novel protein toxin of *Mycobacterium tuberculosis*

Jim Sun[1], Olga Danilchanka[1], Mikhail Pavlenok[1], Alexander Speer[1], Axel Siroy[1], Christian Maueröder[2], Luis E. Muñoz[3], Martin Herrmann[3], Christian Berens[2] and Michael Niederweis[1]

[1]Department of Microbiology, University of Alabama at Birmingham, [2]Department of Biology, Friedrich-Alexander-Universitaet Erlangen-Nuernberg, Erlangen, Germany, [3]Department of Internal Medicine 3 - Rheumatology and Immunology, Friedrich-Alexander-Universitaet Erlangen-Nuernberg, Erlangen, Germany

Mycobacterium tuberculosis (Mtb) is able to control the timing and mode of host cell death as a strategy to subvert the host bactericidal response. However, the exact molecular mechanism by which this process occurs is obscure. Death of Mtb-infected macrophages is characterized by typical features of necrosis which consists of metabolic collapse and loss of membrane integrity, and is therefore a method for Mtb to exit the destroyed cells, evade host defenses, and disseminate to other tissues and uninfected hosts. While Mtb is known to secrete virulence factors which interfere with phagosome maturation, it is unclear how Mtb kills macrophages. Several bacterial pathogens utilize protein toxins to kill host cells in order to evade immune responses, but such toxins are unknown in Mtb. Here, we identify and characterize a Mtb outer membrane protein which consists of an N-terminal channel domain involved in nutrient uptake and a secreted C-terminal toxin domain responsible for virulence. The toxin domain is a major determinant of Mtb cytotoxicity in macrophages, is required for intracellular survival in macrophages, and causes necrotic cell death in eukaryotic cells. Thus, this protein has been named CpnT (channel protein with necrosis inducing toxin), and represents a novel protein toxin in Mtb.

Posters

Poster #1

Development and evaluation of a reduced inoculation method for the Bactec MGIT 960 pyrazinamide drug susceptibility test

Alexandra Dubon Mercante, Glenn P. Morlock, Kelsey L. Hughes, and James Posey

Division of TB Elimination, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention

Pyrazinamide is a crucial first-line drug used to combat infections caused by *Mycobacterium tuberculosis*. Pyrazinamide is a prodrug that diffuses across the mycobacterial cell membrane where the enzyme pyrazinamidase, encoded by the gene *pncA*, converts pyrazinamide to its active form pyrazinoic acid. A major contributor to resistance is the numerous mutations that occur throughout *pncA* which result in a loss of pyrazinamidase activity making accurate detection of drug resistance imperative. The Mycobacterial Growth Indicator Tube (MGIT) system remains the preferred tool for phenotypic detection of susceptibility to pyrazinamide. Yet, the prevalence of false resistance due in part to a large inoculum size which impairs pyrazinamidase activity remains an obstacle. We developed a reduced inoculation procedure and compared this new method to the standard inoculation method by determining the minimal inhibitory concentration (MIC) of 121 isolates harboring a wild type *pncA* gene. Concordance between the two inoculation procedures occurred 86.4% of the time. Importantly the new method reduced false resistance by 13.6%. The reduced inoculation procedure was also used to determine the effect of 149 *pncA* mutations on the MIC for pyrazinamide. While 26.2% of mutations had no effect on the MIC for pyrazinamide, 57% of the mutations resulted in an MIC of >800 and only 9.4% of the mutations demonstrated an MIC between 200-600 µg/ml.

Poster #2

A novel component of SecA2 protein export in *Mycobacterium tuberculosis*

Brittany K. Miller**,** Lauren S. Ligon, Nathan W. Rigel, Miriam Braunstein

Department of Microbiology & Immunology, University of North Carolina, Chapel Hill, NC, USA

SecA2-dependent protein export in *Mycobacterium tuberculosis* is important for virulence and *ΔsecA2* mutants are attenuated in macrophages and mice. SecA2 is an ATPase that provides energy for exporting a small subset of proteins through the canonical SecYEG translocase. In *Mycobacterium smegmatis*, a mutation in the ATP binding region, referred to as SecA2KR, renders SecA2 nonfunctional and dominant negative. Mutations arise spontaneously that suppress *secA2KR* phenotypes. By sequencing extragenic suppressor mutations, our goal is to identify proteins that interact with SecA2. We have sequenced the genomes of six independent, extragenic suppressors of *secA2KR* that have mutations in a conserved, hypothetical protein denoted Msmeg1684. This suppression indicates that Msmeg1684 may be a novel component of SecA2 protein export.

Our preliminary work confirms that an *M. smegmatis Δmsmeg1684* mutant expressing SecA2KR suppresses the SecA2KR phenotype. Additionally, we've shown that we can complement an *Δmsmeg1684* mutant expressing SecA2KR with a plasmid expressing Msmeg1684. Msmeg1684 has a homolog in *M. tuberculosis* denoted Rv3311 (64% identical, 78% similar). Like *secA2, rv3311* was identified in a TraSH screen as an essential gene for survival in macrophages. We have shown that Rv3311 can complement an *Δmsmeg1684* mutant, indicating that these proteins have homologous functions in *M. tuberculosis* and *M. smegmatis* respectively. Our future work will focus on elucidating the function of Rv3311/Msmeg1684 and their role in SecA2-dependent export. Because Rv3311 homologs and SecA2 homologs are conserved together in Actinobactera, we anticipate that the role of Rv3311 in SecA2-dependent export is not limited to *M. tuberculosis* and *M. smegmatis*.

Poster #3

Host Genetics and Immunological Response to *Mycobacterium leprae* Promoting the Use of Monozygotic Twins in Research

L. Stump, G. Balamayooran, M.T. Pena, R. Sharma, V. Marks, G. McCormick, L. Adams and R.W. Truman.

Other than humans, nine-banded armadillos (*Dasypus novemcinctus*) are the only natural hosts of *Mycobacterium leprae*. They produce polyembryonic offspring, making them potentially ideal candidates for research regarding the immunological response to *M. leprae* exposure as it is influenced by genetics. To determine the degree of similarity between nine-banded armadillo twins and amass data that could be valuable in determining their effectiveness as an animal model, seven litters consisting of four naïve sibling armadillos, two litters consisting of two naïve sibling armadillos, and one group of four unrelated armadillos were examined. Gross measurements were taken, microsatellite analysis was performed to assess phylogenetic drift, and Lepromin test was used to determine the form of leprosy in each animal. Immunological response was assessed using mixed lymphocyte reactions and cytokine expression following mitogen stimulation. The percent coefficient of variation for weight of sibling litters 2 and 3 were only 4.13 and 5.46, respectively, while it was 14.89 for the outbred group. In lymphocyte proliferations, the mean stimulation of litter 3 littermates against each other was 1310.504 +/- 540.573 DPM, the mean stimulation of Litter 3 littermates against an unrelated animal was 2431.327+/- 742.25 DPM. For cytokine production, littermates grouped together as high or low responders within 1.5 logs of one another. Analysis confirms that siblings are morphologically and immunologically similar. Although these results indicate a higher degree of congruency among siblings than between sibling units and outbred animals, further research is necessary to draw conclusions on the usefulness of armadillo siblings to model leprosy.

Poster #4

Characterization of the secreted serine protease, Rv2223c of *Mycobacterium tuberculosis*

Erica Bizzell[1,2], Maria Georgieva[1,2] and Jyothi Rengarajan[1,3]

[1]Emory Vaccine Center. [2]Microbiology and Molecular Genetics Program, Graduate Division of Biological and Biomedical Sciences, Emory University. [3]Division of Infectious Diseases, Emory University.

Mycobacterium tuberculosis (*Mtb*) has evolved multiple strategies to evade host immune defenses and replicate within immune cells. These include alteration of its complex cell wall during intracellular growth, and secretion of effectors that modulate immune responses and enhance pathogen survival. Several pathogenic bacteria use extracellularly secreted proteases to regulate processes ranging from repression of cytokine production to degradation of surface-associated host proteins. While Mtb encodes several putative secreted proteases, their functions are poorly understood. We have been interested in characterizing the predicted Mtb protease, Rv2223c. *Rv2223c* is located in a predicted operon with a cell-wall associated serine protease, Hip1 (Rv2224c), with which it shares 52% amino acid identity. Hip1 is involved in modification of the *Mtb* cell wall during infection, and functions by dampening normal immune responses to *Mtb* infection. Using the model system *Mycobacterium smegmatis* expressing *Mtb* Rv2223c, we found that Rv2223c is secreted from mycobacterial cells in a signal-sequence dependent manner and undergoes further autoproteolytic cleavage upon secretion. Additionally, we have observed that Rv2223c interacts with the Hip1 physiological substrate, GroEL2, suggesting potentially overlapping or cooperative functions of these proteases. We hypothesize that Rv2223c is a secreted protease that functions by cleaving *Mtb* substrates and/or host cell substrates to modify the host response during *Mtb* infection. Biochemical analyses of Rv2223c to determine enzymatic activity, as well as pull-down and co-immunoprecipitation assays to identify novel bacterial and host interactors and/or substrates are currently being conducted. These studies will provide insights into the molecular functions of Rv2223c in *Mtb* pathogenesis.

Poster #5

Protein Localization in Mycobacteria by Electron Microscopy

Bradford Buck, Cindy Rodenburg, Terje Dokland, and Michael Niederweis

Department of Microbiology, University of Alabama at Birmingham

The outer membrane of *M. tuberculosis* provides an efficient permeability barrier that protects the bacterium from bactericidal molecules encountered during the course of infection. This barrier is functionalized by outer membrane proteins which are largely unknown for *M. tuberculosis*. The covalent attachment of the mycobacterial outer membrane (OM) to the cell wall and the complex cell envelope architecture impair classical methods to determine the subcellular localization of OM proteins. We have employed ultrathin cryosections and immuno-gold labeling to detect OM proteins in mycobacteria by electron microscopy. Preliminary experiments provided negatively-stained images of the wild-type *Mycobacterium smegmatis* cell envelope with much greater resolution than previously achieved. The inner and outer membranes are clearly visible. The distance between these membranes ranges from 15-26 nm in good agreement with cryo-electron tomography experiments indicating a good preservation of the native ultrastructure of mycobacterial cells. Immuno-gold labeling of ultrathin cryosections shows that MspA, a known outer membrane protein in *M. smegmatis*, is localized to the cell envelope. We anticipate that antibody fragmentation and colloidal gold conjugation will improve the spatial precision of protein detection in these experiments. The results of these preliminary experiments reveal the potential of immuno-gold labeling of cryosections to reliably localize proteins in the mycobacterial cell envelope by electron microscopy.

Poster #6

Detection of SNP in Mycobacterium tuberculosis using MNAzyme technology

Daniel Nierenberg, Yulia V. Gerasimova, Dmitry M. Kolpashchikov, and Kyle H. Rohde

Chemistry Department and Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, 4000 Central Florida Blvd, Orlando, FL 32816

Mycobacterium tuberculosis's capacity to attenuate the efficacy of anti-pathogenic drugs such as rifampicin, isoniazid, capreomycin, amikacin, and kanamycin through acquisition of single-nucleotide polymorphisms (SNPs) is a foremost concern in the treatment of tuberculosis. It is imperative to detect these drug-resistant mutant strains in a timely fashion in order to efficiently prescribe the appropriate antibiotic. Since conventional cell-culturing techniques may require days to weeks to accurately identify an infectious agent, more rapid means of detection should be implemented. An alternative approach involves a Multi-component Nucleic acid enzyme (MNAzyme) that utilizes an RNA-cleaving deoxyribozyme. A measurable fluorescent signal is produced once the deoxyribozyme cleaves a fluorogenic reporter substrate. In this study MNAzymes were employed to discriminate between rifampicin-resistant and rifampicin-susceptible strains of *M. tuberculosis*. Our focus entailed targeting SNPs in either the 526 or 531 codon regions of the *rpoB* gene which when mutated make the bacterium resistant to rifampicin. The MNAzyme's SNP-specific analyte-binding arm was designed to dissociate from its target analyte at 55°C when the analyte contained a single non-complimentary nucleotide. Synthetic analytes used were based upon the H37Rv strain of *M. tuberculosis* while BCG (Bacillus Calmette–Guérin**)** *M. bovis* DNA served as a non-synthetic analyte. MNAzymes demonstrated significant specificity discriminating wild-type and mutant strains. Further MNAzyme advantages include simplicity in design, time and cost-effectiveness, and requiring relatively fewer experimental materials.

Poster #7

Design, synthesis, and structure-activity relationship studies of antitubercular agents

Atefeh Garzan, Sylvie Garneau-Tsodikova

College of Pharmacy, University of Kentucky, 789 S. Limestone St, Lexington, KY 40536

Tuberculosis (TB) remains one of the leading causes of mortality worldwide, with approximately one-third of the world' s population infected with latent TB. Medical treatment for TB is complicated nowadays by the appearance of new multidrug and extensively drug-resistant (MDR and XDR) strains of *Mycobacterium tuberculosis* (*Mtb*), and therefore, new antibiotics are in great need. We recently revealed the unique function and structure of the mycobacterial enzyme Eis and confirmed its role in conferring resistance to aminoglycosides (AGs) used as second-line of defense against TB. We also revealed unprecedented positions being acetylated on a variety of AG scaffolds. We screened over 120,000 compounds *via* high-throughput screening (HTS) and identified new inhibitors of Eis from *Mtb* with high nanomolar to low micromolar activities. We are going to show design, synthesis, and structure-activity-relationship of highly potent antitubercular agents. Our preliminary studies led to the identification of series of molecules possessing an exceptional activity in the low nanomolar range against *Mtb*. These studies have the potential to lead to the better understanding of the mechanism of resistance in TB and to lead to identification of new combination therapy for XDR-TB.

Poster #8

Role of MARCO Scavenger Receptor in Resistance to Tuberculosis

Coulson G.B[1], Bowdish D.M.E[2], and Sakamoto, K[1]

[1]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA. [2]Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

Phagocytosis of *Mycobacterium tuberculosis* (*Mtb*) by human monocytes or macrophages is a fundamentally important step in the pathogenesis of this facultative intracellular parasite. While the role of complement receptors, mannose receptors and Fc-gamma receptors in host-pathogen interactions have been well established, relatively little is known about the role of scavenger receptors (SR) in tuberculosis, particularly the class A SR, MARCO. MARCO (macrophage receptor with collagenous structure) is constitutively expressed on the surface of only a small subset of macrophage populations, such as splenic, peritoneal, and alveolar macrophages. This receptor has been shown to function in the: i) binding and uptake of *Mtb*, ii) production of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) upon stimulation with *Mtb*, iii) restriction of mycobacterial replication, and iv) granuloma formation. Importantly, MARCO has been shown to bind and respond to trehalose dimycolate (TDM), a cell wall glycolipid and key virulence factor of *Mtb* that is linked to phagosomal maturation arrest *in vitro*. Our lab recently identified a number of genetic polymorphisms in the human MARCO gene associated with susceptibility (n=3) or resistance (n=1) to TB, suggesting that MARCO may play a larger role in pathogenesis than previously appreciated. Bioinformatic analysis of the SNP (rs7559955; "rSNP"), which was found to be strongly associated with resistance to disease, suggested that this rSNP sequence may have potential regulatory activity through the introduction of a novel DNA-binding site for the transcription factor, C/EBP (CAAT-enhancer binding protein). We thus hypothesized that this rSNP introduces a new regulatory site that may enhance the binding of C/EBP transcription factors, which would subsequently lead to an alteration in MARCO expression and associated downstream effects on binding/uptake of *Mtb*, cytokine production, and control of infection. To test this hypothesis, we performed a C/EBP pull-down assay and ELISA using a DNA probe specific for the ancestral ("wildtype") allele and compared C/EBP binding to it relative to the probe for the variant ("resistant") allele. Results from these experiments show that the single (C→T) nucleotide transition in the resistant allele significantly enhances DNA binding of two related C/EBP isoforms, C/EBPα and C/EBPβ, by ~30%. We next determined whether this enhanced C/EBP binding had any effect on transcriptional activity using a standard reporter assay. Five identical repeats of the ancestral DNA-binding site (ACTGC) or variant binding site (ATTGC) were cloned into a commercial vector (pGL4.23) containing a copy of the luciferase gene under transcriptional control of a minimal promoter, and the effect of these enhancer sequences on expression of luciferase was recorded. Our data shows that the enhanced binding of C/EBP to the cognate DNA-binding site observed in the variant allele is associated with significant repression of transcriptional activity (~21% activity relative to ancestral allele). Taken together, these findings suggest that in TB-resistant individuals with the specific rSNP (rs7559955) polymorphism, introduction of novel regulatory sequences by the SNP may lead to the down-regulation of MARCO expression, which may have subsequent effects on macrophage binding/uptake of *Mtb*, and associated inflammation and granuloma formation.

Poster #9

Generation of Recombinant Mycobacteria Expressing *M. ulcerans* Ag85A

Bryan E. Hart and Sunhee Lee

Duke Human Vaccine Institute, Duke University

Buruli ulcer, an emerging tropical disease caused by *Mycobacterium ulcerans* (*MU*), is characterized by disfiguring skin necrosis and high morbidity. Much is not understood about the mode of transmission, pathogenesis, or host immune responses to *M. ulcerans* infection. Due to significant reduction in quality of life for those afflicted with extensive tissue scarring, and to the fact that a disproportionately high percentage of those affected are children, a Buruli ulcer vaccine would be greatly beneficial to the worldwide community. Previous studies have shown that mice inoculated with either BCG or a DNA vaccine encoding Ag85A are temporarily protected against footpad swelling caused by intradermal challenge with *M. ulcerans*. Building upon this principle, we have generated live recombinant strains of mycobacteria heterologously expressing the immunodominant antigen, Ag85A, from *M. ulcerans*. These strains are capable of inducing proliferation of antigen-specific CD4+ T cells *in vivo* as shown by flow cytometric analysis of MHCII tetramer staining. Using IFNγ ELISPOT, we can show that mice vaccinated with these recombinant strains elicit functional splenocytes which recognize *MU* Ag85A and heat killed *M. ulcerans* better than BCG alone. To this date, both BCG and the recombinant strains protect mouse models equally. These lengthy challenge studies are still ongoing and future observation will determine if increased protection is conferred by the recombinant vaccine.

Poster #10

Dynamic changes in CD4$^+$ T cells activation markers associated with standard tuberculosis treatment in patients with active pulmonary tuberculosis: One-year follow-up study

Toidi Adekambi[1], Chris C. Ibegbu[1], Stephanie Cagle[2], Ameeta S. Kalokhe[1,2], Susan M. Ray[2], and Jyothi Rengarajan[1, 2]

[1]Emory Vaccine Center, [2]Division of Infectious Disease, Department of Medicine, Emory University, Atlanta, GA

Mycobacterium tuberculosis (Mtb) infection remains one of the world's major causes of illness and mortality with an estimated 2 million deaths each year. Treatment response among active pulmonary TB patients is presently monitored by clinical and microbiologic improvement, as measured by direct smear examination and culture of the respiratory samples. However, the higher rate of smear-negative TB disease (20-66%) and the long culture periods (6-8 weeks) for *Mtb* limit the use of these parameters. These limitations emphasize the need for improved TB diagnostics and surrogate biomarkers of response to TB treatment.
In this study, we investigated the *Mtb*-specific CD4$^+$ T cells activation markers in the peripheral blood mononuclear cells of 10 active pulmonary TB patients during standard tuberculosis treatment for one year. Before treatment, we found that CD38 expression on CD4$^+$IFN-g$^+$ T was high. During the 2 months of the intensive phase of anti-TB treatment (rifampicin + isoniazid + pyrazinamide + ethambutol), CD38$^+$IFN-g$^+$ T cells frequency decline rapidly. During the following 4-month of 2 drugs treatment (rifampicin + isoniazid), CD38$^+$IFN-g$^+$ T cells frequency decline slowly and remains constant after. No relapse was observed.
This data tend to mirror smear and culture conversion in the respiratory samples suggesting that CD38$^+$IFN-g$^+$ T cells may represent a "real time" indicator of *Mtb* load *in vivo* and may be characterized by sensitivity to TB chemotherapies. Thus, measuring activated *Mtb*-specific CD38$^+$ T cells subsets in the blood of TB patients can be used to predict bacillary clearance *in vivo*. These studies highlighted the fact that single biomarker such as CD38$^+$IFN-g$^+$ T cells may provide better understanding of resolution of *Mtb* infection in longitudinal studies.

Poster #11

Heme oxygenase-1 confers protection against *Mycobacterium tuberculosis* by restricting host immunoregulatory responses

Krishna Chaytanya Chinta[1], Vikram Saini[1, 2], Joel N Glasgow[1], Gene P. Siegal[3,] Anupam Agarwal[4], Veena B. Antony[5], Jessy Deshane[5] and Adrie JC Steyn[1, 2, 6]

[1]Department of Microbiology, [2]UAB Center for Free Radical Biology, [3]Department of Pathology, [4]Division of Nephrology, [5]Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, USA. [6]KwaZulu-Natal Research Institute for TB and HIV, Durban, South Africa

Heme oxygenase-1 (HO-1) is a redox sensitive, cytoprotective enzyme that restricts key immune responses and inflammatory reactions via the catabolism of heme into carbon monoxide (CO), iron and biliverdin. We have previously shown that HO-1 transcripts, protein levels, and enzymatic activity are increased in *Mtb*-infected macrophages and the lungs of infected mice. Further, CO generated by HO-1 induces the *Mtb* dormancy regulon via the heme sensor kinases DosS and DosT. While it is clear that HO-1 plays a role in *Mtb* persistence, its function in modulating immune responses to *Mtb* infection and the mechanisms of these responses is not known. We hypothesize that HO-1 is required for mounting specific host immune responses against *Mtb* and that HO-1 regulates *Mtb* disease progression. To test this hypothesis, we infected HO-$1^{+/+}$ and HO-$1^{-/-}$ mice with *Mtb* and monitored disease progression, mortality and host immune responses. We observed increased bacterial burden in lungs and spleens of HO-$1^{-/-}$ mice compared to HO-$1^{+/+}$ mice, with more infiltration of lymphocytes and increased fibrosis in HO-$1^{-/-}$ mice. FACS analysis revealed increased infiltration of myeloid-derived suppressor cells (MDSCs) with elevated percentages of Ly-$6C^{+}CD11b^{+}F4/80^{+}$ subsets which shifted to Ly-$6G^{+}CD11b^{+}F4/80^{+}$ subsets during the chronic stage of infection in HO-$1^{-/-}$ mice. High MDSC levels in HO-$1^{-/-}$ mice were associated with elevated levels of $CD4^{+}Foxp3^{+}$ regulatory T-cells and a high percentage of neutrophils in infected lungs. Our findings demonstrate that HO-1 regulates levels of MDSC, neutrophils and associated T-cell responses, thereby providing protection against *Mtb* disease progression.

Poster #12

High-throughput Spoligotyping on the Ion Torrent PGM platform

Paige Gupton, Lauren Cowan, Melisa Willby and Jamie Posey

National Center for HIV/AIDS, Viral Hepatitis, Sexually Transmitted Diseases and Tuberculosis Prevention, Centers for Disease Control and Prevention, Atlanta, GA

Spoligotyping, a method used for genotyping strains belonging to the *Mycobacterium tuberculosis* complex (MTBC), is based on polymorphisms found in the direct repeat locus. In most MTBC strains, this locus contains up to 68 unique spacer sequences separated by a 36 bp direct repeat. Previous spoligotyping assays consisted of amplification of spacer sequences followed with detection of spacer sequences by hybridization of ampilcons to probes immobilized on membranes or microspheres. Here we demonstrate that the Ion Torrent PGM can be used to detect the spacer sequences by direct sequencing of the amplicons. A set of 96 fusion primers were designed such that the amplicons are tagged with the sequencing primer and a unique barcode**.** To determine amplification variability among barcoded primers, a single sample was used for each of the 96 amplifications. The total number of mapped reads obtained with each barcoded primer ranged from 0–19,437 (avg 12,241). Results suggest that 5,000 reads/sample is sufficient and that spacers with >20 fold coverage are present, spacers with <1 fold coverage are absent. To determine accuracy, 270 previously spoligotyped samples were analyzed. The total number of mapped reads obtained per sample ranged from 1,239 to 28,653 (avg 16,844). The depth of coverage for present spacers ranged from 11 – 3,914 (avg 461.1) and for absent spacers ranged from 0 – 5 (avg 0.12). In conclusion, the Ion Platform is suitable for high-throughput spoligotyping. Future experiments are planned to determine the feasibility of sequencing additional amplicons in combination with spoligotyping.

Poster #13

Longitudinal changes in the functional abilities of BCG vaccination-induced T cells and its association with the protection against *M. tuberculosis*

Subhadra Nandakumar[1], Sunil Kannanganat[2], Bonnie B. Plikaytis[1], James E. Posey[1], Rama Rao Amara[2] and Suraj B. Sable[1]

[1]Division of Tuberculosis Elimination, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA; [2]Department of Microbiology and Immunology, Emory University, Atlanta, GA 30329, USA

Mycobacterium bovis bacille Calmette-Guerin (BCG) is the most widely used live attenuated vaccine. However, the correlates of protection and waning of its immunity against tuberculosis is incompletely understood. Here, we evaluated the magnitude, quality in terms of cytokine secretion, cytotoxic ability, memory and dysfunction of the T cell responses induced after mucosal or parenteral BCG (Copenhagen) vaccination of BALB/c mice in the draining lymph nodes (DLN), lungs and spleen at different time points encompassing entire life span, i.e. till 104 weeks after vaccination and its association with protection against *Mtb*. Our results demonstrate that the WCL-specific CD4 and CD8 T cell responses persisted for more than 52 weeks after BCG vaccination by either route, but wanes by week 78. BCG induced CD4 and CD8 T cell response was dominated by IFN-γ production. The protection in terms of reduction in *Mtb* CFU after challenge correlated with the peak of immune response at week 32, while decrease in T cell proliferative ability and increase in dysfunctional and senescent T cells paralleled waning of BCG induced immunity. Furthermore, BCG vaccination by either route induced compartmentalization of immune response in the respective DLNs which persisted for entire life. Overall, the BCG vaccine induces durable anti-*Mtb* immune responses of dynamic quality in mice and our results have implications for the use of BCG as a preclinical vaccine standard and for the development of effective prime-boost strategies against TB using mouse model.

Poster #14

Investigation of SecA2 export of an acid phosphatase by Mycobacterium tuberculosis

K. Zulauf, J.T. Sullivan, and M. Braunstein

Department of Microbiology and Immunology, University of North Carolina at Chapel Hill

Mycobacterium tuberculosis grows and replicates within the phagosome of alveolar macrophages. The ability of M. tuberculosis to replicate inside of macrophages is critical for virulence and the SecA2-dependent protein export system plays an essential role in this process by secreting a subset of effectors. Recently, we showed that SecA2 is required for phagosome maturation arrest and consequently growth of M. tuberculosis in macrophages. In order to understand the mechanism(s) by which the SecA2 system inhibits phagosome maturation, we are working to identify proteins exported by SecA2 that contribute to phagosome maturation arrest. One potential SecA2-dependent secreted substrate is the secreted acid phosphatase SapM. SapM has a predicted N-terminal Sec signal sequence and is required for virulence. Specifically, SapM dephosphorylates phosphotidylinositol 3-phosphate (PI3P) present on phagosomal membranes preventing the recruitment of early endosome antigen 1 (EEA1) and subsequent phagosome maturation. When SapM secretion was examined, the ΔsecA2 mutant of M. tuberculosis had a partial but significant decrease in the amount of secreted SapM. Furthermore, the ΔsecA2 mutant has a reduction in overall secreted phosphatase activity, which may be due to the reduction in SapM export. To investigate the contribution of SapM secretion by SecA2 in inhibiting phagosome maturation, we will restore SapM secretion in the ΔsecA2 mutant of M. tuberculosis and test if the restoration of SapM secretion can rescue the growth and phagosome maturation arrest defects of the ΔsecA2 mutant.

Poster #15

Generation of nonsynonymous single nucleotide polymorphisms in *pncA* using an *E. coli* mutagenesis system and assessment of their impact on both pyrazinamidase activity and pyrazinamide resistance

Kelsey L. Hughes, Kristin Birkness, Melisa Willby, Alexandra Dubon Mercante, and James Posey

Division of TB Elimination, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention

Pyrazinamide (PZA) is an essential first-line drug used to treat tuberculosis caused by *Mycobacterium tuberculosis*. The enzyme pyrazinamidase (PZase), which is encoded by the gene *pncA*, converts PZA to its active form, pyrazinoic acid (POA). PZA resistance is mostly associated with mutations in *pncA* that disrupt the activity of PncA and prevent the conversion of PZA to POA. Most mutations in *pncA* are nonsynonymous single nucleotide polymorphisms (nSNPs). Unlike other drug targets, PncA does not seem to have a defined resistance determining region, or hotspot. Based on database searches, we have identified mutations in approximately 72% of the codons. Due to this phenomenon, we decided to change each nucleotide to the other three nucleotides, starting at the start codon. This approach resulted in more than 1000 nonsynonymous substitutions possible in the gene. Using a mutagenesis kit and an *E. coli* system, we are creating each of these mutations in *pncA* and assaying for PZase activity. After adding a substrate, strains showing an intense color change are considered to have functional PZase activity and would be considered susceptible to PZA. An enzymatic assay appearing to have little or no color change shows that the mutation reduces or abolishes enzymatic activity and is therefore resistant to PZA. We have analyzed 121 *pncA* mutations (codons 1-36) using this system, and 17 (14%) demonstrate highly reduced or no PZase activity. The remaining mutations that we have tested have no observed effect on the activity of PncA and would not confer resistance to PZA.

Poster #16

Development of a Colorimetric Assay for Detection of Pyrazinamidase Activity of *pncA* Mutants

Kristin Birkness, Kelsey L. Hughes, Melisa Willby, Alexandra M. Mercante, Glenn P. Morlock, and James E. Posey.

National Center for HIV/AIDS, Viral Hepatitis, Sexually Transmitted Diseases and Tuberculosis Prevention, Centers for Disease Control and Prevention, Atlanta, GA

Pyrazinamide (PZA) is a powerful first-line prodrug used in the treatment of tuberculosis (TB). Pyrazinamide is converted to its functional derivative through the action of a pyrazinimidase (PZase) enzyme encoded by the *pncA* gene, and loss of PZase activity results in resistance to PZA. Clinical isolates resistant to PZA often contain mutations in *pncA.* However, mutations are not localized to a discrete region of *pncA* but are found throughout the entire gene. For molecular detection of PZA resistance to be feasible, the relationship between each mutation identified and susceptibility to PZA must be determined. We have developed a simple, colorimetric assay to assess functionality of mutant PncA alleles. An *E. coli* strain with its native *pncA* deleted was engineered to express an *M. tuberculosis pncA* allele of interest. The bacteria are grown in the presence of PZA, a colorimetric substrate is added, and the development of a red color indicates a functional PZase suggesting susceptibility to PZA. Lack of any color change indicates a nonfunctional PZase and suggests resistance to PZA. In this study, we compared the PZA minimal inhibitory concentrations (MIC) of strains harboring *pncA* mutations to the enzymatic assay results. Results correlated for 87.5% (77 of 88) of isolates, while for 10.2% (9 of 88) of isolates they did not. Two isolates had contradictory MIC results, therefore correlation cannot be determined until this is resolved. The enzymatic assay described in this study represents an alternative technique for predicting resistance to PZA.

Poster #17

Prime-boost vaccination strategies against tuberculosis: influence of persisting primed T-cell immunity

Subhadra Nandakumar[1], Sunil Kannanganat[2], Megan Lucas[3], Karen Dobos[3], Bonnie B. Plikaytis[1], James E. Posey[1], Rama Rao Amara[2] and Suraj B. Sable[1]

[1]Division of Tuberculosis Elimination, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA; [2]Department of Microbiology and Immunology, Emory University, Atlanta, GA 30329, USA; [3]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523, USA.

Heterologous prime-boost vaccination regimens using BCG and protein-subunit vaccines are effective strategies to promote durable and stronger T-cell responses against *Mycobacterium tuberculosis* (*Mtb*). Here, we longitudinally followed the expansion and contraction of T-cells in BCG-primed BALB/c mice using *Mtb* Apa and evaluated Apa protein-subunit as a BCG-booster vaccine. Apa induced a strong T-cell response following parenteral (S/C) or mucosal (I/N) BCG vaccination, and the antigen-specific T-cell response was characterized by polyfunctional CD4 T cells and IFN-gamma single-positive CD8 T cells, which peaked at 32-52 weeks but waned significantly by 78 weeks after BCG administration. When the timing of Apa-DDA-MPL booster was compared, a single mucosal (I/N) Apa boost administered during waning of BCG response increased the magnitude of Apa- or whole cell lysate (WCL)-specific T-cells superior than when the booster was administered at the peak of response. Mucosal (I/N) BCG prime-Apa subunit boost was more effective than the mucosal (I/N) Apa subunit prime-BCG boost in enhancing the magnitude of specific T-cell responses. However, parenteral (S/C) BCG prime-Apa protein subunit boost by the homologous route was the most effective strategy in terms of boosting of specific T-cell responses in the lungs and spleen. Two S/C Apa subunit booster doses significantly improved waning BCG immunity in elderly mice and imparted significant protection against *Mtb* challenge, regardless of protein mannose modifications and the form of Apa used. Overall, our results demonstrate the marked influence of time to boost, order and route of prime-boost vaccination on the booster outcome.

Poster #18

Development and Evaluation of a High Throughput Assay for Detection of Drug Resistance in *Mycobacterium tuberculosis* on a Next Generation Sequencing Platform

Melisa Willby and James E. Posey

National Center for HIV/AIDS, Viral Hepatitis, Sexually Transmitted Diseases and Tuberculosis Prevention, Centers for Disease Control and Prevention, Atlanta, GA.

Rapid and accurate detection of drug resistance is a critical tool in the battle to eliminate tuberculosis infection. While growth based susceptibility tests such as agar proportion are regarded as the gold standard, they are far from rapid. In recent years, molecular techniques such as Sanger sequencing, pyrosequencing and real-time PCR based methods including Gene Xpert have emerged that are capable of detecting resistance associated mutations within targeted regions of a microbial genome in a matter of hours. These techniques enable more timely application of treatment regimens tailored to individual patients. Unfortunately, these methods are not practical for country level surveillance of drug resistance trends. We have utilized next generation sequencing technology based on the Ion Torrent Personal Genome Machine platform to develop a multiplexed assay that is efficient and cost-effective for evaluation of up to 96 specimens for the presence of genetic markers associated with resistance to the most common first and second-line drugs. In the initial pilot study we assayed 8 isolates whose resistance profiles had previously been phenotypically and genetically characterized. Reads were generated from all isolates for all targets with a coverage depth ranging from 31-5903 reads. All previously known mutations located within the assay regions were identified within the Ion Torrent data. In a subsequent pilot experiment, 48 well characterized isolates were assayed. Initial evaluation of previously phenotypically and genetically characterized isolates shows promise, but the data also highlight areas for further improvement.

Poster #19

Exploiting Marine Natural Products for TB Drug Discovery

Carolina Rodrigues Felix[1], Amy Wright[2], and Kyle H. Rohde[1]

[1]Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL. [2]Harbor Branch Oceanographic Institute, Florida Atlantic University, Ft. Pierce, FL

The enormous disease burden caused by *Mycobacterium tuberculosis* coupled with ineffective drug treatments and the emergence of multidrug-resistant strains have led the World Health Organization to declare tuberculosis (TB) a global public health emergency. There is an urgent need for new drugs with novel targets able to kill dormant *Mtb* and thus shorten treatment time. Our goal is to exploit the unique chemical diversity of marine natural products (MNP) to identify novel drugs active against latent *Mtb.* Antimicrobial activity of ~5000 MNP peak fractions are being tested using 3 fluorescence-based whole cell screening assays: i) *in vitro* liquid culture, ii) during extended growth inside J774 macrophages, and iii) under multiple-stress, dormancy-inducing *in vitro* conditions. The two fluorescent readouts include *Mtb* expressing mCherry and addition of the viability indicator Alamar Blue dye post-treatment. Subsequent confirmation of primary hit activity, potency, and specificity (lack of cytotoxicity) is conducted using alternate assays. We are also evaluating a new dual color reporter strain *Mtb::pVVRG* (mCherry+, GFP+) for HTS applications. In a pilot screen of 160 MNP peak fractions against *in vitro* and intracellular *Mtb::*mCherry, 10 hits with significant activity and minimal cytotoxicity were identified including two hits with selective activity against intracellular *Mtb.* Detailed characterization of one selective hit for which we have structural data is underway. Full screens of MNP libraries under all three model conditions are also ongoing. In conclusion the MNP have demonstrated potent antimycobacterial activity, possibly targeting *Mtb* intracellular survival pathways which have not yet been explored as antibacterial targets.

Poster #20

Infection of A549 human type II epithelial cells with *Mycobacterium tuberculosis* induces changes in mitochondrial morphology, distribution and mass that are dependent on the early secreted antigen, ESAT-6

K. Fine-Coulson, F. D. Quinn, B. J. Reaves

Department of Infectious Diseases, University of Georgia, Athens, GA

Pulmonary infection by *Mycobacterium tuberculosis (Mtb)* involves the invasion of alveolar epithelial cells, however host-pathogen interactions in these cells are just starting to be characterized. In electron microscopy studies we observed extensive redistribution and remodeling of mitochondria in A549 type II human alveolar epithelial cells infected with the virulent strain *Mtb* Erdman at late stages of infection, specifically 72 and 96 hours post-infection (hpi). In order to further investigate these changes, we used Mitotracker Red® to assess changes in mitochondrial morphology/ distribution and mass from 6-72 hpi by both confocal microscopy and flow cytometry respectively. Early in infection there was no apparent effect on mitochondrial morphology, however, by 36-48 hpi mitochondria appeared fragmented with small spheres clustered in a perinuclear localization. In addition, the median intensity fluorescence (MFI) in flow cytometry experiments decreased by 30% (average of 3 experiments) at 48 hpi with a further decrease at 72 hpi suggesting a reduction in mitochondrial mass. Mitotracker Red ®accumulation is dependent on mitochondrial membrane potential (Ψm), so these results could indicate either a reduction in Ψm or a direct effect on numbers and structure of mitochondria. Extensive colocalization of Mitotracker Red® with the integral membrane protein COXIV at 48 hpi indicated that the decrease in numbers and changes in distribution/morphology of the mitochondria were due to direct effects on structure. In contrast, mitochondria in cells infected with the non-virulent strain *M. bovis* BCG appeared similar to uninfected control cells. A virulence factor present in *Mtb* Erdman that is lacking in *M. Bovis* BCG is the early secreted antigen, ESAT-6 which has been shown to be important for infection in type II pneumoncytes. Therefore, we performed similar experiments using the parental strain *Mtb* Erdman, an ESAT-6 deletion mutant and its complement. MFI decreased in the WT and complemented strains versus uninfected controls by 65% and 45% respectively; no decrease was detected in the deletion mutant. These results indicate an involvement of ESAT-6 in the perturbation of mitochondria by virulent *Mtb*. Current studies are addressing whether this is a direct effect of ESAT-6 on mitochondrial membranes and whether other co-effectors are necessary.

Poster #21

Exploration of a class of small molecule inhibitors of Mycobacterium tuberculosis lipases

Brian Garrett[1], Quentin Bubb[2], and Saskia Neher[1]

[1] Department of Biochemistry and Biophysics, UNC-Chapel Hill, [2] Biophysics Department, Johns Hopkins University

Mycobacterium tuberculosis, the causative agent of tuberculosis, is a slow growing bacterium that can remain dormant in an individual's lungs for decades. With multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains of TB emerging, novel therapeutics and therapeutic targets must be explored. Out of the 4,000 genes that comprise the M. tuberculosis genome, approximately 250 genes are involved in lipid metabolism including lipases and other serine hydrolases. M. tuberculosis relies heavily on this lipid metabolism during growth and re-growth periods. Lipases are important for mobilizing host lipids to be re-esterified and repackaged within the bacteria and utilizing stored lipids for energy. However, only a single triacylglycerol (TAG) lipase, LipY, has been identified as an important but non-essential enzyme for mycobacterial survival. To identify novel inhibitors of LipY and other potential lipases important for M. tuberculosis survival, we tested a library of 120 different small molecules for their ability to inhibit LipY activity. Our screen revealed several compounds as potentially potent inhibitors. We next want to test the activity of select compounds in vivo. Lastly, we are working to identify all of the serine hydrolases secreted by M. tuberculosis using activity based protein profiling (ABPP), with the goal of identifying additional lipases and other important enzymes key to bacterial growth and survival.

Poster #22

A multicopper oxidase is required for copper resistance in *M. tuberculosis*

Jennifer L Rowland and Michael Niederweis

Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL

Copper is required for life but toxic at high concentrations. Mammalian immune systems take advantage of this duality and use copper as a bactericidal weapon to combat infections. Macrophages increase the expression of several genes encoding copper transporters in response to bacterial infections and increase the copper concentration of phagosomes. Resistance mechanisms such as copper extrusion and sequestration are required for virulence of *M. tuberculosis* (*Mtb*). Some bacteria also use multicopper oxidases to detoxify copper. These proteins often oxidize organic substrates, but some are capable of oxidizing metals including copper. Here, we describe a multicopper oxidase encoded by *rv0846c* and show its involvement in copper resistance of *Mtb*. *In vitro* assays showed that Rv0846c oxidizes both organic substrates and Fe(II). Hence, Rv0846c is a multicopper oxidase of *Mtb* and was renamed mycobacterial multicopper oxidase (MmcO). Deletion of *mmcO* increased the susceptibility of *Mtb* to copper by at least ten-fold to a MIC lower than that for other copper-susceptible mutants. Importantly, mutation of the predicted copper coordinating cysteine 486 resulted in inactive MmcO and loss of copper resistance. These results establish MmcO as a major component of copper resistance in *Mtb*. MmcO is membrane associated, likely through lipidation after export across the inner membrane by the TAT system. Loss of lipidation did not alter oxidase activity or copper resistance of *Mtb*. Our study revealed MmcO as an important copper resistance mechanism of *Mtb*, which probably acts by oxidation of toxic Cu(I) to less toxic Cu(II) in the periplasm.

Poster #23

Constructing regulatable mycobacteria mutants to identify and validate Tat specific inhibitors

N. Scott, J.Hayden, M. Braunstein

Department of Microbiology and Immunology, University of North Carolina at Chapel Hill

Tuberculosis, caused by the bacterium *Mycobacterium tuberculosis*, is the second leading cause of infectious disease related death worldwide and the leading cause of mortality in HIV populations. Multidrug resistant (MDR) and extensively drug resistant (XDR) *M. tuberculosis* strains pose a serious risk to tuberculosis control efforts. Developing novel drugs to treat tuberculosis is critical.

The *M. tuberculosis* Twin Arginine Translocation (Tat) pathway is a potential novel drug target. The three main components of the Tat pathway are the membrane proteins TatA, TatB, and TatC. We are developing mycobacterial whole cell assays to identify and validate Tat specific inhibitors. One set of assays requires mycobacterial strains in which Tat expression is regulatable. When a specific Tat component is overexpressed, an inhibitor that targets that Tat component will be less effective. However, when the level of a specific Tat component is reduced, an inhibitor that targets that Tat component will be more effective. Conditional *tat* mutants are being constructed in *M. smegmatis* using tetracycline regulated promoters driving *tat* expression. Strain construction is a multi-step process. Using Gateway® cloning vectors, we have completed the first steps for both, *tatAC* and *tatC.* After validating each Tat inhibitor in *M. smegmatis*, conditional *tat* mutants will be constructed in *M. tuberculosis* to further confirm inhibitor activity as being Tat specific. In addition to identifying and validating Tat inhibitors, the regulatable mutants we are constructing will also be useful for detailed studies of the Tat system.

Poster #24

Development of qRT-PCR Assays to Determine *Mycobacterium leprae* Viability in Tissues and Application to Drug Screening Assessments

Linda B. Adams, Ramanuj Lahiri, and Diana L. Williams

DHHS, HRSA, HSB, National Hansen's Disease Programs, Baton Rouge, LA

Mycobacterium leprae does not grow in axenic medium and has a 12-14 day generation time. Therefore, specialized techniques are required to ascertain viability. Our goal was to develop simple and sensitive qRT-PCR assays to determine *M. leprae* viability in tissues and screen anti-leprosy drugs in the mouse foot pad (MFP) model. *M. leprae* were inoculated into athymic *nu/nu* MFP which allow prolific bacterial growth, and BALB/c MFP where bacilli are killed via an immune response. Infected *nu/nu* were also administered rifampin or rifapentine by 3 regimens (1, 5, or 20 daily doses) or ofloxacin, minocycline, gatifloxacin, moxofloxacin, and rifampin-ofloxacin-minocycline (ROM) by the 5 daily doses regimen. Mice were rested 1 month post treatment completion before harvest. *M. leprae* multiplication and viability were monitored using non-molecular assays (AFB counts, radiorespirometry, viability staining) on freshly harvested bacilli and molecular assays (RLEP PCR, qRT-PCR of *esxA* and *hsp18* transcripts) on nucleic acids purified from ethanol-fixed MFP. *esx*A and *hsp18* were sensitive indicators of *M. leprae* viability. Molecular and non-molecular assays demonstrated bacterial growth and viability in *nu/nu* MFP. In contrast, viability declined in immunocompetent mice. Rifapentine, gatifloxacin, moxifloxacin, and ROM reduced bacterial viability after 5 treatments. Rifampin required up to 20 treatments for similar efficacy. Molecular-based assays are simple, rapid, and reliable indicators of *M. leprae* viability in tissues and facilitate in vivo evaluation of potential leprosy drugs. Moreover, molecular assays allow quantification and viability assessment on ethanol-fixed tissues without bacterial isolation or immediate processing, making them promising for clinical and field applications.

Poster #25

BCG vaccination did not protect armadillos against experimental infection with *M. leprae*

Maria Pena[1,4], Rahul Sharma[1,4], Ray Waters[2], Malcolm Duthie[3], Tom Gillis[4], Linda Adams[4] and Richard W. Truman[4].

[1]Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana-70803, USA. [2]United States Department of Agriculture, agricultural Research Service, National Animal Disease Center, Bacterial Diseases of Livestock Research Unit, PO box 70, Ames, Iowa 50010-0070, USA. [3]Infectious Disease Research Institute, 1124 Columbia Street, Suite 400, Seattle, Washington, 98104, USA. [4]Department of Health and Human Services, Health Resources and Services Administration, National Hansen's Disease Program, Baton Rouge, Louisiana-70803, USA

Although multi-drug therapy has reduced leprosy prevalence rates worldwide, more than 200,000 new cases are reported annually and there is a need for prophylactic interventions such a leprosy specific vaccines and immunotherapies. BCG has been widely used for years in endemic countries with varying protection levels against leprosy in different locales. In this study we vaccinated 12 armadillos with BCG and challenged them along with 10 unvaccinated armadillos with intravenous inoculation of live *M. leprae*. We monitored the armadillos for immune responses and clinical signs of leprosy as disease progressed to dissemination. Assessing survival, we saw that 75% of the vaccinated armadillos survived to 19 months post-challenge compared to only 30% of the non-vaccinated animals. BCG conveyed some protection, although not statistically significant. More importantly, vaccinated and unvaccinated armadillos showed nerve involvement as early as 6-8 months post-challenge. In addition, at 7 months after challenge about 70% of the armadillos in both groups had detectable PGL-1 IgM antibodies levels. The results of this study show that BCG vaccine is not highly effective protecting armadillos against *M. leprae* experimental inoculation as both vaccinated and unvaccinated groups show clinical signs of leprosy such as nerve impairment, seropositivity to PGL-, and dissemination of the bacilli in the RES with similar bacteria load. However, armadillos can be used effectively to model vaccination with *M. leprae* and provide important insights with regards to the most salient features of leprosy pathology. Further studies may help elucidate new intervention strategies to better control leprosy and benefit patients.

Poster #26

Mycobacterium tuberculosis impairs dendritic cell functions through the serine hydrolase Hip1

Ranjna Madan-Lala, Jonathan Kevin Sia, Rebecca King, Toidi Adekambi, Leticia Monin, Shabaana A Khader, Bali Pulendran and Jyothi Rengarajan

Mycobacterium tuberculosis (Mtb) is a highly successful human pathogen that primarily resides in host phagocytes, such as macrophages and dendritic cells (DCs), and interferes with their functions. While multiple strategies used by Mtb to modulate macrophage responses have been discovered, interactions between Mtb and DCs are less well understood. DCs are the primary antigen presenting cells (APCs) of the immune system and play a central role in linking innate and adaptive immune responses to microbial pathogens. In this study we show that Mtb impairs DC cytokine secretion, maturation and antigen presentation through the cell envelope-associated serine hydrolase Hip1. Compared to wild type, a *hip1* mutant strain of Mtb induced enhanced levels of the key T helper 1 (Th1)-inducing cytokine IL-12, as well as other proinflammatory cytokines (IL-23, IL-6, TNF-α, IL-1β, IL-18) in DCs via MyD88- and TLR2/9-dependent pathways, indicating that Hip1 restricts optimal DC inflammatory responses. Infection with the *hip1* mutant also induced higher levels of MHC class II and co-stimulatory molecules, CD40 and CD86, indicating that Mtb impairs DC maturation through Hip1. Further, we show that Mtb promotes sub-optimal antigen presentation, as DCs infected with the *hip1* mutant showed increased capacity to present antigen to OT-II- and early secreted antigenic target 6 (ESAT-6)-specific transgenic CD4 T cells and enhanced Th1 and Th17 polarization. Overall, these data show that Mtb impairs DC functions and modulates the nature of antigen-specific T cell responses, with important implications for vaccination strategies.

Poster #27

Exported *In vivo* Technology (EXIT) to identify *Mtb* proteins exported during infection

Ellen Perkowski and Miriam Braunstein

Department of Microbiology and Immunology, University of North Carolina

Exported proteins of *Mtb* are well positioned to interact with the host, and are important to virulence. Although multiple strategies have been used to identify exported proteins, they are all limited by their use of *in vitro* grown bacteria. Using a β-lactamase reporter, we developed EXIT (Exported *in vivo* Technology) as a system to identify proteins that are exported by *Mtb* during *in vivo* infection. BlaTEM-1 is an exported protein that confers β-lactam resistance when expressed by *Mtb*. When expressed without its signal sequence, the truncated BlaTEM-1 (‘Bla) is not exported and does not confer β-lactam resistance. However, if an exported protein is fused to ‘Bla the resulting fusion protein is exported and confers β-lactam resistance. In EXIT, we adapted this reporter system to select for exported β-lactam resistant ‘Bla fusion proteins during *Mtb* infection of β-lactam treated mice. EXIT identified over 500 *Mtb* proteins as exported during infection. Of these proteins, 94% have a predicted signal peptide or transmembrane domain. Many of these proteins have no prior experimental evidence of export. Additionally, with an average of 16 fusions per ORF, EXIT provides topology information for integral membrane proteins. Finally, we hypothesized that some *Mtb* exported proteins are missed by current methods that rely on *in vitro* grown *Mtb* (either through regulation of expression or export). Comparison of exported *Mtb-‘Bla* fusions *in vivo* and *in vitro* identified 29 proteins as being exported to significantly higher levels during infection. Current experiments are focused on identifying the role of these proteins during infection.

Poster #28

Discovery and characterization of inhibitors of Mycobacterium tuberculosis primase DnaG

Caixia Hou, Tapan Biswas, Esteban Resto-Roldan, Sean K. Sawyer, Irina Artsimovitch and Oleg V. Tsodikov

Department of Pharmaceutical Sciences, University of Kentucky Lexington KY

Bacterial DNA primase DnaG generates RNA primers required for chromosomal DNA replication. In this study, we expressed and purified the previously uncharacterized primase DnaG from Mycobacterium tuberculosis (Mtb DnaG). By coupling the activity of Mtb DnaG to that of another essential enzyme, inorganic pyrophosphatase from M. tuberculosis (Mtb PPiase), we developed the first non-radioactive primase–pyrophosphatase assay. An extensive optimization of the assay enabled its efficient use in HTS (Z′ = 0.7 in the 384-well format). HTS of 2560 small molecules to search for inhibitory compounds yielded several hits, including suramin, doxorubicin and ellagic acid. We demonstrate that these three compounds inhibit Mtb DnaG. Both suramin and doxorubicin are potent (low-μM) DNA- and nucleotide triphosphate-competitive priming inhibitors. The rational discovery of inhibitors of M. tuberculosis DnaG enables one to exploit this attractive enzyme as an anti-tuberculosis drug target.

Poster #29

Evaluating the effects of pro-cell-death Mycobacterium tuberculosis mutants on virulence and immunogenicity

Kristen Smith and Sunhee Lee

Duke University

Mycobacterium tuberculosis (Mtb) is an intracellular pathogen with several known survival mechanisms, one of which is its ability to inhibit apoptosis in host alveolar macrophages. It has been shown that apoptotic bodies from cells infected with *Mtb* are able to induce T cell-mediated immune responses against the pathogen. By screening an *Mtb* H37Rv transposon library, we have identified 22 pro-apoptotic mutants. We hypothesize that these pro-apoptotic mutants will have increased immunogenicity when compared to the parental strain and may have potential as novel vaccine strain candidates.

An *in vitro* screening method was utilized to identify those mutants with enhanced immunogenicity when compared to the parental strain, leading to the selection of fourteen for *in vivo* study. Through intracellular cytokine staining and IFN-gamma ELISPOT, three mutants were found to have enhanced immunogenicity *in vivo.* Not only did the mutants enhance the CD4+ T cell response, but they also induced greater activation of CD8+ T cells. Bacterial survival of these mutants in a C57BL6 mouse model of infection is also reduced, suggesting a loss of virulence. Further immunological evaluation of these mutants is currently ongoing to evaluate their potential as vaccine strains. The mechanisms by which mycobacterial gene products inhibit apoptosis in wild-type H37Rv are also being studied. Taken together, the outcomes of this work will improve our knowledge of how a successful human pathogen subverts an important host response, which may lead to an improved *Mtb* vaccine.

Poster #30

Aerosol Generation of Pyrazinoic Acid Esters for Pulmonary Delivery

Phillip G Durham[1], Linbin Zhong,[2] John T. Welch[2], Anthony J Hickey[1]

[1]RTI International, Research Triangle Park, North Carolina. [2]Department of Chemistry, University at Albany, NY 12222

Pyrazinamide (PZA) is known to act synergistically with other first line antituberculous agents. Unfortunately, PZA efficacy diminishes with use and PZA doses are limited by hepatic toxicity. PZA is a prodrug for the active agent pyrazinoic acid (POA) that is revealed on hydrolysis. Pyrazinoic acid esters(POAE) display lower minimum inhibitory concentrations than PZA however these esters are hydrophobic and exhibit poor oral bioavailability. The purpose of this study is to formulate and deliver POAE as a therapeutic aerosol directly to the lungs to treat pulmonary tuberculosis.

Two liquid esters of pyrazinoic acid (*n*-propyl pyrazinoate, 2-methyl-propyl pyrazinoate) were prepared. Esters dissolved in ethanol (EtOH) and phosphate buffered saline (PBS) were atomized via vibrating mesh nebulizer. Solution nebulization performance was characterized by output rate and emitted dose via timed emittance and filter deposition. Filter was assayed by UV-Vis spectrophotometry. Aerosol particle size was determined by time-of-flight analysis.

The time to dry for a solution of 15:25:60 %v/v (POAE:EtOH:PBS) was 3:18, at an output rate of 90.73 µL/minute. Total drug mass emitted was 26.82 mg for a mass rate of 8.11 mg/minute. Droplets had a mass median aerodynamic diameter of 2.92 µm and a geometric standard deviation of 1.6, with approximately 22% of mass below 2 µm.

Vibrating mesh nebulizers allow the use of small drug quantities to achieve high chamber concentrations. Nebulizer and formulation performance resulted in a practical output rate and droplet size for delivery of therapeutic doses to test animals by passive respiration.

Poster #31

Engineering tools for chemoenzymatic synthesis of novel aminoglycosides

Yijia Li and Sylvie Garneau-Tsodikova

Department of Pharmaceutical Sciences, University of Kentucky, BioPharm Complex, 789 South Limestone Street, 40536-0596, KY, USA.

Tuberculosis (TB), caused by *Mycobacteria tuberculosis*, kills over 2 million people each year worldwide. With the emergence of resistance to all the currently used anti-TB drugs, there is a need for the development of novel molecules for treatment of this devastating lung disease. The aminoglycosides (AGs) are used as second line of defense against TB. However, AGs are inactive in extensively-drug resistant (XDR) strains of *M. tuberculosis*. In this project, we developed mutants of aminoglycoside-modifying enzyme, AAC(6')/APH(2''), to be used as tools for the chemoenzymatic synthesis of novel AGs to be used as potential TB treatment.

Poster #32

Single-chain MspA for nanotechnology

Mikhail Pavlenok and Michael Niederweis

Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL

Nanopore DNA sequencing is a promising next-generation sequencing technology. In this method, individual single-stranded DNA molecules are detected as they are driven through nanometer-scale pore by an external electric field. *Mycobacterium smegmatis* porin A (MspA) is currently the best channel protein for nanopore sequencing of DNA with high specificity of distinguishing the four nucleobases. However, MspA's composition of eight subunits makes it impossible to introduce asymmetric changes in the pore, which would be ideal to optimize the properties of the MspA pore for DNA sequencing. We constructed single-chain MspA (scMspA) where all eight subunits are covalently linked. Expression of *scmspA* in *Mycobacterium smegmatis* was reduced to 7% wt *mspA* level. Western blot showed that scMspA was as stable as wt MspA when protein sample was boiled for 15 min in the presence of 2% SDS. To test if scMspA forms functional channels *in vivo* we performed bilayer experiments. Addition of scMspA resulted in a step-wise increase in the current indicating insertion of the channels into the lipid membrane. Analysis of the current recordings showed that scMspA had a well-defined conductance peak of 1.1 nS. The resistance of scMspA to voltage gating is drastically increased from ±40mV for wt MspA to ±100mV. This is beneficial under high voltage conditions of DNA sequencing. Overall, the ability to introduce asymmetric changes into the pore, the more coherent channel conductance, and the increased voltage resistance of scMspA are important improvements of the physical and chemical properties of the MspA pore for DNA sequencing.

Poster #33

Homologs of a *Mycobacterium tuberculosis* resistance enzyme: Insights into novel antibiotic resistant bacteria

Keith D. Green[1], Rachel E. Pricer[2], Megan N. Stewart[3], Sylvie Garneau-Tsodikova[1]

[1]College of Pharmacy, University of Kentucky, 789 S. Limestone St, Lexington, KY 40536, [2]Chemical Biology Doctoral Program, University of Michigan, Ann Arbor, MI 48109, [3]Department of Medicinal Chemistry, University of Michigan, Ann Arbor, MI 48109

Tuberculosis (TB) has become a worldwide threat, mainly due to the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Part of the resistance problem stems from the over-expression of the enhanced intracellular survival (Eis) protein, due to a mutation in the promoter of the corresponding gene. Previously, Eis was shown to modify kanamycin A, a hallmark of XDR-TB. Recently, we have conducted studies with Eis and several of its homologs to show that they not only acetylates a wide range of aminoglycosides, but also inactivate these drugs by catalyzing multiple acetylations on a single aminoglycoside molecule. In addition to our biochemical and structural studies of Eis, we used high-throughput screening to identify several classes of small-molecule inhibitors of this enzyme, which deter the acetylation of aminoglycosides.

Poster #34

Analysis of the role of *cobK* in vitamin B_{12} biosynthesis in mycobacteria

Samantha Tucker, Benjamin Grosse-Siestrup, Song Kue, Kimberly Romano, Frederick Quinn, and Russell Karls

Department of Infectious Diseases, University of Georgia, Athens, GA

Cobalamin (vitamin B_{12}) is a complex molecule that functions as the cofactor for multiple enzymes; however, its production is exclusive to bacteria. Aerobic synthesis of vitamin B_{12} requires up to 30 genes. *Mycobacterium tuberculosis* appears to have a full complement of B_{12} synthesis genes, however, extracts from *M. tuberculosis* fail to enable growth of a *Salmonella* B_{12} auxotroph. In contrast, extracts from *M. smegmatis* confer growth of the auxotroph. One possibility for the differing results may be due to the diversity between predicted cobalamin biosynthesis homologs in these species. For example, the amino acid sequence of the predicted precorrin-6x reductase enzyme CobK from *M. smegmatis* bears only 70% homology with *M. tuberculosis* CobK. A Δ*cobK* mutant was created in *M. smegmatis* to help to analyze B_{12} biosynthesis in mycobacteria and to compare the functionality of the CobK homologs in *M. smegmatis* and *M. tuberculosis*. Utilizing the *Salmonella* B_{12} auxotroph-feeding assay, the results indicate that *cobK* is necessary for production of vitamin B_{12} in *M. smegmatis*, and that the *cobK* gene from either *M. smegmatis* or *M. tuberculosis* can restore vitamin B_{12} synthesis. Experiments to characterize the form(s) of vitamin B_{12} produced by different *Mycobacterium* species are in progress

Poster #35

Site-directed mutagenesis of a potential metal-binding motif in *Mycobacterium tuberculosis* sigma factor C

Song Kue, Benjamin Grosse-Siestrup, Megan Prescott, Frederick Quinn, and Russell Karls

Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA

Transcription sigma factor SigC is a known *Mycobacterium tuberculosis* virulence factor in small animal models. Our global transcription studies suggest that sigma factor SigC influences transcription of genes involved in trace metal transport. Short-term artificial induction of *sigC* in Middlebrook 7H9 medium resulted in elevated levels of an uncharacterized cation transport gene, while long-term induction led to induction of trace metal efflux and detoxification genes. Surprisingly, after *sigC* induction in 7H9-cultured cells only very low levels of SigC protein was detected by western blot using an antibody to a hexahistidine tag fused to SigC. However, SigC was quite stable when *M. tuberculosis* was cultured in Sauton medium. Trace metals present in 7H9 medium but lacking in Sauton medium support a hypothesis that SigC stability varies inversely with availability of specific trace metals. Examination of the amino acid sequence of SigC revealed the presence of a potential metal-binding motif. Mutagenesis of residues in the motif resulted in increased stability of SigC in *M. tuberculosis* cultured in 7H9 medium. These results suggest that SigC may function as a direct trace metal sensor. Experiments are underway to define which metals are directly sensed by SigC.

Poster #36

Efficacy testing of aerosol delivered CPZEN-45 in the treatment of tuberculosis in guinea pigs.

Ellen Young*, Ellen Perkowski**, Phil Durham[ii], Miriam Braunstein* and Anthony Hickey[ii]

*Microbiology and Immunology Dept., University of North Carolina at Chapel Hill, [ii]RTI International, Research Triangle Park, NC

There is an urgent need to develop new treatments for tuberculosis, as the World Health Organization estimates there were 500,000 new cases of MDR-TB in 2011[1]. CPZEN-45 (caprazene 4-butylanilide), a derivative of the liponucleoside antibiotic caprazamycin B, is a promising new antituberculosis drug candidate. CPZEN-45 has excellent antituberculosis activity and has been shown to work on drug resistant *Mycobacterium tuberculosis* strains[2]. However, CPZEN-45 is poorly bioavailable by conventional routes of administration. As an approach for improving CPZEN-45 bioavailability and treatment potential, we tested the treatment efficacy of delivering aerosolized CPZEN-45 to the lungs of *M. tuberculosis* infected guinea pigs. For this purpose we formulated spray dried CPZEN-45 into particle sizes suitable for aerosol delivery. We hypothesized that direct delivery of CPZEN-45 to the lungs of infected guinea pigs would result in high local concentrations and better eradication of *M. tuberculosis*. We then used the CPZEN-45 particles in the following infection and treatment protocol. Groups of guinea pigs were first infected by low dose aerosol with virulent *M. tuberculosis*. At 4 weeks post-*M. tuberculosis* infection, daily aerosol CPZEN-45 treatment was initiated for 4 additional weeks. At the end of the treatment phase, the guinea pigs were sacrificed. Bacterial burden and histology was assessed in the lung and spleens of untreated versus treated animals. The results of our preliminary experiment showed aerosol delivered CPZEN-45 to be effective in reducing the bacterial burden in the lungs with respect to untreated controls by more than one log. In addition, no adverse effects were observed from the aerosol delivered CPZEN-45.

[1] http://www.who.int/tb/challenges/mdr/MDR_TB_FactSheet.pdf
[2] *Engohang-Ndong, J. (2012) J. Antibiot. 38, 1617-1621.*

Poster #37

Untargeted metabolomic discovery of a redox hierarchy in mycobacteria and human tuberculosis

Loni Guidry[1], Vikram Saini[1], Bridgette Cumming[3], John H. Adamson[3], Joel N. Glasgow[1], Aisha Farhana[1], Amit Singh[4], Anaximandro Gomez-Velasco[5], Yossef Av-Gay[5], Horacio Bach[6], Victoria Kasprowicz[3], Jacques H. Grosset[3,9], Deepak Almeida[3,9], Hyungjin Eoh[7], Kyu Rhee[7], and Adrie J.C. Steyn[1,2,3,8]

[1]Department of Microbiology, [2]Centers for AIDS Research and Free Radical Biology, University of Alabama at Birmingham, Birmingham, AL, USA. [3]KwaZulu-Natal Research Institute for Tuberculosis and HIV, Durban, South Africa. [4]International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067. [5]Division of Infectious Diseases, University of British Columbia, Vancouver, BC, Canada. [6]Department of Medicine, University of British Columbia, Vancouver, BC, Canada V6H 3Z6. [7]Department of Medicine, Weill Cornell Medical College, New York, NY, USA. [8]Department of Pathology, Nelson Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa. [9]Center for Tuberculosis Research, Division of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

A major challenge in the study of *Mycobacterium tuberculosis* (*Mtb)* is to understand how redox balance is maintained during disease. Here, we report the metabolomic and genetic discovery that the antioxidant ergothioneine (EGT) is regulated by the *Mtb* 4Fe-4S redox sensor and virulence factor, WhiB3. EGT is a thiol-based antioxidant, which has long remained enigmatic and has been the subject of much speculation. We demonstrate that EGT is regulated by levels of lipid precursors including acetate and propionate, linked to mycothiol (MSH) production and the sigma factor SigH, and involved in antimycobacterial drug resistance and antioxidant defense. Comprehensive transcriptomic analysis of EGT and MSH-deficient *Mtb* strains identified a subset of 25 commonly regulated genes that constitute the *Mtb* redoxome. These data demonstrate that EGT and MSH regulate overlapping genes involved in free radical-mediated regulation of DNA and sulfur metabolism, secretion, toxin/anti-toxin, lipid metabolism and cytochrome biogenesis. Notably, mass spectrometry analyses of the plasma of TB and HIV patients show that active TB is associated with lower ERG levels whereas no difference was observed between healthy and TB/HIV patients. This study demonstrates that EGT is a component of a previously unrecognized hierarchy of redox buffers to help protect *Mtb* against a graded spectrum of host-generated free radicals. We also showed that EGT is essential for *Mtb* survival upon infection and that *Mtb* is not an exogenous source of EGT *in vivo*. The findings have significant clinical implications considering the lack of knowledge of how *Mtb* maintains redox balance during long-term persistence.

Meeting Attendees

1. **Rodrigo Abreu**
   Graduate Student
   Quinn Lab
   UGA
   rbabreu@uga.edu
2. **Linda B. Adams**
   Senior Research Scientist
   DHHS, HRSA, HSB
   National Hansen's Disease Programs
   ladams@hrsa.gov
3. **Toidi Adekambi**
   Research Associate
   Rengarajan Lab
   Emory University
   tadekam@emory.edu
4. **Ahmad Al-Mestarihi**
   Postdoctoral Fellow
   Garneau-Tsodikova Lab
   University of Kentucky
   ahmad.almestarihi@uky.edu
5. **Veena Antony**
   MD
   Pulmonary, Allergy & Critical Care Medicine
   UAB
   vantony@uab.edu
6. **Gayathriy Balamayooran**
   Postdoctoral Fellow
   Truman Lab
   gmahad1@tigers.lsu.edu
7. **Rebecca Beerman**
   Postdoctoral Fellow
   Tobin Lab
   Duke University
   rebecca.beerman@duke.edu
8. **Hillary Bengtson**
   Graduate Student
   Kolpashchikov/Rohde Labs
   University of Central Florida
   hillary.bengtson@knights.ucf.edu
9. **William Benjamin**
   Professor
   Clinical Pathology
   UAB
   bbenjami@uab.edu
10. **Kristen Birkness**
    Posey Lab
    CDC
    kab1@cdc.gov
11. **Erica Bizzell**
    Graduate Student
    Rengarajan Lab
    Emory University
    ebizzel@emory.edu
12. **Miriam Braunstein**
    Professor
    Dept. of Microbiology and Immunology
    UNC-Chapel Hill
    miriam_braunstein@med.unc.edu
13. **Brad Buck**
    Graduate Student
    Niederweis Lab
    UAB
    brbuck@uab.edu
14. **Sam Cartner**
    Animal Research Services and Director
    Assistant VP ARP
    UAB
    scartner@uab.edu
15. **Gail Cassell**
    Senior Lecturer
    Senior Research Scientist
    HMS/Brigham and Women's Hospital
    gail.h.cassell@gmail.com
16. **Martin Cheramie**
    Graduate Student
    Ennis Lab
    U. Louisiana-Lafayette
    mnc1680@louisiana.edu
17. **Krishna Chinta**
    Research Assistant
    Steyn Lab
    UAB
    chait@uab.edu
18. **Barry Cochran**
    Research Assistant
    Wolschendorf Lab
    UAB
    cochrjb@uab.edu

19. **Garry Coulson**
    Postdoctoral Fellow
    Sakamoto Lab
    University of Georgia
    gcoulson@uga.edu

20. **Marilyn Crain, MD**
    Department of Pediatrics
    UAB School of Medicine
    mcrain@Peds.uab.edu

21. **Mark Cronan**
    Postdoctoral Fellow
    Tobin Lab
    Duke University
    mark.cronan@duke.edu

22. **Kathryn Doornbos**
    Graduate Student
    Niederweis Lab
    UAB
    doornbos@uab.edu

23. **Phillip Durham**
    RTI International
    pdurham@rti.org

24. **Don Ennis**
    Professor
    U. Louisiana-Lafayette
    dge5893@louisiana.edu

25. **Susan Farmer**
    Director
    Zebrafish Research Facility
    UAB
    sfarmer@uab.edu

26. **Carolina Rodrigues Felix**
    University of Central Florida
    carolinarodriguesfelix@knights.ucf.edu

27. **Marina Fosso**
    Postdoctoral Fellow
    Garneau-Tsodikova Lab
    University of Kentucky
    marina.fosso@uky.edu

28. **Sylvie Garneau-Tsodikova**
    Associate Professor
    University of Kentucky
    sylviegtsodikova@uky.edu

29. **Brian Garrett**
    Graduate Student
    Neher Lab
    UNC-Chapel Hill
    cbgarret@email.unc.edu

30. **Atefeh Garzan**
    Postdoctoral Fellow
    Garneau-Tsodikova Lab
    University of Kentucky
    atefeh.garzan@uky.edu

31. **Maria Georgieva**
    Graduate Student
    Rengarajan Lab
    Emory University
    mgeorgi@emory.edu

32. **Pramod Giri**
    Associate Research Scientist
    Infectious Diseases/ Vet. Med.
    University of Georgia
    pgiri@uga.edu

33. **Keith Green**
    Senior Research Associate
    Garneau-Tsodikova Lab
    University of Kentucky
    kgr234@uky.edu

34. **Tuhina Gupta**
    Assistant Research Scientist
    Infectious Diseases
    UGA
    tgupta@uga.edu

35. **Paige Gupton**
    Associate Service Fellow
    Posey Lab
    CDC
    pgupton@cdc.gov

36. **Debra Haas**
    Graduate Student
    Infectious Diseases
    UGA
    dhass@uga.edu

37. **Mehri Haeili**
    Graduate Student
    Wolschendorf Lab
    UAB
    m_haeli@yahoo.com

38. **Bryan Hart**
    Postdoctoral Fellow
    Lee Lab
    Duke University
    bryan.hart@dm.duke.edu

39. **Jenny Hayden**
    Postdoctoral Fellow
    Braunstein Lab
    UNC-Chapel Hill
    jenny_hayden@med.unc.edu

40. **Tony Hickey**
    Distinguished Fellow
    RTI International
    ahickey@rti.org

41. **Caixia Hou**
    Research Scientist
    Tsodikov Lab
    University of Kentucky
    chou2@uky.edu

42. **Kelsey Lauren Hughes**
    ORISE Fellow
    Posey Lab
    CDC
    wye0@cdc.gov

43. **Daniel Kalman**
    Associate Professor
    Emory University
    dkalman@emory.edu

44. **Suman Karki**
    Postdoctoral Fellow
    Antony Lab
    UAB
    sumank@uab.edu

45. **Russell Karls**
    Associate Research Scientist
    University of Georgia
    rkarls@uga.edu

46. **Ed Khan**
    MD
    Jefferson County Health Dept.
    edward.khan@jcdh.org

47. **Hardy Kornfeld**
    Professor
    U. Massachusetts
    hardy.kornfeld@umassmed.edu

48. **Konstantin Korotkov**
    Assistant Professor
    University of Kentucky
    kkorotkov@uky.edu

49. **Song Kue**
    Research Technician
    Quinn/Karls Labs
    University of Georgia
    song_kue@yahoo.com

50. **Karen LaCourciere**
    Program Officer
    NIAID
    karen.lacourciere@nih.gov

51. **Monica LaGatta**
    Research Technician
    Quinn/Karls Labs
    University of Georgia
    mlagatta@uga.edu

52. **Kajal Larson**
    Postdoctoral Fellow
    Acosta Lab
    UAB
    kbuck@uab.edu

53. **Alasdair Leslie**
    Assistant Investigator
    K-RITH
    al.leslie@k-rith.org

54. **Yijia (Selina) Li**
    Graduate Student
    Garneau-Tsodikova Lab
    University of Kentucky
    selina.li@uky.edu

55. **Ranjna Madan-Lala**
    Research Associate
    Rengarajan Lab
    Emory University
    rmadanl@emory.edu

56. **Seidu Malik**
    Postdoctoral Fellow
    Braunstein Lab
    UNC-Chapel Hill
    maliks@med.unc.edu

57. **James Mazorodze**
    Graduate Student
    Steyn Lab
    K-RITH
    hove@uab.edu

58. **Virginia Meikle**
    Research Associate
    Niederweis Lab
    UAB
    vmeikle@uab.edu

59. **Alexandra Dubon Mercante**
Postdoctoral Fellow
Posey Lab
CDC
wip2@cdc.gov

60. **Brittany Miller**
Graduate Student
Braunstein Lab
UNC-Chapel Hill
bcreeden@email.unc.edu

61. **Ninell P. Mortensen**
Research Biologist
Discovery, Science, Technology
RTI International
nmortensen@rti.org

62. **Subhadra Nandakumar**
Research Microbiologist
CDC
snandakumar@CDC.gov

63. **Michael Niederweis**
Professor
Dept. of Microbiology
UAB
mnieder@uab.edu

64. **Daniel Nierenberg**
Post-Baccalaureate
Kolpashchikov Lab
University of Central Florida
danielnierenberg@gmail.com

65. **Oliver Shey Njila**
Graduate Student
Quinn/Karls Labs
University of Georgia
osnjila@uga.edu

66. **Simon Owino**
Postdoctoral Fellow
Quinn/ Karls Labs
UGA
sowinoo@pathens.com

67. **Ashish K. Pathak**
Advanced Research Scientist
Southern Research Institute
pathaka@southernresearch.org

68. **Mikhail Pavlenok**
Graduate Student
Niederweis Lab
UAB
pavlenok@uab.edu

69. **Maria Pena**
Scientist
NHDP LRB/SVM LSU
Louisiana State University
maria65@lsu.edu

70. **Ellen Perkowski**
Graduate Student
Braunstein Lab
UNC-Chapel Hill
foot@email.unc.edu

71. **James Posey**
Team Leader
Division of Tuberculosis Elimination
CDC
jposey@cdc.gov

72. **Megan Prescott**
Graduate Student
Quinn/Karls Labs
University of Georgia
mp01031@uga.edu

73. **Fred Quinn**
Department Head, Professor
Infectious Diseases
University of Georgia
fquinn@uga.edu

74. **Lalita Ramakrishnan**
Professor
Microbiology and Medicine
U. Washington
lalitar@uw.edu

75. **Barbara Reaves**
Associate Research Scientist
University of Georgia
bjreaves@uga.edu

76. **Vineel Reddy**
Postdoctoral Fellow
Steyn Lab
UAB
vineel@uab.edu

77. **Jyothi Rengarajan**
Assistant Professor
Emory Vaccine Center
Emory University
jrengar@emory.edu

78. **Kyle Rohde**
Assistant Professor
Burnett School of Biomedical Sciences
University of Central Florida
kyle.rohde@ucf.edu

79. **Jennifer Rowland**
Graduate Student
Niederweis Lab
UAB
rowlandj@uab.edu

80. **Suraj Sable**
Senior Staff Scientist
Division of Tuberculosis Elimination
CDC
dpt7@cdc.gov

81. **Vikram Saini**
Postdoctoral Fellow
Steyn Lab
UAB
vsaini@uab.edu

82. **Ninecia Scott**
Post-Baccalaureate
Braunstein Lab
UNC-Chapel Hill
nrscott@email.unc.edu

83. **Santosh Shah**
Postdoctoral Fellow
Wolschendorf Lab
UAB
sshah142@email.arizona.edu

84. **Rahul Sharma**
Postdoctoral Fellow
Truman Lab
Louisiana State University
rsharm4@lsu.edu

85. **Kevin Sia**
Graduate Student
Rengarajan Lab
Emory University
jsia@emory.edu

86. **Kristen Smith**
Graduate Student
Lee Lab
Duke University
kristen.jurcic@duke.edu

87. **Alex Speer**
Postdoctoral Fellow
Niederweis Lab
UAB
speerax@uab.edu

88. **Adrie Steyn**
Associate Professor, UAB
Investigator, K-RITH
asteyn@uab.edu

89. **Jim Sun**
Postdoctoral Fellow
Niederweis Lab
UAB
jsun14@uab.edu

90. **Ranu Surolia**
Postdoctoral Fellow
Antony Lab
UAB
rsurolia@uab.edu

91. **David Tobin**
Assistant Professor
Dept. of Mol. Gen. and Microbiology
Duke University
david.tobin@duke.edu

92. **Oleg Tsodikov**
Associate Professor
Dept. of Pharmaceutical Sciences
University of Kentucky
ovts222@uky.edu

93. **Samantha Tucker**
Graduate Student
Karls Lab
UGA
sltucker@uga.edu

94. **Melisa Willby**
Microbiologist
CDC
mwillby@cdc.gov

95. **Frank Wolschendorf**
Assistant Professor
Dept. of Medicine
UAB
fwolsche@uab.edu

96. **Ellen Young**
Research Associate
Braunstein Lab
UNC-Chapel Hill
younge@med.unc.edu

97. **Kate Zulauf**
Graduate Student
Braunstein Lab
UNC-Chapel Hill
kzulauf@email.unc.edu

Notes