6th Southeastern Mycobacteria Meeting

Keynote Speaker: David Russell, Ph.D.
Cornell University, Ithaca, NY

Organizing Committee:
Miriam Braunstein, Ph.D. (University of North Carolina, Chapel Hill)
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Christopher Whalen, M.D. (University of Georgia, Athens, GA)
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Meeting Program

Friday, February 3, 2017

6:30 p.m. – 9:30 p.m.  Meeting registration and welcome reception

All meeting events are in the UGA Center for Continuing Education and Hotel, 1197 South Lumpkin Street, Athens, GA 30602.

Stop by the Conference Registration Desk to pick up meeting materials and name badges.

Please wear your name badge to the welcome reception located in the Pecan Tree Galleria and to all other meeting events.

Saturday, February 4, 2017

7:00 a.m. – 8:30 a.m.  Breakfast  (Magnolia Ballroom)

8:30 a.m. – 12:00 p.m.  Oral presentations  (Mahler Hall)

12:00 p.m. – 1:00 p.m.  Lunch  (Magnolia Ballroom)

12:30 p.m. – 1:45 p.m.  Poster session I  (Hill Atrium and Mahler Hall)

2:00 p.m. – 3:35 p.m.  Oral presentations  (Mahler Hall)

3:35 p.m. – 4:25 p.m.  Keynote Address: David Russell  (Mahler Hall)

4:30 p.m. – 6:15 p.m.  Poster session II  (Hill Atrium and Mahler Hall)

7:00 p.m. – 11:00 p.m.  Dinner and entertainment  (Magnolia Ballroom)

Sunday, February 5, 2017

9:00 a.m. – 11:35 a.m.  Oral presentations  (Mahler Hall)

11:35 a.m. – 12:00 p.m.  Awards and meeting survey  (Mahler Hall)

12:00 p.m. – 1:00 p.m.  Lunch  (Hill Atrium)
Detailed Meeting Schedule

Friday, February 3, 2017

6:30 – 9:30 p.m.  Registration (Conference Registration Desk)
UGA Center for Continuing Education and Hotel,
1197 South Lumpkin Street, Athens, GA 30602

6:30 – 9:30 p.m.  Welcome reception (Pecan Tree Galleria)
UGA Center for Continuing Education and Hotel

Saturday, February 4, 2017

7:00 – 8:30 a.m.  Breakfast (Magnolia Ballroom)
A full breakfast will be served.

Registration (Conference Registration Desk)
For anyone who did not pick up their packet on Friday

8:00 – 8:30 a.m.  Powerpoint presentation setup (Mahler Hall)
Speakers should upload their talks to the podium computer in Mahler Hall.
Posters can also be set up at this time in the Hill Atrium and Mahler Hall.
Poster numbers are listed above the abstracts in this booklet.

8:30 – 8:45 a.m.  Opening remarks (Mahler Hall)
Dr. David Lee, UGA Vice President for Research
Dr. Harry Dickerson, Assoc. Dean for Research, UGA College of Vet. Med.
Dr. Frederick Quinn, Chair, Dept. of Infectious Diseases

Oral presentations session I – Moderator: Russell Karls (Mahler Hall)

8:45 – 9:05 a.m.  Tuhina Gupta, University of Georgia
Ferrets as a transmission model for tuberculosis

9:05 – 9:25 a.m.  Carolina Rodrigues Felix, University of Central Florida
Killing dormant Mycobacterium tuberculosis with marine-derived natural products

9:25 – 9:45 a.m.  Martin Cheramie, St. Jude Children’s Research Hospital
In with the old: modifying and repurposing antibiotics to confront emerging and re-emerging infectious diseases

9:45 – 10:05 a.m.  Avishek Mitra, University of Alabama at Birmingham
PPE surface proteins are required for heme utilization by Mycobacterium tuberculosis
10:05 – 10:30 a.m. Coffee Break (Hill Atrium)

Oral presentation session II – Moderator: Kaori Sakamoto (Mahler Hall)

10:30 – 10:50 a.m. Rebecca Edgar, University of Kentucky  
*Mycobacterial NadD enzymes are inhibited by NADP*

10:50 – 11:10 a.m. Katelyn Zulauf, University of North Carolina  
*Export of phagosome maturation arrest effectors by the SecA2-dependent protein export system of Mycobacterium tuberculosis*

11:10 – 11:30 a.m. Rodrigo Abreu, University of Georgia  
*Looking into host iron metabolism as novel therapeutic target during Mycobacterium tuberculosis infection*

11:30 – 11:50 a.m. Vikram Saini, University of Alabama at Birmingham  
*Host-generated hydrogen sulfide stimulates Mycobacterium tuberculosis respiration and growth to exacerbate TB disease*

Lunch and Poster session I

12:00 – 1:00 p.m. Lunch (Magnolia Ballroom)

12:30 – 1:45 p.m. Poster session I – Posters 1-20 (Hill Atrium and Mahler Hall)  
All posters should be set up by this time.

Oral presentations session III – Moderator: Fred Quinn (Mahler Hall)

2:00 – 2:20 p.m. Kathryn Doornbos, University of Alabama at Birmingham  
*Outer membrane localization of CpnT and toxin translocation is dependent on type VII secretion in Mycobacterium tuberculosis*

2:20 – 2:40 p.m. Jonathan Sia, Emory University  
*Th17 immunity to tuberculosis requires CD40-CD40L interaction and is enhanced by CD40 engagement on Mycobacterium tuberculosis-infected dendritic cells*

2:40 – 3:00 p.m. Samantha Tucker, University of Georgia  
*Investigating the effects of copper on sigma factor C in Mycobacterium tuberculosis*

3:00 – 3:20 p.m. Mark Cronan, Duke University  
*Macrophage epithelial reprogramming underlies mycobacterial granuloma formation and promotes infection*

3:20 – 3:35 p.m. Coffee Break (Hill Atrium)
3:35 – 4:25 p.m.  Keynote Address  (Mahler Hall)
David Russell, Cornell University
*Mycobacterium tuberculosis: Here today....and here tomorrow*

**Poster session II – Posters 21 and higher**  (Mahler Hall)

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

**Dinner and entertainment**  (Magnolia Ballroom)

7:00 – 11:00 p.m. Dinner and dancing

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**Sunday, February 5, 2017**

7:00 – 8:30 a.m.  Breakfast  (Magnolia Ballroom)

**Oral presentations session IV – Moderator: Christopher Whalen**  (Mahler Hall)

9:00 – 9:20 a.m.  Kaitlyn Schaaf, University of Alabama at Birmingham
*Targeting the PPM1A signaling pathway to trigger apoptosis of M. tuberculosis-infected macrophages*

9:20 – 9:40 a.m.  Taylor Foreman, Tulane National Primate Research Center
*CD4+ T cell-independent mechanisms suppress reactivation of latent tuberculosis in a macaque model of HIV co-infection*

9:40 – 10:00 a.m.  Tesia Cleverley, Emory University
*Imatinib (Gleevec) stimulates myelopoiesis and alters T cell responses*

10:00 – 10:20 a.m.  Alan Pang, University of Kentucky
*Discovery of allosteric and selective inhibitors of Mycobacterium tuberculosis inorganic pyrophosphatase*

10:20 – 10:35 a.m.  Coffee Break  (Hill Atrium)

10:35 – 11:05 a.m.  Matthew Ezewudo, Critical Path Institute
*The Unified Variant Pipeline: a whole genome sequence data approach to deciphering antibiotic resistance and lineage classification of Mycobacterium tuberculosis*

11:05 – 11:35 a.m.  Christopher Whalen, University of Georgia
*Transmission dynamics of tuberculosis*

11:35- 12:00 p.m.  Awards and Meeting survey  (Mahler Hall)

12:00 – 1:00 p.m.  Lunch  (Hill Atrium)
Other Information

Meeting location
All meeting events will take place in various locations within the University of Georgia Center for Continuing Education and Hotel, 1197 South Lumpkin Street, Athens, GA 30602. The phone number for the hotel is (706) 548-1311.

Registration
Registration badges and meeting materials should be picked up from the Conference Registration Desk within the University of Georgia Center for Continuing Education and Hotel. Badges and materials can be picked up prior to and during the Welcome Reception (6:30 – 9:30 p.m.) on Friday evening (February 3\textsuperscript{rd}) or on Saturday morning (February 4\textsuperscript{th}). The UGA Center requires badges/nametags to be worn for access to meeting events.

Parking
The parking deck behind the UGA Center for Continuing Education and Hotel charges 24 hours/day. Those staying in this hotel receive 1 free single-use parking pass per room per day. Free parking should also be available after 5 p.m. on Friday and on the weekend in the UGA surface parking lot S10 located at the corner of Carlton St. and Sanford Drive. There is a gymnastics meet at Stegeman Coliseum at 7 p.m. on Friday, so this lot may fill up quickly. Parking in S10 on Saturday and Sunday should not be a problem.

Oral presentations
All Powerpoint presentations should be uploaded onto the computer in Mahler Hall on Saturday, February 4\textsuperscript{th}, between 8:00 and 8:30 a.m. Presentations should not exceed 20 minutes including 5 minutes for questions.

Poster sessions
Each poster has been assigned a number (see poster section of the abstract book). Posters 1-20 will be presented during Poster session I (12:30 – 1:45 p.m.). Posters assigned numbers 21 and higher will be presented during Poster session II (4:45 – 6:15 p.m.).

Posters should be set up on February 4\textsuperscript{th} between 8:00 and 8:30 a.m. or between 11:50 a.m. and 12:30 p.m. in Mahler Hall. Poster boards are 5’ x 5’. Push pins will be provided.

Prizes will be awarded for the top oral and poster presentations by students or post-doctoral fellows.
Talks

Talk #1

Ferrets as a transmission model for tuberculosis

Tuhina Gupta¹, Cheryl Day⁶, Thomas Rowe⁵, Monica LaGatta¹, Shelly Helms¹, Wayne Jacobs³, Simon Odera Owino¹, Jarrett Sweenly³, Kaori Sakamoto², Steve Harvey³, Christopher Whalen⁴, Russell Karls¹, Biao He¹, Ted Ross¹ and Frederick Quinn¹

¹Department of Infectious Diseases, ²Department of Pathology, ³Animal Resources Program, and ⁴Department of Epidemiology and Biostatistics, University of Georgia, Athens, GA, ⁵Influenza Division, Virology, Surveillance, and Diagnosis Branch, Centers for Disease Control and Prevention, Atlanta, GA, and ⁶Emory Vaccine Center, Atlanta, GA.

Effective control of the global tuberculosis (TB) burden requires interrupting transmission. Due to the lack of a suitable animal model, this important component of the disease process is incompletely understood. Cattle and non-human primate models that mimic human transmission of Mycobacterium tuberculosis (Mtb) are costly and logistically complex. In comparison, ferrets (Mustela putorius furo) mimic the human respiratory epithelium, are social, can cough, sneeze and have been used as transmission models for influenza virus. Here we present results demonstrating ferrets are an effective Mtb transmission model.

Ferrets were intratracheally infected with variable doses of Mtb strain Erdman and followed by assays to measure viable bacilli in the upper airways, delayed hypersensitivity responses to purified protein derivative (PPD), post-necropsy organ bacterial burdens and histopathology. All infected animals developed characteristic symptoms of acute TB seven weeks post infection (pi). All animals receiving high doses (10⁴ CFU), some receiving medium (100 - 200 CFU) or low doses (10 - 50 CFU) of the Mtb became PPD positive within 4 - 6 weeks pi with evident pulmonary and extra-pulmonary dissemination of bacilli. Transmission was assessed by co-housing infected transmitter ferrets (1,000 CFU/ animal) with naïve sentinels (1:1) for 6 months. By study termination, all six of the naive sentinels were infected based on at least two of the following criteria: >20% weight loss, fever, PPD skin test conversion, positive-culture/PCR from nasal washes or throat swabs and/or positive-culture/PCR from tissues (lung, spleen). These data validate ferrets as a successful transmission model for Mtb. Subsequent studies will assess vaccine efficacy in protecting sentinels or reducing transmission efficiency.
Killing Dormant *Mycobacterium tuberculosis* With Marine-Derived Natural Products

Carolina Rodrigues Felix¹, Amy Wright², Rashmi Gupta¹, Sandra Geden¹, and Kyle H. Rohde¹

¹Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL; ²Harbor Branch Oceanographic Institute, Florida Atlantic University, FL

The dormant phenotype acquired by *Mycobacterium tuberculosis* during infection imposes a major challenge in disease treatment since these bacilli are tolerant to front line drugs. Therefore, it is imperative to find novel compounds that effectively kill dormant bacteria. By screening 4400 marine natural product peak fractions in 2 fluorescence-based whole cell assays (replicating in 7H9 and under dormancy-inducing *in vitro* conditions) we have identified compounds that are selectively active against dormant *Mtb*. This validates our strategy of screening all compounds in both assays as opposed to using the dormancy model as a secondary screen. Overall, twenty five hits were confirmed and shown to be non-cytotoxic. The activity of peak fractions against dormant *Mtb* was confirmed by CFU to rule out false positives. Hits were further deconvoluted to identify unique and novel chemical structures active in each screening model. Five compounds were purified and their structures defined by NMR. Interestingly, 2 lipid compounds were identified with potent activity towards dormant and actively growing *Mtb*. One of these was commercially obtained and showed similar potent activity against *Mtb* in both screening models. Follow up studies to determine the molecular target of this compound are currently underway. In conclusion we have identified and characterized novel chemical structures from marine organisms with antimycobacterial activity, possibly targeting *Mtb* pathways conditionally essential for dormancy survival.

This project is supported by NIH R21 AI105977-01 and R33AI105977-03.
CD4+ T cell-independent mechanisms suppress reactivation of latent tuberculosis in a macaque model of HIV co-infection

Taylor W. Foreman a,b, Smriti Mehra a,c,d, Denae N. LoBato a, Adel Malek e, Xavier Alvarez a, Nadia A. Golden a, Allison N. Bucșan a,b, Andrew A. Lackner a,b, John Chan e, Shabaana A. Khader f, William R. Jacobs, Jr. e, and Deepak Kaushal a,b

aTulane National Primate Research Center, Covington, LA; bDepartment of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA; cCenter for Biomedical Research Excellence, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA; dDepartment of Pathobiological Sciences, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA; eDepartment of Microbiology and Immunology, Howard Hughes Medical Institute, Albert Einstein College of Medicine, Bronx, New York, USA; fDepartment of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri

The synergy between Mycobacterium tuberculosis (Mtb) and HIV in co-infected patients has profoundly impacted global mortality due to tuberculosis (TB) and AIDS. HIV significantly increases rates of reactivation of latent TB infection (LTBI) to active disease, with the decline in CD4+ T cells believed to be the major causality. In this study, non-human primates (NHPs) were co-infected with Mtb and simian immunodeficiency virus (SIV), recapitulating human co-infection. A majority of animals exhibited rapid reactivation of Mtb replication, progressing to disseminated tuberculosis and increased SIV-associated pathology. Although a severe loss of pulmonary CD4+ T cells was observed in all co-infected macaques, a subpopulation of the animals was still able to prevent reactivation and maintain LTBI. Investigation of pulmonary immune responses and pathology in this cohort demonstrated that increased CD8+ memory T cell proliferation, higher granzyme B production, and expanded B cell follicles correlated with protection from reactivation. Our findings reveal novel mechanisms that control SIV- and TB-associated pathology. These CD4-independent protective immune responses warrant further studies in HIV co-infected humans able to control their TB infection. Moreover, these findings will provide insight into the natural immunity to Mtb and will guide development of novel vaccine strategies and immunotherapies.
PPE surface proteins are required for heme utilization by *Mycobacterium tuberculosis*

Avishek Mitra, Alexander Speer, and Michael Niederweis

University of Alabama at Birmingham, Birmingham, AL

*Mycobacterium tuberculosis* (Mt) is primarily taken up in the lung by alveolar macrophages. Acquisition of iron is essential for Mt replication, but iron is efficiently sequestered in the human host. More than 90% of iron in the human body is bound in heme. Not surprisingly many bacterial pathogens including Mt are able to acquire iron from heme and/or hemoglobin. However, the mechanism of heme uptake by Mt is poorly understood. To identify proteins involved in heme acquisition by Mt a high-density transposon library was screened for resistance to the toxic heme-analog gallium(III)-porphyrin (Ga-PIX). Inactivation of the *ppe36*, *ppe62* and *rv0265c* genes resulted in complete resistance to Ga-PIX. Growth of isogenic Mt deletion mutants was either completely absent or strongly reduced in medium with heme as the sole iron source, but no phenotype was observed with hemoglobin. None of the genes restored growth of the heterologous Mt mutants. These results showed that these genes have separate functions in heme acquisition but are not involved in hemoglobin utilization by Mt. While PPE36 is essential for heme acquisition by Mt, the functions of PPE62 and Rv0265c are partially redundant. Surface plasmon resonance spectroscopy showed that PPE36, PPE62 and Rv0265c bind heme. Subcellular localization and surface detection experiments indicated that both PPE36 and PPE62 proteins are cell surface-accessible membrane proteins. PPE36 and PPE62 are, to our knowledge, the first PPE proteins of Mt known to be involved in nutrient acquisition. These findings constitute a major advancement of our understanding of heme utilization by Mt.
Mycobacterial NadD enzymes are inhibited by NADP

Jan Abendroth\textsuperscript{1,2}, Rebecca J. Edgar\textsuperscript{3}, Robert W. Reed\textsuperscript{3}, David M. Dranow\textsuperscript{1,2}, Donald D. Lorimer\textsuperscript{1,2}, Thomas E. Edwards\textsuperscript{1,2}, Konstantin V. Korotkov\textsuperscript{3}

\textsuperscript{1}Seattle Structural Genomics Center for Infectious Disease, \textsuperscript{2}Beryllium Discovery, Inc., Bainbridge Island, WA; \textsuperscript{3}Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY

In \textit{Mtb}, NadD occupies a central position in NAD biosynthesis mediating the formation of nicotinic acid adenine dinucleotide (NaAD) from nicotinic acid mononucleotide (NaMN) and ATP. Surprisingly, we identified NADP as a bound ligand in several crystal forms of \textit{Mtb} NadD. A mass-spectrometry analysis of \textit{Mtb} NadD protein samples confirmed the identity of the ligand. Similarly, NADP was found in the structure of \textit{M. abscessus} NadD. The follow-up biochemical experiments confirmed that NADP is a natural metabolite inhibitor of mycobacterial NadD homologs, with an IC\textsubscript{50} in the nM range. This property of mycobacterial NadDs appears to be unique, as no other bacterial or mammalian NadD homologs show inhibition by NADP, a downstream metabolite. Interestingly, the conformation of NadD in NADP-bound form is different from the previously solved structures of bacterial NadDs, because the adenine moiety adopts a non-native orientation compared to the ATP or NaAD structures. Surprisingly, in the recent structure of \textit{Mtb} NadD in apo form, the protein also adopts a similar conformation, corresponding to the NADP-bound form. We developed a protocol for NADP removal; thereafter, we have been able to obtain structures of \textit{M. abscessus} NadD in complexes with Mg-ATP and NaAD, a substrate and a product of the enzymatic reaction, respectively. These structures are overall similar to the previously reported structures of bacterial NadDs and display the ATP moiety in the productive orientation. What is the functional consequence of NadD inhibition by NADP? It is possible that this inhibition reflects a low activity metabolic state corresponding to the dormancy conditions. Clearly, this unusual conformational state of the enzyme would be useful to exploit for the structure-based inhibitor design.
Talk #6

Export of phagosome maturation arrest effectors by the SecA2-dependent protein export system of *Mycobacterium tuberculosis*

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

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

*Mycobacterium tuberculosis* (*Mtb*) grows and replicates within the phagosome of macrophages. The ability to replicate in macrophages is critical for virulence and *Mtb* creates a suitable niche for growth by arresting phagosome maturation. Recently, we showed that the SecA2 protein export system is required for phagosome maturation arrest and consequently growth of *Mtb* 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 this process. While the process of phagosome maturation arrest by *Mtb* remains to be fully understood, the *Mtb* secreted phosphatase SapM is known to be involved. SapM dephosphorylates 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 *Mtb* had a partial but significant decrease in the amount of secreted SapM compared to wild-type. Since SapM phosphatase activity prevents the recruitment of EEA1 to phagosomal membranes, we also examined EEA1 levels on phagosomes containing the ΔsecA2 mutant. We determined that the ΔsecA2 mutant co-localized significantly more with EEA1 when compared to wild-type indicating that SecA2 is required for *Mtb* to inhibit EEA1 recruitment. To determine if SapM secretion can explain the role of the SecA2 pathway in phagosome maturation arrest and growth in macrophages, we built a ΔsecA2 strain with increased SapM secretion (ΔsecA2+SapM). We then asked if the increased levels of SapM secretion in ΔsecA2+SapM could counteract the phenotypes of a ΔsecA2 mutant in macrophages. When SapM secretion was increased in ΔsecA2+SapM, EEA1 recruitment to the phagosome was reduced to wild-type levels indicating that SecA2 secretion of SapM is required for *Mtb* to inhibit EEA1 recruitment. When we examined growth and phagosome acidification in macrophages infected with ΔsecA2+SapM, we saw increased SapM secretion partially rescued the phagosome maturation arrest and the replication defects of the ΔsecA2 mutant. These results indicate that SecA2 secretion of SapM contributes to phagosome maturation arrest and growth of *Mtb* in macrophages but the partial restoration of defects suggests additional SecA2 effectors are required for inhibition of phagosome maturation by *Mtb*.
Looking into host iron metabolism as novel therapeutic target during *Mycobacterium tuberculosis* infection

Rodrigo Abreu, Frederick Quinn, and Pramod Giri

Dept. of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA

*Mycobacterium tuberculosis* (Mtb) is a siderophilic bacterial lung pathogen that persists in alveolar macrophages leading to extensive lung inflammation and pathology. The importance of iron in disease progression is well reported and Mtb mutants deficient in iron sequestration are severely attenuated both *in vivo* and *in vitro*. Despite promising results with iron chelators in *in vitro* macrophage infections studies, the impact of these agents *in vivo* has not been reported and the impacts of iron chelation in the host immune response are unknown.

Hepcidin (Hepc) is the major regulator of iron serum levels in mammals promoting intracellular iron sequestration in hepatocytes and macrophages. Furthermore, we have previously shown that Hepc is highly expressed during inflammation, and is one of the mechanisms by which Mtb bacilli gain access to the host iron pool. It is then important to understand the role of ferroportin inhibition in the course of Mtb infection, and its impact on intra-macrophage bacterial replication.

In this study, we examine Hepc and modulation of macrophage iron metabolism as a novel therapeutic target during Mtb infection. Moreover, we suggest that heparin can act as a Hepc inhibitor and prevent intracellular bacterial replication during Mtb infection in human macrophages.

Mtb and attenuated *Mycobacterium bovis* BGC bacilli induce up to 30 fold Hepc expression in human macrophages, and this response can be inhibited by 50 μg/ml heparin *in vitro*. In accordance with our hypothesis of Hepc promoting intracellular bacterial growth, heparin-treated macrophages have significantly lower bacterial burden and increased cell viability at 24 and 48h post infection compared to untreated controls. In a similar way, Hepc blocking with a Hepc-capture antibody, also decreases bacterial burden at 48 and 72h post infection with minimal intracellular replication. These results support the importance of iron metabolism during Mtb infection and highlight a novel therapeutic target for tuberculosis.
Host-generated hydrogen sulfide stimulates *Mycobacterium tuberculosis* respiration and growth to exacerbate TB disease

Vikram Saini\(^1\), Krishna C. Chinta\(^1\), Vineel P. Reddy\(^1\), Asaf Stein\(^2\), Dirk A. Lamprecht\(^6\), Joel N. Glasgow\(^1\), Shannon M. Bailey\(^2\), Douglas R. Moellering\(^3\), Jack R. Lancaster Jr.\(^7\) and Adrie J.C. Steyn\(^{1,4,5,6}\)

Departments of \(^1\)Microbiology, \(^2\)Environment Health Sciences and \(^3\)Nutrition Sciences, \(^4\)Centers for AIDS Research and \(^5\)Free Radical Biology, University of Alabama at Birmingham, Birmingham, AL, USA, \(^6\)Africa Health Research Institute, Durban 4001, South Africa, \(^7\)Departments of Pharmacology and Chemical Biology, Medicine, and Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.

Recent studies have linked intracellular gases such as carbon monoxide (CO) and nitric oxide (\(\cdot\)NO) to the induction of TB dormancy. Hydrogen sulfide (H\(_2\)S), which has emerged as an important signaling molecule, plays critical roles in numerous pathophysiological processes in mammals, and shares several overlapping functions with CO and \(\cdot\)NO. However, the role of H\(_2\)S in bacterial pathogenesis and tuberculosis (TB) disease is unknown. We hypothesized that host-derived H\(_2\)S influences the intracellular survival of *Mycobacterium tuberculosis* (*Mtb*) by altering its metabolic and bioenergetic functions. To test this hypothesis, we used high-resolution respirometry to measure bioenergetic functions of *Mtb* following exposure to H\(_2\)S and performed infection studies in H\(_2\)S-deficient mice. We demonstrate that *Mtb*-infected mice lacking the sulfide-producing enzyme cystathionine β-synthase (CBS) survived longer with reduced organ burden and inflammation. Using respirometry, transcriptomics and mass spectrometry, we establish that exposure of *Mtb* to H\(_2\)S increases its respiration, growth, and bioenergetics. Notably, we found that like CO, \(\cdot\)NO and hypoxia, H\(_2\)S also induces expression of genes associated with *Mtb* dormancy regulon. Further, we demonstrate that sulfide reverses the effect the inhibitory effect of \(\cdot\)NO on *Mtb* respiration. Our findings reveal a previously unknown aspect of *Mtb* physiology wherein the bacillus exploits host-generated gas to stimulate growth and promote disease. Thus, modulation of sulfide levels may hold promise as a host-directed therapeutic approach against TB disease.
Outer membrane localization of CpnT and toxin translocation is dependent on type VII secretion in \textit{Mycobacterium tuberculosis}

K. S. Doornbos\textsuperscript{1}, O. Danilchanka\textsuperscript{2}, J. Sun\textsuperscript{1}, M. Niederweis\textsuperscript{1}

\textsuperscript{1}Microbiology, University of Alabama at Birmingham, Birmingham; \textsuperscript{2}Epiva Biosciences, Cambridge,

\textit{Mycobacterium tuberculosis} (Mtb) secretes a NAD-glycohydrolase which causes necrotic death in host cells. This tuberculosis necrotizing toxin (TNT) constitutes the C-terminal domain of the outer membrane protein (OMP) CpnT and is a major cytotoxicity factor of Mtb. The absence of classical export signals within CpnT raised the question of how CpnT is integrated into mycobacterial outer membranes and how TNT is secreted. In this study we demonstrate that localization of CpnT depends on an N-terminal Esx motif which target proteins to the type VII secretion systems of Mtb known as the Esx systems. A separate, C-terminal Esx motif is required for TNT translocation across the outer membrane, but not for the channel activity of CpnT in the outer membrane. Thus, CpnT is the first OMP whose assembly is controlled by type VII secretion. Esx motifs similar to those in CpnT are found in 29 other Mtb proteins. Taken together, this study not only advances our mechanistic understanding of toxin secretion by Mtb but also reveals a previously unknown function of Esx systems in OMP assembly.
Th17 immunity to tuberculosis requires CD40-CD40L interaction and is enhanced by CD40 engagement on *Mycobacterium tuberculosis*-infected dendritic cells

Jonathan Kevin Sia¹, Ranjna Madan-Lala¹, and Jyothi Rengarajan¹

¹Emory Vaccine Center and Division of Infectious Diseases, Emory University, Atlanta GA

Generation of a robust CD4 T cell response is a key facet of immunity against *Mycobacterium tuberculosis* (Mtb) infection. Th17 cells are an important CD4 subset protecting mucosal surfaces and involved in immunity against Mtb. However, mechanisms leading to the generation of Th17 cells during Mtb infection remains limited. We have previously shown that Mtb infection does not optimally mature dendritic cells (DCs). We now demonstrate that CD40-CD40L interaction between DCs and CD4 T cells is required and sufficient to induce IL-17 responses during Mtb infection. Disrupting the CD40-CD40L interaction, genetically or by antibody blockade, attenuates antigen specific IL-17 responses after Mtb infection in vitro. CD40 knockout DCs are incapable of inducing IL-17 responses after infection despite the presence of innate polarizing cytokines including IL-6, IL-1β, and IL-12. Additionally, CD40L knockout CD4 T cells, or T cells treated with CD40L-blocking antibody, are incapable of producing IL-17 after interaction with Mtb infected DCs. Using a CD40L trimer to engage CD40 on DCs, we demonstrate that activating Mtb infected DCs through CD40 can enhance the production of polarizing cytokines, notably IL-6 and IL-23, and enhance the IL-17 response from CD4 T cells. Intratracheal instillation of Mtb infected DCs activated through CD40 improves antigen specific IL-17 responses in the lung and mediastinal lymph nodes compared to Mtb infected DCs without CD40 activation, suggesting that the CD40 pathway plays a critical role in the induction of the IL-17 response against Mtb in vivo. Our work identifies the CD40-CD40L interaction between DCs and CD4 T cells as a key pathway in the generation of antigen specific IL-17 responses during Mtb infection.
Investigating the effects of copper on sigma factor C in *Mycobacterium tuberculosis*

Samantha Tucker, Benjamin Grosse-Siestrup, Russell Karls

Dept. of Infectious Diseases, University of Georgia, Athens, GA

Tuberculosis (TB) typically caused in humans by the bacterium *Mycobacterium tuberculosis* (*M.tbc*) results in roughly 1.5 million deaths each year. It is estimated that one third of the population harbors *M.tbc* as a latent infection. To survive in the host, *M.tbc* must adapt to changing environmental conditions. Bacteria use transcription sigma factors to bind RNA polymerase and initiate transcription of genomic targets specific to each sigma factor. Each of the 13 identified sigma factors in *M.tbc* is likely uniquely regulated in response to physiological signals sensed by the bacteria. Sigma factor C (SigC) is conserved among pathogenic mycobacteria, but not in soil saprophytes such as *M. smegmatis*. SigC is required for full *M.tbc* virulence in mice and guinea pig infection models. An in vitro growth defect of *M.tbc* *sigC* deletion mutant Δ*sigC* has been detected in copper-limited Sauton medium. In the current studies, we use the copper-specific chelator ammonium tetrathiomolybdate (TTM) to confirm the copper-dependence of Δ*sigC*. Western blot analyses following artificial induction of *sigC* show increased levels of SigC protein in the presence TTM, but decreased amounts of the protein after copper addition. Additionally, results of mutational analysis of potential metal-binding residues within SigC will be presented. Understanding the regulatory effects of copper ions on SigC will lead to a better understanding of homeostasis of this metal in *M.tbc* bacilli.
Macrophage Epithelial Reprogramming Underlies Mycobacterial Granuloma Formation and Promotes Infection

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*Mycobacterium tuberculosis* infection in humans triggers formation of granulomas, which are tightly organized immune cell aggregates that are the central structure of tuberculosis. During granuloma formation, infected and uninfected macrophages interdigitate together, assuming an altered, flattened appearance. Although pathologists described the morphological changes of the granuloma over a century ago, the molecular and cellular programs underlying the morphological changes of the granuloma are unclear. Here, using the zebrafish-*Mycobacterium marinum* model, we found that mycobacterial granuloma formation is accompanied by macrophage induction of canonical epithelial molecules and structures. We identified fundamental macrophage reprogramming events that parallel developmental mesenchymal-epithelial transitions. Macrophage-specific disruption of E-cadherin-dependent epithelialization resulted in disordered granuloma formation, enhanced immune cell access, decreased bacterial burden, and increased host survival, suggesting that the granuloma can also serve a bacteria-protective role. Granuloma macrophages in humans with tuberculosis were similarly transformed. Thus, during mycobacterial infection, granuloma macrophages are broadly reprogrammed by engagement of an epithelialization program, and this reprogramming alters the trajectory of the infection and immune response.
Talk #13

*Mycobacterium tuberculosis:* Here today....and here tomorrow

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The interaction between *Mycobacterium tuberculosis* (Mtb) and its host cell is highly complex and extremely intimate. The metabolic activity and physiology of both host and microbe are shaped by this co-existence. We believe that where this appreciation has greatest significance is in the field of drug discovery. Evolution rewards efficiency, and Mtb has evolved to utilize the environmental cues within its host cell to control large genetic programs or regulons. But these regulons may represent chinks in the bacterium’s armor because they include off-target effects, such as the constraint of Mtb’s metabolic plasticity. A prime example is how the presence of cholesterol within the host cell appears to limit the ability of Mtb to co-metabolize or assimilate other carbon sources. We believe firmly that to understand the physiology of Mtb, and to identify new drug targets, it is imperative that the bacterium be interrogated within the context of its host cell. The constraints induced by the environmental cues present within the host cell need to be preserved and exploited. Furthermore, host immune responses modulate drug efficacy *in vivo* and can induce tolerance responses in the infecting bacilli. We need to incorporate as many of these host environmental components as possible in our discovery platforms.

We will discuss how the design and outcome of high-throughput screens of large chemical libraries validate this approach and identify new targets for drug development. The data support our contention that we need to consider the incorporation of the host in new approaches for therapeutics and bridges the divide between antibiotics and host-directed therapies.
Targeting the PPM1A signaling pathway to trigger apoptosis of *M. tuberculosis*-infected macrophages

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The capacity of *Mycobacterium tuberculosis* (*Mtb*) to reprogram host macrophages to suppress apoptosis is essential to its ability to evade the cell-mediated immune response while also protecting it from antibiotic treatment. We recently demonstrated that *Mtb* infection upregulates expression of the host phosphatase PPM1A, which significantly impairs the antibacterial response of macrophages. Here we establish PPM1A as a checkpoint in the apoptotic response of macrophages that is hijacked and exploited by *Mtb*. We showed that overproduction of PPM1A inhibits apoptosis of *Mtb*-infected macrophages by a mechanism dependent on inactivation of the c-Jun N-terminal kinase (JNK). Consistent with this, targeted depletion of PPM1A by shRNA or pharmacological inhibition of PPM1A activity by sanguinarine restored JNK activation, resulting in increased apoptosis of *Mtb*-infected macrophages. We also demonstrated that activation of JNK by subtoxic concentrations of anisomycin induced selective apoptotic killing of *Mtb*-infected human macrophages, a phenotype that was completely blocked in the presence of the specific JNK inhibitor SP600125. Finally, we described that the release of bacteria following selective killing of *Mtb*-infected macrophages enabled rifampicin to effectively kill *Mtb* at concentrations that were insufficient to act against intracellular *Mtb*, delivering proof of principle for the efficacy of a “release and kill” strategy. As anti-*Mtb* drug treatment is primarily hampered by long treatment periods lasting over 6 months leading to patient non-adherence, such a strategy when used as adjunctive TB therapy has the potential to significantly shorten the antibiotic treatment period for TB. These findings show for the first time that drug-induced selective apoptosis of *Mtb*-infected macrophages is achievable.
Talk #15

**In with the Old: Modifying and Repurposing Antibiotics to Confront Emerging and Re-emerging Infectious Diseases**

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*Mycobacterium abscessus* (Mab) is an emerging, difficult-to-treat pathogen with few viable treatment options. The recommended therapy of amikacin and clarithromycin fails in over 50% of cases, frequently requiring burdensing invasive procedures to resolve infection. Tethered with unclear modes of transmission and high intrinsic resistance to most antibiotics, Mab represents a severe public health risk to immunocompromised populations. The need for the development of novel antibiotics to combat Mab and other non-tuberculosis mycobacteria (NTM) infections is pressing.

To approach this challenge, we have explored the potential of spectinomycin analogues to treat NTM infections, including Mab. Profiling experiments identified a subclass of aminomethylspectinomycins (amSPC) with enhanced antimicrobial activity against Mab. The amSPC leads were shown to exhibit activity against *M. tuberculosis* and to inhibit ribosomal protein synthesis in mycobacteria. The activity of the lead compounds was validated against Mab clinical isolates from St. Jude and multi-drug resistant isolates from University of Zurich. Our initial leads demonstrated potent anti-Mab activity with comparable minimum inhibitory concentrations to recommended therapeutic agents used to treat Mab infections. The structure activity relationship studies on the series will be reported along with corresponding in vitro safety, pharmacology testing results, and sequencing results. The results of this study suggest that amSPC analogues have the potential to be advanced as novel Mab therapeutics.
Imatinib (Gleevec) stimulates myelopoiesis and alters T cell responses

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With existing anti-tubercular therapies, treatment success rates for multi- and extensively-drug resistant tuberculosis (MDR-TB, XDR-TB) are dismal, highlighting the urgent need for new drugs. Imatinib mesylate (Gleevec), a cancer drug used in humans for chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GISTs), is a potential “host-directed therapeutic (HDT)” for drug resistant TB infections and HIV/TB co-infections. We found that imatinib limits mycobacterial infections in culture and animal models by targeting the host response to the bacteria. By reducing both entry into macrophages and augmenting phago-lysosomal fusion, imatinib facilitates antigen presentation and pathogen killing. Additionally, imatinib increases myeloid cell numbers (myelopoiesis) by mimicking the “emergency response,” an innate response to infection suppressed by Mtb. Though the effects of imatinib on the myeloid cell compartment have been characterized, we reasoned that the increased bacteriocidal activity of innate immune cells might also affect antigen presentation, and as a consequence augment adaptive responses. To test this idea, we first used pathogen-specific tetramers, together with direct measurements of TCR affinity and T cell activation, to characterize T cell responses against two pathogens: Mycobacterium marinum (Mm), and Lymphocytic choriomeningitis virus (LCMV; Armstrong strain). Whereas LCMV induces a strong T cell response that is rapidly sterilizing, the T cell response to Mm is much less robust. We next assessed whether Imatinib altered the T cell response to these pathogens. Our results suggest a mechanism by which enhancing myelopoiesis can augment adaptive responses to pathogens that engender suboptimal immune responses.

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Talk #17

Discovery of allosteric and selective inhibitors of *Mycobacterium tuberculosis* inorganic pyrophosphatase

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Innovative strategies are needed to discover novel anti-tuberculosis therapeutics, as alternatives to existing antibiotics that suffer from resistance, toxicity and ineffective mechanisms of action. Discovery of inhibitors of novel targets in *Mycobacterium tuberculosis* (*Mtb*) that could be useful as leads for antibiotic development is one such strategy. Bacterial inorganic pyrophosphatase (PPiase) is an essential enzyme that drives a multitude of vital metabolic processes, including fatty and nucleic acid biosynthesis. PPiase breaks down inorganic pyrophosphate (PPi), which is toxic to cell when accumulated. The active site of the PPiase is conserved from bacteria to human, and decades of PPiase inhibitor discovery have produced only toxic low-potency competitive inhibitors of PPiase of no therapeutic relevance.

A high-throughput assay of a library of small molecules, followed by medicinal chemistry optimization, yielded low-μM inhibitors of *Mtb* PPiase. We obtained crystal structures of *Mtb* PPiase in complex with two inhibitors. The structures reveal that the inhibitors bind in the intersubunit interface of the hexameric *Mtb* PPiase that is distant from the active site, while the active site is occupied by the PPi substrate. This interface is not present in the dimeric eukaryotic PPiases and is weakly conserved in bacteria. Indeed, we show that the inhibitors are selective and inhibit *Mtb* PPiase, but not other bacterial or eukaryotic PPiases. Our kinetic analysis shows that the inhibitors are uncompetitive, consistent with the crystal structures. Further efforts are underway to develop these compounds into therapeutic candidates.
Talk #18

The Unified Variant Pipeline: A whole genome sequence data approach to deciphering antibiotic resistance and lineage classification of Mycobacterium tuberculosis

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Drug-resistant Mycobacterium tuberculosis (Mtb) poses a global and persistent public health threat. Lately, the analysis of whole genome sequence (WGS) data has been used to elucidate details on genetic variants that underlie drug resistant phenotypes. One challenge in utilizing WGS is the difficulty in analyzing the vast amount of data and the lack of a standardized and curated repository of variants containing genomic and phenotypic drug resistance data for Mtb.

We developed a unified variant bioinformatic pipeline (UVP) that is openly sourced to identify variant positions and lineage classification across genomes of Mtb isolates using stringent thresholds and quality checks to establish confidence in the polymorphisms inferred. These quality checks includes ensuring the input data is in acceptable fastq format, a 90 percent Mycobacterium tuberculosis complex (MTBC) proportional representation in the input sequence reads, a minimum Q20 quality score for accepting read sequences and calling variants based on the H37Rv Mtb genome and a minimum genome coverage depth of 20X coverage. UVP generates a number of reports from the analysis of each Mtb genome; an annotation file that includes variant positions across the genome of the isolate, a lineage classification file that assigns the strain to one of the major Mtb lineages, a coverage report that maps the depth of coverage of all the genes and features of interest in the genome and detailed quality metrics sub-folders that provides a summary of the quality of the input sequencing data. The list of variants for each isolate is aggregated into the ReSeqTB database platform that is capable of standardizing, curating, storing and aggregating WGS, phenotypic drug susceptibility (DST) and clinical data within a single platform. The UVP was validated using a well characterized Mtb dataset that comprised both phenotypic testing results and conventional DNA sequencing data of 90 diverse Mtb isolates. The UVP was 100% concordant with conventional methods in assigning lineage classification to Mtb isolates and was in 98% agreement on the variants previously detected by conventional DNA sequencing. Once validated, we analyzed more than 3500 isolates representing all seven major Mtb lineages. Empirical statistical analysis of the results corroborates the strong association between mutations known to confer antibiotic resistance, with the corresponding antibiotic resistance as previously described in Mtb literature. The aggregation of variants over time in the ReSeqTB platform will lead to the development of high confidence mutations statistically linked to drug resistance which will be invaluable to DST assay developers, researchers and clinicians and be especially relevant for the new and repurposed drugs being deployed.
Talk # 19

Transmission Dynamics of Tuberculosis

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According to the World Health Organization, the global burden of tuberculosis peaked in 2000 and has since declined by 1.5% per year. Although encouraging, this modest progress falls short of the Millennial Development Goals for tuberculosis elimination. Moreover, this average decline hides in the incidence and prevalence of tuberculosis around the world today. This variation in tuberculosis disease burden highlights that the determinants of incidence or prevalence may differ by regions or populations, so a ‘one size fits all’ approach to tuberculosis control is not tenable and that interventions need to be tailored to the local epidemiology of disease.

In most tuberculosis control programs, tuberculosis disease is the target of control measures. This approach, however, may have limited effectiveness in areas with a high tuberculosis burden because transmission of Mycobacterium tuberculosis is also high. One unaddressed challenge in tuberculosis control is that by the time a case is properly diagnosed and treated, many susceptible contacts have already been newly infected. These newly infected individuals represent the cases of the future.

To make progress with the tuberculosis epidemic in these areas, new cases must be prevented, either by reducing transmission or by preventing disease once infected. Taking the latter strategy first, we already have effective ways to prevent tuberculosis once infected, using BCG vaccine or treatment of latent tuberculosis infection. As for reducing transmission, this challenge remains, especially in the community.

Over the past 25 years two lines of research have emerged pertaining to M. tuberculosis transmission. One line focuses on the contacts of infectious cases and has quantified transmission in different community settings; the other focuses on the genetics of the isolate causing disease and has clarified the role of recent transmission in epidemics and revealed the global lineages of M. tuberculosis. More recently, whole genome sequencing of M. tuberculosis has been used to infer the order and timing of infections within disease clusters.

Despite the remarkable progress along these two lines of intertwining research, there is still a critical gap. We do not know where M. tuberculosis is transmitted in a given community – not just the settings of transmission, or the likelihood of undetected cases, but the geographic locales. From multiple household contact studies of tuberculosis, we know that the household of an index case is a location with intense transmission. However, several studies have recently shown that only about 20% of transmission in an area may be attributed to the household. Stated another way, close to 80% of M. tuberculosis transmission occurs outside of the households of index cases. Therein lies another challenge of tuberculosis; that is, to understand the local epidemiology of tuberculosis and implement targeted and tailored interventions to reduce M. tuberculosis transmission, which would, in turn, reduce the number of new infections.

In an ongoing project in Kampala, Uganda, we are identifying transmission niches that represent geographic locations and built environments where infectious tuberculosis cases and susceptible contacts mix to sustain transmission of M. tuberculosis in a community. We study the relational and mobility networks that link cases with contacts and then use whole genome sequencing to infer the order and timing of infections among cases. In this way, we can model the transmission dynamics of tuberculosis in an urban African setting.
Response to hypoxia may govern the fitness and virulence of the tubercle bacilli

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*Mtb*, the causative agent of tuberculosis, demonstrates different virulence not only between lineages but between strains in the same lineage as well. Genomes of any two strains of *Mtb* regardless of lineage are genetically very close with an average difference of 0.03%; thus it is unlikely that genetic differences could explain differential virulence. While the immune response to strains of *Mtb* with different virulence has been studied, the underlying bacterial mechanism of differential virulence between strains has not. We hypothesized that differential response to in-vivo stress may be responsible for differential virulence. We studied two strains, one known to exhibit low virulence, CDC1551, and the other, generally considered to exhibit high virulence, Erdman, to determine mechanisms of differential responses to in vivo-relevant stress in vitro. Erdman exhibited greater fitness upon culturing in a hypoxic environment relative to CDC1551 and replicated significantly better relative to CDC1551 in an in-vitro model of reactivation (re-aeration). In order to determine a possible mechanism for these differences, we characterized the early hypoxia/re-aeration transcriptome. During early hypoxia, the expression of the DosR regulon was induced to significantly higher levels, both in magnitude and breadth, in Erdman relative to CDC1551. The DosR signaling pathway is critical for the persistence of Mtb in lungs. We postulate that one or more DosR-regulated proteins mediate the survival of Mtb in hypoxia and its transition to an environment of normoxia; The greater fitness of Erdman during hypoxia and reaeration may result from its ability to invoke the expression of this pathway to much higher levels. Erdman also exhibited significantly higher resistance to thiol-oxidative stress and intra-phagosomal growth relative to CDC1551.
Inhibition of tryptophan catabolism by host-directed therapy radically reorganizes the tuberculoma and augments the immune-mediated control of *M. tuberculosis*

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*Mycobacterium tuberculosis* (*Mtb*) continues to cause catastrophic levels of tuberculosis (TB) related mortality. The failure to control TB stems from incomplete understanding of the highly specialized strategies that *Mtb* utilizes to modulate host immunity in order to persist in host lungs. In a macaque model of inhalation TB, the expression of Indoleamine dioxygenase (IDO), a powerful immunosuppressant of activated CD4⁺ T cells, is induced in myeloid cells. Amelioration of IDO activity *in-vivo* resulted in significant reduction in clinical signs of TB, pathology and bacterial burden, and increased survival. This was accompanied by increased lung T cell proliferation, induction of bronchus associated lymphoid tissue (iBALT) and bacterial killing correlates; reduced checkpoint inhibitor signaling and the relocation of T cells to the center of the granulomata. The profoundly better killing of *Mtb* in macrophages by CD4⁺ T cells could be modeled *in-vitro*, in a novel macaque macrophage:CD4⁺ T cell co-culture system. Inhibition of IDO activity also increased Type I interferon (IFN) signaling. Since the latter is already a target of host-directed therapy (HDT) approaches against TB, additional concurrent intervention to inhibit Tryptophan (Trp) catabolism, thereby modulating a complex immune/metabolic signaling network that promotes bacterial survival, immune dysfunction and disease, may be beneficial in TB patients. Our results strongly suggest that inhibition of IDO is a novel and prime target for adjunctive HDT against the multidrug resistant TB epidemic.
Poster #3

Investigating the effect of deletion of the Hip1 protease on BCG vaccine-induced protective immunity to tuberculosis

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In 2015, 1.8 million people died of tuberculosis (TB) globally. This was despite the availability of a TB vaccine as well as an effective drug treatment regimen. The Bacille Calmette-Guerin (BCG) vaccine to tuberculosis derived from Mycobacterium bovis provides children with protection against disseminated TB but not the most prevalent pulmonary form of the disease. Unfortunately, the protective capability of BCG does not extend to adults. This inefficient performance of BCG highlights the necessity for a better TB vaccine. Previous studies have shown that the Mycobacterium tuberculosis (Mtb) cell wall-associated serine protease, Hip1 plays a major role in pathogenesis of the bacterium on host cells. When mice or bone marrow derived macrophages (BMDMs) and dendritic cells (BMDCs) are infected with Mtb harboring a disruption in hip1, mice live longer than those infected with wild-type Mtb, and the innate immune response is significantly higher. These data indicate that during infection with WT Mtb, Hip1 assists in suppressing the innate immune response. As the BCG genome contains an identical homolog of hip1, we hypothesized that deleting this gene in BCG would produce a vaccine strain capable of eliciting a stronger innate immune response, which would then lead to better protection against challenge than vaccination with WT BCG. We have observed that compared to WT BCG, our Δhip1 BCG strain induces an augmented pro-inflammatory cytokine response from BMDMs and BMDCs. We have also observed that infection with Δhip1 BCG increased maturation of BMDCs. Studies to determine whether the increase in innate immune responses leads to better cellular immunity and to determine the protective capacity of this strain are ongoing.
Poster #4

Whole genome sequencing identifies circulating Beijing-lineage *Mycobacterium tuberculosis* strains in Guatemala and an associated urban outbreak

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Limited data are available regarding the molecular epidemiology of *Mycobacterium tuberculosis* (Mtbb) strains circulating in Guatemala. Beijing-lineage Mtbb strains have gained prevalence worldwide and are associated with increased virulence and drug resistance, but there have been only a few cases reported in Central America. Here we report the first whole genome sequencing of Central American Beijing-lineage strains of Mtbb. We find that multiple Beijing-lineage strains, derived from independent founding events, are currently circulating in Guatemala, but overall still represent a relatively small proportion of disease burden. Finally, we identify a specific Beijing-lineage outbreak centered on a poor neighborhood in Guatemala City.
MAIT cell characterization and responses during *Mtb*/SIV infection in Rhesus macaques

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Tuberculosis (TB) is a catastrophic infectious disease, affecting roughly one third of the world’s population. In 2015, TB caused 10.4 million acute cases and 1.4 million deaths, of which 400,000 of those infected were also HIV positive. While only 5-10% of infections will develop into active TB, co-infection with HIV drastically increases the risk of TB reactivation, leading to life-threatening active infection. Recent work has demonstrated that nonclassical T cells possess highly conserved TCRs and recognize non-peptide antigens. Mucosal-associated invariant T (MAIT) cells recognize vitamin B metabolites produced by bacteria, possess a potent effector memory phenotype, and express tissue-homing markers driving migration to sites of infection. Although MAIT cells are conserved across many mammalian species and comprise up to 10% of the CD8+ T cell compartment in humans, these have been poorly characterized as regulators of the immune system. Previous research has shown that MAIT cells are depleted in the periphery during both *Mtb* infection and HIV infection. We hypothesized that MAIT cells may be migrating to the site of infection where their mycobacterial effector functions during the early stages of *Mtb* infection may play an important role in protective long-term TB immunity. Early in infection, MAIT cells increased predominantly in the blood of actively infected rhesus macaques compared to those with LTBI. Interestingly, the frequency of these cells increased at the site of infection in animals with latent TB. Furthermore, following infection, the chemokines expressed on MAIT cells reflected a shift towards a Th1 phenotype from a shared Th1/Th17 phenotype. Better understanding the role of nonclassical T cell populations, such as MAIT cells, may enable us to develop better vaccines that target early TB pathogenesis by involving the innate immune response.
Discovery and development of inhibitors *Mycobacterium tuberculosis* primase and inorganic pyrophosphatase

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New strategies need to be explored to discover novel anti-tuberculosis therapeutics, in the face of a looming global epidemic of drug-resistant tuberculosis. Recently we developed a novel coupled primase-pyrophosphatase assay for discovery of inhibitors of two essential bacterial enzymes, primase (DnaG) and inorganic pyrophosphatase (PPiase). DnaG is responsible for synthesis of RNA primers for chromosomal DNA replication, and PPiase breaks down inorganic pyrophosphate (PPI) to drive all DNA and RNA synthesis reactions in the cell. We applied this assay to discovery of inhibitors of DnaG and PPiase from *Mycobacterium tuberculosis*. The high-throughput assay of a library of small molecules yielded low-μM selective inhibitors of primase and inorganic pyrophosphatase. We obtained crystal structures of the RNA polymerase domain of *M. tuberculosis* DnaG and inorganic pyrophosphatase (the latter both alone and in complex with its inhibitors). These structures clarify the mechanisms of catalysis and inhibition of these enzymes and drive medicinal chemistry efforts to develop these compounds into therapeutic candidates.
Examining the properties of the kanamycin-resistance enzyme, Eis, from *Mycobacterium tuberculosis*

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One mechanism of resistance found in *Mycobacterium tuberculosis* (*Mtb*), the causative agents of tuberculosis (TB), is an acetyltransferase (Eis) that acetylates aminoglycosides, most importantly kanamycin (KAN). Eis acetylates many of the free amine groups of KAN prohibiting the antibiotic from binding to its biological target. Utilizing a high-throughput screen of 123,000 compounds, several compounds containing an oxidized isothiazole heterocyclic core were identified as inhibitors of Eis. A combination of structure-activity relationships, kinetics studies, and the crystal structure of Eis in complex with two potent inhibitors revealed that the compounds bind to the expansive aminoglycoside-binding pocket, preventing KAN from becoming acetylated. Importantly, when the compounds are used in combination with KAN, Eis-induced resistant *Mtb* cells are efficiently killed. This approach opens the door for new therapies against aminoglycoside-resistant TB.
An Inhaled Pyrazinoic Acid and n-Propyl Ester Dry Powder Aerosol for Tuberculosis Therapy

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Tuberculosis causes over one million deaths each year. Pyrazinamide (PZA), a first line agent, is administered orally and is converted by a microbial enzyme pyrazinamidase (PncA) to pyrazinoic acid (POA), the active moiety (Zhang et al, 2003). The largest category of PZA resistance mutations map to the pncA gene. Delivery of POA and its ester, n-propyl POA (PAE) directly to the lungs as an aerosol might mitigate resistance and require a lower drug dose to treat local infection and prevent transmission. An efficacy study combining inhaled therapy with oral rifampicin (+ RIF) was conducted in a Mycobacterium tuberculosis strain H37Rv infected guinea pig model. A combination POA-PAE dry powder aerosol delivered in conjunction with oral rifampicin (+ RIF) significantly lowered the bacterial burden in the lungs and showed significant improvement in histopathology compared with untreated control animals or animals treated with oral RIF alone. Notably, the estimated aerosol powder dose delivered to each animal was approximately 1-2 mg which is lower than the dose of oral PZA currently given to treat TB patients. Additionally, systemic effects were observed in animals receiving inhaled drug, with a 3.0 log reduction in the spleen bacterial burden and significant improvement in histopathology. Comparatively, the RIF alone group showed a 1.4 log reduction in the spleen bacterial burden. As oral PZA may result in hepatotoxic side effects, the relatively low doses of inhaled POA-PAE used in this study has tremendous potential to benefit patients worldwide. Delivering POA and esters of POA has demonstrated efficacy and may be a strategy to mitigate bacterial resistance to PZA.
Discovery of novel acetyltransferase Eis inhibitors as kanamycin adjuvants in *Mycobacterium tuberculosis*

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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. One mechanism of resistance in *Mtb* is the upregulation of the enhanced intracellular survival (Eis) gene of *Mtb*. Establishing the unique function and structure of the mycobacterial enzyme Eis and determining its role in conferring resistance to aminoglycosides (AGs) used as second-line of defense against TB have been focuses of studies in our group. Our 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. Several libraries of small molecules were tested in our lab via HTS in combination with kanamycin (KAN) looking for compounds that abolished the acetyl transfer. Several compounds showed potent inhibitory activity against Eis. The screening of >123,000 compounds against purified Eis enzyme led to the identification of several new Eis inhibitors. We are going to show design, synthesis, and structure-activity-relationship of highly potent antitubercular agents as adjuvants of KAN. To investigate the promise of our compounds as potential Eis inhibitors for use in combination with KAN, we determined their IC₅₀ values against purified Eis enzyme, and most of these compounds displayed high Eis inhibitory activities in the low nanomolar ranges. The biological (effect on the MIC values of KAN in KAN-sensitive *Mtb* H37Rv and in *Mtb* K204, which is KAN-resistant due to *eis* upregulation) properties were also studied. Seeking an understanding of the binding mode of our inhibitors to Eis, crystal structures of Eis in complex with CoA and inhibitor for our scaffold is also determined.
Novel inhibitors of the acetyltransferase Eis to combat aminoglycoside resistance in *Mycobacterium tuberculosis*

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Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtb*) is the second deadliest infectious disease. About 10.4 million new cases and 1.4 million TB-related deaths were reported in 2015. Drug resistance is a major threat when it comes to trying to treat TB. In 2009, from XDR- *Mtb* clinical isolates, the enhanced intracellular survival (*eis*) gene was reported to be upregulated, causing resistance to the aminoglycoside (AG) kanamycin (KAN). Eis is a unique acetyltransferase capable of acetylating multiple amine functionalities on AG scaffold presenting a major obstacle for designing novel AGs. Analogous to beta-lactamase inhibitors and penicillin, we rationalize that Eis inhibitors combined with KAN could overcome resistance in *Mtb* where Eis is upregulated. To prove this hypothesis, 123,000 compounds were evaluated in a high-throughput screening (HTS) campaign and several Eis inhibitors were identified, which displayed great structural diversity. Here, we are reporting the synthesis of a series of compounds belonging to a novel Eis inhibitor scaffold and their structure activity relationships (SAR). We further show that our best inhibitors are not only active in enzymatic assays, but also able to restore the antibacterial activity of KAN. Additionally, our best inhibitors are non-toxic to mammalian cells at therapeutic concentrations. We show how these compounds bind to Eis by presenting the crystal structure of Eis in complex with coenzyme A (CoA) and one of the best inhibitors in this series. Consequently, the data support these Eis inhibitors as potential novel anti-TB adjuvants that can be used to in conjunction with KAN to combat drug-resistant TB.
Investigation of mycolactone gene modulation on exposure to various abiotic and biotic factors to decipher pathogenesis of *Mycobacterium ulcerans*

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Buruli ulcer disease (BUD), characterized by necrotizing skin lesions, is the third most common mycobacterial disease after tuberculosis and leprosy. The etiologic agent of the disease is *Mycobacterium ulcerans* (MU) whose major virulence factor is mycolactone, a lipid toxin. An exact mode of transmission of BUD has yet to be determined although several modes of transmission such as inoculation through abrasion sites or infection followed by bite of mosquito or giant water bugs has been postulated. In order to understand the mode of MU, it is important to understand how bacteria regulate virulence factors in natural environments, and hosts during infection. The objective of the study was to observe modulation of mycolactone expression and production on exposure to various biotic and abiotic factors such as UV, quorum sensing molecule, mosquitoes and varied oxygen and temperature conditions *in vitro*. In order to accomplish the objective, MU was exposed to these parameters to observe growth and the mycolactone gene expression compared to controls. Results showed that mycolactone gene was upregulated under microaerophilic conditions and at 37°C, an environment that MU also encounters during infection to human host. Mycolactone was upregulated on exposure to mosquitoes, suggesting possible role of mosquitoes-in dispersal and/or transmission. Mycolactone gene was not modulated on exposure to UV at our tested time intervals suggesting that MU may be protected from UV in the environment or that UV protection is through regulation of other genes. Mycolactone gene was downregulated on exposure to quorum sensing molecule of *Pseudomonas aeruginosa* (N-(3-oxo-dodecanoyl)-L- homoserine lactone), however, this needs further investigation. Results from this work suggest a possible role for mycolactone providing a fitness advantage to MU in environment and in hosts and is an important step in elucidating the role of mycolactone in natural environments in order to prevent transmission.
**Poster #12**

**Identification and validation of new mechanisms of isoniazid resistance in *Mycobacterium tuberculosis***

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**OBJECTIVE:** Globally, approximately 14% of all tuberculosis (TB) cases are isoniazid (INH)-resistant, and ~10-12% of INH resistance is unexplained. The validation of novel INH resistance mechanisms can enhance the accuracy of rapid molecular tests for INH resistance, including those used for detection of both INH- and rifampin-resistant tuberculosis (i.e., multidrug resistance).

**STUDY DESIGN:** We identified 13 *M. tuberculosis* clinical isolates from archived samples that were previously classified as INH-resistant by agar proportion testing but lacked mutations commonly associated with INH resistance. The minimum inhibitory concentration (MIC) for INH was determined using broth microdilution assays, and whole genome sequencing (WGS) was performed to identify mutations associated with INH resistance.

**RESULTS:** A few strains (4/13) were susceptible to INH while most exhibited low-level (4/13) or high-level (5/13) resistance. We identified numerous distinct mutations in the INH resistance-associated genes *katG*, *ahpC*, *ahpD*, *fabG1*, and *fabG3* but none in *furA*, *inhA*, or Rv1910c. One high-level INH-resistant strain did not harbor mutations in any of the aforementioned loci. We used recombineering to generate a novel *katG* V1A mutation in the pansusceptible strain H37Rv. We confirmed the mutation was successfully introduced into H37Rv by both Sanger sequencing and WGS, and found that it allows growth on 7H10 agar containing INH at 0.2 µg/mL.

**CONCLUSION:** A mutation discovered by WGS (*katG* V1A) was shown by functional genetics to confer INH resistance, likely due to the loss of the *katG* translational start codon. Additional analyses will be conducted to quantify the level of resistance conferred by the *katG* V1A mutation and determine the contribution of other mutations identified in our dataset for INH resistance. Examination of isolates with unknown mechanisms of antibiotic resistance, coupled with WGS data, could aid assay development by identifying novel resistance markers for INH and other drugs.
Analysis of protein-protein interaction networks in *Mycobacterium tuberculosis* \(H37Rv\)

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*Mycobacterium tuberculosis* is an extremely successful pathogen causing tuberculosis which remains a significant world health burden and one of the major causes of human suffering. The genome sequencing project of *M. tuberculosis H37Rv* was completed in 1998. Since then it has been possible for biologists to explore its genome. Presently, besides experimental data, genome sequences of more than 60 Mycobacterial species are available in public domain which can be analyzed to unravel hidden genomic attributes. To extract useful information from sequenced genomes, large-scale genome comparison is very useful. It can also be systematically employed to build protein interaction network which in turn help to identify high degree nodes or gene that are critically involved in the cellular physiology of bacterium. In order to address such issues, our present study employs computational methods such as Gene neighborhood, Gene fusion, Phylogenetic profiles, Mirror tree etc. Subsequently, with the implementation of these approaches, protein-protein interactions were predicted. Further, these were utilized to develop protein-protein interaction network and to explore various other attributes. The interaction network was observed to comprise 82,350 edges connected across 3946 nodes. It is observed that the built interaction network is a scale-free network possessing 583 crucial hubs with the threshold of 50 degree. Our present protein network analysis will have potential implications to understand the role of essential genes in the cellular physiology of *M. tuberculosis*. 
Heme oxygenase-1 suppresses myeloid cell inflammation and oxidative/nitrosative stress-mediated immunopathology in human tuberculosis

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Heme oxygenase-1 (HO-1) is a cytoprotective enzyme that controls inflammatory responses and redox homeostasis; however, the function of HO-1 in regulating host immune responses to Mycobacterium tuberculosis (Mtb) infection and tuberculosis (TB) disease progression is poorly defined. Further, the clinical significance of animal studies is difficult to access since these models do not represent the full spectrum of human TB. To address these gaps, we used freshly-resected lung tissue from TB patients in addition to mice with complete HO-1 knockout (HO-1−/−) or myeloid cell-specific HO-1 knockout (HO-1LysM−/−). Flow cytometric analysis of pathologically distinct regions of human TB lung revealed that neutrophils and macrophages are the primary source of HO-1, and that HO-1 levels decrease with advanced disease. Interestingly, myeloid cells with reduced HO-1 levels produced significantly more reactive oxygen (ROI) and nitrogen intermediates (RNI), suggesting that HO-1 protects the host from redox-mediated tissue damage during active TB. Transcriptomic analysis of Mtb-infected WT and HO-1−/− mice revealed that pathways involved in myeloid cell migration are significantly regulated by HO-1 during Mtb infection. Consistently, Mtb-infected HO-1−/− and HO-1LysM−/− mice showed significant increases in the accumulation of macrophages and neutrophils. Further, the lack of HO-1 also resulted in dysregulated T-cell responses, including reduced Th1 (CD4+IFNγ+) and T-cell activation responses as well as increased CD4+FoxP3+ regulatory T-cells. Together, these altered immune responses in both HO-1-deficient mouse models resulted in increased bacterial loads and disease pathology, as well as significantly reduced survival. Taken together, our human and animal data show that HO-1 is essential for effective immune and oxidative stress-mediated control of severe immunopathology during TB.
Ferritin H chain deficiency dysregulates iron homeostasis and acutely increases susceptibility to *Mycobacterium tuberculosis* infection

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Iron is an essential factor in host regulatory mechanisms as well as for the growth and virulence of *Mycobacterium tuberculosis* (*Mtb*). Ferritin H chain (*FtH*) is a major intracellular iron storage protein in host that reduces free iron levels following *Mtb* infection. We hypothesized that perturbations in host iron storage mechanisms could alter the outcome of *Mtb* infection. To test our hypothesis, we used knockout mice lacking *FtH* expression (*Fth*⁻⁻) in myeloid-derived cell populations and studied *Mtb* disease progression. Following *Mtb* infection, we observed that *Fth*⁻⁻ mice were highly susceptible to infection and all the mice succumbed by 85 days compared to none in WT mice. Bacillary burden in the lungs and spleens of *Fth*⁻⁻ mice were significantly increased, and we observed dissemination of *Mtb* into the brain and eyes. Immune analysis revealed, a stronger Th-1 response was generated in *Fth*⁻⁻ mice compared to WT mice upon infection. In addition, we observed significantly increased numbers of neutrophils and CD4⁺ in lungs and spleen of *Fth*⁻⁻ mice compared to WT mice. RNA sequencing of mouse lungs and extracellular flux analysis of peritoneal macrophages suggests that *FtH* expression is required for the mitochondrial function during *Mtb* infection. CE-MS based metabolomic analysis shows that amino acid levels and polyamine synthesis are significantly altered in lungs of *Mtb* infected *Fth*⁻⁻ mice compared to WT mice. In response to *Mtb* infection, *Fth*⁻⁻ mice upregulate Ferroportin levels in the lungs in order to reduce availability of iron for *Mtb* growth. *Fth*⁻⁻ mice have increased serum Hepcidin levels which degrades the Ferroportin in duodenal enterocytes, thereby reducing iron absorption from the gut leading to increased iron in feces of *Fth*⁻⁻ mice. We conclude that *FtH* expression in macrophages is required to control the *Mtb* infection in a mouse model of tuberculosis.
Screening for and Cheminformatic Analysis of Copper-dependent Tuberculosis Inhibitors

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One potential source of new antibacterials is through probing chemical libraries for copper-dependent inhibitors (CDIs), i.e., compounds that are highly inhibitory in the presence of copper but impotent in its absence. Recently, we demonstrated that these CDIs are readily found in existing chemical libraries. However, actively screening whole collections is a costly and laborious task that often produces few hit compounds. To make drug screening more efficient, we sought to utilize machine learning to generate a cheminformatic algorithm enabling prediction of new hit compounds in an unscreened library. We first performed a small-scale screen for CDIs against Mycobacterium tuberculosis. Of the 5280 compounds screened, 99 compounds were able to inhibit growth of M. tuberculosis, but only in the presence of copper. To build our model, we used naïve Bayesian classification of fragments from molecular fingerprints. Each fragment found in the 5280 compounds was differentiated and scored based upon relative over- or under-representation in the hit population compared to the non-hit population. Individual compounds were then scored using their constitutive fragments. A cutoff retaining at least half of the hits gave a predictive accuracy of 91% on a test set. We then applied this model to a library of 13,000 unscreened compounds and manually assayed a subset of the top scoring molecules. This filtered set offered a hit rate of 14%, a seven-fold increase over the original screen’s 2% hit rate. Already a dramatic increase, this hit rate can likely be further improved through consideration of additional molecular properties.
Phenotypic characterization of *Mycobacterium tuberculosis*-specific CD4 T cells in individuals with HIV co-infection

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Approximately 90% of immunocompetent individuals infected with *Mycobacterium tuberculosis* (*Mtb*) never develop symptoms of clinical disease and are considered to have latent *Mtb* infection (LTBI). However, co-infection with HIV greatly increases the risk of reactivation of LTBI and progression to TB disease. Although *Mtb*-specific T cell immunity is clearly important in maintaining successful immune control of *Mtb*, the immune parameters that are perturbed by HIV infection and result in loss of immune control of LTBI have not been defined. We hypothesize that *Mtb*-specific T cell function is impaired in the setting of HIV co-infection, which may contribute to increased risk of TB disease in HIV co-infected individuals. One mechanism contributing to impaired T cell function is upregulation of immunoregulatory receptors, including PD-1, BTLA, and CTLA-4. Using blood samples from HIV-infected and uninfected adults with LTBI, we tested the hypothesis that immunoregulatory receptors are upregulated on *Mtb*-specific T cells in the setting of LTBI/HIV co-infection. PBMCs were stimulated with *Mtb* CFP-10 and ESAT-6 peptide pools, and analyzed by flow cytometry to evaluate expression of inhibitory receptors by *Mtb*-specific CD4 T cells producing IFN-γ and TNF-α. Understanding the characteristics of *Mtb*-specific CD4 T cell responses that are associated with a high risk of reactivation of *Mtb*, compared to durable control of LTBI, is needed to develop new tools to evaluate protective immunity and vaccine efficacy.

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Poster #18

A new model for ex vivo analysis and manipulation of mycobacterial granulomas

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Macrophages infected with pathogenic mycobacteria recruit uninfected macrophages and other immune cells to form characteristic structures called granulomas. However, studying these structures in animal models has proved difficult because of limitations in optical and experimental accessibility. We have paired *Mycobacterium marinum* (Mm), a close relative of the *Mtb* complex and a natural pathogen of ectotherms, with the zebrafish to establish a validated surrogate model for human mycobacterial granulomas. Granulomas that form in the zebrafish-*Mycobacterium marinum* model of infection recapitulate important features of human granulomas (organization, hypoxia, caseation necrosis) that are absent from standard mouse models. Here, we have established a new 2.5D ex vivo granuloma culture model by dissecting mature granulomas from infected adult zebrafish. With this model we have unprecedented visibility of this complex structure. We genetically label and manipulate the host immune cells that comprise the granuloma to investigate how this central structure of mycobacterial infection is assembled and remodeled.
Host-Directed Therapies for Tuberculosis: Discoveries from a Zebrafish Chemical Screen

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*Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis, kills over one million people annually. Drug resistance to current therapies is rapidly increasing and novel therapeutic strategies are required for treatment. We use zebrafish infected with *Mycobacterium marinum* (*Mm*), the closest genetic relative of the *Mtb* complex, to identify drug treatments that may potentiate host responses to mycobacterial infection. From an *in vivo* chemical screen using zebrafish larvae, we uncovered six novel FDA-approved compounds that reduce *Mm* burden through host-dependent processes. Using CRISPR/Cas9-mediated disruption of candidate target genes, we have identified a host target of one small molecule that controls *Mm* infection by potentiating host innate immune signaling pathways. We have shown that this drug requires cytosolic mycobacterial signals to induce cell death and downstream inflammatory processes. This drug, and the mechanisms it invokes in zebrafish larvae, may help to inform the development of host-directed therapies for tuberculosis in humans.
SatS: A novel component of the specialized SecA2 protein export pathway in *Mycobacterium tuberculosis*

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Bacteria have a variety of general and specialized systems to export proteins from the cytoplasm to extracytoplasmic spaces. One specialized pathway, denoted the SecA2-dependent export pathway, exports a subset of proteins important for virulence in several pathogens, including *Mycobacterium tuberculosis*. *M. tuberculosis* ∆secA2 mutants are attenuated in macrophages and mice. SecA2 is an ATPase that provides the energy to export a subset of proteins across the cytoplasmic membrane. Our data thus far support a model where SecA2 uses the canonical SecYEG translocase to transport these proteins. In the non-pathogenic model *Mycobacterium smegmatis*, a mutation in the ATP binding region, referred to as SecA2KR, renders SecA2 nonfunctional and dominant negative. We predict that SecA2KR is dominant negative because it is locked in a complex with SecY. Mutations arise spontaneously that suppress secA2KR phenotypes. By identifying extragenic suppressor mutations of secA2KR, our goal is to identify proteins that interact with SecA2. Whole-genome sequencing of six extragenic suppressors revealed unique mutations affecting the msmeg1684 gene, which we have renamed satS for secA2 (two) suppressor. SatS is a hypothetical protein with no informative homology to known proteins. We constructed a satS mutant and showed that it suppresses the SecA2KR phenotype. Additionally, we showed that we can complement the suppressor phenotype of a satS mutant by expressing either satSmsm or satSmtb indicating that these proteins have homologous functions in *M. tuberculosis* and *M. smegmatis* respectively.

We hypothesize that SatS works with SecA2 to export substrates, and that in its absence the dominant negative secA2KR phenotypes are suppressed because SecA2KR is no longer recruited to SecY. Like secA2, satS is predicted to be an essential gene for survival in macrophages and mice. Our current work is focused on elucidating the function of SatS. We have constructed mutant *M. tuberculosis* lacking satS which we are using to study its role in export and its contribution to the virulence of *M. tuberculosis*. 

Poster #21

Characterization of Orphaned Mce Associated Membrane Proteins of Mycobacterial Mce Transporter Systems

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Multiple aspects of Mycobacterium tuberculosis (Mtb) pathogenesis depend upon lipids. Not only is the Mtb cell wall composed of complex lipids with roles in infection, but Mtb also metabolizes host lipids during infection. Mtb has numerous metabolic pathways to degrade lipids and systems to import lipids, including Mce transporters. Mtb encodes four Mce transporters (Mce1-4), each of which are implicated in Mtb virulence. Mce4 is known to import cholesterol and it is speculated that Mce1 re-imports mycolic acids. Each Mce operon is comprised of two predicted permeases and six Mce proteins (MceA-F) predicted to function as solute binding proteins (SBPs). An unlinked ATPase, MceG, is predicted to function in all four Mce systems. At first glance, Mce systems resemble ABC transporters. However, Mce transporters appear to be much more complex. ABC transporters that import solutes involve a single SBP, whereas Mce transporters have six putative SBPs. Adding to their complexity, genes encoding Mce Associated Membrane (Mam) proteins are linked to Mce operons, although their function remains unknown. Furthermore, our lab identified five Orphaned Mce Associated Membrane proteins (OmamA-E), which share predicted structural similarity to Mam proteins but are not linked to mce operons. Mam and Omam proteins also share predicted structural similarity to VirB8, a component of type IV secretion systems (T4SS). VirB8 acts to stabilize and assemble the T4SS. We hypothesize that Omam and Mam proteins function as VirB8 equivalents in Mtb, serving to stabilize the Mce transporter complex. Our lab demonstrated that OmamA impacts multiple Mce systems. OmamA is necessary for Mce4 cholesterol import and the stability of multiple members of the Mce1 transporter system. As a means of better understanding OmamA function, we are identifying interacting proteins. VirB8 functions as a dimer, and our studies using Bacterial Two Hybrid indicate that OmamA can form homodimers. Interestingly, OmamA also interacts with an additional Omam encoded by the gene downstream of omamA (OmamB), suggesting that multiple Omams may come together to stabilize Mce transporters. In attempt to gain further insight in the assembly and mechanism of Mce transporters, we are continuing to map protein-protein interactions within Mce transporters and examine the effect of individual components (Omam, Mam, and Mce proteins) on Mce transporter function.
Disrupting Vital Signaling Pathways through Inhibition of Protein-Protein Interactions in *Mycobacterium tuberculosis*

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*Mycobacterium tuberculosis* (*Mtb*) is the organism responsible for tuberculosis, which is the second leading cause of death by single infectious agent worldwide. In addition to being deadly, TB is becoming increasingly difficult to treat; drug resistant *Mtb*, especially multi-drug resistant *Mtb*, are an increasing threat globally. TB is currently treated with a cocktail of drugs for at least six months, and these drugs are becoming less effective as drug resistance develops. For this reason, new drugs are needed.

The M-PFC (*Mycobacterial* protein-fragmentation complementation) assay, is used to study protein-protein interactions (PPI) in *Mtb*. In this assay, proteins are fused to split domains of dihydrofolate reductase (DHFR), which confers resistance to trimethoprim (TRIM); interaction between the proteins of interest results in enzyme reassembly and survival of *M. smegmatis* when TRIM is present. The M-PFC system is used here to explore two protein interactions, DosR homodimerization, which is essential for *Mtb* survival in hypoxia, and SigA-WhiB interaction, which is involved in transcription of many genes and is believed to be an essential interaction. In a separate project, we have developed and executed an M-PFC based high-throughput screen to identify novel inhibitors of a well-validated PPI. The main goal of this project is to exploit M-PFC to identify and validate new druggable PPI in *Mtb*. By expressing full-length and truncated versions of interactor proteins not fused to DHFR, we will identify the minimal domain necessary to block the PPI of interest, resulting in sensitization to TRIM. Overexpression of these blocker peptides in *Mtb* will then verify that inhibition of PPI involved in critical signaling pathways leads to impaired survival of *Mtb* under *in vivo* conditions. We have also explored a novel genetic tool, a theophylline responsive riboswitch, to regulate blocker peptide expression for inducer dose-dependent control of signaling pathways. The use of “riboswitchable” PPI blocking peptides may prove useful for prioritizing PPIs based on vulnerability to inhibition. These studies, if successful, will provide unique tools for studying *Mtb* signaling pathways as well as proof-of-principle for targeting of PPIs as a novel approach to TB drug discovery.
Poster #23

Role of the transcriotinal regulator, WhiB7\textsubscript{Mab} in inducible drug resistance in \textit{Mycobacterium abscessus}

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\textit{Mycobacterium abscessus} (\textit{Mab}) is a fast growing non-tuberculous mycobacterium that causes lung damage in cystic fibrosis patients. It is inherently resistant to many chemotherapeutic agents. The impermeable lipid rich cell wall, drug efflux pumps, and transcriptional regulators are thought to contribute to drug resistance. Our lab is interested in one such transcriptional regulator known as WhiB7. It is implicated in regulating multiple mechanisms of inducible drug resistance in \textit{M. tuberculosis}. The role of WhiB7 in \textit{Mab} has not yet been documented. However, it is known that the ribosome methylase gene, \textit{erm} (41), confers inducible resistance to the macrolide clarithromycin in \textit{Mab} but the underlying mechanism of regulation is not known. We hypothesize that the WhiB7\textsubscript{Mtb} homolog in \textit{Mab} contributes to the incredibly high intrinsic drug resistance of this pathogen by activating \textit{erm} and perhaps additional factors as well.

First, we examined the similarity between WhiB7\textsubscript{Mtb} and Mab\textsubscript{3508c}, a WhiB7 homolog in \textit{Mab} (WhiB7\textsubscript{Mab}) by performing a Blast P analysis. It showed 75% identity and conserved signature motifs- 4 cysteines involved in Fe-S cluster coordination, tryptophan rich region and an AT hook indicating that WhiB7\textsubscript{Mab} is a probable WhiB7 transcriptional regulator. Analysis of the promoter region showed a putative WhiB7 binding site which is consistent with positive autoregulation of WhiB7. Then, we demonstrated induction of WhiB7\textsubscript{Mab} by macrolide antibiotics (clarithromycin and azithromycin) using transcriptional mCherry reporter fusions. To understand the connection between WhiB7\textsubscript{Mab} and inducible resistance, we examined expression of \textit{whiB7}, \textit{erm} and other drug resistance associated genes by qRT-PCR in response to clarithromycin. We observed induction of both \textit{whiB7} and \textit{erm} in the presence of clarithromycin with similar kinetics. To fully define the role and importance of this protein, we are constructing and characterizing a WhiB7\textsubscript{Mab} knock-out mutant using recombineering.

The study indicates that WhiB7\textsubscript{Mab} is a functional homolog of the WhiB7\textsubscript{Mtb} transcriptional regulator that likely mediates inducible resistance to clarithromycin. Understanding the inducible resistance mechanisms in \textit{Mab} will improve macrolide-based therapy outcome in lung patients.

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Development of TB Diagnostics using Deoxyribozyme Sensors

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Current TB diagnostics have many disadvantages including high failure rates, slow turnaround times, and high cost. To address this problem, we are developing novel molecular diagnostic tools for the detection and genotypic drug susceptibility testing of *Mycobacterium tuberculosis* (*Mtb*) based on deoxyribozyme (DNAzyme) sensors. DNAzyme sensors are designed to allow for the sensitive and specific detection of RNA or DNA analytes by targeting regions containing single nucleotide polymorphisms (SNPs). We are developing a portfolio of assays which are capable of species-specific identification of *Mtb* and detection of drug resistance. To enable use at the point of care (POC), we aimed to develop a diagnostic tool which can detect the presence of *Mtb* in several hours without the need for specialized equipment. Therefore, we have developed an assay for the detection of *Mtb* RNA directly from clinical samples. Alternatively, we have developed two strategies for the detection of drug resistance conferring SNPs in DNA amplicons generated either by multiplex PCR or loop-mediated isothermal amplification (LAMP). In our proof-of-concept design, analysis of amplicons using a panel of DNAzyme sensors allows detection of resistance to 3 anti-TB drugs – rifampin, isoniazid, and fluoroquinolones. In addition, we have expanded our multiplex PCR assay to include up to 15-loci to enable strain typing and the detection of additional drug resistance profiles. Our technology provides the foundation for the development of cost-effective and sensitive alternatives for molecular POC diagnostics.

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Discovery of novel anti-TB lead compounds by phenotypic and target-based screening of positional-scanning combinatorial libraries

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There is an urgent need for potent new drugs with novel modes of action able to shorten treatment duration and kill intracellular, dormant, and drug-resistant M. tuberculosis (Mtb). Diversity oriented synthesis offers a unique opportunity to screen large numbers of compounds with less effort, time, and resources than traditional HTS campaigns. In this project, we screened combinatorial libraries of >30 million compounds built around 81 molecular scaffolds systematically arranged in scaffold ranking and positional scanning (PS) formats in both target-based and phenotypic whole cell assays. The high density coverage of chemical space aids in the identification of activity cliffs and provides a rapid understanding of the structure-activity relationships (SAR) associated with novel leads. First, using a dual-fluorescent reporter Mtb strain in a 384-well assay, we identified multiple libraries capable of inhibiting replicating and dormant Mtb. Bioactivity-guided deconvolution of the PS libraries directs the synthesis of candidate individual compounds with all possible combinations of active functional R groups for further activity screening. Secondly, these mixture-based combinatorial libraries were screened to identify novel bacterial topoisomerase I (Top1) inhibitors. We identified four compounds that inhibit mycobacterial Top1 in vitro, exhibit selective bactericidal activity against M. smegmatis and Mtb, with overexpression of Mtb Top1 in M. smegmatis yielding increased MIC consistent with inhibition of Top1 activity as the mode of action. These results highlight the potential of combinatorial library screening combined with target-based and phenotypic assays that mimic in vivo conditions to discover novel anti-TB lead compounds.
The Role of a Macrophage- and Acid-Inducible Hemerythrin Homolog in *Mycobacterium tuberculosis* Pathogenesis

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mt*)b, is responsible for ~1.5 million deaths and ~9 million new infections annually. The success of *Mt* as a pathogen is largely ascribed to its ability to survive inside the host’s own immune system, specifically alveolar macrophages. Additionally, *Mt* can adapt and survive within the hostile environment of a granuloma, where it encounters a variety of host-generated stress conditions, such as acidic pH, hypoxia, nutrient starvation, and reactive oxygen & nitrogen species (ROS, RNS). Our study focuses on a putative virulence factor encoded by Rv2633c which preliminary microarray data shows is part of a macrophage- and acid-inducible regulon. Based on its regulation and proposed hemerythrin-like domain structure, we hypothesize that Rv2633c may function in oxygen storage, oxidative stress survival, and/or as an environmental sensor during transitions into hypoxic, hostile host environments. Therefore, we have used RT-PCR to define the transcriptional unit of Rv2633c, and we have used qRT-PCR and fluorescent reporter strains to characterize the regulation of this gene under stress conditions. Several lines of evidence suggest that Rv2633c is controlled by at least three different transcriptional regulators. We will use regulator mutants to determine their role in Rv2633c regulation. Additionally, we have created an Rv2633c knock-out mutant to determine its role in pathogenesis by assessing phenotypes under *in vitro* stress conditions and in a macrophage infection model. Initial *in vivo* analyses of ∆Rv2633c in a mouse model are currently underway to assess its role during infection. These data will lend support to our assertion that Rv2633c plays a key role in the pathogenesis of *Mt* during survival within macrophages and hypoxic granulomas.
Poster #27

Expanding the genetic toolbox for mycobacteria: Constructing the *pheS* counterselection marker

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Recently surpassing HIV/AIDS, Tuberculosis is the leading cause of death from a single infectious agent, infecting an estimated 1 in 3 individuals and killing ~1.5 million globally in 2015. Despite the causative agent, *Mycobacterium tuberculosis* (*Mtb*), being discovered over a century ago, scientists are still lacking reliable and efficient genetic tools to investigate genes suspected to be involved in its notable virulence. *Mycobacterium abscessus* (*Mab*), an increasingly prevalent cause of death in Cystic Fibrosis patients, lacks usable counterselection methods entirely, limiting the ability to genetically manipulate this pathogen. The goal of this project is to develop a novel counterselection marker utilizing the *pheS* gene, to further enhance the mycobacterial gene KO strategy used to study uncharacterized genes that may be linked to their virulence.

Counterselection, a necessary component of reverse genetics, confers an inability to grow on a selection medium for any bacteria that have not undergone a double crossover to successfully create a gene KO. In this case, we employ the use of *pheS*; encoding for the alpha subunit of phenylalanyl-tRNA synthetase. PheS plays an essential role in microbial physiology by incorporating the amino acid phenylalanine during protein synthesis. Through site-directed mutagenesis we will create a PheS variant (PheS*) that will exhibit reduced substrate specificity, readily misincorporating the toxic analog, 4-chlorophenylalanine (4CP). The addition of a PheS* counterselection marker into our current suicide vector, pFCKO, will provide optimized selection of desired KOs, as cells that have eliminated the PheS*-containing vector will be able to grow on media containing 4CP.
Development of a targeted enrichment strategy for the rapid identification and characterization of Mycobacterium tuberculosis from clinical samples

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Globally, tuberculosis disease (TB), caused by Mycobacterium tuberculosis (MTB), kills more people annually than any other infectious disease. Drug-resistant MTB poses obvious challenges when treating TB disease with the standard antibiotic regime. Characterization of MTB is difficult due to slow growth of mycobacterial cultures, which subsequently explains the slow process of evaluating the susceptibility to antibiotics. Molecular tests can provide a rapid alternative to determine drug susceptibility. However, these tests generally only detect a few specific drug resistance factors unlike whole genome sequencing (WGS) which would allow for broader analysis. In this study, we evaluated the SureSelect (Agilent) and NimbleGen (Roche) for enrichment of specific gene targets as potential culture-free methods to allow for rapid detection and characterization of MTB directly from sputum samples using WGS.

To evaluate targeted enrichment kits, we developed a synthetic dilution set using human derived sputum DNA spiked with MTB H37Rv genomic DNA at various levels, ranging from 10% (Dilution 1) to 0% (Dilution 7) loads. Sequencing of the non-treated dilution set produced mostly human reads at all concentrations. SureSelect treatment of Dilution 1 resulted in 71% of the sequencing reads aligning to the MTB reference, with 100% average reference coverage, and 392X average read depth. The percent MTB read alignment, average reference coverage, and read depth for dilution 2 through dilution 5 were lower than that of dilution 1. Dilution 6 and 7 produced no reads aligning to MTB. While SureSelect did increase the number of reads mapping to MTB, the depth of coverage required for characterizing strains was lacking at lower dilutions. Like SureSelect, NimbleGen treatment increased sequencing reads aligning to MTB, but with improved reference coverage and read depth at lower dilutions. Even at dilution 5 (.001% MTB), NimbleGen treatment provided enough depth for drug resistance to be characterized for MTB. These preliminary results provide a promising foundation and will serve as a platform for further investigation and optimization of targeted enrichment methods.
Copper ions sensitize mycobacteria to select clinical antibiotics

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Copper is an increasing interest in the bacterial community. Recent studies have demonstrated its use by the innate immune system as an antimicrobial metal to fight bacterial infections [1]. Additionally, we previously demonstrated that copper ions can boost the antimicrobial activity of novel bacterial inhibitors through direct copper-compound interactions [2-6]. In this study, we identified the mycobacterial cell envelope as a potential target of copper toxicity. Uptake of ethidium bromide by *Mycobacterium smegmatis* and *M. tuberculosis* increased in a dose-dependent manner upon copper treatment suggesting that copper ions modulate cell-wall permeability in these bacteria. This observation is further supported by a copper-induced drug-sensitivity phenotype in *M. smegmatis*, which is defined as hyper-sensitivity to several antibiotics known to cross the mycobacterial cells envelope in a porin-independent manner. Importantly, we show that cells pre-treated with physiological copper concentrations for 8 hours display this drug-sensitivity phenotype even when assayed in copper deprived Middlebrook 7H9 medium, suggesting that enhanced inhibition does not result from the formation of a copper-antibiotic complex. In conclusion, our findings indicate that free copper ions damage the mycobacterial cell envelope in a manner that is not lethal to *M. smegmatis* but impairs its innate ability to resist select clinical antibiotics. This information is valuable to potential copper-based microbial treatments, and holds implications for enhanced anti-tuberculosis drug therapies in the future.
The NAD⁺ glycohydrolase activity of the tuberculosis necrotizing toxin

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*Mycobacterium tuberculosis* (*Mtb*) secretes the tuberculosis necrotizing toxin (TNT) into the cytosol of infected macrophages. TNT is a β-NAD⁺ glycohydrolase which depletes cellular NAD⁺ pools within the macrophage leading to necrosis and bacterial dissemination. *Mtb* produces an Inhibitory Factor to TNT (IFT) to prevent self-poisoning by TNT. TNT is unusual in that it shares structural homology with other NAD⁺ utilizing enzymes including bacterial ADP-ribosyltransferases, yet functions as a pure β-NAD⁺ glycohydrolase. This suggests that *Mtb* utilizes TNT solely for the purpose of depleting host NAD⁺ to induce primary necrosis. Here we further investigate the enzymatic properties of TNT. We demonstrated that TNT is also capable of hydrolyzing NADP⁺ but not the reduced dinucleotides. Analysis of product formation by NMR indicated that TNT does not produce cyclic-ADP ribose and is not inhibited by the hydrolysis products. Structural inspection of TNT docked with NAD⁺ revealed residues predicted to be involved in binding and/or dinucleotide hydrolysis. We identified several key residues which upon mutation abolished the enzymatic activity of TNT. Overall our study showed that TNT is a β-NAD(P)⁺ glycohydrolase and utilizes a novel mechanism of dinucleotide hydrolysis. The molecular consequences of TNT-mediated NAD(P)⁺ hydrolysis in cells infected with *Mtb* are yet to be determined, but ultimately result in an atypical, necrosis-like cell death.
Sugar Import by Msmeg1704 and Msmeg1712: Two SecA2-dependent sugar-binding lipoproteins of *Mycobacterium smegmatis*

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In bacteria, including mycobacteria, the highly conserved Sec and Tat pathways are responsible for the majority of protein export that occurs (i.e. protein transport from the cytoplasm to the cell envelope or extracytoplasmic space). Mycobacteria additionally possess a specialized SecA2-dependent pathway that exports a more limited set of proteins. *Mycobacterium smegmatis* is a non-pathogenic and fast growing mycobacteria that serves as a model for studying SecA2-dependent export. In *M. smegmatis*, two solute binding proteins (SBPs), denoted Msmeg1704 and Msmeg1712, are known to require SecA2 for their export to the cell wall. Msmeg1704 and Msmeg1712 are predicted to be components of two different ABC transporters, both of which are predicted to import sugars. The substrate specificity of an ABC transporter is determined by its SBP. *In silico* data predicts that Msmeg1704 imports xylose and Msmeg1712 imports arabinose; however, this has not been addressed experimentally. We constructed a *M. smegmatis* mutant that lacks the genomic region spanning *msmeg1704* and *msmeg1712*. This strain is missing Msmeg1704 and Msmeg1712 as well as their respective permeases and ATPases (additional components of their ABC transporters). We hypothesized that this mutant would be unable to grow on sugars that are imported by the ABC transporters involving Msmeg1704 and Msmeg1712. Using resazurin conversion as a marker of metabolism and growth, we found the *msmeg1704-msmeg1712* deletion mutant to be defective in growth on both mannose and L-arabinose when compared to wild type *M. smegmatis*, suggesting that these sugars are imported by either Msmeg1704 and/or Msmeg1712.

Since Msmeg1704 and Msmeg1712 are SecA2-dependent substrates, we also tested a *secA2* mutant of *M. smegmatis* for growth on mannose and L-arabinose. A *secA2* mutant was also defective for growth in mannose or L-arabinose media, and these phenotypes could be complemented by adding back a copy of *secA2* on a plasmid. Together, these results indicate that the role of the SecA2 pathway in exporting Msmeg1704 and Msmeg1712 is required for *M. smegmatis* to import and grow on mannose and L-arabinose as sole carbon sources. Future studies are planned to identify which of these sugars is imported by the respective ABC transporters.
Poster #32

Monitoring the response to TB treatment using T cells biomarkers in TB/HIV patients undergoing ART

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TB is the leading cause of death in HIV-infected persons. Anti-TB treatment in TB/HIV co-infected persons is therefore a public health priority. In patients undergoing anti-TB treatment and ART, ART initiation is often deferred by 2-8 weeks at the physician’s discretion in patients presenting CD4 counts >350 cells/ml to sufficiently reduce the Mtb burden in order to minimize potential drug interactions and complications related to the immune reconstitution inflammatory syndrome (IRIS). However, we currently lack reliable “real time” diagnostic methods to assess Mtb clearance during TB treatment. Isolation of Mtb from sputum can take 4-6 weeks and the rate of sputum smear-negative/active TB cases is upto 66% higher in HIV(+) than in HIV (-) patients. PCR-based technology while sensitive, does not differentiate between live and dead Mtb. We have recently identified specific host immune activation markers (CD38 and HLA-DR) on Mtb-specific CD4+ T cells that correlate with Mtb clearance during and after TB treatment of HIV (-) pulmonary active TB using flow cytometry. We have extended these studies to assess the performance of these biomarkers in diagnosing ATB in HIV(+) patients. Further, we are exploring the value of these biomarker profiles on T cells from TB/HIV co-infected patients undergoing ART and TB treatment, to evaluate their potential to serve as clinical correlates of Mtb clearance in ART treated TB/HIV+ patients.
Poster #33

Development of Imatinib (Gleevec) as a host-directed therapeutic for antibiotic-resistant tuberculosis

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In 2015, there were an estimated 10.4 million new cases of TB, resulting in 1.8 million TB-attributable deaths, 0.4 million of which were associated with HIV. Furthermore, approximately 480,000 cases of multi-drug resistant TB (MDR-TB), which is resistant to the first line drugs isoniazid and rifampin, occur annually, and extensively-drug resistant (XDR-TB) made up approximately 10% of all MDR-TB cases reported by 117 countries (WHO Global TB Report 2016). Treatment success rates in MDR-TB and XDR-TB are dismally low (52% and 28%, respectively) despite the use of toxic and costly regimens patients are required to take for 18 months or longer. Transmission of increasingly resistant forms of TB is ongoing, therefore there is an urgent need to identify new and effective treatments for drug resistant TB disease. We found that the cancer drug imatinib mesylate (trade name, Gleevec) is a “host-directed therapeutic (HDT) that limits mycobacterial infections in culture and animal models by targeting the host response to the bacteria. By reducing both entry into macrophages and augmenting phago-lysosomal fusion, imatinib facilitates antigen presentation and pathogen killing. Additionally, imatinib increases myeloid cell numbers (myelopoiesis) by mimicking the “emergency response,” an innate response to infection suppressed by Mycobacterium tuberculosis (Mtb). Using a Rhesus macaque model of human TB infection, we have tested efficacy of Gleevec as a treatment against acute lethal TB infection, and as or against SIV-induced reactivation. Preliminary data from these experiments will be presented and discussed.

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Mycobacterial lipid modifications regulate vascularization and infection outcome

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Vascularization of granulomas during infection with pathogenic mycobacteria has been shown to be beneficial to bacterial viability, implying that this angiogenesis may be driven in part by the pathogen itself. We have identified a set of transposon mutants of the zebrafish pathogen *Mycobacterium marinum* (*M. marinum*) that produces largely avascular infection foci during otherwise stereotypical infections of zebrafish larvae. These mutants harbor a disruption of the proximal cyclopropane synthase of alpha mycolates (PcaA) gene, and as a result are incapable of catalyzing the addition of a cis-cyclopropyl modification to the mycolic acid tails of trehalose dimycolate (TDM). Additionally, we have demonstrated that purified TDM containing the full set of mature mycolic acid tails is capable of inducing robust angiogenesis independent of infection. Thus, TDM containing this modification appears critical for driving blood vessel formation during infection, and may be acting independently to engage or significantly enhance host angiogenic pathways.
Towards the structure of PE5-PPE4-EspG3 heterotrimer from \textit{Mycobacterium smegmatis} to elucidate PE-PPE dimer recognition by cognate EspG

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Mycobacteria use a specialized Type VII Secretion (T7S) system in order to translocate protein substrates across their highly hydrophobic didermal cell envelope. In the pathogenic species the T7S system is crucial for the secretion of various virulence factors. There are five T7S loci in the mycobacterium genome, classified ESX-1 through ESX-5. ESX-1, ESX-3, and ESX-5 have been previously shown to secrete substrates, while there is lacking evidence that ESX-2 and ESX-4 are functional systems. All three of the functional systems secrete substrates from the large PE and PPE protein families, named for their Pro-Glu and Pro-Pro-Glu residues respectively. Nearly all PE proteins are found in a heterodimer with a specific PPE protein. A system-specific chaperone, EspG, for the PE/PPE dimers is encoded by each functional ESX. Each EspG protein interacts with the PPE substrate of the dimer via a hh motif that is conserved within secreted PPE proteins. Yet, the PPE substrates are only recognized by their cognate EspG partners. The goal of this study is to solve the structure of the PE5/PPE4/EspG3 trimer from ESX-3 and perform structural comparison to the homologous ESX-5 trimer to uncover key residues that elicit the specificity of the EspG interaction.
The frequency and prevalence of mutations in the RDDR of *M. tuberculosis* isolated from TB cases diagnosed in the United States

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The standard treatment regimen for tuberculosis (TB) combines four first-line antibiotics: isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (EMB). Effective treatment and prevention of transmission requires accurate diagnosis of resistance to these antibiotics. Conventional growth-based drug susceptibility testing can require weeks to months depending on the methods and testing algorithm. In comparison, molecular-based tests can provide results within a few hours starting directly from a sputum sample. However, to design this type of test, the molecular basis of resistance must be known. The molecular mechanism of resistance to RIF is the most completely understood of the four first-line drugs. 95% or more of RIF-resistant isolates contain mutations within the rifampin resistance determining region (RRDR) of *rpoB*. Commercial hybridization assays have been developed to detect mutations in the RDDR, but in some cases, the tests only distinguish if the RRDR is wildtype or mutant and cannot identify the exact mutation that is present. The positive predictive value of these tests, especially in countries such as the United States with low prevalence of RIF resistant TB, can be significantly reduced by the presence of silent mutations in the RDDR. The goal of this project was to describe the frequency and prevalence of mutations in the RDDR of *M. tuberculosis* isolated from TB cases diagnosed in the United States. The RDDR was amplified and sequenced using the IonTorrent PGM platform from 9,888 samples. Variants were detected in 314 (3%) samples. Double mutations were detected in 12 samples and a single mutation was detected in 302 samples. 29 distinct mutations resulting in 28 alleles were identified at 18 positions within the RDDR. Twenty-four nonsynonymous mutations were detected in 220 (70%) samples. The most prevalent mutation, S531L, was present in 130 (41%) samples. Five silent mutations were detected in 94 (30%) samples. F514F was detected in 63 (20%) samples, and R528R was detected in 27 (9%) samples; genotypes of these samples suggest that these silent mutations are lineage specific. Silent mutations were not identified in the 12 samples with double mutations. Future plans for this study include correlating these sequencing results with conventional culture-based drug susceptibility results and patient outcomes.
Targeting Fatty Acid Synthase-II Protein-Protein Interactions: A New Way to Kill TB

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Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), is the second most lethal infectious disease in the world and remains the leading cause of HIV/AIDS-related deaths. Current treatment of active TB involves a drug cocktail of oral pills taken daily for a minimum of six months (and potentially up to two years). This strict drug regimen has led to patient non-compliance and the rise of multi-drug resistant (MDR) and extremely drug resistant (XDR) strains. An unexploited target for tuberculosis drug discovery is mycobacterial protein-protein interactions (PPIs). PPIs are a critical component of biosynthetic, metabolic, and regulatory processes essential for bacterial viability and in vivo survival. We are using the M-PFC (Mycobacterial-Protein Fragment Complementation) two hybrid system as a basis for discovering PPI inhibitors, as it allows us to monitor protein interactions via fluorescence. We aim to create a library of M-PFC assay strains using M. smegmatis, a safe surrogate to Mtb, that monitor PPIs previously validated in literature as important for pathogenesis. These strains will then be used as a drug screening platform to identify inhibitors of PPIs. Our current project focuses on the FAS-II complex of proteins that sequentially working together to synthesize mycolic acids. Out of our four selected FAS-II proteins of interest, MabA:MabA PPI have been previously validated as essential for survival. Current frontline drugs isoniazid and ethambutol also target this TB-specific cell wall component. Our first aim is to develop M-PFC strains that exhibit robust interactions between selected proteins. We can then overexpress domains of interactors to identify peptides that block PPI, and assess whether PPI inhibition can actually lead to death of the pathogen. We predict that overexpression of MabA fragments can 1) interrupt MabA-MabA dimerization in our MPF-C strain and 2) lead to loss of viability in Mtb. These studies will provide proof of principle that inhibition of this PPI would lead to the desired killing of TB. M-PFC based high-throughput screening could then identify small molecule inhibitors of validated PPIs.
Mycobacterium tuberculosis modulates host iron metabolism to increase intracellular iron availability

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Mycobacterium tuberculosis (Mtb) is the world’s number one infectious disease killer, with over 9 million cases and 1.5 million deaths in 2014. Iron is a crucial micronutrient for all eukaryotes and most bacteria, and upon infection, Mtb bacilli must fight the host for the same iron pool. Extensive literature has shown that Mtb mutants deficient in iron sequestration are severely attenuated. Alternatively, increased dietary iron or hemochromatosis has long been associated with a worse disease prognosis. Hepcidin (Hepc) is the major regulator of serum iron levels in mammals, and promotes intracellular iron sequestration in hepatocytes and macrophages. Furthermore, Hepc has been shown to be highly expressed during inflammation. It is then important to understand the inflammatory signals leading to increased Hepc expression in innate immune cells such as macrophages, along with the role of Hepc in the innate immune response against Mtb and other intracellular pathogens.

Here we hypothesize a possible mechanism by which Mtb bacilli cause an increase in intracellular iron bioavailability to facilitate replication.

The Bacillus Calmette–Guérin (BCG) vaccine is an attenuated strain of M. bovis and was used to infect THP-1 macrophages. These BCG-infected cells show a 30-fold increase in Hepc expression and 95% reduction of the iron exported by ferroportin (FPN), dependent on MyD88 signaling. While LPS- (TLR4 ligand) treated macrophages present similar upregulation of Hepc, without any significant impact on FPN transcriptional levels, the addition of Pam3Csk4 (TLR2 ligand) promotes direct FPN transcriptional downregulation independent of Hepc expression, revealing two redundant and independent mechanisms by which Mtb bacilli can induce intracellular iron sequestration.

To assess if this response is protective to the host or if it benefits intracellular bacterial replication, THP-1 macrophages were infected in iron supplemented medium and bacterial burden was determined by gentamicin protection assay. Iron supplementation significantly increases intracellular replication. To confirm that Hepc is responsible for the increased intracellular iron availability, THP-1 cells were infected with Mtb bacilli in iron supplemented medium in the presence of an Hepc capture antibody. A significant decrease in intracellular bacterial replication was observed when compared to the untreated control.

These results elucidate a new mechanism by which Mtb bacilli evade the innate immune system and uncover iron metabolism as a novel therapeutic target for Mtb infection.
Investigation of Fluoroquinolone Heteroresistance in Serial Patient Isolates using Ultra-deep Amplicon Sequencing

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Fluoroquinolone (FQ) antibiotics are important for successful treatment of multidrug-resistant tuberculosis (MDR TB). Resistance to FQs is most often associated with specific mutations within a region of gyrA known as the Quinolone Resistance Determining Region (QRDR). Different mutations lead to low or high-level resistance. Samples from individual patients often contain mixtures of FQ-susceptible and FQ-resistant Mycobacterium tuberculosis (Mtb) or mixtures of Mtb expressing different gyrA alleles (heteroresistance).

We used molecular methods to investigate heteroresistance in serial sputum culture isolates from MDR TB patients who acquired resistance to a FQ during the Preserving Effective TB Treatment Study (PETTS). From 2005 to 2010 consecutive, consenting adults from study sites in Estonia, Latvia, Peru, Philippines, Russia, South Africa, South Korea, and Thailand with locally confirmed pulmonary MDR TB were enrolled in PETTS at the initiation of second-line treatment. Baseline and monthly sputum specimens were cultured and tested for phenotypic resistance to FQs. We sequenced the gyrA QRDR from 80 individuals who acquired FQ resistance during PETTS using IonTorrent Personal Genome Machine™. Here we present preliminary analysis of data from 21 of these individuals with cultures from at least four timepoints and with evidence of FQ heteroresistance. Individual patients were culture positive for as long as 24 months, and the number of isolates from each patient ranged from 4 to 18. A total of 27 unique QRDR alleles were identified among these patients. The number of alleles present at least once ranged from 3 to 16 per patient. For any given time point, the number alleles present ranged between 1 and 10. These data highlight the complexities of FQ heteroresistance within this patient population. Future plans include further analysis of this data taking into account treatment regimens and evaluating mixed infections through whole genome sequencing.
Characterization of the *Mycobacterium tuberculosis* deletion mutant ΔRv3351c

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*Mycobacterium tuberculosis* (*Mtb*) has recently been shown to invade and replicate in alveolar epithelial cells (AECs). Deletion of the *Rv3351c* gene from *Mtb* impacts survival of both the bacteria and the AEC. In order to characterize the *Rv3351c* gene and define the mechanism of interaction between ΔRv3351c and the AEC, studies examining bacteria and host cell viability, localization of the Rv3351c protein, and trafficking of bacteria in host cells were conducted with ΔRv3351c and parent strain Erdman. ΔRv3351c bacteria show less replication in A549 AECs and THP1 monocyte derived macrophages by CFU counts, and host cells infected with ΔRv3351c exhibit less lactate dehydrogenate (LDH) release. Confocal images show more labelling of ΔRv3351c bacteria with lysosomal markers Lamp-2 and Cathepsin-L, as well as the autophagy marker LC3 in A549 cells, indicating trafficking differences between the mutant and parent strain in these cells. Monoclonal antibodies to Rv3351c localize the protein to membrane fractions of *Mtb* cultures. These data suggest that trafficking differences between ΔRv3351c bacteria and the parent strain lead to reduced survival of the bacteria and increased survival of the host cell.
In or out? Examining the role of *Mycobacterium tuberculosis* CtpB in copper transport

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Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*M.tb*) is the leading cause of mortality by a single infectious agent in humans. As bacteria require biological trace elements for growth, metal transport systems such as the P-type ATPases might serve as novel drug or vaccine targets. One such target may be cation transport protein B (CtpB), a putative copper-transporting ATPase that is fully conserved in *M.tb* and in *Mycobacterium bovis* BCG. To determine if *ctpB* functions in copper acquisition, recombineering was used to replace *ctpB* with a hygromycin resistance cassette in BCG. Preliminary analysis indicates slower growth of the ∆*ctpB* mutant relative to BCG in naturally copper-deficient Sauton’s medium suggesting that CtpB may function as a copper influx pump. An alternate approach to examine CtpB function has been to express *ctpB* or other cation transport protein genes from an anhydrotetracycline (aTc)-inducible promoter in *Mycobacterium smegmatis*. In nonshaking Middlebrook 7H9 broth (a copper-containing medium), *M. smegmatis* expressing *ctpB* grows faster than an empty vector control strain; however, the growth phenotypes reverse when the strains are induced with 50 ng/ml aTc. Such phenotypes might be expected if leaky expression results in a low level of CtpB to aid in copper acquisition, but after aTc induction too much CtpB may result in toxic levels of imported copper. For comparison, we also examined *M. smegmatis* expressing Cu(II) efflux protein gene *ctpV*. Induction of *ctpV* resulted in a slower growth rate than the empty vector control strain in 7H9 medium, but the growth rate differences narrowed in cultures supplemented with 30-60 µM CuCl₂, as would be expected if CtpV is exporting excess copper levels. Interestingly, above 125 µM CuCl₂, growth of all strains is similarly restricted, suggesting that copper toxicity is impacting vital function. Taken together, these data support a role for CtpB as a copper import protein.
Transmission Potential of HIV Seropositive Tuberculosis Patients in sub-Saharan Africa

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INTRODUCTION: Policy recommendations on contact tracing of HIV seropositive tuberculosis patients have changed several times. Current epidemiological evidence informing these recommendations is considered low-quality and prior studies have shown heterogeneous results. We assessed latent tuberculosis infection (LTBI) in household contacts of HIV seropositive and seronegative tuberculosis patients, evaluating three-way interaction with other variables. We also compared co-prevalent and incident tuberculosis in these contacts.

METHODS: Adults laboratory diagnosed with either sputum culture or smear positive tuberculosis were identified from Old Mulago Hospital in Kampala, Uganda. Field workers visited case homes and enrolled consenting household members. LTBI in contacts was measured through tuberculin skin testing and a positive result was defined as an induration ≥10 millimeters for HIV seronegative contacts and ≥5 millimeters for HIV seropositive contacts. Relative risks (RR) were calculated using modified Poisson regression models with robust error variance. Standard assessments of interaction between LTBI, the HIV serostatus of the index case, and third variables were performed. Contacts were evaluated for tuberculosis disease at baseline and at six month intervals for 24 months.

RESULTS: In total, 1912 household contacts of 499 tuberculosis index cases were enrolled. LTBI was found in 623/908 (68.6%) and 752/1004 (74.9%) contacts of HIV seropositive and seronegative tuberculosis cases (RR=0.92, [95% CI, 0.85–0.99]). Upon further stratification, an interaction was found between LTBI, the HIV status of index cases, and both cavitary disease (p<0.0001 for interaction) and smear status (p=0.009 for interaction) of indexes. A multivariable model, controlling for family size, age and alcohol use of the contact, and smear and cavitary status of index cases, continued to show interaction. No other variables modified this relationship including cough duration of the index (p=0.6138 for interaction). Rates of co-prevalent and incident disease were similar amongst contacts.

CONCLUSIONS: This study suggests that HIV seropositive tuberculosis cases are only less infectious than seronegative patients when smear-negative or without cavitary disease. This finding may explain variability found in prior studies and, coupled with equal rates of disease in both groups of contacts, provide evidence suggesting that active case finding through contact tracing should include HIV seropositive index cases in high burden settings.
Poster #43

Permanent polymer coatings with antibacterial activity against aquatic mycobacteria

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Mycobacteriosis is an important, devastating, costly, bacterial disease of aquarium fish. This disease is also zoonotic, as it can infect people via skin wound exposure. A quaternary ammonium compound with antimicrobial activity was developed into a polymer coating that can be applied to a variety of surfaces and does not leach into the surrounding medium. We hypothesized that exposing the aquatic mycobacterial species, *Mycobacterium marinum*, to this polymer would inhibit growth. For the experiments, 1 × 10⁸ colony forming units of *M. marinum* were exposed to a polymer-coated, uncoated, or no fabric strip in 10 ml of 7H9 broth in T25 flasks. After one week, the optical density of each flask was determined. Each flask that contained the polymer-coated strips grew a minimal amount of bacteria (mean optical density = 0.0268, SD = 0.0275). For the flasks with the uncoated strip (negative control) or no strip, the optical density was approximately 70 times greater (1.426 and 1.463, respectively; SD = 0.188 and 0.266, respectively). We concluded that this polymer does inhibit the growth of *M. marinum* in broth over the course of one week. Using these results, we plan to test this polymer in an aquarium setting as a means to prevent outbreaks of mycobacteriosis.
Prevalence of latent tuberculosis infection in the community by usual setting of social interaction

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Background: In areas with high-burden of tuberculosis, public places may be the main source of tuberculosis transmission. However, specific venues where this transmission occurs in the community are not fully understood. Comparison of latent tuberculosis infection (LTBI) prevalence in different congregate locations may inform efforts to target tuberculosis prevention activities.

Design/Methods: From 2012 to the present, we conducted a cross-sectional study in patients with active tuberculosis in Kampala, Uganda. Trained health workers interviewed subjects with tuberculosis to assess the nature of their relationship to their community contacts and to obtain a list of the usual settings of social interaction for each of their contacts. A tuberculin skin test was done on each contact, and LTBI was defined as an induration ≥ 10 mm.

Results: We analyzed 245 community contacts of 63 tuberculosis cases. The median age of the contacts was 25 years (range 1-61 years) and 53% of them (131/245) were male. Their most usual setting of social interaction was the workplace (31%), followed by the home of the tuberculosis case (30%), home of contact/friend/neighbor (21%) and congregate settings (18%).

The prevalence of LTBI among community contacts was higher when the usual setting of social interaction was a congregate setting (23/44, 52%), and lower in the workplace (29/74, 39%), home of a tuberculosis case (28/74, 38%), and the home of contact/friend/neighbor (17/52, 33%). Among the congregate settings, worship centers, neighborhood bars and trading centers were the ones accounting for the highest prevalence of LTBI.

Conclusions: In community contacts of tuberculosis cases, congregate settings appear to be associated with a substantial risk of latent tuberculosis infections as compared to other settings. A prospective cohort study is currently underway to determine the role of these settings in the risk of incident tuberculosis infection.
The Development of Novel Cephalosporins for the Treatment of *Mycobacterium tuberculosis*

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Results from a high-throughput screening campaign revealed cephalosporin derivatives bearing an ester at the C-2 position with activity against *M. tuberculosis* (Mtb), which prompted our interest in developing novel cephalosporins for the treatment of tuberculosis (TB). A series of cephalosporins bearing an ester or an oxadiazole isostere at C-2, a position that is almost exclusively a carboxylic acid in clinical drugs, are reported to have selective activity against Mtb under non-replicating (NR) conditions. Representatives of the series were identified to be stable in cell-free medium, stable at both pH 2 and 7, soluble at pH 7.4, predicted to be membrane-permeable and active in macrophages without toxicity. These results support the emerging view that β-lactams may prove clinically useful in the treatment of TB.
Four Degrees of Separation: Social and Provider Networks Influence the Steps to Diagnosis of Active Tuberculosis in Urban Uganda

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Background: Delay in tuberculosis (TB) diagnosis adversely affects patients’ health outcomes and prolongs transmission in the community. The steps taken by active TB patients to seek a diagnosis are not well understood.

Methods: Using a retrospective cohort design, TB patients on treatment for 3 months or less and aged ≥18 years were enrolled from three public clinics in Kampala, Uganda, between March and July 2014. Structured face-to-face interviews were used to evaluate information about the patients’ social and health provider networks. Steps, or degrees of separation, were defined as the number of contacts a patient made to seek help since recognition of symptoms to the time of final diagnosis. Social contacts included family members, friends, and coworkers. Health providers included public and private hospitals, private clinics, public health centers, pharmacies, village health workers, and herbal healers. Step sequence analyses, followed by Cox regression analysis to determine factors associated with steps and time to final diagnosis. Hazard ratios and 95% confidence intervals represented the likelihood of timely TB diagnosis.

Results: Of 294 TB patients, 58% were male and median age was 30 (IQR; 24-38) years. The median number of steps was 4 (IQR: 3, 7) corresponding to 70 (IQR: 28,140) days to diagnosis. New patients had more steps and time to diagnosis compared to retreatment patients (5 vs. 3, P<0.0001; 84 vs. 46 days P<0.0001). Fifty-eight percent of patients first contacted persons in their social network. The first step to seeking care accounted for 41% of the patients’ time to diagnosis while visits to non-TB providers accounted for 34%. New TB patients vs. retreatment (HR: 0.66, 95% CI; 1.11, 1.99), patients first contacting a non-TB provider vs. social contact (HR: 0.72 95% CI; 0.55, 0.95) and HIV seronegative vs. seropositive patients (HR: 0.70, 95% CI; 0.53, 0.92) had a significantly lower likelihood of a timely final diagnosis.

Conclusions: On average, there were four steps or degrees of separation between the onset of symptoms in a TB patient and a final diagnosis. The patients’ social and provider networks influenced their respective diagnostic pathways. Most delays occurred in the first step, representing the patients’ initial decisions to seek help, or through interactions with non-TB providers. TB control programs should strengthen community education and TB screening as well as continuing medical education to health providers to ensure timely diagnosis of TB.
Tuberculosis Super-spreaders: Duration of cough as proxy for transmission

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Background: Heterogeneity in transmission plays an important role in the dynamics and control of infectious diseases. Super-spreaders infect disproportionately more secondary cases. In tuberculosis, the role of super-spreaders is controversial because transmission chains are difficult to measure given the long and variable latent period of infection. We evaluate the use of cough duration as a proxy measure of transmission, and evaluate the impact of super-spreaders.

Methods: We performed a secondary data analysis of cough duration in 868 tuberculosis patients from 3 independent studies, recruited in Uganda between 1995 to 2016. We examined the distribution of self-reported cough duration to determine the proportion of patients that contributed the most cough days, also referred to as “super-coughers”. To determine the factors associated with longer durations of cough, we examined patient characteristics such as age, sex, BMI, smear grade, previous TB, Karnovfsky score and type of provider using linear regression.

Findings: We found heterogeneity in cough duration, ranging 1 to 730 days. All three studies consistently showed that 50% of patients contributed 80% of cough days, and 20% of patients contributed 50% of the cough days. Clinical characteristics were weakly predictive of cough duration.

Conclusions: Patterns of cough duration are remarkably consistent among tuberculosis patients, even from different times and settings. Super-coughing may be a proxy for super-spreading transmission of M. tuberculosis, but we need to know more about the epidemiologic characteristics of super-coughers before we can use them to guide targeted public health interventions for effective tuberculosis control.
Using reduced *Mycobacterium tuberculosis* inoculum densities in MGIT pyrazinamide susceptibility testing to prevent false-resistant results and improve accuracy: a multi-center evaluation

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BACKGROUND: The primary platform used for pyrazinamide (PZA) susceptibility testing of *Mycobacterium tuberculosis* is the MGIT culture system (Becton Dickinson). False-resistant results have been associated with the use of this system. We conducted a multi-center evaluation to determine the effect of using a reduced cell density inoculum on the rate of false-resistance.

Methods: Two reduced inoculum densities were compared with that prescribed by the manufacturer (designated as “BD” method). The reduced inoculum methods (designated “A” and “C”) were identical to the manufacturer’s protocol in all aspects with the exception of the cell density of the inoculum. Twenty *M. tuberculosis* isolates, whose pncA gene had been sequenced and PZA Minimal Inhibitory Concentration determined, were tested in duplicate by nine independent laboratories using the three inoculum methods.

RESULTS: False-resistant results declined from 64 (21.1%) using the standard “BD” method to 17 (5.7%) using the intermediate (“A”) inoculum and further declined to 8 (2.8%) using the most dilute (“C”) inoculum method. The accuracy of the test results improved from 78.3% for the “BD” inoculum method to 90.5% and 94.1%, using the “A” and “C” methods, respectively. The percentages of resistant results that were false-resistant declined from 55.2% for the “BD” test to 28.8% and 16.0% for the “A” and “C” tests, respectively. Both test accuracy and precision were markedly improved using the two reduced cell density methods.

CONCLUSION: These results represent compelling evidence that the occurrence of false-resistant MGIT PZA susceptibility test results can be mitigated through the use of reduced inoculum densities.
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