Rev. salud pública. 12 sup (2): 53-65, 2010

Poster Presentation

Basic research in mycobacteria

Three-dimensional structure prediction of putative chloride channel of *Mycobacterium tuberculosis*

Nelson Enrique Arenas[1], Luz Mary Salazar[1], Edgar Reyes[1], Luis Ramón Osses[1], Arley Gómez[2], and Carlos Yesid Soto[1,3]

1 Departamento de Química.
2 Unidad de Enfermedades Infecciosas y Medicina Tropical, Facultad de Medicina, Universidad del Rosario, Bogotá, Colombia.
3 Instituto de Genética, Universidad Nacional de Colombia.

Mycobacterial factors that allow inhibit phagolysosomal destruction and bacterial surviving into host macrophages remains elusive, therefore the studies of proteins and pathways involved in these specific evasion mechanisms during mycobacterial infections represent a challenge for understanding of *Mycobacterium tuberculosis* biology.

In this study, a topology protein prediction (TPP) using three transmembrane helix servers (TMHMM, TMpred and TopPred) was performed on hypothetical Chloride channel sequence (Rv0143c). Multiple alignment and structure threedimensional prediction was carried out on ClustalW and Insight II (Asselryx) respectively. Results shown a membrane protein composed by eleven transmembrane helices containing 15 specific domains (GXXXG) which probably allows helix–helix interactions and dimerization. The presence of Glycine and Alanine residues inside this domain suggests that they might be important for the packaging of the chloride channel. Homology comparison allowed the identification of specific amino-acids and common regions among chloride channels (E_{135}, Y_{469} residues and $_{170}$GREGP$_{174}$, $_{379}$GLFGP$_{383}$ consensus sequences) mainly associated to anion selectivity which are present and brought together near the membrane centre to form an ion-binding site. The protein model revealed a structure similar to a channel-forming unit which exhibits an antiparallel architecture with positively charged residues within the membrane environment and the opposite ends of the helices (away from the active site) were to be directed into the aqueous environment outside the membrane.We suggest that this protein could be involved in setting and restoring the resting membrane potential, furthermore they could play important role in solute concentration mechanisms and pH regulation as previously demonstrated in other microorganisms.

Key Words: Chloride channel, Structure prediction, Membrane protein, Tuberculosis.

Molecular modeling and characterization of *Mycobacterium tuberculosis* 3-ketoacyl-acyl carrier protein reductase (FabG2)

Nelson Enrique Arenas[1], Ana Silvia Ramírez[1], Luz Mary Salazar[1], Arley Gómez[2] and Carlos Yesid Soto[1, 3]

1 Departamento de Química and
2 Unidad de Enfermedades Infecciosas y Medicina Tropical, Facultad de Medicina, Universidad del Rosario, Bogotá, Colombia.
3 Instituto de Genética, Universidad Nacional de Colombia.

The mechanism of lipid biosynthesis in *Mycobacterium tuberculosis* comprises a complex pathway with a defined function of mycolic acid biosynthesis, acid-fastness,

and pathogenesis, and represents an opportunity to develop new mycobacterial drug targets. FabG2 is an essential gene which encodes a polyketide synthase that catalyses the first step in the reductive modification of the beta-carbonyl centers in the growing polyketide chain. It uses NADPH to reduce the keto group to a hydroxy group and would act as a â-hydroxyacyl-coenzyme A dehydrogenase.

We built a partial structural model from FabG2 (Rv1350) aminoacid sequence by using bioinformatic homology servers; refinement and minimization structure was carried out in SwissPDBViewer (http://www.expasy.org/spdbv/) and Insight II (accelrys) according to a secondary structure prediction. Domains and transmembrane helices were predicted by SMART and TopPred respectively.

Our results show a high structural similarity among FabG2 and eukaryotic hydroxysteroid dehydrogenase. The protein contains a typical Rossmann fold structure, with a twisted, parallel á-sheet composed of seven â-strands flanked on both sides by a total of eight á helices. Structural comparison with *E. coli* FabG2 structure without cofactor suggest that substantial conformational change occurs in the enzyme upon cofactor binding and it contains a conserved catalytic triad (Tyr155, Lys159 and a Ser142). FabG2 lacks transmembrane regions; their hydrophobicity profile and KR domain suggests strong membrane association.
Deciphering clues on enzymes involved in mycobacterial lipid biosynthesis is relevant for its role in the constitutive physiological process of lipid metabolism/trafficking for mycobacterial viability and virulence.

Key Words: Lipid biosynthesis, FabG2, Polyketide synthase, Tuberculosis, Structural modeling.

Variations on a theme: different targets for isoxyl and thiacetazone depending on the mycobacterial species tested?

Juan M. Belardinelli[1] and Héctor R. Morbidoni[1]

1 Cátedra de Microbiología, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Rosario, Argentina.

Isoxyl (ISO) and Thiacetazone (TAC) are thiocarbamide containing anti-mycobacterial pro-drugs. Both compounds have been used for clinical treatment of tuberculosis but then discontinued because of poor performance or toxicity, thus, little is known on the mechanism(s) of action of these drugs. We analyzed the “in vitro” activity of both drugs against several mycobacterial species. *M. tuberculosis* and *M. kansasii* were the most sensitive species, with Minimal Inhibitory Concentrations (MICs) ranging from 0.1 to 2.5µg/ml to both drugs. *M. avium* was sensitive only to TAC (MIC=100 µg/ml), while the other species tested were resistant to both drugs. Analysis of fatty and mycolic acid biosynthesis, judged by [1-14C] acetate incorporation, showed that ISO inhibited synthesis of both oleic and mycolic acids in *M. tuberculosis* but only a partial inhibition of mycolic acids in *M. kansasii*, suggesting different susceptibility of the postulated targets or different targets in both species. Conversely, TAC inhibited mycolic acid synthesis (but not oleic acid) in all species analyzed. Unexpectedly, TAC treatment of *M. tuberculosis* didn´t lead to

any evident accumulation of unsaturated mycolate precursors as opposite to recently reported results in *M. bovis* BCG which led to suggest a TAC-mediated inhibition of cyclopropane mycolic acid synthases (CMASs). These discrepancies indicate that TAC may have a different target in *M .tuberculosis*, *M. avium* and *M. kansasii* other than CMASs.
Understanding the mechanism(s) of action and identifying the target(s) of both drugs will help gaining knowledge on the essential mycolic acid synthesis pathway and designing novel, more effective drugs.

Key Words: anti-mycobacterial drugs, mechanisms of action, tuberculosis, thiacetazone, isoxyl.

Antimycobacterial activity of essential oils from colombian medicinal plants

Juan Gabriel Bueno-Sánchez[1], Elena E. Stashenko[2], Jairo René Martínez[2], Wellman Ribón[1]

1 Grupo de Micobacterias, Instituto Nacional de Salud, Bogotá, D.C.; Centro Colombiano de Investigación en Tuberculosis CCITB, Colombia.
2 Centro de Investigación en Biomoléculas, CIBIMOL, CENIVAM, Universidad Industrial de Santander, Bucaramanga, Colombia.

INTRODUCTION: Tuberculosis caused by *Mycobacterium tuberculosis* is one of today's most pressing public health problems. Although there is an effective treatment against it, the emergence of broadly multidrug-resistant isolates has decreased healing possibilities. The search and development of new antituberculosis agents against such type of isolates useful for short duration therapies has, therefore, become urgent. OBJECTIVE: To determine in vitro activity of five essential oils (EOs) on the *M. tuberculosis* H37Rv reference strain and its four ATCC resistant variants. METHODS: EOs were obtained through hydrodestilation from *Swinglea glutinosa, Achyrocline alata, Salvia aratocensis, Turnera diffusa* and *Lippia americana*. Their minimum inhibitory concentration (MIC) was determined with regard to five *M. tuberculosis* strains using an MTT colorimetric assay. A time kill curve analysis was later done to evaluate their bactericide or bacteriostatic activity over the H37Rv strain. Isoniazid and rifampicine were used as control drugs. RESULTS: The most active EOs (MIC<100 µg/mL) against the five strains were those obtained from *Achyrocline alata*, *Salvia aratocensis* and *Turnera diffusa*, these last two registering bactericide activity (decrease of >3 log UFC/mL). CONCLUSIONS: EOs are a promising source for new antimycobacterial drugs; this is the first study undertaken in the country in this field. *Salvia aratocensis* and *Turnera diffusa* are promising plants to carry out tests on drug resistant clinical isolates.

Key Words: Essential oils, *M. tuberculosis*, medicinal plants.

Molecular markers of tuberculosis disease progression in patients with or without Diabetes Mellitus type 2

Benjamin Gandara[1,3] ,Noe Macias[1], Yolanda Garcia[1], Leonor Enciso[1], Ema Valenzuela[1], Nicolas Hernandez[1], Elisa Venegas[1],Carmen Serrano[1], Bruno Rivas[1], Gerardo Martinez[2], Martha Moran[2,] Jesus Guerrrero[2], Antonio Enciso[1]

1 Unidad de Investigacion Medica,IMSS-Zacatecas, Mexico.
2 Unidad de Investigacion Medica,IMSS-Durango, Mexico.
3 ENCB del IPN, Mexico.

INTRODUCTION: No molecular methods are available to identify tuberculosis disease progression in human risk groups, including diabetic patients. Transcriptome analysis has been used to identify differential gene expression associated to several human pathologies. Thus, microarrays represent a good strategy to identify molecular markers of disease progression in tuberculosis. OBJECTIVES: To identify the transcriptional profile associated to active human pulmonary tuberculosis using microarray technology. METHODS: Oligonucleotides custom microarrays (CombiMatrix 4X2K, USA) were designed and build with gene sequences related to active pulmonary tuberculosis (TBPA), inflammatory response, antimicrobial peptides and DM2 pathogenesis. Blood from individuals with TB latent infected (TBLT), TBPA and non-TB infected (Control), from DM2 y non-DM2 subgroups previously selected by clinical and laboratory criteria was collected (6 individual per group, 36 in total), in PAXgene tubes and total RNA extracted, enriched and concentrated. Globin transcripts reduction and biotynilated-cRNA synthesis was done. Fragmented labelled-cRNA transcripts were hybridized to microarrays according to manufacturer's instructions (CombiMatrix, USA). Statistical analysis was performed using SAM 1.15 software. Cluster analysis for gene expression levels was done with "Gene Cluster 2.11 and processed by TreeView 1.60 software. RESULTS Specific transcriptome including 103 TBPA associated genes were identified in non-DM2 patients. Only 11 of them were associated to TBPA from both DM2 and non-DM2 patients, and have statistical significance according to SAM analysis. CONCLUSIONS: We identified a specific transcriptional signature that distinguishes DM2 or non-DM2 patients with active TBPA from those with TBLT or non TB infected individuals.

Key Words: Microarrays Analysis, Diabetes mellitus2, Tuberculosis progression.

Antituberculosis activity of β-lapachones derivates

Tatiane Silveira Coelho[1,3], Daniela Fernandes Ramos[1,3], Antonio Ventura Pinto[2], Raphael Salles Ferreira Silva[2], Maria do Carmo Freire Ribeiro Pinto[2], Kelly Cristina Gallan de Moura[2] and Pedro Eduardo Almeida da Silva[1,3]

1 Universidade Federal do Rio Grande – FURG.
2 Universidade Federal do Rio de Janeiro – UFRJ.
3 Programa de Pós-Graduação Ciências da Saúde – FURG, Brasil.

The increase of tuberculosis (TB) incidence with resistant strains and HIV co-infection has reinforced the necessary to develop of new drugs to treatment. These

molecules should be useful to treat these TB forms, shorten treatment regime and to kill persistent strains. Derivatives imidazoles of natural quinones as β-lapachones, have been synthesized and their antiTB activity evaluated.against *M. tuberculosis* H37Rv (ATCC 27294) and rifampicin resistant strain (*rpo*B 526 His-Tir). Using Resazurin Microtiter Assay method was observed molecules actives with MIC 1,56 to 6.25 µg/mL to susceptible and resistant strains. Have been showed that conversion quinone to imidazole function increase biological activity and decrease toxicity. These results show the potentiality these molecules as prototype to futures drugs antiTB.

Key Words: β-lapachones, *Mycobacterium tuberculosis*, drugs.

Characterization of *Rv2624c* AND *Rv2625c* genes of *Mycobacterium tuberculosis* during vegetative growth and non replicating persistence state

Elba Guerrero and Leiria Salazar

Laboratorio de Biología Molecular, Departamento de Biología Estructural, Instituto Venezolano de Investigaciones Científicas (IVIC).

Mycobacterium tuberculosis can persist in the human host, in a latent state, that represents an obstacle for the control of this disease. It has been suggested that, between other factors*, M. tuberculosis* persistence can be caused by oxygen limitation and low doses of nitric oxide. It has been observed by microarrays that a great number of genes are up regulated under these conditions. The expression of *Rv2624c* and *Rv2625c,* which encode for proteins with homology to universal stress proteins and homology to metalloproteases respectively, are increased at least 5 times under conditions mimicking in vitro persistance. In order to determine the possible role of *Rv2624c* and *Rv2625c*, we determined the expression of these genes by quantitative PCR during exponential growth and persistence state, *in vitro. Rv2625c* and *Rv2624c* transcripts were detected only in cultures treated with nitric oxide. A mutant strain of *M. tuberculosis* (Δ*Rv2625c*) that does express neither *Rv2625c* nor *Rv2624c* was constructed. *M. tuberculosis* Δ*Rv2625c* showed to be more sensitive to the treatment simultaneous with nitric oxide and hydrogen peroxide, suggesting that these genes could play an important role in bacterium survival under these conditions.

Key Words: persistence, expression, mutant.

Functional evaluation of C-terminal domain of the protein serin kinase G, PknG, of *Mycobacterium tuberculosis*

Rossana Jaspe and Leiria Salazar

Molecular Biology Laboratory, Department of Structural Biology, Venezuelan Institute of Scientific Research (I.V.I.C.). Caracas, Venezuela.

Mycobacterium tuberculosis uses several strategies to circumvent lisosomal delivery into macrophages. It has suggested that the secretion of the serine protein

kinase G (PknG) inhibits the phagosome-lysosome fusion allowing the survival of the mycobacteria. PknG has, in addition to the kinase domain, two Thioredoxin (Trx) motif and one tetratricopeptide (TPR) motif. With the aim to determine the possible role of the TPR motif, a *M. tuberculosis* PknG mutant, which expressed a C-terminal truncated PknG protein (PknGÄTPR), was constructed (*M. tuberculosis* pknGÄTPR::hyg). Western blot analysis, using anti-PknG specific antibodies against *M. tuberculosis* cellular fractions, showed that a polypeptide of 30 kDa, presumably containing the TPR motif, remains into the membrane and cell wall, while that the Trx and kinase motifs are exported as a 52 kDa polypeptide. Immunodetection assays showed that, in contrast to H_{37}Rv strain, high fluorescent signals were observed in the cellular envelope of *M. tuberculosis* pknGÄTPR*::hyg* during stationary phase of growth, suggesting that PknGÄTPR accumulated in this region. We propose that the C-terminal region of PknG, which contains the TPR motif, could be involved in the export pathway of 52 kDa PknG polypeptide.

Key Words: Tuberculosis, protein kinase, mutant.

Pathogenic and non-pathogenic mycobacteria infects B cell by macropinocytosis

Julieta Luna-Herrera, Juan José De la Cruz-López , Blanca Estela García-Pérez

Departamento de Inmunología, Laboratorio de Inmunoquímica II, Escuela Nacional de Ciencias Biológicas, IPN, México.

INTRODUCTION: Most of the studies on the pathogenesis of mycobacterial diseases rely on the macrophage-mycobacteria interaction. However, recently, other cells have been implied in the mycobacterial diseases, among them are epithelial, endothelial and fibroblast cells. Studies related on the interactions micobacteria-host cell are necessary.

PURPOSE OF THE STUDY: Human B lymphocytes (Raji cell line), were analyzed for their ability of performing macropinocytosis, the endocytic pathway responsible of bacteria internalization in non-phagocytic cells. METHODS: Early times of B-cell infection with *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* were study and PMA was used as a chemical inductor of macropinocytosis. Intracellular survival was evaluated by CFU determination; structural changes were studied by scanning electron and transmission microscopy. Fluid phase uptake triggered by mycobacterial infection was evaluated by plate spectrofluorometry. Nitric oxide production was observed by fluorescence microscopy. RESULTS: Scanning electron microscopy revealed significant changes on cell membrane topography with both bacteria and PMA, abundant lamellipodia surrounding the bacteria and all around the cell was observed. Cytoskeleton rearrangements were observed, with filamentous actin accumulation beneath lamellipodia in infected and PMA treated cells. Confocal microscopy and transmission microscopy revealed intracellular *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*. Fluid phase uptake induced by the infection and by PMA was quantified and several inhibitors were tested along to the inductors; amiloride, cytochalasin and wortmannin abolished totally fluid phase uptake induce by bacteria

and PMA. *Mycobacterium smegmatis* was efficiently eliminated contrary to *Mycobacterium tuberculosis*.
CONCLUSIONS: This report gives evidences that: a) B-lymphocytes are able of uptake micobacteria being macropinocytosis the mechanism responsible of this capability; b) *M. tuberculosis* survives intracelullary in this cell, contrary to *M. smegmatis*; c) B cell produce nitric oxide in response to mycobacterial infection.

A possible role of insulin-like growth factor I (IGFI) on the functional deactivation of macrophages infected with Mycobacteria

Leonardo Ribeiro Batista Silva[1], Luciana Silva Rodrigues[2],
Maria Cristina Vidal Pessolani[1]

1 Laboratório de Microbiologia Celular.
2 Instituto Oswaldo Cruz, Rio de Janeiro, Brasil.

Mononuclear phagocytes are target cells for pathogenic mycobacteria such as *Mycobacterium tuberculosis* and *Mycobacterium leprae.* These bacteria are able to subvert macrophage microbicidal mechanisms and survive and replicate within these cells. However, the molecular mechanisms involved in this deactivation are not completely understood. Recently, we have reported that *M. leprae* induces the expression of insulin-like growth factor I (IGF-I) in Schwann cells. In addition, recent studies have observed that IGF-I can inhibit inducible nitric oxide synthase (iNOS) expression and consequently nitric oxide (NO) production induced by *Leishmania amazonensis* in macrophages. Based on these data, we have investigated the involvement of IGF-I on macrophage deactivation observed during mycobacterial infection. For this purpose, RAW 264.7 murine macrophages were pretreated or not with IGF-I and the expression of iNOS and production of NO estimated after stimulation with different mycobacterial species and classical agonists. NO production was evaluated by determination of nitrite concentration in the culture media using the Griess reagent, and iNOS expression was monitored by Western Blot. IGF-I pre-treated cells showed a significant reduction in nitrite levels after infection with mycobacteria. The decrease in NO production was accompanied by a downregulation of iNOS expression, suggesting that IGF-I may play a role in the regulation of host response during bacterial infection. Currently experiments are underway to analyze the modulation of macrophage IGF expression upon mycobacterial infection.

Key Words: Mycobacteria, nitric oxide, IGF-I.

Mycobacterium tuberculosis susceptibility to antimicrobial peptides: proposal for new therapies in pulmonary Tuberculosis

Santiago Bruno-Rivas[1], Irma E González-Curiel[1], Ruben Mendez-García[1],
Leonor Moreno-Enciso [1,] j. Antonio Enciso-Moreno[1], Robert Hancock [2]

1 Medical Research Unit, IMSS, Zacatecas, Mexico.
2 Laboratory of Microbiology. University of British Columbia. Vancouver, Canada.

To determine *Mycobacterium tuberculosis susceptibility* to different types of antimicrobial peptides. *M. tuberculosis* H37Rv was cultured in Middlebrook 7H9 me-

dium for 21 days. Antimicrobial peptides SMAP – 29, CP-26, Endolicidin, E2, polyphemusin, E6, LL-37, CRAMP y Beta-defensin, were obtained by isolation from culture medium or by chemistry synthesis, each peptide was used at different dilutions on 96 wells plates. We added 1.65 x10^7 CFU/well and were incubated at 37°C for 5 days, after this time resazurine was added. These plates were reincubated at 37°C for 24 hours; subsequently, viability's lecture was taken. The results of viability were confirmed for dilutions and cultured on plates with Middlebrook 7H10 solid medium, it was incubated for 21 days more and take viability's lecture.

Results showed that *Mycobacterium tuberculosis* is susceptible to most of peptides used during this study; the minimal inhibitory concentrations vary from 8 µg to 128 µg. However, due these peptides have immunomodulatories activities in vivo, it's plausible that antimicrobial activity might be improved in vivo, we propose to assess these antimicrobial peptides in an infection model in vivo. Since multidrugs-resistance have increase lately it is necessary to implement new therapeutic strategies. Because of antimicrobial peptides have either antimicrobial and immunomodulatory function might be a good candidate for dug development.

Key Words: Antimicrobial peptides, therapeutics.

Role of the diguanylate cyclase of *Mycobacterium tuberculosis*: a protein putatively involved in multicellular behavior

Juan Germán Rodríguez , Monica Natalia Gonzalez , Maria Mercedes Zambrano and Patricia Del Portillo

Corporación Corpogen.

Mycobacterium tuberculosis is easily adapted to environmental changes; this is evident in the process of infection in which the bacilli confront different stress conditions. One possible mechanism of adaptation is multicellular behaviour which can result in mycobacteria with different phenotypes inside the host. The diguanylate cyclase protein (DCG) synthesizes c-di-GMP, a signal molecule that has gained importance as a novel second messenger that regulates cell surface adhesiveness in bacteria. The objective of this work was to explore the role of the putative DGC of *M. tuberculosis* and its possible involvement in biological process such as biofilm formation, cell-cell aggregation, and cell wall modification.

To analyze its function, we overexpressed the GGDEF domain of the putative DGC from *M. tuberculosis* in *M. smegmatis* using the pMV261 expression vector. Phenotypic analyses of transformants included evaluation of colony morphology by microscopy and growth on LB solid medium containing Congo red/Coomassie blue and Calcofluor, as well as motility assays using semisolid agar medium. Biofilm formation was also determined both in PVC plastic 96-well microtitre plates, and on glass surfaces. Our results show that overexpression of DGC changed colony morphology, induced the production of extracellular polysaccharides and affected motility. Biofilm formation was also altered on glass surfaces but not on PVC. In

conclusion, the putative DGC of *M. tuberculosis* has an active domain that affects multicellular behaviour in mycobacteria.

Key Words: diguanylate cyclase, multicellularity, *M. tuberculosis*.

Ciprofloxacin enters *Mycobacterium smegmatis* through porin MspA

Rosalva Rodriguez[1], Anelin Pazos[1], Michael Neiderweis[2], Howard Takiff[1]

1 Laboratorio de Genética Molecular, Instituto Venezolano de Investigaciones Científicas (I.V.I.C) Altos de Pipe, Venezuela.
2 Department of Microbiology, University of Alabama at Birmingham, 609 Bevill Biomedical Research Building, 845 19th Street South, Birmingham, AL 35294, U.S.A.

Scientific interest in mycobacterias is propelled not only by the extraordinary medical importance of *Mycobacterium tuberculosis* as a clinical entity, but also by the properties that distinguish them from other bacteria. The most outstanding characteristic is their unique cell wall and envelop. The permeability of mycobacteria is several orders of magnitude lower than that of Gram negative bacteria, which likely explains much of the intinsic resistance of the mycobacteria to most antibiotics. It is believed that some chemotherapeutic agents such as ciprofloxacin can use porins as a entry route. These proteins function as channels in cellular membrane and in mycobacteria they mediate import of the hydrophilic solutes, but the efficiency of this route is 100 to 1000 times lower than in *Escheri*chia *coli.*To determine whether ciprofloxacin entry is mediated by porins, we used three previously described *Mycobacterium smegmatis* mutants deleted in the three porins (MspA, MspC y MspD) and then determined the intracellular accumulation of ciprofloxacin by measuring fluorescence intrinsic to the drug. We found that ciprofloxacin entry route is mediated by MspA, the principal porin of *M. smegmatis.*

Key Words: Ciprofloxacin, porins, mycobacteria.

In vitro assays to determine *Mycobacterium tuberculosis* H37RV growth by spectrofluorometry and flow cytometry

María Elena Ramírez Agudelo[1, 2], Luis Fernando García[1], Mauricio Rojas[1, 2]

1 Grupo de Inmunología Celular e Inmunogenética, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia.
2 Unidad de Citometría. Sede de Investigación Universitaria, Universidad de Antioquia, Medellín, Colombia.

CFSE and SNARF-1 are fluorescent probes commonly used to metabolically label cells and determine replication of mammalian cells based on the fluorescence reduction associated with replication cycles.

AIM: To test changes in the fluorescence of CFSE- and SANRF-1-labeled Mtb associated with extracellular and intracellular replication.

METHODS: Mtb (10^5 CFU) were labeled with CFSE and SNARF-1, Mtb was fixed to determine the spontaneous decrease of CFSE and SNARF1 fluorescence. Mtb was

seeded in Middlebrook 7H9 in the presence of anti-mycobacterial drugs or used to infected B10R and B10S murine macrophages. Changes in fluorescence were studied by spectrofluorimetry and flow cytometry.

RESULTS: Viable but not paraformaldehyde inactivated CFSE- and SANRF-1-labeled Mtb H37Rv underwent a time-depending decrease in the fluorescence intensity when cultured in Middlebrook 7H9 that correlated with mycobacterial replication. Changes in the fluorescence of CFSE- and SANRF-1-labeled Mtb H37Rv were sensitive enough to distinguish the influence of the genetic background on bacterial control. Testing the 620/570 emission it was possible to detect Mtb outside of the acid vesicles, but only with very high number of Mtb per macrophages (20:1). Changes in the fluorescence of CFSE- and SNARF-1-labeled Mtb correlated with incorporation U^3HTdR in the presence of anti-TB drugs, however, unidentified interactions between the drugs and the probes make this strategy usefully only for some pharmacologycal agents.

CONLUSION: Extracellular replication of Mtb, and its senstitivity to pharmacological agents is detectable with both CFSFE, SNARF-1, however, these probes are no sensitive enough to determine the replication of Mtb insde specific cell compartments.

Key Words: CFSFE, SNARF-1, spectrofluorometry.

Synthesis of antibiotic peptides and *in vitro* activity against Mycobacteria

Luz Mary Salazar[1], Paola Santos[1], José Manuel Lozano[2], Luis Ramón Osses[1] and Carlos Yesid Soto[1, 3]

1 Departamento de Química.
2 Departamento de Farmacia.
3 Instituto de Genética, Universidad Nacional de Colombia, Bogotá, Colombia.

Infections caused by mycobacteria remain an important worldwide threat for public health. The design of new drugs against mycobacteria has been one of the most important research fields; however, antimycobacterial peptides still remain an unknown therapeutic tool.

In this work, nine peptides with well-demonstrated antimicrobial activity against gram positive and negative bacteria were synthesized by using Fmoc protocol. All peptides were purified by Reversed Phase-High Performance Liquid Chromatographic (RP-HPLC) and characterized by mass spectroscopy MALDI-TOF. Peptide antimicrobial activity against *Mycobacterium smegmatis* was determined using a radial diffusion assay. Three out of eighteen peptides (Maganin I, Mastoparan and Cecropin B) showed antimicrobial activity against *M. smegmatis* and *M. tuberculosis* H37Ra which was confirmed by a microplate colorimetric-based test using [3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide] (MTT) as a bacterial growth indicator. The possible mechanism of action of these peptides will be discussed based upon their structural-functional relationship.

Key Words: Maganin I, Mastoparan, Cecropin B, *Mycobacterium*, radial diffusion, peptide synthesis.

Oral Presentation

Massive parallel sequencing of the flanking regions of IS*6110* insertion element of *Mycobacterium tuberculosis*

Andrea Sandoval[1], Alejandro Reyes[1,2], Viviana Ritacco[3], Jaime Robledo[4], Sofia Samper[5], Maria Mercedes Zambrano[1], Rob Mitra[2] and Patricia Del Portillo[1]

1 Corporación CorpoGen, Bogotá, Colombia. Centro Colombiano de Investigación en Tuberculosis CCITB.
2 Universidad de Washington, Saint Louis, MO, USA.
3 Instituto Nacional de Enfermedades Infecciosas INEI ANLIS "Carlos G Malbran", Buenos Aires, Argentina.
4 Corporación para Investigaciones Biológicas CIB, Medellín, Colombia, CCITB.
5 Universidad de Zaragoza, España.

Mycobacterium tuberculosis (MTB) is one of the most successful bacterial pathogen in history. One third of world population being infected and the acquire resistance to all know antibiotics confirm it. Contrastingly, the genetic diversity is only due to DNA mutations as rearrangements, insertions or deletions. Mobile genetic elements such as insertion sequences (IS) are known to be responsible for some of these changes. IS*6110* is unique of the MTB complex, its copy number and position has been used for years as the gold standard for typing and epidemiological studies; however, little is known about the precise insertion site. Mapping the exact insertion is fundamental in the understanding of the role of IS*6110* in the biology of mycobacteria. In this study we developed a method to amplify and sequence all IS*6110* flanking regions in 579 clinical isolates from collections of Colombia, Argentina and Spain. In brief, shredded DNA was ligated to specific adapters that harbor a sample specific barcode. Multiplex PCR was made and massive parallel sequencing (illumina) is being carried out on the amplicons. Preliminary results obtained from traditional cloning / Sanger sequencing of 10 samples showed a 100 % efficiency of the method; all clones evaluated contained the appropriate primer sequences and barcodes. 89.5 % of the sequences from reference strain H37Rv corresponded to known insertion sites, allowing the identification of 6/16 of the total sites. Illumina sequencing would allow an average of 400 sequences per insertion site on each sample, assuring the necessary coverage to identify all IS from all samples.

Key Words: *Mycobacterium tuberculosis*, insertion sequence, flanking regions.

Mce3R a tetr-type transcriptional repressor, controls de expression of a large regulon involved in lipid metabolism and oxidation in *Mycobacterium tuberculosis*

María de la Paz Santangelo[1], Javier Nunez-Garcia[3], Federico Blanco[1] Marcelo Soria[2], Maria del Carmen García Pelayo[3], Verónica Bianco[1], Laura Klepp[1], Angel Cataldi[1], Steve Gordon[3] and Fabiana Bigi[1]

1 Instituto de Biotechnology, CICVyA-INTA, Los Reseros y Las Cabañas, 1712 Castelar, Argentina.
2 Microbiología Agrícola, Facultad de Agronomía. Universidad de Buenos Aires, Av. San Martin 4453, 1417 Buenos Aires. Argentina.
3 Veterinary Laboratories Agency (Weybridge), New Haw, Addlestone, Surrey KT15 3NB, United Kingdom.

INTRODUCTION: *mce3* is one of the four virulence-related *mce* operons of *M. tuberculosis*. The biological function of Mce proteins is not known, but they are related to the virulence of *M. tuberculosis*. We have previously characterized a transcriptional regulator, Mce3R that represses *mce3* transcription. This regulation is exclusive for the *mce3* operon among *mce* genes and regulates its own expression. AIMS: The identification of the *mce*3R regulon could help to decipher the function of *mce*3 genes and would provide valuable insight into their role on *M. tuberculosis* virulence. METHODS: We performed whole-genome microarray technology to study expression profiling on wild type H37Rv and mutant *mce*3*R* strains and validate these results by QRT-PCR. By analysis *in silico* we identify a *tet* motif recognized by Mce3R within *mce*3 promoter. RESULTS: 81 genes were significantly differentially expressed between strains. 67 genes were over-expressed in mce3R, while 14 genes were down regulated in this strain. Genes whose expression was significantly altered were analysed. Many of the genes were predicted to be involved in electron transport, fatty acid metabolic process and redox reactions. We identified a *tet* motif within *mce*3 promoter region and define a consensus, a variant of which is located upstream of nearly all genes of Mce3R regulon. CONCLUSIONS**:** Mce3R regulates a large number of genes involved in lipid metabolism and redox reactions. The identification of the *mce3R* regulon would help to decipher the function of *mce3* genes and provides valuable insight into their role on *M. tuberculosis* virulence.

Key Words: mce, tuberculosis, regulation.

Oral Presentation

Change in lipid composition, decreased metabolism and low virulence induced by hypoxia in mycobacteria

Ana Silvia Ramírez[1] and Carlos Yesid Soto[1,2]

1 Departamento de Química, Facultad de Ciencias, and
2 Instituto de Genética, Universidad Nacional de Colombia, Bogotá, Colombia.

Approximately a third of the worldwide population is latently infected with *Mycobacterium tuberculosis*. Although latency has been linked to low oxygen tension within the host, and latent *Mycobacterium tuberculosis* has been correlated with distinct cell-wall alterations, the metabolic and structural response of mycobacteria to hypoxia and its implications in latency remains poorly characterized.

In this work, mycobacteria were grown under hypoxic conditions to determine its electrochemical activity, lipid profiling and virulence level induced by oxygen starvation. The current produced by hypoxic mycobacteria was measured in the presence of the electron acceptor 2, 6-dichlorophenol indophenol (DCIP) showing that the transition of mycobacteria to hypoxic state accompanies an electrochemical decrease of cell activity. This electrochemical behavior of hypoxic mycobacteria indicates a decreased metabolism as expected in latent bacilli. On the other hand, lipid analysis by thin layer chromatography showed that hypoxic *M. tuberculosis* has an impaired capacity to synthesize Sulfolipid-I (SL-I) and also over-express

Diacylthehaloses (DAT). These methyl-branched long-chain fatty acids have long been thought to play an important role in host-pathogen interactions. In addition, we found a negative neutral-red staining of hypoxic *M. tuberculosis* indicating a diminished virulence of tubercle bacili grown in an oxygen-limited environment. The results indicate an interesting correlation between metabolic decrease, glycolipid composition and impaired virulence of *M. tuberculosis* growing in an oxygen-limited environment similar to the mycobacterial latent state.

Key Words: Mycobacteria, hypoxia, cell wall.

Promoter sequence characterization of *Mycobacterium tuberculosis pe_pgrs33*

Antonio J. Vallecillo and Clara Espitia

Departamento de Inmunología. Instituto de Investigaciones Biomédicas Universidad Nacional Autónoma de México. C.P. 04510 México, D.F. México. Apartado Postal 70-228 04510 D.F. México D.F.

The sequencing of the *M. tuberculosis* genome reveals two enigmatic multigenic families, the PPE and the PE. Recent work shows that the expression of the PE/PE_PGRS genes family occur both *in vitro* and *in vivo* under variable environmental conditions, but very little is known about their promoter sequences and how they are regulated. We found that *pe_pgrs33* a typical member of PE_PGRS family is transcribed in a monocystronic mRNA. In addition, the gene promoter functionality was assayed in *Mycobacterium smegmatis* transformed with the green fluorescent protein reporter gene fused to different lengths of *pe_pgrs33* 5´ upstream sequences. The GFP was down-regulated in the stationary phase, under nutrient starvation and oxygen depletion, when the longest 5´ upstream regions were assayed, suggesting these results that, in stress conditions, regulation of the gene could be under control of a repressor molecule. A 52 -RACE assay of transcriptional fusions evaluated in *M. smegmatis* and in *M. tuberculosis* mRNA revealed a transcription start point 75 nt upstream of the ATG codon and a -10 like-SigA box. Furthermore, a transcription run assay confirmed that SigA mediates *in vitro* transcription of *pe_pgrs33*. Interestingly, conserved -10 SigA boxes were found in the intergenic region of several PE_PGRS genes. These results suggest that expression of some PE_PGRS genes may be mediated by SigA, and the differences in expression observed in the gene family could be explained by the participation of additional regulatory genetic elements.

Key Words: PE, PPE, SigA, PE_PGRS genes.