NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS

Research Activities

April 2010 – March 2011

CONTENTS

PREFACE

This Annual report presents various research activities undertaken at NIRT (formerly TRC) during the year 2010 - 2011. NIRT's activities were closely linked to the felt needs of the Indian TB and AIDS Control programmes, hence the priority of the institute continued to be the identification and testing of the efficacy of new regimens useful to national and global programme for control of TB & HIV-TB. The main focus continued to be shortening the duration of treatment of TB, management of MDR-TB; and treatment of HIV/TB co-infection. Socio behavioural evaluation and research remains an integral part of chemotherapy trials to ensure treatment completion and better patient retrieval. Studies to understand the link between HIV & sexually transmitted diseases and risk factors for HIV in vulnerable groups were also undertaken.

Periodic disease surveys to obtain data on the incidence and prevalence of TB in the community are an important activity of the institute. The upgraded Bacteriology department is well equipped with rapid methods such as MGIT, LPA for diagnosis of drug resistant TB. The laboratory is equipped to undertake drug susceptibility testing of both 1st and 2nd line anti-TB drugs. In addition, the department provides technical support to other ICMR institutes in mycobacteriology and accreditation of laboratories. The HIV laboratory is accredited by the NIH, USA for the performance of viral load and HIV drug resistance testing and is a national referral laboratory for HIV DNA PCR for early infant diagnosis. Laboratory studies in Immunology, Pathology, Clinical Pharmacology, Molecular Epidemiology and Molecular Virology are other important research activities of the institute.

The institute continues to be recognized for its expertise in the field of TB research as a WHO collaborating Centre and International Centre of Excellence in Research (ICER) by NIH. This report is a documentation of inspired and committed work of scientific and technical staff, well supported by grass root workers.

The centre celebrated its Foundation day on Aug 01, 2011, and on that occasion it was renamed as the National Institute for Research in Tuberculosis (NIRT) by the Secretary, Department of Health Research, Govt. of India and the Director General, ICMR. NIRT will continue to provide new knowledge and tools that are worthy of our status as a flagship institute of ICMR.

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Appellate Authority	:	Dr.P. Venkatesan Scientist 'E' Department of Statistics Phone: 2836 9638

Distinguished Visitors

Date	Name	Organization / Place	Remarks
16.12.2010	Dr Grady Hanscom	NIAID, NIH	"Thank you for sharing your work and remarkable scientific efforts. NIAID is very pleased with our on-going partnership".
23.12.2010	Mr. Andrew T. Simkin	Consul General of the USA	"I am impressed by this facility and the important work done here. We at the US Consulate General wish to extend any possible support that we may provide. Thank you".
11.02.2011	Dr. Nata Menabde	WHO Representative to India	"It has been a great pleasure to meet with the core team of this scientifically highly credible institution. Your work helps WHO and the Govt. of India in resolving important health problem and we count on our collaborations in the future".

ABBREVIATIONS

2D-liquid phase electrophoresis (2D-LPE)
Acquired Rifampicin Resistance (ARR)
Alcohol Use Disorders (AUDs)
Alcohol use disorder identification test (AUDIT)
Anti-TB treatment (ATT)
Antiretroviral therapy (ART)
Chennai Urban Rural Epidemiology Study (CURES)
Chlamydia trachomatis (CT)
Circulating immune complexes (CIC)
Complementary DNA (cDNA)
Confidence interval (CI)
Contact specific (CS)
Coronary arterial disease (CAD)
C-reactive proteins (CRP)
Culture filtrate antigen (CFA)
Culture filtrate protein-10 (CFP-10)
Cytotoxic T-lymphocytes (CTL)
Data Safety & Monitoring Board (DSMB)
Delayed type hypersensitivity (DTH)
Dendritic cells (DC)
Diagnostic LRP assay (DLRPA)
Directly Observed Treatment Short-course (DOTS)
Drug susceptibility testing (DST)
Early secreted antigenic target-6 (ESAT-6)
Electronic Data Processing (EDP)
Electro Mobility Shift Assay (EMSA)
Endemic normal (EN)
Ethambutol (EMB)
External quality assurance (EQA)
Extrapulmonary TB (ETB)
Fixed dose combination (FDC)
Glyceraldehyde-3 phosphate dehydrogenase (GAPDH)
Healthy control subjects (HCs)
Healthy house hold contact (HHC)
High performance liquid chromatography (HPLC)
Hunter-Gaston discriminatory index (HGDI)
Huner-Gaston index (HGI)
Immune reconstitution inflammatory syndrome (IRIS)
Inducible protein (IP)
Infected individuals (INF)
Integrated Behavioural and Biological Assessment (IBBA)
Interferon γ (IFN-γ)
Interferon γ release assay (IGRA)
Interleukin (IL)
Intemediate reference laboratory (IRL)
Isoniazid (INH)
KG-1 cell derived DC (KGDC)
Lowenstein Jensen (LJ)

Luciferase reporter phage (LRP)
Lymphatic filariasis (LF)
Lymphatic pathology (CP)
Mannose-binding lectin (MBL)
Men who have sex with Men (MSM)
Mitogen-Activated Protein Kinases (MAPKs)
Monocyte chemoattractant protein-1 (MCP-1)
Mothers living with HIV (MLH)
Moxifloxacin (MFX)
Multi drug resistant *M. tuberculosis* **(**MDR-TB)
Multiplicity of infection (MOI)
Mycobacteriophage genome database (MGDB)
Neisseria gonorrhea (NG)
Odd's ratio (OR)
Ofloxacin (OFX)
Onsite evaluation (OSE)
Pantothenate synthetase (PS)
Polymerase chain reaction (PCR)
Pleural mesothelial cells (PMC)
Propidium iodide (PI)
Pulmonary tuberculosis (PTB)
Pyrazinamide (PZA)
QuantiFERON TB-Gold In tube (QFT-IT)
Regulated upon activation normal T-cell expressed and secreted (RANTES)
Real time PCR (RT-PCR)
Region of deletion-1 (RD1)
Restriction fragment length polymorphism (RFLP)
Revised National Tuberculosis Control Programme (RNTCP)
Rifabutin (RBT)
Rifampicin (RMP)
Ritonavir (RTV)
Serine threonine protein kinases (STPK)
Serum Amyloid protein-A (SAA)
Sexually transmitted infections (STIs)
Short course chemotherapy (SCC)
Signal transducers and activators of transcription (STAT)
Stromal cell-derived factor-1 (SDF-1/CXCL12)
Suppressor of cytokine signaling (SOCS)
Time to detection (TTD)
Tibotec medicinal compound (TMC 207)
Toll-like receptors (TLR)
Treponema pallidum (TP)
Tuberculin skin test (TST)
Untranslated region (UTR)
Vascular endothelial growth factor-A (VEGF-A)
Vitamin D receptor (VDR)

DEPARTMENT OF CLINICAL RESEARCH

Completed studies:

(i) Efficacy and safety of immunomodulator *(Mycobacterium W)* as an adjunct therapy in Category-II pulmonary tuberculosis

(Principal Investigator: Dr.R. Balambal; Funding: Department of Biotechnology, India)

Background: The immunomodulator containing *Mycobacterium w* developed by the National Institute of Immunology, New Delhi in 1980, was found to be useful in the prevention of tuberculosis (TB) in experimental animals. Studies have documented faster sputum conversion when *Mycobacterium w* was added to the short course chemotherapy (SCC). Immunomodulators work against persistors, which may result in reducing the relapse rates. The addition of immunomodulator to chemotherapy is well tolerated and does not increase adverse reactions to the therapy.

Aim: To study the cure rate in Category-II pulmonary TB (PTB) patients after addition of *Mycobacterium w* vaccine to standard anti-TB drugs

Methods: This study was a double blind, randomized, placebo controlled multicentric clinical trial. The patients were randomly chosen to receive either the vaccine or placebo along with the standard Category-II Revised National Control Programme (RNTCP) regimen.

Results: Of the 59 patients enrolled to the study, between March 2006 and April 2008, 45 completed treatment and follow up, 4 defaulted for treatment - 2 in the intensive phase and 2 in the continuation phase, 4 developed serious adverse events – 2 renal and 2 hepatic. Eight patients required change of chemotherapy – 4 for multidrug resistance, one for clinical complication, one for pregnancy and 2 for hepatotoxicity. The study period was over on 31, March 2011,

and the data has been handed over to the funding agency.

(ii) Changes in HIV viral load in patients undergoing treatment for filarial infection

(Principal Investigator: Dr. Soumya Swaminathan / Dr. Pradeep Aravindan Menon; Funding: NIAID, NIH)

Background: Evidence is beginning to accumulate that the course of HIV infection and progression to AIDS may be influenced by co-existing infections, other than the classic opportunistic infections. The interaction of helminth infections with HIV has not been well established. Some studies have shown that patients with HIV and concomitant helminth infections have higher viral loads which decrease upon treatment whereas others have shown no effect of helminths on viral load, CD4 count or disease progression.

Aims: (i) to determine the changes in HIV viral loads that occur in patients co-infected with HIV and filaria, over 1 year following treatment with DEC/Albendazole and (ii) to compare those changes with HIV infected patients without filarial co-infections

Methods: A total of 53 HIV-infected patients, 14 with filariasis (7 males and 7 females) and 39 controls (20 males and 19 females) were recruited at NIRT and YRG Care. The number of subjects was reduced as the number of positives screened was very low in that population. Statistical changes were made to achieve the confidence level.

Results: At baseline, there was no difference in mean CD4 counts (370/ul [95%CI 271-503] vs. 468/ul [95%CI 396-553]; p=0.17) or viral loads (6202 copies/ul [95%CI 1702-13286] vs. 4403 copies/ul [95%CI 2154-9000]; p=0.90) between the HIV/FIL+ and HIV/Fil- groups. Following DEC/Alb, there were no differences noted in clinical outcomes between the groups. There also was no difference in the HIV viral load at 12 months (5064 copies/ml [95%CI 1497-17127] vs. 3880 copies/ul [95%CI 1860-8094];

p=0.78) between the two groups. Furthermore, there were no significant differences found in either the change in viral load at 1, 3, or 6 months (p>0.5 for all comparisons) or in the CD4 count at 3, 6, or 12 months (p>0.5 for all comparisons).

Conclusions: There was no significant effect of *W. bancrofti* infection on HIV clinical course or parameters associated with HIV infection. However, the study was limited by the numbers of study participants—the prevalence of lymphatic filariasis (LF) having diminished in south India.

Studies in progress:

(i) Randomised Clinical Trial to study the efficacy and tolerability of 3- and 4-month regimens containing moxifloxacin in the treatment of patients with sputum positive pulmonary tuberculosis

(Principal Investigator: Dr.M.S. Jawahar)

(PROVCTRI/2008/091/000024)

During the year under review patient enrollment to this clinical trial continued in both Chennai and Madurai. In this RCT the efficacy and safety of 3- and 4-month moxifloxacin (MFX) containing regimens are being compared with that of a standard 6-month regimen in the treatment of patients with sputum positive pulmonary TB. The primary aim of this study is to shorten the duration of easibility of shortening the duration of TB treatment by supplementing the standard 4-drug TB regimen with MFX, a fluoroquiniolone with potent bactericidal and sterilising activities against *M. tuberculosis*. Patients with newly diagnosed sputum positive, HIV sero-negative pulmonary TB, resident in Chennai and Madurai are being randomly allocated to 3-month or 4-month MFX regimens, or a control 6-month regimen. Treatment is given under direct observation and response to treatment is assessed with sputum examinations. The patients are also closely monitored for adverse drug reactions which are critically

documented. After completion of treatment, successfully treated patients are being followed up with monthly evaluations for assessing recurrence of TB.

The regimens being tested in this study are shown in table 1.

Table-1: Study regimens

Regimen	Months						Duration (Months)
	1	2	3	4	5	6	
Test Reg. 1	RHZEM						3
Test Reg. 2	RHZEM		RHM				4
Test Reg. 3	RHZEM		RHM3				4
Test Reg. 4	RHZEM		RHEM3				4
Control Reg.	RHZE3		RH3				6

R – rifampicin; H – isoniazid; Z – pyrazinamide; E – ethambutol; M - moxifloxacin

Intensive phase Continuation phase

A total of 480 patients have been enrolled as of 31st March 2011. The baseline characteristics of these patients are shown in table 2.

Table-2: Baseline characteristics of 480 patients enrolled in study

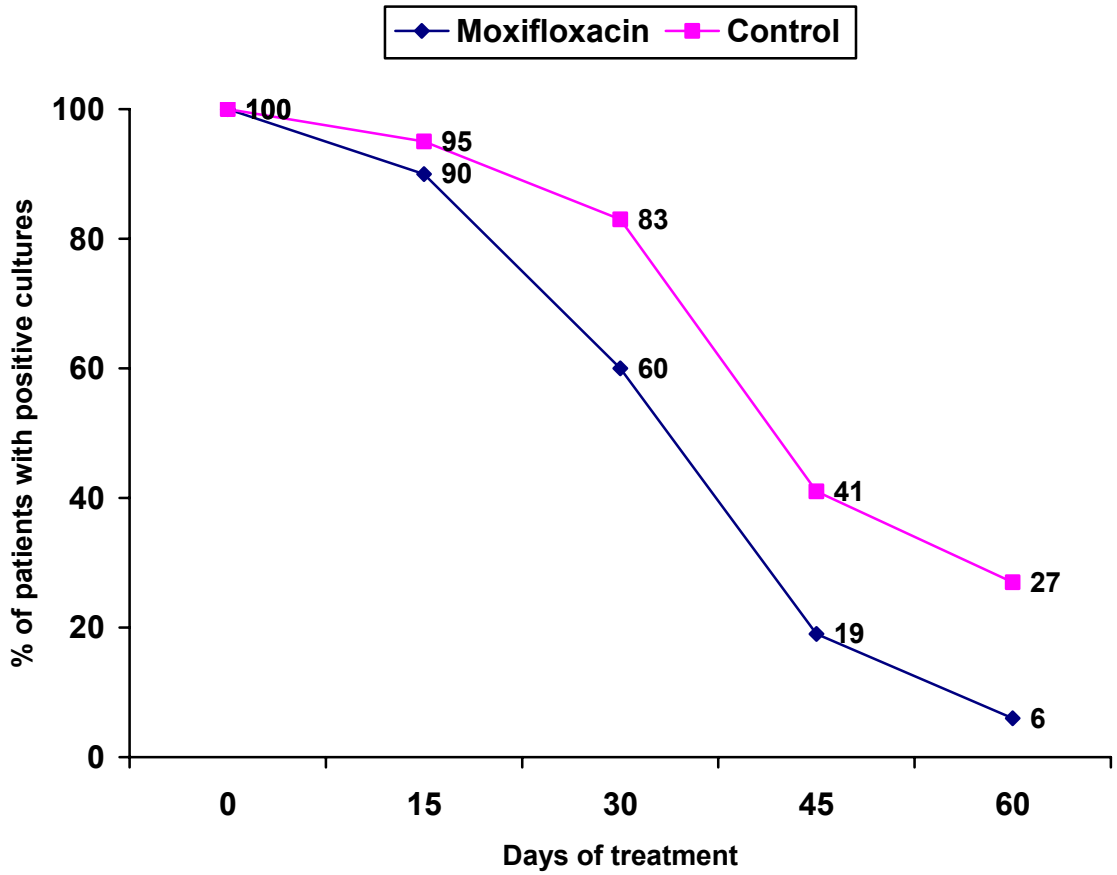
Regimen	Test Reg. 1 (n = 91)	Test Reg. 2 (n = 95)	Test Reg. 3 (n = 105)	Test Reg. 4 (n = 94)	Control Reg. (n = 95)	Total (n = 480)
Sex						
Male	72	75	80	65	75	367 (77%)
Female	19	20	25	29	20	113
Age						
<40 years	62	56	74	66	63	321 (67%)
≥40 years	29	39	31	28	32	159
Initial sputum culture						
1+	2	5	4	4	2	17
2+ or 3+	89	90	101	90	93	463 (97%)

Extent of Initial X-ray involvement (zones)						
≤ 2	18	18	20	21	20	97
> 2	73	77	85	73	75	383 (80%)

Sputum culture conversion with treatment:

The earlier observation (Annual Report 2009-10) that the proportion of patients who became sputum culture negative after treatment was significantly higher in the MFX arm (consolidated for all four test regimens) compared to the control arm was sustained with the larger sample of patients. Fig.1 illustrates the proportion of patients with positive sputum cultures at 15, 30, 45 and 60 days of treatment. At 2 months (60 days) 94% of the patients treated with the MFX regimens were sputum culture negative compared with 73% of the aptients treated with the control regimen. This is a significant finding as it shows that patients treated with the test regimens become less infectious earlier and to a greater degree.

Fig.1: Sputum culture conversion with treatment in 480 patients



Results at end of treatment:

Table 3 describes the results at the end of treatment in 452 patients. Among patients treated with MFX regimens, 90% to 96% had all cultures negative at the end of treatment compared to 88% in the control regimen. Six patients required change of treatment for either drug toxicity or pregnancy.

Table 3: Response at the end of treatment

Regimen	Patients	Favourable	Unfavourable			Defaulted
			Bacteriological	Treatment changed	Death	
Test Reg. 1	86^	81 (94%)		2*		3
Test Reg. 2	91	85 (93%)	1	1*	1	3
Test Reg. 3	99	89 (90%)	4	1*		5
Test Reg. 4	88	84 (96%)		1***	1	2
Control Reg.	88	77 (88%)	1	1**	2	7

^ 3 patients who received < 80% of treatment excluded

* for jaundice

** for rifampicin induced skin lesions

*** for pregnancy

The study is registered in the Clinical Trials Registry of India (CTRI) (ctri.nic.in).

(ii) Management of patients who fail on Category-II regimen of the TB control programme

(Principal Investigator: Dr. Aleyamma Thomas)

Background: The centre had earlier undertaken a drug resistance surveillance study in patients treated with RNTCP regimens in a TB Unit in Tiruvallur district in Tamil Nadu, during 1999-2005. This study showed that 52 patients failed to Category-II regimen. The drug susceptibility testing (DST) results showed that 32% had multi drug-resistant TB (MDR-TB), 26% had mono or poly drug resistant organisms (i.e. resistant to isoniazid (INH) / streptomycin / streptomycin + INH / EMB), 29% harboured fully susceptible organisms, and in 13% there was no growth in the culture. Though Directly Observed Treatment Short-course (DOTS) using standardized Category-IV regimen (DOTS plus) is being implemented in India in a phased manner, limited information is available on the feasibility, effectiveness and profile of adverse reactions to the Category-IV regimen. There are no guidelines available for the management of patients who fail to Category-II

regimen but are non-MDR. As the detection and treatment of drug resistant TB is essential to stop primary transmission, a prospective study was initiated on the management of patients who failed to Category-II regimen of RNTCP in Chennai corporation area and Tiruvallur District.

Aims: To assess the feasibility, effectiveness and adverse reaction profile of two regimens for the treatment of:

- Category II failures with MDR-TB and
- Category II failures with non MDR-TB

Study Design: This study was planned as a randomized clinical trial, in which the following treatment regimens were tested:

Treatment regimens:

MDR TB:

Regimen 1	6(9) (K, Of, Eth, Z, E and C) $_7$ / 18 (Of, Eth, E & C) $_7$.
Regimen 2	6(9) K_3, (Of, Eth, Z, E and C) $_7$ / 18 (Of, Eth, and E & C) $_7$.

Non-MDR TB:

Regimen 1	6 K_3 (REZ) $_7$/ 3 (REZ) $_7$.
Regimen 2	6 K_3 (REZH) $_7$/ 3 (REZH) $_7$

(K-kanamycin; Of-ofloxacin; Eth-ethionamide; Z-pyrazinamide; E-ethambutol; C-cycloserine; R-rifampicin, H-isoniazid)

Outcome measures:

1) Sputum smear conversion at 3 and 6 months of treatment

2) Sputum culture conversion at 3 and 6 months of treatment

3) Favourable response (bacteriological) at the end of treatment

4) Adverse reactions to anti-TB drugs

Results

The study was initiated in 2007 with a plan to recruit 300 patients, consisting of equal numbers of MDR-TB and non-MDR-TB patients. Till December 2009, 75 patients were recruited, 54 and 21 MDR-TB and non-MDR-TB patients respectively. Intake to the study was stopped in January 2010. The demographic

details of patients admitted to the study are given in the previous Annual Report. The patients admitted to the study are being followed up at regular intervals.

Drug resistance pattern among the MDR-TB patients showed 24% HR + 1 drug, 41% HR + 2 drugs, 33% HR+ 3 drugs and 2% HR+4 drug resistance. None had XDR pattern. Drug resistance pattern in non MDR-TB patients showed 13% -1 drug, 53% -2 drugs, 27% 3 drugs and 7% 4 drug resistance. The treatment details and treatment response is given in table 4.

Table-4: Treatment details and response of 75 patients

	MDR	NMDR	Total
Admitted	54	21	75
Favorable response	28	9	37
Failure	9	7	16
Defaulted	5	4	9
Died	6	0	6
Relapse	0	1	1
Still on treatment	6	0	6

(iii) A Phase II, placebo-controlled, double-blind, randomized trial to evaluate the anti-bacterial activity, safety and tolerability of TMC207 in participants with sputum smear-positive pulmonary infection with multi-drug resistant *M. tuberculosis*

(Principal Investigator: Dr. Aleyamma Thomas; Funding: Tibotec BVBA)

The primary objective of this ongoing double-blind, stratified, randomized, placebo-controlled, phase II trial was to demonstrate the superiority in the anti-bacterial activity of the new anti-TB drug, Tibotec medicinal compound (TMC207) compared to placebo when added for 24 weeks to a background regimen in participants with newly diagnosed sputum smear-positive pulmonary MDR-TB infection. TMC 207 was given at 400mg dosage once a day for first 2 weeks followed by 200 mg thrice a

week for the next 22 weeks along with the background regimen consisting of kanamycin, ethionamide, pyrazinamide, ofloxacin and cycloserine in doses based on body weight. The patients were monitored as per protocol requirements weekly for 2 months, fortnightly for 4 months and monthly for 18 months. There were three patients recruited to this study which was initiated in August 2009. One patient is a diabetic. All the three patients had culture conversion and have completed TMC 207/ Placebo and currently receiving the background regimen. They have completed 18 months of treatment with the background regimen and have remained culture negative. The study is on-going.

(iv) Randomized controlled clinical trial of TB preventive therapy in HIV-infected individuals starting on antiretroviral therapy

(Principal Investigators: Dr. C. Padmapriyadarsini / Dr. Sheik Iliayas; Funding: United States Agency for International Development (USAID) through WHO Model DOTS Project)

A parallel-arm, double blind, placebo controlled, prospective randomized clinical trial among HIV-positive individuals, without active TB, starting on antiretroviral therapy (ART) was initiated.

The main objectives of this study were: (i) to study the efficacy of TB preventive therapy in reducing the incidence of TB and mortality among HIV-infected individuals starting highly active ART and (ii) to assess the role of symptoms, clinical examination, sputum microscopy and chest X-ray in ruling out active TB

The study regimens were INH (H - 300mg) daily vs placebo daily for 6 months – self administered, collected once a month – along with the background ART regimen.

The study was initiated in October, 2009, and terminated in January, 2011 in view of the global policy and NACO's proposed feasibility study of INH preventive therapy. Fifty four patients were recruited to the study, 42 had completed treatment and 12

patients were receiving study regimen.

As per instructions from the Institutional Ethics Committee, study blind was broken and all patients who received placebo were started on INH 300mg daily for 6 months. Follow up of patients is ongoing.

(v) A randomized controlled clinical trial comparing daily vs. intermittent 6 – month SCC in reducing failures & emergence of Acquired Rifampicin Resistance in patients with HIV and PTB (CTRI Registration No: 476/09, NCT No: 933790)

(Principal Investigator: Dr. G. Narendran, Co-PI Dr. Soumya Swaminathan; Funding Agency: United States Agency for International Development (USAID) through WHO Model DOTS Project)

Background: There is a high risk of development of Acquired Rifampicin (RMP) Resistance (ARR) in regimens using thrice weekly RMP throughout the treatment period among patients with HIV-associated TB. Whether, daily administration of RMP could overcome this phenomenon by achieving a better and sustained concentration in the blood in the setting of ART is the research question. The study, while comparing daily and intermittent regimens of anti-TB treatment (ATT) is also looking into various dimensions of HIV/TB management like sputum conversion, Immune reconstitution inflammatory syndrome (IRIS), toxicity profile etc.

Objective: To compare the efficacy of three anti–TB regimens among HIV-infected TB patients, namely; (1) daily regimen ($2EHRZ_7/4HR_7$), (2) partly intermittent ($2EHRZ_7/4HR_3$) (3) a fully intermittent regimen ($2EHRZ_3/4HR_3$), given for 6 months specifically in (a) reducing bacteriological failures and (b) decreasing the emergence of ARR

Methodology: This was an open labelled, prospective, parallel arm, active comparator, randomized controlled clinical trial; patients were stratified based on sputum smear grading and CD4 cell counts at baseline. HIV-positive patients (regardless of ART status) with newly diagnosed PTB were

randomized to receive one of the three regimens, treated for a period of 6 months and followed up for a period of one year. Patients were investigated and suitably referred to NACO ART centres for ART initiation as per national guidelines. The primary outcomes were failure and emergence of ARR. Secondary outcomes included overall failures, recurrences, death due to TB and IRIS. Both the efficacy analysis and intent to treat analysis will be undertaken.

Results: The study started in September, 2009 after an initial pilot study to establish the logistics and the manual of operations prepared based on the inputs. As on 31.03.2011, 213 patients who were HIV-positive with symptoms suggestive of PTB were screened. Sixty seven patients were registered and 51 among them were randomly allocated to the study. The baseline characteristics of the study subjects are given in table 5. Three patients were excluded since their baseline sputum culture was resistant to INH and RMP (MDR-TB). Of the 48 cases, 17 were allocated to the daily regimen, 17 to the part daily regimen and 14 to the intermittent regimen. The study is ongoing.

Table 5: Baseline demographics (n=51)

Mean age ± SD in years	37 ± 8
Mean Weight ± SD in Kg	41± 6
Median CD4 (IQR) cells/mm^3	128 (68-220)
Median VL (IQR) copies/ml	226500 (41600- 433000)
Male : Female ratio	35:16

(vI) Anaemia and nutrition among children with perinatally acquired HIV infection in south India

(Multi centric study PI: Dr Anita Shet, St Johns Medical School, Bangalore)

(NIRT Principal Investigators: Dr P.K. Bhavani / Dr Soumya Swaminathan; Funding Agency: ICMR Task Force)

Objectives: The main objectives of this study were: (i) to assess the prevalence of anemia and micronutrient deficiencies (namely

iron, vitamins A, B12, and folic acid) among HIV-infected children in south India, (ii) to examine nutritional and non-nutritional etiological factors contributing to anemia among HIV-infected children, (iii) to compare the effect of therapeutic iron supplementation in those with nutritional anemia and anemia of inflammation, using haematological endpoints (such as hemoglobin, markers of iron status) and measurable endpoints for HIV disease progression (CD4 counts, viral load, opportunistic infections, hospital admissions, death) and (iv) to assess the effect of baseline anemia on growth and HIV disease progression status in children with HIV infection

Methodology: Children with perinatally acquired HIV infection aged 2 to 12 years at three sites in south India, with equal proportions of children who were ART-naïve and on ART have been enrolled to the study. All eligible children underwent clinical and physical examination. Basic demographic data, clinical history and dietary history were collected.

All the children underwent baseline investigations and were divided into anemic and non-anemic groups based on the hemoglobin level (Hb<11 g/dl was considered as anemia). All anemic children were given iron, folate and B_{12} supplementation for 3 months. At the end of 3rd month, investigations were repeated, to find the effect of iron, folate and B_{12} supplementation. Follow-up was once in every 3 months. During the follow-up period, clinical examinations, dietary intake history, anthropometry and blood tests were done.

The study was initiated in February 2010. As on 31st March 2011, the intake to the study was completed. A total of 80 children were recruited; their baseline characteristics are given in table 6. Forty children have completed their 1 year follow up. The study is ongoing.

Table 6: **Baseline characteristics of the children recruited at NIRT**

Characteristics of study population	ART NAIVE (40)	ON ART(40)
Sex		
Male	21 (55%)	20 (55%)
Female	19 (40%)	20 (50%)
Hemoglobin <11 g/dl	19 (47.5%)	4 (10%)
Average (Mean)		
Age (months)	78.8	83.5
Height (cm)	108.4	109.2
Weight (kg)	17.2	17.5
BMI	14.4	14.5

(vli) Study to evaluate the effect of Physician's advice in quitting smoking in HIV and TB patients in south India - A pilot study

(Principal Investigator: Dr.S. Ramesh Kumar (Funding: Fogarty grant, Miriam Hospital, Brown University, USA)

(No.: CTRI / 2009 / 091 / 000962 dt.14.12.2009)

Background: HIV burden and TB burden in India is high. Association of TB and smoking is evident. Smoking in HIV poses additional risks. Smoking cessation initiation by Physician's advice has shown to be useful in previous studies.

Objectives: (i) to determine the efficacy of Physician's advice using "modified 5 A" strategy in quitting smoking in patients with HIV and patients with TB and (ii) to compare the effectiveness of Physician's advice by administering a brochure containing smoking cessation information and counselor's counseling to brochure and counselor counseling alone in quit rate in patients with HIV and patients with TB

Sample size:

HIV patients (smokers) = 80;

TB patients (smokers) = 80

Methods: Patients with TB or HIV with history of current smoking referred to the clinic were randomized to receive Group A (Physician's advice + Counselors counseling + Brochure/ educative material) or Group B (Counselors counseling + Brochure/ educative material) strategy of smoking cessation, stratified based on Nicotine dependence assessed by using Fagerstrom dependence scale. In Group A, in addition to the administering of brochures and a standard counseling by counselor (strategy in Group B), Physician's advice using 'modified 5 A' strategy was systematically approached in the five standard steps namely "Ask, Advise, Assess, Assist and Arrange". In addition, a 'modified 5A' approach is also being adopted wherein the physician delivers a brief structured advice to the study subject and his/her family member.

Recruitment: The study was initiated in March 2010. At the end of March 2011, 61 subjects (42 TB and 19 HIV) were recruited. Among them, 33 were allocated to Group A and 28 to Group B. Intake of subjects to the study is in progress. The study has been registered in Clinical trials registry of India.

HIV Vaccine Trial

Phase I double-blind, placebo-controlled, randomized trial to evaluate the safety and immunogenicity of TBC-M4, a multigenic MVA HIV vaccine vs ADVAX, a multigenic DNA HIV vaccine followed by TBC-M4, a multigenic MVA HIV vaccine

(Principal Investigator: Dr.V.D. Ramanathan)

The second phase I HIV vaccine trial to be conducted by this Institute was completed during 2010-11. This study, which was in the follow up phase was wound up after the last scheduled volunteer visit and completion of the accompanying laboratory tests. The study volunteers also were unblended during this period and information regarding whether they received the vaccine or the placebo was intimated to them.

Briefly, this trial consisted of two arms: a) heterologous prime-boost using 2 injections of DNA followed by 2 injections of MVA at 0,1,3 and 6 months, respectively (Group A - 6 volunteers) and b) homologous prime-boost with 3 injections of MVA at 0,1 and 6 months (Group B - 6 volunteers). There were 4 volunteers who were given placebo injections.

It was found that both the vaccine regimens were safe and immunogenic in more than 90% of the volunteers. However, the response was modest though it persisted in many of the volunteers even 12 months after the last vaccination.

Laboratory studies:

Completed studies:

(i) Determination of MFX concentrations in saliva of healthy subjects (Principal Investigator: Dr. Geetha Ramachandran)

Background: Salivary concentrations of drugs have been employed for therapeutic drug monitoring and for calculation of pharmacokinetic variables. Collection of saliva is non-invasive, involves minimal discomfort and can be collected at multiple time points. It is particularly suitable in geriatric and pediatric populations.

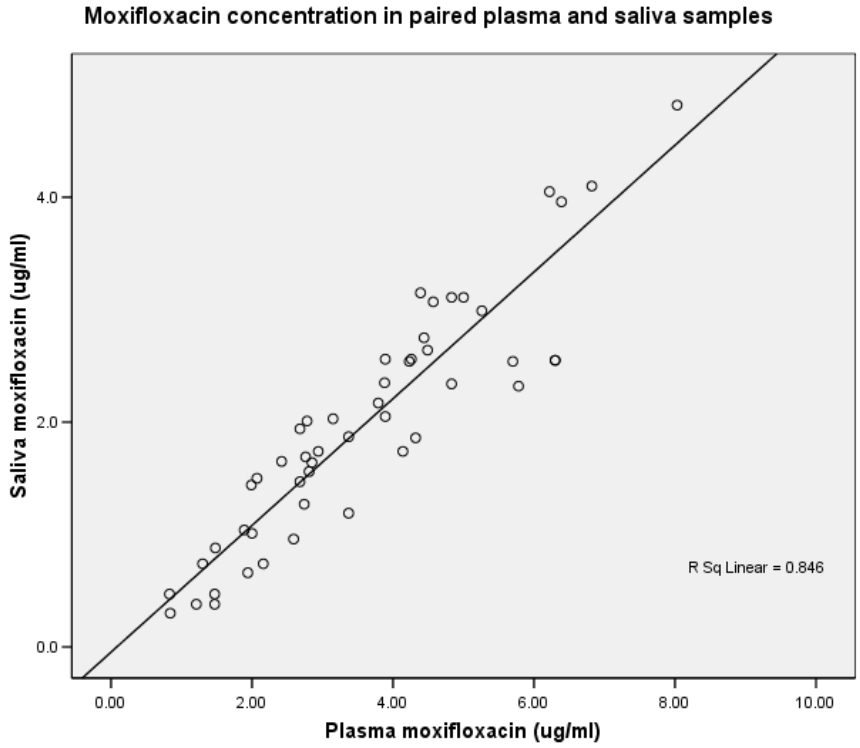
Aims: (i) to develop and validate a simple and rapid assay procedure for estimation of MFX in saliva and
(ii) to apply this method to determine its correlation with plasma concentrations

Methods: The method involved deproteinisation of the sample with perchloric acid and analysis of the supernatant using a reversed-phase C_{18} column (150mm) and fluorescence detection at an excitation wavelength of 290nm and an emission wavelength of 460nm. Forty eight paired saliva and plasma samples were collected from 24 healthy volunteers at different time points after administration of a single oral dose of 400mg MFX.

Results: The assay was specific for MFX and linear from 0.25 to 10.0µg/ml. The relative standard

deviation of intra- and inter-day assays was lower than 10%. The average recovery of MFX from saliva was 101%. MFX concentrations in 48 paired plasma and saliva samples were highly correlated (r^2 = 0.847; $p < 0.001$) (Fig.2). The mean saliva and plasma concentrations were 2.01 and 3.65µg/ml respectively, the saliva to plasma ratio being 0.54. The plasma protein binding of MFX was about 46%.

Fig. 2: **MFX concentration in paired plasma and saliva samples**



Conclusions: A sensitive, specific and validated method for quantitative determination of MFX in saliva was developed. This method has the advantages of being rapid (run time is only 8 minutes) and using a small sample volume (100 microlitres), without any loss of analyte. The use of ofloxacin (OFX) as internal standard helped in monitoring the recovery of MFX from plasma. A good correlation between plasma and saliva concentrations of MFX, suggest that estimation of salivary levels could be used in therapeutic monitoring and pharmacokinetic studies.

(ii) Lamivudine testing in HIV-infected individuals – a retrospective study

(Principal Investigator: Dr. Geetha Ramachandran; Funding: Karnataka Health Promotion Trust)

Background: The *Avahan* project was initiated with the goal of reducing HIV prevalence among high-risk groups, and to stabilize

infection rates among the general population. Cross sectional surveys, termed Integrated Behavioural and Biological Assessments (IBBA) in 5 districts were designed to evaluate the effectiveness of the *Avahan* project in India, by assessing changes in HIV and sexually transmitted infection prevalence over time. Measurement of change in HIV prevalence over time is confounded by recent increase in the use of antiretroviral medications that are known to prolong life and improve the quality of life. The International Evaluation Advisory Group of the *Avahan* project, which met in Mumbai in June 2010, recommended that information on the number of HIV-positive individuals receiving ART can be obtained. Lamivudine could potentially serve as a marker for ART, since it is present in all the fixed dose combination pills that are commonly used by HIV-infected individuals.

Aim: To undertake testing for the presence of lamivudine in stored serum samples collected from HIV-infected individuals, during the course of a survey undertaken in different parts of Karnataka state

Methods: As part of the IBBA and general population surveys, blood samples were collected to test for HIV and other sexually transmitted diseases. Among a total of about 5000 samples collected, 720 tested seropositive for HIV. A total of 627 serum samples were transported to NIRT for lamivudine testing. This was undertaken by HPLC (Shimadzu Corporation, Kyoto, Japan) with UV detection according to a validated method.

Results & Conclusions: Of the 627 samples, results were available for 601 samples. Among these, 335 tested positive for lamivudine (55.7%). This finding suggests that lamivudine testing in serum could be used to check adherence to ART. However, one main limitation of the study was that the blood samples collected from the study participants were untimed; one or two missed doses prior to the day of blood draw could have given a negative result.

Studies in progress:

(i) Predictors and immunologic characterization of TB-associated IRIS in a prospective clinical trial cohort

(Principal Investigator: Dr.G. Narendran / Sudha Subramanyam; Funding Agency: Intramural to India Grant (NIAID), National Institute of Health)

IRIS is the paradoxical worsening or unmasking of infection, tumor-associated pathology, or auto-immune disease in HIV-infected patients following the initiation of ART, and is particularly common in AIDS patients with baseline mycobacterial disease, including TB. However, the pathophysiology of TB-IRIS remains incompletely understood which has hampered efforts at prevention and the use of targeted therapeutic approaches. This is a prospective cohort study nested within a parent TB-treatment trial titled "A randomized controlled clinical trial comparing daily vs. intermittent 6 – month SCC in reducing failures & emergence of ARR in patients with HIV and PTB".

In this study, clinical predictors are evaluated and compared between patients who develop TB-IRIS and those who do not, based on baseline characteristics. Markers of T-cell activation (e.g., PD-1, CD69, intracellular Ki67, co-expression of HLA-DR and CD38) are evaluated for predicting TB-IRIS by comparing values at baseline and during TB-IRIS episodes. The effector responses of CD4 T-cells to TB are studied by longitudinal follow-up of T-cell stimulation assays and a panel of serum cytokines (Th1/Th2/Th17) is measured.

Results: Fifty one patients who were allocated to the RCT were screened; 23 who were ART naïve at baseline and who required ART during TB treatment were included in the IRIS study. Ten of them experienced symptoms and signs suggestive of IRIS confirmed by viral load decline of at least 1log from baseline. The median days from the start of ART to IRIS clinically were 8 days and the median time for resolution of symptoms was 9 days. Six of the 10 patients who experienced IRIS had an extrapulmonary focus in the form of lymphadenopathy. ART was not

withheld in any of the cases. The study is ongoing.

(ii) Pharmacokinetics of anti-TB drugs in children: impact of age, nutritional status and HIV infection

(Principal Investigator: Dr. Geetha Ramachandran; Collaboration: Institute of Child Health, Chennai, Govt. Hospital of Thoracic Medicine, Chennai, Kilpauk Medical College & Hospital, Chennai and Govt. Rajaji Hospital, Madurai; Funding: ICMR Task force on Pediatric HIV)

Background: TB is an important public health problem and among the 10 major causes of mortality in children. In the RNTCP in India, children diagnosed with TB receive first-line anti-TB drugs with dosages based on body weight. Currently recommended dosages of RMP, INH, pyrazinamide (PZA) and ethambutol (EMB) are extrapolated from pharmacokinetic studies performed in adults and have not been adequately evaluated in children. Children exhibit age-related differences in drug pharmacokinetics because of enzyme maturation and other factors. The effect of malnutrition on blood levels of key anti-TB drugs is not well described.

Aim: To study the impact of age, nutritional status and HIV infection on the pharmacokinetics of RMP, INH and PZA in children with TB

Methods: This was a multi-centric study done at four hospitals in south India. Children with TB (group I) or with TB & HIV (group II) formed the study populations. Eighty four HIV uninfected children aged 1 to 12 years with pulmonary or extra-pulmonary TB, on anti-TB treatment for at least 2 weeks were recruited. Assessment of nutritional status was done using z scores calculated from the child's weight & height (CDC). The INH acetylator status was determined using saliva. On the day of the study, blood samples were collected pre-dosing and at 2, 4, 6 and 8 hours after supervised administration of anti-TB medications. Plasma RMP, INH, and PZA were estimated by HPLC and pharmacokinetic variables calcula-ted.

Results: Recruitment of children with TB was completed and results are presented here. Recruitment of HIV-infected children with TB is continuing. So far, 28 such children have been included into the study.

Children aged 1-3 years had significantly lower peak concentration and exposure of RMP, INH and PZA compared to other age groups (Table 7).

Table 7: PK of RMP, INH & PZA among different age groups

Drugs	PK Variables	Age Groups (years)			
		1 – 3 n = 17	**3.1 – 6 n =22**	**6.1 – 9 n = 22**	**9.1 – 12 n = 23**
RMP	Cmax	2.9 ± 1 .4*	5.4 ± 1.6	6.3 ± 2.2	5.8 ± 2.4
	AUC(0-8)	13.6 ± 6.9*	25.5 ± 10.0	29.4 ± 11.3	28.1 ± 12.7
INH	Cmax	3.3 ± 1.2*	6.7 ± 3.4	6.4 ± 2.4	7.5 ± 2.3
	AUC(0-8)	14.0 ± 6.7*	27.5 ± 14.8	22.8 ± 9.0	30.7 ± 11.4
PZA	Cmax	28.4 ± 7.2*	38.6 ± 14.8	42.1 ± 8.6	38.0 ± 10.3
	AUC(0-8)	156.6 ± 41.8*	219.2 ± 66.3	232.5 ± 48.8	220.3 ± 55.8

The proportion of children with sub-therapeutic RMP, INH and PZA were 90%, 12% and 37% respectively. All children < 3 years had sub-therapeutic RMP which was not significant from those who were aged 3 years and above (100% vs. 88%). However, the proportion of children with sub-therapeutic INH (38% vs. 6%) and PZA (88% & 25%) was significantly higher in those < 3 years than those above 3 years of age. Children with stunting and underweight had significantly lower peak concentration and exposure of RMP and PZA than normally nourished children. Multivariable linear regression analysis showed age to be independently associated with peak concentration and exposure of RMP, INH and PZA.

Conclusions: Younger children have lower blood levels of key first-line anti-TB drugs. These findings have important clinical implications and suggest that dose modifications of anti-TB drugs may be needed, especially in young children, in order

to achieve optimal blood levels. However, the blood levels have to be correlated with TB treatment outcomes.

Recruitment of HIV children with TB is ongoing.

(iii) Comparative pharmacokinetics of RMP during daily and intermittent dosing in HIV-TB patients

(Principal Investigator: Dr.A.K. Hemanth Kumar; Collaboration: Govt. Hospital of Thoracic Medicine, Chennai)

Background: TB is the most common opportunistic infection in patients with HIV infection worldwide. The use of RMP is pivotal for the effective control of TB. It has been reported that intermittent RMP therapy in HIV-infected patients significantly increases the risk of acquired RMP resistance among patients who fail. Sub-therapeutic RMP concentrations in blood have been associated with poor clinical response.

Aim: To study the pharmacokinetics of RMP in HIV-TB patients who are receiving daily and intermittent anti-TB regimens

Methods: The pharmacokinetic study is conducted in a sub-set of 48 patients (24 each in the daily and intermittent dosing arms) who are taking part in a randomized controlled clinical trial that will compare daily vs. intermittent 6 – month SCC in patients with HIV and PTB. Eligible subjects are identified by the Study investigators. On the day of the study, blood samples are collected at 0, 1, 2, 6, 8, 12 and 24 hours after dosing. Plasma concentrations of RMP are estimated by HPLC according to a validated method.

Pharmacokinetic study of RMP will be undertaken in those patients who fail anti-TB treatment. So far, 13 patients (10 daily & 3 intermittent) have been admitted to the study.

The study is in progress.

(iv) Development of a simple and accurate method for estimation of plasma rifabutin by high performance liquid chromatography

(Principal Investigator: Dr. Geetha Ramachandran)

Concomitant use of rifabutin (RBT) with PI-based antiretroviral therapy has been reported to lead to successful treatment outcomes. However, ritonavir (RTV), being a CYP3A4 inhibitor markedly increases serum concentrations and toxicity of RBT. It therefore becomes necessary to decrease the dose of RBT when co-administered with RTV. There have been conflicting reports regarding the dose of RBT during concomitant RTV administration. RBT has become available in India and is recommended for use in HIV-infected TB patients who receive second-line ART regimens containing LPV/ RTV. The dose of RBT is reduced by 50% in patients receiving concomitant LPV/RTV. There is not much pharmacokinetic data available to support this reduction. It has been planned to undertake pharmacokinetic studies to decide the appropriate dose of RBT to be used during concomitant administration of LPV/RTV.

Prior to undertaking the pharmacokinetic study, we are standardizing a simple and accurate method to estimate plasma RBT by high performance liquid chromatography **(**HPLC). Experiments are in progress to establish certain method validation parameters such as sensitivity, specificity, precision, accuracy, linearity, inter- & intra - day variations and recovery.

(v) Molecular characterization of the envelope gene and co-receptor usage of Indian HIV-1 subtype C isolates

(Principal Investigator: Dr. Luke Elizabeth Hanna)

Molecular determinants of co-receptor usage are known to be present on the HIV envelope. Changes in cellular tropism by HIV-1 *in vivo* seems to be a key event in disease pathogenesis, and broadening of the co-receptor usage profile of HIV-1 may be associated with accelerated CD4 T-cell loss and disease progression to AIDS. The

present study attempts to identify pertinent amino acid differences in the envelope gene of CCR5, CXCR4 and dual tropic phenotypes with a view to identify genetic factors responsible for expanded co-receptor usage in HIV-1 subtype C isolates circulating in the Indian population. Nucleotide sequencing of the HIV-1 subtype C env and analysis of the predicted envelope will provide information on the relationship between immunologically relevant sites and the impact of viral diversity on these sites, which will have important implications for both vaccine and drug development studies.

Thirty clinical isolates have been co-cultured and typed for co-receptor usage by means of a phenotypic assay using U87.CD4 cell lines expressing either CXCR4 or CCR5. Full length envelope genes of the viruses were sequenced and subjected to preliminary bioinformatics analysis. The study is ongoing.

(vi) Prevalence and incidence of sexually transmitted infections among men who have sex with men

(Principal Investigator: Dr. Beena E Thomas / Dr. Luke Elizabeth Hanna)

NACO has estimated the prevalence of HIV among men who have sex with men (MSM) as 6.4%. HIV prevention efforts among this group include screening and care for sexually transmitted infections (STIs). We studied the prevalence and incidence of STIs in a cohort of MSM in Chennai, south India. This study is nested within an intervention study "Addressing the psychosocial needs and HIV risk among MSM". Ninety six MSMs were recruited for this study. Serum samples were tested for HIV and *Treponema pallidum* (TP) infection. Urine and rectal swab specimens were tested for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhea* (NG) infection at baseline and 6 months. At baseline, 8.3% (8/96) tested positive for HIV and no added HIV infection was reported at the 6th month visit. At baseline, 19% (18/96) tested positive for STI. At the 6th month follow up, 23% (19/82) tested

positive for STI with 5 recurrent STIs. Our findings highlight the need for ongoing monitoring and treatment of STIs among MSMs. Early diagnosis and treatment of STIs is an important HIV prevention activity.

(vii) Screening of anti-HIV activity of transitmycin

(Principal Investigator: Dr.Luke Elizabeth Hanna)

A novel brominated compound isolated from an actinomycete obtained from the coral reef ecosystem in Rameshwaram showing anti-mycobacterial activity was evaluated for its anti-HIV activity. The compound demonstrated antiviral activity on a laboratory adapted strain of HIV-1 (HIV-1 IIIB). The activity of this compound on different subtypes of HIV (subtypes A, B, C, D, A/C, A/E, as well as a NVP-resistant and zidovudine-resistant strains) was also ascertained. The compound was found to have an inhibitory effect on all the subtypes of HIV tested including the resistant forms. Studies have been planned to investigate the mechanism of action of transitmycin.

(viii) Characterization of neutralization specificities of sera from HIV-1 patients in south India

(Principal Investigator: Dr. Luke Elizabeth Hanna)

The few neutralizing antibodies isolated so far from HIV-infected persons are believed to have limited inhibitory activity on HIV-1 subtype C. Identification of cross-reactive neutralizing antibodies that can neutralize HIV-1C and most other circulating virus strains will have the potential to protect against HIV infection and thus be of great value in the design of a vaccine for HIV. The aim of the present study was to determine the incidence of similar or yet to be uncharacterized specificities in HIV-1 subtype C infected individuals. Seventy patients have been recruited for the study till date. The study is ongoing.

Socio-behavioral studies:

Completed studies:

(i) Alcohol use disorders among TB patients: A study from Chennai, south India

(Principal Investigator: Dr. Mohanarani Suhadev)

Summary: Alcohol Use Disorders (AUDs) among TB patients are associated with non adherence and poor treatment outcomes. This study was done to estimate prevalence of AUD among TB patients using the Alcohol use disorder identification test (AUDIT) scale and their perceptions of a feasible intervention programme. This was a cross-sectional cohort study conducted in four zones which were randomly selected from 10 corporation zones in Chennai. It included situational assessment followed by screening of TB patients using an AUDIT scale developed by WHO. All TB patients treated during July to September 2009 were screened with AUDIT scale for alcohol consumption.

Results: Out of 490 patients, 66% were males, 66% were 35 years and above, 57% were married, 58% were from the low monthly income group of < Rs 5000 per month. No females reported alcohol use. Out of 490 TB patients, 29% (141) were found to consume alcohol. Among 141 current drinkers, 52% (73) had an AUDIT score of >8. The factors significantly influencing AUD were age (>35 years), education (less educated), income (<Rs 5000 per month), marital status (separated/ divorced) and treatment category (Category 2). The qualitative findings of this study stressed the need for an intervention program. This was also expressed both by patients and the health providers who were not equipped to deal with patients with AUD. The findings of this study endorse the need for alcohol intervention strategies to be integrated into the TB control program.

(ii) Community based approach in designing an AIDS program for mothers living with HIV

(Principal Investigator: Dr. Beena E. Thomas; Indo-US collaboration- TRC-ICMR/UCLA University of California, Los Angeles)

The purpose of this qualitative study was to explore a feasible intervention based on the perceptions and needs of mothers living with HIV (MLH). A qualitative study was done with 60 MLHs who were recruited from the sites with the help of counselors, medical social workers and MLH themselves.

A total of 11 focus group discussions were conducted, lasting 60– 75 min, with 60 MLHs (3 to 7 women in an group). These discussions were conducted by a medical social worker and a psychologist who were well trained in methods of qualitative focus group design. The topic guide was used for each focus group. All discussions were tape recorded with permission of the MLH. During the focus group sessions, the facilitators also captured observations such as nonverbal interactions, gestures and emotional content of the women by means of field notes.

Interim findings:

Five general themes that have come out from the discussions thus far are:

1. Health care needs of MLH
2. Sites they seek care
3. Disclosure issues
4. Stigma
5. Strategies for improving access to health care

This study has been innovative in that the MLHs are often not addressed in HIV prevention and intervention programs. The study has provided multidimensional portrayal of the impact of HIV/AIDS on MLH. The salient finding was the stigma experienced from health care providers. The study also covered their perceptions of a feasible intervention program and the focus areas for the intervention.

We also plan to have individual interviews with 40 MLHs. Furthermore with these interim findings we are planning to develop an intervention manual covering these topics in detail separately. This manual will be pilot tested with MLH to test the feasibility and impact on

MLH to have a better quality of life. We are also in the process of The study is ongoing.

producing a short documentary film based on the study findings.

Studies in progress:

Addressing psychosocial needs and HIV risk in Indian MSM in India (Principal Investigator: Dr. Beena E. Thomas, Source of Funding- Indo-US Joint Working group Collaborators: Harvard Medical School/MGH and Fenway Community Health)

MSM in India are a marginalized population in need of evidence based HIV prevention efforts. MSMs are considered a “bridge" population. Our initial study on the Behavioral risk factors among 210 MSMs has provided a background that is relevant to the conduct of the present study. The salient finding of the study was that more than three quarters of the respondents remained unreached in any HIV prevention intervention program. Findings from the qualitative part of the study reflected that MSM were saturated with messages on HIV/AIDS and condom use and felt that there was dearth of an intervention program to address their psychosocial concerns. The innovativeness of this study was that it explored the possibility of providing an intervention that targets psychosocial problems concurrent with HIV risk reduction behaviors among the MSM population.

Significance and Outcome

This study is different from the usual intervention programs that concentrate on HIV prevention through HIV/STI messages, condom distribution and HIV testing programs. The findings of this study would help to provide an innovative intervention model addressing the psychosocial needs of MSM and thereby work towards sexual risk reduction sexually transmitted infections (STI) and HIV/AIDS prevention and control.

Progress of the study:

Phase 1–Intervention Development: During phase 1, the team collected additional formative data

from 59 MSMs to inform the content and scope of an intervention manual and the subsequent pilot behavioral intervention. They included 9 Key informants (e.g., NGO leaders of MSM groups) and 5 MSMs focus group discussions with 10 participants in each group (50 MSMs) from August 2009 – November 2009. They provided input about the intervention's perceived effectiveness, participant acceptability, feasibility of delivery, and strategies to recruit a diverse and representative sample.

Based on phase-1 findings, training of staff on intervention was done. The findings of the study helped to prepare the guide for the open pilot intervention.

Phase- 2-Open Pilot:

Following Phase 1, the team conducted an open-phase non-randomized pilot of the intervention. The open pilot took place from December 2009 to January 2010. Seventeen participants were screened, 10 were eligible and enrolled in the study after obtaining their informed consent.

Each participant attended 6 facilitated group sessions and 3 individual counseling sessions over 3 months. Immediately following completion of all sessions, participants again completed an interviewer-administered questionnaire, and during the second 3-month follow-up, participants completed a final assessment with an interviewer-administered questionnaire, HIV counseling and testing, and STI testing. The group was open to participants who were both HIV-infected and uninfected and of any MSM identity, unless Phase 1 findings indicated otherwise. Participants were asked to provide extensive feedback during exit interviews regarding the usefulness of the format, content, and group logistics.

Based on the findings from the open pilot we have modified the format of the interventions. Each participant goes through a base line interview, HIV/STI testing, 4 group sessions and 4 individual sessions, 3rd monthly and 6th monthly follow up sessions. We have shortened the group sessions from 6 to 4 sessions

and increased the individual sessions from 3 to 4 sessions based on the experiences from the pilot group sessions.

We are in the process of screening participants for Phase 3.

Department of Bacteriology

Completed studies:

(i) Luciferase reporter phage assay for the detection of *M. tuberculosis* using phage lysin to control the overgrowth of normal flora in processed sputum samples

(Principal Investigator: Dr. Vanaja Kumar)

Background: Use of phage lysin as a substitute for antibiotics to control the overgrowth of normal flora in processed sputum samples has been established. Phage lysin was evaluated in comparison with antimicrobial supplement PANTA in sputum samples processed by modified Petroff's method for the detection of *M. tuberculosis* using BACTEC MGIT 960 system.

Aim: To evaluate phage lysin to control the overgrowth of normal flora in processed sputum samples for the sensitive detection of *M. tuberculosis* using luciferase reporter phage (LRP) assay

Methods: A total of 129 sputum samples were processed by modified Petroff's method. Two Lowenstein Jensen (LJ) slopes were inoculated from the processed sputum deposit thus obtained. The remaining deposits were transferred to 7 ml of Middlebrook 7H9 complete medium supplemented with phage lysin and incubated at 37^{o}C. LRP was done using phAE129 at days 7, 9, 14 and 21. At the end of day 21, the samples were centrifuged and the pellets were inoculated on to 2 more LJ slopes to cross check LRP results.

Results: The sensitivity and specificity of diagnostic LRP assay (DLRPA) in detecting *M. tuberculosis* from sputum specimens was 90% and 81% respectively compared to conventional LJ culture. The agreement between the methods was 87% (Table 8). The average time to detection for detecting culture negatives, few-colony-growth, 1+, 2+ and 3+ by DLRPA was 19, 19, 15.45,

10.88 and 8.14 days respectively with a median time to detection (TTD) of 14.5 days (Table 9). The rate of contamination for LRP using phage lysin was 9.3%.

Conclusion: Phage lysin can be used to decontaminate processed sputum samples for the detection of *M. tuberculosis* using LRP assay.

Table 8: Comparison of DLRPA with Petroff's culture

		Petroff's culture		
		Pos	Neg	Total
DLRPA	Pos	63	8	71
	Neg	7	34	41
	Total	70	42	112
Sensitivity 90%; Speficifity 81%; Agreement 87%, PPV 89%; NPV 83%				

Pos: Positive; Neg: Negative; PPV: Positive predictive value; NPV: Negative predictive value

Table 9: **DLRPA for the detection of *M. tuberculosis* from sputum samples**

Culture grade	No. of samples	LRP		Early time of detection (days)				Average TTD (in days)	
		Pos (%)	Neg (Cont)	7	9	14	21	LRP	LJ
Negatives	48	8 (16.6)	34 (6)	0	1	1	6	19	NA
Colonies	9	6 (66.6)	3	1	0	0	5	19	38.5
1+	13	11 (84.6)	2	3	1	1	6	15.45	29.27
2+	26	24 (92.3)	2	8	8	5	3	10.88	20.41
3+	22	22 (100)	0	17	2	3	0	8.14	14
NTM	10	0	5 (5)	0	0	0	0	NA	23.1
Contamination	1	0	0(1)	0	0	0	0	NA	NA
Total	129	71	58						

Pos: Positives; Neg: Negatives; Cont: Contaminated; NTM: Non-tuberculous mycobacteria; TTD: Time-to-detection; NA: Not applicable; LJ: Petroff's culture

(ii) Mycobacteriophage genome database

(Principal Investigator: Dr. Vanaja Kumar)

Background: Mycobacteriophage genome database (MGDB) is an exclusive repository of the 64 completely sequenced mycobacteriophages with annotated information. It is a comprehensive compilation of the various gene parameters captured from several databases pooled together to empower mycobacteriophage researchers which was developed by the department. The MGDB (Version No.1.0) comprises of 6086 genes from 64 mycobacteriophages classified into 72 families based on ACLAME database.

Aims: (i) to collect and organize the complexity inherent to mycobacterio-

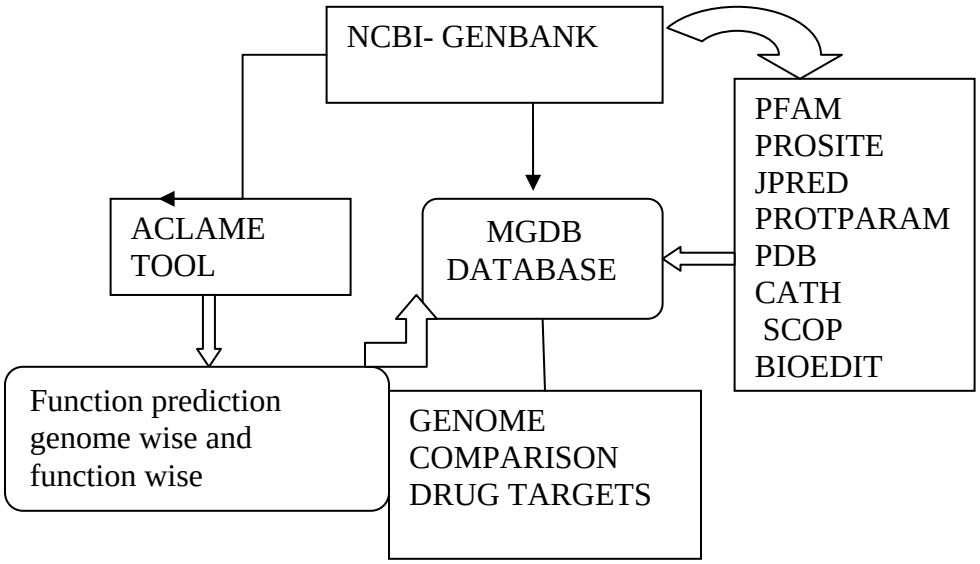
phage protein classification in a rational way and

(ii) to browse the existing and new genomes and describe their functional annotation

Methodology:

We performed the manual curation which was aided by information available from public databases. ACLAME software package was used for comparative genomic analysis within the 64 mycobacteriophages. This was enriched further by analysis (Fig.3). Its web interface allows browsing as well as querying the classification (Fig.4).

Fig.3: **Data flow diagram for MGDB**



Database Statistics:

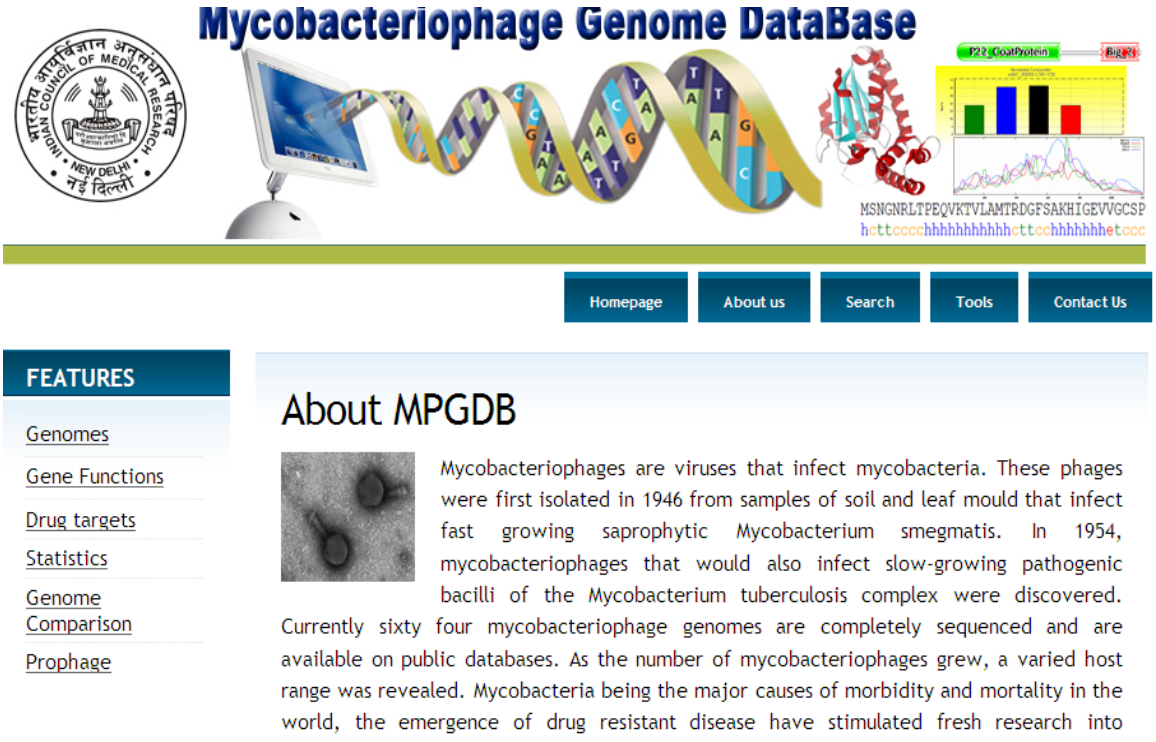
The data mining and genome annotation has been completed for all the 64 mycobacteriophages for which sequence details are available in the public domain and linked to the database. This includes 7325 protein sequences and their functions.

Results and Conclusion:

List of the data types can be enumerated as genome, protein and

structural details. It consists of about 25 parameters for each gene. They were classified into 72 functional families and incorporated into the database. These were further broadly classified and clustered into 8 groups.

Fig.4: **The screen shot of the home page of the database**



[Contact person: Dr. Vanaja Kumar (E-Mail ID: vanajakumar@trcchennai.in)]

(iii) Performance of GenoType MTBDR*plus* assay in a 'non-exclusive facility'

(Principal Investigator: Dr. Vanaja Kumar)

Background: GenoType MTBDR-*plus* assay developed by Hain's Laboratories, Germany has been recommended by World Health Organization for rapid detection of MDR-TB. An 'exclusive facility' is being established in many laboratories for performing the assay. The current work studies the performance of the test in a 'non-exclusive facility' in molecular biology.

Aim: To study the performance of the GenoType MTBDR*plus* assay in a 'non exclusive facility'

Method: A panel of 113 cultures with known resistance pattern obtained by conventional DST were selected and coded. The panel consisted of 41 MDR and 72 non-MDR. Thirteen of these cultures were duplicates. DNA from these cultures was extracted by boiling method. The assay was performed as per manufacturer's protocol. The 'non exclusive facility' included an air-conditioned room with a common clean hood for master mix preparation in one corner and a thermocycler for amplification in another. Hybridization was done in another common air-conditioned facility. Results were decoded and compared with the reference results.

Results: The GenoType MTBDR*plus* assay identified 35 (85%) of the 41 MDR strains. Among 72 non-MDR strains, false resistance to RMP was reported in 3 strains. Agreement between the assay and the gold standard was 92%. Reproducibility of the assay was 100%.

Conclusion: The GenoType MTBDR*plus* assay performed in a non-exclusive facility has exhibited good correlation (92%) with the conventional assay. The study suggests that a non-exclusive facility may be further explored for performing the test.

(iv) Recovery of *M. tuberculosis* from Lowenstein-Jensen media contaminated with other organisms

(Principal Investigator: Dr. Vanaja Kumar)

Background: Growth of contaminating organisms along with *M. tuberculosis* on LJ medium is common. However, there is no documented evidence on the decontamination procedure adopted in mycobacteriology laboratories to recover *M. tuberculosis* from the contaminants grown on LJ medium. Cetrimide, was explored to recover *M. tuberculosis* from the contaminated LJ slopes.

Aim: To retrieve *M. tuberculosis* from LJ medium contaminated with other organisms using cetrimide

Methods: A total of 1,048 LJ slopes with *M. tuberculosis* were received at NIRT from four states (Andhra Pradesh, Gujarat, Kerala and Tamil Nadu) between July 2009 and March 2011 through courier service. The LJ slopes were incubated over night at 37^{o}C to check for the presence of any contaminating organisms. The contaminated LJ slopes were subjected to decontamination procedure by 1% cetrimide method.

Results: Of the 1,048 LJ slopes with *M. tuberculosis* received from intermediate reference laboratories, 98 (9%) were found contaminated. Of these 98, 87 (89%) *M. tuberculosis* cultures were retrieved after decontamination with 1 % cetrimide.

Conclusion: In this study, significant proportion (89%) of cultures was retrieved from contaminated LJ slopes. The advantages of this method are that reagent is inexpensive and shelf life at room temperature is long. It is also cost saving in avoiding processing of additional samples or recalling the patients for repeat examination. The 1% cetrimide method is being practised over a decade in NIRT to recover *M. tuberculosis* from LJ slopes contaminated with other organisms. This method is easily adaptable and similar to the one used to sub- culture *M. tuberculosis* on LJ medium.

RNTCP activities as supra national reference laboratory
(Contact person: Dr.N. Selvakumar)

The NIRT is one of the National Reference Laboratories (NRL) working closely with intermediate reference laboratories (IRLs) to monitor RNTCP activities in India. National referemce laboratory (NRL) microbiologists and laboratory supervisors/technicians visit each state at least once a year for 2 to 3 days as a part of onsite evaluation (OSE) under the RNTCP external quality assurance (EQA) protocol. Eight states and 5 union territories viz. Andhra Pradesh, Chhattisgarh,

Goa, Gujarat, Dadra Nagar Haveli, Daman & Diu), Kerala, Lakshadweep, Sikkim, Tamil Nadu, Andaman & Nicobar, Punjab and Puducherry are being supervised. The institute is monitoring EQA in sputum smear microscopy for 150 districts in total and culture & DST activities in 10 IRLs and 5 private laboratories. During OSE visit, NRL microbiologists provide technical support for establishing quality assured smear microscopy, culture & DST services including facility design for introduction of newer diagnostic tools such as liquid culture and molecular tests for rapid diagnosis of MDR-TB. NRL also undertakes periodic proficiency testing for IRLs as part of the accreditation process for culture & DST under RNTCP. Till date, 5 IRLs viz. Gujarat, Andhra Pradesh, Kerala, Tamil Nadu and Puducherry have been accredited and all these labs are already undertaking culture & DST activity for MDR-TB patients. Five private laboratories such as Blue Peter Health Research Centre, Hyderabad, PD-Hinduja Hospital Mumbai, Christian Medical College (CMC) Vellore, Regional Medical Research Centre (RMRCT) for Tribals (ICMR) – Jabalpur, Damien Foundation of India Trust (DFIT) –Nellore are also accredited to perform culture & DST for MDR-TB services. Accreditation is initiated in 16 other laboratories and 3 of them were visited for pre-accreditation assessment. Intermediate reference laboratories in Gujarat and Andhra Pradesh are in the process of accreditation for liquid culture as well as for second line DST. During 2010-2011, the institute conducted second round of proficiency testing in 7 laboratories for panel of 30 cultures for susceptibility testing for anti-TB drugs namely INH, RMP, EMB and streptomycin. Retesting of cultures for first line drugs for accreditation was done in eight laboratories including one IRL and 6 private laboratories. As a NRL, the institute is supporting second line drug DST for MDR-TB patients. During this period a total of 897 cultures were received from different states and processed. Four states were visited for OSE of sputum microscopy and 420 panel slides were used to

assess 84 laboratory personnel. The institute is conducting periodical training programmes for culture & DST of *M. tuberculosis*. A total of 204 microbiologists / laboratory techni-cians from 31 different national and international institutes were trained. Electronic system (e-PROCULTB) was developed for monitoring proficiency test results of DST of *M. tuberculosis* and this system was gifted to other three NRLs in India as requested by RNTCP.

Department of Immunology

Completed studies:

(i) Identification of immunoreactive T-cell antigens of *M. tuberculosis* through proteomic techniques

(Principal Investigator: Dr. Alamelu Raja)

Background: During our efforts to define immunodominant T-cell antigens from the secreted proteome of *M. tuberculosis*, it was identified that out of 350 2D-liquid phase electrophoresis (2D-LPE) fractions, 10 were specifically recognized by healthy contacts alone. Proteomic analysis revealed that 16 proteins were present in these 10 "contact specific" (CS) fractions. In our previously reported studies, IFN-γ was measured as a marker of recognition. In the present study, other biomarkers were also measured in the antigen stimulated supernatants.

Aim: To understand the immune response induced by the CS fractions and to identify the additional biomarkers which will be helpful in the differentiation of healthy house hold contacts (HHC) from TB patients

Methods: The levels of cytokine produced by whole blood culture supernatants were measured using a Bioplex multiplex cytokine assay system by following manufacturer's instructions. Twenty four cytokines analysed were IL-1β, IL-1Ra, IL-2, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (P70), IL-13, IL-15, IL-17, Eotaxin, FGF basic, G-CSF, GM-CSF, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF, RANTES and VEGF.

Results: In the unstimulated cultures the level of IL-1Ra was significantly higher in the TB group compared to the HHC group. Two contact specific fractions (9-24 and 11-24) induced significantly higher IL-6 levels in contacts, compared to the TB group. Fraction 11-24 induced significantly higher level of five cytokines (G-CSF, IL-7, IL-8, IL-9 and PGDF) in TB patients compared to HHC. Other immune parameters measured in this study (IL-1β, IL-2, IL-5, IL-10, IL-12 (P70), IL-13, IL-15, IL-17, Eotaxin, FGF basic, GM-CSF, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, VEGF) were not significantly different between the HHC and TB groups (Figs.5a & b).

Conclusion: Upon stimulation with CS fractions, 6 cytokines (IL-1Ra, G-CSF, IL-6, IL-8, IL-9 and PGDF) levels were differentially expressed between HHC and TB. Further studies in this direction may help to identify the potential bio markers for the differentiation of HHC from TB patients.

Fig.5a: Concentration of IL-1RA in nil antigen cultures

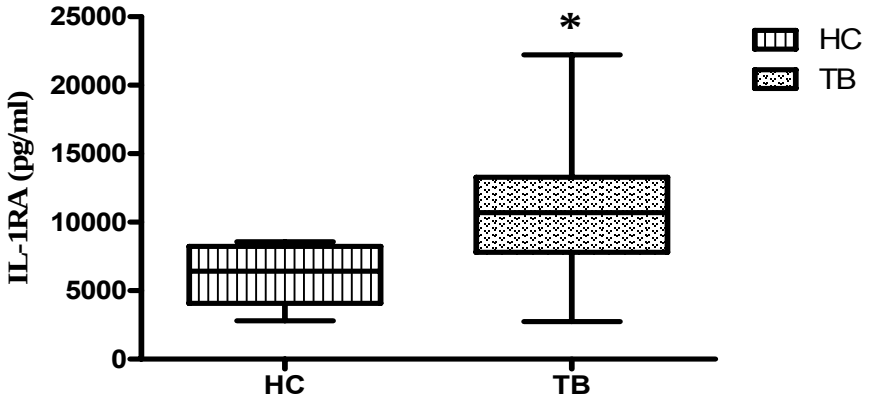
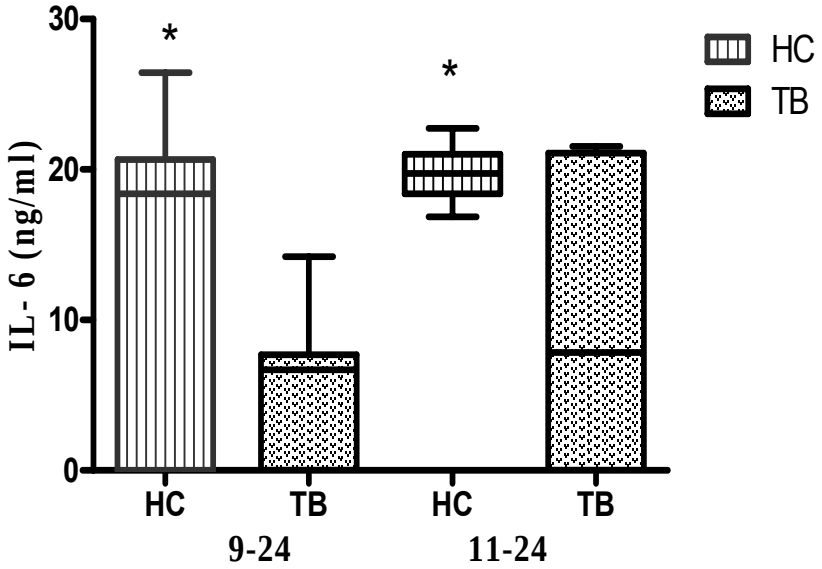


Fig.5b: IL-6 level after CS fraction 9-24 and 11-24 stimulation



* refers to significant value ($p<0.05$) when compared with TB,

- Statistical analysis was performed using unpaired t-test.
- 9-24 and 11-24 - two contact specific fractions
- Vertical bars denote standard error of mean

(ii) Cytotoxic cell response in *M. tuberculosis* infection

(Principal Investigator: Dr. Alamelu Raja)

Background: The proteins, early secreted antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10), are reported as potent T-cell antigens. The region encoding these proteins is referred to as region of deletion-1 (RD1) which is present in *M. tuberculosis*, but not in *M. bovis* BCG. Although implicated in virulence, they also have a role in affording protection against TB. Screening minimal putative epitopes in these proteins may aid using them in diagnostics or vaccine design strategies.

Aim: To analyse the cytokine and chemokine response to the selected ESAT-6 and CFP-10 peptides by flow cytometry

Methods: Cytokine [tumor necrosis factor (TNF)-α, interleukin-2 (IL-2), IL-4] and chemokine [regulated upon activation normal T-cell expressed and secreted (RANTES), monocyte chemoattractant protein (MCP) -1] responses to selected peptides were studied in HHC and patients with PTB.

Results:

ESAT-6 peptides:

CD4: It was observed that Th1 cytokines, TNF-α positive CD4 T-cells were elevated significantly ($p<0.05$) in response to the peptides Esp1, Esp6 in HHC, as compared to TB (Fig.6a). Cytokine IL-2 and chemokine RANTES levels were not different between the two groups. Th2 cytokine IL-4 positive T-cells were enhanced by Esp1 and Esp6 in PTB (Fig.6b).

CD8: Esp1 and Esp6 peptides significantly ($p<0.05$) elevated number of TNF-α+ CD8+ cells in HHC, when compared to PTB. Similar results were obtained with IL-2+ CD8+ cells too. Esp6 showed a significant increase ($p<0.05$) in IL-4+CD8+ cells in PTB than HHC. A higher response for the chemokine RANTES was found for Esp1 and ESAT-6 ($p<0.05$) in HHC as compared to PTB (Table 10).

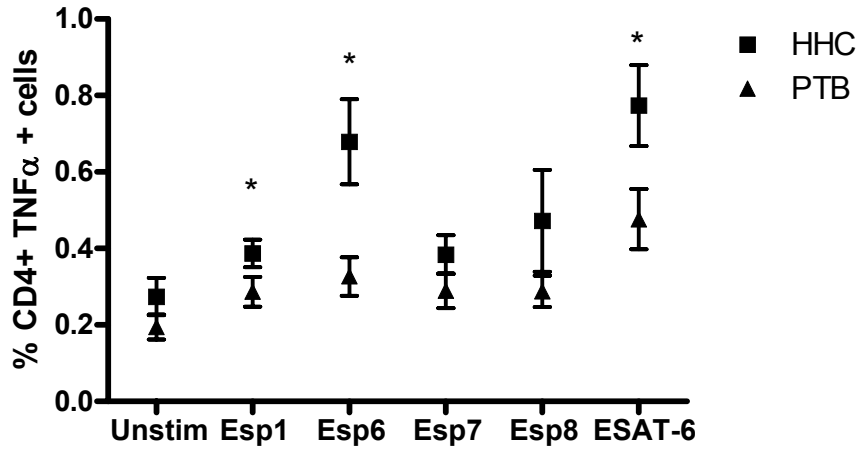
CFP-10 peptides: The peptide Cfp8 significantly increased ($p<0.05$) IL-2 positivity in HHC in comparison to PTB. The peptides Cfp6 ($p<0.05$) and Cfp8 ($p<0.01$) elicited an increase in TNF-α positive cells in HHC compared to PTB. No significant difference was observed in response between groups, for the peptides, with respect to another Th1

cytokine TNF-α, Th2 cytokine IL-4 or chemokine RANTES.

MCP-1 positive monocytes increased in response to the peptides Esp1, Esp6, Cfp8, and Cfp9 in PTB compared to HHC (Table 11).

Conclusion: Because of their role in proliferation and cytokine secretion, and also *in silico* predictions, these peptides deserve attention for further immune studies.

Fig.6a: **CD4+TNF-α+ cell responses to ESAT-6 peptides**

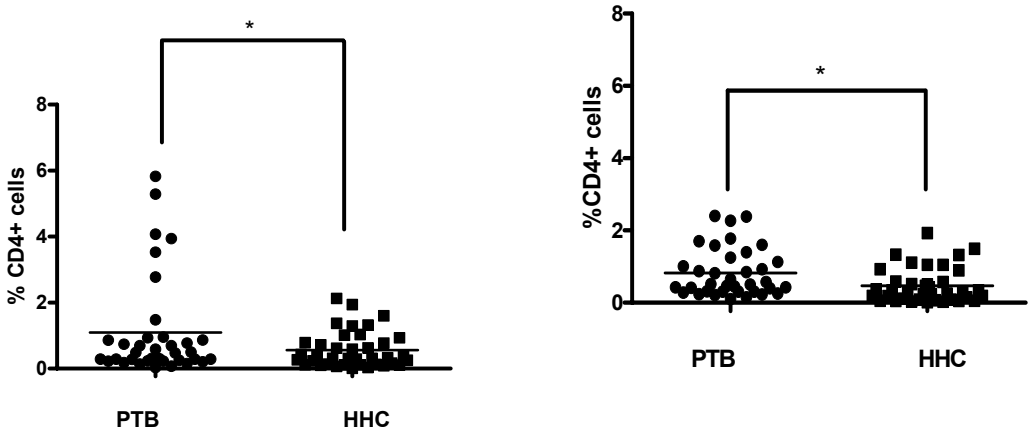


Percentage of TNF-α positive CD4+ cells were ascertained by flowcytometry. Each filed square or triangle refers to the mean ± standard error of mean of a particular group for a stimulus.

Statistical comparison was made between PTB and HHC groups by Mann-Whitney test.

A *p* value <0.05 was considered significant and represented by the symbol *.
HHC – Healthy household contacts
PTB – Pulmonary TB patients.

Fig.6b: **CD4+ IL-4+ cell responses to ESAT-6 peptides**



Percentage of IL-4 positive CD4+ cells were ascertained by flow cytometry. Each filled circle or square represents an individual.
Statistical comparison was made between PTB and HHC groups by Student's t test. A p value of <0.05 was considered significant and represented by the symbol *.
HHC – Healthy household contacts
PTB – Pulmonary TB patients.

Table 10: **Cytokine and chemokine responses in CD8+ cells to ESAT-6**

Stimulants	Groups	% of Positive cells			
		TNF-α	IL-2	IL-4	RANTES
Control	HHC	0.22±0.09	0.19±0.03	0.38±0.05	0.21±0.05
	PTB	0.14±0.03	0.25±0.08	0.65±0.08	0.23±0.05
Esp1	HHC	**0.64 ±0.10***	**0.78±0.11***	0.77±0.10	**0.60±0.08***
	PTB	0.37±0.06	0.41±0.09	0.57±0.08	0.34±0.04
Esp6	HHC	**1.15±0.38***	0.56±0.19	0.56±0.08	0.53±0.18
	PTB	0.35±0.07	0.34±0.06	**0.82±0.10***	0.36±0.07
Esp7	HHC	0.47±0.08	0.53±0.08	0.66±0.14	0.37±0.08
	PTB	0.39±0.07	0.40±0.08	0.54±0.08	0.39±0.08
Esp8	HHC	0.57±0.13	0.58±0.16	0.60±0.11	0.46±0.11
	PTB	0.40±0.07	0.48±0.15	0.53±0.06	0.31±0.04
ESAT-6	HHC	**0.88±0.13***	**0.80±0.09***	1.20±0.21	**1.00±0.22***
	PTB	0.55±0.13	0.46±0.10	1.01±0.12	0.40±0.08

Table 11: **MCP-1 responses in CD14+ cells to ESAT-6 and CFP-10**

Stimulants	% of Positive cells	
	HHC	PTB
Control	0.07±0.02	0.08±0.02
Esp1	0.58±0.18	**0.69±0.35***
Esp6	0.56±0.15	**1.12±0.14***
Esp7	0.58±0.27	0.63±0.24
Esp8	0.40±0.16	1.12±0.60
Cfp6	0.26±0.09	1.04±0.81
Cfp7	0.56±0.32	0.97±0.90
Cfp8	0.58±0.23	**1.37±0.28***
Cfp9	0.23±0.08	**1.35±0.36***
ESAT-6	0.88±0.16	**3.19±0.53****
CFP-10	0.58±0.35	**2.43±0.63****

Percentage of MCP-1 positive CD14+ cells were ascertained by flow cytometry.

Statistical comparison was made between the groups by Mann-Whitney test.
A value <0.05 was considered significant. *p<0.05, **p<0.01

HHC – Healthy household contacts; PTB – Pulmonary TB patients.
MCP-1 – Monocyte chemoattractant protein -1

(iii) Innate immunity in HIV infection

(Principal Investigator: Dr. Alamelu Raja)

Background: Chemokines are small multi-functional proteins that bind to G protein-coupled receptors on target cells and are involved in leukocyte trafficking and homing, organ development, angiogenesis, tumorigenesis and metastasis, as well as in immune responses to microbial infection including

HIV. The orderly recruitment of immune cells to inflammatory or infected sites during TB or HIV infection is critical and is mediated through the secreted chemokines and their receptors. The molecular basis of NK cell chemokine response and its subsequent recruitment is still being elucidated.

Aim: To investigate the effect of IL-15+IL-12 stimulation on the NK cell chemokine response and chemokine receptor expression among HIV-infected patients with/without TB

Method: The study included normal healthy subjects, PTB patients, HIV-infected individuals and patients with HIV and TB co-infection. The expression of CC-chemokines (MIP-1α, MIP-1β, and RANTES) and chemokine receptors (CCR1, CCR4, CCR5 and CXCR4) on NK subsets was measured by flow cytometry.

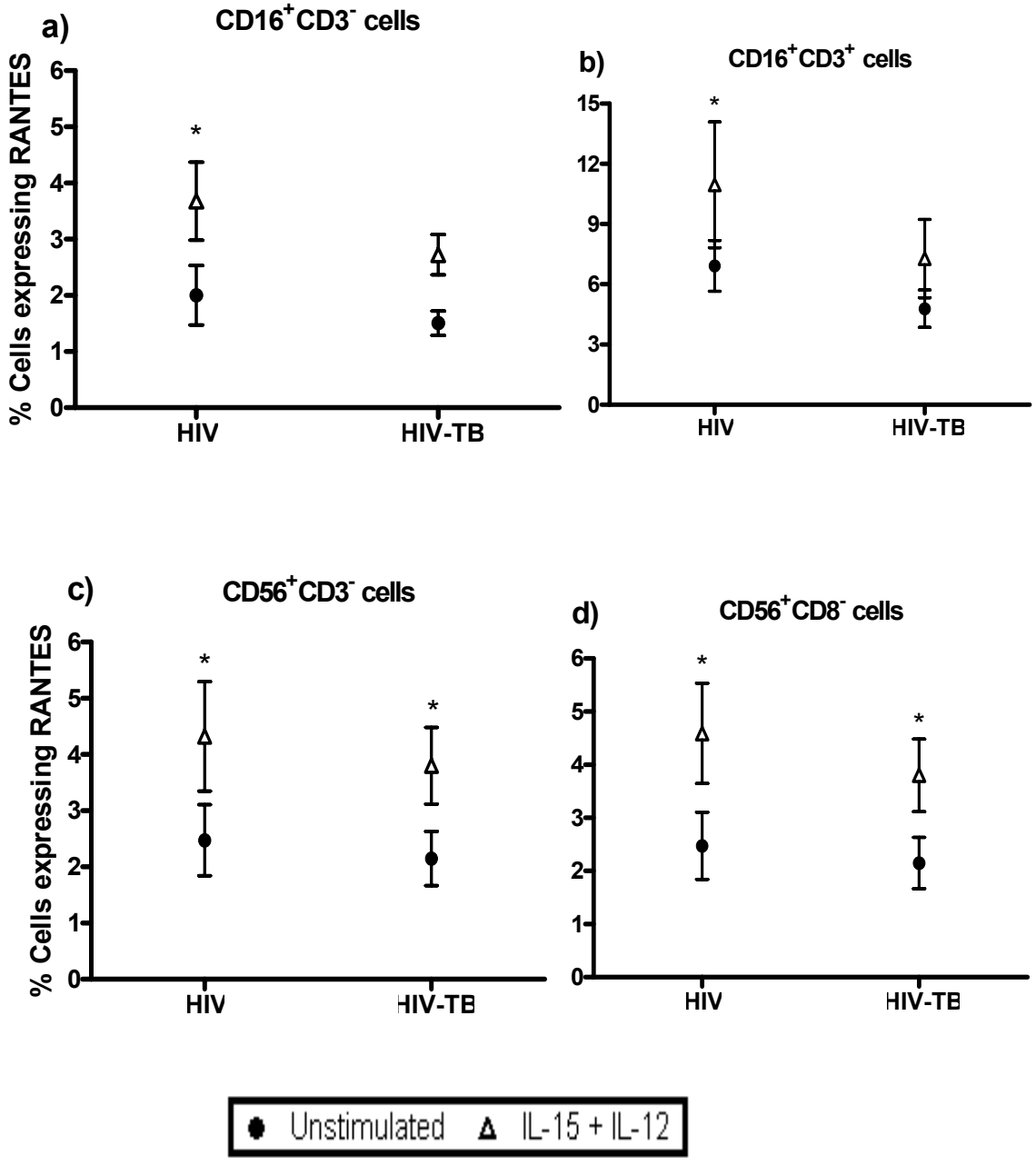
Results: The CD56+CD3- NK subset showed elevated (p<0.05) basal RANTES and MIP-1β in HIV (Table 12), but only MIP-1β in HIV-TB. IL-15+IL-12 stimulation enhanced the chemokines in HIV, notably for CD16+CD3- and CD56+CD3- subsets (Fig.7). But in HIV-TB, RANTES was elevated on CD56+ cells only. MIP-1α and MIP-1β also showed exactly similar results.

Table 12: Basal expression of chemokines by NK cell subpopulation

NK cell subpopulation	HIV	HIV-TB
RANTES		
CD16+CD3-	2.0 ± 0.1	1.5 ± 0.1
CD56+CD3-	**2.5 ± 0.2***	2.2 ± 0.2
MIP-1α		1.6 ± 0.1
CD16 +CD3-	3.4 ± 0.2	2.0 ± 0.1
CD56+CD3-	4.0 ± 0.1	
MIP-1β		
CD16+CD3-	4.0 ± 0.1	3.6 ± 0.2
CD56+CD3-	5.3 ± 0.2*	5.1 ± 0.3*

Data are presented as percentage of mean ± SEM. Statistical analysis performed between NHS and other groups was carried out using one-way ANOVA followed by Tukey's multiple comparison test. A p value of <0.05 was considered significant which is represented by *.

Fig.7: Effect of IL-15 + IL-12 stimulation on the expression of RANTES



Data are represented as mean ± SEM. Statistical analysis between unstimulated and IL-15 + IL-12 stimulated cultures were performed and the significance is denoted by *. A *p* value <0.05 was considered significant.

Chemokine receptors CCR1, CCR4, CCR5 and CXCR4 studied on resting NK subsets, were found to be down-regulated (p<0.05) in HIV and HIV-TB compared to NHS. Upon stimulation, the chemokine receptors in HIV, but neither TB nor HIV-TB, were down-regulated (p<0.05) on NK subsets. Furthermore, a negative correlation was found upon stimulation suggesting that increased MIP-1α and decreased CCR4 expression might be a beneficial effect of IL-15+IL-12 during HIV infection (Table 13).

Conclusion: IL-15+IL-12 have the potential to improve the CC-chemokine expression and down-regulate chemokine receptors on NK subsets primarily in HIV, but less effective in HIV-TB.

Table 13: **Chemokine receptor expression on NK subsets**

Chemokine receptors	Groups	Stimulant	% Cells expressing chemokine receptors (Mean ± SEM)			
			CD16+CD3-	CD56+CD3-	CD16+CD8-	CD56+CD8-
CCR1	HIV	Unstimulated	13.0 ± 3.7	10.9 ± 3.2	12.8 ± 3.5	10.6 ± 1.7
		IL-15 + IL-12	11.2 ± 4.1	**2.4 ± 2.1 #**	14.5 ± 5.6	11.9 ± 2.7
	HIV-TB	Unstimulated	4.9 ± 0.9	7.5 ± 8.3	6.1 ± 1.5	18.5 ± 6.4
		IL-15 + IL-12	3.5 ± 0.6	7.8 ± 8.7	6.1 ± 1.2	26.9 ± 6.6
CCR5	HIV	Unstimulated	9.9 ± 3.2	5.2 ± 1.8	11.1 ± 3.1	10.0 ± 2.0
		IL-15 + IL-12	9.7 ± 4.0	**1.6 ± 1.4 #**	10.1 ± 3.6	3.8 ± 3.4
	HIV-TB	Unstimulated	2.6 ± 0.7	3.1 ± 1.6	4.0 ± 0.8	16.8 ± 3.9
		IL-15 + IL-12	3.2 ± 0.6	3.3 ± 5.7	7.5 ± 3.7	20.9 ± 4.6
CXCR4	HIV	Unstimulated	21.9 ± 6.0	17.9 ± 5.8	24.8 ± 8.1	19.2 ± 6.8
		IL-15 + IL-12	**9.1 ± 3.0 #**	18.8 ± 7.6	**11.7 ± 4.7 #**	12.2 ± 4.0
	HIV-TB	Unstimulated	33.0 ± 6.8	36.8 ± 5.8	33.6 ± 8.9	35.1 ± 5.2
		IL-15 + IL-12	23.5 ± 5.2	37.7 ± 7.1	26.5 ± 6.9	38.2 ± 5.9

Data are given as percentage of mean ± SEM. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's post test. # refers to the significant comparison (p<0.05) between basal and IL-15 + IL-12 stimulation.

(iv) Role of Interferon gamma assay for latent TB in HIV infection

(Principal Investigator: Dr. Alamelu Raja; Funding: RO3 Grant, NIAID, NIH)

Background: A major break through in TB diagnosis is the advent of Interferon gamma (IFN-γ) release assay (IGRA) with *M. tuberculosis* specific antigens. The suboptimal sensitivity of IFN-γ based *in vitro* assays, especially in immunocompromised individuals, emphasizes the need for alternative markers for diagnosing TB.

Aims: To evaluate whether interferon-inducible protein (IP)-10, monocyte chemotactic protein (MCP)-2 and interleukin (IL)-2 can be useful biomarkers for evaluating a specific response to region of deletion-1 (RD1) antigens associated with active disease in HIV-infected and uninfected TB patients

Methods: Active PTB patients with and without HIV infection and controls (household contacts and community controls with and without HIV infection) were prospectively enrolled. A number of HLA-Class-II restricted epitopes were predicted out of ESAT-6 and CFP-10 RD1 proteins and were synthesized. A whole blood assay based on RD1 selected peptides was performed. Soluble factors were evaluated by ELISA in plasma harvested at day 1 post-culture. Enrolled individuals were also tested by QuantiFERON TB-Gold In tube (QFT-IT) and tuberculin skin tests (TST).

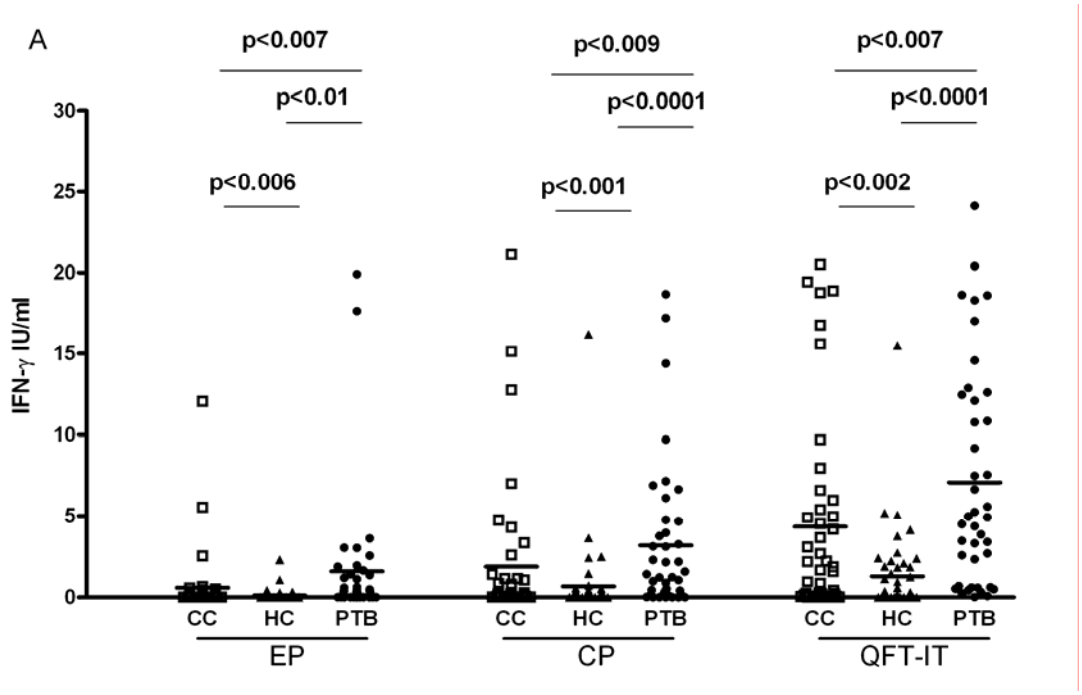
Results: IFN-γ response to RD1 selected peptides was significantly higher in active TB patients than in HHC and community controls. IP-10 and MCP-2 response did not differ between active TB patients and HHC, although it was higher in these groups compared to community controls (Figs.8A & B). Conversely IL-2 response did not differ among the three groups. When IFN-γ response to RD1 selected peptides was scored based on receiver-operator-characteristic analysis, active TB was predicted with 68% sensitivity and 86% specificity. QFT-IT and TST showed sensitivity of 90% and 68% and specificity of 58% and 59%, respectively for active TB.

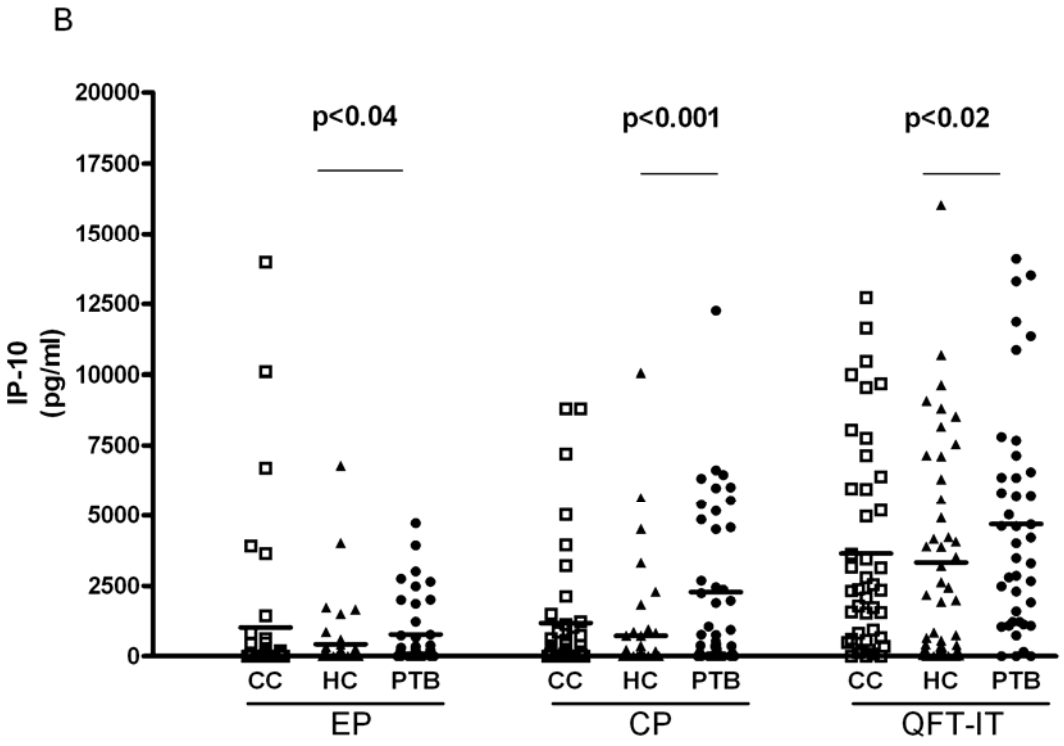
The results indicate that by detecting IP-10, the sensitivity of the experimental test and QFT-antigen for HIV-TB was higher compared to the same assays based on IFN-γ. On the other side, *in vitro* IL-2 and MCP-2 responses were not significantly associated with active TB.

Conclusions: IFN-γ (but not IP-10, MCP-2 and IL-2) response to RD1

selected peptides is associated with active TB. HIV infection does not impair RD1-specific response detected by IP-10, while it significantly decreases IFN-γ mediated responses.

Fig.8: IFN-γ and IP-10 response to selected peptides





(v) Development of Recombinant BCG based epitope vaccine candidate for TB

(Principal Investigator: Dr. Sujatha Narayanan)

Background: BCG, the only available vaccine for TB provides variable efficacy in protection against adult PTB. Developing better vaccines using novel approaches is a major goal for the TB research community. Epitope-based vaccines designed to induce T-cell responses specific for *M. tuberculosis* antigens are being developed as one of the means of improving vaccine potential.

Aims: (i) to construct recombinant BCG (rBCG) based epitope vaccines for TB using the epitope delivery system constructed by our group earlier and

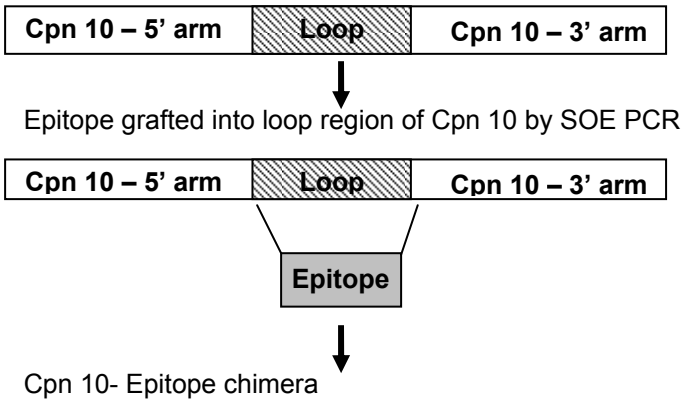
(ii) to evaluate the immunogenicity of the recombinant BCG vaccines in a mouse model

Materials and Methods: Epitope grafting was done by Splicing Overlap Extension polymerase chain reaction. Expression of the chimeric antigens in BCG was proved by

Western blotting. For the immunogenicity experiments, BALB/c mice were immunized subcutaneously with BCG or individual rBCGs. Cell - mediated immune response to specific mycobacterial antigens was studied by evaluation of delayed type hypersensitive (DTH) response, *in vitro* splenocyte proliferation (MTT assay) and cytokine estimation (ELISA). Humoral immune response was studied by measuring the serum antibody titre.

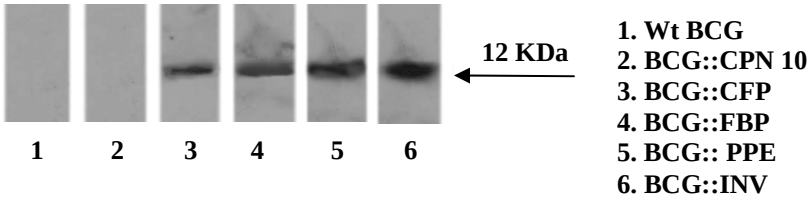
Results: Immunodominant epitopes were chosen from four well defined *M.tuberculosis* antigens, Ag85C (Rv2903c), 10-kDa culture filtrate protein (CFP-10) (Rv3874), PPE68 (Rv3873) and INV 2 (Rv1478). The epitope encoding genes were grafted into a Cpn 10 based epitope delivery system (Fig.9).

Fig. 9: Schematic representation of Epitope grafting - Cpn 10 ORF



The Cpn 10-epitope chimeras were further cloned and expressed in BCG to obtain four rBCGs (BCG::CFP, BCG::FBP, BCG::PPE and BCG::INV2) (Fig.10).

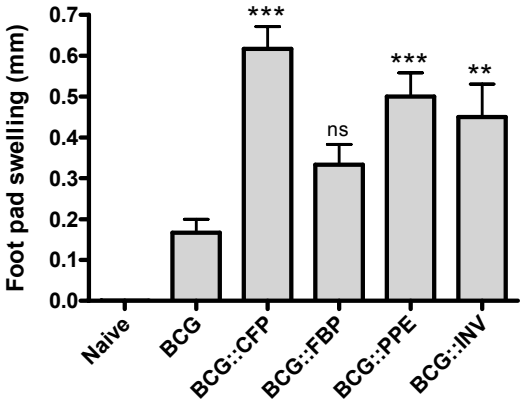
Fig.10: Western blot analysis to verify the expression of the CPN 10-epitope chimeras



BCG Pastuer was electroporated with the pMV306 harbouring the Cpn 10-epitope chimera and the cell lysates were subjected to western blot with Anti-His antibody.

Both cellular and humoral immune responses induced by these r-BCG strains were evaluated in BALB/c mice after subcutaneous injection of a single dose of 1×10^6CFU of the Individual rBCGs. rBCG vaccination elicited strong DTH responses, proving the *in vivo* priming of T- cells against the targeted epitopes (Fig.11).

Fig.11: DTH response elicited by rBCG vaccination



Five weeks after immunization, BALB/c mice (n=6) immunized rBCG were injected with 10 µg/ml of the respective recall antigens in 20 µl PBS on the left footpad and 20 µl PBS on the right footpad. BCG immunized animals were injected with 20 µl PPD on left footpad and 20 µl PBS on right foot pad. Naïve animals were injected with 20 µl PBS on both footpads. Footpad swelling was measured after 24 h and results were calculated as difference in the swelling between right and left footpads. The results shown are the mean ± SD for six mice in each group. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS- $P > 0.05$ (compared to BCG).

Compared to the parent BCG immunized animals the splenocytes derived from rBCG vaccinated groups showed greater antigen specific proliferation, characterized with higher IFN-γ response and reduced IL-4 secretion (Figs.12a&b).

Fig.12:(a) ***In vitro*** **splenocyte proliferation and cytokine responses elicited by rBCG immunization**

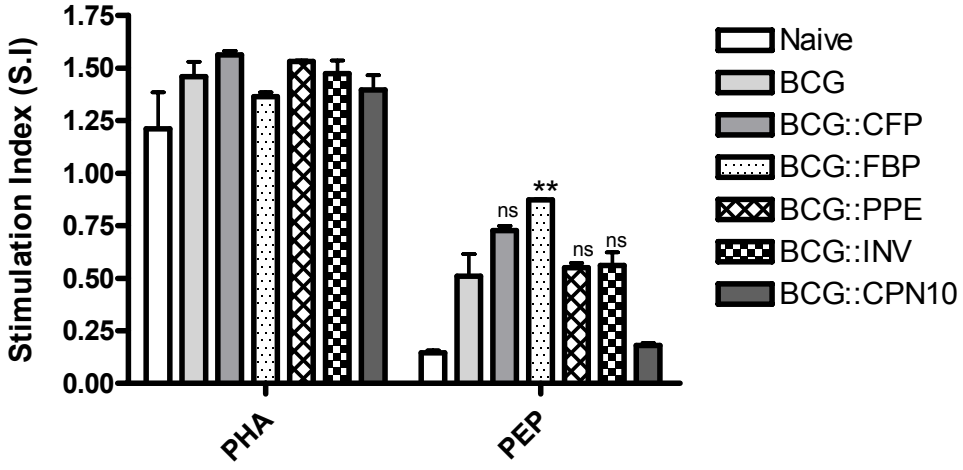
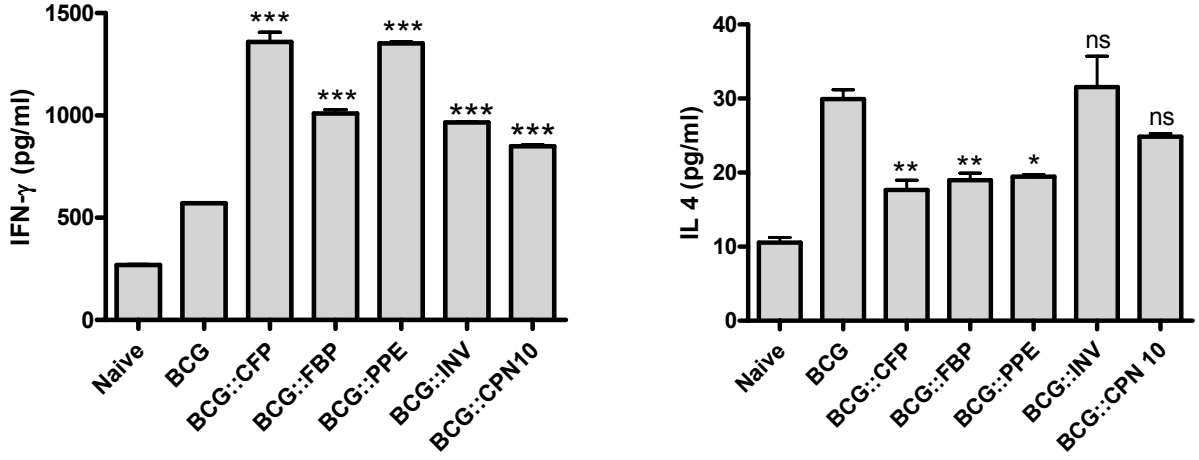


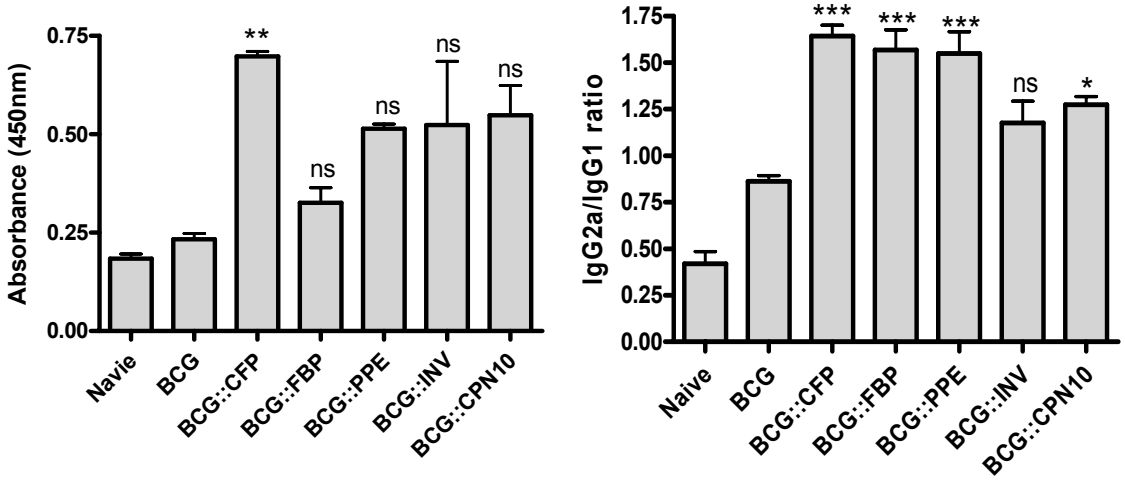
Fig.12:(b)



At 5 weeks post immunization, splenocytes were prepared from Naïve/BCG/rBCG groups, and stimulated with PHA or recall peptides for 68 h. Splenocytes from rBCG immunized animals were stimulated with the respective recall peptides (5 µg/ml) while Naïve/BCG/BCG::Cpn10 groups were stimulated with 10 µg/ml of peptide cocktail. PHA (5 µg/ml) was used as a polyclonal stimulator for positive control. MTT (10 µg/ml) was added for 4 h and the proliferation induced by the recall peptide was measured. Cytokines in the culture supernatant were estimated by sandwich ELISA. The results presented are the mean ± SD of S.I. The experiment was repeated thrice with similar results. *** P < 0.001; ** P < 0.01; * P < 0.05; NS - P > 0.05 (compared to BCG).

Also rBCG vaccination was able to induce specific humoral immune response with an enhanced IgG2a/IgG1 ratio (Fig.13).

Fig.13: **Epitope -specific humoral immune responses elicited upon rBCG immunization**



BALB/c mice were immunized subcutaneously with BCG, rBCG or PBS. Five weeks after immunization sera from two mice per group (n=6) were pooled together. (A) Epitope-specific serum IgG responses was analyzed by ELISA. (B) Subclasses of serum IgG were detected at the same time. The peptide-specific antibody levels were assayed in triplicates. The vertical bars denote average ± SD *** P<0.001; ** P<0.01; * P<0.05; NS- P>0.05 (compared to BCG).

Conclusion: Our results indicate that the rBCGs favour a Th1 type response, which is known to be important for mycobacterial immunity and are thus promising TB vaccine candidates.

(vi) Profiling of molecular heterogeneity and identification of Region-specific gene segments in the field strains of *M. tuberculosis*

(Principal Investigator: Dr. Sujatha Narayanan; Funding: Department of Biotechnology, New Delhi)

Back ground: Emergence of MDR-TB, HIV and poor TB control programs have all contributed to the dramatic increase in the TB burden. In order to monitor TB control, epidemiological studies are extremely important. DNA fingerprinting techniques identify specific strains of mycobacteria and facilitate better understanding about the geographic distribution of strains, dynamics of dissemination,

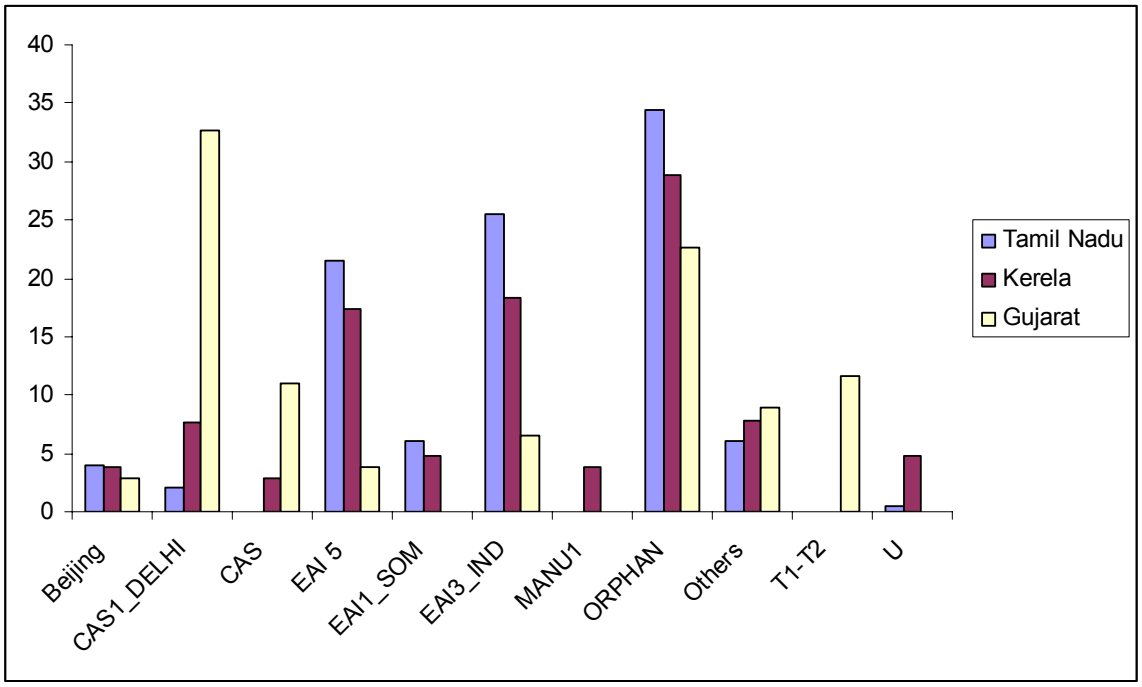
identification of populations at risk and to determine the risk factors for TB transmission in a community

Aim: To study the genetic diversity of *M. tuberculosis* isolates present in three geographical regions of India (Tamil Nadu, Kerala and Gujarat)

Methods: The project covered three different geographical regions, namely Tamil Nadu, Gujarat and Kerala in India. A total of 485 *M. tuberculosis* isolates, 200 from Tamil Nadu, 181 Gujarat and 104 from Kerala were subjected to spoligotyping. RFLP was done on the samples obtained from Tamil Nadu and Gujarat. Genomic DNA was extracted from *M. tuberculosis* cultures by standard CTAB-NaCl extraction method. Spoligotyping was performed with *M. bovis* BCG P_3 and H37Rv as positive controls. The DR region was amplified using the DRa (5′ biotinylated) and DRb primers. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each correspond-ing to a unique spacer sequence within the DR locus. The results were documented in the form of a binary code. The data was analyzed on a global scale through comparison with the international SpolDB4 database. IS6110 DNA fingerprinting was done according to internationally accepted guidelines. Briefly, 2 µg of genomic DNA was digested with PvuII. DNA fragments were separated by electrophoresis on agarose gels, denatured, and blotted onto nylon membrane by the alkaline transfer procedure. Hybridization was performed on PvuII-restricted genomic DNA with a chemiluminescence-labeled 254-bp IS6110 fragment.

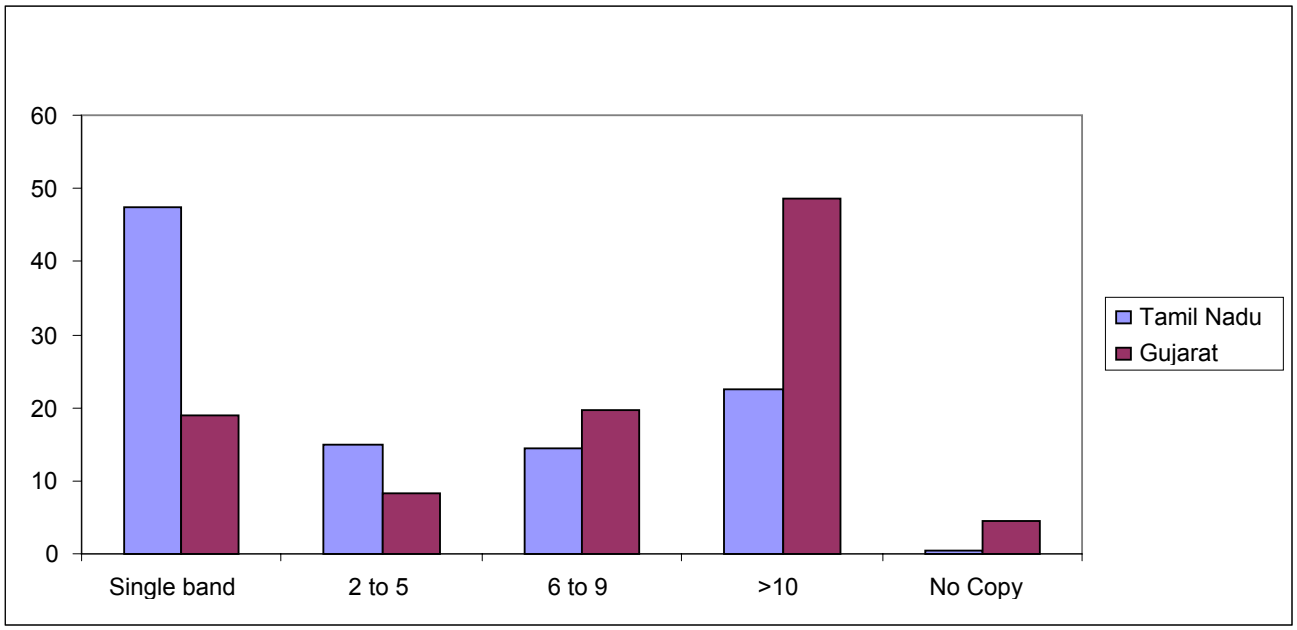
Results: Spoligotyping: Spoligotyping results of 485 *M. tuberculosis* isolates from Tamil Nadu, Kerala and Gujarat were matched with SpolDB4 data base. EAI strains were predominant in Tamil Nadu and Kerala compared to Gujarat, where as CAS and T strains where predominant in Gujarat compared to Kerala and Tamil Nadu (Fig.14).

Fig.14: **Spoligotyping of *M. tuberculosis* from Tamil Nadu, Kerala and Gujarat**



IS6110 RFLP: IS6110 RFLP results of 381 *M. tuberculosis* isolates from Gujarat and Tamil Nadu showed predominance of low IS6110 copy (0 to 5) isolates in Tamil Nadu, where as high IS6110 copy (>10) to be predominant in Gujarat (Fig.15).

Fig.15: **IS6110 RFLP of *M. tuberculosis* from Tamil Nadu and Gujarat**



Conclusions: The regional differences in distribution of spoligotypes and IS6110 RFLP pattern may be linked to the different ethnic subpopulations in North (Gujarat) versus South (Tamil Nadu and Kerala), and their respective migration histories. Such analysis clearly indicates the prevalence of historical versus recently imported "modern" clones of TB in regions of India. Despite the presence of predominant shared types, in each region, *M. tuberculosis* strains showed a high degree of genetic diversity. This diversity may be due to reactivation of past disease or individual clones appearing or disappearing over time.

(vii) AmiA acts as a repressor in the regulation of acetamidase operon of *M. smegmatis*

(Principal Investigator: Dr. Sujatha Narayanan)

Background: We have been working on the gene regulation of the inducible acetamidase of *M. smegmatis* for the past several years. Since the mechanism of regulation is complicated, we are trying to unravel step by step. The acetamidase promoter has been used to over-express mycobacterial proteins. However, the system is imperfect because of leaky expression and the large size occupied by the promoter which makes the system unstable to express certain proteins. The acetamidase operon of *M. smegmatis* has four predicted open reading frames (AmiC-AmiA-AmiD-AmiS) upstream to the structural gene AmiE. In order to improve the system, we were interested in characterizing the gene regulation of this operon. Previously, we reported that AmiA binds near the P2 promoter and it might negatively regulate the acetamidase operon. In this report, we show the negative regulation in transcript level and protein level to prove that AmiA is a negative regulator.

Objective: To characterize the regulatory elements of the

acetamidase operon of *M. smegmatis* and in particular to prove the role of AmiA as a negative regulator

Results: Electro Mobility Shift Assay (EMSA) and DNase I protection assays from our previous report showed that AmiA binds in a direct repeat sequence of GGGTGA spaced by eight bases (P2 Promoter).

AmiA over-expressing strain was constructed by cloning *amiA* in pMV261 vector under *hsp* promoter and electroporated in *M.smegmatis*. Heat shock was given to induce AmiA expression. Wild type and AmiA over-expressing cells were induced with 25mM, 35mM and 50mM of acetamide respectively.

Total RNA was isolated from the wild type and AmiA over-expressing cells and qRT-PCR was performed to quantify the Acetamidase transcript. After the addition of acetamide at increasing concentrations (25mM and 35mM), there was a reduction in acetamidase transcript level observed in AmiA over-expressing strain at 4h and 18h time point as comparing to wild type strain (Figs. 16 & 17).

Fig.16: **qRT-PCR of mRNA abundance in wild-type and *amiA* over-expression strains**

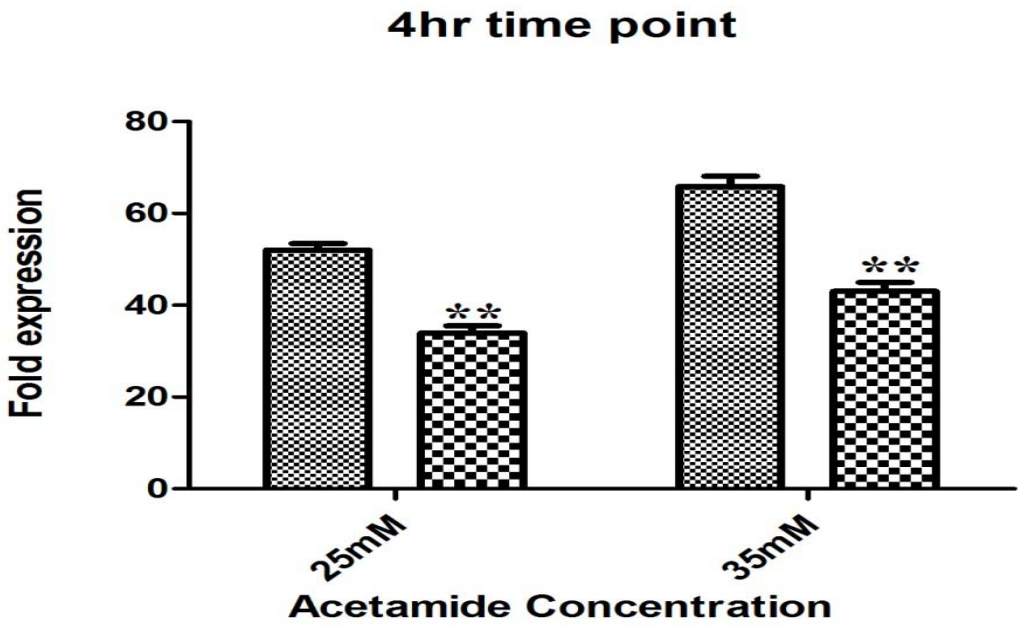
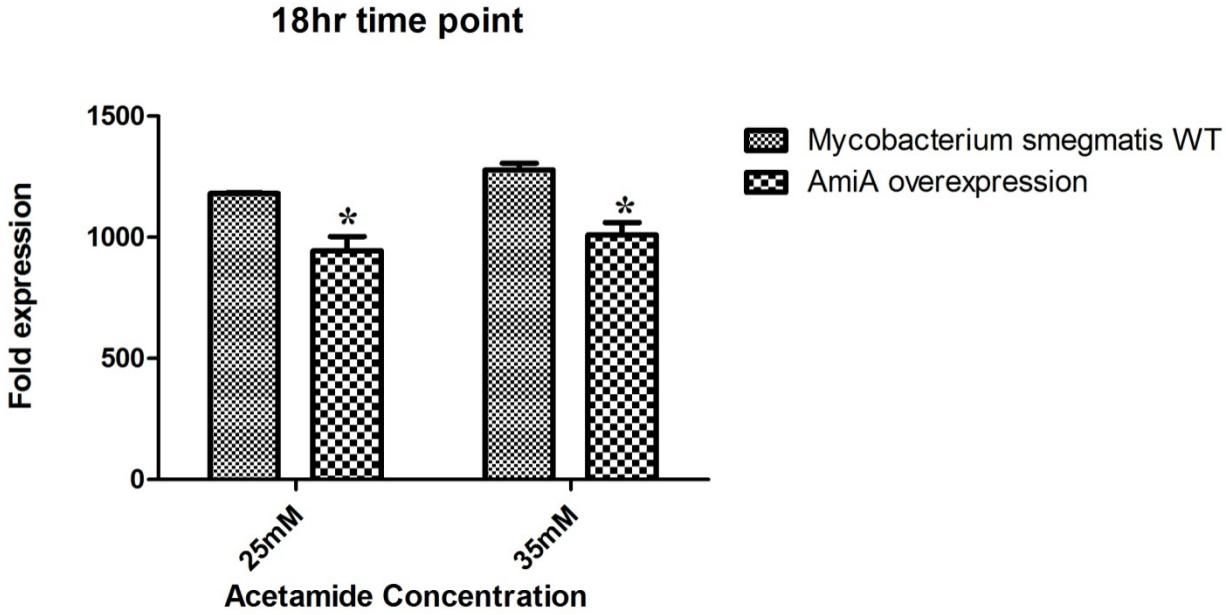


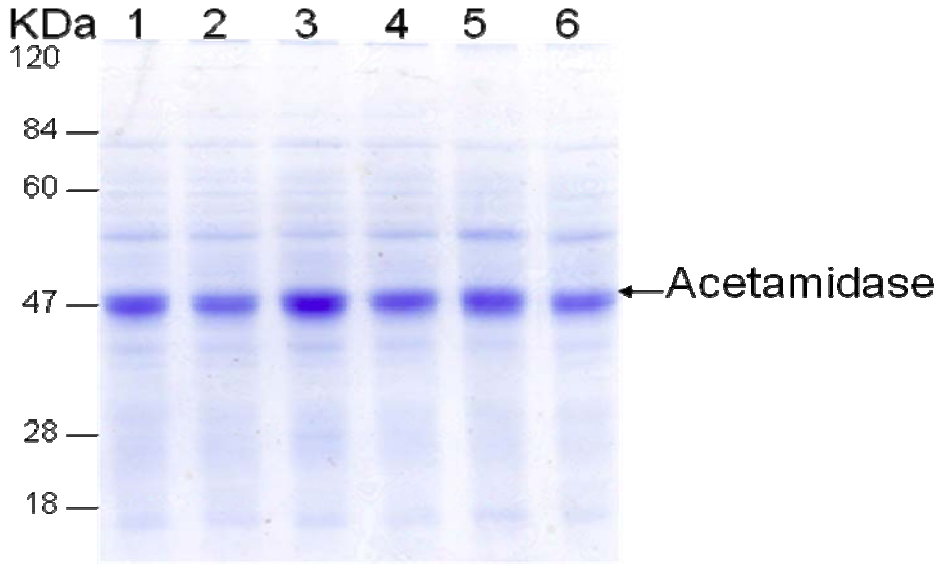
Fig.17:



Relative mRNA abundance was determined for Acetamidase (*amiE*) using total RNA at 4h and 18h after acetamide induction. The data represent average values and standard error measurements from three technical replicates, shown as percent relative abundance normalized to the *16s rRNA* gene. The symbols (*,**) indicate P<0.05 and P<0.01 respectively.

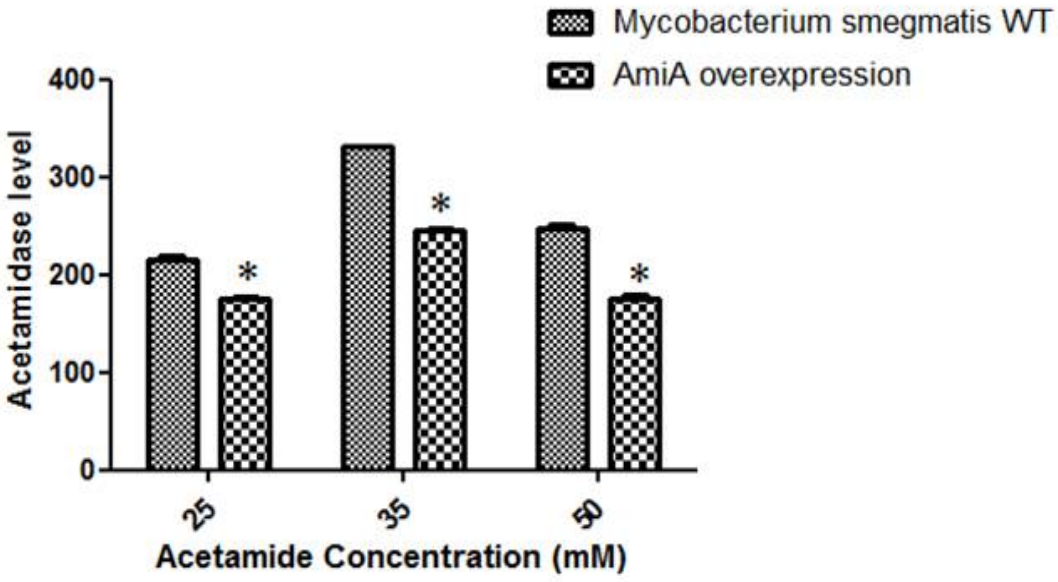
Wild type and AmiA over-expressing cells were induced with 25mM, 35mM and 50mM of acetamide at 18h after induction. The cells were lysed and acetamidase was estimated using SDS-PAGE and Densitometry analysis. As observed in SDS-PAGE, wild type cells harboring no extra copy of AmiA expressed high level of acetamidase. However the cells over expressing AmiA expressed significantly lower levels of acetamidase than the wild type as observed in the protein band intensity at 47kDa by densitometry analysis (Figs.18 & 19).

Fig.18: **Over expression of AmiA and its effect on the expression of acetamidase**



M. smegmatis wild type cells and AmiA over-expressing cells were grown and induced with acetamide for 18h. Bacterial proteins were extracted and analyzed by SDS-PAGE. Lane 1, 3 and 5 are crude extracts of wild type cells and Lane 2, 4 and 6 are crude extracts of AmiA over expressing cells induced with 25mM, 35mM and 50mM acetamide respectively. Molecular weight marker is indicated by KDa.

Fig.19: **Effect of AmiA over expression on Acetamidase level**



Acetamidase level of wild type and AmiA over-expressed strains were measured using Densitometer scanning and concentration was determined using Quantity One software in

various acetamide induced concentrations (25mM, 35mM and 50mM).The symbol (*) indicates mean values that are significant (P<0.05).

Conclusion: qRT-PCR revealed that acetamidase transcript level is significantly decreased in AmiA over-expressed strain compared to wild type. This decrease in transcript level was well correlated with protein expression by densitometry analysis. These data strongly suggest that AmiA acts as a repressor in the regulation of acetamidase operon of *M. smegmatis.*

(viii) *PknE* a serine / Threonine kinase from *M. tuberculosis* plays a role in adaptive responses

(Principal Investigator: Dr. Sujatha Narayanan)

Background: TB caused due to *M. tuberculosis* thwarts the host immune response to survive. The survival response leads to shifting from active infection to a persistent, metabolically dormant state. This distinctive life cycle of *M. tuberculosis* encompasses unique developmental adaptations to the changing environmental cues. *M. tuberculosis* infected macrophages fail to acidify and prevent phagosome maturation. Hence those genes which regulate resistance towards this acidification are of greater importance for a putative drug target. Serine threonine protein kinases (STPK), the novel signal transduction system identified in *M. tuberculosis* plays a role in physiology and pathogenesis of this organism. Among the 11 STPKs, *pknI*, *pknK* and *pknG* were reported to play a role in the survival responses. Previously we have shown that *pknE* induces intrinsic pathway of apoptosis. The present work analysis the function of *pknE* in adaptive responses encountered within the phagosome.

Aim: To examine the role of *pknE*

- in growth in 7H9, pH (5.5 and 7.0) and SDS
- in Biofilm formation
- in morphological defects

Methods: The strains *M. tuberculosis* H_{37}Rv (Rv), *M. tuberculosis* H_{37}RvΔ*pknE*(Δ*pknE*) and complemented *M. tuberculosis* H_{37}RvΔ*pknE*(CΔE) were grown in middlebrook 7H9 containing albumin dextrose catalase enrichments for growth curve analysis. Log phase cultures of Rv, Δ*pknE* and CΔE adjusted using Mc Farland standards were used to carry out the pH sensitivity experiment at pH5.5 and 7.0 using Sauton's media buffered with 100 mM 4-morpholine ethanesulfonic acid. SDS sensitivity was carried out using 7H10 agar plates containing SDS at 0.1%, 0.01% or 0.001% concentrations and colony forming units (cfu) were counted after 10 days. Biofilms were grown using saturated planktonic cultures by inoculating into polystyrene petri dishes and in 24 well costar plates containing Sauton's medium without Tween 80. The morphological examination of cells was determined using differential interference contrast (DIC) microscopy in 100X DIC lens using Zeiss AxioObserver Z1.

Results: Survival kinetics of Rv, Δ*pknE* and CΔE in 7H9 medium did not show any growth differences (data not shown). However Δ*pknE* showed increased growth compared to Rv and CΔE at pH5.5 (Fig.20). In contrast Δ*pknE* showed reduced cfu at pH7.0 after 16 days compared to Rv and CΔE (Fig.21). In the presence of SDS, a detergent Δ*pknE* exhibited resistance at 0.01% while Rv and CΔE were susceptible (Fig. 22). The susceptibility at 0.1% SDS was growth inhibitory for all the strains, while 0.001% SDS promoted growth invariably for all the strains (data not shown). Δ*pknE* resistant at 0.01% SDS concentration also showed colonies with altered morphology (Fig.23). Assessment of bioflim formation did not differ in Rv, Δ*pknE* and CΔE strains (data not shown). Morphological assessment of cells by DIC showed Δ*pknE* to be smaller in size compared to Rv (Fig.24).

Conclusion: Our data for the first time suggests a role for *pknE* in adaptation responses. This is exemplified by the growth of mutants in acidic pH and in SDS, both

constituting the host response for pathogen clearance. The morphological defect exhibited by the mutant in terms of reduced cell size and altered colony morphology shows the role of *pknE* in cell integrity. In conclusion, *pknE* contributes to the resistance of host microbicidal responses by modulating the adaptive response genes in *M. tuberculosis*.

Fig.20: **Growth analysis in pH5.5**

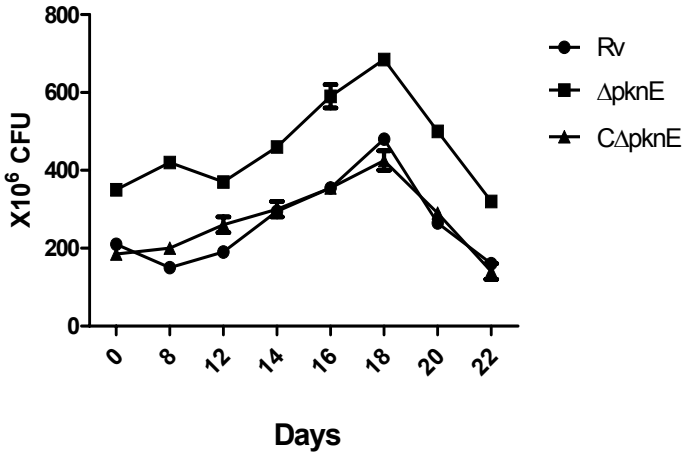


Fig.21: **Growth analysis in pH7.0**

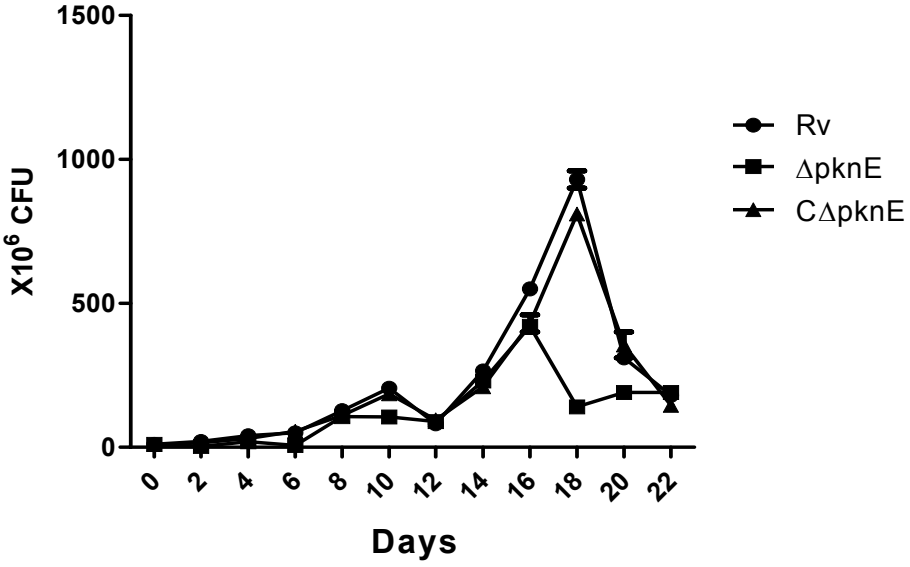
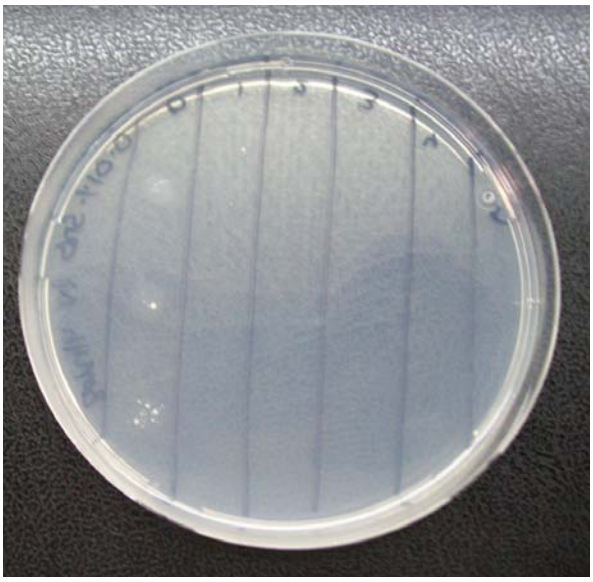
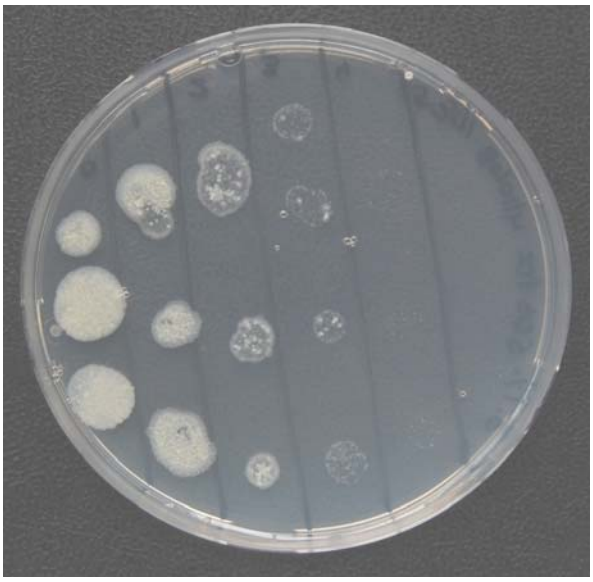


Fig.22: **ΔpknE is resistant to (SDS) stress**

Rv **Δ*pknE***

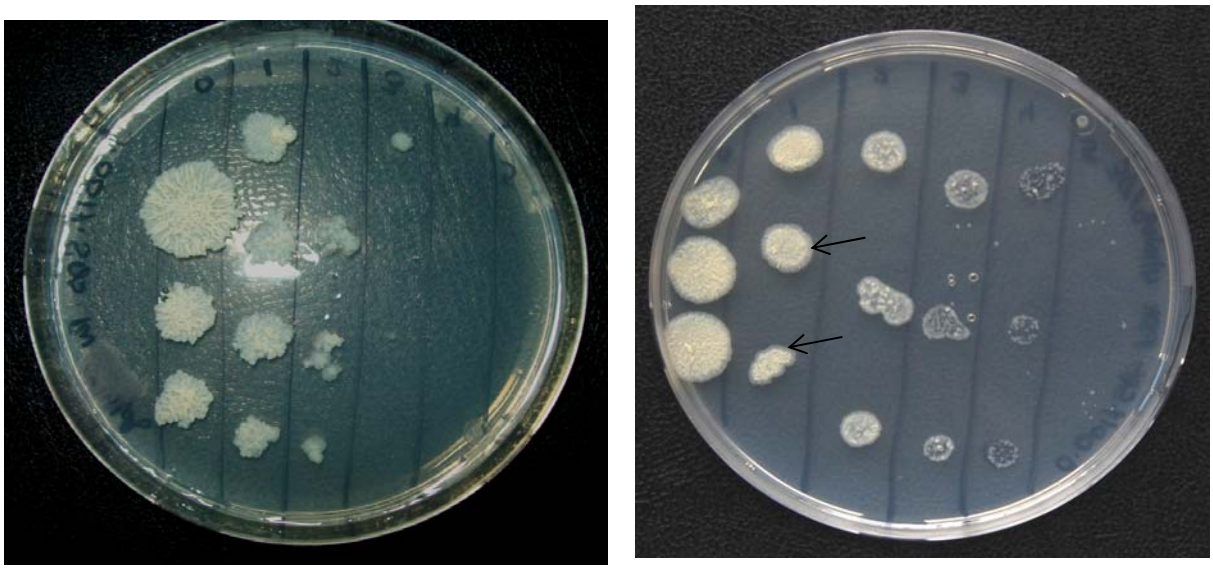




CΔE

Fig.23: **Δ*pknE* exhibits altered colony morphology during SDS stress**

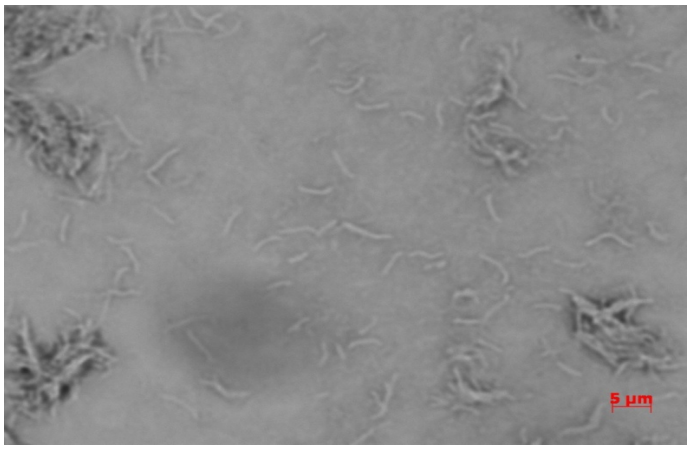
Rv ***ΔpknE***



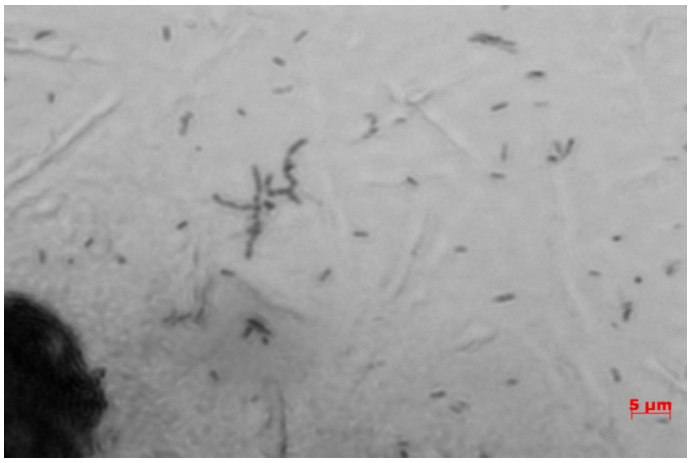
***Arrows indicate altered colony morphology**

Fig.24: **Δ*pknE* reduced cell size**

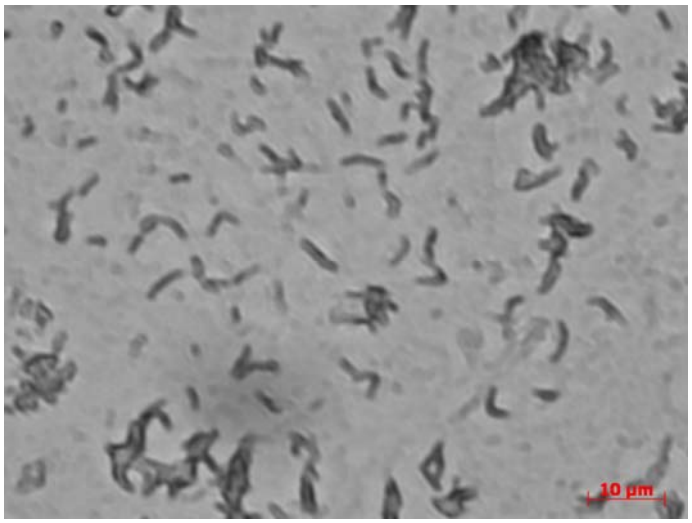
Rv



Δ*pknE*



CΔE



(ix) Polymorphism in RD1 locus and its effect on transcription of down stream genes among south Indian isolates of *M. tuberculosis*

(Principal Investigator: Dr. Sujatha Narayanan)

Background: RD1, the region of difference between the virulent clinical strains of *M. tuberculosis* and *M. bovis,* and attenuated vaccine stains of *M. bovis* BCG, is the most explored region in the genome for the better understanding of mycobacterial virulence and vaccine design. RD1 encompasses nine genes of which, three are predicted to form a secretory apparatus for the transport of other two immunodominant T-cell antigens, ESAT6/CFP10. During screening of RD1 region, we found a polymorphic intergenic region between the two genes, Rv3870 and Rv3871, which are obligatory for the secretion of ESAT6/CFP10.

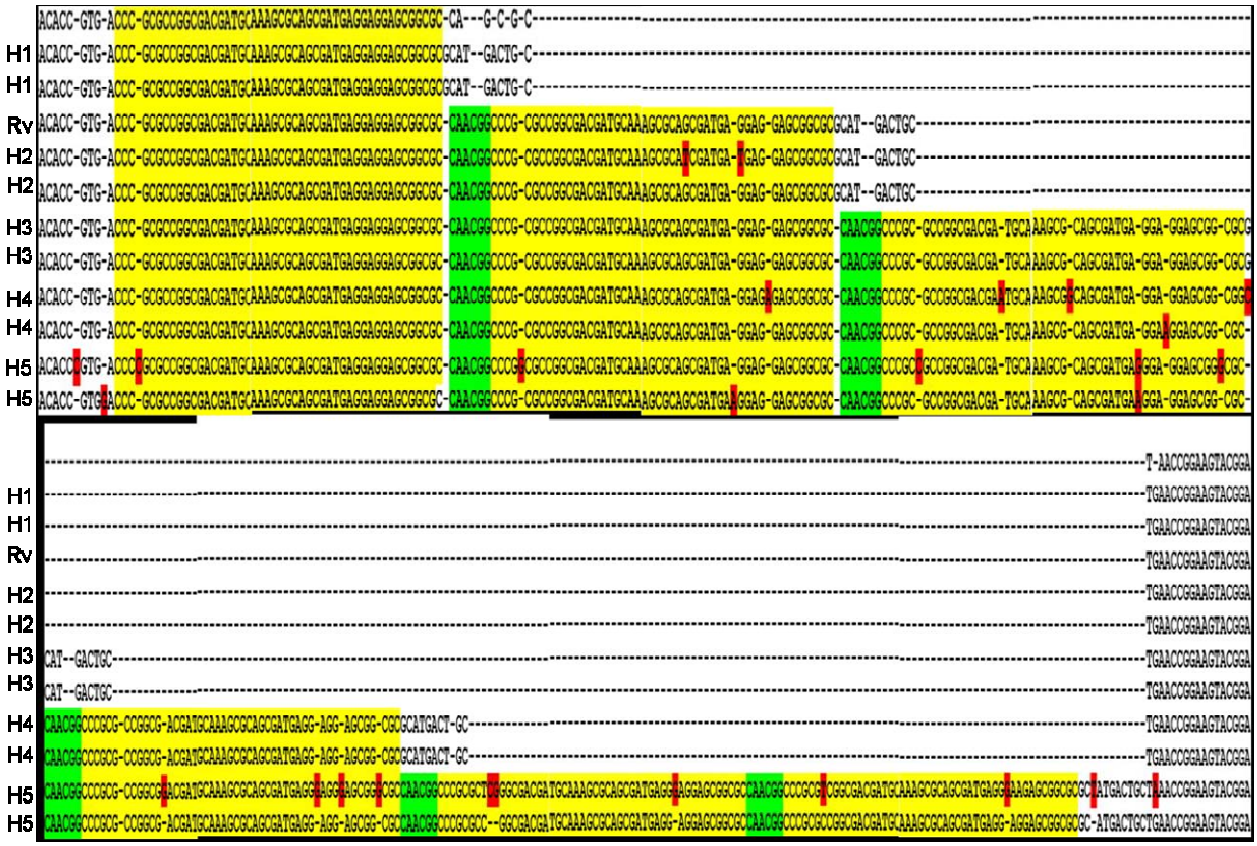
Aims: (i) to identify the presence or absence of polymorphism in the intergenic region among the clinical isolates and (ii) to investigate the functional role of the repeat variability in transcription by measuring the transcription level of downstream genes by qRT-PCR

Methodology: A total of 407 mycobacterial isolates were used for this study with *M. tuberculosis* H37Rv and *M. bovis* BCG as reference strains. Genomic DNA extracted was used for spoligotyping and PCR. Total RNA was extracted and cDNA synthesized was used for RT-PCR experiments. The Hunter-Gaston discriminatory index (HGDI) and one way ANOVA table was used for statistical analysis.

Results: From sequencing results, the polymorphism was observed only in the intergenic region of Rv3870 and Rv3871. A set of primers were designed exclusively for this region and the expected amplification product for standard *M. tuberculosis* H37Rv strain was 230bp and named as RD-INS. RD-INS primers differentiated the samples into five groups namely, H1(~180bp), H2(~230bp), H3(~280bp), H4(~330bp) and H5(~420bp) when resolved in 2% agarose gel. The polymorphism was due to the insertion of a repeat element (MIRU

39) in different copy numbers in the intergenic region of Rv 3870 and Rv 3871. This was further confirmed by sequencing two samples from each group (Fig.25).

Fig.25: **Multiple sequence alignment showing polymorphisms among different groups**



We analysed a total of 407 clinical isolates with RD1-INS primers to look for the frequency of polymorphism among the different clades of *M. tuberculosis* in south India. The discriminatory power of this locus was found to be high for EAI strains as indicated by Hunter Gaston Index (HGI) of 0.58 and low for Beijing (0.26) and CAS (0.29) strains. The results suggest that the polymorphism created by different loci and the discriminatory power of MIRU varies among strains of diverse geographical origin.

The presence and variability of MIRU 39 in the intergenic region led us to investigate the functional role of the polymorphism in transcription by measuring the transcription level of

the downstream genes. Since Rv3870 and Rv3871, are obligatory for the secretion of ESAT6/CFP10 (Rv3874/Rv3875), we selected the immediate downstream gene Rv3871 and one gene Rv3874 from the ESAT6/CFP10 complex to measure the transcriptional level by quantitative RT-PCR in these five groups.

The expression of Rv3871 was higher in the group containing four copies and Rv3874 was higher in the group containing six copies as measured by quantitative RT-PCR (Figs.26 & 27).

Fig.26: Transcriptional level of Rv3871 among different groups

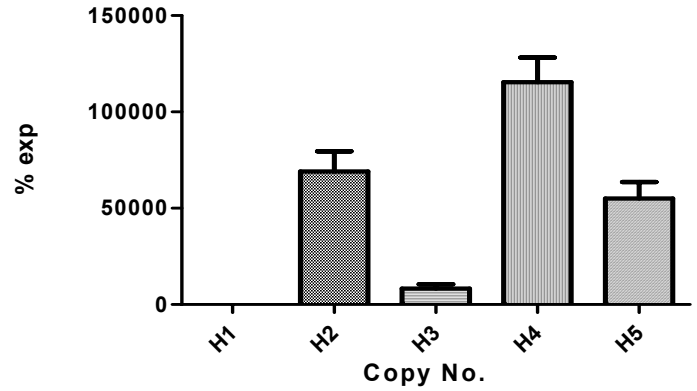
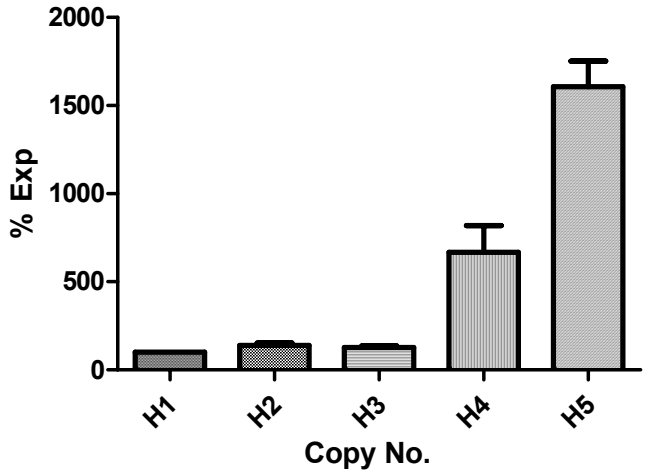


Fig.27: Transcriptional level of Rv3874 among different groups



Conclusion: The results suggest that the polymorphism created by different loci and the discriminatory power of MIRU varies among strains of diverse geographical origin. Change in transcription level due to these repeat elements may also play a role in virulence of *M. tuberculosis.*

(x) Toll-Like receptor and TIRAP gene polymorphisms in PTB

(Principal Investigator: Dr.P. Selvaraj)

Background: Toll-like receptors (TLRs) are pattern recognition receptors and play an important role in innate immunity. Changes in TLRs and signaling molecules that result from polymorphisms are often associated with susceptibility to various infectious diseases.

Aim: To find out whether TLR-1,-2,-4,-6,-9 and TIRAP gene polymorphisms are associated with susceptibility or resistance to PTB

Methods: Genotyping of TLR-1 1805T/G (Ile602Ser), TLR-2 2258G/A (Arg753Gln), TLR-4 896A/G (Asp299Gly), TLR-4 1196 C/T (Thr399Ile), TLR-6 745C/T (Ser249Pro), TIRAP 975C/T (Ser180Leu) genes and TLR-9 promoter region polymorphisms at positions -1237C/T and -1486C/T were performed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) method in 212 healthy control subjects (HCs) and 206 PTB patients.

Results: The allele and genotype frequencies of various TLR genes were not different between the HCs and PTB patients. The frequency of T allele of TIRAP 975C/T (Ser180Leu) polymorphism was significantly increased among PTB patients as compared to HCs [p = 0.026; Odds ratio (OR) 1.49, 95% Confidence interval (CI) 1.049 - 2.22]. A trend towards an increased frequency of TT genotype of TIRAP 975C/T was also observed in PTB patients [p = 0.078, OR 3.10 95% CI (0.96- 10.05)].

Conclusion: The present study suggests that T allele of TIRAP 975C/T polymorphism may be associated with susceptibility to PTB in south Indian population.

(xi) ROLE OF CHEMOKINES IN TUBERCULOUS IMMUNITY: *M. tuberculosis* antigen induced proliferation and apoptosis of pleural mesothelial cells

(Principal Investigator: Dr.D. Sulochana)

Background: The balance between proliferation and apoptosis in infected cells is critical to understand the mechanisms of acute lung injury and fibrogenesis. Many studies showed asbestos induced proliferation and apoptosis in mesothelial cells, but no information is available on how these two phenomena are altered in TB. Hence, in this study, proliferation and apoptosis of pleural mesothelial cells (PMC) in response to mycobacterial antigens and bacilli was evaluated.

Objective: To study *M. tuberculosis* antigen induced proliferation and apoptosis of PMC at the site of infection

Methodology: Mesothelial cell proliferation was determined with a [^{3}H] thymidine uptake assay. Cell cycle analysis and apoptosis of infected PMC was performed by TACS™ annexin V-FITC and propidium iodide (PI) staining.

Results: A significant increase in the proliferation was observed at 24hrs, in response to PPD and CFA compared to control (Table 14A). However, at 72hrs after infection with H37Rv, the cells showed significantly decreased proliferation. The cell cycle analysis showed significant increase in the S-phase of stimulated or infected cells after 24hrs thus correlating well with proliferation (Table 14B).

No apoptosis was observed till 24hrs either in control or in infected conditions (Table 15A). At the later time points of 48 and 72hrs, significant increase in apoptosis under all stimulated and infected conditions was observed. In general, the percentage of cells undergoing necrosis was higher than apoptosis (Table 15B). Interestingly, live *M. tuberculosis* (H37Ra and H37Rv) triggered more necrosis than apoptosis in mesothelial cells.

Conclusion: The mycobacterial antigens showed the innate ability to trigger the diametric pathways of proliferation and apoptosis. Thus

dual signal phenomenon is operative in TB pleuritis. The ability of the live *M.tuberculosis* to cause necrosis is well evidenced by the occurrence of caseating necrosis during granuloma formation. Thus, PMC might undergo necrosis during infection with live *M.tuberculosis,* whereas they respond to mycobacterial antigens by proliferation.

Table 14: ***M. tuberculosis*** **and its antigen induced proliferation**

A] Proliferation

	Control	PPD	CFA	H37Ra	H37Rv
18hr	4912±287	3611±217	3115±231	4062±470	3494±228
24hrs	5099±220	5307±114*	6088±138*	4377±107	4429±329
48hrs	5149±121	4424±132	4128±273	4353±265	4012±395
72hrs	4157±103	3344±167	3667±224	3883±231	2640±346*

B] Phase of cell cycle

S phase	Control	PPD	CFA	H37Ra	H37Rv
18hr	31±2	30±5	31±2	32±8	30±6
24hr	38±6	46±4*	49±7*	41±3*	52±5*
48hr	35±2	39±6	40±5	35±5	39±5
72hrs	36±5	30±3	39±6	33±9	30±7

Proliferation of *M.tb* and its antigen in Met-5A cells by 3[H] thymidine uptake (A) and cell cycle analysis by PI staining [B] performed in triplicates. The statistical significance* $p<0.05$ represents comparison with respective control.

Table 15: ***M. tuberculosis*** **and its antigen induced apoptosis**

A] Apoptosis

	Control	PPD	CFA	H37Ra	H37Rv
18hr	2.9±0.6	2.7±0.1	1.5±0.1	2.5±0.9	3.6±0.4
24hrs	2.2±0.2	1.3±0.1	1.9±0.4	1.3±0.1	6.4±4.0
48hrs	6.5±0.1	12.1±2.3*	11.1±4.9*	13.7±2.5*	12.9±1.1*
72hrs	8.8±2.7	18.4±1.1*	18.8±2.9*	18.4±7.8*	17.1±3.5*

B] Necrosis

	Control	PPD	CFA	H37Ra	H37Rv
18hr	6±0.5	13±0.8	10±1.2	27±0.5	10±0.1
24hrs	9±0.6	12±0.30	11±0.5	8±0.8	3±0.3
48hrs	15±0.9	20±0.6	21±0.3	30±2.0*	28±0.2*
72hrs	22±1.0	23±0.5	18±0.6	25±0.2	28±0.8

Apoptosis and necrosis of *M.tb* and its antigen in Met-5A cells by annexin V staining (A) and necrosis by PI staining [B] performed in triplicates. The statistical significance * $p<0.05$ represents comparison with respective control.

(xii) ROLE OF DENDRITIC CELLS IN MYCOBACTERIAL IMMUNITY: Differential activation of signaling molecules involved in maturation of dendritic cells during *in vitro M. tuberculosis* infection

(Principal Investigator: Dr.D. Sulochana)

Background: Maturation of dendritic cells (DC) is crucial for its biological functions like the ability to polarize distinct T-cell subsets. Mounting evidence indicates that intracellular signaling pathways that regulate DC maturation have key roles in patho-physiological processes during TB infection.

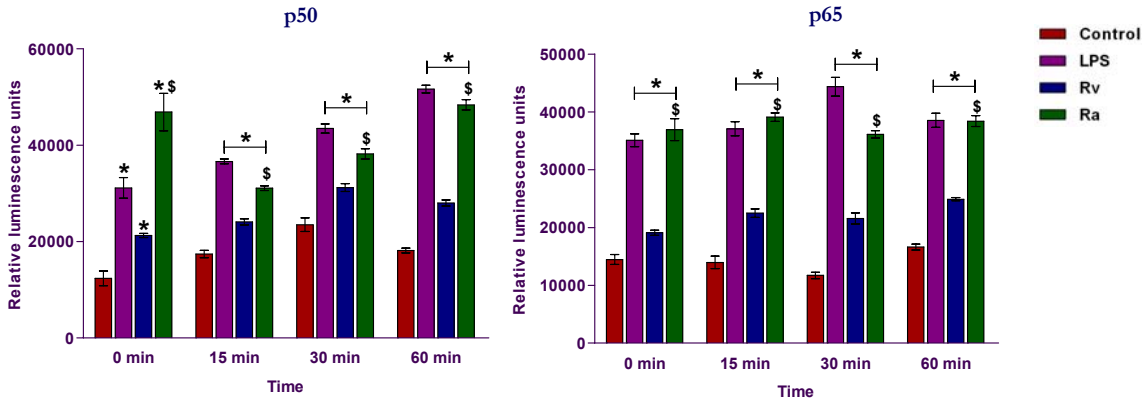
Aims: (i) to study the activation of PI3-Kinase, Mitogen-Activated Protein Kinases (MAPKs) and expression of transcription factors AP-1 and NF-kappaB in *M.tb* infected KG-1 cell derived DC (KGDC) and (ii) to evaluate the expression of negative regulators like suppressor of cytokine signaling (SOCS) and Signal transducers and activators of transcription (STAT)-3 molecules

Methodology: The differentiated KGDC were infected with H37Rv and H37Ra at multiplicity of infection (MOI) of 10. The nuclear extract, cDNA and cell lysates were prepared at different time points. The levels of AP-1, NF-κB and STAT-3 were measured by ELISA. The signaling and SOCS molecules were measured using Bio-Plex and Real Time PCR assays.

Results: The levels of AP-1 and NF-κB (Fig.28) were increased under all infections but were low in Rv compared to Ra. The increased activation of PI3kinase, ERK pathway (Fig.29), STAT-3, SOCS-1 and 3 (Fig.30) molecules but reduced MAPkinase activity was observed in Rv infected KGDC.

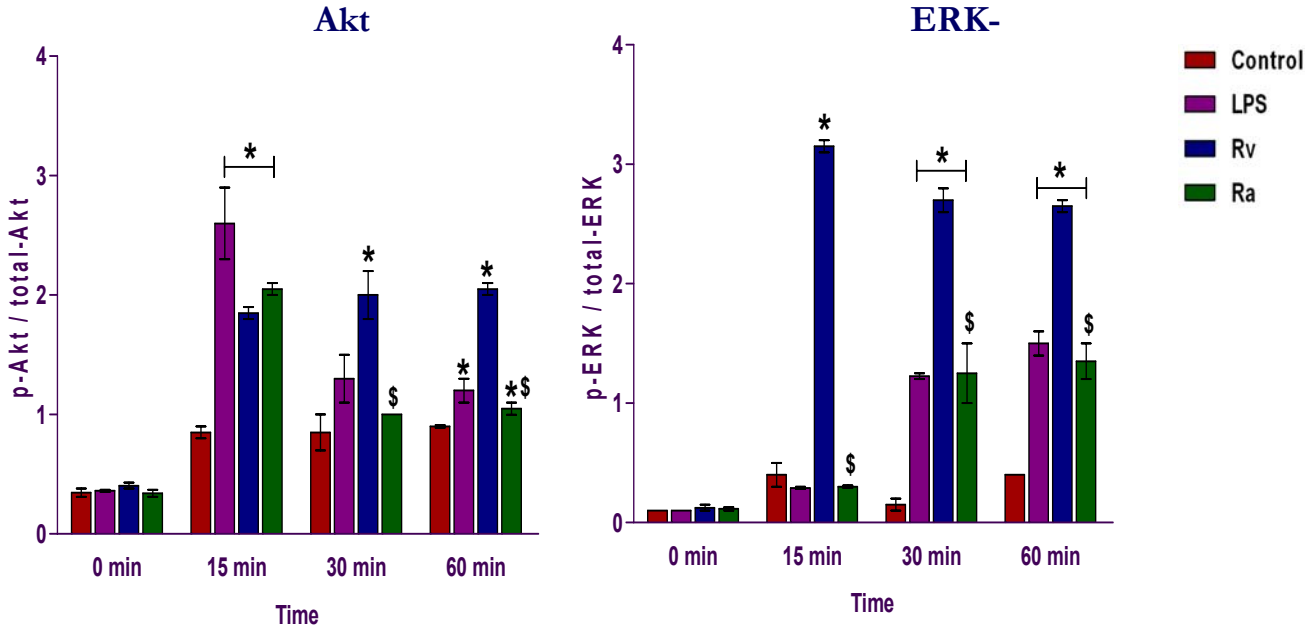
Conclusion: The data altogether suggest that signaling pathways are differentially activated when infected with Ra and Rv. Activation of ERK and PI3 kinase are probable mechanisms involved in the suppression of DC functions when infected with Rv. The induction of SOCS expression and STAT-3 activation are the ways by which Rv inhibits the maturation of KGDC.

Fig.28: **Activation of transcription factors (NF-κB) in infected KGDC**



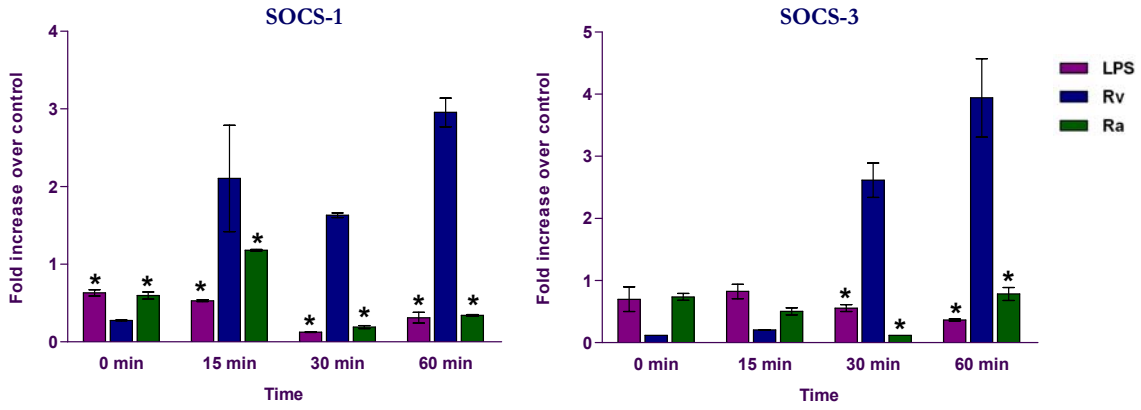
* compared to uninfected control, $ compared to Rv

Fig.29: **Activation of Signaling molecules (PI3kinase and ERK pathway) in infected KGDC**



* compared to uninfected control, $ compared to Rv

Fig.30: **Expression of SOCS-1 & 3 molecules**



* compared to Rv

Studies in progress:

(i) Role of Chemokine, DC-SIGN and TLR gene variants on immunity to TB

(Principal Investigator: Dr.P. Selvaraj)

Background: Invasion of the host by microbial pathogens causes activation of the innate immune response (first line defense) and triggers the secretion of various cytokines and chemokines and initiation of adaptive immunity. Chemokines along with cytokines are involved in the recruitment of T-cells to the inflammatory sites, activation of T-cells and inhibition of intracellular growth of *M.tuberculosis*. DC-SIGN (dentritic cell-specific ICAM-3 grabing nonintegrin), a C-type lectin, is the major *M. tuberculosis* receptor on human dentritic cells and involved in phagocytosis and cellular interactions. TLRs recognize lipid carbohydrate, peptide and nucleic acid structures expressed by various microorganisms. Polymorphisms of Chemokine, DC-SIGN and TLR genes have been shown to be associated with susceptibility or resistance to various infectious diseases.

Aims: (i) to find out whether Chemokine, DC-SIGN and TLR gene polymorphisms are associated with susceptibility or resistance to TB (Part-I) and (ii) to understand the role played by these gene variants on the innate and adaptive immunity to TB (Part-II)

Part-I of the study is carried out using stored DNA samples of 200 PTB patients and 200 healthy controls, collected earlier for various immunogenetic studies. Part-II of the study is carried out in a prospective manner using freshly drawn blood.

Polymorphisms of various chemokine genes are being studied. During the year RANTES (CCL5) and Stromal cell derived factor-1 (SDF-1 / CXCL12) gene polymorphisms were studied.

(ii) RANTES (CCL5) gene polymorphisms in PTB patients of south India (Principal Investigator: Dr.P. Selvaraj)

Background: The chemokine CCL5 is known to play an important role in the formation of granuloma during infection with *M. tuberculosis*. Production of CCL5 is influenced by polymorphisms in the CCL5 gene.

Aim: To find out whether polymorphisms in the promoter and intron region of CCL5 gene are associated with susceptibility or resistance to PTB in south Indian population

Methods: Polymorphisms in the promoter (-403 G/A- and -28C/G) and intron (In1.1T/C) regions of CCL5 gene were studied in 212 PTB patients and 213 HCs, using PCR based RFLP methods.

The allele and genotype frequencies of various CCL5 genes are being analysed.

(iii) Stromal cell derived factor-1 (SDF-1/CXCL12) gene polymorphisms in PTB patients of south India

(Principal Investigator: Dr.P. Selvaraj)

Background: The chemokine, Stromal cell-derived factor-1 (SDF-1/CXCL12) regulates the trafficking of various types of leucocytes to areas of injury and infection. Polymorphisms in the CXCL12 gene influence CXCL12 levels and might be associated with the outcome of infection.

Aim: To find out whether polymorphisms in the intron and 3'untranslated region (UTR) of SDF-1/CXCL12 gene are associated with susceptibility or resistance to PTB

Methods: Genotyping of In2 +5887 (rs2839693) In2 +6201(rs266085) 3' UTR +12197 (rs1801157) 3' UTR +14478 (rs1065297) polymorphisms were investigated among 184 PTB patients and 187 healthy controls of south India using PCR based RFLP methods.

Results: The allele and genotype frequencies of various SDF-1 genes are being analysed.

(iv) Effect of vitamin D_3 on neutrophil Cathelicidin, Defensin-1α and TLR gene expression in PTB

(Principal Investigator: Dr.P. Selvaraj)

Background: Neutrophils are essential components of the human innate immune system and associated with the first line defense mechanism against invading microorganisms. Vitamin D_3, a potential immunomodulator, is known to influence innate and adaptive immunity. In the present study, the effect of vitamin D_3 on the innate immune functions of neutrophils in PTB is explored at the molecular level using real-time PCR.

Aim: To find out the vitamin D_3 effect on cathelicidin, defensin-1α and TLR gene expression in neutrophils of PTB patients

Methods: The study was carried out in 20 PTB patients and 20 healthy control subjects. Neutrophils

isolated from heparinized blood by Ficoll-Hypaque gradient centrifugation followed by sedimentation in 3% Dextran. Neutrophils were cultured for 18hrs with live *M. tuberculosis* and its culture filtrate antigen (CFA) in the presence and absence of vitamin D_3. The total RNA extracted was used for complementary DNA (cDNA) synthesis. The relative quantification for the target genes cathelicidin, defensin-1α, vitamin D receptor (VDR), Cyp27B1,TLR-2,4,8,9 and TIRAP and house keeping gene, β-actin was done using real time PCR (RT-PCR) with TaqMan assay primers and probes.

The results are being analysed.

(v) Effect of vitamin D_3 on chemokine expression in PTB

(Principal Investigator: Dr.P. Selvaraj)

Background: Chemokines are a family of small cytokines with molecular weight of 8–10 kDa. They are responsible for the activation of monocytes, macrophages and other leucocytes. Vitamin D_3, induces antimicrobial peptide cathelicidin expression and increases cell migration and secretion of signaling molecules such as cytokines and chemokines from activated cells. *M. tuberculosis* infection of macrophages results in the induction of various chemokines that are required for the formation of the tuberculous granuloma and inhibition of its growth. The present study is attempted to understand the effect of vitamin D_3 on various chemokine gene expression in TB.

Aim: To find out the effect of vitamin D_3 on chemokine expression in PTB

Methods: The study was carried out in 20 PTB patients and 20 healthy control subjects. Peripheral blood mononuclear cells were cultured with live *M. tuberculosis* and its CFA in the presence and absence of vitamin D_3 for 48 hrs. Chemokines such as MCP-1, Macrophage inflammatory protein-1α and -1β (MIP-1α, MIP-1β), inducible protein-10 and RANTES were estimated using commercially available cytometric bead array kit. Total RNA extracted from 48 hrs old macrophages was used for cDNA

synthesis. The relative quantification for the target genes MCP-1, MIP-1α, MIP-1β, IP-10 and RANTES and house keeping gene, glyceraldehyde-3 phosphate dehydrogenase (GAPDH) was done using RT-PCR with TaqMan assay primers and probes.

The study results are being analysed.

(vi) Effect of vitamin D_3 on intracellular expression of perforin, granulysin and regulatory T-cells in PTB

(Principal Investigator: Dr.P. Selvaraj)

Background: Protective immunity in TB is dependent on the co-ordinated release of cytolytic effector molecules from effector T-cells and the subsequent granule-associated killing of infected target cells. Vitamin D_3 is a potent modulator of macrophage and lymphocyte functions and enhances the exocytosis of cytolytic granules like perforin and granzymes and antimicrobial molecules such as granulysin from cytotoxic T-lymphocytes (CTL). T-regulatory (Treg) cells have been shown to suppress antimicrobial immune responses against intracellular pathogens and protect the host by preventing collateral damage from excessive inflammation. The present study was aimed to understand the effect of vitamin D_3 on regulatory T-cells and intracellular expression of various cytolytic molecules in PTB.

Aim: To find out the effect of vitamin D_3 on intracellular expression of perforin, granulysin and regulatory T-cells in PTB

Methods: The study is carried out in 20 PTB patients and 20 healthy control subjects. Peripheral blood mononuclear cells are cultured for 72 hrs with live *M. tuberculosis* and its CFA in the presence and absence of vitamin D_3. After 72 hrs, the cells were processed for immunostaining of CD4, CD8, CD25 and CD56 cell surface markers and intracellular perforin, granulysin and $Foxp3^+$ regulatory T-cells by using specific monoclonal antibodies and analyzed in flow cytometry.

The study is in progress.

Department of Pathology

Studies in progress:

Protein engineering of self-assembly systems for applications in nanoscience and nanotechnology

(Principal Investigator: Dr.V.D. Ramanathan; Funding: Department of Biotechnology)

Protein engineering studies of self-assembly systems for applications in nanoscience and nanotechnology have been initiated. Standardization experiments to test the immunogenicity of the vaccine constructs have been done. Further studies are in progress.

Department of Statistics

Studies in progress:

Modelling the spread of HIV/AIDS – A Stochastic approach

(Principal Investigator: Dr.P. Venkatesan)

The past six decades have witnessed unprecedented success in controlling infection disease, an achievement that has created great confidence in medicine's ability to conquer sickness. But AIDS has shaken the confidence and revived fears at least as old as the medieval plagues. Epidemics of fatal infection diseases are not unique in human history, but each is a unique event in its own time. Also there are important differences between AIDS and past epidemics, and between AIDS and other disease of our own time that exact a heavy human toll. It is a fatal infection, disease for which there is now no cure, and its sufferers appear to remain infectious for life.

The HIV/AIDS epidemic in not only the most important public health problem affecting large part of world, but also an unprecedented threat to the regions development. It is therefore, a development crisis. HIV/AIDS is also surpassing malaria as the leading cause of the death in many countries. The most disturbing long-term features of HIV/AIDS epidemic is its impact on life expectance making HIV unprecedented catastrophe in the world's history. Worldwide, AIDS hits people hardest in their most productive years. This profoundly disrupts the economic and social bases of families, when a family loses its primary income earner its very survival is threatened.

The epidemic evolves mainly by the infecting of CD4+ T- cells by HIV. When a person is infected with HIV the virus enters the body and lives and multiplies primarily in the white blood cell. These are the immune cells that normally protect us from disease. Hall mark of the HIV infection is the progressive loss of a CD4 cells. As the virus grows, it damages or kills these and other cells, weakening the immune system

and leaving the individual vulnerable to various diseases. By five years nearly all patients have abnormally low CD4+T lymphocyte levels and by 10 years, about half of those infected have developed AIDS.

Spread of the epidemic has varied considerable between developed and developing countries, depending on the culture as well as other social and behavioral patterns. Incidence rates have been the highest in developing countries where heterosexual transmission is most common. Poor awareness and knowledge about AIDS, increase in Sexually Transmitted Disease, long incubation period, and low social status of woman, poor medical services, associated social stigma and non availability of effective vaccine are the main reasons for the fast spread of HIV to all section of the population and across the country.

This disease has become a big social and medical burden in many countries. Various treatments have been developed with good success in slowing down disease progression. However, the high cost makes treatments unaffordable for most patients in developing countries. Moreover, long-term therapy is associated with several harmful side-effects and drug-resistance. As AIDS is incurable disease prevention is the best approach to protecting population at risk from infection.

The epidemic appears to have stabilized in most regions, although prevalence continues to increase in Eastern Europe and Central Asia and in often parts of Asia due to a high rate of new HIV infections. Sub-Saharan Africa remains the most heavily affected region, accounting for 69% of all new HIV infections.

India has a population of more than one billion, around half of whom are adults in the sexually active age group. The first AIDS case in India was detected in 1986 and since then HIV infection has been reported in all states and union territories. Demographically the second largest country in the world, India has also the third largest number of people living with HIV/AIDS. As per the HIV estimate of 2009, there are an estimated 24 lakhs people living with

HIV/AIDS in India. The HIV prevalence rate in the country is 0.29 percent. Most infection occurs through heterosexual route of transmission. However in the north-eastern region, injecting drug use in the major cause for the epidemic spread.

Information from persons testing positive for HIV at the integrated counseling and testing centers across the country during 2009-10 shows that 87.1 percent of HIV infections are still occurring through heterosexual routes of transmission while parent to child transmission accounts for 5.4 percent of HIV cases detected, injecting drug use 1.6 percent, men who have sex with men 1.5 percent and contaminated blood and blood products account for one percents.

The spread of HIV in India has been uneven. Although much of India has a low rate of injection, certain places have been more affected than others. HIV epidemics are more sever in the southern half of the country and far north east. The highest HIV prevalence rates are found in Andhra Pradesh, Maharashtra, Tamil Nadu, and Karnataka in the south, and Manipur and Nagaland in the north-east.

Available evidence on HIV epidemic in India shows a stable trend at national level. Number of people living with HIV in India 2009 is 24 lakhs. The primary drives of HIV epidemic in India are unprotected paid sex, unprotected sex between men and injecting drug use. The HIV prevalence in the country is .029% in 2008.

STOCHASTIC MODELS: Stochastic models assume that some of the important parameters are random variables. Since nature is basically stochastic and many variables are subject to stochastic variations. Stochastic models are more realistic than deterministic models. Stochastic models are range of values for variables in the form of probability distributions. It advantageous over both the stochastic model and statistical model when used alone since it combines information and advantages from both of these models.

Projections of HIV/AIDS using the statistical modeling approach are done based on the following three methods: The first method is based on fitting a model to the incidence of HIV/AIDS and extrapolating the curves into the future (Public Health Service 1986; Morgan and Curran, 1986). The estimates obtained using this method depends on the mathematical function used and hence some function can produce anomalous results. This model is also less efficient as this does not include important information on the epidemic like incubation period, infection density and nature of the spread of the epidemic. The second approach is based on modeling the dynamics of the epidemic (Gonzales et al 1987). This approach requires certain knowledge about mixing pattern of HIV individual with probabilities of infection per contact, size of high risk behavior group, probabilities of infection through blood product, needle sharing etc. In developing counties, knowledge about these key parameters is incomplete. Also stochastic modeling of the epidemic demands many parameters, which are generally difficult to estimate due to limitation of appropriate data especially in the Indian context. There is a lot of literature on deterministic and stochastic models for spread of HIV epidemic. The third most popular method is the back calculation method (Brookmeyer et al., 1986, 1988). This method is used to reconstruct the past pattern of HIV infection and to predict the future number of AIDS cases, apart from knowing the present infection status. This method depends on three important factors namely, the incubation period distribution, incidence curves and the observed number of AIDS cases over a time period. There are also uncertainties associated with this approach because lack of certain information about incubation period distribution, the effect of intervention therapy on incubation period and errors in reported AIDS incidence.

There are three types of people regarding HIV epidemic in the population: The susceptible (S) people, the infective (I) people and the clinical AIDS (A) People. A

person in S does not carry the AIDS virus, but can contact it through sexual contact with I or A or by sharing needles in IDU or through blood transfusion of contaminated blood. A person in I carries AIDS virus and can transmit the virus to people in S. There is a chance that the person will develop AIDS symptoms to become an AIDS case. A person in A can develop AIDS symptoms or CD4+ T – Cell counts falling below a threshold level (usually below 200 / mm^3).

Let S(t) , I(t), and A(t) denote the number of susceptible, infected and AIDS cases at time t. Let

$$\underset{\sim}{X}(t) = \{S(t), I(t), A(t)\}.$$

Let t_0=0 be the time at which a few HIV were introduced in to the population to start the AIDS epidemic. Then $\{(\downarrow\sim\uparrow X)(t), t \geq 0\}$ is a three dimensional stochastic process with parameter space T = {t>0} and with state space$(= \{(i, j, K), i, j, k \geq 0\}$.
This is a multidimensional stochastic process with discrete state space and continuous parameter space.
Similarly for the HIV pathogenesis a four dimension stochastic processes can be formulated: In an HIV infected individual, the time of HIV infection is zero. Then there are three types of CD4+ T- cells, the uninfected T cells (T_1), the latently infected T cells (T_2) and the productively HIV infected T cells (T_3).

Let $T_i(t)$ (I= 1,2,3) denote the number of t-cells T_i(i=1,2,3) at time t and let V(t) denote the number of free HIV at time t. Let

$$\underset{\sim}{X}(t) = \{T_1(t) \; i = 1, 2, 3, \quad V(t)\}.$$

Then $\{(\downarrow\sim\uparrow X)(t), t \geq 0\}$ is a four dimension stochastic process with parameter space T = {t ≥ 0} and with discrete state S = {(i, j, k), i, j, k ≥ 0}
In AIDS stochastic system, the processes can be characterized by a dependence condition referred to as the Markov condition. These processes are called as Markov processes.

Let {X(t), t∈ T}be a stochastic process with parameter space T and state space S. then X(t) is called a Markov process iff for every n and for every n and for every t_1<...<t_n≤t in T.

$$P\left\{X(t) \in \frac{A}{X(t_1)} = x_1, ..X(t_n) = x_n\right\} = P\left\{X(t) \in A \frac{\square}{\square} X(t_n) = x_n\right\}$$

for any event A $\subset$ S.

The above result states that the probability distribution of X(t) depends only on the results of the most recent time and is independent of history. Most of the processes in AIDS epidemiology are stochastic in native

A Markov process $\{\underset{\sim}{X}(t)$, t $\in$T} with state space S is called a Markov chain iff S in Discrete; A Galton – Watson process is a homogenous Markov chain.

A staged model of the AIDS epidemiology can be constructed by considering the effects of infection duration. The stage I is usually divided into sub stages I_1,….I_k with stochastic transitions between three stages (Longini et al 1991; 1996).

The stages can be classified in to 6 sub stages given by I_1 (CD4> 900/mm^3), I_2 (700 $\leq$ CD4 <900/mm^3), I_3 (500 $\leq$ CD4 <700/mm^3), I_4 (350 $\leq$ CD4<500/mm^3), I_5 (200 $\leq$ CD4 <350/mm^3), I_6 (CD4 < 200/mm^3). In general we have a (K+2) dimensional stochastic process. If the transition probabilities between the I_k are independent of the state S, the (K+2) dimensional stochastic process in Markov. This is also a Markov chain since the number of states is countably infinite. On the other hand if the transition probabilities defined on S, the process is not Markov. The non-Markovian process arises because of treatment of HIV infected individual by anti-viral drugs. The Markov models are elaborately studied in this work.

In this work we concentrate mainly on understanding the current states of epidemic and forecasting the future path via stochastic model. The broad objectives of the present research study are (i) to collect and unify the methods of construction Stochastic model in the projection of HIV/AIDS epidemic, (ii) to propose and investigate new models for HIV/AIDS projections and (iii) to provide HIV/AIDS estimates using surveillance date for India.

The work is in progress.

Biomedical Informatics

Completed studies:

(i) Molecular docking of azole drugs and their analogs on CYP121 of *M. tuberculosis*

(Principal Investigator: Dr. Luke Elizabeth Hanna)

The *M. tuberculosis* genome codes for 20 different cytochromes. These cytochromes are involved in the breakdown of recalcitrant pollutants and the synthesis of polyketide antibiotics and other complex macromolecules. It has been demonstrated that CYP121 is essential for viability of the bacterium by gene knock-out and complementation studies. CYP121 could therefore be a probable target for the development of new drugs for TB. It has been widely reported that orthologs of CYP121 in fungi are inhibited by azole drugs. We evaluated whether these azole drugs or their structural analogs could bind to and inhibit CYP121 of *M. tuberculosis* using molecular docking. Six molecules with known anti-CYP121 activity were selected from literature and PubChem database was searched to identify structural analogs for these inhibitors. Three hundred and sixty three molecules were identified as structural analogs and used in docking studies. Five molecules were found to consistently score better than the known azole drugs by two different scoring functions. These molecules may be further tested by *in vitro* experimentation for their activity against CYP121 of *M. tuberculosis*.

(ii) *In silico* identification of potential antigenic proteins and promiscuous CTL epitopes in *M. tuberculosis*

(Principal Investigator: Dr. Luke Elizabeth Hanna)

Cellular immune responses help to control *M. tuberculosis* infection. We hypothesized that those proteins of *M. tuberculosis* that do not have homologs in humans as well as human gut flora, would mount a

good antigenic response in man. A bioinformatics approach was employed to identify MTB antigens capable of inducing a robust cell-mediated immune response in humans. In the first step, we identified 624 MTB proteins that had no homologs in humans. Comparison of this set of proteins with the proteome of 77 different microbes that comprise the human gut flora narrowed down the list to 180 proteins unique to MTB. Further analysis of the 180 unique proteins revealed that a large number of these proteins belonged to the PE and PPE family. Fifty four of these proteins have been reported by other investigators as well, adding to their significance. Further, 19 promiscuous epitopes were identified from four representative antigenic proteins, and nine of them were observed to have >65% of population coverage. The shortlisted antigenic proteins can be further investigated for their immunological relevance and the use of the promiscuous epitopes as vaccine candidates may be considered.

(iii) Structural comparison of fold usage in *M. tuberculosis* proteins (Principal Investigator: Dr. Luke Elizabeth Hanna)

Emergence of strains of *M. tuberculosis* that have developed resistance to the present suite of antibiotics, has resulted in a dire need for development of novel drugs, which necessitates the identification of good targets. Advancement in technology has helped us to solve structures of several *M. tuberculosis* proteins and thereby aid in studying the structural and active site similarity between *M. tuberculosis* proteins. All 843 structures available for proteins encoded by 323 genes of *M. tuberculosis* with entries in SCOP database were analyzed. For structures with SCOP entries, each of the SCOP domains were analyzed for conserved fold usage within the genome of *M. tuberculosis*. We also identified 21 paralogs and 27 analogs of *M. tuberculosis* based on domains and EC classification. Of the 78 SCOP domains, 65 domains shared structural similarity to

proteins having similar class and fold whereas 13 proteins shared similarity to protein structures belonging to same class but different fold groups. Majority of the protein folds in *M. tuberculosis* proteins belonged to the α/β class proteins. Of the 23 different protein folds in *M. tuberculosis*, TIM barrel fold was identified to be highly conserved at very low sequence identity. From a practical perspective, understanding the structural homologues within the genome will help in selecting appropriate drug targets and designing small molecules.

Studies in progress:

(i) *In silico* screening of small molecules against Pantothenate Synthetase of *M. tuberculosis*

(Principal Investigator: Dr. Luke Elizabeth Hanna)

There is an urgent need for new drugs for TB since it is one of the global emergencies due to the emergence of MDR-TB and XDR-TB, co-infection with HIV and relapse after treatment. Virtual screening plays an important role in identifying lead compound in the process of drug discovery. We identified a set of prioritized targets and performed virtual screening to identify potential lead molecules.

Pantothenate is a metabolic product only in plants and microorganisms but not synthesized in human. Therefore, the pantothenate biosynthesis pathway could provide potential targets in *M. tuberculosis*. Pantothenate synthetase (PS), one of the enzymes from this pathway has been reported to be important for latency and virulence of *M. tuberculosis*. PS catalyzes the last step of pantothenate biosynthesis, the ATP-dependent condensation of pantoate and β-alanine to form pantothenate. The absence of an equivalent enzyme in humans makes it an attractive target for TB. We made an attempt using *in silico* methods to identify potential inhibitors of pantothenate of *M. tuberculosis*.

Two hundred and twenty eight molecules were selected from PubChem based on different criteria

and docked with PS using CDOCKER. Using scoring function PLP1, the docked compounds were ranked. Totally 37 molecules were found to rank higher than the positive controls (known substrates and reported inhibitors). The highly ranked molecules were energy minimized. Studies are on going to further refine the results.

(ii) Ligand-induced conformational changes in protein structures of *M. tuberculosis*

(Principal Investigator: Dr. Luke Elizabeth Hanna)

All protein/enzymes are flexible molecules due to the non-covalent nature of their folded 3D structure and therefore exist in a range of conformations often with relatively small energy differences between them. Advances in structural and molecular biology, as well as in biophysics, have led to the determination of high resolution atomic structures of many proteins. Currently, of the 828 structures encoded by 328 mycobacterial genes, 683 structures are in complex with ligands. The holo-complexes of proteins are further grouped based on functional (cognate) and non-functional ligands. In this study, we aim to study the conformational changes induced due to binding of the ligand and the nature of those changes.

(iii) *In silico* characterization of novel proteins of *M. tuberculosis*

(Principal Investigator: Dr. Alamelu Raja)

The present study involved *in silico* characterization of Rv1177, Rv1292, Rv2504c, Rv3503c and Rv2675c proteins. Three dimensional models, functional details and the active site information for these five genes were predicted. Of these, Rv1177, Rv3503c and Rv2675c were found to have no close homologues in the human genome. Rv1177 and Rv2675c are already reported to be essential genes for *M. tuberculosis* and are therefore important candidates for further studies. The predicted models will help in further understanding the function of these

proteins and can be used for screening small molecule database to identify probable lead molecules.

(iv) *In silico* characterization of Che12 Lysin A

(Principal Investigator: Dr. Vanaja Kumar)

Mycobacteriophages are viruses that infect mycobacterial host such as *M. tuberculosis*. The difficulty of treating TB and the emergence of drug resistance has encouraged investigations on whether mycobacteriophages could provide a complementary means of therapy. At the end of the lytic life cycle, mycobacteriophages express at least two enzymes: lysin which hydrolyses the cell wall components, and holin a small membrane protein that defines the time of lysis and allows the lysin to cleave its target. This leads to mycobacterial host cell lysis.

This study focused on Lysin A produced by mycobacteriophage Che12 (isolated in Chennai). *In silico* analysis of Che12 Lysin A revealed the presence of Chitinase class I domain in the C-terminal region of lysin A. The three dimensional structure for lysin A was modeled and its probable substrate was predicted. This will be further confirmed experimentally.

Epidemiology

Completed study:

(i) One time survey (Disease survey)

(Principal Investigator: Dr.C. Kolappan)

The One time survey was conducted in a sample of villages selected from villages in the BCG trial area not covered under epidemiological impact study. The study was started in March 2008 with a sample population size of 54110 (i.e. adult population is 41935 (77.5%) (Table16).

Table 16: Coverage for one time disease survey

Activities	Coverage
Enumeration	41983
Symptom screening	38827 (93%)
X-ray screening	37893 (90%)
Sputum eligible	4408
Sputum collection	4211 (96%)

152 Individuals were identified as cases through examination either by smear, culture or both.

Studies in progress:

(i) Estimation of prevalence of PTB in Chennai city

(Principal Investigator: Dr.C. Kolappan)

Since the prevalence of PTB is high in urban areas, this sample survey was started in the Chennai metropolitan city in August 2009. Assuming a prevalence of 400/100000 population, 25% precision, design effect of 1.3 and 25% loss to coverage, the sample

size was estimated to be 26,529. The sample size was distributed among 50 clusters in a cluster size of 531. The adult population (>15 years) to be enumerated in each ward was 600 (Slum 200, Non Slum 400). The coverage up to the period March 2011 is shown in table 17.

Table 17: Chennai disease survey

Activities	Coverage
Enumeration	13638
Symptom screening	12690 (93%)
X-ray screening	12472 (92%)
Sputum eligible	1298
Sputum collection	1154 (89%)

Twenty eight Individuals were identified as cases through examination either by smear or culture or both.

(ii) Sample survey to estimate tobacco use in urban, semi-urban and rural areas from Tamil Nadu

(Principal Investigator: Dr.C. Kolappan)

Tobacco use is considered to be one of the chief preventable causes of death in the world. The proposed survey attempts to collect detailed data on tobacco use from individuals 15 years and above through a community based sample survey in urban, semi-urban and rural areas in Tamil Nadu.

The national estimate of prevalence of consumption of tobacco in any form among men aged 15-54 years was 57% and that among women was 11% (source NFHS 3). With an overall prevalence of 28.3% required, precision of 10%, 90% coverage and design effect of 2, the required sample size was 2141. The same sample size was used for all the three centers namely urban, semi urban and rural.

Urban (Chennai) - No. of Wards : 31, adult population >= 15 years to be enumerated in each ward 90 (Slum

30 and Non Slum 60). Semi Urban (Ambattur) - No. of Wards: 10, adult population >= 15 years to be enumerated in each ward 240 (Slum 80 and Non Slum 160). Rural (Sriperumbuthur)- No. of villages:30; adult population >= 15 years to be enumerated in each village 90 (colony 30 and main village 60). The survey was started in August 2009. A written consent was obtained from all the participants. The registered population was screened for PTB and tobacco use by a structured questionnaire. Two sputum specimens were collected from all the study participants. At present rural and semi urban surveys have been completed. The over all coverage (up to March 2011) is shown in table 18.

Table 18: Tobacco use survey

Activities	Tobacco use survey
Enumeration	6818
Screened for tobacco use and TB	6397 (94%)
Tobacco users	1391 (22%)
Sputum collection	5793 (91%)
Smear and/or culture positives	49

Electronic Data Processing

The Department of Electronic Data Processing (EDP) provides computerized services for all departments in NIRT. NIRT departments have direct access to the data with their personal computers. The EDP division is continuing to give data management support including data entry/verification to various studies undertaken in the Centre. Also, this division generates reports and prepares pre-printed forms for field activity of large scale epidemiological studies and supply data tabulations for monitoring the studies and publication of research work.

Data entry, information process and e-mailing are the key requirements for our research organization. The existing IT equipments are being maintained under comprehensive annual maintenance contract. This includes managing the installation of the facilities and ensuring that the computers are maintained and kept up to-date.

The existing LAN facility has been well utilized by the researchers, students and trainees. The wireless internet facility was provided in the main building of NIRT and at Madurai unit of NIRT, Madurai.

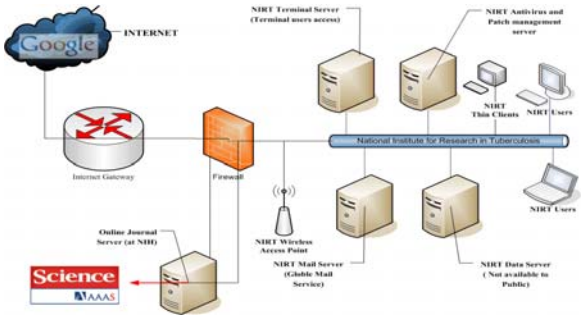
The quantum of documents of epidemiological, clinical, laboratory and program based studies entered and verified from April, 2010 to March, 2011 is shown below.

No. of documents entered	**1,00,506**
No. of documents verified	**1,01,316**

A total of 74,226 records were processed for the on-going one-time prevalence survey, Chennai disease prevalence survey and Tobacco use survey undertaken by epidemiology unit. The data base of the third repeat disease prevalence survey was cleaned and kept ready for data analysis during the period.

Data processing section

Computer networking



(Contact person: Mr.R. Subramani)

International Centre of Excellence in Research

Completed studies:

(i) Filarial lymphatic pathology reflects augmented Toll-like receptor-mediated, mitogen-activated protein kinase-mediated proinflammatory cytokine production

(Principal Investigators: Dr.S. Subash Babu / Dr.V. Kumaraswami)

Background: LF can be associated with the development of serious pathology in the form of lymphedema, hydrocele, and elephantiasis in a subset of infected patients. Toll-like receptors (TLRs) are thought to play a major role in the development of filarial pathology.

Objectives: To elucidate the role of TLRs in the development of lymphatic pathology, we examined cytokine responses to different Toll ligands in patients with lymphatic pathology (CP), infected patients with subclinical pathology (INF), and uninfected, endemic normal (EN) individuals

Results: TLR 2, 7 and 9 ligands induced significantly elevated production of Th1 and other pro-inflammatory cytokines in CP patients in comparison to both INF and EN. TLR adaptor expression was not significantly different among the groups; however, both TLR2 and TLR9 ligands induced significantly higher levels of phosphorylation of ERK1/2 and p38 MAP kinases as well as increased activation of NF-κB in CP individuals. Pharmacologic inhibition of both ERK1/2 and p38 MAP kinase pathways resulted in significantly diminished production of pro-inflammatory cytokines in CP individuals.

Conclusion: Our data, therefore, strongly suggest an important role for TLR2- and TLR9-mediated pro-inflammatory cytokine induction and activation of both the MAPK and NF-κB pathways in the development of pathology in human LF.

(ii) TLR- and filarial antigen-mediated, MAPK- and NF-κB-dependent regulation of angiogenic growth factors in filarial lymphatic pathology

(Principal Investigators: Dr.S Subash Babu / Dr.V. Kumaraswami)

Background: Filarial lymphatic pathology is of multifactorial origin, with inflammation, lymphangio-genesis, and innate immune responses all playing important roles. The role of TLRs in the development of filarial pathology has been well characterized. Similarly, the association of pathology with elevated levels of plasma angiogenic factors has also been documented.

Objectives: To examine the association between TLR function and the development of lymphangiogenesis in filarial infections, we examined TLR- and filarial antigen-induced expression and production of various angiogenic growth factors. We demonstrate that TLR ligands (specifically TLR2 and 5 ligands) induce significantly increased expression/production of vascular endothelial growth factor-A (VEGF-A) and angiopoietin-1 (Ang-1) in the peripheral blood mononuclear cells of individuals with lymphatic pathology (CP) compared with those of asymptomatic, infected individuals (INF)

Results: Similarly, filarial antigens induce significantly enhanced production of VEGF-A and VEGF-C in those with CP compared with INF. TLR2-mediated enhancement of angiogenic growth factor production in those with CP was shown to be dependent on MAPK and NF-κB signaling, as pharmacologic inhibition of either ERK1/2, p38 MAP, or NF-κB signaling resulted in significantly diminished production of VEGF-A and Ang-2.

Conclusion: Our data, therefore, strongly suggest an important association between TLR signaling and lymphangiogenesis in the development of pathology in human LF.

(iii) Role of biomarkers in pathogenesis of lymphatic filarial disease

(Principal Investigators: Dr.S. Subash Babu / Dr.V. Kumaraswami)

Background: LF can be associated with development of serious pathology in the form of lymphedema, hydrocele, and elephantiasis in a subset of infected patients. Dysregulated host inflammatory responses, lymphatic dysfunction, endothelial activation and extracellular matrix remodeling play central roles in filarial disease pathogenesis.

Objectives: To identify factors contributing to pathogenesis of disease in LF, we examined the role of microbial translocation markers (LPS, LBP, EndoCAb and sCD14); acute phase proteins [α-2 Macroglobulin (α-2 m), Haptogloblin, C-reactive proteins (CRP) and Serum Amyloid protein-A (SAA)]; angiogenic factors (VEGF – A, C, D, R1, R2 and R3 and Angiopoietin -1 and 2); pro- and/or anti-fibrotic factors (MMP – 1, 7, 8 and 9 and TIMP – 1, 2, 3 and 4) and pro-inflammatory cytokines (IFNγ, TNFα, IL-12, IL-1β, IL-6, IL-17 and GM-CSF) in chronic filarial pathology with (CP Ag+ (n=24)) or without (CP Ag- (n=65)) active infection as well as in asymptomatic, infected (INF (n=84)); and uninfected, endemic normal (EN (n=64)) individuals

Results: Markers that were significantly elevated in CP Ag+ compared to INF but not in CP Ag- compared to EN individuals were considered to truly reflect biomarkers of pathogenesis. CPAg+ individuals had significantly elevated plasma levels of LPS ($p=0.0001$) [Fig.31A], α2m ($p=0.0003$), haptogloblin ($p<0.0001$) and SAA ($p=0.0385$) [Fig.31B] among the microbial translocation and acute phase panels. Among the angiogenic growth and fibrotic factors, we found significantly elevated levels of VEGF-A ($p=0.0031$) and C ($p<0.0001$), VEGF-R1 ($p=0.0033$), R2 ($p<0.0001$), R3 ($p=0005$) and Angiopoietin-1 ($p=0.0481$) but not the MMP/TIMP family. In addition, a variety of pro-inflammatory cytokines including IFNγ, IL-12, GM-CSF ($p<0.0001$ for all) and IL-1β ($p=0.0073$) were significantly elevated in CP Ag+ individuals.

Conclusion: The elevated levels of these factors suggest quite strongly that the alteration of lymphatic integrity and peri-lymphatic inflammation should be implicated in the pathogenesis of lymphatic filarial pathology.

Fig.31A

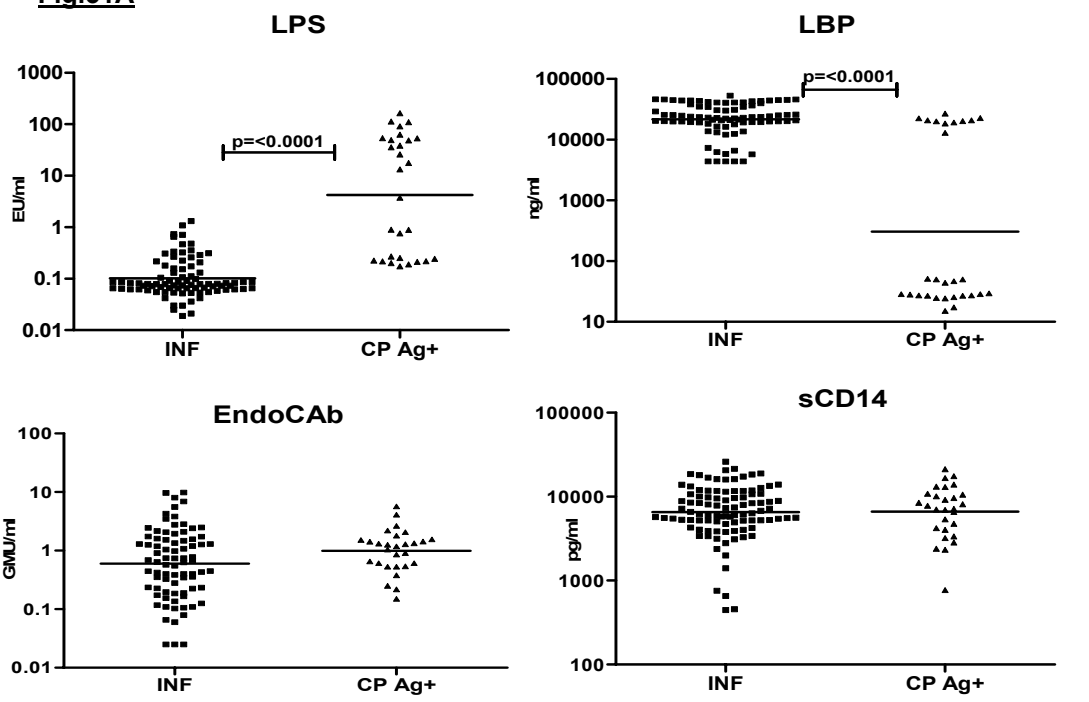
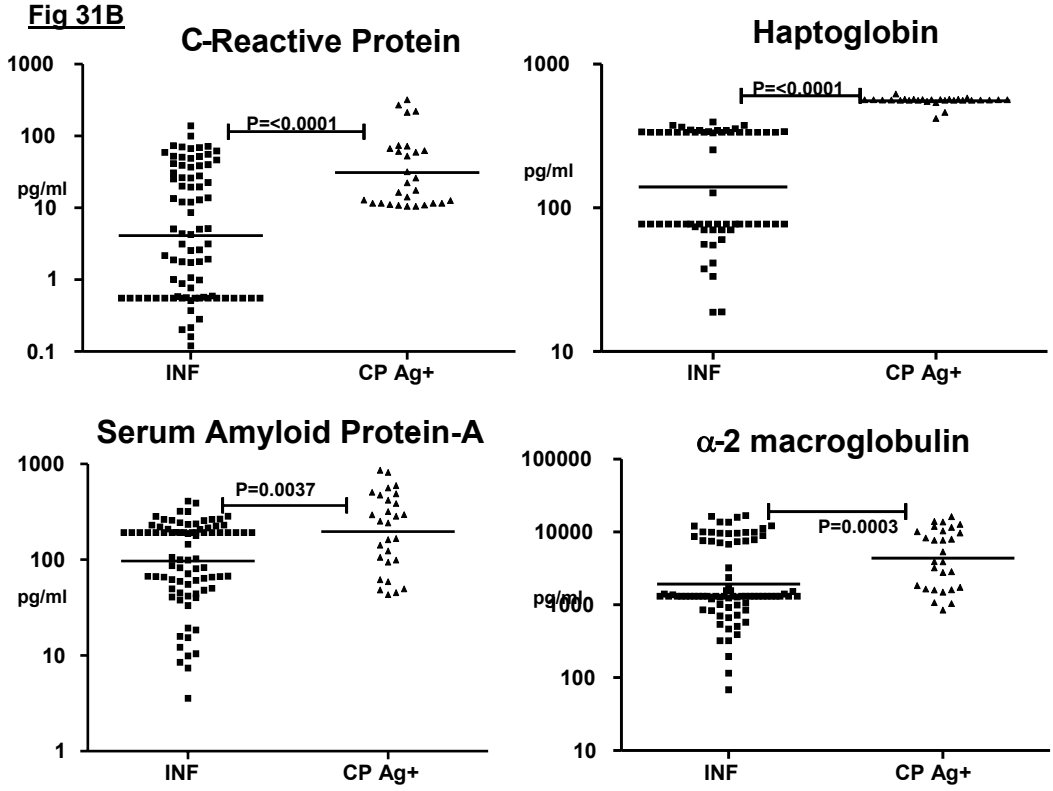


Fig 31B



(iv) Heightened measures of immune complex and complement function and immune complex-mediated granulocyte activation in human LF

(Principal Investigators: Dr.S. Subash Babu / Dr.V.D. Ramanathan)

Background: The presence of circulating immune complexes (CIC) is a characteristic feature of human LF. However, the role of CIC in modulating granulocyte function and complement functional activity in filarial infection is unknown.

Objective: The levels of CIC in association with complement activation in clinically asymptomatic, filarial-infected patients (INF); filarial-infected patients with overt lymphatic pathology (CPDT) and uninfected control individuals (EN) were examined.

Results: Significantly increased levels of CIC and enhanced functional efficiency of the classical and Mannose-binding lectin (MBL) pathway of the complement system was observed in INF compared to CPDT and EN. PEG-precipitated CIC from INF and CPDT patients induced significantly increased granulocyte activation compared to those from EN, determined by the increased production of neutrophil granular proteins as well as a variety of pro-inflammatory cytokines.

Conclusion: Thus, CIC mediated enhanced granulocyte activation and modulations of complement function are important features of filarial infection and disease.

(v) Decreased prevalence of LF among diabetic subjects

(Principal Investigators: Dr.S. Subash Babu / Dr.V. Kumaraswami / Dr.V. Aravindhan)

Background: Epidemiological and animal studies have shown an inverse correlation between the incidence of helminth infections including LF and the incidence of atopy and autoimmunity.

Objective: However, the interrelationship between LF and Type-1 and Type-2 diabetes (T1DM and T2DM, respectively) in humans is not known and hence, two cross sectional studies to assess the baseline prevalence and the

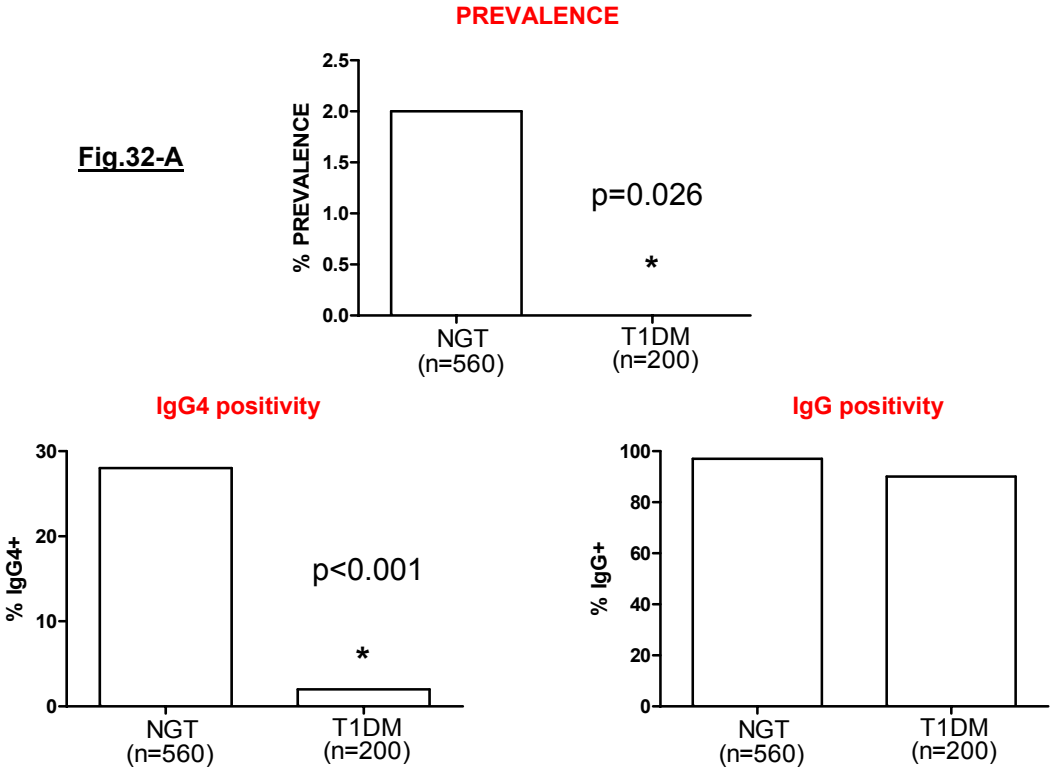
correlates of sero-positivity of LF among diabetic subjects were undertaken in Chennai, India

Results: In the first study, there was a significantly lower prevalence (p=0.026) of LF among T1DM subjects (0%; n=200) compared to non-diabetic subjects (2.6%; n=500) [Fig.32A] providing validation for animal data showing the protective effect of filarial infection on T1DM. More importantly, in the second study, there was a significantly lower prevalence of LF among T2DM subjects (both newly diagnosed [5.7%; n=158] and those under treatment [4.3%; n=161]) compared to pre-diabetic subjects [9.1%; n=154] (p=0.0095) and non-diabetic subjects [10.4%; n=943] (p=0.0463). Among those with filarial infection, there were significantly lower filarial antigen loads among T2DM subjects compared to non-diabetic subjects (Geometric Mean of 354 U/ml in T2DM vs. 1594 U/ml in non-diabetic subjects; p=0.04) [Fig.32B]. Serum levels of the pro-inflammatory cytokines - IL-6 and GM-CSF were significantly lower in T2DM subjects who were LF positive compared to those who were LF negative. There were, however, no significant differences in serum levels of the anti-inflammatory cytokines, IL-10, IL-13 and TGF-β between the two groups.

Conclusion: Thus, there appears to be a striking inverse relationship between the prevalence of LF and diabetes, which is reflected by a diminished serum pro-inflammatory cytokine response in subjects with diabetes and concomitant LF.

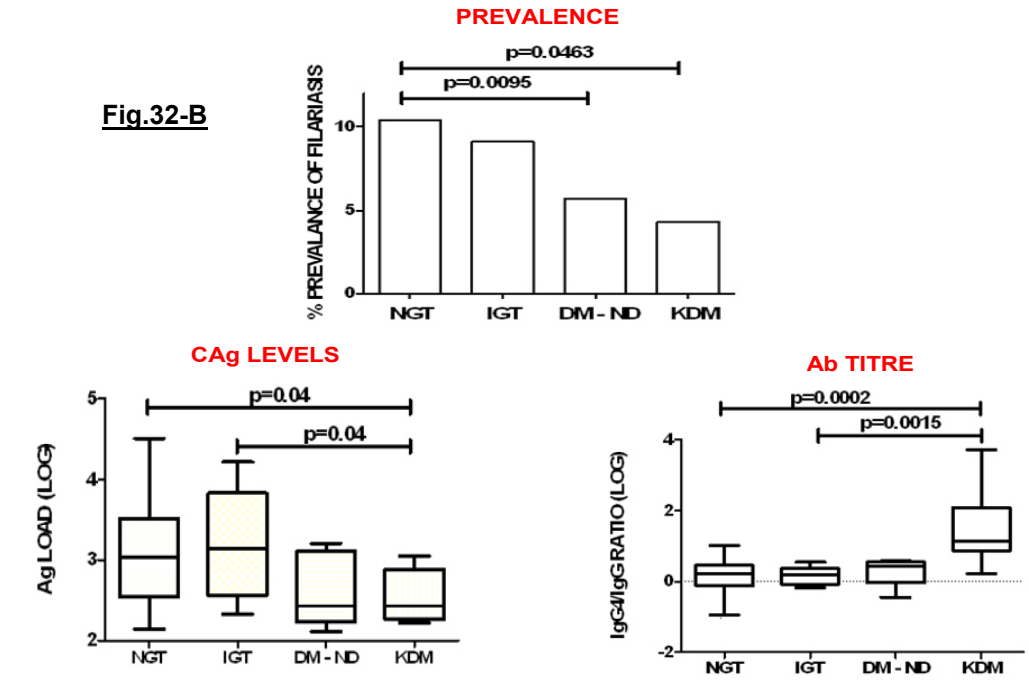
Prevalence of filarial infection in Type 1 DM

Fig.32-A



Prevalence of filarial infection in Type 2 DM

Fig.32-B



(vi) Prevalence of LF among subjects with coronary artery disease

(Principal Investigators: Dr.S. Subash Babu / Dr.V. Kumaraswami / Dr.V. Aravindhan)

Background: Helminth infections, in contrast to viral and bacterial infections can confer protection against metabolic disorders like diabetes by immunomodulation. Previously, we have shown decreased prevalence of LF among type-2 diabetic subjects which was associated with significant immunomodulation.

Objective: In the present study, the baseline prevalence and the correlates of sero-positivity of LF among subjects without (n=236) and with (n=217) coronary arterial disease (CAD) was carried out as part of the Chennai Urban Rural Epidemiology Study (CURES)

Results: In contrast to diabetes, the prevalence of LF was not significantly different between control and CAD subjects. LF antigen load and antibody levels in the serum indicated comparable levels of infection and exposure between the groups. Serum cytokine profile showed a moderate upregulation of inflammatory markers in LF positive compared to LF negative CAD subjects. Further, defects in serum pancreatic and gut hormones and adipocytokines were seen in CAD subjects which was unaffected by the LF status.

Conclusion: Although a direct causal link is yet to be shown, unlike type-2 diabetes there appears to be a no association between the prevalence of LF and CAD, in the Asian Indian population.

(vii) Suppressed type 1, type 2 and type 17 cytokine responses in active TB in children

(Principal Investigators: Dr.S. Subash Babu / Dr.V. Kumaraswami)

Background: Type 1 cytokine responses are known to play an important role in immunity to TB in children, although little is known about other factors that might be important. In addition, children are more prone to develop

extrapulmonary manifestations of TB compared to adults.

Objective: To identify the immune responses important both in control of infection and in extrapulmonary dissemination, we examined mycobacteria–specific cytokine responses of children with PTB and extrapulmonary TB (ETB) and compared them with those of healthy control children (HC)

Results: Although there were no differences between those with PTB and ETB, children with active TB compared with HC showed markedly diminished production of Types 1 (IFN-γ and TNF-α), 2 (IL-4 and IL-13), and 17 (IL-17A, IL-21, and IL-23) -associated cytokines at homeostasis as well as in response to mycobacterial Ags. [Figs.33A–33D) This was not associated with significantly altered production of IL-10 or TGF-β. Among children with ETB, those with neurologic involvement exhibited more significantly diminished Ag-driven IFN-γ and IL-17 production.

Conclusion: Pediatric TB is characterized by diminished Types 1, 2 and 17 cytokine responses, with the most profound diminution favoring development of neurologic TB, suggesting a crucial role for these cytokines in protection against pediatric TB.

Fig.33-A Non-stimulated

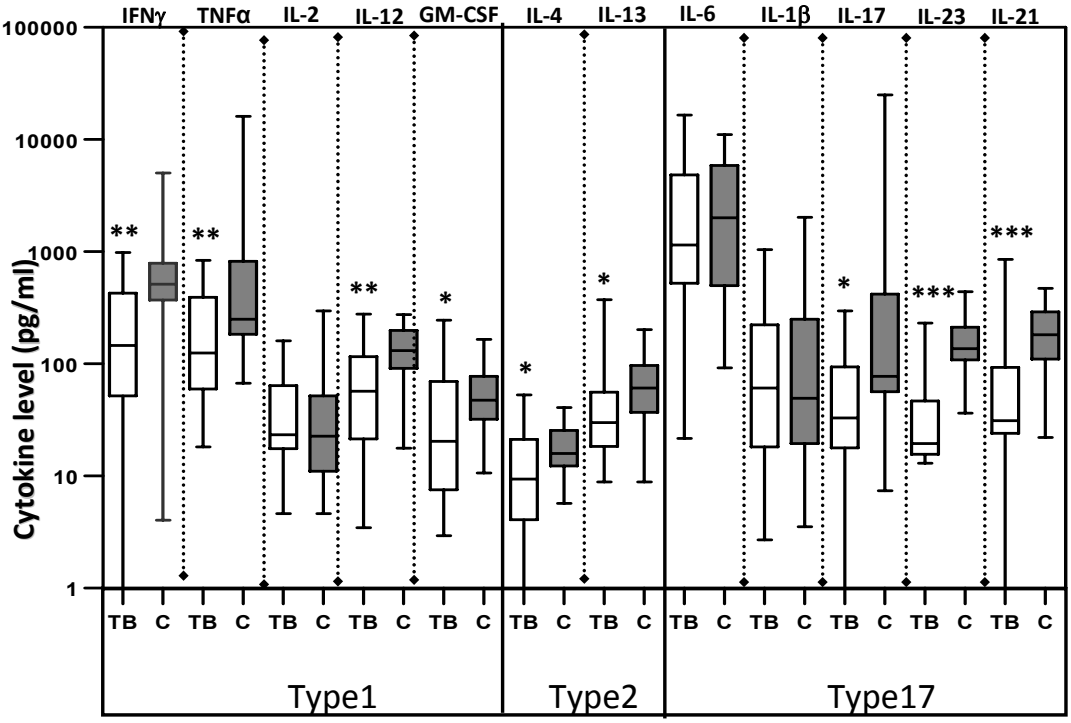


Fig.33B αCD3

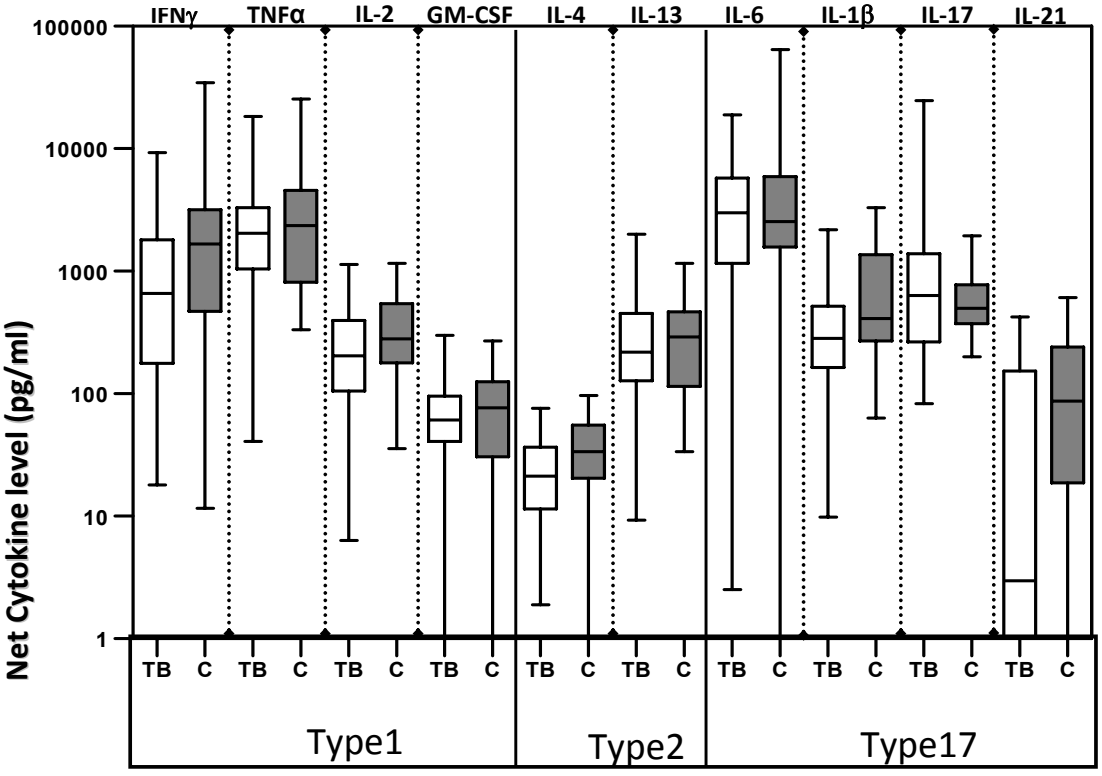


Fig.33C PPD

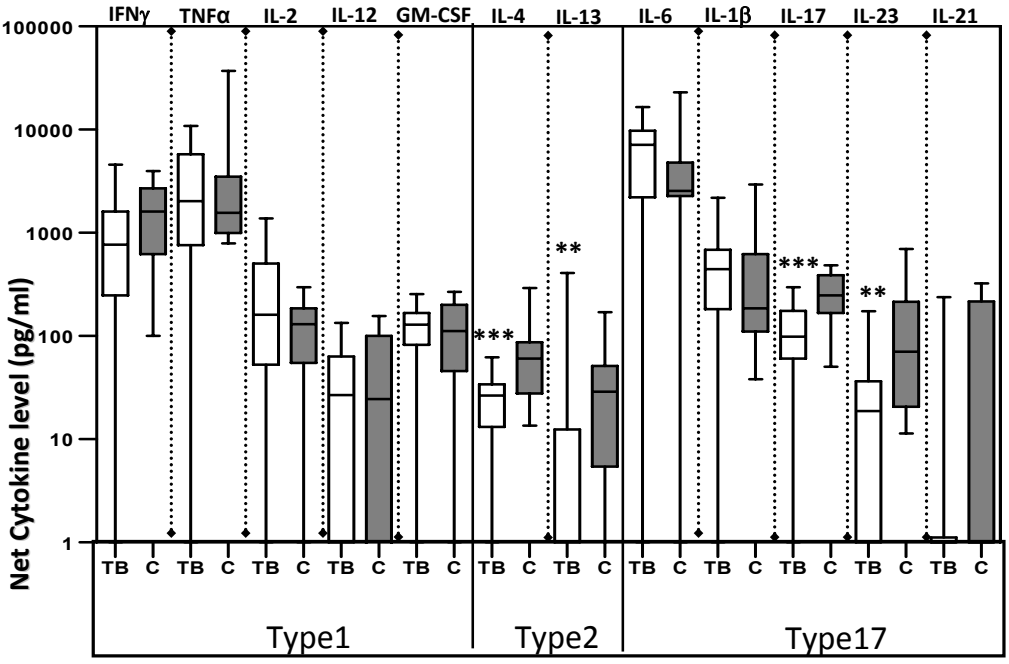
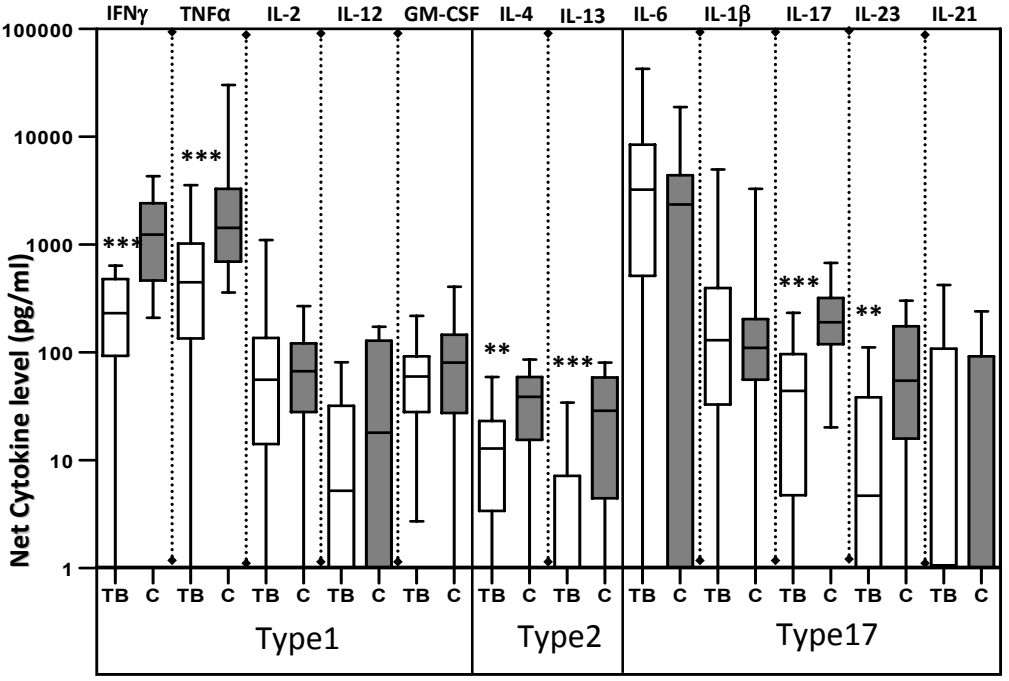


Fig.33D CFP



(viii) Biomarkers and pediatric TB

(Principal Investigators: Dr.S. Subash Babu / Dr.V. Kumaraswami)

Background: TB in children is not only more likely to cause more severe disease than seen in adults; it is also more likely to be extra-pulmonary. Moreover, pediatric TB is very difficult to diagnose and suffers from a lack of understanding of host biomarkers to monitor progression of disease.

Objective: We sought to identify the immune responses important in control of infection as well as extra-pulmonary dissemination we examined a variety of plasma biomarkers in children with active TB (n=36) and compared them to those of healthy children (HC) (n=19)

Results: Children with active TB, when compared to HC, showed markedly elevated plasma levels of the acute phase proteins – CRP (p=0.010) and a2-macroglobulin (p=0.037) but not haptoglobin or serum amyloid P. In addition, children with TB also had significantly elevated levels of matrix metalloproteinases (MMP-1 (p=0.006), 7 (p=0.042) and 8 (p=0.025) among the various MMPs examined) and TIMP-1 (p=0.005). [Fig.34A] Although there were no significant differences in the levels of Type 1- (IFNγ, TNFα, IL-2 and GM-CSF), Type 2- (IL-4, IL-5, IL-10, IL-13, IL-20 and IL-33), Type 17- (IL-17A, IL-23, IL-21, IL-1β and IL-6) or Type 1 interferon- (IFNα and IFNβ) associated cytokines between the two groups, pediatric TB was associated with elevated plasma TGFβ (p=0.036), and markedly depressed IL-22 (p<0.0001), compared to HC. In addition, those children with TB and extra-pulmonary dissemination, exhibited significantly diminished levels of IL-20 (p=0.009), TGFβ (p=0.0005) and MMP-7 (p=0.031) [Fig.34B] in comparison to children with active PTB.

Conclusion: Thus, pediatric TB is characterized by elevated levels of acute phase proteins, MMPs/TIMP-1, IL-22 and TGFβ, suggesting that these responses may play a crucial role in protection against disease. It is also possible that these analytes

may be used as biomarkers to monitor the progress of disease.

Fig. 34A

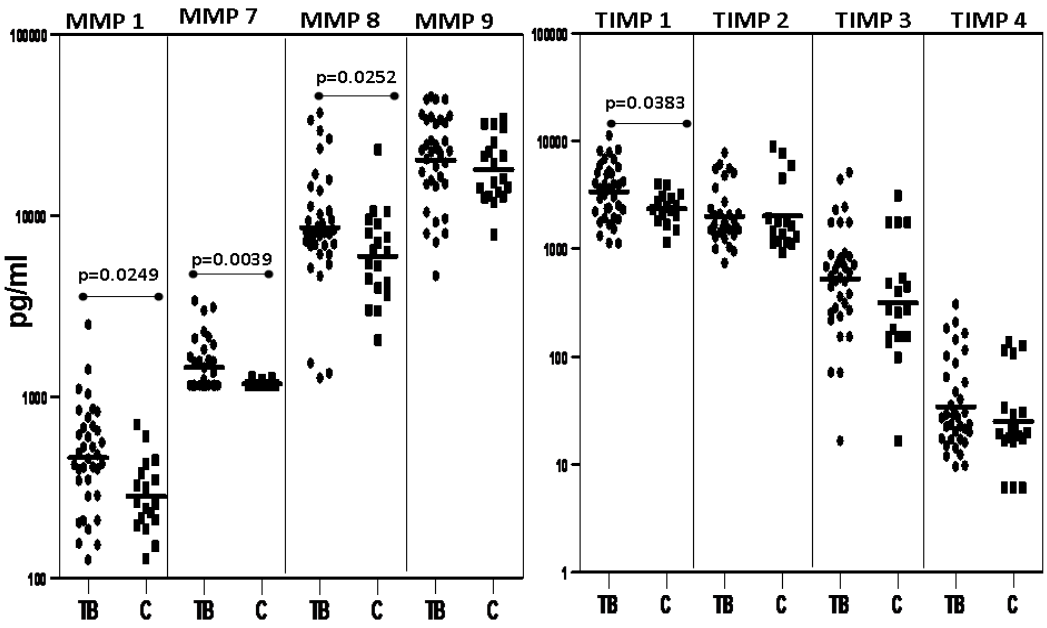
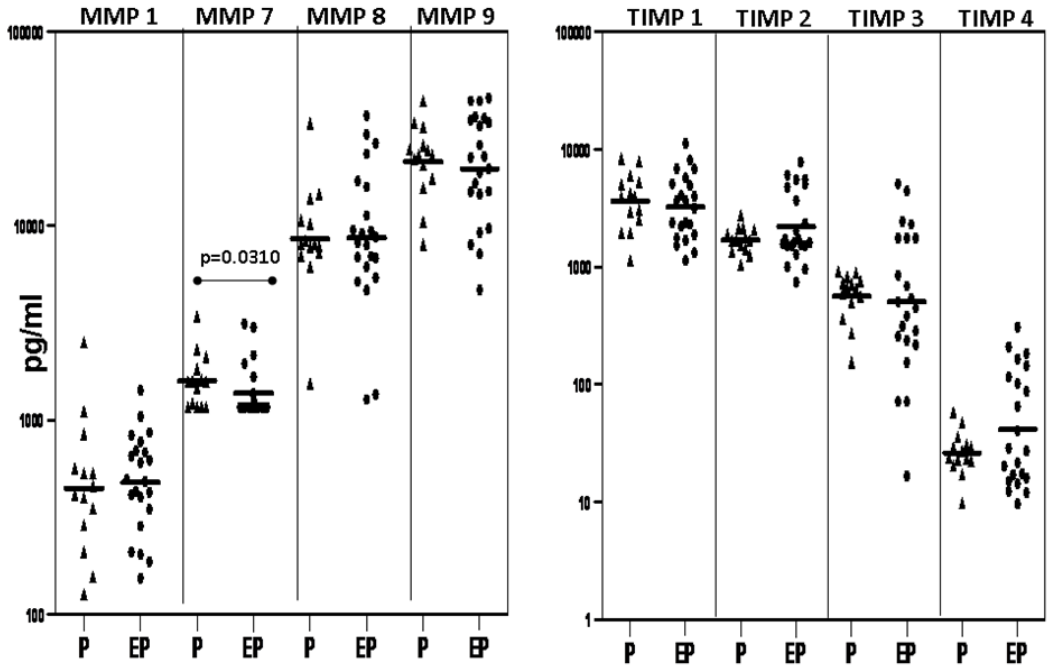


Fig. 34B



Studies in progress:

Characterization of immune response in pulmonary and extra-PTB

(Principal Investigators: Dr.S. Subash Babu / Dr.M.S. Jawahar)

The immune responses in latent TB are poorly understood. While it is difficult to define the onset of latency during natural infection, patients undergoing treatment for TB are driven into a state of latency or cure. The present study on the effect of 3 and 4 month regimens containing MFX in sputum smear and culture positive PTB (TRC Study number 24) offers us the opportunity to study definitive immune responses pre and post treatment. We will evaluate a variety of innate and adaptive immune responses in patients before and after treatment and our study will compare the differences in immuno-phenotype (eg. Markers of T, B and NK cell activation, proliferation and regulatory phenotype) and function (eg. Production of cytokines, proliferative responses to TB antigens) at different time points following treatment. In addition, since a small percentage of patients will undergo relapse following treatment, the kinetics of immune responses in these patients will used to assess immunological predictors of relapse in TB. In addition, we are also trying to determine immunological differences between PTB, extrapulmonary TB, latent TB and uninfected individuals. We have performed ex vivo phenotyping on a variety of T, B, NK, DC and monocyte markers including regulatory T-cells, plasmacytoid and lymphoid dendritic cells, regulatory B cells and inflammatory monocytes on more than 70 PTB, 40 ETB, 20 LTB and 40 uninfected individuals, thus far.

Tuberculosis Research Centre (ICMR)
INSTITUTIONAL ETHICS COMMITTEE
Mayor V R Ramanathan Road, Chetput, Chennai – 600 031, Tamil Nadu, India

Annual Report 2010

Tuberculosis Research Centre Institutional Ethics Committee conducted five meetings (four scheduled and one unscheduled) during the calendar year 2010. All the meetings satisfied the quorum requirements.

The Chair, the Member Secretary, and two members were present during all the 5 meetings; out of the remaining six members, two members were present for 4 meetings, two members were present for 3 meetings, one member was present for 1 meeting, and one member missed all the 5 meetings.

Seven new protocols and five re-submissions were reviewed during the year 2010. Thirty five ongoing reviews and four expedited reviews (four protocols) were also conducted.

Projects reviewed	Tuberculosis	HIV	HIV TB	Filariasis	Vaccine	**Total**
Clinical Trials	12	4	4	1	2	**23**
Public Health						
Epidemiology	2					**2**
Programme OR	4		1			**5**
Laboratory						
Bacteriology	2	1				**3**
Immunology	6	3	1			**10**
Pharmacology		2	1			**3**
Social Sciences	2	3				**5**
Total	**28**	**13**	**7**	**1**	**2**	**51**

Five case study files were closed as the projects were completed and summary report submitted.

Out of the 7 new protocols, six protocols were approved in one submission and one protocol was approved in two submissions.

Some of the salient features that figured during the year 2010 were:

1. 'Continued Bioethics Education' discussions were continued during the course of the meetings and via electronic mail
2. In continuation with TRC's US Federal Wide Assurance, an annual report on possible research misconduct for the year 2009 was filled with the Office of Research Integrity (ORI), US Department of Health & Human Services

Selected highlights of ethical concerns:

- *Over the years, both during initial and continuing review, the Committee had addressed ethical issues regarding conduct of randomized controlled trials for shortening tuberculosis treatment duration, phase I vaccine trials, pediatric studies, and multi centric industry driven research, to name a few*
- *The Committee was concerned about the inordinate delay between the SAC approval and submission for ethical clearance as evident by the low protocol submission rate during 2010*

Member Secretary **Chair**

Library & Information Centre

The Library makes significant investiments in acquiring e-journals. It has developed and improved its core services and products this year. It strives to keep pace with a dynamic and technology-enabled information environment to meet the expectations of its users. For providing access to the e-journal literature a customized Digital Library portal has been evolved. It provides web-based access to full-text journals and databases being subscribed by the library and open access journals. It enhances a customized integrated (24hrs) access (browse; search; locate; download) facility to our patrons; all it needs few mouse click.

e-Resources @ Library

Individual Titles	**Resource Sharing** ▪ JCCC@ICMR
Cumulative Collection ▪ American Society for Microbiology	**e-Bundle** ▪ Annual Reviews Life Science
Data Bases ▪ ERMED ▪ IndiaHealthStat ▪ J-Gate ▪ MD Consult ▪ OvidSP	**Archives** ▪ AIDS ▪ ASM ▪ Annual Reviews Life Science ▪ JAIDS ▪ Nature ▪ Science Classic ▪ Scientific American
Consortia BMJ; Lancet; Nature; NEJM; Science	**Package** Nature Life Science Package
Subject Collection ▪ ScienceDirect: o Immunology & Microbiology	**e-Books** ▪ Books@MDConsult ▪ Books@OvidSP

SERVICES

The library offers a range of value added services including:

- access to electronic resources
- circulation
- current awareness service
- document delivery service (Print&Soft)
- e-Mail co-ordination
- Inter Library Loan
- Internet Lab
- Publication (in-house)
- Reference assistance
- Resource Sharing
- Facilitating digital medial resources and web based services.

The TRC Web Site is developed and being maintained by the library.

PUBLICATION

As part of Value Added Services, a monthly publication, "**TB Alert**" is being published among ICMR institutes. The publication comprises of bibliographical details of the latest tuberculosis related articles published internationally. Each article has been given pointer/interlinks to URL.

(Contact person: Mr.R. Rathinasabapati)

APPENDICES

LIST OF PUBLICATIONS

Publications in Journals : 49

Published	i)	International	: 34
	ii)	National	: 15
Others - Books			: 4
Accepted	i)	International	: 30
	ii)	National	: 5

International:

2010:

1. Aravindhan V, Mohan V, Surendar J, Rao MM, Pavankumar N, Deepa M, Rajagopalan R, Kumaraswami V, Nutman TB, Babu S. Decreased prevalence of lymphatic filariasis among diabetic subjects associated with a diminished pro-inflammatory cytokine response (CURES 83). PLoS Negl Trop Dis.2010;4:1-6. (IF:4.752) (Cit.1)

2. Aravindhan V, Mohan V, Surendar J, Rao MM, Ranjani H, Kumaraswami V, Nutman TB, Babu S. Decreased prevalence of lymphatic filariasis among subjects with type-1 diabetes. Am J Trop Med Hyg.2010;83:1336-1339. (IF:2.446)

3. Basirudeen S, Venkatesan P, Paulkumaran P, Raja A. Yield of QuantiFERON-TB gold in tube assay and tuberculin skin test in healthy persons from a tuberculosis endemic population. J Pediatr Infect Dis.2010;5:125-129.

4. Basirudeen S, Balambal R, Thomas A, Venkatesan P, Raja A. Role of QuantiFERON-TB gold, interferon gamma inducible Protein-10 and tuberculin skin test in active tuberculosis diagnosis. PloS ONE.2010;5:1-7. (IF:4.411)

5. Bennuru S, Maldarelli G, Kumaraswami V, Klion AD, Nutman TB. Elevated levels of plasma angiogenic factors are associated with human lymphatic filarial infections. Am J Trop Med Hyg.2010;83:884-890. (IF:2.446)

6. Charles N, Thomas BE, Watson B, Raja Sakthivel M, Chandrasekeran V, Wares F. Care seeking behavior of chest symptomatics: A community based study done in south India after the implementation of the RNTCP. PLoS ONE;2010:5:1-6. (IF:4.411)

7. Goletti D, Raja A, Basirudeen S, Rodrigues C, Sodha A, Butera O, Carrara S, Vernet G, Longuet C, Ippolito G, Thangaraj S, Leportier M, Girardi E, Lagrange PH. IFN-γ, but not IP-10, MCP-2 or IL-2 response to RD1 selected peptides associates to active tuberculosis. J Infect.2010;61:133-143. (IF:3.805)(Cit.9)

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9. Hassan S, Dusthackeer A, Subramanyam B, Ponnuraja C, Sivaramakrishnan GN, Kumar V. Lytic efficiency of mycobacteriophages. The Open Systems Biol J.2010;3:21-28.

10. Joseph J, Hassan S, Rajendran V, Kumar V. Microbial genome databases: A user's perspective. Int J Pharma and Bio Sciences.2010;V1:1-9.

11. Kumar M, Raja A. Cytotoxicity responses to selected ESAT-6 and CFP-10 peptides in tuberculosis. Cell Immunol.2010;265:146-155. (IF:2.575)

12. Muniyandi M, Rajeswari R, Balasubramanian R, Thomas A, Santha T, Narayanan PR. India's Revised National Tuberculosis Control Programme (RNTCP): Budget and performance. J Health Management.2010;12:261–271.

13. Ponnuraja C, Venkatesan P. Survival models for exploring tuberculosis clinical trial data-an empirical comparison. Ind J Sci Technol.2010;3:755-758.

14. Rama R, Swaminathan R, Venkatesan P. Cure models for estimating hospital-based breast cancer survival. Asian Pacific J Cancer Prev.2010:11:387-391.

15. Ramana Rao PV, Ramanavelan S, Rajasekaran S, Raja A. Natural-killer cell-derived cytolytic molecules in HIV-associated pulmonary tuberculosis—Role of exogenous interleukins. J Clin Immunol.2010;30:393-401. (IF:3.326) (Cit.1)

16. Rao VG, Gopi PG, Bhat J, Selvakumar N, Yadav R, Tiwari B, Gadge V, Bhondeley MK, Wares F. Pulmonary tuberculosis: a public health problem amongst the Saharia, a primitive tribe of Madhya Pradesh, Central India. Int J Infect Dis.2010;14:713-716. (IF:2.529) (Cit.1)

17. Selvaraj P, Harishankar M, Singh B, Jawahar MS, Banurekha VV. Toll-like receptor and TIRAP gene polymorphisms in pulmonary tuberculosis patients of south India. Tuberculosis.2010;90:306-310. (IF:2.650) (Cit.5)

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24. Swaminathan S, Padmapriyadarsini C, Narendran G. HIV-associated tuberculosis: Clinical update. Clin Infect Dis.2010;50:1377-1386.(IF:8.186) (Cit.9)

25. Swaminathan S, Rekha B. Pediatric tuberculosis: Global overview and challenges. Clin Infect Dis.2010;50:S184–S194. (IF:8.186) (cit.20)

26. Swaminathan S, Padmapriyadarsini C, Yoojin L, Sukumar B, Iliayas S, Karthipriya J, Sakthivel R, Gomathy P, Thomas BE, Mathew M, Wanke CA, Narayanan PR. Nutritional supplementation in HIV-infected individuals in south India: A prospective interventional study. Clin Infect Dis.2010;51:51-57. (IF:8.186) (Cit.7)

27. Uhler LM, Kumarasamy N, Mayer KH, Saxena A, Losina E, Muniyandi M, Stoler AW, Lu Z, Walensky RP, Flanigan TP, Bender MA, Freedberg KA, Swaminathan S; CEPAC International investigators. Cost-effectiveness of HIV testing referral strategies among tuberculosis patients in India. PLoS One.2010;5:1-9. (IF:4.411)

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2. Hemanth Kumar AK, Sudha V, Swaminathan S, Ramachandran G. Comparison of HPLC & spectrophotometric methods for estimation of antiretroviral drug content in pharmaceutical products. Indian J Med Res.2010;132:390-394. (IF:1.826)

3. Muruganathan A, Thomas A, Muniyandi M, Chandrasekaran V. Revised National Tuberculosis Control Programme (RNTCP). J Indian Med Assoc.2010;108:868-70.

4. Nalini S, Lakshmi R, Devika K, Ravikumar D, Ramachandran R. Combined drug medium with isoniazid and rifampicin for identification of multi-drug resistant *Mycobacterium tuberculosis.* Indian J Med Microbiol.2010;28:162-163. (IF:1.006) (Cit.1)

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1. Madhan Kumar M, Tamilmani K, Raja A. Cytokine and chemokine responses to selected early secreted antigenic target-6 and culture filtrate protein-10 peptides in tuberculosis. J Interferon Cytokine Res.2011;31: 299-307. (IF:2.576)

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6. Suhadev M, Mahadevan U, Dilip M, Suryanarayanan D, Sikhamani R, Thomas B. Percentages, process, and patterns of HIV disclosure among the spouses of HIV-infected men in south India. J Int Assoc Physicians AIDS Care (Chic).2011;10:26-29. (Cit.1)

7. Swaminathan S, Kabra SK. Childhood tuberculosis challenges and way forward. Indian J Pediatr.2011;78:319-320. (IF:0.502)

National:

1. Radhakrishnan M, Kumar V, Selvakumar N. Mycobacteria in environmental clean-up. ENVIS.2011;2-5.

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1. Prabhu Anand S, Selvaraj P. Vitamin D3 and Immunity to Tuberculosis. Chapter 3 - In: Vitamin D: Nutrition, Side Effects and Supplements. E-Book. 2010:89-104. 2010. Edited by: Stephanie R. Malone. Nova Science Publishers, Inc.

2. Selvaraj P. Vitamin D, Vitamin D Receptor, and Cathelicidin in the treatment of Tuberculosis. Chapter 13 - In: Vitamins and Hormones: Vitamins and the Immune system. 2011:86;307-325. Edited by: Gerald Litwack. Elsevier, Inc. Academic Press.

3. Jaggarajamma K, Thomas BE. Challenges in dealing with the problem of Tuberculosis-Experiences from Tuberculosis Research Centre, Chennai, India'. Proceedings of 2010 International Conference on Humanities, Historical and Social Sciences (CHHSS2010) ISBN:978-1-84626-025-4;474-477.

4. Suhadev M, Mahadevan U, Thomas BE, Swaminathan S. Women as carers: Burden of the wives of HIV infected men in South India. Proceedings of 2010 International Conference on Humanities, Historical and Social Sciences (CHHSS2010) ISBN: 978-1-84626-025-4; 454-458.

Accepted:

International:

1. Azger D, Balaji S, Gomathi NS, Selvakumar N, Vanaja K. Diagnostic luciferase reporter phage assay for active and non replicating persistors to detect tubercle bacilli from sputum samples. Clin Microbiol Infect.

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mediated, Mitogen-activated protein kinase-mediated proinflammatory cytokine production. Infect Immun.

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5. Devi NP, Chandrasekaran K, Bhavani PK, Thiruvalluvan C, Swaminathan S. Persistence of stunting after highly active antiretroviral therapy in HIV-infected children in south India. Indian Pediatr.

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29. Venkatesan P, Sundaram N. Exponentiated exponential modes for survival data. Indian J Sci Technol.

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National:

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2. Raghu B, Venkatesan P. Relationship between Cigarette Smoking and Novel Risk Factors for Cardiovascular Disease. Biomedicine.

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Awards/Honours

- Patent filed for “Improved luciferase reporter phage assay for rapid detection of dormant and active tubercle bacilli from clinical samples: Indian Patent Application No.: 2530/DEL/2010 dt.22.10.2010 – Dr. Vanaja Kumar.

- Patent filed for “New antituberculous antibiotic from marine actinomycete strain R2 – Indian Patnet Application No. 247/DEL/2011 dt. 02.02.2011 – Dr. Vanaja Kumar.

- ‘Dr.P.K. Sen TAI Gold Medical Oration’ during NATCON 2010 held at Bengaluru during January 2011 – Dr.N. Selvakumar.

- Tamil Nadu Tuberculosis Association (Medicine) Endowment lecture (2010-11) – Newer approved diagnostic tools in tuberculosis during February 2011 – Dr.N. Selvakumar.

- Best poster award for the paper titled, “Evaluation of Capilia TB Neo kit for the identification of *M. tuberculosis* from MGIT system” at NATCON 2010 held at Bengaluru during January 2011 – Dr.N.S. Gomathi.

- Life-time achievement award of the Society for Immunology and Immunopathology in December 2010 – Dr.V.D. Ramanathan.

Special Assignments

Dr. Soumya Swaminathan

Membership of Committees

1) Adjunct Associate Clinical Professor, Dept. of Public Health and Family Medicine at Tufts University School of Medicine, USA.
2) Chair, HIV section, International Union against Tuberculosis and Lung Disease (The Union).

Membership of Project Review Committee

1) Department of Bio-Technology, New Delhi.
2) Indian Council of Medical Research, New Delhi.

Dr.M.S. Jawahar

Membership in expert committees:

1. Member, Ethics Committee of Madras Diabetes Research Foundation (MDRF), Chennai.
2. Member, DOTS Plus Committee, Central TB Division of the Ministry of Health, Government of India to formulate guidelines for the DOTS Plus Programme.
3. Member of the DOTS Plus Writing Committee, Central TB Division of the Ministry of Health, Government of India.
4. Member, Institutional Review Board of Fetal Care Research Foundation, Chennai.
5. Member of ICMR Project Review Committee for tuberculosis, leprosy and chest diseases.
6. Member, Operational Research Committee (South Zone) of the Central TB Division, Govt. of India.
7. Member, Ethics Committee of REACH, Chennai.
8. Member of Working Group to develop National Strategic Plan on Research (2012-2017) for RNTCP.
9. Member of Expert Group on Open Source Drug Development (OSDD) of Council of Scientific and Industrial Research (CSIR).
10. Organising Committee member of 'An intensive course on Tuberculosis Diagnostic Research – Beyond the Basics', jointly organized by the Tuberculosis Research Centre, McGill University, TDR, Stop TB Partnership and FIND, at Tuberculosis Research Centre, Chennai, December 2010, as part of the ICMR Centenary Celebrations.

FACULTY POSITIONS:

1. Faculty member for MAE and FETP programmes of National Institute of Epidemiology (NIE), Chennai.

2. Faculty member for International Short Course in Clinical Tropical Medicine, organized by Christian Medical College, Vellore.

Reviewer for Indian Journal of Tuberculosis.

Dr. Alamelu Raja

Training provided:

Post graduates students under supervision:
Training and guidance have been provided for 5 students (M. Sc) for their dissertation during 2009-2010, as part of their syllabus during the last semester (6 months). Training given to 5 students (post M. Sc) – on cloning and over expression of *M. tuberculosis* genes, as well as Real-time PCR analysis.

Membership in Committees:

- Expert Member, Institutional Review Board of Sri Kanchi Kamakoti CHILDS Trust Hospital, Chennai
- Member, Editorial Board, Indian Journal of Medical Research
- Life Member, Indian Immunology Society

Reviewer of manuscripts submitted to for national & international journals.

Reviewed Project Proposals submitted to funding agencies:
- Short Term Studentship, ICMR
- DBT
- DST
- RNTCP

Student Advisory Committee:
- Member in doctoral and up-gradation committees for M. Phil Dissertation and Ph.D for candidates

Dr. Sujatha Narayanan

Training provided:
We have given training to 6 students (M.Sc., and B.Tech., Biotechnology) from Anna University, Madras University, VIT (Vellore) and Bharathidasan University in Immunology and Molecular Biology for 6 months.

Reviewer of manuscripts submitted to for national & international journals.

Reviewer of project proposals submitted to ICMR.

External Examiner for Ph.D viva- voce Examination, conducted at Jadavpur University, Kolkotta.

Dr.P. Selvaraj

- Recognized Guide/Supervisor –Madras University, Chennai for guiding research work on candidates leading to PhD degree
- Executive council member, Indian society for Histocompatibility and Immunogenetics, New Delhi
- External Examiner for Ph.D viva- voce Examination, conducted on 09-04-2010, at Lady Doak College, Madurai-2

Reviewer of manuscripts submitted to for national & international journals.

Dr.P. Venkatesan

1. Adjunct Professor - Manipal University, Manipal
2. Honorary Visiting Professor - Sri Ramachandra Medical University, Chennai
3. Chairman - Institute Ethics Committee/Institute Review Board, Sri Ramachandra Medical University, Chennai. Reviewed more than 50 proposals during this period
4. Chairman - Board of Studies – M.Sc., (Bioinformatics) B.Tech (Biomedical informatics) - Sri Ramachandra Medical University, Chennai
5. Expert Member - Scientific Advisory Board, Sai's Biosciences Research Institute, Chennai.
6. Member- Board of Studies – M.Sc (Statistics) & M.Sc (Biostatistics), University of Madras, Chennai
7. Member- Board of Studies – M.Sc., (Statistics) & B.Sc (Biostatistics), Manormaniam Sundaranar University, Tirunelveli
8. Member- Board of Studies – M.Sc., (Mathematics), M.Phil (Mathematics) M.Tech (Computer Science) Periyar University, Salem
9. Member- Board of Studies - M.Tech., (Bioinformatics) and BTech (Bioinformatics), Sathyabama University, Chennai
10. Member- Board of Studies - M.Sc., (Biotechnology & Bioinformatics), SRM University, Chennai
11. Member- Board of Studies – M.Sc., (Mathematics), Meenakshi College for Women (Autonomous), Chennai
12. Member- Board of Studies – M.Sc., (Biostatistics) & B.Sc (Statistics) SDNP Vaishnav College for Women, Chennai
13. Member- Board of Studies – M.Sc., (Bioinformatics), Stella Maris College for Women (Autonomous), Chennai
14. External Examiner - University of Madras, Tamil Nadu Dr.MGR Medical University and Sri Ramachandra University, Chennai
15. External Examiner - PhD Viva-voce Examination, Madras University, Dr. MGR University, Chennai

16. External examiner for evaluation of four PhD theses for the award of Ph.D. Degree
17. Nominated for question paper settings for graduate and post-graduate courses in University of Madras, The Tamilnadu Dr MGR Medical University, Sri Ramachandra University, SRM University and Sathyabama University
18. Member - Editorial Board: Journal of Pure and Applied Spectrophysics
19. Member - Editorial Board: Indian Journal of Science and Technology
20. Joint Organizing Secretary - 2nd international Symposium on Global Trends in Biomedical Informatics, Research and Education, Chennai
21. General Secretary - Indian Society for Medical Statistics (ISMS)
22. General Secretary - International Biometric Society (IR)
23. Guide for Ph.D students

Dr.D. Sulochana

- Doctoral committee member for two Ph.D. students from the Department of Biochemistry, University of Madras, Chennai

Reviewer of manuscripts submitted to for national & international journals.

Training provided:

Training provided to B.Tech and Post graduate students from Bio-technology, Biochemistry and Molecular Biology disciplines.

Dr. Geetha Ramachandran

- Member of the Project Review Committee of ICMR
- Mentor to review research proposals submitted to the ICMR for funding under Pediatric HIV
- Invited as Chief Guest by Jamal Mohammed College, Tiruchi on the occasion of their Centenary celebrations
- Reviewer of research proposals submitted to ICMR
- Member of the Expert committee appointed by CMC, Vellore to inspect the Clinical Pharmacology department and assess their infrastructure and preparedness to undertake a course titled "Fellowship in Analytical Clinical Pharmacology"

Reviewer of manuscripts submitted to for national & international journals.

Training provided:

- Project guide for M.D. (Pharmacology), M.Sc., (Biochemistry) and B.Tech (Biotechnology) students

Dr. Beena E Thomas

- Technical Consultant for Hindustan latex Family Planning Promotion Trust (HLFPPT) is a trust promoted by HLL life care ltd, a Public sector under taking of Government of India
- Member of Project Advisory Committee (PAC) - Family Planning Association of India (FPA) for people living with HIV stigma Indexed roll out in Tamil Nadu
- Abstract reviewer for the 6th IAS Conference on HIV Pathogenesis 2011-
- Member of the National Consultation committee on HIV social Research Priorities in India TISS (Tata Institute of Social Sciences), Mumbai, India-
- Member of the Academic Counsel in Madras School of social work
- Board Member for AroGyan
- Consultant/Resource person for ITECH-International Training Education Center
- Member of the IRB (Institutional Review Board)-Government Hospital of Thoracic Medicine (GHTM)
- Member of the TORCH panel –TANSACS
- Member of Advisory Committee in APAC – VHS for BSS Wave XII

Dr.N S Gomathi

- Team member for evaluating Super Religare Laboratories, New Delhi for pre accredition assessment in June 2010
- Team member for lab up-gradation-evaluation of Intermediate Reference Laboratory, Pondicherry, by FIND Team, in August 2010
- Visited Super Religare Laboratories, Kolkata for pre-accredition assessment in February, 2011

Mrs. Niruparani Charles

- Resource person for Training Program on TB for Hello Plus at Hyderabad organized by APAC
- External Examiner for the Post Graduate Students of Department of Social Work at School of social work

Conferences / Workshops /training programs attended

1. Disease prevalence survey workshop - Preparation of a common template for reporting the data analysis, organized by CTD & NTI during April, 2010 held at NTI, Bengaluru – R. Subramani.

2. Presentation of study titled "Comparison of Interferon gamma and Interferon gamma inducible protein-10 in the diagnosis of latent tuberculosis infection in children" at the 28th Annual Meeting of the European Society for Pediatric Infectious Diseases, held in Paris during May, 2010 – S. Basirudeen.

3. Bi-regional Asia Pacific Workshop on 'HIV Drug Resistance Genotyping' held at Ho Chi Minh City, Vietnam, during May, 2010 – L E Hanna.

4. CME on Current trends in diagnosis and management of tuberculosis organised by Department of Microbiology, Kasturba Medical College held at Manipal during May, 2010 - N. Selvakumar.

5. Paper Presentation and chaired a session at VI International Conference on social work in Health and Mental Health held at Dublin, Ireland during June - July 2010 - Beena E Thomas.

6. XIV International Congress of Immunology held at Japan during August, 2010 – Kaustuv Nayak.

7. '3rd Annual Science Symposium on HIV/AIDS – HIV Science 2010', held at Chennai during August, 2010 – S. Jagadish Chandrabose.

8. International Conference on Aquatic Microbiology (*Status, Challenges and Opportunities*) held at Annamalai University, Parangipettai during September, 2010 - N. Selvakumar.

9. Paper titled "Safety and Immunogenicity of DNA Prime and Modified Vaccinia Ankara Virus HIV Subtype C boost in Indian Volunteers" presented at the AIDS Vaccine 2010 Conference held at Atlanta, USA during September - October 2010 – V.D. Ramanathan.

10. 'Workshop and Conference on Modeling Infectious Diseases' held at The Institute of Mathematical Sciences, Chennai during September, 2010 – S. Jagadish Chandrabose.

11. Oral presentation of study titled "Pharmacokinetics of anti-TB drugs in children" at the Third International Workshop on Clinical Pharmacology of Tuberculosis Drugs held at Boston during September, 2010 – Geetha Ramachandran.

12. Paper titled "IFN-γ, IP-10 and MCP-2 response to RD1 selected peptides are associated with active tuberculosis" at the European Respiratory Society Congress held at Barcelona, Spain during September, 2010 - S. Basirudeen.

13. Poster Presentation titled "A Pilot study on screening for Alcohol use disorder among tuberculosis patient" in International conference on Alcohol and HIV held at New Delhi during September, 2010 - Mohana Suhadev.

14. Invited speaker at the 2nd International conference on Alcohol and HIV: Insights from Interventions organized by the International Center for Research on Women, Institute for Communiy Resesrch, Public Health Foundation of India and National Institute on Alcohol abuse and alcoholism held at New Delhi during September, 2010 – M Muniyandi.

15. Guest lecture at the National Symposium on Molecular Diagnosis of Communicable Diseases organized by Adhiparasakthi College of Arts and Science, Kalavai during October, 2010 - Vanaja Kumar.

16. Guest lecture at the National Seminar on Recent Trends in Microbial Technology with Reference to Extremophiles organized by Periyar University, Salem during October, 2010 - Vanaja Kumar.

17. 42nd Union World Conference on Lung Health held at Berlin, Germany during November, 2010 – Soumya Swaminathan.

18. Invited speaker at the Science writing workshop held at Saveetha University, Chennai during December, 2010 – M. Muniyandi.

19. Keynote address on "Complement system and mycobacterial diseases" at the Convention of the Society for Immunology and Immunopathology at Institute of Biotechnology held at Padwadangar, Uttarkhand during December, 2010 - V.D. Ramanathan.

20. Proceedings of 2010 International Conference on Humanities, Historical and Social sciences: (i) Challenges in dealing with the problem of Tuberculosis-Experiences from TRC - K Jaggarajamma, Beena Thomas and (ii) Women as carers: Burden of the wives of HIV infected men in South India - Mohanarani Suhadev, Udaya Mahadevan, Beena E Thomas & Soumya Swaminathan.

21. The following papers were presented at the International symposium on "Tuberculosis diagnostics: Innovations making an impact" held at ICGEB, Delhi during December, 2010:
    (i) Proteomic Analysis of culture filtrate proteins of *M. tuberculosis* by a Combination of Preparative Two-Dimensional Liquid-Phase Electrophoresis and mass spectrometry - D. Anbarasu

(ii) Pkn E, aserine threonine kinase from M tb modulates apoptosis and Arginase signaling in Macrophages - P. Dinesh Kumar
(iii) Development of recombinant BCG based epitope vaccine candidates for tuberculosis – K.D. Karthika
(iv) Effect of over expression of signal recognition particle receptor in mycobacteria (FtsY) – V. Malini

22. Workshop titled "Tuberculosis Diagnostic Research: Beyond the Basic" organized with support from TDR, European Commission, EDCTP, McGill University, Stop TB Partnership's New Diagnostics Working Group and the Foundation for Innovative New Diagnostics (FIND) held at TRC, Chennai during December, 2010 - Vanaja Kumar, Alamelu Raja, Sujatha Narayanan.

23. Participated in the National Consultative Meeting organized by WHO (SEARO) held at New Delhi during December, 2010 - Vanaja Kumar.

24. Plenary talk in session "Agriculture, Biotechnology and Nutrition" at the 98th session of Indian Science Congress held at Chennai, during January, 2011 – Soumya Swaminathan.

25. Chaired a session at the 65th National Conference on TB & Chest Diseases, Bengaluru, during January, 2011 - N. Selvakumar.

26. International Conference on 'Translational Research in HIV/AIDS in India' held at Goa during January, 2011 – L E Hanna.

27. Invited by NACO to present the Clinical Pharmacology studies undertaken at TRC at the National Conference on HIV/AIDS held in New Delhi during January, 2011 - Geetha Ramachandran.

28. "Symposium on Digital Library Initiatives in India" organized by the Department of Library & Information Science held at University of Madras, Chennai during January, 2011 – R. Rathinasabapati.

29. Presented the following papers at the 65th National Conference on Tuberculosis and Chest Diseases – NATCON 2010 organized by Tuberculosis Association of India, held at NIMHANS, Bengaluru, during January, 2011:

(i) Comparison of panel slides prepared by phenol ammonium sulphate sediment and acetyl-L-crysteine methods for proficiency testing - R Radhakrishnan.
(ii) Development of an electronic system for monitoring proficiency test results of drug susceptibility testing (DST) of *M. tuberculosis* - Prabu Sreenivasan.

(iii) Evaluation of phage lysine to decontaminate sputum samples for the detection of *M. tuberculosis* using BACTEC MGIT 960 system -Balaji Subramanyam.

(iv) *M. tuberculosis* proteasome – a novel drug target - Jerrine Joseph.

(v) Standardization of DST for ethionamide by phenotypic methods - R Lakshmi.

(vi) Evaluation of Capilia TB Neo kit for the identification of *M. tuberculosis* from MGIT system - N.S. Gomathi.

30. Participated in consultative meeting titled "Galvanizing evidence for HIV management "held at NARI Pune during January, 2011 – A Thomas, Vanaja Kumar, Geetha Ramachandran, Pradeep Menon, G Narendran, C Padmapriyadarsini.

31. Paper titled "Diminished micobacterial-specific Th1, Th2, Th17 and increased Type-1 IFN responses in active pediatric tuberculosis" presented at the Keystone Symposia held at Vancouver, British Colombia, Canada during January 2011 - N. Pavan Kumar.

32. Presented the following papers at the 37th Annual Conference of Indian Immunology Society, held at Jammu University, Jammu during February, 2011 –

(i) *In silico* prediction of antigenic epitopes from human T-cell antigens of *M. tuberculosis* – D Santhi.

(ii) Differential activation of signaling molecules involved in maturation of dendritic cells during *in vitro M. tuberculosis* infection – D. Sulochana.

(iii) Increased frequency of circulating myeloid and plasmacytoid dendritic cells and decreased frequency of natural regulatory T-ells in lymphatic filarial disease – P. Jovvian George.

(iv) Elevated levels of microbial translocation markers, acute phase proteins and angiogenic factors but not pro-inflammatory cytokines in lymphatic filarial disease – R. Anuradha.

(v) Enhanced production of MMPs, TIMP1, TGFβ and acute phase proteins in the systemic immune response of active pediatric tuberculosis infection - N. Pavan Kumar.

33. Invited talk at the National Symposium on Therapeutic Drug Monitoring organized by CMC, Vellore, held in Vellore during February, 2011 - Geetha Ramachandran.

34. 'Workshop on Development of Technology Commercialisation and Transfer Specialists' held in Chennai during February, 2011 – S. Jagadish Chandrabose.

35. 18th Conference on Retroviruses and Opportunistic Infections (CROI 2010)" held at Boston, during February – March, 2011 – Soumya Swaminathan.

36. Seminar on 'An overview of RNTCP' at Karpaga Vinayaga Institute of Medical Science and Research Centre, Chinnakolambakkam during March, 2011 - N. Selvakumar.

37. CME on WHO approved diagnostic tools in tuberculosis, during World TB day celebrations held at Stanley Medical College, Chennai during March, 2011 - N. Selvakumar.

Conferences/Workshops/Training Programmes Organized:

1. Bio-Informatics Centre - Workshop on 'Computer Aided Drug Designing' at Chennai during May 11-12, 2010.

2. Dept. of Bacteriology - National symposium on 'Antituberculosis drug discovery: Current status and future prospects" at Chennai on October 30, 2010.

3. Social Workers Division - Training program on "Awareness of Tuberculosis & the possibilities of curing it" for the students from Gojan School of Business And Technology on February 14, 2011.

4. Library & Information Services - Co-ordinated the ICMR-ELSEVIER Workshop on "Information and Analytical Tools for the Medical Science Researchers" on February 17, 2011.

5. Social Workers Division - Dissemination workshop on the clinical and socio-behavioral studies on TB for RNTCP clinicians, STS, STLS and HV on March 12, 2010 as part of the World TB day celebrations.

6. Social Workers Division - Recreation and sensitization program for children in Shelter home – an NGO which refers children with HIV for our pediatric studies.

7. Social Workers Division - Training program for students from Medical social work and Medical sociology to undergo fieldwork training on converting theory in to practice.

8. Dept. of Clinical Research – Co-ordinated the Workshop titled “Tuberculosis Diagnostic Research: Beyond the Basic” organized with support from TDR, European Commission, EDCTP, McGill University, Stop TB Partnership's New Diagnostics Working Group and the Foundation for Innovative New Diagnostics (FIND) during December, 2010.

Ph.D. Scholars

List of staff / students who have obtained their Ph.D. degree from the University of Madras

Sl. No.	Name of the candidates	Title of the Ph.D. thesis	Supervisor/Guide
1.	Dr. Prabhu Anand S.	Regulatory effects of vitamin D_3 & vitamin D receptor genotypes on VDR expression & cytokine production in PTB	Dr.P. Selvaraj
2.	Dr. Alagarasu K.	Studies on Mannose binding lectin, CD209 and vitamin D receptor gene polymorphisms in south Indian HIV-1 infected patients with and without TB	Dr.P. Selvaraj
3.	Dr. Raghavan S.	Human Leucocyte Antigen polymorphism studies in HIV and HIV-TB patients	Dr.P. Selvaraj
4.	Dr. Anbarasu D.	Identification & characterization of immunoreactive T-cell antigens of *M. tuberculosis*	Dr. Alamelu Raja
5	Dr. Mohanarani Suhadev	Sociological aspects of HIV/AIDS	Dr. Udaya Mahadevan
6.	Dr. Ramana Rao P.V.	Innate immunity in HIV infection	Dr. Alamelu Raja
7.	Dr. Rajashree P.	Role of dendritic cells in tuberculous immunity	Dr.D.S ulochana
8.	Dr. Lakshmi S.	HIV drug resistance	Dr.P.R. Narayanan

List of staff/students who have submitted their Thesis and waiting for their Ph.D. degree from the University of Madras

Sl.No.	Name of the candidate	Title of the Ph.D. thesis	Supervisor/Guide
1.	Mr.Madhan Kumar M.	Cytotoxic cellular response in TB	Dr. Alamelu Raja
2.	Mr. Azger Dusthackeer V.N.	Mycobacterial latency & TB diagnosis	Dr. Vanaja Kumar
3.	Mr. Basirudeen S	Inferferon gamma assay for latent TB infection in HIV patients	Dr. Alamelu Raja
4.	Ms. Aparna J Christy	Development of epitope delivery system for construction of recombinant BCG vaccine for TB	Dr. Sujatha Narayanan

List of students who have registered (full-time) for their Ph.D. programme with the University of Madras

Sl.No.	Name of the Candidate	Source of Funding	Title of the Ph.D. thesis	Supervisor/Guide
1.	Ms. Neema Boruai	CSIR	Penicillin binding protein from *M. tuberculosis* & *M. smegmatis*	Dr. Sujatha Narayanan
2.	Ms. Malini V.	ICMR	Functional characterization of FtsY, a signal recognition particle receptor from *M. tuberculosis*	Dr. Sujatha Narayanan
3.	Mr. Balaji S.	ICMR	Rapid diagnosis of *M. tuberculosis*	Dr. Vanaja Kumar
4.	Mr. Brijendra Singh	CSIR	Chemokine gene polymorphism and chemokine expression in PTB	Dr.P. Selvaraj
5.	Mr. Afsal K.	ICMR	Effect of vitamin D3 on innate and adaptive immunity in pulmonary tuberculosis	Dr.P. Selvaraj
6.	Mr. Dinesh Kumar P.	ICMR	A molecular approach to patho-genesis role of serine/ threonine kinase PknE in signal transduction involved in host pathogen interactions	Dr. Sujatha Narayanan
7.	Mr. Jagadish Chandra Bose	ICMR-Biomedical Inf. Centre	Immunodominant epitopes against HIV subtype C	Dr. Luke Elizabeth Hanna
8.	Ms. Lakshmi R.	ICMR	Molecular studies on mycobacteria	Dr. Vanaja Kumar
9.	Ms. Karthika K.D.	CSIR	Recombinant BCG based vaccine for TB	Dr. Sujatha Narayanan
10.	Mr. Pawan Kumar N.	ICER	Pediatric TB	Dr. Luke Elizabeth Hanna
11.	Ms. Anuradha R.	ICER	Role of TLR in filarial pathology	Dr. Luke Elizabeth Hanna
12.	Mr. Pugazhvendhan P.	ICMR	Immunoproteomic identification of B-cell antigens of *M. tuberculosis*	Dr. Alamelu Raja
13.	Mr. Radhakrishnan M.	DST	Anti-TB drugs from actinomycetes	Dr. Vanaja Kumar
14.	Mr. Sameer Hassan	ICMR-Biomedical Inf. Centre	Genome analysis of phages and viruses	Dr. Vanaja Kumar
15.	Ms. Suba S.	MSSRF	Characterization of Lipoproteins of *M. tuberculosis*	Dr.Sujatha Narayanan
16.	Mr. Srinivasan K.	NIH	Comparative genomics and pathogenesis of TB	Dr.Sujatha Narayanan
17.	Ms. Yamuna N.	UGC	Classification and regression trees	Dr.P. Venkatesan

Staff registered (part-time) for their Ph.D. programme with the University of Madras, Chennai

Sl.No.	Name of the staff	Title of the Ph.D. thesis	Supervisor/Guide
1.	Ms. Amudha N.	Antimycobacterial compounds	Dr. Vanaja Kumar
2.	Mr. Anbalagan S.	Innate & adaptive immunity in HIV	Dr. Luke Elizabeth Hanna
3.	Mr. Arunkumar N.*	Causal analysis	Dr.P. Venkatesan
4.	Mr. Harishankar M.	Role of vitamin D receptor promoter & 3'UTR gene variants on vitamin D modulated immune functions in TB	Dr.P. Selvaraj
5.	Mr. Muthusamy M.	Antimicrobial and antimycobacterial agents	Dr. Vanaja Kumar
6.	Mr. Rathinasabapati R.	Institutional repository for the Tuberculosis Research Centre	Dr.A. Amudhavalli, University of Madras
7.	Mr. Sekar L.	Survival analysis	Dr.P. Venkatesan
8.	Mr. Sivakumar S.	Molecular epidemiology of TB	Dr. Sujatha Narayanan
9.	Mr. Srinivasan R.	Spatial analysis	Dr.P. Venkatesan
10.	Mr. Sukumar B.*	Statistical methods for micro array data analysis	Dr.P. Venkatesan
11.	Ms. Vasantha M.	Structural equation modeling	Dr.P. Venkatesan

* Ex-staff

STAFF LIST

(As on 1 April, 2010)

SCIENTIST 'G'

1. Dr.V.Kumaraswami, M.D., MNAMS, Ph.D.,**Tec**
2. Dr.Aleyamma Thomas, M.D., Dipl. Lep.
3. Dr.Soumya Swaminathan, M.D., DNB
4. Dr.V.D.Ramanathan, M.B.B.S., Ph.D.,

SCIENTIST 'F'

1. Dr.M.S.Jawahar, M.D., M.Sc., DLSHTM
2. Dr.N.Selvakumar, PhD.,
3. Dr.Vanaja Kumar, Ph.D.,
4. Dr.Alamelu Raja, Ph.D.,
5. Dr.Sujatha Narayanan, Ph.D., CT.,

SCIENTIST 'E'

1. Dr.K.Rajaram, M.B.B.S., DTRD
2. Dr.P.Selvaraj, Ph.D.,
3. Dr.R.Balambal, M.D.,
4. Dr.P.Venkatesan, MPS, Ph.D., PGCDM, DSQCOR (ISI), SDS (ISI)
5. Dr.D.Sulochana, Ph.D.,

SCIENTIST 'D'

1. Dr.K.C. Umapathy, M.B.B.S.,
2. Dr.P.Paul Kumaran, M.B.B.S., M.P.H.,
3. Dr.Ranjani Ramachandran, M.D.
4. Dr.D.Baskaran, M.B.B.S.,
5. Dr.Pradeep Aravindan Menon,MBBS,DPM
6. Dr.Sudha Subramanian, Ph.D.,
7. Dr.C.PadmaPriyadarshini, M.B.B.S.,DNB.,

SCIENTIST 'C'

1. Dr.Geetha Ramachandran, Ph.D.,
2. Dr.C. Ponnuraja, Ph.D.,
3. Dr. Luke Elizebath Hanna, Ph.D.,
4. Dr.V.Chandrasekaran, Ph.D.,
5. Dr.A. Sheik Illiayas, M.B.B.S.,
6. Dr.S. Ramesh Kumar, M.B.B.S.,
7. Dr.G.Narendran, M.B.B.S., DTRD, DNB.,

SCIENTIST 'B'

1. Dr. Beena E Thomas, Ph.D.,
2. Dr.V.V.Banurekha, M.B.B.S.,
3. Dr.P.K. Bhavani, M.B.B.S.,
4. Dr.M.Makesh Kumar, M.B.B.S.,
5. Dr.P. Kannan, M.V.Sc., Ph.D.,

Technical Officer - B

1. Dr.K.Jayasankar, Ph.D.,
2. Mr.S. Ramanujam, B.Sc.,
3. Ms. Niruparani Charles, M.A.,
4. Mr.K. Sankaran, M.Sc.,

Technical Officer – A

1. Ms.K. Padmavathy
2. Mr.M. Ponnambalam, B.Sc.,
3. Mr.S. Jagadeesan
4. Mr.A. Syam Sundar
5. Ms. Jemima Shiela Fredrick
6. Dr. Subhas Chandra Bose, Ph.D.,
7. Dr.M. Mohana Rani Suhadev, Ph.D.,
8. Mr.J. Samuel Vasanthan Good Will, B.Sc.,
9. Mr.A.S. Kripasankar, B.Sc.,
10. Ms.K. Silambu Chelvi, M.Sc.,
11. Dr.A.K. HemanthKumar, Ph.D.,
12. Ms.K. Sampooranam
13. Mr.R.K. Rajendran
14. Mr.S. Manoharan, B.Sc.,
15. Mr.K.Rajagopal, B.Sc.,
16. Ms. Padma Prakash
17. Mr.D. Suryanarayanan, M.Sc.,
18. Mr.M. Rajasakthivel, M.A.,
19. Ms.K.J. Jaganatha Rao, M.A.,
20. Mr.E. Thiruvalluvan, M.A.,
21. Ms. Meenalochani Dilip, M.A.,
22. Ms. Chandra Suresh, M.A.,
23. Ms.D.Kalaiselvi, M.A.,
24. Mr.P. Murugesan, M.A.,

Technical Assistant

1. Mr.L. Sekar, M.Sc.,
2. Dr.D. Vijayabhaskara Rao, Ph.D.,
3. Dr.K. Chandrasekaran, Ph.D.,
4. Dr.M. Muniyandi, Ph.D.,
5. Mr.R. Srinivasan, M.Sc.,
6. Mr.S. Anbalagan, M.Sc.,
7. Mr.M Harishankar, M.Sc.,
8. Ms. Lucia Precilla, M.Sc.,
9. Mr.K. Ramesh, M.Sc.,
10. Mr.S. Sivakumar, M.Sc.,
11. Mr.M. Anandan
12. Ms. Mariam George, M.Sc.,
13. Dr.K. Ramakrishnan, Ph.D.,
14. Ms.D. Saraswathi, M.Sc.,
15. Ms.M. Vasantha, M.Phil.,
16. Mr.M Tamizhselvan, M.Sc.,
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34. Mr.D Thangaraj
35. Dr.L. Prabakaran, Ph.D.,
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2. Ms.M. Rathinam
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4. Ms.P. Kowsalya
5. Ms.M. Mohana
6. Ms.R.Vetrichselvi, M.A.,
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8. Ms.A. Vijayalakshmi
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12. Ms. Senthamizhselvi
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3. Mr.G. Kabirdass
4. Ms.K. Shanthi

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2. Mr.K. Chandran
3. Mr.S. Mookkan
4. Mr.N. Ramakrishnan
5. Mr.A. Manoharan
6. Mr.P. Chandran

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2. Ms.B.V.S. Chalapathi Rao, B.Sc.,
3. Ms.A.Gunasundari, M.Sc.,

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2. Ms. Valarmathi Nagarajan, M.Sc.,
3. Ms.C. Kavidha, B.Sc.,
4. Ms.S. Chellam
5. Ms.K. Sureswari
6. Ms. Mary Eunice George
7. Ms.K. Rosily
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15. Ms.S. Stella Mary
16. Ms.A. Stella Mary

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3. Ms. Usha Devi Gopalan

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2. Ms.A. K. Vijayal
3. Ms.L. Vijayakumari
4. Mr.B. Durai Raj
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8. Mr.R. Hariharan, B.Com.,

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1. Mr.V. Adiseshan
2. Ms. Molly Joseph
3. Mr.K. Ganesan

Senior Receptionist cum Telephone Operator
1. Ms.K.S. Anusuya

Senior Telephone Operator cum Receptionist
1. Ms. Kanchana Udayakumar

Telephone Operator
1. Ms.V. Shailaja Devi

Motor Driver (Special Grade)
1. Mr.S. Rajkumar

Motor Driver (Grade - I)
1. Mr.R. Arthur Sundar Singh
2. Mr.K. Vadivel
3. Mr.K. Ayyasamy
4. Mr.C. Krishnamurthy
5. Mr.K. Jayaraman
6. Mr.P. Anbu

Motor Driver (Grade - II)
1. Mr.S. Sri Ramachandran
2. Mr.A. Ravi

Motor Driver (Ordinary Grade)
1. Mr.A. Elangovan
2. Mr.I. Seenivasan
3. Mr.P. Sivakumar
4. Mr.N. Rajan Babu
5. Mr. Thiyagarajan

Technical Officer – A (Engineering Support)
1. Mr.R. Manoharan

Technical Assistant (Engineering Support)
1. Mr.K. Palayandi

Technician – C (Engineering Support)
1. Mr.B. Kanaga Sabapathy, M.A.,

Technician – B (Engineering Support)
1. Mr.K. Parthiban
2. Mr.S. Iyyappan
3. Mr.D. Shanmugam

Technician – A (Engineering Support)
1. Mr.K.S. Venkatesan
2. Mr.E.A. John Washington
3. Mr.R. Anbulingam

CANTEEN
1. Mr.C. Kanagamani, Assistant Manager cum Store Keeper
2. Mr.K. Vijayan, Clerk
3. Mr.K. Sundaram, Bearer
4. Mr.B. Venkateswaralu, Asst. Halwai cum Cook
5. Mr.A. Govindarajan, Safai Wala
6. Mr.B. Balakoti, Dish Cleaner

Attendant (Services)
1. Mr.R.S. Soma Sundaram
2. Mr.J. Loganathan
3. Mr.V. Mohan
4. Ms. Padmavathi Asaithambi
5. Ms. Rosily Edwin
6. Mr.K. Jayavel Anandan
7. Ms.D. Sundari
8. Mr.M. Kannan, B.Sc.,
9. Mr. D Srinivasa Raju
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38. Mr.D. Sugumar
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64. Mr. Jeevanath Sharma
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Epidemiology Unit

Scientist 'F'

1. Dr C. Kolappan, M.B.B.S., M.Sc.,

Scientist 'E'

1. Mr.R. Subramani, M.Sc.,

Technical Officer – B

1. Mr.H. Dhanasekaran

Technical Officer – A

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2. Mr.T. Krishnamoorthy, M.Sc.,
3. Mr.L. Ranganathan, M.Sc (Stats), M.Sc., (Maths), M.Tech (IT)
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5. Mr.S Gopalakrishnan
6. Mr.T. Raman
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23. Mr.A. Vedachalam
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25. Mr.D. Pachayappan
26. Dr. Gomathi Sekar, M.Sc., Ph.D.,

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36. Mr. John Arokiya Doss Y, M.Sc, D.M.L.T.,
37. Mr.C.V. Mohan
38. Mr.J. David Silver Durai

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2. Mr.A. Vasudevan, M.Sc(Bio-Chemistry) M.Sc(Clinical Microbiology)
3. Mr.M. Mahesh Kumar, M.Sc, D.M.L.T.,
4. Mr.K. Anbarasan, M.Sc, D.M.L.T.,
5. Mr.M. Karthikesan, M.Sc.,
6. Mr.P. Chandrasekaran, M.Sc, M.B.A,
7. Mr.P. Kumaravel, M.A, D.M.L.T.,
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11. Mr.T.K. Bharath, M.Sc.,
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14. Mr.P.C. Nagaraja, B.A.,
15. Mr.R. Levelin David Raj Kumar, M.A.,
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17. Mr.S.S. Jeganathan, B.Sc.,
18. Ms.N. Lakshmi, B.B.A,
19. Mr.M. Mohan
20. Mr.A. Durairaj, M.A.,

Technician-B

1. Mr.K. Munusamy
2. Mr. Srikant Davani

3. Mr. Ishwori Dhakal
4. Mr.J. Udhaya Kumar
5. Mr.V. Raja

Technician-C (Engg Support)
1. Mr.T.S. Mahadevan
2. Mr.G. Vasu

Technician-B (Engg Support)
1. Mr.R. Balu
2. Mr.B. Vijayakumar

Technician-A (Engg Support)
1. Mr.D. Balraj
2. Mr.B. Anbudoss
3. Mr.K. Poongavanam
4. Mr.L. Venkatesan
5. Mr.W. Wilingson Mathew

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2. Mr.K. Sampath Kumar, B.Sc.,
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Private Secretary
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Personal Assistant
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Assistant
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2. Mr.R.S. Dayalakumar, B.A.,
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3. Ms.A. Uma, B.Com.,
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5. Mr.M. Senthil Kumar, B.Sc.,
6. Mr.V. Navalan
7. Ms.B. Manjula, M.Com, M.C.A.,

Driver Spl. Grade
1. Mr.K.G. Kanagasabhapathy

Driver Grade-I
1. Mr.K. Karunakaran
2. Mr.N. Govindarajulu
3. Mr.J. Prakash
4. Mr.K. Venugopal
5. Mr.P. Soundararajan
6. Mr.B. Kalaiselvam
7. Mr.V. Thanigaivel

Driver Grade-II
1. Mr.M. Manoharan
2. Mr.K. Saravanan
3. Mr.A.S. Dayalan
4. Mr.P. Subbaiah
5. Mr.B. Suresh Kumar
6. Mr.K Thulasingam

Driver Ordinary Grade
1. Mr.J. Loganathan
2. Mr.K. Jagadesan
3. Mr.V. Babu
4. Mr.V.S. Senthil Kumar
5. Mr.G. Vasu
6. Mr.S. Dass
7. Mr.L. Gunalan

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