4th ANNUAL TRAINEE RESEARCH DAY
June 3, 2016
RI MUHC Glen Site Auditorium

11:30 Poster Set Up
Registration & lunch
12:00 Poster Judging
13:00 Opening Remarks
13:05 Keynote Speaker
14:00 Coffee & Snacks
14:15 Poster Session
15:00 Oral Presentations
16:00 Wrap Up, Photos, Prizes
Wine & Cheese

Image Courtesy Clifton Barry – In Vivo Imaging of Tuberculosis
# 4th ANNUAL TRAINEE RESEARCH DAY

**1001 boul. Décarie, RI Auditorium, Room ES1.1129**  
**Friday June 3, 2016**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:30 - 12:00</td>
<td><strong>Poster set-up, Registration and Lunch boxes</strong></td>
</tr>
</tbody>
</table>
| 12:00 - 13:00 | **Poster Judging**  
Hannah Alsdurf, *Supervisor: Dr. Dick Menzies*, Poster # 1  
Marwan Ghanem, *Supervisor: Marcel Behr*, Poster # 2  
Nargis Khan, *Supervisor: Dr. Maziar Divangahi*, Poster # 3  
Zhiyi Lan, *Supervisor: Dr. Dick Menzies*, Poster # 4  
Emily MacLean, *Supervisor: Madhu Pai*, Poster # 5  
Joe Mangiapane, *Supervisor: John White*, Poster # 11  
Jeremy Manry, *Supervisor: Erwin Schurr*, Poster # 6  
Laura Mendonca, *Supervisor: Dr. Maziar Divangahi*, Poster # 7  
Leighanne Parkes, *Supervisor: Dr. Marcel A. Behr*, Poster # 8  
Hugo Randanne, *Supervisor: Frederic Veyrier*, Poster # 9  
Jason Zou, *Supervisor: Dr. Michael B. Reed*, Poster # 10 |
| 13:00 - 13:05 | **Opening Remarks:** *Dr. Marcel Behr, Centre Director*              |
| 13:05 - 14:00 | **Keynote speaker:** *Dr. Clifton E. Barry III, Ph.D.*  
Chief, Tuberculosis Research Section  
Laboratory of Clinical Infectious Diseases  
National Institute of Allergy and Infectious Diseases  
*In vivo imaging of Tuberculosis*                           |
| 14:00 - 14:15 | **Coffee and snacks**                                                |
| 14:15 - 15:00 | **Poster Session**                                                   |
| 15:00 - 15:15 | **Oral Presentations – Moderated by Dr. Madhukar Pai**               |
Jean-Yves Dube, *Supervisor: Marcel Behr*  
Vinicius Fava, *Supervisor: Erwin Schurr*  
Catherine Hogan, *Supervisor: Madhukar Pai*  
Joaquín Sanz, *Supervisor: Luis B. Barreiro*  
15:30 - 16:00 | **Wrap up, Photos, Prizes, Wine and Cheese**                         |
|             | o Research Day                                                       |
|             | o 2015 Publication Prizes                                             |
|             | o 2015-2016 Travel Awards                                            |

*In partnership with:*
KEYNOTE SPEAKER

In vivo imaging of Tuberculosis

Clifton E. Barry III, Ph.D.
Chief, Tuberculosis Research Section
Laboratory of Clinical Infectious Diseases
National Institute of Allergy and Infectious Diseases

Biography
Dr. Barry received his Ph.D. in organic and bio-organic chemistry in 1989 from Cornell University. He joined NIAID following postdoctoral research at Johns Hopkins University. In 1998, he was tenured as chief of the TBRS. Dr. Barry is a member of several editorial boards, has authored more than 250 research publications in tuberculosis, and is the most cited researcher in the field, according to ScienceWatch.com. Dr. Barry is also an Honorary Professor at the Institute for Infectious Diseases and Molecular Medicine at the University of Cape Town where he operates laboratory and clinical research facilities.

Program Description
Tuberculosis (TB) is one of the leading infectious diseases in the world, with approximately one-third of the world’s population harboring the causative agent, Mycobacterium tuberculosis (Mtb). Though previously a disease associated with aristocratic societies, TB is now predominantly a third-world disease, particularly affecting Asian communities and sub-Saharan Africa. Mtb isolates are increasingly resistant to drug therapies: multidrug-resistant TB (MDR TB) or more severely, extensively drug-resistant TB (XDR TB). As a consequence of these emerging strains, it is becoming increasingly apparent that novel drugs are necessary to combat Mtb infections.

Tuberculosis Research Section
The Tuberculosis Research Section (TBRS) is a multidisciplinary group of research scientists comprised of biologists, chemists, and clinical researchers who share a common interest in TB. TBRS projects focus on understanding the scientific issues that facilitate the development of drugs that will make a genuine difference in the outcome for TB subjects globally. TBRS scientists are highly interactive worldwide in this endeavor, and as a result of our outstanding collaborators, TBRS has the distinction of being the most highly-cited research group in the world in the field of TB over the past 10 years. They were cited in Nature Medicine’s “The top twenty research papers on tuberculosis” (13(3):276-7, 2007). Learn more about TBRS’s ranking among TB researchers worldwide.
The Cascade of Care in Latent Tuberculosis Infection, Diagnosis and Treatment - A Systematic Review and Meta-Analysis

Hannah Alsdurf - McGill University
Dr. Dick Menzies - Montreal Chest Institute; Global TB Programme, World Health Organization (WHO)
Dr. Phillip C Hill - Centre for International Health, University of Otago, Dunedin, New Zealand
Dr. Alberto Matteelli - Global TB Programme, WHO; Clinic of Infectious and Tropical Diseases, WHO Collaborating Center for TB/HIV and TB Elimination, University of Brescia, Brescia, Italy
Dr. Haileyesus Getahun - Global TB Programme, WHO

Background: The WHO has estimated that one-third of the world’s population has latent tuberculosis infection (LTBI), and that less than 5% are diagnosed and treated to prevent tuberculosis (TB). We conducted a systematic review of studies reporting the steps in the process from initial TB screening through to LTBI treatment, which we termed the LTBI Cascade of Care.

Methods: Studies were included if they reported primary data from a cohort investigated and treated for LTBI. The search was conducted using key words related to LTBI in three electronic databases. Meta-analysis was performed using random and fixed effects analyses in SAS.

Results: The review included 58 studies, describing 70 distinct cohorts, comprised of a total of 748,572 persons. Steps in the Cascade associated with greater losses included: 72% of those intended for screening completed testing, 66% of those with a positive LTBI test completed medical evaluation, of which only 66% were recommended therapy, and 69% completed treatment if started. Steps with fewer losses included: TST reading or receiving an IGRA result, referral for evaluation if test positive, and accepting to start therapy if recommended. Overall, of those estimated to have LTBI, less than 20% completed LTBI treatment. Factors associated with fewer losses were an immune-compromising medical indication, being part of contact investigations and use of rifamycin-based regimens.

Conclusions: This review identified major losses at several steps in the LTBI Cascade of Care. Improving management of LTBI will require programmatic approaches to address the losses at each step in the Cascade.
Complementation of Mycobacterium tuberculosis-specific genes in M. kansasii to investigate the evolution of TB as a human pathogen

Marwan Ghanem1, 2, 3, Joyce Wang1, 2, 3, Fiona McIntosh2, 3, Branch Moody4 and Marcel Behr1, 2, 3, 5.

1 Department of Microbiology and Immunology, McGill University
2 Research Institute of the McGill University Health Centre
3 McGill International TB Centre
4 Brigham and Women’s Hospital, Boston
5 Department of Medicine, McGill University Health Centre

Background:
Our group has recently executed a genomic comparison between Mycobacterium tuberculosis and a low-virulence, closely related environmental species, M. kansasii, both of which having diverged from a common predecessor. These findings support the hypothesis that the latter can be used as a pertinent model to assess the genetic requirements that led to the emergence of M. tuberculosis as a professional human pathogen.

Objectives:
In the present project, we will perform a gain-of-function study to determine the effect of complementing M. kansasii with MTBC-specific genes on the behavior of the microorganism during infection. The genes of interest in this study, Rv3377-8c, are implicated in the synthesis of 1-tuberculosinyladenosine (1-TbAd), a lipid found exclusively in M. tuberculosis. 1-TbAd makes up 1% of all lipids produced by the pathogen, is constitutively synthesized and its absence leads to impeded growth of M. tuberculosis in macrophages, at least partially due to problems with phagosome acidification. We hypothesize that the production of 1-TbAd will provide M. kansasii with the means to become a more robust pathogen.

Results and Future Directions:
We have found that one of our clones has successfully gained the ability to produce adenosine-labeled lipids through TLC chromatography. We have since then confirmed the identity of these lipids as 1-TbAd by sending extracts from our clones for analysis by liquid chromatography–mass spectrometry. Next, we will observe the in-vitro growth of the successfully engineered M. kansasii mutants before moving on to assess their abilities to thrive in macrophages and in-vivo murine infection models. This study will shed light on the role of horizontal gene transfer during the emergence of M. tuberculosis as a professional pathogen.
The impact of the microbiome in shaping host defense against tuberculosis

Nargis Khan, Irah King, Dick Menzies, and Maziar Divangahi
Department of Medicine, Department of Microbiology and Immunology, Department of Pathology, McGill International TB Centre, McGill University Health Centre, Montreal, Quebec, Canada.

RATIONALE: Mycobacterium tuberculosis (Mtb) remains one of the most successful pathogens in human history. World Health Organization (WHO) estimated approximately 1.6 million people died of active tuberculosis (TB) and more than 2 billion people, are asymptomatic carriers of latent or dormant Mtb. Although anti-mycobacterial drugs are effective in controlling Mtb growth, prolonged antibiotic (Abx) treatment still comes with a significant risk of disease redevelopment.

The gut microbiota directly interfaces with the largest reservoir of leukocytes of any tissue in the body. Acute alteration of the microbiota can profoundly affect innate and adaptive immunity. Anti-mycobacterial drugs may also have off-target effects that result in microbial dysbiosis in the host. Therefore, this study is designed to understand the effects of anti-mycobacterial therapy on commensal microbial species and its consequences on the immune response to Mtb infection.

METHODS: C57BL/6 sex and age-matched mice (n=5/group) were infected with Mtb (H37Rv; ~50-100 CFU/mouse) and 6 wks later some of the mice were treated with either Rif or INH in the drinking water. After 4 weeks of Abx treatment (10 weeks post-infection), lung and intestinal tissues were harvested and prepared for both microbial and immunological endpoints.

RESULTS: We observed a robust expansion of Mtb-specific CD44hi CD8+ T cells in the lungs of infected mice. Unexpectedly, a significant population of Mtb-reactive CD8+ T cells in the colon of infected mice was observed. INH treatment led to a decrease in the effector CD8+ T cell response, RIF-treated mice were similar to infected mice. Importantly, there was a remarkable shift towards Th2 immunity in the INH-treated mice.

CONCLUSIONS: Collectively these data indicate that although INH treatment controls Mtb growth, it may cause gut microbial dysbiosis that simultaneously promote a Th2 response and ultimately prevent the generation of sterile immunity. Thus a novel combination therapy targeting both host Th2 immunity (immunotherapy) as well as Mtb growth (antibiotics) may greatly improve to control TB.
An Updated Systematic Review and Meta-Analysis for Treatment of Multidrug-Resistant Tuberculosis

Mayara Lisboa Bastos1, Zhiyi Lan2, Dick Menzies2
1. Internal Medicine Graduate Program, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
2. Respiratory Epidemiology and Clinical Research Unit, Montreal Chest Institute, McGill University, Montreal, Canada

In the past 7 years, many studies have reported new drugs or regimens for the treatment of multidrug-resistant tuberculosis (MDR-TB); this systematic review aimed to update the current evidence for MDR-TB treatment. We searched three databases for studies that reported treatment information and clinical characteristics for at least 25 patients with microbiologically confirmed pulmonary MDR-TB, and either: end-of-treatment outcomes, six-month culture conversion, or serious adverse events (SAEs). We assessed the association of these outcomes with patients' characteristics or treatment parameters. We identified 74 studies, mostly observational studies, including 17,494 participants. Information regarding diagnostic, treatment and outcome information was incomplete in most studies. No treatment parameter, including number or duration of drugs, and individual drugs were associated with improved 6-month conversion, or end of treatment outcomes. When reports from 20 cohorts were pooled, rates of SAE ranged from as little 0.5% attributed to Ethambutol to 12.2% attributed to PAS. The lack of significant associations of treatment outcomes with specific drugs or regimens may reflect the limitations and difficulties of pooling this data rather than true lack of differences in efficacy of regimens or individual drugs. This review highlights the need for stronger evidence from better designed and reported studies.
Biomarkers for active tuberculosis and prediction of active disease: a scoping landscape review

Emily MacLean (1), Tobias Broger (2), Claudia Denkinger (3), Madhukar Pai (4)
(1) Infectious Diseases and Immunity in Global Health & McGill International TB Centre;
(2) FIND, Geneva, Switzerland;
(3) FIND, Geneva, Switzerland;
(4) McGill International TB Centre, Department of Epidemiology, Biostatistics, and Occupational Health

Background: Traditional diagnostic methods for tuberculosis (TB), such as culture, are often slow or, as with smear microscopy, low in sensitivity; more modern techniques, such as GeneXpert MTB/RIF, do not reach patients where they present early or are very expensive. Simple, low-cost, biomarker-based tests, used to identify active TB or to predict those latent infections at risk of progressing to active disease, have been profiled by the WHO as priority target products.

Methods & Results: In collaboration with FIND, we are conducting a landscape study to review the state of TB biomarkers research and identify the most promising diagnostic candidates; ultimately, we aim to accelerate their translation into urgently-needed diagnostic products. A comprehensive search strategy was composed and used in multiple databases. Initially, 7909 publications were identified, of which 5549 were published between 2010 and 2015, the relevant time period for study inclusion. After removing duplicates, 3424 records remained which were then screened by title and, subsequently, by abstract. We further stratified publications based on the target condition the biomarker diagnosed, focusing on active TB and prediction of infection progression to active TB. Ultimately, 469 publications fit the inclusion criteria for full-text data extraction. There were 419 articles concerning biomarkers to diagnose active TB and 51 concerning progression of latent infection to active disease. Concerning active TB biomarkers, hematologic markers, including cell surface proteins and lymphocyte ratios, microRNAs, and cytokine signatures are being investigated. Lymphocyte signatures are also being probed to predict TB progression, but many groups continue to study interferon-gamma release assays despite their poor predictive abilities.

Conclusions & Perspective: This project is on-going, but it is apparent that the landscape of TB biomarkers is full of activity, with a range of markers showing good diagnostic promise. This avenue of research may provide much-needed tools in the global fight against tuberculosis.
Macrophage transcriptomic response to M. Tuberculosis infection

Joe Mangiapane, John White
Physiology Dept., McGill University

Mycobacterium tuberculosis (Mtb) is the etiological agent of tuberculosis (TB), one of the most infectious diseases worldwide with close to 10 million new cases annually. The primary targets for Mtb are alveolar macrophages. Our lab aims to further understand macrophage transcriptional and metabolic responses to Mtb infection. Nuclear receptors play a role in maintaining cellular homeostasis as well as responding to stimuli by activating or inhibiting gene transcription in a ligand dependent or independent manner. Nuclear receptors Retinoic Acid Receptor (RAR) and Retinoid X Receptors (RXRs) form heterodimers, which can then bind to retinoic acid response elements (RARE) on DNA to initiate gene transcription. Retinoic Acid Receptors (RARs) are non-permissive nuclear receptors that activate gene transcription when bound to ligand all trans retinoic acid (AtRA). AtRA signaling is known to play a role in cholesterol metabolism and innate immunity. Mtb uses host cholesterol as its main carbon source and when infected cells are deprived of cholesterol M.tb fails to establish chronic infection. Our previous work has shown that activation of LXR signaling during infection increases cholesterol efflux and is critical for pathogen clearance. This highlights the importance of cholesterol metabolism during the early stages of M.tb infection.

Using gene expression profiling techniques, the lab aims to understand the role of retinoids and RAR in macrophages responses to Mtb infection. Macrophage like cells were infected with H37Ra at an MOI of 5 for 48 h, and mRNA and protein expression of genes implicated in AtRA production such as ALDH1A1, CYP26B1 and DHRS3 was analyzed. Our preliminary results suggest that macrophages gain the capacity to activate RAR signaling in response to Mtb infection by inducing endogenous AtRA production which cooperates with LXR signaling to potentiate the innate immune response of Mtb infected macrophages.
Natural selection has shaped gene expression level control involved in Mycobacterium leprae infection

Jérémy Manry1,2,3, Yohann Nédélec4,5, Vinicius M. Fava1,2,3, Aurélie Cobat6, Marianna Orlova1,2,3, Guillaume Laval7,8, Luis B. Barreiro4,5,9, Erwin Schurr1,2,3
1Program in Infectious Diseases and Immunity in Global Health, The Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada;
2McGill International TB Centre, McGill University, Montreal, Quebec, Canada;
3Departments of Medicine and Human Genetics, McGill University, Montreal, Quebec, Canada;
4Sainte-Justine Hospital Research Centre, Montreal, Quebec H3T 1C5, Canada;
5Department of Biochemistry, Faculty of Medicine, University of Montreal, Montreal, Quebec H3T 1J4, Canada;
6Laboratory of Human Genetics of Infectious Diseases, Necker Branch, Institut National de la Santé et de la Recherche Médicale, U.1163, Paris 75015, France;
7Institut Pasteur, Unit of Human Evolutionary Genetics, Department of Genomes and Genetics, Paris 75015, France;
8Centre National de la Recherche Scientifique, URA3012, Paris 75015, France;
9Department of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Quebec H3T 1J4, Canada.

Leprosy is a human infectious disease caused by Mycobacterium leprae. A strong host genetic contribution to leprosy susceptibility is well established. However, the modulation of the transcriptional response to infection and the mechanism(s) of disease control are poorly understood. To address this gap in knowledge of leprosy pathogenicity, we conducted a genome-wide search for expression quantitative trait loci (eQTL) that are associated with transcript variation — before and after stimulation with M. leprae sonicate in whole blood cells. Such stimulation is expected to trigger an immune response. Indeed, among the genes upregulated by M. leprae antigen stimulation we observed a striking enrichment of immune related genes. We identified cis-eQTL for 818 genes. In addition, we found cis-eQTL for 260 genes as being specific to either stimulated or to non-stimulated cells but not under both conditions. Hence, such cis-eQTL show significant evidence for interaction with the stimulus and are termed response eQTL (reQTL). reQTL correspond to regulatory variations that affect the interaction between human white blood cells and M. leprae sonicate, and thus between the human host and M. leprae. Our evolutionary genetics approach allowed us to identify reQTL under positive selection and thus likely having a major role in the host defense against infection. Specifically, the expression of ADCY3, previously identified as a leprosy type-I reaction signature gene, and IRF4, a key inflammatory mediator gene, were modulated by reQTL that exhibited signatures of positive selection. The strongest signature of selection we identified concerned a reQTL for the UBA7 gene whose protein is known to have a critical role in immunity by conjugating with ISG15, a major type-I interferon stimulating protein. reQTL under natural selection emerge as critical mediators of the protective anti-M. leprae immune response.
Cracking Macrophages Code in Immunity to TB: Ontogeny & Metabolism

Laura Mendonça and Maziar Divangahi
Meakins-Christie Laboratories, Department of Medicine, Department of Microbiology and Immunology, Research Institute of the McGill University Health Centre, Center for Translational Biology, McGill International TB Centre, Montreal, Quebec, Canada

Rationale: Mycobacterium tuberculosis (Mtb) is a parasite of the intracellular milieu of the pulmonary macrophage (Mφ), where it not only survives but replicates in the naturally hostile environment of Mφ. Bone marrow derived (BMD) monocytes are the main source of tissue macrophages during infection/inflammation. However, a recent fate-mapping study has demonstrated that under steady state conditions, tissue macrophages from the brain, spleen, peritoneal cavity, and lung are all of prenatal origin and do not require BMDMφ for self-renewal. As the functional role of residential alveolar Mφ (AMφ) versus recruited BMDMφ during Mtb infection has not been studied, we plan to delineate the contribution of AMφ and recruited Mφ in regulation of pulmonary host defense and tissue repair mechanisms.

Methods: Bone marrow was collected from C57BL/6 and differentiated into macrophages using 30% L929 media for 6 days, prior to a 24hr polarization into M1 (using LPS and IFNγ) or M2 (using IL-4) Mφ. AMφ were harvested from the bronchoalveolar lavage fluid of C57BL/6 mice. M0 (naïve), M1 and M2 BMDMφ and naïve AMφ were then infected with H37Rv. After 48h of infection, culture supernatants, RNA and macrophages were collected for ELISA, RT-qPCR and CFU assays, respectively. Additionally, macrophages were subjected to Seahorse assays to measure basal levels of glycolysis and oxidative phosphorylation.

Results: Our data indicate that at steady state, AMφ are more reliant on mitochondrial oxidative phosphorylation, while BMDMφ depend more on glycolysis, which is in line with their anti-inflammatory and proinflammatory phenotypes, respectively. Additionally, after in vitro infection, AMφ produce more TNFα but less IL-6 and IL-10 compared to M1 and M2 BMDMφ.
Isoniazid resistance in BCG Danish: It isn’t mmA3

Leighanne O. Parkes 1†; Matthew P. Cheng 1†; Fiona McIntosh 2, 3; Marcel A. Behr 1, 2, 3
1 McGill University Health Centre, McGill University, Montreal, QC, Canada, H4A 3J1;
2 The Research Institute of the McGill University Health Centre, Montreal, QC, Canada, H4A 3J1;
3 McGill International TB Centre, McGill University Health Centre, Montreal, QC, Canada, H4A 3J1; † These authors contributed equally to this paper.

Background: Originally derived from a virulent strain of Mycobacterium bovis, Bacillus Calmette-Guerin (BCG) primary attenuation has been ascribed to the loss of RD1 locus, with subsequent in vitro propagation resulting in further genetic alterations. Although the phenotypic consequences of these alterations have yet to be fully elucidated, it has been demonstrated that strains obtained from the Pasteur Institute after 1927 do not produce methoxymycolates, which has been attributed to a point mutation at position 293 in the mmA3 gene. As the mechanism of action of isoniazid (INH) is to inhibit the synthesis of β-mycolate, methoxymycolate and α-mycolate, it has been postulated that this mutation might be associated with decreased susceptibility to INH. This carries significant clinical implications, as ‘late’, potentially INH-resistant, BCG substrain groups (Danish, Prague, Tice and Connaught) are used in vaccination against tuberculosis as well as bladder cancer immunotherapy, and as such have the capacity to adversely cause clinical disease (“BCG-osis”). Accordingly, the objective of our study was to determine if mmA3 mutant strains demonstrate phenotypic INH resistance.

Methods: BCG strains Pasteur and Danish, with and without mmA3 complementation, were grown to an OD600 of 0.5 and then diluted to an OD600 of 0.01 in 7H9 Glycerol stock. Mycobacteria were grown for seven days at 37°C in 96 well plates (Greiner 96 Flat Bottom Transparent Polystyrene) in the presence of serial dilutions of three antibiotics, including rifampin, isoniazid, and ethionamide (Sigma-Aldrich Co.). They were also grown in the antibiotic control vectors of 7H9 glycerol stock, dimethyl sulfoxide (DMSO) and pyridine. Growth was measured as the increase in OD600 in a multimode microplate reader (Tecan Infinite® 200 Pro, Tecan LifeSciences) as previously validated. The experiment was performed in triplicate.

Results: BCG Danish was not inhibited by the presence of increasing concentrations of INH in an mmA3 impendent manner. This resistance was uncoupled from rifampin or ethionamide susceptibility. In contrast, BCG Pasteur, both with and without complementation by mmA3, was fully susceptible to all three antibiotics.

Conclusion: In our analysis, BCG mmA3 mutant strains did not confer phenotypic susceptibility to INH. BCG Danish, but not BCG Pasteur, demonstrated decreased sensitivity to INH. This suggests possible underlying mutations in BCG Danish other than mmA3 that might be associated with INH resistance, distinguishing this substrain phenotypically from BCG Pasteur.
Specific regulation of mycobacteria cell cycle by small non-coding RNA

Hugo Randanne, Vincent Charron-Lamoureux, Kassen Patten, Jonathan Perreault, Frederic J. Veyrier
INRS-Institut Armand Frappier

Mycobacteria evolved a quasi-unique way of cell-elongation that, unlike other bacilli, take place at the pole. This polar-elongation is highly specific and may be particularly advantageous, in the context of infections, as newly translocated proteins, such as the well-known Exs1 type VII secretion system, primarily populate growing poles. In our efforts to understand mycobacterial evolution and its atypical cell cycle, we searched for specific differences with other bacteria. We looked closely at the dcw cluster that encodes components of the divisome and that is conserved in most bacteria. We found that a C-rich ncRNA (that we called B11.2) separates the universally conserved dyad mraZ-mraW that often starts the dcw cluster. MraW has been shown to methylate cytosine in 16s RNA. Interestingly, the terminal loop characteristic of B11.2 is composed of multiple conserved cytosines. By searching for similar ncRNA in the Mycobacteria genomes, we found another specific ncRNA (called B11) that harbours a similar structure with two C-rich loops. This RNA is surrounded by ssgB and ssd genes, that respectively encode a factor implicated in cell division by promoting FtsZ assembly [52], and a factor that has been suggested to determine the septum site [53].

Altogether, we believe that the presence of these C-rich ncRNAs (and their methylation by MraW) may represent a specific way of regulating the expression of the dcw cluster and the cell cycle. We also believe that this regulation may be important in the context of M. tuberculosis (or other Mycobacteria) human respiratory tract colonization.

As a first step to understand this regulation, we have constructed a reporter plasmid harbouring a gfp-B11.2-luxAB fusion to measure the effect of the presence of B11.2 on the mraW-mraZ operon. We also over-expressed the B11.2, B11, MraW and measure the effect on cell-morphology, gene expression and virulence.
The functional consequences of constitutive DosR over-expression in the Mycobacterium tuberculosis complex

Jason Zou, Pilar Domenech, and Michael B. Reed; Dept. of Medicine, McGill University & The Infectious Diseases and Immunity in Global Health Program, Research Institute of the McGill University Health Centre

Background:
It has been suggested that bacterial stress response pathways in M. tuberculosis (MTB) may be linked to the development of phenotypic antibiotic tolerance exhibited by certain MTB strains. One stress response pathway in MTB involves the DosR regulon. The ~50 genes composing this regulon are coordinately controlled by the DosR/S two-component system, and are believed to be associated with the metabolic adaptation to the host environment. Interestingly, upregulation of the DosR regulon has been observed alongside the development of phenotypic drug tolerance in in vitro as well as in vivo models, and strains of the Beijing MTB lineage have been shown to constitutively overexpress this regulon. The conserved nature of this phenotype within Beijing strains suggests that DosR overexpression may confer an evolutionary advantage, perhaps in the form of decreased drug sensitivity.

Objectives:
We aimed to evaluate the effect of DosR overexpression on antibiotic sensitivity and to characterize unknown regulators of the DosR promoter.

Methods:
(1) Recombinant M. bovis BCG expressing DosR under control of either the Beijing or non-Beijing DosR promoters were generated and employed in a range of assays to assess differences in drug sensitivity. (2) Reporter M. smegmatis strains containing the hygromycin resistance cassette under control of the Beijing or non-Beijing DosR promoters were subjected to resazurin cell viability assays to confirm differences in hygromycin resistance.

Results:
We report that the Mtb Beijing DosR overexpression phenotype can be recapitulated in BCG. Additionally, DosR overexpression in BCG conferred a slight decrease in isoniazid sensitivity at very specific concentrations of drug within preliminary in vitro assays. We also describe the generation of reporter M. smegmatis strains that exploit the variability in DosR promoter strength to drive striking (32-fold) differences in hygromycin resistance, which can be used in later mutagenesis screens to identify novel regulators of DosR promoter function.
Mycobacterial N-Glycolyl Muramyl Dipeptide Drives Cell-Mediated Immunity from Complete Freund’s Adjuvant

Jean-Yves R.J. Dubé (1,2,3), Damien J.C. Montamat-Sicotte (2,3), Marcel A. Behr (1,2,3,4)

Authors’ Affiliations
1, Department of Microbiology and Immunology, McGill University
2, Research Institute of the McGill University Health Centre
3, McGill International TB Centre
4, Department of Medicine, McGill University Health Centre

Background:
The capacity for the mycobacterial cell to act as an adjuvant has long been recognized, notably in complete Freund’s adjuvant (CFA, killed Mycobacterium tuberculosis in mineral oil). However, work remains to define the molecular entities unique to mycobacteria essential for CFA adjuvancy, particularly in their capacity for cell-mediated immunity (CMI) first demonstrated by Chase and Landsteiner circa 1940. Subsequent work by Lederer and colleagues suggested the peptidoglycan fragment N-acetyl muramyl dipeptide (MDP) was the minimal component for CFA adjuvancy. However, later studies highlighted the rare ability of mycobacteria to catalyze N-glycolylation of the muramic acid. N-glycolyl MDP was shown by our group to superiorly elicit pro-inflammatory cytokine production in macrophages, but how this occurs remains unknown. We hypothesize that N-glycolyl MDP more strongly elicits CMI than N-acetyl MDP, thereby contributing to CFA adjuvancy.

Objectives:
We wish to determine the contribution of N-glycolyl MDP to CMI elicited by CFA, and understand the mechanisms of MDP recognition at the level of cellular architecture important during natural mycobacterial infection (i.e. the phagosome) and at the molecular level regarding MDP structure and host molecules involved.

Methods:
In vitro, we are examining murine and human macrophages, neutrophils and dendritic cells (DCs) to understand MDP adjuvancy at the cellular level. With an in vivo murine model of immunization, we are ‘completing’ Freund’s incomplete adjuvant (mineral oil without mycobacteria) by adding MDPs and other mycobacterial pathogen-associated molecular patterns to fully describe CFA adjuvancy.

Results:
N-glycolyl MDP uniquely augments DC functions, explaining CFA adjuvancy. Conversely, neutrophils do not discriminate between N-acetyl and N-glycolyl MDPS, highlighting a cell-type-specific complexity in MDP recognition, and suggesting differential MDP recognition may involve more than just quantitatively different ligation to the supposed host receptor NOD2. The majority of CMI from CFA is dependent on Nod2, strongly implicating mycobacterial MDP in CFA adjuvancy.
A genome wide association study of pathological inflammatory responses in leprosy

Vinicius M Fava,1,2 Aurélie Cobat,3,4 Jeremy Manry,1,2 Marianna Orlova,2 Nguyen Van Thuc,6 Milton O Moraes,7 Mariane M A Stefani,8 Ana Carla P. Latini,9 Andrea Belone,9 Vu Hong Thai,6 Laurent Abel, 3,4,5 Alexandre Alcais,3,4,5 and Erwin Schurr,1,2,*
1 Infectious Diseases and Immunity in Global Health program, Research Institute of the McGill University Health Centre, and
2 The McGill International TB Centre, Departments of Human Genetics and Medicine, McGill University, Montreal, Quebec, Canada.
3 Laboratory of Human Genetics of Infectious Diseases, Necker Branch, Institut National de la Santé et de la Recherche Médicale U1163, and
4 University Paris Descartes, Imagine Institute, Paris, France.
5 Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, Rockefeller University, New York, New York.
6 Hospital for Dermato-Venerology, Ho Chi Minh City, Vietnam.
7 Laboratório de Hanseníase, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil.
8 Tropical Pathology and Public Health Institute, Federal University of Goiás, Goiânia, Brazil.
9 Lauro de Souza Lima Institute, Bauru, Brazil.

Background: Leprosy is a chronic dermato-neurological infectious disease caused by Mycobacterium leprae. Among communicable diseases, leprosy is a leading cause of permanent disabilities mainly due to pathological inflammatory responses coined Type-1 Reactions (T1R). Clinical and environmental factors have been associated with T1R susceptibility; however, studies of the host genetic contribution to T1R are scarce. Here we evaluated the association of host genetic factors with T1R using a genome wide association approach (GWAS).

Methods: A GWAS scan followed by imputation resulted in approximate 6 million genetic variant to be tested for association with T1R. Evidence of association was evaluated in two Vietnamese family-based samples: A set of T1R-affected and a second set of T1R-free families. Only SNPs significant for T1R-affected families with significant evidence of heterogeneity relative to T1R-free families were considered T1R-specific. T1R-specific loci with P < 1.0 e-6 were selected for a stepwise replication in independent population samples from Vietnam and Brazil.

Results: A total of 1490 subjects were used for the GWAS while 1880 samples were used for the replication phase. In the T1R GWAS, a suggestive signal was observed in chromosome10p21.2 (Pmin = 8.2e-7). SNPs representing seven SNP bins in linkage disequilibrium (r2 < 0.8) were selected for further analyses. Two T1R risk SNP bins in chromosome region 10p21 were replicated in two independent case-control samples from Vietnam and Brazil (P < 0.01 for both). A pooled analysis resulted in a strong association with T1R (Pmin = 5.5e-9; OR = 1.55, 95% CI = 1.34 - 1.80). The region associated with T1R is located between two recombination hot spots where a single lncRNA gene was located.

Conclusion: Our findings denote the first hypothesis free approach in the study of excessive inflammatory responses in leprosy. We identified a regulatory gene as a potential mediator in T1R pathogenesis.
Impact of fluoroquinolone treatment on the delay of tuberculosis diagnosis: a systematic review

Catherine Hogan, McGill Department of Infectious Diseases and Medical Microbiology, McGill International TB centre, Lekha Puri, McGill International TB centre, Genevieve Gore, McGill University, Madhukar Pai, McGill International TB centre

Background:
Fluoroquinolones are among the most commonly used antibiotics for the treatment of respiratory infections. Because quinolones show bactericidal activity against Mycobacterium tuberculosis complex, there is concern that their use can delay the diagnosis of tuberculosis. We conducted a systematic review to assess whether empiric treatment with fluoroquinolones delays the diagnosis and treatment of tuberculosis in patients with respiratory tract infections.

Objectives:
The primary objective was to assess the delay in days in the diagnosis and treatment of tuberculosis, among patients who received quinolones, compared to those who did not.

Methods:
We included studies of adult patients treated with fluoroquinolones prior to a confirmed diagnosis of tuberculosis. We performed a literature search of 7 databases (including PubMed, Embase and Cochrane Library) with no language restrictions. We excluded articles for which either the full text or information on diagnostic delay was not available. A meta-analysis was not conducted because of variations in comparison groups and study methodologies.

Results:
A total of 2261 citations were identified from the literature search; of these, 15 articles were selected for full-text review. A total of 8 studies were retained for the narrative synthesis. These included 5 retrospective cohort studies and 3 case-control studies. 1 of the 8 studies was from a high TB burden country. The most commonly used fluoroquinolones were levofloxacin, gemifloxacin and moxifloxacin. The median delay in time of presentation to time of diagnosis of tuberculosis was 22.5 days (IQR 13.5-26.2) in the fluoroquinolone group compared to the non-fluoroquinolone group. When stratified by acid-fast smear status, the delay was consistently greater in the smear-negative group.

Conclusion:
The use of fluoroquinolones in patients with respiratory infections delays the diagnosis of active pulmonary tuberculosis. Consistent with the International Standards for TB Care, their use should be avoided when tuberculosis is suspected.
Design principles for tuberculosis vaccines’ clinical trials based on spreading dynamics

Sergio Arregui 1,2, Joaquín Sanz 1,3,4, Dessislava Marinova 5,6, Carlos Martín 5,6,7, Yamir Moreno 1,2,8
1 Institute for Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza, Spain
2 Department of Theoretical Physics, University of Zaragoza, Spain
3 Sainte-Justine Hospital Research Centre, Montreal, Canada.
4 Department of Pediatrics, University of Montreal, Canada.
5 Microbiology Department Faculty of Medicine, University of Zaragoza, Spain
6 Networked biomedical research center on respiratory disease CIBERES,
7 Service of Microbiology, Miguel Servet Hospital. IIS Aragon.
8 Complex Networks and Systems Lagrange Lab, Institute for Scientific Interchange, Turin, Italy.

Among the different epidemiological interventions for reducing tuberculosis burden worldwide, the introduction of new vaccines aimed to improve or substitute BCG constitutes one of the most promising and cost-effective possibilities nowadays under consideration. Consequently, tens of disparate vaccine candidates are currently being developed at different preclinical and clinical stages, some of which like the MVA85A vaccine from Oxford, have already been tested for efficacy, or are to be tested soon, in large and challenging efficacy clinical trials. This kind of trials, in the lack of reliable immunological correlates of protection, constitute the only feasible alternative for efficacy estimation of TB vaccines, either at the level of protection against infection (VE_inf) or against progression to disease (VE_dis). In this work, we identify a conceptual limitation of the classical approach to estimate vaccine efficacy from them, which consists on a degeneracy in the different mechanisms through which a vaccine can disrupt the natural cycle of the disease that are in turn compatible with a single trial observation of VE_dis. In this sense, once measured VE_dis, we identify an entire family of compatible vaccines in which the mechanism of action is arbitrarily distributed between 1) a reduction of the fraction of the individuals with fast progression after infection and 2) a deceleration of the fast progression rate to disease. Furthermore, we find that the mentioned –and so far neglected– degeneracy encompasses the introduction of critical levels of uncertainty when it comes to estimate the expected vaccine’s impact in terms of reduction of disease burden; compromising our very ability to make meaningful impact forecasts. Finally, we propose an alternative approach to solve the degeneracy problem, based on the analysis of the individual transition times of the individuals between the different end-points in the trial, an observable whose retrieval is compatible with state-of-the-art protocols. The new method contributes to a more detailed description of vaccines’ features and unlocks more reliable impact forecasts, a crucial need for researchers and funding partners to ensure optimal allocation of resources in the TB vaccine development pipeline.