

# TUBERCULOSIS 2006

*Paradise Suites Hotel, Kololi, The Gambia*

*Monday 24th to Wednesday 26th, 2006*

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### **Running Order of Oral Presentations**

1st – Horacio Bach	11th – Kalifa Manneh	21st – Rajpal Singh Kashyap
2nd – Renan Goude	12th – Anne Lenaerts	22nd – Paulo Rabna
3rd – Robert Hunter	13th – Ken Duncan	23rd – Rajnish Kumar
4th – Ana Luisa Santos (1)	14th – Morten Bjerregaard-Andersen	24th – Tanya Parish
5th – Helen McIlleron (1)	15th – Abdulrahman Hammond	25th – Ana Luisa Santos (2)
6th – Yossi Av-Gay (1)	16th – Sarah Parker	26th – Helen McIlleron (2)
7th – Thomas Keller	17th – Kathleen McDonough	27th – Yossi Av-Gay (2)
8th – Alex Aiken	18th – Basudev Bhattacharya	28th – Oyebode Olakanmi
9th – Gilly Dean	19th – Claire Geoghegan	
10th – Paul Carroll	20th – Mariangela Biava	

### **Titles/Abstracts of Presentations / Summaries of Participants**

Listed Alphabetically by Family Name [ Number and/or Type of Presentation(s) in Parentheses ]

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**Alex Aiken [ Oral 8 ]**

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**Title: Reversion of the ELISPOT Test After Treatment in Gambian Tuberculosis Cases**

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**Anita Amin [ Poster x 2 ]**

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**Poster A**  
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**Antituberculous drug discovery: *In vitro* and *In vivo* screening to facilitate evaluation of novel compounds on the path to drug development**

**Anita G Amin, Shiva K Angala, Michael R McNeil, Delphi Chatterjee,**

**Department of Microbiology, Immunology & Pathology, Colorado State University, Fort Collins, Colorado, USA**

Tuberculosis is one of the most important infectious disease, killing 2 million people and causing disease in about 10 million people each year. Treatment of the disease is prolonged and the multidrug resistance cases are ever increasing. Efforts to discover and develop new drugs have increased in recent years and improvements over the existing therapies is urgently needed. The challenge of any drug discovery effort is to identify and develop compounds with properties that show good efficacy on the disease causing organism and safety in humans. We have access to a library of lead compounds that seem to inhibit the growth of *Mycobacterium tuberculosis (M.tb)*. An estimated 3000 compounds have been tested so far for their MIC values against *M.tb* of which 22 nucleoside sugar analogues consistently showed decent MICs. These are further being evaluated for cytotoxicity (IC50) in mammalian cell lines. Compounds with a selectivity index (SI), MIC/IC50 >10 will move forward for studies in the animal model. The information obtained from evaluating these compounds will then be used to enable better targeted compound synthesis.

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## Poster B

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**Identification and characterization of *emb* promoter elements of *Mycobacterium tuberculosis* using *Mycobacterium smegmatis* as the genetic host**

**Anita G Amin\*\***, Renan Goude\*, Tanya Parish\*, Delphi Chatterjee\*\*

**\*\*Department of Microbiology, Immunology & Pathology, Colorado State University, Fort Collins, Colorado, USA.**

**\*Institute of Cell & Molecular Science, Barts & The London, Queen Mary's School of Medicine and Dentistry, London, UK.**

The *emb* genes are conserved among different mycobacterial species. In *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* the three *emb* genes, *embCAB*, belong to an operon where *embA* and *embB* are involved in the synthesis of arabinogalactan (AG) and *embC* is shown to be involved in synthesis of arabinan in lipoarabinomannan (LAM). Since *embCAB* is likely organised as an operon, it would be reasonable to expect that these three genes are transcribed as a single polycistronic mRNA from a unique promoter. In this study we have identified two promoter regions, a 39 bp region upstream of *embC* and an 85bp region immediately upstream of *embA* which induced expression of the *LacZ* gene in *M.smegmatis*. A strong promoter found upstream of *embC* just before *Rv3790* is induced during the early log phase of the growth. It also shows strong induction under oxidative stress but is turned off under hypoxia. Mutations in the putative -10 region confirm the presence of the promoter and also that it is essential for the promoter activity in *M.smegmatis*. LAM extracted from cells under various growth conditions does not show any significant change in the synthesis. Also it is interesting to see LAM being synthesized in the cells under hypoxic stress when the promoter is shut down.

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**Zaida Araujo [ Poster x 2 ]**

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## Poster A

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**DIAGNOSTIC UTILITY OF ADENOSINE DEAMINASE (ADA) IFN- $\gamma$  AND IL-12 IN PLEURAL TUBERCULOSIS**

C. Fernandez de Larrea<sup>1</sup>, A. Duplat<sup>1</sup>, Z. Araujo<sup>2</sup>, F. Giampietro<sup>2</sup>, J.H. de Waard<sup>3</sup>, I. Rivera<sup>3</sup>, M. Comengna<sup>4</sup>

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Pleural tuberculosis (PTB), a frequent form of presentation of extrapulmonary tuberculosis, approximately represents the 5 percent of all the diseases associated infection by *Mycobacterium tuberculosis*. The diagnosis in many occasions

is prolonged in the time, as in the case of the delay of the pleural liquid culture, which takes weeks. Other tests are bloody and expensive, like the pleural biopsy or the accomplishment of the Polymerase Chain Reaction (PCR). Due to this, strategies related to enzyme determination have been designed as adenosine deaminase (ADA), or cytokines such as IFN- $\gamma$  or TNF- $\alpha$  in pleural liquid and serum, to improve the yield diagnose cost-benefit. The objective of the present study consisted of comparing the diagnostic utility from the determination of IL-12, IFN- $\gamma$  and ADA in pleural liquid as well as in serum of 60 patients with pleural effusion: 20 with pleural tuberculous effusion and 40 with non-tuberculous pleural effusion. There were significant differences between the diminished levels of IL-12 in serum in the patient groups with PTB in comparison with the controls ( $p < 0.03$ ). For IFN- $\gamma$ , there were significant differences between the increased levels of IFN- $\gamma$  produced by the TB patients in pleural liquid in comparison with the controls ( $p < 0.0001$ ). According to analysis ROC, the point of cut-off considered for IL-12 in serum was 497 pg/ml, for IFN- $\gamma$  in liquid was 612 pg/ml and 29 UI/L for ADA, also in pleural liquid. The greater diagnostic utility was obtained with ADA in pleural liquid showing a sensitivity of 95% and a specificity of 92.5%. The determination of IFN- $\gamma$  in liquid showed the second best diagnostic utility, with a sensitivity of 95% and a specificity of 82.5%. Unlike the IFN- $\gamma$ , IL-12 in serum, a better correlation was obtained than in liquid. It showed a sensitivity of 95% and a lower specificity of 50%. Conclusions: The determination of IFN- $\gamma$  in liquid and of IL-12 in serum would allow to add a value diagnosis to ADA in liquid. The differential role of IL-12 and IFN- $\gamma$  related to the greater production of these in serum and liquid, respectively, it could be in correlation with the immune response forehead to *Mycobacterium tuberculosis* that settles down at systemic and local level.

Keywords: Tuberculosis, adenosine deaminase (ADA), IL-12, IFN- $\gamma$ , TNF- $\alpha$ , pleural liquid.

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## Poster B

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### REACTIVITY OF THE IgG ISOTYPES AGAINST ANTIGEN OF *MYCOBACTERIUM TUBERCULOSIS*

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The diagnosis of tuberculosis (TB) in rural zones such as the Amacuro Delta is hampered by the use of low-sensitivity methods such as bacilloscopy, which renders around 60%. The aim of this study was to evaluate the sensitivity and specificity of the IgG isotypes against the PPD antigen of *Mycobacterium tuberculosis* in the sera of patients from indigenous and non-indigenous populations with pulmonary and extra-pulmonary TB, by means of an ELISA assay. The study included 166 patients and 190 controls. The former groups consisted of 87 Warao indigenous patients with pulmonary TB; 58 non-indigenous patients with pulmonary TB; and 21 non-indigenous patients with extra-pulmonary TB, presenting pleural discharge. The control groups were composed of 75 healthy Warao indigenous controls; 76 healthy non-indigenous controls; and 39 non-indigenous controls with pleural discharge unrelated to TB. The results of the assay using IgG1 and IgG2 isotypes were lowly specific and sensitive for all groups. However, even though that the assay using the IgG3 isotype revealed sensitivities of 20.7% (IC 95%, 12.7-30.7) for the pulmonary form in the indigenous population, compared with 32.8% (IC 95%, 21.0-46.3) and 4.8% (IC 95%, 0.12-23.8) for the TB pulmonary and extra-pulmonary forms respectively in the non-indigenous population. The results of the method were highly specific, especially in the indigenous group 100% (IC 95%, 95.5-100), the other specificities were 89% (IC 95%, 80.3-95.3) and 90% (IC 95%, 75.8-97.1) for the TB pulmonary and extra-pulmonary forms respectively in the non-indigenous population. For this isotype, the positive predictive values (PPV) were 100% (IC 95%, 81.5-100), 70.4% (IC 95, 49.8-86.2) and 20% (IC 95%, 0.5-71.6) respectively, while the negative predictive values (NPV) were 52.1% (IC 95%, 43.6-60.5), 63.6% (IC 95%, 53.7-72.6) and 63.6% (IC 95%, 49.6-76.2) respectively. In conclusion, the assay using IgG3 is highly specific and therefore could be of use in confirming TB when a positive method has been previously applied, especially among the Warao population.

Keywords: Tuberculosis, Warao indigenous, non-indigenous, IgG1, IgG2 and IgG3 isotypes.

**Comparison of Rapid Tests for Screening Sputum Specimens for Rifampicin-resistant Tuberculosis in Uganda.**

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**Introduction:** Rifampicin and Isoniazid resistant tuberculosis (MDR-TB) is increasing in developing nations. MDR-TB is high in retreatment cases, and about 50% of patients admitted to the TB ward at the national referral hospital, Mulago in Uganda, are re-treatment cases. Over 90% of Rifampicin resistance among MTB isolates are coupled with Isoniazid resistance, making Rifampicin resistance a surrogate marker for MDR-TB. In this study, 3 direct rapid tests namely; 1) Direct BACTEC 460 drug susceptibility test, 2) Direct phage susceptibility assay and 3) INNO-LiPA Rif TB Assay were compared against the Gold standard, the indirect BACTEC 460 drug susceptibility test. Each test was evaluated for its percentage agreement with the Gold standard, turn around time and cost. In addition, the usefulness of rifampicin resistance as a marker for MDR-TB in the study setting was also evaluated.

**Methods:** Early morning spot smear-positive sputum specimens from retreatment patients were selected as they potentially have a higher probability of being resistant. Specimens were processed using 1% NaOH/NALC method. The sediment was reconstituted to 2.5 mls, using phosphate buffer pH 6.8, and the resulting suspension was used to prepare smears and cultures. A routine Bactec culture that was used for indirect susceptibility testing and MTB identification was set up. Simultaneously, the direct tests were set up using part of the remaining inoculum.

**Results:** The number of samples that were analyzable by the different direct methods was 34 for LiPA, 59 for direct phage, and 38 for direct Bactec. The percentage agreements with the gold standard for the tests were 74% for LiPA, 83% for direct phage and 100% for direct Bactec. The mean turn around time in days for the tests was 2 for LiPA, 3 for direct phage and 9 for direct Bactec, compared to an average of 24 for the indirect Bactec. The cost in US \$ of each of the rapid tests was 52 for LiPA, 24 for direct phage and 30 for direct Bactec. Finally, direct Bactec correctly discerned MDR's 100% of the time, while LiPA's forecast was 94%.

**Conclusion:** All the three methods offered more rapid detection of rifampicin resistance than the conventional method, and were good predictors of MDR-TB. However, the sample tested in this pilot study was small and contained a high percentage of heavy smear positive specimens. Further studies to determine accuracy are to be undertaken.

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**Oral 6**  
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**Title: *M. tuberculosis* Protein Kinase; Translating External Signals into Adaptive Gene Expression (Cognate Substrates and Down Stream Effectors)**

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**Oral 27**  
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**Title: Mycothiol; Unique Thiol Metabolism in Mycobacteria**

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**Horacio Bach [ Oral 1 ]**

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**Title: Substrate Identification of the Protein-Tyrosine Phosphatase A (PtpA) from Mycobacterium Tuberculosis**

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**Basudev Bhattacharya [ Oral 18 ]**

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**Development of a New Sensitive and efficient Multiplex PCR for for identification and differentiation of different mycobacterial species and Molecular Diagnosis of MDR-TB applying some basic molecular tools** Basudev Bhattacharya<sup>1</sup>, Debashis Banerjee<sup>1</sup>, Siddartha Gupta<sup>1</sup>, Vishmadeb Pramanik<sup>2</sup>, Sekhar Chakrabarti<sup>3</sup>

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For early detection and species differentiation of mycobacteria, polymerase chain reaction (PCR) techniques are currently in wide use. However, individual techniques using amplification of different targets with appropriate primers still have some limitations, which have to be overcome. The ideal technique would use DNA sequences which should be present in all mycobacteria and absent in others and would be able to discriminate one species from the other, as non-tuberculous mycobacteria (NTM) are on rise in terms of frequency of detection. We developed a multiplex PCR based on amplification of 165, 365 and 541 bp target fragments of unrelated genes, *hsp 65* coding for 65 kDa antigen, *dnaJ* gene of mycobacteria and insertion element *IS 6110* of *Mycobacterium tuberculosis*, respectively. This multiplex PCR was tested over 10 years from 1996 to 2005 with 411 clinical specimens from suspected cases of tuberculosis and mycobacterioses and compared with standard laboratory techniques. The multiplex PCR was positive for 379 cases compared with 280 cases by standard techniques ( $P < 0.0001$ ). It could distinguish between strains of the *M. tuberculosis* complex and NTM; the results are comparable with standard techniques. Thus the multiplex PCR can be useful in early detection, species differentiation and epidemiology.

The drug resistance tuberculosis (MDR-TB) has frequently been encountered in India and its presence has been known from the time anti-tuberculosis drugs were introduced for the treatment of tuberculosis. Much of the drug resistance is presumed clinically, because lack of proof of drug resistance is mainly due to limited facilities for sputum culture and susceptibility tests if and it is also time consuming. At present multidrug resistant tuberculosis scenario in India is not clear because of paucity of survey data.

We have developed and standardized by PCR-SSCP (Single strand conformation polymorphism) followed by sequencing of *rpoB* gene, *KatG* gene and *gyrA* gene for identification of Rifampicin, Isoniazid and Fluoroquinolone.

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**Mariangela Biava [ Oral 20 ]**

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**New 1,5-diphenyl pyrrole derived from BM 212: a new class of antimycobacterial agents.**

**Biava M,** <sup>1</sup> Porretta GC, <sup>1</sup> Poce G, <sup>1</sup> Supino S, <sup>1</sup> Pompei R, <sup>2</sup> Manetti F<sup>3</sup>, Botta M<sup>3</sup>

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*Mycobacterium tuberculosis* (MTB), responsible for tuberculosis (TB) in humans, causes the death of almost 3 million people each year, and it is positioned as the leading bacterial infectious agent. According to a recent report compiled by the World Health Organization (WHO), the total number of new cases of TB worldwide in 2002 had risen to approximately 9 million and it is estimated that between 2002 and 2020, approximately 1000 million people will be

newly infected, over 150 million people will get sick, and 36 million will die of TB if proper control measures are not instituted. Increased infection with the *M. avium* complex (MAC) is also contributing to the morbidity and mortality in AIDS patients. The most urgent goal of chemotherapy of tuberculosis infections should be the development of highly active and low-cost drugs, which should be used not only in industrialized countries but also in developing ones in which both these infections are now rapidly increasing.

As active molecules already introduced in therapy very soon generate resistance, scientists have focused their attention on the development of new antimycobacterial compounds acting with a mode of action without cross-resistance.

In this way many other new antimycobacterial compounds, belonging to different chemical classes, have been synthesized and developed; some of them appear to be promising candidates for further development.

The communication will deal with our studies on antimycobacterial new drugs: in pyrrole antibacterial area, a subclass with a good potent *in vitro* activity against mycobacteria and fungi was identified. Moreover, the salient structural feature was individuated and **BM 212** was classified as the lead compound. SAR studies allowed us to synthesize several analogue derivatives. Some of them revealed to be more active than **BM 212** against mycobacteria, but they lost antifungal activity. In particular the Protection Index (PI) for many derivatives was comparable to that of reference compounds, Isoniazid, Streptomycin and Rifampin. Many of the synthesized compounds revealed also to be active against intracellular mycobacteria and they showed to inhibit drug-resistant mycobacteria of clinical origin. On the base of microbiological results we have hypothesized a pharmacophore model that was also optimized. The rational design, and the evaluation of the *in vitro* activity against mycobacteria will be described.

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**Morten Bjerregaard-Andersen [ Oral 14 ]**

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**Title: TB Prevalence in a Low Resource Setting**

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**Paul Carroll [ Oral 10 ]**

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The development of a conditional expression system in *Mycobacterium smegmatis*

Paul Carroll, Amanda Claire Brown and Tanya Parish

Defined mutations are central to the understanding of individual gene function in any organism. However, essential genes cannot be deleted since a lethal phenotype is generated, so the ability to elucidate their functional roles is more difficult. Increasing numbers of essential genes are being discovered in *Mycobacterium tuberculosis*. Thus methods for studying essential genes in mycobacteria are urgently required.

One technique that has been used is the generation of conditional mutants which can express the gene only under defined conditions. Often this has been achieved using inducible promoters which can be switched on and off in a controlled manner. To date, only the tetracycline-dependent and the acetamide-inducible systems have been used in mycobacteria. However, these systems are not always tightly controlled and therefore may not be suitable for the study of essential genes.

The P<sub>BAD</sub> promoter system, derived from the arabinose operon of *Escherichia coli*, has been used to tightly regulate gene expression. Expression is induced by the presence of arabinose and can be tightly switched off in the presence of glucose by catabolite repression. We investigated this system as a ideal candidate for studying gene

essentiality in mycobacteria. We adapted the P<sub>BAD</sub> system for use in mycobacteria and assayed its use in *M. smegmatis* using a reporter gene and a toxin from *M. tuberculosis*.

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**Delphi Chatterjee [ Poster ]**

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*Title is Awaiting Confirmation by the Author*

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**Bouke de Jong [ Poster ]**

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Title: *M. africanum* phenotypes compared with *M. tuberculosis*

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**Ken Duncan [ Oral 13 ]**

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Dr Ken Duncan  
Imperial College, London  
Consultant to the Bill & Melinda Gates Foundation

The future of TB drug discovery

A number of new TB drug candidates are being evaluated in clinical trials. However, a new approach is needed in order to discover lead compounds that have the potential to decrease radically the duration of therapy for active tuberculosis or to treat latent infection. The lack of understanding of basic biology that underpins the long-term survival of *Mycobacterium tuberculosis* in humans must be addressed and linked directly to discovery of new TB drugs. In particular, we need to reassess what is currently known to establish a firm baseline of knowledge, attempt to understand the biology of persistent *M. tuberculosis*, improve screening and lead compound identification efforts, and create new tools for preclinical evaluation of drug candidates. This requires the engagement of a broad range of expertise in a collaborative and coordinated research program. As an example, researchers in the U.K., U.S., Singapore, Korea, and Mexico are collaborating in a project that addresses one of the "Grand Challenges in Global Health" identified by the Bill & Melinda Gates Foundation. The team is led by Prof Douglas Young at Imperial College, London, and is attempting to understand the fundamental biology of latency and use this knowledge to develop drugs that are effective against latent TB. Their work could also lead to improved treatments for active TB disease. This project exemplifies co-operation between academic groups and an industrial partner, the Novartis Institute for Neglected Diseases.

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**Gilly Dean [ Oral 9 ]**

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**Isoniazid Treatment of Bovine Tuberculosis : Development of a Memory Model**

Dean GS\*, Rhodes SG, Coad M, Whelan AO, Cockle PJ, Wheeler P, Villareal-Ramos B♦, Mead E♦, Clifford DJ, Hewinson RG and Vordermeier HM

TB Research Group, Veterinary Laboratories Agency, Weybridge,  
United Kingdom KT15 3NB and ♦Institute for Animal Health, Compton, Berkshire RG20 7NN, UK

Isoniazid is widely used in the treatment of infection with *Mycobacterium tuberculosis* in humans. It has also been shown to be effective in the treatment of cattle infected with *M. bovis* although this procedure is illegal in the United Kingdom. This series of experiments aimed to determine whether animals infected with virulent *M. bovis* and then treated with isoniazid were protected against subsequent challenge. A preliminary experiment showed that cattle infected with *M. bovis* and treated with isoniazid were significantly protected in that they displayed a much reduced pathology on post mortem examination. Treatment with isoniazid did not affect either the growth of individual animals or liver function (hepatotoxicity is known to be a potential side effect of isoniazid treatment). This infection / treatment regime was then repeated in a larger experiment. 24 calves were divided into 3 groups of 8 ; Group 1 received a primary infection with *M. bovis* (spoligotype 9), treatment with isoniazid, and re-challenge with *M. bovis* (spoligotype 35) ; Group 2 received infection with *M. bovis* (spoligotype 9) and treatment with isoniazid only ; Group 3 received

M.bovis (spoligotype 35) only. Immunological parameters were measured throughout the experiment and detailed post mortem examination carried out 13 weeks after the M.bovis spoligotype 35 challenge. Differences in cytokine profile, pathology and histopathology between the three groups will be discussed and the potential of this regime as a model for protective immunity in bovine tuberculosis.

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**Claire Geoghegan [ Oral & Poster ]**

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**Oral 19**  
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**Title: Bovine Tuberculosis: Shedding Light on the Zoonotic Interface Between Human and Animal Health**

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**Poster**  
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**Title: Bovine Tuberculosis: Examining the Interface Between Human and Animal Health**

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**Renan Goude [ Oral 2 ]**

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Lipoarabinomannan biosynthesis in *Mycobacterium tuberculosis*

Renan Goude, Anita G Amin, Delphi Chatterjee and Tanya Parish.

The cell wall of *Mycobacterium tuberculosis* (Mtb) is a complex, lipid rich structure unique to mycobacteria. As well as its role in maintaining the structural integrity of the cell, it forms a major permeability barrier and so a great deal of effort has gone into elucidating its molecular structure and biosynthetic machinery, in order to identify targets for new drugs. Lipoarabinomannan (LAM) is one of the major constituents of the cell wall. In addition to a structural role, it has potent immunomodulatory effects. Although the structure and biosynthesis of LAM have been the focus of many studies, relatively little is yet known about the synthesis of the arabinan core.

In the related but non-pathogenic species *Mycobacterium smegmatis* (Msm), EmbC has been identified as an arabinosyl transferase involved in LAM biosynthesis. Although the role of Msm EmbC has been elucidated, there is no information for EmbC in Mtb. Our preliminary results indicate that *embC* is an essential gene in Mtb, in contrast to Msm. This striking observation leads to the hypothesis that LAM has a much more critical role in the biology of Mtb. Since we cannot isolate a traditional deletion mutant of *embC*, we are using an alternative approach to make a conditionally-expressing strain. The expression of the antisense strand of *embC* under control of the tetracycline-inducible promoter should allow us to switch off *embC* expression.

Promoter analysis of the *embCAB* gene region indicates that promoters are found upstream of Rv3790, as well as upstream of *embA*, so that *embC* could be expressed independently from *embAB*. Neither promoter was induced in response to ofloxacin or ethambutol treatment. However, growth phase-dependant expression was observed for the *embC* promoter in Mtb. Interestingly, under the same conditions no effect on the lipomannan/lipoarabinomannan ratio was observed.

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**Abdulrahman Hammond [ Oral 15 ]**

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**Title: Investigating Mycobacterial -Specific Immune Responses in HIV-Infected Patients**

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**Robert Hunter [ Oral 3 ]**

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Robert L. Hunter M.D., Ph.D, Professor and Chairman  
Department of Pathology and Laboratory Medicine  
University of Texas-Houston Medical School  
Houston, TX 77030 Robert.L.Hunter@UTH.TMC.EDU

## Personal Background:

Robert Hunter is a pathologist with long standing interest in the biology of surfactants and amphiphilic molecules as vaccine adjuvants and immunomodulating agents. TDM (cord factor) was a prototype surface active immunoadjuvant. Studies on cord factor led us to read the work of Hubert Bloch who discovered cord factor and produced evidence that it is a virulence factor of tuberculosis. This work was rapidly repeated, but soon discredited because TDM was found in avirulent organisms and the requirement for oil for expression of toxicity was considered 'unphysiologic'. Even though generations of investigators reported the conclusion that cord factor had been discredited, the data implicating it in the pathogenesis of tuberculosis was never contradicted or repeated.

We reported that the toxicity of cord factor is due to a particular crystalline monolayer that it forms at hydrophobe-water interfaces. This was a new mechanism of toxicity that involved crystalline lipids. As such, it lay outside the domains of molecular biology and protein chemistry and has been generally ignored. In attempting to reproduce Bloch's observations that TDM is able to enhance acute and chronic tuberculosis in mice, we found that it could induce caseating granulomas. This stimulated the present studies.

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## Oral 3

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### Pathogenesis of Pulmonary Tuberculosis: Off Course for 50 years.

Robert Hunter, Margaret Olsen, Jeffrey Actor and Chinnaswamy Jagannath. The University of Texas – Houston Medical School, Houston, TX

Tuberculosis is an obligate human parasite whose survival depends upon transmission from person to person. It is one of the most studied diseases in history and has been declared a public health emergency by the WHO. Nevertheless, several fundamental questions remain. Since *M. Tuberculosis* is a prototype intracellular pathogen, how can most bacterial multiplication in immunocompetent adults occur extra cellular in cavities? How does tuberculosis turn immune responses against us to produce caseating granulomas and cavities? Why do individual lesions in the lung develop independently from one another and why don't they heal? We propose that essential clues have been largely ignored for decades.

It is widely reported that mice are a flawed model of tuberculosis because they fail to produce caseating granulomas. However, we recently reported that appropriately sensitized mice can produce typical caseating granulomas following injection of virulent organisms with a sufficient dose of trehalose 6,6' dimycolate (TDM or cord factor) (Am J Path, April 06). Caseating granulomas resulted from interaction of TDM with lipid in an immune host. These findings stimulated inquiry into pathology of developing pulmonary tuberculosis in humans. We found that textbooks have abandoned older descriptions of human tuberculosis in favor of the disease produced in rabbits, especially the more severe disease produced by *M. bovis*. Unfortunately, rabbits do not develop secondary tuberculosis.

Current textbook descriptions of developing pulmonary tuberculosis report that macrophages are continuously recruited to developing granulomas and are activated by cell mediated immunity. If insufficiently activated to kill ingested organisms, the macrophages are destroyed by cell mediated immunity and accumulate as slowly expanding caseating granulomas. This is a reasonable description of progressive primary tuberculosis or disease in immunocompromised people. However, as reported by Osler in 1892 and subsequently by many others, adult or secondary human pulmonary tuberculosis typically begins in immunocompetent people an exudative broncho or lobar pneumonic process. There are no granulomas. Rather infection is confined to alveolar macrophages in a sensitized host. Host lipids and mycobacterial antigens progressively accumulate within foamy alveolar macrophages. The lesions may smolder for an extended period before developing severe inflammation and rapidly undergoing necrosis of large sections, or an entire lobe, of a lung to form caseous masses and cavities. With the advent of chemotherapy that rapidly alters histopathology, decline in human disease and advancement of research in animal models, this description was gradually dropped from most literature. However, we located tissue sections of 4 cases of untreated early adult human pulmonary tuberculosis. They clearly show the pneumonic changes and lipid accumulation leading to caseation and cavities as described many years ago.

This revived understanding of the pathologic precursors of pulmonary tuberculosis in humans together with data from recent studies on caseating granulomas in mice suggests a new hypothesis for the pathogenesis of secondary pulmonary tuberculosis. TDM, the most abundant lipid extractable from viable organisms, may be the key driver of this process. It can switch between two different sets of biologic activities depending on its environment. When located on organisms, TDM is non toxic, inhibits phagosome-lysosome fusion, acidification of phagosomes and protects organisms from killing by macrophages. If TDM comes off organisms and attaches to the surface of sufficiently large lipid droplets, it becomes orders of magnitude more toxic than any other mycobacterial lipid, induces large amounts of TNF, kills macrophages, and induces CD1D restricted T cell mediated immune granulomas and caseation necrosis.

We propose that the two sets of biologic activities of TDM, far from being unphysiologic, are central to the pathogenesis of tuberculosis. TDM on organisms facilitates their survival in alveolar macrophages in immune hosts. The infection quietly stimulates accumulation of host lipid, TDM and other mycobacterial products in these cells. Eventually conditions develop for the activation of the toxicity of TDM. At this point, TDM is instantly transformed from a non toxic protector of organisms to the highly toxic driver of caseating granulomas. It rapidly kills macrophages and immune cells producing caseating lesions and cavities that are impervious to immune responses and facilitate transmission of infection to new hosts.

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**Rajpal Kashyap [ Oral 21 ]**

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**Demonstration of Components of Antigen 85 Complex in Cerebrospinal Fluid of Tuberculous Meningitis Patients.**

**Rajpal S. Kashyap**, Karen M. Dobos<sup>1</sup>, John T. Belisle<sup>1</sup>, Hemant J. Purohit<sup>2</sup>, Nitin H. Chandak, Girdhar M. Taori, and Hatim F. Daginawala.

Biochemistry Research Laboratory, Central India Institute of Medical Sciences, 88/2 Bajaj Nagar, Nagpur-440010, India

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2. Environmental Genomics Unit, National Environmental Engineering Research Institute, Nehru Marg, Nagpur-440020, India<sup>3</sup>

Tuberculous meningitis (TBM) is the most common form of chronic infection of the central nervous system. Despite the magnitude of the problem, the general diagnostic outlook is discouraging. Specifically, there is no generally accepted early confirmative diagnosis protocol available for TBM. Various *Mycobacterium tuberculosis* antigens are now recognized as potential markers for diagnosis of TBM. However, their presence remains questionable, and many of these antigens are reported in the blood but not in the cerebrospinal fluid (CSF). This study identifies a specific protein marker in CSF which will be useful in early diagnosis of TBM. We have demonstrated the presence of a 30-kDa protein band in CSF of 100% ( $n = 5$ ) of confirmed and 90% ( $n = 138$ ) of suspected TBM patients out of 153 TBM patients. The 30-kDa band was excised from the gel, destained extensively, and digested with trypsin. The resulting peptides were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Partially purified proteins from CSF samples of TBM were analyzed by two-dimensional polyacrylamide gel electrophoresis and Western blotting. Immunoblotting and enzyme-linked immunosorbent assay (ELISA) were performed to confirm the presence of proteins in the 30-kDa protein band. The antigen 85 (Ag 85) complex was detected in CSF of TBM patients by indirect ELISA using antibodies against Ag 85 complex. The results of this study showed the 30-kDa protein band contained MTB proteins Rv3804c (Ag85A) and Rv1886c (Ag 85B), both members of the Ag85 complex. This was also confirmed by using immunotechniques such as indirect ELISA and the dot immunobinding assay. Detection of Ag85 complex was observed in CSF of 89% (71 out of 80) of suspected TBM patients that were 30-kDa protein positive. The observed 30-kDa protein in the CSF is comprised of the MTB Ag85 complex. This protein was earlier reported to be present in the blood of patients with extra-central nervous system tuberculosis. Therefore, this finding suggests that this protein can be used as a molecular marker for any type of tuberculous infection. It also provides a more sensitive immunoassay option for the early and confirmatory diagnosis of TBM.

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**Thomas Keller [ Oral 7 ]**

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**Title: TB Drug Discovery at Novartis Institute for Tropical Diseases (NITD)**

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**Rajnish Kumar [ Oral 23 ]**

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**Assessment of protective potential of narrow molecular mass culture filtrate fractions of *M.tuberculosis* H<sub>37</sub>Rv**

Rajnish kumar, Indu Verma, and G. K. Khuller.

### **Background**

In view of the failure of BCG, more potent vaccine against TB is desperately needed. Culture filtrate proteins of *Mycobacterium tuberculosis* have been shown to contain protective antigens based on the induction of immune responses in various animal studies. However, the direct immunological correlate of protection against TB is yet unknown. Hence, screening of mycobacterial secretory proteome on the basis of protective efficacy is essential.

### **Objective**

The study was designed to evaluate the protective efficacy of fractionated culture filtrate of *M. tuberculosis* H<sub>37</sub>Rv and study the immunogenicity of protective fractions and their individual polypeptides.

### **Methods**

Total culture filtrate of *M. tuberculosis* was fractionated into fifteen narrow molecular mass fractions by multielution technique. Each fraction was evaluated for protective efficacy in Balb/c mice using DDA (Di-methyldioctadecylammonium bromide) as adjuvant against intravenous challenge with *M. tuberculosis*. The protective fractions were further evaluated in guinea pig model. Lymphocyte proliferation and cytokines induction were monitored in response to *in vitro* stimulation with immunoprotective fractions.

### **Results**

Out of fifteen fractions, fraction F7 and F11 in the molecular weight range of 20-24kDa and 38-43 kDa respectively, were found to be significantly more protective ( $p < 0.05$ ) than BCG in both Mouse as well as Guinea pig model. However, F8 comprising the polypeptides in the molecular weight range of 25-30kDa showed protective efficacy comparable to BCG. T cell responses following immunization with the three protective fractions revealed maximum proliferation and release of TH1 cytokines, IFN- $\gamma$  and IL12 against F7 as compared to F8 and F11. The selected individual proteins of the protective fractions showed encouraging results.

### **Conclusion**

Culture filtrate proteins in the molecular weight range of 20-43kDa appear to have immunoprotective significance against tuberculosis and need further exploration to constitute a subunit vaccine.

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**Anne Lenaerts [ Oral 12 & Poster ]**

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### **Oral 12**

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Promising compound series with activity in different *in vitro* and *in vivo* models of tuberculosis

Anne J. Lenaerts<sup>1</sup>, Veronica Gruppo<sup>1</sup>, Christine Johnson<sup>1</sup>, Karen Marietta<sup>1</sup>, Donald Hoff<sup>1</sup>, Heather Brookshire<sup>1</sup>, Barbara Laughon<sup>2</sup>, Robert Goldman<sup>2</sup> and Ian M. Orme<sup>1</sup>

<sup>1</sup>Dept. Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO;

<sup>2</sup>NIAID/NIH, Bethesda, MD

As part of the overall research effort of the NIH to develop new treatment strategies to combat tuberculosis, the NIAID has established a screening program at several institutions to efficiently screen large numbers of compounds for activity against *M. tuberculosis* (MTB). The compounds are received as part of the TAACF (Tuberculosis Antimicrobial Acquisition and Coordinating Facility, [www.TAACF.org](http://www.TAACF.org)) which provides no-cost screening of compounds from industrial and academic sources. To date over 80,000 compounds have been provided to the program. Of these compounds 0.3% performed well enough during the extensive initial *in vitro* testing to be subsequently tested *in vivo* using mouse models.

There is complete consensus in the field that animal models, in particular the mouse, are predictive of the effects of anti-mycobacterial drugs. This is assuming pharmacodynamic parameters such as metabolic rate, body surface area, etc, are taken into account. Although mice fail to develop the advanced lung pathology observed in TB patients (as necrosis and cavating disease), nor do they develop a latent disease stage, mice remain the most

economical and practical animal for initial steps of in vivo testing of experimental compounds. Crucial characteristics as acute toxicity, oral bioavailability, permeability across cell membranes, as well as efficacy against actively replicating TB bacilli can be efficiently tested in mouse models. Under the NIH screening Contract, a series of several highly pertinent in vitro and in vivo assay systems have been developed for this purpose.

To date more than 180 compounds from the TAACF were tested in vivo, of which 10 compounds showed significant antituberculosis activity in the mouse. Four series of promising compounds are currently being pursued by the TAACF. Data from in vivo studies showed that two compound series, quinolone and nitro-imidazole, reduced the bacterial load significantly in a rapid and long term mouse infection models. One compound series reduced the bacterial load in the lungs to a similar extent as INH, the second series showed activity similar to that of moxifloxacin. This work is funded by NIH/NIAID contract NO1 AI-95385.

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## Poster

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### Promising Compound Series with Activity in Animal Models of Tuberculosis

Anne J. Lenaerts<sup>1</sup>, Veronica Gruppo<sup>1</sup>, Christine Johnson<sup>1</sup>, Karen Marietta<sup>1</sup>, Diane Driscoll<sup>1</sup>, Nicholas Tompkin<sup>1</sup>, Karen A. Near<sup>2</sup>, Barbara Laughon<sup>2</sup> and Ian M. Orme<sup>1</sup>.

(1) Mycobacteria Research Laboratories, Dept. Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO;

(2) NIAID/NIH, Bethesda, MD

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## Kalifa Manneh [ Oral 11 ]

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**Title: The Gambia Experience – West Africa TB Research Initiative (WATBRI)**

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## Kathleen McDonough [ Oral 17 ]

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### cAMP Signaling as a Global Gene Regulatory Mechanism in TB-Complex Mycobacteria

Guangchun Bai<sup>1</sup>, Michaela A. Gazdik<sup>2</sup>, Damen D. Schaak<sup>1</sup>,  
and Kathleen A. McDonough<sup>1,2</sup>

<sup>1</sup>Wadsworth Center, New York State Department of Health, 120 New Scotland Avenue  
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<sup>2</sup>Department of Biomedical Sciences, University at Albany, Albany, NY 12222

A greater understanding of the biology and gene regulation of *Mycobacterium tuberculosis* gene regulation is needed to combat TB disease. We previously showed that the Mtb genome encodes 15 adenylate cyclases, and provided direct proteomic evidence for cAMP-mediated gene regulation in TB-complex mycobacteria. We also demonstrated that the CRP homolog in Mtb (CRP<sub>Mt</sub>), encoded by Rv3676, is a CRP-like gene regulator. CRP<sub>Mt</sub> binding sites were identified in a total of 73 promoter regions regulating 114 genes in the *M. tuberculosis* genome, which are being explored as a regulon. Specific CRP<sub>Mt</sub> binding caused DNA bending, and substitution of highly conserved nucleotides in the binding site resulted in complete loss of binding to CRP<sub>Mt</sub>. cAMP enhanced CRP<sub>Mt</sub>'s ability to bind DNA and caused allosteric alterations in CRP<sub>Mt</sub> conformation. These results provided the first direct evidence for cAMP binding to a transcription factor in *M. tuberculosis*, suggesting a role for cAMP signal transduction in *M. tuberculosis* and implicating CRP<sub>Mt</sub> as a cAMP-responsive global regulator. Defects are predicted in both the DNA- and cAMP-binding sites of the *M. bovis* BCG CRP homolog, CRP<sub>BCG</sub>, based on two amino acid changes in the BCG protein. The present study investigates this possibility of CRP<sub>BCG</sub> dysfunction. We purified and compared the activities of both CRP<sub>Mt</sub> and CRP<sub>BCG</sub> with respect to dimerization, DNA binding in vitro and in vivo, interactions with cAMP, and gene regulation. CRP<sub>BCG</sub> bound all tested DNA target sequences as well as, or better than, CRP<sub>Mt</sub> during

in vitro electrophoretic mobility shift assay (EMSA). However, proteolysis studies in the presence and absence of cAMP indicated structural differences between CRP<sub>Mt</sub> and CRP<sub>BCG</sub>, and showed that the proteins varied in their interactions with cAMP. Both proteins bound to most expected DNA targets in vivo when assayed by chromatin immunoprecipitation (ChIP), although their DNA binding profiles differed. Several targeted genes were found by qRT PCR to regulate differently in response to starvation signals in Mtb versus BCG. These gene regulatory differences were consistent with the variability we observed with *in vivo* DNA binding of specific gene targets, suggesting a direct correlation between gene expression and *in vivo* target DNA binding by these CRP-like proteins. We conclude that both CRP<sub>Mt</sub> and CRP<sub>BCG</sub> are functional transcription factors in their respective bacterial hosts, but they differ in their gene regulatory patterns. These gene regulatory differences may have significant impact on the virulence potential of each bacterium, and could contribute to the attenuation of BCG relative to *M. tuberculosis*.

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**Helen McIlleron [ Oral 5 and 26 ]**

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**Oral 5**  
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**Inter-patient variation in TB drug PK.**

**Helen McIlleron, Peter Wash, Andre Burger, Peter I Folb and Pete Smith**

Evaluation of sources of pharmacokinetic variation can facilitate optimization of tuberculosis treatment regimens by identification of avoidable sources of variation and of risk factors for low or high drug concentrations in patients. The results of a pharmacokinetic study in 142 patients with drug-sensitive pulmonary tuberculosis will be presented. Our objective was to describe the pharmacokinetics of rifampicin, isoniazid, pyrazinamide and ethambutol in a cohort of tuberculosis patients established on first-line treatment regimens and to evaluate determinants of pharmacokinetic variation. Plasma concentration–time profiles were determined for each of the drugs in after 2 months of daily treatment in hospital. Pharmacokinetic measures were described using noncompartmental analysis. Multiple linear regression was used to evaluate patient and treatment factors associated with variation of the area under the concentration-time curve from 0 to 8 hours. Several factors independently associated with variations in antituberculosis drug concentrations were identified: HIV-infection was associated with 39% and 27% reductions for rifampicin and ethambutol, respectively; formulation factors were determinants of rifampicin and isoniazid bioavailability; females had increased rifampicin and isoniazid, but reduced ethambutol concentrations; older patients had higher levels of isoniazid and ethambutol; patients with a history of previous antituberculosis treatment had lower ethambutol concentrations; and the dose per kilogram of body weight was associated with the concentrations of all four agents.

[REF: Helen McIlleron, Peter Wash, André Burger, Jennifer Norman, Peter I Folb, Pete Smith. Determinants of rifampicin, isoniazid, pyrazinamide and ethambutol pharmacokinetics in a cohort of tuberculosis patients. *Antimicrob Agents Chemother* 2006; 50(4): 1170-7.]

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**Oral 26**  
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**Co-treatment of TB and HIV: Pharmacokinetic dilemmas.**

**Helen McIlleron**

Access to antiretroviral treatment is rapidly expanding in resource-limited settings, where tuberculosis is the most common opportunistic infection. Co-administration of antitubercular and antiretroviral agents is therefore occurring commonly, and is associated with important complications. This presentation will focus on pharmacokinetic issues. Some preliminary data of PK studies of adults and children on antiretroviral regimens with and without concomitant rifampicin-based antitubercular treatment will be presented, and research priorities highlighted.

[REF: Helen McIlleron, Graeme Meintjes, William J. Burman, Gary Maartens. Complications of antiretroviral therapy in patients with tuberculosis - drug interactions, toxicity and immune reconstitution inflammatory syndrome. *Journal of Infectious Diseases*. Suppl. In press.]

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**Oyebode Olakanmi [ Oral 28 ]**

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**INTRAPHAGOSOMAL MYCOBACTERIUM TUBERCULOSIS GROWS LESS IN MACROPHAGES FROM PATIENTS WITH HEREDITARY HEMOCHROMATOSIS.**

Oyebode Olakanmi, Larry S. Schlesinger, and Bradley E. Britigan. VA Medical Center-Cincinnati, OH; University of Cincinnati, and The Ohio State University, Columbus, OH

Iron (Fe) is critical to the growth of most microbes, including *M. tuberculosis* (*M.tb*). In humans, extracellular Fe is chelated to transferrin (TF) and lactoferrin (LF), in part to limit Fe availability to pathogenic microorganisms. Hereditary hemochromatosis (HH) is a disorder characterized by mutations in the HFE gene. One consequence is low Fe content of peripheral blood macrophages from individuals with HH. Hepcidin is a 25-amino acid peptide synthesized in the liver and which regulates Fe homeostasis. Hepcidin binds to ferroportin on target cells to reduce Fe efflux from macrophages. We reported (*JBC* 277:29727, 2002) that HH monocyte-derived macrophages (MDM) acquire similar amounts of Fe from TF over 24h, but *M.tb* residing within HH MDM acquire significantly less Fe (60-70%) compared to bacilli within MDM from healthy donors. We have extended these studies to LF, and also assess intracellular growth of *M.tb* in HH MDM. HH and control MDM exhibited similar <sup>59</sup>Fe uptake from TF, LF Fe uptake by the MDM was twice as much from LF compared to TF. However, Fe acquisition by intracellular *M.tb* was five times from LF as from TF. We also compared Fe uptake by macrophages from hemochromatosis patients and healthy control subjects. For both Tf and Lf, no significant difference was observed in Fe uptake by MDM of both phenotypes. However, Fe acquisition by intracellular *M.tb* residing within hemochromatosis MDM was only 25% of that from healthy control irrespective of the chelate. The difference in Fe uptake appeared to reflect intracellular bacterial growth. The number of *M.tb* phagocytosed by hemochromatosis and healthy control cells were equivalent based on initial CFUs. There was a steady increase in the number of intracellular *M.tb* over time, irrespective of the chelate or the phenotype of the macrophage. However, the growth was slower in hemochromatosis (~50%) than in healthy control macrophages. The slower growth rate was demonstrable and significant at later time points (> day 2). IFN- $\gamma$  treatment of MDM decreased <sup>59</sup>Fe uptake from TF by *M.tb* within HH MDM by 50%, but had no impact on uptake from LF. Both TF and LF trafficked to the *M.tb* phagosome as assessed by confocal microscopy. The number of positively stained phagosomes in which the bacilli co-localized with TF or LF (16-23%) was similar for HH and control cells, irrespective of the chelate. Interestingly, incubation of macrophages with hepcidin had no impact on Fe acquisition by MDM or the intraphagosomal *M.tb* irrespective of the phenotype of the cells. In summary, the ability of intraphagosomal *M.tb* to acquire Fe from both the extracellular environment and sites within the macrophage varies with the Fe chelate. IFN- $\gamma$  decreased Fe acquisition from TF. Fe acquisition and intracellular growth of *M.tb* decreased in MDM from HH patients. *M.tb* has developed efficient and unique mechanisms for acquiring Fe from a variety of Fe forms that it would potentially encounter within the human lung. More investigation is required to define these mechanisms and the possible role of the HH phenotype as an evolutionary strategy of host defense against *M.tb*.

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**Jacob Kweku Otu [ Poster ]**

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*Title is Awaiting Confirmation by the Author*

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**Tanya Parish [ Oral 24 ]**

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**Sarah Parker [ Oral 16 & Poster ]**

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**Oral 16**

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Purification and partial characterization of mycobacterial phospholipase A: an activity associated with mycobacterial cutinase

Parker, Sarah K. and Vasil, Michael L., University of Colorado Health Sciences Center, Denver, Colorado, USA

Since discovering phospholipase A (PLA) activity in mycobacteria, we have purified the active protein and used reverse genetics to identify gene candidates; our results suggest the PLA activity is associated with mycobacterial cutinase. Phospholipase As (PLAs), which hydrolyze fatty acids on phospholipids, play a significant role in human inflammatory states and disease pathogenesis. In prokaryotes, their recognition in virulence is more recent. We purified the PLA activity from *M. smegmatis* culture supernatant, utilizing liquid phase chromatography and native gel electrophoresis, to a single band. This band was analyzed with mass spectrometry which identified two proteins in *M. smegmatis*, one of which has a cutinase motif. Cutinases hydrolyze plant cutin and are classically found in phytopathogenic fungi. They bridge the functional properties between esterases and lipases and, like PLAs, have an

alpha/beta hydrolase fold. Evidence that mycobacterial cutinase confers PLA activity includes 1) though cutinases were not previously known to be active on phospholipids, fungal cutinase from *Fusarium solani* has PLA activity on various substrates by radiolabel C14 assay, 2) our purified preparation is active on nitrophenylbutyrate and inhibited by phenylboronic acid, consistent with described cutinases, and 3) PLA activity increases in culture supernatant spiked with the cutinase substrate polycaprolactone. Mycobacterial PLA activity is present in all mycobacteria tested, including *M. smegmatis* mc2 155 and ATCC 14468, *M. bovis*, *M. marinum* ATCC 25039, *M. tuberculosis* H37Rv and CSU93. In *M. smegmatis* and *M. marinum* PLA activity is secreted in the culture supernatant, while in *M. tuberculosis* strains the activity is localized to the cell wall fraction. Initial characterization of purified enzyme from *M. smegmatis* indicates the activity hydrolyzes the Sn-2 fatty acid of phospholipids, indicating it is a PLA<sub>2</sub>. Purified fractions have PLA activity on phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingomyelin. The homologues of the purified protein were identified in *M. tuberculosis* database, and we are currently pursuing overexpression of active *M. tuberculosis* cutinase. We hypothesize that mycobacterial PLAs/cutinase play a unique role in cell wall remodeling, cell-to-cell trafficking, inflammatory signaling and/or nutrient acquisition. The phospholipase and cutinase activities will be further characterized and deletion mutants made in *M. tuberculosis* to assess possible contributions to virulence.

We gratefully acknowledge funding from 1-K08-AI50646-01A1, and materials and mass spectrometry data from Drs. John Belisle and Karen Dobos at Colorado State University, and technical assistance from Kathryn Curtin.

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## Poster

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### **Purification and Partial Characterization of Mycobacterial Phospholipase A: An Activity Associated with Mycobacterial Cutinase**

Sarah K. Parker and Michael L. Vasil, University of Colorado Health Sciences Center

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**Sharon Perry [ Poster ]**

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**Title: IFN-gamma Responses to Mycobacterial Antigens in *H. pylori*-*M. tuberculosis* Co-infection**

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**Paulo Rabna [ Oral 22 ]**

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**Title: Soluble Urokinase Plasminogen Activator Receptor (suPAR) as a Marker of TB Treatment Efficacy and HIV Disease State. A prospective, Longitudinal Cohort Study in Tuberculosis Patients in Guinea-Bissau**

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**Ana Luisa Santos [ Oral 4 & 25 & Poster ]**

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## Oral 4

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***Why don't physicians and paleopathologists see the same? rib lesions as possible evidences of pulmonary tuberculosis***

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According to historical documents, pulmonary tuberculosis (TB) was the most common form of TB in the past, as it is today; however, there is a low frequency of evidence in archaeological skeletons. Nevertheless, in the last 20 years, studies on human identified skeletal collections, with known biographical data such as cause of death, age and sex, reported a significant relationship between new bone formation on the visceral surface of ribs and pulmonary TB. In Portugal, investigations done on individuals of both sexes who lived during the late 19th century and the first half of the 20th century, a time after Koch's discovery of the bacillus and before antibiotics were widespread, revealed:

- when pulmonary TB was recorded as cause of death, new bone formation on ribs was present in 90.9% (10 out of 11) of the juveniles and 85.7% (54/63) of the adults in the Coimbra Identified Skeletal Collection (CISC). Further,

90.5% (76/84) of the individuals between 13 and 88 years of age in the Human Identified Skeletal Collection of the Museu Bocage at Lisbon (HISC-MB) also showed new bone formation.

- when examining individuals that died due to other causes of death, the percentage of individuals showing new bone formation was lower. When pulmonary non-TB causes of death were considered, rib lesions were apparent in only 15.4% (4/26) of individuals from the CISC and 36.7% (18/49) of individuals from the HISC-MB. For extrapulmonary non-TB, only 18.8% (12/64) of the individuals in the CISC and 25.0% (16/64) of the individuals in the HISC-MB demonstrated new bone formation.

Assuming that the causes of death for both collections were accurate, and that the lesions observed in the skeletons were caused by pulmonary TB, it is suggested that the new bone formation, although not pathognomonic, can be a criterion for differential diagnosis of pulmonary TB. This is strongly supported by previous studies performed on other documented collections. However, these subtle bone lesions commonly seen on ribs from archaeological populations are not mentioned in the clinical diagnostic criteria, probably because they are not seen on radiographs and are not relevant to the diagnosis. Despite this, Eyer *et al.* (1996:925) concluded that in living patients “the most common condition associated with rib enlargement was pulmonary TB.”

Examining living individuals suffering from TB in countries where access to drug therapy is not yet available could be another approach to confirm this relationship between periosteal reaction and TB. In the study of paleopathology overall, and tuberculosis in particular, more fundamental and multidisciplinary research is necessary. It is time for researchers, such as those in medicine and paleopathology, not only to consider traditional diagnostic criteria for identifying skeletal diseases, but also to develop a more cooperative way of conducting research which may help in the prevention of this disease that has crossed the millennia.

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## Oral 25

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### ***Tuberculosis in retrospective: from pre-Koch epochs to antibiotics***

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Tuberculosis (TB) continues to be poorly understood at several levels despite the identification of its causative pathological agent in 1882 by Robert Koch. Important and apparently simple questions are: when did it first appear in humans and in animals? Did it arise in a single region and from there spread worldwide? Or did it arise *de novo* in several regions of the world? Still there are not yet satisfactory answers. The paleopathological studies give important clues to past TB understanding. In this presentation a balance of the evidences is done from antiquity to modern era using skeletal, mummified, documentary and iconographic sources. Medical progress and the Coimbra practice in the 19<sup>th</sup> to 20<sup>th</sup> century's transition will be also focus in relation to transmission, prevention, diagnostic and therapeutic approaches.

The profile of TB victims changed from the early part of the 20<sup>th</sup> century. New cases have arisen due to recent increase of forced sedentism of formerly pastoral populations, due to humanitarian crises due to wars, in HIV/AIDS patients, due to multi drug resistance and augment of homeless in “western” countries, among many other factors. But looking to the past we can advance problems and solutions, namely related to methodology to adopt to fight the white plague that still goes on in a world wide scale.

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## Poster

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