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Meeting Report: World Tuberculosis Day Symposium 2012

World Tuberculosis Day Symposium Boston University School of Medicine March 22 – March 23, 2012

Since 2009, the Tuberculosis research community of the Northeast United States has commemorated World TB Day, the anniversary of Robert Koch's discovery in 1882 of the tubercle bacillus with a Scientific Symposium. It seems consistent with the spirit of the day that researchers that have committed their careers to the study of Tuberculosis have an occasion for high level scientific interaction in an informal setting. The rules are brief: 1) PI - only presentations; and 2) mostly of unpublished research with ample time for discussion. The ***Potts Memorial Foundation*** has generously subsidized the cost of the meeting. The conveners extended invitations to all within a dayapos;s drive of the conference center. The first conference was at the ***Trudeau Institute*** (Saranac Lake, NY), the second at the ***Public Health Research Institute/University of Medicine and Dentistry of New Jersey (UMDNJ)***, (Newark, NJ) and the third at the ***Wadsworth Center, New York Dept of Health*** (Albany, NY). This year the World TB Day Symposium was held at ***Boston University School of Medicine*** and ***Boston Medical Center*** with sponsorship by the ***Potts Memorial Foundation***, the ***National Emerging Infectious Diseases Laboratory*** and the ***Section of Infectious Diseases at Boston Medical Center***. The program committee consisted of Andrea Cooper, Marila Genarro, Kathleen McDonough, Igor Kramnik and Jerrold Ellner.

The conference was current and the discussions exciting. Boston has a strong academic record in research on TB and investigators and laboratories from the local institutions were well-represented. In addition to the PIs there were an unusually large number of participants from diverse schools and disciplines reflective of the scientific draw of TB - as the co-editors in chief can testify, this was not always the case. Because most of the presentations consisted of unpublished data some presenters were unwilling to submit abstracts. You can, however, get a sense of the depth and breadth of material from the Program and the submitted abstracts. On behalf of the scientific community, I want to thank Tuberculosis for providing access to the larger community of TB researchers.

Abstracts

Global and translational science

NIAID TB research program - Priorities, funding and collaborations in global TB research

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The National Institute of Allergy and Infectious Diseases (NIAID), a component of the National Institutes of Health (NIH), is a major global funder of biomedical research in tuberculosis, a recognized global health issue. NIAID's goal in supporting this research is to enhance understanding of TB as an infectious disease and facilitate transition of fundamental knowledge into new tools and approaches to combat both drug-sensitive and drug-resistant TB in all relevant patient populations. NIAID also offers support and provides resources for the discovery, advancement and clinical evaluation of novel vaccines, drugs, and diagnostics.

This presentation will provide an overview of the NIAID extramural TB program and its interaction with domestic and global partners, and will summarize areas of particular emphasis for TB research, funding opportunities and available resources to assist researchers in advancing biomedical and clinical science in this area.

For more information:

NIAID Research on Tuberculosis:
www.niaid.nih.gov/topics/tuberculosis/Pages/Default.aspx

NIAID Research in Global Health:
http://www.niaid.nih.gov/topics/globalresearch/Pages/default.aspx

NIAID Global Research: Improving Health in a Changing World:
http://www.niaid.nih.gov/topics/globalResearch/Documents/niaidglobal508.pdf

NIAID Research Agenda for Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis:
www.niaid.nih.gov/topics/tuberculosis/research/documents/mdrxdrresearchagenda.pdf

NIAID Funding Opportunities:
http://www.niaid.nih.gov/researchfunding/ann/Pages/opps.aspx

NIAID Research Resources
www.niaid.nih.gov/labsandresources/resources/Pages/default.aspx

New drugs for TB treatment

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No new classes of antituberculosis agents were brought to market between 1970 and 2000. However, in 2000 the Declaration of Cape Town began a movement that has resulted in clinical trials

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of six new anti-TB drugs, representing four new antibiotic classes. In addition, renewed efforts to shorten treatment and of TB infection and disease using existing agents have resulted in a large number of clinical studies. Phase 2 EBA studies to establish dosing have been or soon will be completed for PA-824, OPC-67683, AZD-5847, SQ-109, and PNU-100480. Phase 2b trials of TMC-207 and OPC-67683 in patients with MDR-TB have demonstrated efficacy in this important population, and 3 phase 3 studies in MDR-TB have been initiated (STREAM, TMC-207 and OPC-67683). Three phase 3 treatment-shortening trials of drug-susceptible TB are also nearly complete (OFLOTUB, ReMox and RIFAQUIN). A three-month, once weekly regimen of INH and Rifapentine for LTBI has been shown to be as effective as 9 months of INH. Regimens including these new agents hold the promise of shorter and more effective regimens for both drug-susceptible and drug-resistant infection and disease. Such regimens would greatly expand the capacity of global health programs to deliver TB treatment. As additional new drugs become available, regimen design will need to be refined to arrive at the simplest and most effective regimens. However, a major challenge will be preventing the emergence of resistance to the new agents.

Inoculum-dependent differences in tuberculin skin test (TST) and interferon- γ release assay (IGRA) responses.

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Despite established evidence that *Mycobacterium tuberculosis* is transmitted via fine aerosols, the sputum acid-fast bacilli (AFB) smear has defined tuberculosis (TB) infectiousness for decades. This prolonged reliance on sputum studies to measure TB transmission has distracted from the importance viable *M. tuberculosis* aerosols may play in modulating human immunopathology following an infectious exposure. We have developed a method to culture and quantify cough-generated aerosols from patients with pulmonary TB. We performed a prospective household contact study in Kampala, Uganda to measure TST and IGRA responses in household contacts exposed to TB patients producing no aerosols, low aerosols (1-9 colony forming units of *M. tuberculosis* in aerosols) or high aerosols (≥10 CFU). From May 2009 to January 2011, we enrolled 96 sputum culture-positive index TB cases and their 442 contacts. Compared to contacts of negative aerosol and low aerosol patients, contacts of high aerosol TB cases had: 1) a higher rate of both prevalent (baseline) and incident (six weeks) latent TB infection by either TST or IGRA criteria; 2) a larger TST induration size and higher interferon-γ levels, and; 3) larger proportion with concordant TST/IGRA results. We propose that, compared to sputum AFB microscopy, cough aerosols are a new and improved quantitative surrogate for inhaled dose. In future studies, cough aerosols may fulfill an important role as an exposure biomarker to predict infection and, possibly, progression to TB disease in contacts of pulmonary TB patients.

Post-initiation regulation of mRNA biogenesis in cells infected with *M. tuberculosis* and in active tuberculosis

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M. tuberculosis infection alters macrophage gene expression and macrophage response to interferon gamma (IFNg), a critical host defense cytokine. Understanding the regulation of changes in host gene expression is likely a prerequisite to developing any effective immunomodulatory therapy. Toward this end, we used an in vitro macrophage infection model to investigate the expression of the transcription factors STAT1 and IRF1, which mediate cellular response to IFNg and host defense against *M. tuberculosis*, and we analyzed transcriptome data for the model and for clinical studies. In differentiated THP-1 cells infected with *M. tuberculosis* and stimulated with IFNγ, we found evidence for negative post-initiation regulation of mRNA biogenesis based on changes in the abundance of nascent transcripts, total nuclear RNA, nuclear poly-A+ RNA and total cellular poly-A+ RNA, together with lack of change in the half-life of STAT1 and IRF1 transcripts in total nuclear RNA and total poly-A+ RNA. In contrast, no such regulation appeared to occur upon infection with *M. bovis* BCG. Consistent with the molecular biology of expression for STAT1 and IRF1, analysis of transcriptome data for the in vitro model and for two clinical studies comparing peripheral blood mononuclear cells demonstrated that expression of post-initiation pathway genes differed significantly with infection in vitro and between active tuberculosis and latent infection. Moreover, most significantly regulated genes associated with those annotations were repressed by infection / active disease. Collectively these results demonstrate a novel means by which *M. tuberculosis* may curb the immune response, not least by limiting the induction of STAT1 and IRF1, and perhaps other genes.

Nitric oxide controls inflammatory pathology in tuberculosis

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Proinflammatory cytokines are important mediators of innate immunity, but can also promote tissue destruction. During persistent infections, such as tuberculosis, the beneficial antimicrobial role of these cytokines must be balanced with the need to prevent immunopathology. By exogenously controlling the replication of *Mycobacterium tuberculosis in vivo*, we obviated the requirement for antimicrobial immunity and discovered that inflammatory cytokine production, neutrophil recruitment, and infection-induced immunopathology were all suppressed the adaptive immune response. In the absence of this immunoregulatory pathway, animals succumbed to infection even if bacterial replication was arrested. We propose that this anti-inflammatory activity of adaptive immunity plays an indispensable role in modulating destructive innate inflammatory responses that are elicited by chronic infection.

Repurposing parasite drugs for TB

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New targets for TB antibiotic drug discovery include bacterial processes that take place in both replicating and non-replicating TB. Promising candidates are related to essential membrane functions and basal energy metabolism, such as maintaining an activated membrane state. A second trend is to repurpose known antifungal and antihelminthic drugs, as outlined by Zhang's early paper screening known antiparasitic compounds. Among those discussed was the antiparasitic Nitazoxanide (Alinia®) (2-acetolyloxy-N-(5-nitro-2-thiazolyl) benzamide, or NTZ). NTZ a thiazolide noted for its use in the effective treatment of cryptosporidium and giardia, among other protozoa. Although in use worldwide since 1996, NTZ was approved by the FDA in 2002 for the treatment of pediatric diarrhea caused by *Cryptosporidium* species and *G. intestinalis*. In 2004 the drug was approved for similar treatment of adults. The primary mechanism of action of NTZ is believed to be the inhibition of pyruvate ferredoxin oxidoreductase (PFOR), an essential enzyme in anaerobic metabolism. Thus, in addition to the parasitic organisms mentioned above, anaerobic bacterial species such as *Clostridium* and *Bacteroides,* and microaerobic organisms such as *Helicobacter pylori*), are susceptible to NTZ. Since a number of anaerobic metabolic pathways are induced in non-replicating mycobacteria, antimicrobial agents known to target anaerobic metabolism, such as NTZ, are of recent interest for use in TB treatment. De Carvalho et al. first published the effects of NTZ on replicating and non-replicating *M. tuberculosis* (Mtb) in 2009, followed by studies on pH homeostasis and membrane potential disruption (2011). Single step resistant mutants were not recovered. Here we describe a method of NTZ^R mutant recovery and characterization of these mutants by high throughput sequencing.

High-throughput screening approaches to TB drug discovery

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There is an urgent need for new drugs for treating tuberculosis (TB), particularly in the setting of rising drug and multi-drug resistance in *M. tuberculosis*. Despite extensive genetic efforts that have identified essential genes in *M. tuberculosis*, one of the major challenges of anti-TB drug discovery continues to be the identification of novel, validated *in vivo* targets that can be chemically disrupted, thus moving beyond genetic validation to bearing true therapeutic potential. One approach to this difficulty is to target novel targets in validated pathways that are known to be required for *M. tuberculosis* survival during infection. New molecules that hit novel targets in validated pathways have the dual advantage of a high likelihood of therapeutic efficacy based on a validated mechanism of action while overcoming the high levels of resistance to current inhibitors of the pathway. As mycolic acid biosynthesis is one of the relatively few well-validated pathways for the development of TB drugs, intensive interest and effort has focused on the identification of small molecule inhibitors of new enzymes in this pathway, with increasing urgency in the setting of rising isoniazid resistance. We will discuss our efforts to identify a small molecule inhibitor of mycolic acid biosynthesis with *in vivo* efficacy in a mouse model of TB.

A complementary approach to conventional TB drug discovery is to exploit the host response to TB infection. As 1/3 of the world's population is infected with TB but only 9 million people have active disease, the host response is remarkably effective with the additional advantage that targeting the host is unlikely to engender the same rapid resistance that is observed when targeting the bacilli. We will discuss screening methods for targeting the macrophage-*M. tuberculosis* interaction as a parallel therapeutic approach.

Cyclic nucleotides in mycobacteria: beyond 3′,5′- cAMP

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Mycobacterium tuberculosis causes nearly nine million new tuberculosis cases annually. Although this pathogen was identified over a century ago, the biology of this bacillus is still not fully understood. A better understanding of the signaling cascades by which *M. tuberculosis* adapts to new environmental niches is essential for eradication of tuberculosis. It is well known that bacteria are able to utilize several cyclic nucleotides as signaling molecules, including cyclic adenosine 3′,5′-monophosphate (cAMP), cyclic guanosine 3′,5′-monophosphate (cGMP), cyclic di-adenosine monophosphate (c-di-AMP) and cyclic di-guanosine monophosphate (c-di-GMP). *M. tuberculosis* is exceptional in prokaryotes that it possesses a unique cAMP network, which includes 16 adenylyl cyclases and 10 binding proteins. Our previous studies have revealed that *M. tuberculosis* increases cAMP production and secretes significant amounts of cAMP into macrophages upon phagocytosis. In addition, mycobacterial genes associated with cyclic nucleotides other than 3′,5′-cAMP have been reported by several research groups. Rv0805 is a cyclic nucleotide phosphodiesterase that cleaves 2′,3′-cNMP (where N is an A or a G) more effectively than 3′,5′-cNMP. Rv1354c encodes a diguanulate cyclase and both Rv1354c and Rv1357c have c-di-GMP phophodiesterase

activity. In addition, we have also shown that Rv3586 is a diadenylate cyclase, which forms c-di-AMP from either ATP or ADP. Nevertheless, the biological functions of these nucleotides in *M. tuberculosis* remain largely unexplored. Our future studies will decipher the impact of these small molecules in the biology and the pathogenicity of *M. tuberculosis*.

Role of iron storage in iron homeostasis, physiology and virulence of *Mycobacterium tuberculosis*

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Like most living organisms, *Mycobacterium tuberculosis* (*Mtb*) requires iron for essential cellular functions ranging from respiration to DNA replication. Because iron is insoluble at neutral pH in the presence of oxygen, there is essentially no free iron available in the host. Pathogens require elaborated mechanisms to scavenge iron during infection. Iron can also catalyze the formation of harmful hydroxyl radicals from normal products of aerobic metabolism. Therefore, both host and pathogen must regulate intracellular iron levels to prevent iron-mediated toxicity. *Mtb* controls iron metabolism through the action of IdeR, an indispensable transcriptional regulator that represses the synthesis of mycobactins (the main siderophores produced by *Mtb*) and its transporter IrtAB, while induces iron storage genes when the cell has obtained sufficient iron. In general, iron storage proteins allow iron dependent organisms to deal with the problems of iron limitation, insolubility and potential toxicity. *Mtb* expresses two ferritin-like proteins BfrA and BfrB. The distinct roles of these proteins in the physiology and virulence of *Mtb* will be discussed.

Protein acetylation as a regulatory mechanism in Mycobacterium tuberculosis

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The post-translational modification of prokaryotic and eukaryotic proteins has been recognized for many decades. However, there are few reports of the post-translational modification of proteins in *Mycobacterium tuberculosis*. As part of our genome-wide investigations of the 20 Gcn5-related N-acetyltransferases in mycobacteria, we cloned and expressed the MSMEG_5458 (PAT) gene from *Mycobacterium smegmatis*. Using an activity-based reagent, chloroacetyl-CoA, we identified a protein from crude extracts that was chloroacetylated by MSMEG_5458 (which we refer to as Protein AcetylTransferase, PAT). Trypsin digestion followed by MS/MS identified this protein as acetylCoA synthetase (ACS). We cloned, expressed and purified the TB ACS, and showed that it was also a substrate for PAT, and that the rate of acetylation was completely dependent on the addition of 3',5'-cyclic AMP. The TB ACS protein was monoacetylated on K617, and acetylation of K617 abolished catalytic activity. Since post-translational protein modification must be reversible, we then sought a protein similar to other prokaryotic sirtuins, NAD^+-dependent acetyl-lysine deacetylases. We cloned and expressed the TB Rv1151c gene and purified the protein. We could demonstrate that Rv1151c was capable of deacetylating acetylated ACS, and that deaceylation restored catalytic activity. Together, these data argue that mycobacteria contain both a cAMP-dependent protein acetyltransferase that can modify proteins with a loss of activity and a sirtium-like deacetylase capable of restoring this activity. By using the sequence of the peptide surrounding K617, we have been able to identify a number of other proteins with similar sequence. We are expressing and purifying these enzymes, developing enzymatic assays for them and determining whether they are also reversibly acetylated with functional consequences.

Molecular mechanisms of *Mycobacterium tuberculosis* membrane biogenesis

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Mycobacterium tuberculosis (Mtb) possesses complex cell wall structures, including unusual outer membrane lipids that have been implicated in virulence. Two such species are polyacyltrehalose (PAT) and sulfolipid-1 (SL-1). Both comprise a trehalose core elaborated with four or five multiply methylated fatty acid chains. The biosynthetic pathways of SL-1 and PAT had been characterized up to the diacyl intermediates. The role of a novel acyltransferase in the final steps of SL-1 biosynthesis was recently characterized*. It will be shown that a homologous acyltransferase is involved in PAT biosynthesis. Also, as in the SL-1 pathway, PAT biosynthesis and transport are linked through a putative lipid transporter, perhaps through the interaction of multiple proteins at the membrane-cytosol interface.

* Seeliger, J. C., Holsclaw, C. M., Schelle, M. W., Botyanszki, Z., Gilmore, S. A., Tully, S. E., Niederweis, M., Cravatt, B. F., Leary, J. A., and Bertozzi, C. R. Elucidation and Chemical Modulation of Sulfolipid-1 Biosynthesis in Mycobacterium tuberculosis, *J. Biol. Chem*. In press.

Efferocytosis: A new mechanism of innate antibacterial defense

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Mycobacterium tuberculosis persists within macrophages in an arrested phagosome and depends upon necrosis to avoid both innate and adaptive immunity and to disseminate. Conversely, apoptosis of an *M. tuberculosis*-infected macrophage restricts bacterial replication via a hitherto unknown mechanism. We find that apoptosis itself is not intrinsically Following apoptosis, *M. tuberculosis*-infected macrophages are rapidly engulfed by uninfected macrophages through the process of efferocytosis. Engulfment of *M. tuberculosis* sequestered within an apoptotic macrophage further compartmentalizes the bacterium and delivers it along with the apoptotic cell debris to the lysosomal compartment. *M. tuberculosis* is killed following efferocytosis, implicating this process as the link between macrophage cell death and reduction in bacterial viability.

Thus efferocytosis is not just a constitutive housekeeping function of macrophages, but is also as an antimicrobial effector mechanism.

T cell priming during chronic *Mtb* infection

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T cells are required for long-term control of *Mycobacterium tuberculosis* (Mtb) infection, but we do not understand how fully functional T cell responses are maintained during chronic infection. Many factors, such as antigen availability, the types of antigen presenting cells, costimulatory molecules, the cytokine environment, and regulatory T cells, all likely modulate protective T cell responses. We have addressed how new thymus-derived T cells contribute to the maintenance of the peripheral CD4 and CD8 T cell response, during both acute and chronic infection. Although newly-generated T cells contribute to the peripheral response, especially during acute infection, the contribution of recent thymic emigrants declines during chronic infection. The decline is correlated with an apparent decrease in antigen presentation, or in the capability of antigen-presenting cells to drive the activation of naive T cells, because the priming and expansion of newly-generated T cells is less efficient during chronic infection. In contrast, *in vitro*-generated effector T cells readily responded to antigen when introduced during chronic infection. These findings demonstrate that although naive thymus-derived T cells can contribute to the maintenance of immunity, this process is not a major mechanism. Instead, other processes are likely responsible, such as antigen-driven T cell proliferation, perhaps originating from a population of yet undefined T cells capable of self-renewal. Our findings also reveal that antigen is not limiting for driving the activation of naive T cells during chronic infection; we propose instead that the context of antigen presentation (i.e., the type of APC, or the quality of co-stimulatory signals) changes throughout infection. Our data also reveal that thymic tolerance is not a major factor in limiting protective T cell responses to chronic Mtb infection. These studies extend our understanding of how T cell responses are maintained during persistent bacterial infections.

Pulmonary immune pathology in TB

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Morbidity and mortality in TB is largely attributable to tissue-destructive and fibrosing immune pathology. Despite its significance, the mechanisms of lung injury and fibrosis in TB are poorly understood, reflecting a gap in knowledge that has not been widely addressed in basic research. Clinical studies have shown that, after adjusting for confounders including smoking, people with prior TB disease are at increased risk for airflow limitation. This underscores the functional significance of dysregulated wound repair in TB. Lung injury can progress in some cases during the course of TB treatment that cures the infection but leaves the surviving patient with permanently impaired respiratory function. The drug pipelines for idiopathic pulmonary fibrosis and for emphysema are extensive but have so far produced disappointing results. These pipelines may, however, be productively exploited for treatments to protect lung structure and function in TB patients receiving antimicrobial therapy. Drugs that failed for other indications may be more effective in TB where treatment can be initiated early in the course of disease and where the underlying factor driving immune pathology is understood and in most cases can be eliminated. The rational selection of lung-protective drugs for interventional trials and the identification of TB patients at risk for lung can both be supported by preclinical studies in animal models. We propose that neutrophils recruited to the lung in response to cytokines and death-associated molecular patterns are the primary host cells responsible for damaging immune pathology in TB. Conditions predisposing to elevated rates of infection-induced cytolysis, and therefore increased neutrophilic inflammation will be discussed.

Heterogeneity of lung lesions in mice infected with Mycobacterium tuberculosis: a broad perspective on the mouse model of TB

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Following infection with *Mycobacterium tuberculosis* (*M.tb*), humans develop microscopic lesions in the lungs that range from well-encapsulated granulomas, to poorly delineated sheets of mixed cellular inflammation, to large areas of necrosis. Experimental animal models also show variability in the microscopic appearance of *M.tb*-induced lung lesions. Although mice do not readily form well-encapsulated granulomas, published and unpublished results from *M.tb*-infected immunocompetent inbred mice and genetically manipulated mice, demonstrate a spectrum of microscopic lesions that may be similar to humans, especially to patients with TB disease. We will review the heterogeneity of *M.tb* lesions in mice with controlled primary infection and in mice that developed TB disease, and discuss possibilities for broadening the usefulness of the mouse model of *M.tb* infection.

TB-vis: Visualizing TB patient-pathogen relationships

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DNA fingerprints of *Mycobacterium tuberculosis* complex bacteria (MTBC) are routinely gathered from tuberculosis (TB) patient isolates for all tuberculosis patients in the United States to support TB tracking and control efforts, but few tools are available for visualizing and discovering host-pathogen relationships. We present a new visualization approach, host-pathogen maps, for simultaneously examining MTBC genotyped by multiple molecular typing methods such as spoligotypes, mycobacterial interspersed repetitive units (MIRU) typing, and restriction fragment length polymorphisms (RFLP) along with associated patient surveillance data. The host-pathogen maps are dynamically coupled with spoligo-forests or other phylogenetic tree approaches to allow easy navigation within the pathogen genotyping space. Visualization of New York State and New York City (NYC) TB patient data from 2001-2007 is used to illustrate how host-pathogen maps can be used to potentially identify potential instances of uncontrolled spread of tuberculosis versus disease resulting from latent reactivation of prior infection, a critical component of tuberculosis

control. Host-pathogen maps also reveal trends and anomalies in the relationships between patient groups and MTBC genetic lineages which can provide critical clues in epidemiology and contact investigations of TB as well as relationships between MTBC lineages and antibiotic resistance. The TB-Vis host-pathogen map tool and associated tools for determining MTBC lineages are freely available for use as part of the TB-Insight suite of tools at http://tbinsight.cs.rpi.edu/.

Trailing CD8 + T cell vaccine development for TB

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Resistance to *Mtb* is dependent on both CD4+ and CD8 + T cell responses. Several vaccine candidates and formulations that induce preferentially CD4 + T cell responses have been described and shown to induce partial protection in mouse, guinea pig and monkey models of TB. Some of them are being tested in clinical trials. However, none of them contains *Mtb* candidate molecules that directly target the CD8 + T cell compartment of the immune response during the infectious process. To achieve this goal, we infected C57BL/6 mice with *Mtb* and 15 days later the animals were sacrificed and their adherent spleen cells were obtained followed by affinity purification of their MHC Class I molecules. Peptides bound to these molecules were acid eluted and sequenced by mass spectroscopy. Approximately 500 sequences could be assigned with high degree of certainty. As expected, most of them matched murine proteins. However, four sequences matched well with *Mtb* proteins. One of such molecules (5'-phosphoribosylglycinamide transformylase 2) was chosen for further studies because the discovered peptide (DGYVGAPAH) had a high predicted binding affinity to both H2-Db and H2-Kb molecules of the murine MHC. Initial experiments were performed to test the CD8 + T cell immunogenicity of this protein using a prime/boost vaccination protocol. DNA / Adenovirus construct vectors containing the full length protein sequence were used. A potent CD8 + T cell response to the peptide DGYVGAPAH was detected in spleen cells and in lung mononuclear cells isolated from the vaccinated mice. Moreover, spleen cells and lung mononuclear cells from *Mtb* infected mice also strongly recognized the peptide. Studies are in progress to verify the protection potential of this CD8 + T cell vaccine formulation against TB.

Comparative lipid profiling of *Mycobacterium tuberculosis* to identify virulence factors and biomarkers

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The lipidic envelope of *Mycobacterium tuberculosis* promotes virulence in many ways, so we developed a liquid chromatography-mass spectrometry lipidomics platform that organizes data around two new databases, *MycoMass, MycoMap*. This system can detect more than 10,000 molecular events in triplicate within one day, generating aligned datasets that define all molecular events that differ (2-fold, corrected $p < 0.05$) between any two bacteria. The low error rates allowed the first organism-wide chemotaxonomic analyses of mycobacteria, which describe the extent of chemical change in each strain and identified particular strain-specific molecules for use as biomarkers. Further, by interrogating lipids with structural relationships to mycobactin siderophores, we have identified new molecules involved in iron scavenging. New results show how conversion of deoxymycobactins to their oxygenated state by genetically defined hydroxylation enzymes represents a mechanism for activation presiderophore activation to an iron-binding state, facilitating iron import into *M. tuberculosis*.

Mycobacterial metabolomics: Chemical biology at the interface of pathogen biology and drug development

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Despite the urgent need for better drugs, TB drug development remains stalled by a lack of pharmacologically tractable targets and compounds. The causes of this shortfall are multifactorial. However, one notable deficiency of the current TB drug development toolbox is the lack of biochemical readouts of potential antimycobacterial compounds and targets. Recent advances in liquid chromatography and mass spectrometry have made it possible to measure hundreds, if not thousands, of metabolites in parallel. Metabolites are the integrated product of the genome, proteome and environment and thus the most phenotypic reporters of a cell's physiologic state. In addition, most antibiotics are themselves metabolite-like molecules that perturb bacteria at the level of their biochemical networks. The advent of such technologies has thus opened the door to global biochemical readouts of a cell's physiologic state and drug activity. We have developed and applied what, to our knowledge are, the first analytical tools to capable of providing global biochemical (metabolomic) readouts of Mtb and its response to antimycobacterial drugs. Herein, we present specific applications and case studies that illustrate the unique potential for metabolomics-based approaches to overcome current roadblocks in rationally based TB drug development efforts.

The evolution of drug resistance in M. tuberculosis

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Our research group has addressed two questions relevant to the emergence of drug resistance in M. tuberculosis. The first is to measure the frequency of drug resistance mutations among strains with known drug resistance phenotypes and to identify new mutations that confer resistance among resistant strains that do not harbor a known drug resistance phenotype. The second is to identify compensatory or fitness conferring mutations that abrogate any potential fitness costs that might be associated with the acquisition of a drug resistance mutation. We have approached these issues in multiple ways, including the systematic sampling of drug resistant and sensitive strains, whole genome sequencing and the development of methods to detect positive selection in M. tuberculosis. Through these methods, we have identified a series of candidate genes under positive selection in drug resistant strains.

Two single-tube multiplex assays for M(X)DR-TB and NTMs using LATE-PCR & Thermalight™ probes

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Background: We have constructed two rapid, single-tube assay for M(X)DR tuberculosis and non-tuberculosis mycobacteria, NTM's, using novel technologies invented at Brandeis University.

Methods: LATE-PCR generates single-stranded amplicons; PrimeSafe™ enhances polymerase specificity and multiplexing; Thermalight™ probes make it possible to scan long DNA targets for mutations. Amplification occurs in standard fluorescent thermocyclers. All amplicons are analyzed simultaneously at end-point at temperatures below the annealing temperature.

Current Results: These assays are sensitive down to ten TB genomes in the presence to 10,000 human genomes. Unique "fluorescent signatures" were obtained for 75 of 76 different rif-resistant strains tested, plus 7 drug sensitive strains containing different neutral mutations. Each of the other drug resistance targets also has its own fluorescent signature. In mixtures containing both drug resistance and drug sensitivity genomes the assay is sensitive down to 10% resistance. A second assay distinguishes all NTMs tested, as well as all clinically important MTBCs from each other. All additional tests thus far conducted for each of the other drug resistance targets have given correct answers with no false positives or negatives.

Conclusions: The single-tube assays we are constructing are robust, sensitive, and highly informative for M(X)DR-TB, MTBCs and NTMs.

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Previously unrecognized effects of the antituberculous agent pyrazinamide and analogs of pyrazinamide on the host

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While pyrazinamide(PZA) is well-known as an essential constituent of short course antituberculous therapy, the *in vitro* activity of the compound is limited and best observed at acidic pH. This modest activity is in sharp contrast to the role PZA plays in disease treatment. It is known that PZA is uptaken by *M. tuberculosis* by passive diffusion and is then subject to PZase-promoted hydrolysis to form pyrazinoic acid (POA) the putative toxic agent. It has been shown that POA can disrupt the membrane potential and membrane transport as well as decrease cellular ATP levels. PZA has furthermore been reported to have least two additional effects on *M. tuberculosis*; inhibition of FASI and binding to RpsA to block trans-translation.

In recent experiments PZA was shown to have a dramatic effect on mitigating both visceral and cutaneous leishmaniases in a murine model of infection. Similar to experiments on the inhibition of *M. tuberculosis in vitro*, PZA had only limited activity against both promastigotes and amastigotes in culture. These findings prompted a search for possible interactions of PZA and POA with host cells or essential enzymes. We will discuss our most recent observations on the inhibition of regulatory enzymes found in the human host along with the effect of PZA on leishmania-infected murine macrophages.