TUBERCULOSIS RESEARCH CENTRE

Research Activities

April 2009 – March 2010

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Preface

The Annual Report for the period 2009-10 (2010) is a compilation of the research activities carried out by this centre during the period. The priority of the centre has been, as before, to identify regimens that will be of use in national as well as global programmes for the control of TB and HIV-TB. The results (interim as well as final) of these studies are reported in this document. Closely linked to the evaluation of chemotherapy is the study of behavioral aspects of patients and health systems. The centre has been carrying out several studies to improve the completion of treatment, ensure better retrieval of patients and strengthen monitoring and evaluation of the programme. The Epidemiology division continuing its earlier studies on the burden of illness as well as the assessment of risk factors for TB is now carrying out studies to identify additional risk factors such as use of tobacco, in populations where TB is endemic.

The Bacteriology department has recently upgraded its facilities and is now fully geared to tackle the challenge of not only all forms of TB but also provide expert services in the field of MDR and XDR-TB.

The Centre continues to be recognized for its expertise in the field of Tuberculosis Research and Training for which it is recognized as a WHO Collaborating Centre and also as an International Centre of Excellence in Research (ICER) by the National Institutes of Health which has funded a laboratory at TRC through an intramural grant.

The studies carried out by this centre fully support the national and international control programmes for the control of TB and the members of the centre play significant roles both scientifically and technically in strengthening TB control globally and nationally. This report is a documentation of their inspired and hard work during the year.

As usual, your comments and suggestions are valuable for us to improve our pursuit of excellence.

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ABBREVIATIONS

2D-liquid phase electrophoresis (2D-LPE)
Acid fast bacilli (AFB)
Acquired Rifampicin Resistance (ARR)
Alcohol use disorders identification test (AUDIT)
Alcohol use disorders (AUD)
Anti-TB treatment (ATT)
Anti-retroviral treatment (ART)
Cardamom mosaic virus (CMV)
Colony forming units (CFU)
Community Advisory Board (CAB)
Complementary DNA (cDNA)
Culture filtrate antigens (CFA)
Culture filtrate proteins (CFP)
Cytotoxic T-lymphocytes (CTL)
Data Safety Monitoring Board (DSMB).
Database of drug targets for resistant pathogens (DDTRP).
Dendritic cells (DC)
Diethylcarbamazine (DEC)
Directly Observed Treatment Short-course (DOTS)
Dug-resistant (DR)
Drug resistant mutations (DRM)
Drug susceptibility testing (DST)
Efavirenz (EFV)
Enzyme linked immunosorbent assay (ELISA)
Ethambutol (EMB)
Ethionamide (Eto)
Electronic Data Processing (EDP)
Event free survival (EFS)
Fatty acid synthesis (FAS),
Granulocyte macrophage colony stimulating factor (GM-CSF)
Green fluorescent protein (GFP)
Healthy household contacts (HHC)
High performance liquid chromatography (HPLC)
Immune reconstitution inflammatory syndrome (IRIS)
Inducible protein-10 (IP-10)
Isoniazid (INH)
Interferon gamma (IFN-γ)
KG-1 derived DC (KGDC)
Latent TB infection (LTBI)
Locally advanced breast cancer (LABC)
Lowenstein-Jensen (LJ)
Luciferase reporter phage (LRP)
Matrix involving matrix metalloproteinases (MMPs)
Men who have sex (MSM)

Mothers living with HIV/AIDS (MLH)
Moxifloxacin (MFX)
Multi drug-resistant TB (MDR-TB)
Mycobacterial interspersed repeat unit (MIRU)
Myeloid DC (mDC)
National AIDS control organization (NACO)
Natural Killer (NK)
Nevirapine (NVP)
Nitric oxide (NO)
Normal healthy subjects (NHS)
Peripheral blood mononuclear cell (PBMC)
Prevention of parent to child transmission (PPTCT)
Plasmacytoid DC (pDC)
Pleural mesothelial cells (PMC)
Phorbol myristyl acetate (PMA)
Proportional hazard (PH)
Protease (PR)
Pulmonary tuberculosis (PTB)
Pyrazinamide (PZA),
QuantiFERON-TB Gold in-tube (QFT-IT)
Reactive oxygen species (ROS)
Resiquimod (RSQ)
Restriction fragment length polymorphism (RFLP)
Revised National Tuberculosis Control Programme (RNTCP)
Reverse transcriptase (RT)
Real time PCR (RT-PCR)
Rifampicin (RMP)
Selective Kirchner's liquid medium (SKLM)
Serine/Threonine Protein Kinases (STPK's)
Short course chemotherapy (SCC)
Signal recognition particles (SRP)
T-regulatory (Treg)
Tibotec medicinal compound (TMC)
Toll like receptors (TLRs)
Thin Layer Chromatography (TLC)
Tissue inhibitor of metalloproteinase (TIMP-1)
T_N (naïve T-cells)
T_{EM} (Effector Memory T-cells)
T_E (Effector T-cells)
T_{CM} (Central Memory T-cells)
Transmission electron microscopy (TEM)
Tuberculosis (TB)
Tuberculin skin test (TST)
Variable number tandem repeat (VNTR)
Vitamin D receptor (VDR)

CLINICAL RESEARCH

Ongoing studies

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

(PROVCTRI/2008/091/000024)

Background

Moxifloxacin (MFX), a fluoroquinolone has promising anti mycobacterial activity, and has a potential to shorten tuberculosis (TB) treatment. The randomized clinical trial to study the efficacy and safety of 3- and 4-month MFX containing regimens for treatment of patients with sputum positive pulmonary tuberculosis (PTB) is continuing in Chennai and Madurai.

Aim

- To shorten the duration of TB treatment by supplementing the standard 4-drug TB regimen with MFX

Methods

Newly diagnosed sputum smear positive, HIV seronegative PTB patients, resident in Chennai and Madurai were randomly allocated to 3-month or 4-month MFX regimens, or a control 6-month regimen. Treatment was given under direct observation and response to treatment was assessed with sputum examinations. The patients were also closely monitored for adverse drug reactions which were critically documented. The regimens being tested in this trial are shown in Table 1.

Table 1: Study regimens

Regimens	Intensive phase	Continuation phase	Duration (months)
Test Reg. 1	3 RHZEM daily		3
Test Reg. 2	2 RHZEM daily	2 RHM daily	4
Test Reg. 3	2 RHZEM daily	2 RHM thrice weekly	4
Test Reg. 4	2 RHZEM daily	2 RHEM daily	4
Control Reg.	2 RHZE thrice weekly	4 RH thrice weekly	6

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

Results

A total of 381 patients have been enrolled as of 31st March 2010. The baseline characteristics of these patients are shown in Table 2.

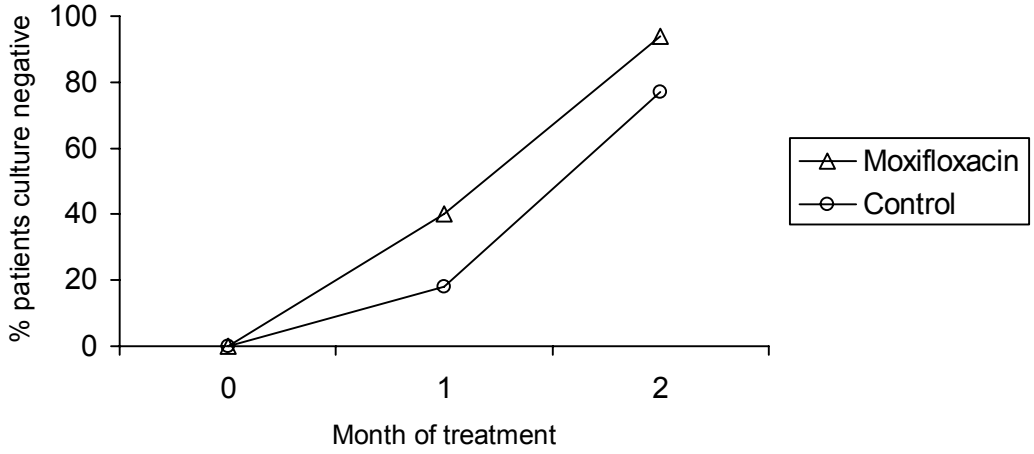
Table 2: Baseline characteristics of 381 patients enrolled in study

Regimen	Test Reg. 1 (77)	Test Reg. 2 (76)	Test Reg. 3 (79)	Test Reg. 4 (73)	Test Reg. 5 (76)	Total (381)
Sex						
Male	61	58	57	53	60	289
Female	16	18	22	20	16	92
Age						
<40 years	51	47	60	49	54	261
≥40 years	26	29	19	24	22	120
Initial sputum smear						
0 or 1+	14	15	16	19	13	77
2+ or 3+	63	61	63	54	63	304

The proportion of patients who became sputum culture negative at two months of treatment was significantly higher (94%) in the MFX arm (consolidated for all four test regimens) compared to the control arm (77%) (Fig.1).

Recruitment of patients to the trial is continuing.

Fig. 1: Sputum culture conversion to negative in patients treated with MFX and control regimens



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Prospective randomized clinical trial to study the efficacy of two different preventive therapies for TB among HIV-infected individuals

(Funded by United States Agency for International Development)

Background

This prospective randomized clinical trial was designed to study the efficacy of two different TB preventive therapy regimens, in HIV-infected persons without active TB. Available evidences indicate that preventive therapy for TB reduces the frequency of active TB in HIV-infected individuals by about 50% to 60%, with protection being greatest in adults with a positive tuberculin skin test (70% reduction, mortality reduced 25%).

Aim

- To compare the efficacy of two different TB preventive therapy regimens in HIV-infected persons, in reducing the incidence of TB and overall mortality

Methods

The treatment regimens tested in this trial were:

1. Ethambutol (EMB) (800 mg) and Isoniazid (INH) (300 mg) daily for six months (EH arm)
2. INH (300 mg) daily for 3 years (in lieu of lifelong prophylaxis) (INH arm)

The primary outcome measures of the trial were development of pulmonary or extra-pulmonary TB or death due to TB, at any time point after starting prophylaxis. The efficacy of these regimens with respect to tuberculin skin test (TST) and relate the efficacy to the stage of HIV infection as assessed by the CD4 cell counts and viral load was also studied.

Patients in both study groups received 10 mg of pyridoxine daily during treatment. All study medications were self administered and collected once in 15 days. The patients were followed up for a period of three years from the time of admission to the study. Clinical examination and relevant investigations were done once in 3 months. Patients suspected to have TB at any time point were investigated and treated appropriately. Any positive culture was subjected to drug susceptibility testing. The cause of death was ascertained in all cases.

Results

A total of 712 patients were admitted to the study from March 2001 – September 2006. Out of this, 632 (229 males and 403 females) were eligible for analysis. The mean age, body weight and CD4 cell counts were comparable in both the groups. A total of 311 patients from the INH arm and 321 from the EH arm have completed 36 months of follow-up as of 31st March 2010 (Table 3).

Table 3: Demographics of study population

Characteristics	EH – 6 months (n =321)		INH- 36 months (n = 311)	
	Mean ± S.D	Range	Mean ± S.D.	Range
Age (yrs)	29 ± 7	18 - 57	30 ± 6	18 -30
Body weight (kg)	51 ± 10	30 - 79	50 ± 10	30 - 97
CD4 cell counts / mm^{3*}	314	208 - 522 (IQR)	322	187 - 463 (IQR)
Mantoux reaction (mm)	8 ± 9	0.0 - 40.0	7 ± 9	0.0-35.0

*Median values

Intent to treat analysis was carried out. Twenty two and 14 patients respectively in the EH and INH arms developed active TB. The TB breakdown rates were 2.44 / 100 person years in the EH arm and 1.55/100 in the INH arm; this difference was not statistically significant. The number of deaths in the EH and INH arms were 25 and 20 respectively (Table 4). Incidence of TB was 4.4 times higher among patients with a baseline CD4 count counts < 200 cells/mm^3, while rates among TST positive and negative patients were similar.

Table 4: Incidence of TB and death by regimen, rate/100py

	EH – 6 months	INH- 36 months
TB Incidence	2.44 (1.42 – 3.46)	1.55 (0.73 – 2.36)
Rate ratio	1.55 (0.79 – 3.03)	
Death (all – cause)	2.77 (1.68 – 3.86)	2.22 (1.24 – 3.18)
Rate ratio	1.26 (0.69 – 2.27)	

Both the TB preventive therapy regimens used in this trial were well tolerated with a very low incidence of adverse events. Most of these events were mild and managed with symptomatic treatment. Treatment had to be terminated in two patients belonging to the INH arm due to severe peripheral neuropathy.

Conclusions

Our findings suggest that

- Preventive therapy with 6 months of EH is as effective as 3 years of INH
- Mantoux positivity is not associated with greater TB breakdown rates
- Patients with lower CD4 cell counts are at higher risk of TB breakdown and death

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Prospective randomized clinical trial to study the efficacy of two different once-daily anti-retroviral regimens along with anti-TB treatment, in patients with HIV-1 and TB

(Funded by United States Agency for International Development)

Background

This randomized clinical trial was designed to study the efficacy and safety of two different once-daily antiretroviral regimens along with anti-TB treatment (ATT) in the treatment of HIV-1 infected TB patients.

Aim

- To compare a once-daily regimen of didanosine+lamivudine+efavirenz (EFV) with didanosine/lamivudine/nevirapine (NVP) when given along with standard ATT in patients with HIV and TB with CD4 cell counts <250 cells/mm^3

Outcome measures

- Suppression of viral load to <400 copies/ml at 24 weeks of anti-retroviral treatment (ART)
- Utility of directly observed vs. self-administered ART

Intake to the main trial was initiated in May 2006 and was stopped in June 2008 as per recommendation of the Data Safety Monitoring Board (DSMB). As of June 2008, 564 patients had been screened for the study at 5 centres (3 sites in Chennai, 1 in Vellore and 1 in Madurai) and 116 patients were admitted to the

study. At the end of intensive phase (2 months) of ATT, 59 patients were randomized to EFV arm and 57 to the NVP arm. The demographic details of these patients are given in Table 5.

Table 5: Demographic details of patients

	EFV regimen N = 59	**NVP regimen N = 57**
Age (years)	34.5 ± 7.5	37.6 ± 7.8
Body weight (kg)	43.0 ± 8.6	42 ± 7.3
Body mass index	16.3 ± 2.6	16.4 ± 2.4
CD4 cell counts/mm^3	85 (47 - 85)	83 (33 - 135)
Viral load copies/ml	3,62,000 (41,575-7,50,000)	2,82,000 (1,28,500-6,49,500)

All values are mean ± SD except CD4 and viral load, which are median and IQR

The favourable responses to ATT were 81% & 92% in the NVP regimen and EFV arms respectively. Only one patient had an adverse reaction to anti-TB drugs, which required a change of treatment.

Overall, the immunological response to ART was satisfactory with good improvement in CD4 counts (Fig.2), with no difference between regimens.

Fig. 2: Immunological response in patients' upto 24 weeks (values are expressed as median)

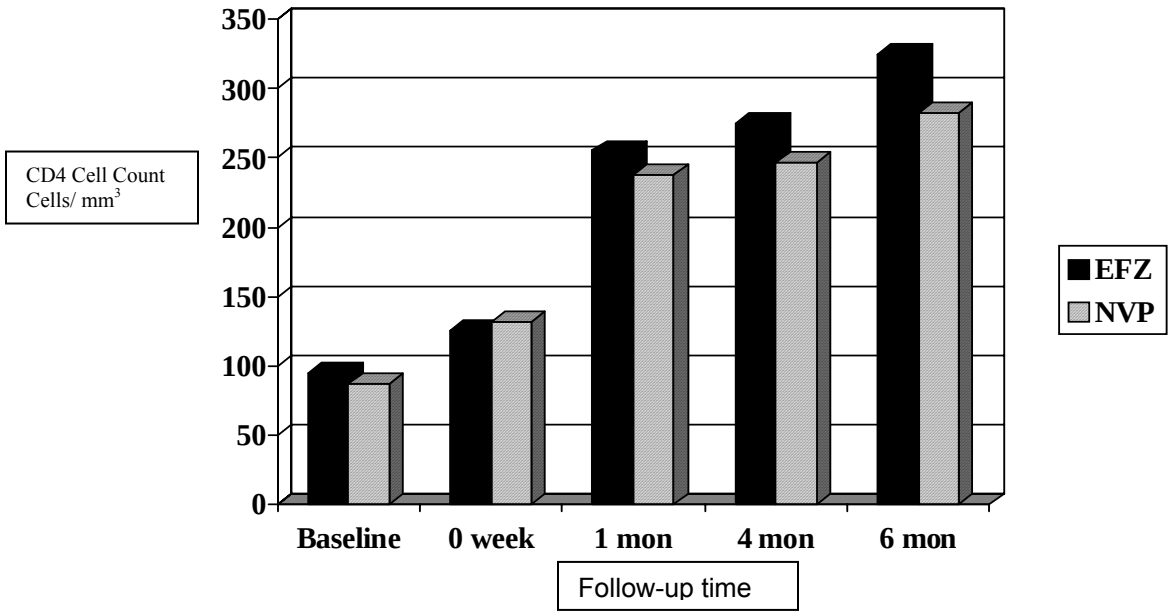

Table 6 shows the outcome, by intent to treat analysis at 24 weeks of ART.

Table 6: Outcome at 24 weeks in the study population

Variable	Patients on EFV regimen (n = 59)	Patients on NVP regimen (n = 57)
Plasma HIV-RNA < 400 (copies/ml)	50 (85%)	37 (65%)*
Virological failure	6	12
Death	0	5
Lost to follow-up	3	3

* denotes p<0.05 vs EFV regimen

In view of the high failure and death rate in the NVP arm as compared to the EFV arm, the DSMB recommended withholding intake to the NVP arm as on December 15, 2007 (favourable response 65% in NVP arm vs. 85% in EFV arm, p=0.038). The 2nd interim analysis was done and presented to the DSMB on June 14, 2008. The DSMB recommended stopping the study, as the primary outcome had been determined and was found to be significantly different between the two regimens. Follow-up of all patients will continue till 24 months when final outcomes will be determined.

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Innate and adaptive immunity in children starting antiretroviral drugs in India

(Funded by Indo-US JWG Maternal and Child Health (NIH and ICMR)

Background

Currently, CD4 counts are the mainstay of immunologic assessment for HIV-infected adults and children on which treatment decisions are made. This study was carried out to identify innate and adaptive immunological markers that can be predictive of disease outcome of perinatally HIV-infected children who are

treatment naïve but now have access to ART as per National AIDS Control Organisation (NACO) guidelines.

Aim

To test the hypothesis that one or more of the following:

- Loss of naïve cells (CD45RA+ CD62L+)
- Increased activation markers (CD38+ HLA-DR+)
- Loss of CD127 expression or
- Impaired DC maturation and function

could provide a sensitive measure of immunologic deterioration/immune reconstitution as compared to changes in CD4 T-cells alone

Methodology

Sixty two ART naïve HIV-infected children (28 males / 34 females) were recruited to the study. Their age ranged from 9 months – 13 years, and had a median body mass index (BMI) of 14.4 (6.4 – 24.1). Median CD4 counts were 777 cells / mm^3 (IQR 131 – 2396) and CD8 counts were 1625 cells / mm^3 (IQR 573 – 6086). Patients were followed-up every 3 months for one year. Nine patients were started on ART during the study and the treatment was given as per NACO guidelines.

For this analysis, patients were classified in to three groups based on their CD4%: Immune Category-1 (IC-1) [CD4 % > 25], Immune Category-2 (IC-2) [CD4% 15 - 25] and Immune Category-3 (IC-3) [CD4 % < 15]. Twenty age-matched healthy controls were also included in the study. Several immunological markers were studied in all the study subjects using flowcytometry. The markers were estimated at regular time points in patients and at a single time point in control subjects.

Immune characteristics in patients were compared to healthy age-matched controls at study entry and follow-up results (with and without ART) were compared with the baseline values of HIV- infected children. Statistical analysis was performed using a general linear model with planned contrasts to compare means among the three immune categories. SAS version 9.1 was used for all analysis.

Results

Table 7 summarizes the immunological characteristics in the different groups of children.

Table 7: Summary of immunological characteristics

Immunological markers	At entry, HIV+ children				At 12 month, HIV+ children				Change		
	Compared to controls				Compared to controls				0 vs 12 month		
	Total	IC1	IC2	IC3	Total	IC1	IC2	IC3	0 vs 12	0 vs 12	0 vs 12
DC%	↓	↓	↓	↓	↓	↔	↓	↔	↑	↑	↑
DC Count	↓	↓	↓	↓	↓	↓	↓	↔	↑	↑	↔
mDC%	↓	↓	↓	↓	↔	↔	↔	↔	↑	↑	↑
mDC Count	↓	↓	↓	↓	↓	↓	↓	↔	↑	↑	↑
pDC%	↓	↓	↓	↓	↓	↓	↓	↔	↔	↔	↔
pDC Count	↓	↓	↓	↓	↓	↓	↓	↔	↔	↔	↔
mDC CD80	↓	↓	↓	↓	↓	↓	↓	↓	↔	↔	↔
pDC CD80	↔	↔	↓	↓	↓	↓	↓	↓	↓	↓	↓
mDC CD83	↓	↓	↓	↓	↓	↓	↓	↓	↔	↔	↔
pDC CD83	↓	↓	↓	↓	↓	↓	↓	↓	↔	↔	↔
mDC CCR7	↓	↔	↔	↔	↓	↔	↔	↔	↔	↔	↔
pDC CCR7	↓	↓	↓	↓	↔	↔	↔	↔	↔	↔	↔
mDC TNF-α	↓	↓	↓	↔	↓	↓	↓	↔	↔	↔	↔
pDC TNF-α	↓	↓	↓	↔	↓	↓	↔	↔	↔	↔	↔
pDC IFN-α	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
CD4 %	↓	↔	↔	↔	↔	↔	↔	↔	↓	↔	↓
CD4 Count	↓	↔	↔	↔	↔	↔	↔	↔	↓	↓	↔
CD4 T_{CM}	↑	↑	↑	↑	↔	↔	↔	↔	↓	↓	↓
CD4 T_N	↓	↓	↓	↓	↓	↔	↓	↔	↔	↔	↓
CD4 T_{EM}	↑	↔	↔	↑	↑	↑	↑	↔	↔	↔	↑
CD4 T_E	↑	↑	↔	↔	↑	↑	↑	↔	↑	↔	↑
CD8 T_{CM}	↔	↔	↔	↔	↔	↔	↔	↔	↔	↑	↑
CD8 T_N	↓	↓	↓	↓	↓	↓	↓	↓	↔	↔	↓
CD8 T_{EM}	↑	↑	↑	↑	↑	↑	↑	↑	↔	↔	↔
CD8 T_E	↑	↑	↑	↔	↑	↑	↑	↔	↔	↔	↔
CD4 DR+ 38+	↓	↓	↓	↔	↔	↓	↔	↔	↔	↔	↑
CD8 DR+ 38+	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
CD4 127+	↓	↓	↓	↓	↓	↓	↔	↔	↔	↔	↔
CD8 127+	↓	↓	↓	↓	↓	↓	↓	↓	↔	↑	↔
↔ No difference ↑ Increase ↓ Decrease											

The following observations were made:

Innate immunity:

1) Dendritic cells (DC), (plasmacytoid DC (pDC) and myeloid DC (mDC)) were deficient quantitatively and qualitatively at entry.
2) Resiquimod (RSQ), which stimulates DC through Toll like receptors (TLR) 7 and 8 resulted in (i) decreased upregulation of maturation markers CD83, CD80 and CCR7 and (ii) decreased induction of intracellular cytokines TNF−α.(mDC and pDC) and IFN-α (pDC).
3) Collectively these defects were evident in analysis of all patients and were variably expressed in patients in immune categories 1, 2 & 3.
4) These defects persisted over follow-up at 3 and 6 months and were evident at 12 months, with further decline in some of the characteristics.
5) Specifically, CD80 and IFN-α induction in pDC was further decreased at 12 months.

Adaptive immunity:

1) At baseline, patients had decreased frequencies of T_N (naïve T-cells) and relatively increased T_{EM} (Effector Memory T-cells) and T_E (Effector T-cells) in both CD4 and CD8 T-cells, with increased T_{CM} (Central Memory T-cells) in CD4 T-cells.
2) CD127, a marker of immunologic memory was decreased in CD4 and CD8 T-cells.
3) Immune activation markers CD38+ HLA-DR+ were increased in CD8 T-cells.
4) Collectively, these defects were evident in analysis of all patients and were variably expressed in patients in immune categories 1, 2 & 3.
5) These defects persisted over follow-up at 3 and 6 months and were evident at 12 months with further exaggeration of some defects.
6) Specifically at 12 months as compared to entry, there was a further decline in naive T-cells, a decrease in memory T-cells and further expansion of terminally differentiated T-cells.

Conclusions

The study findings showed majority of perinatally HIV-infected children who survive infancy without ART remain clinically stable in the short term, but have demonstrable immunologic abnormalities indicative of defects in innate and adaptive immune system with impairment of DC activation and function, along with skewing of maturation T-cell subsets, altered immune activation and elevated immune exhaustion. Children initiated on ART showed improvement in CD4 counts but did not show improvement in DC function or change in CD8 immune activation. These immunological abnormalities point to a need for early initiation of ART.

[Contact person: Dr.Soumya Swaminathan (E-Mail ID: soumyas@trcchennai.in); Dr. P.K Bhavani (E-Mail ID: bhavani.pk@trcchennai.in)]

Efficacy and safety of immunomodulator *(Mycobacterium w)* as an adjunct therapy in category-II PTB

(Funded by Department of Biotechnology, Govt. of India)

Background

The immunomodulator containing *Mycobacterium w* developed by the National Institute of Immunology, New Delhi in 1980, has been found useful in the prevention of TB in experimental animals. Studies have documented faster sputum conversion with *Mycobacterium w*, when 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 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 RNTCP regimen.

Results

Of the 59 patients enrolled to the study, between March 2006 and April 2008, 45 have completed treatment and are on 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. Intake to the study has been completed and patients are being followed up.

The study is expected to be over in December 2010.

[Contact person: Dr.R.Balambal (E-Mail ID: balambal.r@trcchennai.in)]

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

Background

The drug resistance surveillance of patients treated with Revised National Tuberculosis Control Programme (RNTCP) regimens in a TB Unit in Tiruvallur district in Tamil Nadu, south India (1999 to 2005) conducted by TRC 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 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, TRC initiated a prospective study 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-ethionomide; 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 patietns were recruited, 54 and 21 are MDR-TB and non-MDR-TB patients respectively. The demographic details of patients admitted to the study are given in Table 8. The baseline drug resistance patterns of MDR-TB and non-MDR-TB patients are shown in Figs. 3a & b respectively. The patients admitted to the study are being followed up at regular intervals.

Table 8: Details of patients recruited to the study

	MDR (n = 54)			Non-MDR (n = 21)		
	R1	**R2**	**Total**	**R1**	**R2**	**Total**
Patients admitted	26	28	54	11	10	21
Sex Female	5	8	13	0	2	2
Male	21	20	41	11	8	19
Age ≤ 45 years	16	23	39	9	9	18
> 45 years	10	5	15	2	1	3
X-ray Bilateral involvement	24	23	47	10	10	20
Unilateral involvement	2	5	7	1	0	1
X-ray Cavity Yes	11	15	26	6	4	10
No	15	13	28	5	6	11
X- ray zones affected						
2-4	16	15	31	6	5	11
5-6	10	13	23	5	5	10
Weight ≤45kg	15	8	23	8	6	14
>45kg	11	20	31	3	4	7

Fig. 3a: Drug resistance pattern in MDR-TB patients

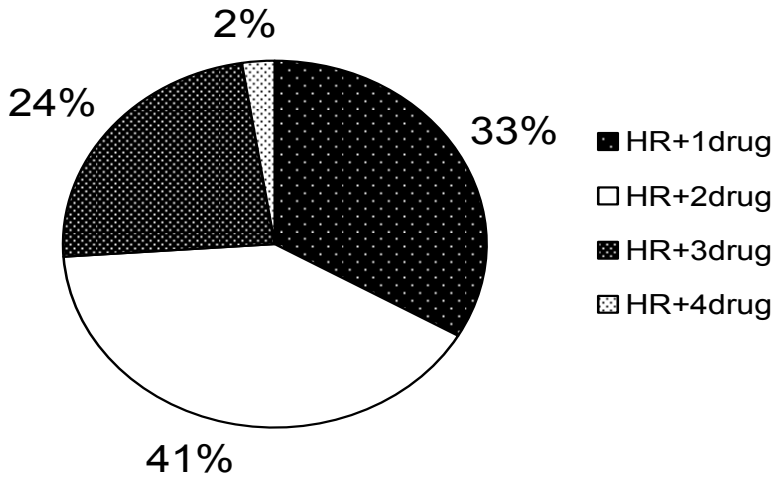
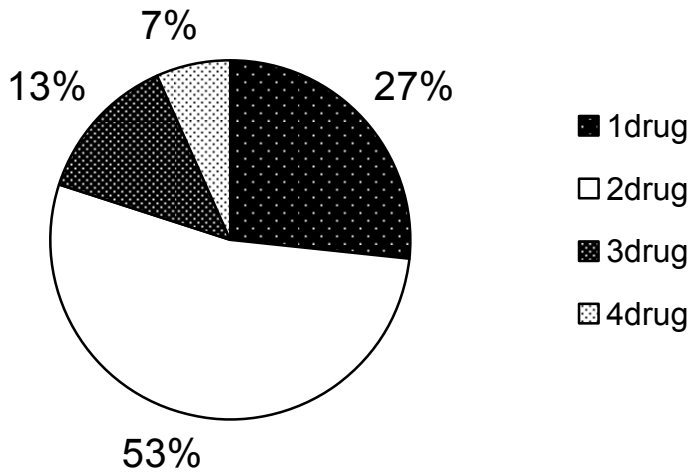


Fig.3b: Drug resistance pattern in non MDR-TB patients



[Contact person: Dr.Aleyamma Thomas (E-Mail ID: aleyammat@trcchennai.in)]

Utility of two antibiotic algorithms and repeat sputum smear microscopy to improve the efficiency of diagnosis in smear negative TB

Background

The diagnosis of smear negative PTB is vital, since such patients are likely to break down to smear positive cases if left untreated. A break down rate of about 28% in six months and 40% in two years has been reported. Importantly, nearly half of smear negative cases who require treatment develop active disease with in the first three months.

Objectives

Primary objectives

- To assess the utility of two antibiotic algorithms to improve the efficiency of diagnosis of smear negative TB
- To study the utility of repeat sputum microscopy in chest symptomatics with persistent symptoms after a course of antibiotics

Secondary objectives

- To study the radiological involvement, among smear negative, but culture positive patients
- To obtain information on the etiological profile of respiratory infections and their sensitivity pattern and studying appropriateness of antibiotic algorithm

Methods

Patients referred with cough of >3 weeks and having three smears negative for acid fast bacilli (AFB) were eligible to get recruited to the study. At TRC, the patients underwent three sputum examinations for AFB by smear and culture, and a chest X-ray was taken. They were randomly allocated to one of the following antibiotic regimens for treatment duration of 10 days:

- Co-trimoxazole (sulphamethoxazole-800 mg, trimethoprim-160 mg) twice daily for 10 days
- Doxycycline 100 mg twice a day on first day then once a day for 4 days followed by Amoxicillin 500 mg three times a day for 5 days

At the end of the antibiotics course, chest X-ray and sputum examinations were repeated, and patients were assessed for persistence of symptoms. Patients were started on Category-III regimen, if repeat sputum remains negative by smear and X-ray is still suggestive of TB. In cases of chest X-ray being abnormal but not suggestive of TB, they were followed up for 6 months with monthly sputum examination. If smears or cultures turned positive, the patients were started on Category-I regimen.

All the patients were reviewed with culture results. It was proposed to admit 700 patients to each antibiotic arm. The study was initiated in February 2007. Till March 2010, 423 patients have been enrolled in the study, whose details are given in Table 9.

Table 9: Details of patients recruited to the study

	Regimen		**Total**
	Co-trimoxazole	**Doxycycline-Amoxicillin**	
Total	212	211	423
Female	69	57	126
Male	143	154	297
Chest X-ray at baseline			
Abnormal	40	47	87
Normal	172	164	336
Patients started on ATT			
Category I	5	2	7
Category III	4	3	7
Culture positive at baseline	15	9	24

The study is in progress.

[Contact person: Dr.Aleyamma Thomas (E-Mail ID: aleyammat@trcchennai.in)]

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 MDR-TB

Background

Development of new TB drugs is a research priority. Tibotec medicinal compound (TMC) 207 is a methoxyquinoline with potential value in combination treatment of TB. It has a unique structure with high *in vitro* activity against wild-type and drug-resistant (DR) strains of *M. tuberculosis*. TMC207 inhibits the proton pump of the bacterial ATP synthase. TMC207 was found to be generally safe and well tolerated in phase I studies. In the exploratory (Stage I) of the trial completed in South Africa, the interim analysis results showed that addition of TMC207 to a 5-drug MDR-TB regimen resulted in significantly shorter time to culture conversion compared to placebo.

Aim

- To demonstrate the anti-bacterial activity of 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

Methods

Study design

Tuberculosis Research Centre was involved in Stage II of the trial. This was a stratified, randomized, double-blind, multi-centric, placebo-controlled Phase II study.

TMC 207 was given at 400mg dosage once a day for first two weeks followed by 200 mg thrice a week for the next 22 weeks along with background regimen consisting of kanamycin, ethionamide, pyrazinamide, ofloxacin and cycloserine in doses based on body weight. The patients were monitored weekly for two months, fortnightly for four months and monthly for 18 months.

Results

The study was initiated on August 2009. We have enrolled three patients among whom one is a diabetic. All three patients had culture conversion and have completed intake of TMC 207/ Placebo. Currently patients are receiving the background regimen.

The study is on-going.

[Contact person: Dr.Aleyamma Thomas (E-Mail ID: aleyammat@trcchennai.in)]

Changes in HIV viral load in patients undergoing treatment for filarial infection

(Collaborative study with National Institute of Health, USA)

The goal of this study is to determine changes in HIV viral loads that occur in patients co-infected with HIV and filaria, over one year following treatment with Diethylcarbamazine (DEC)/Albendazole, and to compare with changes in viral loads among HIV infected patients without filarial co-infections. The target sample size is 138, and two groups of patients are being recruited to the study. The first group (n=46) comprises of individuals with HIV and filarial infections (detected by serum antigen test), while the second or control group (n=92) consists of persons with HIV infection, but not filariasis. Both groups are matched by age, gender, HIV viral load and CD4 cell counts. Patient recruitment is being done in both TRC and YRG Care. Screening of patients for this study started in May 2007 at TRC. A total of 254 patients were screened, of whom 53 (positives – 14, controls – 39) patients were recruited to the study. All were treated with single dose DEC/Albendazole and followed up. HIV viral load measurement, CD4 cell counts and other safety parameters are examined during each follow-up visit. The study is in progress.

[Contact person: Dr.Soumya Swaminathan (E-Mail ID: soumyas@trcchennai.in); Dr. Pradeep Menon (E-Mail ID: pradeepamenon@trcchennai.in)]

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)

(Funded by United States Agency for International Development)

Background

There is paucity of data on development of Acquired Rifampicin Resistance (ARR) in regimens using thrice weekly Rifampicin (RMP) throughout the treatment period. Whether daily administration of RMP could overcome this phenomenon, by achieving a better and sustained concentration in the blood

and/or when ART is started (for those who need it) during ATT, will be our primary research question.

Aim

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

Methodology

An open labelled, prospective, parallel arm, active comparator, randomized controlled clinical trial has been initiated, 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. These patients were investigated for ART requirement and suitably referred to NACO ART centres for ART procurement. The primary outcome was bacteriological failure and the emergence of ARR. Secondary outcomes included overall failures, recurrences, Immune reconstitution inflammatory syndrome (IRIS), and death due to TB.

Results

After conducting a pilot study, the main study was started in September 2009. As on 1st April 2010, 196 patients were screened and 44 patients were registered after preliminary clinical examination and evaluated by sputum smear and chest skiagram. Among these patients, 20 patients have been allocated into the study after getting the informed consent, 14 from Chennai and 6 from Madurai. Seventeen patients were allocated based on smear positivity and 3 cases based on rapid culture by BACTEC. The median CD4 cell counts in this group of patients was 79 cells/mm^3 (IQR 39-145). Four patients who successfully completed ATT have been cured. Patient recruitment to the study is in progress. [Contact person: Dr.G. Narendran (E-Mail ID: nareng@trcchennai.in)]

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

(Funded by United States Agency for International Development)

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

Aims

- To study the efficacy of TB preventive therapy in reducing the incidence of TB and mortality among HIV-infected individuals starting highly active antiretroviral therapy
- To assess the role of symptoms, clinical examination, sputum microscopy and chest X-ray in ruling out active TB

Study regimens:

The 2 study arms will be

1) Background ART regimen + (placebo daily for 6 months) – self administered, collected once a month
2) Background ART regimen + (Isoniazid (H -300mg) daily for 6 months) - self administered, collected once a month.

The pilot study was initiated in October 2009. As of 31st March 2010, 20 cases have been recruited in the pilot study and the study is ongoing.

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

(Funded by ICMR Task Force)

Background

Anaemia is common during HIV infection, and is associated with increased morbidity and mortality. In India, the profile and impact of anaemia during childhood has not been adequately studied.

Aims

- To assess the prevalence of anaemia and micronutrient deficiencies among HIV-infected children in south India
- To examine nutritional and non-nutritional etiological factors contributing to anaemia among HIV-infected children
- To compare the effect of therapeutic iron supplementation in those with nutritional anaemia and anaemia of inflammation
- To assess the effect of baseline anaemia on growth and HIV disease progression status in children with HIV infection

Methodology

A total of 240 children with perinatally acquired HIV infection aged between 2 and 12 years at three sites in south India, with equal proportions of children who are ART-naïve and on ART were planned to be enrolled for this study. All eligible children underwent clinical and physical examination. Basic demographic data, clinical history and dietary history were collected.

Baseline investigations included complete blood count, CD4 cell counts, HIV viral load, markers of iron status (serum ferritin, transferrin saturation, total iron binding capacity, zinc erythrocyte protoporphyrin) and markers of micronutrient status (retinol, vitamin B_{12}, folic acid). Children with hemoglobin level below 11 g/dl were classified as anaemic and those with hemoglobin level more than 11 g/dl as non-anaemic. All anaemic children were given iron, folate and vitamin B_{12} supplementation for 3 months, and investigations such as complete blood count and markers of iron status were repeated, at the end of third month, to find the effect of iron, folate and B_{12} supplementation. The children were followed up every 3 months.

The study was initiated in February 2010. As on 31st March 2010, 11 children have been recruited to the study.

[Contact person: Dr.P.K. Bhavani / Dr. Soumya Swaminathan (E-Mail ID: bhavani.pk@trcchennai.in / soumyas@trcchennai.in)]

Study to evaluate the effect of physician's advice in quitting smoking in HIV and TB patients in south India – A pilot study

(Registered in Clinical trials registry of India No.: CTRI / 2009 / 091 / 000962)

(Funded by Fogarty AITRP, Miriam Hospital/Brown University, USA)

Background

The burden of HIV and TB in India is high, and the association of TB and smoking is evident. Smoking cessation initiation by physician's advice has been shown to be useful in previous studies.

Aims

- To determine the efficacy of physician's advice using "modified 5 A' strategy in quitting smoking in patients with HIV and TB and to compare various strategies of counseling in enabling patients to quit smoking

Methods

It has been estimated to include about 80 smokers each having TB and HIV infections. Eligible individuals are randomized into two groups: Group A will receive Physician's advice and counseling with Brochure/educative material, while Group B will receive only Counselors counseling and Brochure/ educative material for adopting smoking cessation. The study population will be stratified based on nicotine dependence as assessed by the Fagerstrom dependence scale. Physician's advice will follow the 'modified 5 A' strategy namely "Ask, Advise, Assess, Assist and Arrange".

The study commenced in March 2010. As on 31 March 2010, four subjects have been recruited.

Intake of patients to study is in progress.

[Contact person: Dr.S. Ramesh Kumar (E.mail ID: ramesh@trcchennai.in)]

SOCIOLOGICAL RESEARCH

Ongoing studies

Addressing psychosocial needs and HIV risk in Indian Men who have sex with men

(Funded by Indo-US Joint Working group)

Background

Men who have sex with men (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 this present study. The innovativeness of this study is that it explores the possibility of providing an intervention that targets psychosocial problems concurrent with HIV risk reduction behaviours among the MSM population. This is different from the usual intervention programs that concentrate on HIV prevention through HIV/STI messages, condom distribution and HIV testing programs. The intervention study was planned against this background. The study is done in collaboration with Harvard Medical School/MGH and Fenway Community Health.

Aims

- To develop a behavioural prevention intervention for MSMs in Chennai
- To conduct an open pilot randomized controlled trial of the intervention in MSMs in Chennai with an outcome of risk sexual behaviour
- To conduct a pilot randomized controlled pilot of the intervention in MSM with an outcome of risky sexual behaviour

Progress of the study

- Phase I of the study carried out and 59 MSMs enrolled (August 2009-November 2009) after formation of the Community Advisory Board (CAB)
- The findings of Phase 1 helped to prepare the FGD guide for the open pilot initiated from December 2009 till January 2010. Seventeen participants were screened and 10 enrolled in the study. The sessions

included 6 group sessions and 3 individual sessions. The sessions were conducted once a week based on a preliminary intervention manual

- Based on the findings from the open pilot we have initiated the randomized control intervention-Phase 3 initiated in March 2010. The participants go through a base line interview, HIV /STI testing, 4 group sessions and 4 individual sessions, follow-up sessions at 3 and 6 months

We have completed 2 groups of 8 in each group and follow-up sessions are in progress.

[Contact person: Dr.Beena Thomas (E-Mail ID: beenathomas@trcchennai.in)]

Community based approach in designing an AIDS program for HIV+ mothers in India

(Funded by Indo-US Joint working group)

Background

The study is done in collaboration with University of California, Los Angeles, USA. In India, an increasing number of monogamous married women are becoming infected with HIV and the number of children infected with this deadly virus is on the rise. Mothers living with HIV/AIDS (MLH) therefore carry a triple burden of being HIV-infected, mothers of children who may or may not be positive themselves, and care givers to their infected spouses.

Aim

- To explore the perceptions and needs of mothers living with HIV, to gain greater insights into the challenges they face in relation to their health seeking behavior, fears around disclosure, and issues related to stigma and discrimination

Methods

This study has adopted a qualitative approach utilizing focus group discussions with MLH who have children aged 0-10 years. The MLH are recruited from the Maternity hospitals, STD clinics and with the help of Positive Women's Network. To maintain uniformity in discussions a semi-structured interview guide was developed based upon the comprehensive health seeking and coping paradigm.

A CAB (Community Advisory Board) was initiated to direct and review the study. So far, 11 focus group discussions have been conducted with 60 MLH.

NVIVO, a qualitative software program is being used to store and organize the focus group interview data. Content analysis was carried out with the transcripts.

Results

The following five general themes have come out from the discussions so far:

1. **Health care needs of MLH**
    - Women sought care when symptoms were unbearable
    - Delay in seeking care.
    - Problems in going to different clinics for themselves and their children
2. **Sites they seek care**
    - Private clinics are accessed first as their timings are convenient and closer but they are expensive and feeling of being exploited "I spent Rs.7000 in a private clinic for HIV drugs; I had no idea that drugs were free in a government hospital"
3. **Disclosure Issues**
    - Majority expressed fears on negative reactions from in laws and children if disclosure was done.
    - More than one third felt it was better to disclose to their children at an age when they could understand
4. **Stigma**
    - Stigma was pronounced among health care workers and physicians "When they (physicians) see our case records and our reactive status they make us wait till the others finish. They ask us very sensitive and embarrassing questions"
5. **Strategies for improving access to health care**
    - Better heath care to be offered in general hospitals with the other specializations instead of having a separate unit for HIV

We also plan to have individual interviews with 40 MLH. 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.
The study is ongoing.
[Contact person: Dr. Beena Thomas (E-Mail ID: beenathomas@trcchennai.in)]

The alcohol use disorders identification test (AUDIT) among TB patients attending Chennai Corporation Health Centers, south India
(Funded by Model DOTS Project)

Background

It is of great concern that most health care providers across many nations do not screen for alcohol abuse and its associated impact on the prognosis while treating major illnesses, particularly in TB management. In India, there is a dearth of information on prevalence of alcohol use disorders (AUD) among TB patients.

Aims

- To examine factors associated with alcohol abuse in TB patients
- To study the perception of TB patients with alcohol use disorder to an alcohol intervention programme

Methods

This cross-sectional cohort study covering 10 corporation zones in Chennai was carried out in two phases. Four zones were randomly selected out of 10 and all TB patients treated during July to September 2009 were screened using AUDIT scale for alcohol consumption. The first phase was a situational assessment and the second one was screening of TB patients by a WHO developed AUDIT scale. The second phase of the study used qualitative research methodology to find out the perception of TB patients with AUD on a feasible alcohol intervention program.

Interim findings

A total of 490 TB patients were screened of whom 141 (29%) were found to consume alcohol, among the 141 TB patients 73 (52%) had an AUDIT score of >8. Some of the factors associated with alcohol abuse were age above 35 years,

inadequate education, low income, being separated or divorced and receiving Category-II treatment of the RNTCP. The difference between the TB patients with AUD and those without AUD was statistically significant ($p < 0.01$). Further analysis of the study data is in progress.

[Contact person: Mrs. Mohanarani Suhadev (E-Mail ID: mohana@trcchennai.in)]

VACCINE TRIAL

A 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

This prime-boost strategy follows the successful completion of an earlier phase I HIV preventive vaccine trial. A total of 16 volunteers (7 females) who were healthy, HIV uninfected and at low risk for HIV were recruited. Eight of them were allotted to Arm A and six received DNA (ADVAX) prime (2 injections at 0 and 1 month) followed by two booster injections of a vector vaccine (TBC-M4) at 3 and 6 months and two received placebo. Six individuals in Arm B received 3 injections of TBC-M4 at 0, 1 and 6 months and two received placebo. All the scheduled injections have been completed and follow up visits are taking place. The study is expected to be completed in December 2010.

[Contact person: Dr.V.D.Ramanathan (E-Mail ID: ramanathanvd@trcchennai.in)]

EPIDEMIOLOGICAL & OPERATIONAL RESEARCH

Studies completed

Epidemiological impact study: Disease survey

Background

The DOTS programme was implemented in Tiruvallur district of Tamil Nadu in May 1999. The centre is carrying out a series of sample surveys with a duration of 2.5 years, between surveys, to assess the epidemiological impact of DOTS strategy and to estimate the prevalence of the disease in this district, (2 Taluks only) that covers a population of 5,80,000.

Aim

- To study the trend over time for disease occurrence and thereby to measure the impact of DOTS strategy in Tiruvallur

Methods

All adults aged ≥15 years included for the disease survey were screened by two screening methods namely, elicitation of chest symptoms and X-ray examination. Two sputum samples were collected from those who were either symptomatics and/or having an abnormal X-ray suggestive of TB. The sputum specimens were processed for smear and culture and those who became bacteriologically positive were referred for anti-TB treatment.

Results

Three serial disease prevalence surveys have already been completed. The fourth survey was completed in May 2009. Coverage in the survey was above 90% for all examinations, namely, symptoms, X-ray and sputum examination. Table 10 shows the coverage for the fourth survey.

Table 10: Coverage in the fourth survey

Activities	Fourth survey (n)
Enumeration	110905
Symptom screening	101263
X-ray screening	99432
Sputum eligible	11461
Sputum collection	11025

In this survey, 439 individuals were identified as TB cases through examination either by smear, culture or both.

[Contact person: Dr.C.Kolappan (E-Mail ID: kola155@trcchennai.in)]

Studies in progress

One time survey (Disease survey)

The one time disease survey was conducted in a sample of villages selected from those in the BCG trial area that are not covered under the epidemiological impact study. The study started from March 2008 in a sample population of 54110, of which 41935 comprises of adults (77.5%). The coverage up to March 2010 is shown in Table 11.

Table 11: Coverage for the one time disease survey

Activities	One time survey (n)
Enumeration	39963
Symptom screening	37089
X-ray screening	36219
Sputum eligible	4415
Sputum collection	4217

A total of 130 individuals were identified as TB cases through examination either by smear, culture or both. The survey is in progress.

[Contact person: Dr.C.Kolappan (E-Mail ID: kola155@trcchennai.in)]

Estimation of prevalence of PTB in Chennai city

In view of a high prevalence of PTB in urban areas, a sample survey was started in the Chennai Metropolitan city in August 2009.

The sample size has been estimated to be 26,529. This is distributed among 50 clusters with the cluster size of 531; the adult population (>15 years) to be enumerated in each ward is about 600 individuals (slum 200, non slum 400). The coverage in this survey upto March 2010 is shown in Table 12.

Table 12: Coverage in the survey in Chennai

Activities	Survey in Chennai (n)
Enumeration	1200
Symptom screening	1084
X-ray screening	1060
Sputum eligible	79
Sputum collection	67

Two Individuals were identified as TB cases through examination either by smear or culture or both.

[Contact person: Dr.C.Kolappan (E-Mail ID: kola155@trcchennai.in)]

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

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 15-54 years old men was 57% and that among women was 11% (source NFHS 3). The required sample size for this survey has been calculated as 2141. The same sample size is used for the urban, semi urban and rural centres.

The survey commenced from August 2009 in adults aged ≥ 15 years in 31 wards in Chennai (urban) and 10 wards in Ambattur (semi-urban). This included slum and non-slum residents. A written consent was obtained from all the participants. The registered population was screened for pulmonary tuberculosis and tobacco use by a structured questionnaire. Two sputum specimens were collected from all the study participants. The coverage upto March 2010 is shown in Table 13. The survey is in progress.

Table 13: Coverage for tobacco use survey

Activities	Tobacco use survey (n)
Enumeration	2418
Screened for tobacco use and TB	2403
Tobacco users	463
Sputum collection	2007
Smear and/or culture positives	8

[Contact person: Dr.C.Kolappan (E-Mail ID: kola155@trcchennai.in)]

The impact of HIV infection on recurrence of TB

Background

There is limited information on the relative proportion of re-activation and re-infection at the time of recurrence among HIV-infected and uninfected patients successfully treated for TB in India.

Aim

- To compare the genotypic profile of pretreatment isolates and recurrence isolates of *M. tuberculosis* from HIV-infected TB patients and HIV-uninfected TB patients using three different genotypic tools

Methods

HIV-infected and uninfected patients with sputum culture positive PTB were treated with short-course regimens and followed for 36 months, at TRC. Bacteriologic recurrences were documented, and typing of strains done using

three different genotypic techniques: Restriction fragment length polymorphism (RFLP) by IS6110, spoligotyping and mycobacterial interspersed repeat unit (MIRU)-variable number tandem repeat (VNTR). The DNA fingerprints of paired (baseline and at recurrence) *M. tuberculosis* isolates were compared.

Results

DNA fingerprints of 44 HIV-infected and 30 HIV uninfected patients with recurrent TB were available, of which 25 and 23 were paired isolates (Fig.4). Recurrence was due to exogenous re-infection in 80% of HIV-infected and 9% of HIV uninfected patients. The EAI3 clade was the commonest (40-50%) (Fig.5a), and over 40% of *M. tuberculosis* isolates had a single copy of IS6110 in both groups (Fig.5b). Clustering of recurrent strains was more common in the HIV-infected group (Figs.6a & b).

Conclusions

In India, a TB-endemic country, most recurrences after successful treatment for TB are due to exogenous re-infection in HIV-infected and endogenous re-activation in HIV uninfected persons. Strategies for prevention and treatment of TB must take these findings into consideration.

Fig. 4: Profile of patients enrolled in clinical trials in HIV-infected patients

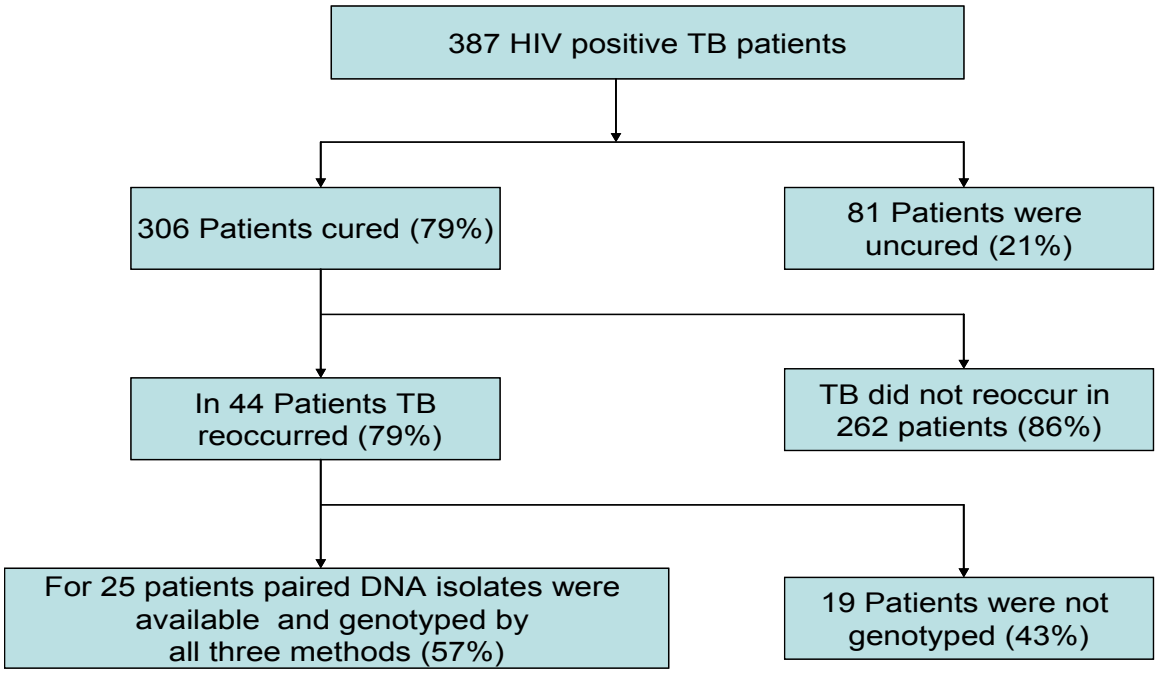


Fig. 5a: Spoligotyping pattern of TB isolates from HIV-infected and HIV-uninfected patients, at baseline and at recurrence

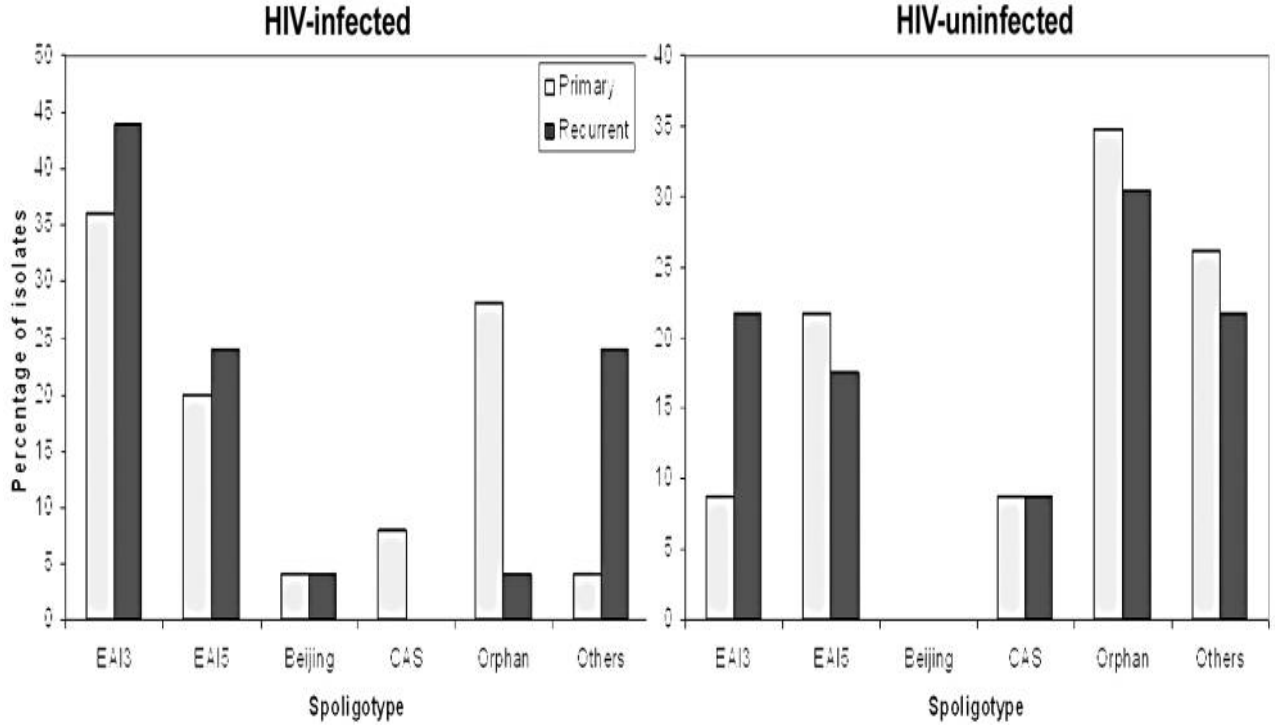


Fig. 5b: IS6110 RFLP of TB isolates from HIV–infected and HIV-uninfected patients, at baseline and at recurrence

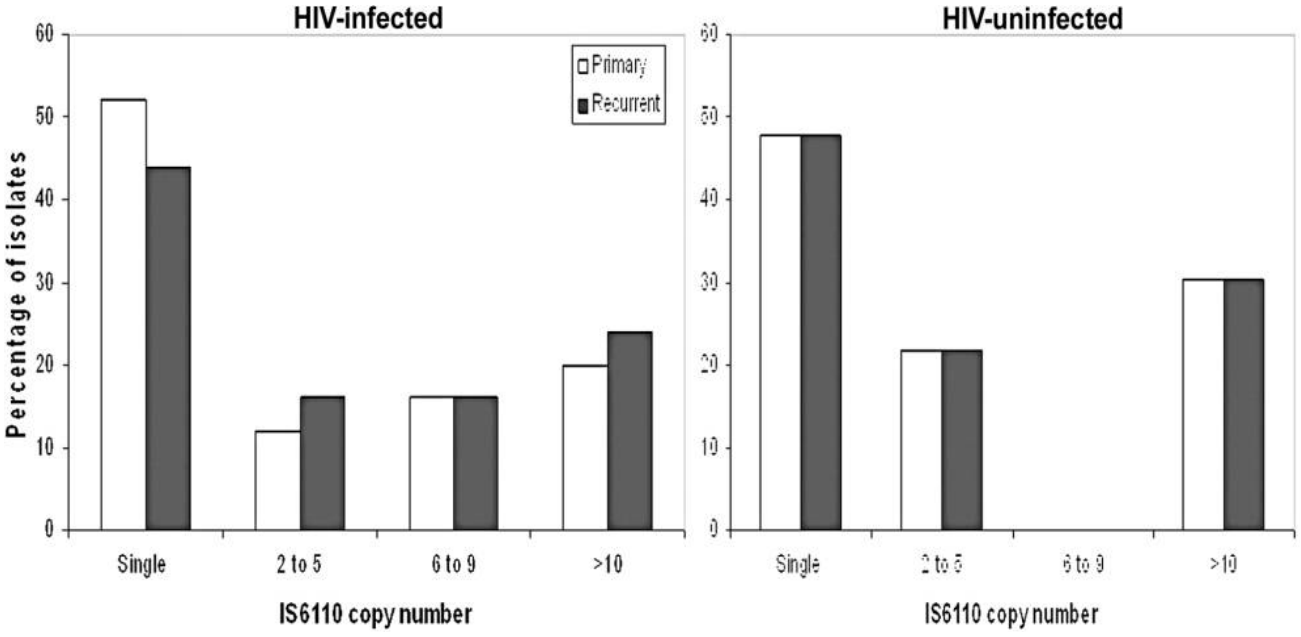
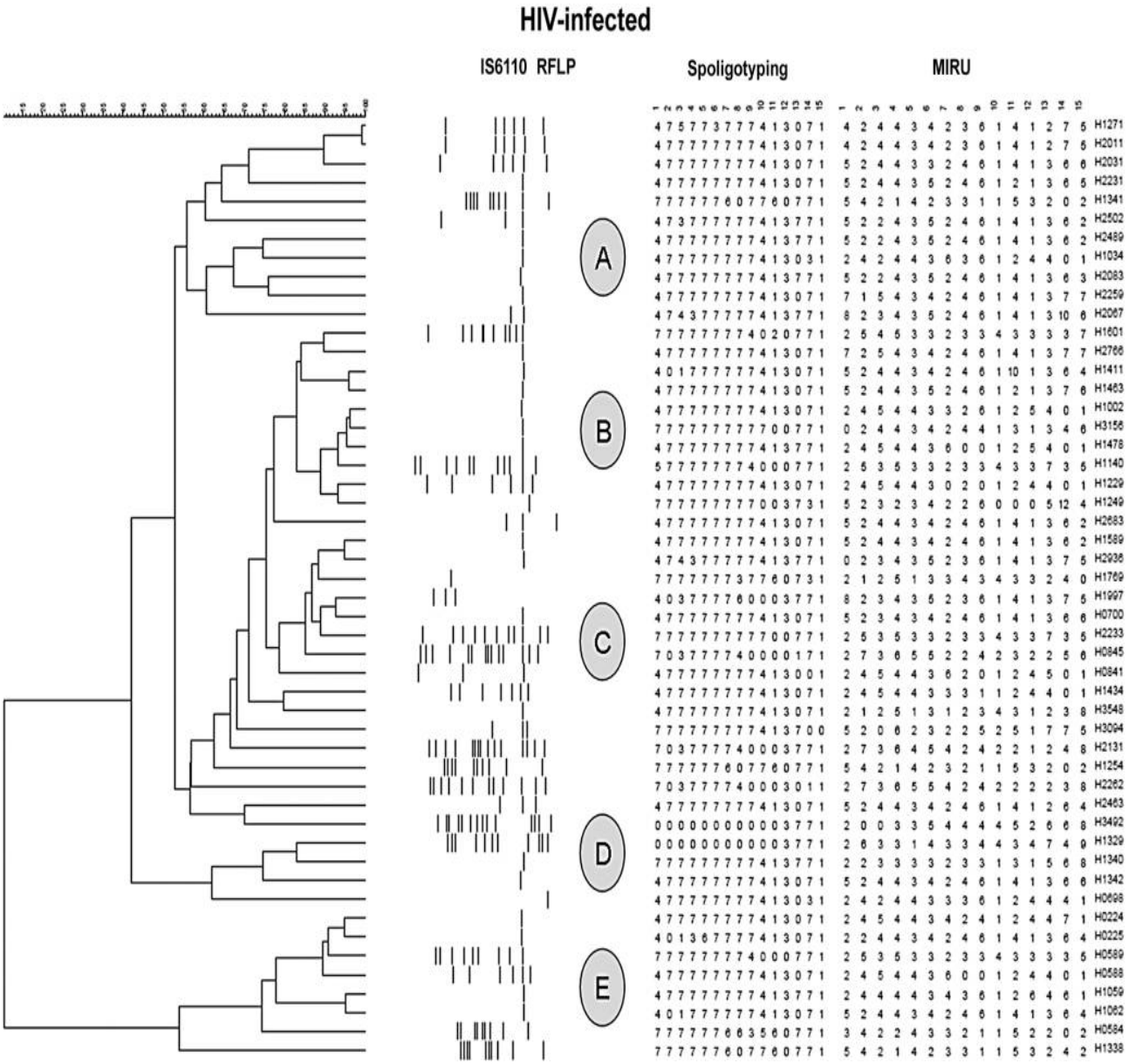
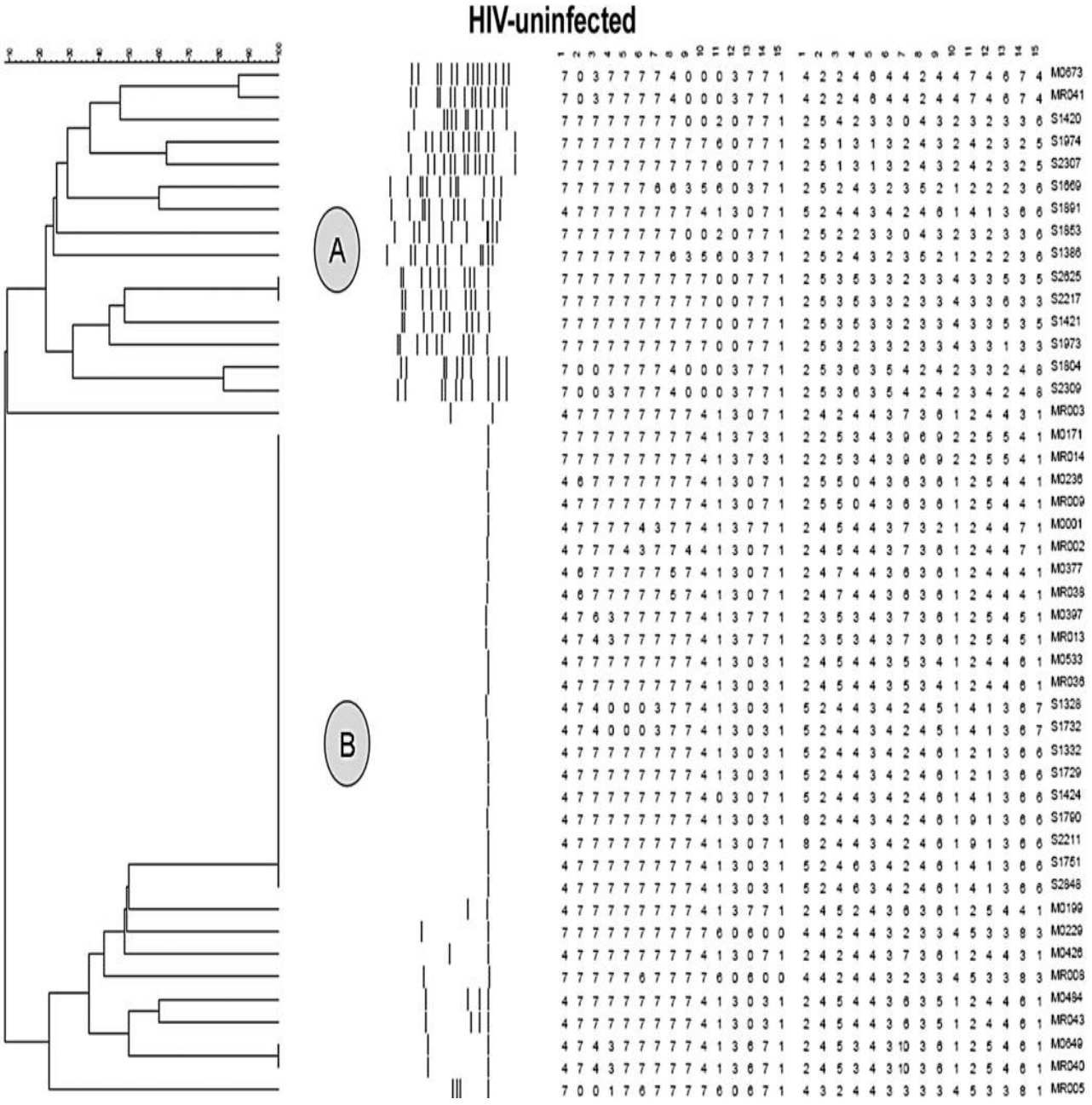


Fig. 6: Combined dendrogram of HIV-infected (a) and HIV-uninfected (b) patients based on similarities of isolates, using IS6110 RFLP, spoligotyping, and MIRU

a:



b:



[Contact person: Dr.Sujatha Narayanan (E-Mail ID: sujathan@trcchennai.in)]

APPLIED RESEARCH

Studies completed

Plasma Efavirenz in HIV-infected children treated with generic antiretroviral drugs in India

Collaboration: Kilpauk Medical College & Hospital, Chennai, Govt. Rajaji Hospital, Madurai and BJ Wadia Hospital, Mumbai

Background

The NACO has developed a weight-based dosing card used in ART centres, which aims to provide the correct dose of antiretroviral drugs to children. EFV is available as a single drug which is given along with lamivudine and stavudine, usually to patients on concurrent anti-TB medications and those who cannot tolerate NVP. It is recommended that EFV be administered based on body weight.

Aim

- To measure the steady state 12-hour plasma concentration of EFV in HIV-infected Indian children receiving treatment with generic drugs

Methods

Sixteen HIV-infected children receiving an EFV-containing ART regimen for at least two weeks from Government ART centres at Kilpauk Medical College & Hospital, Chennai, Government Rajaji Hospital, Madurai and B.J. Wadia Hospital, Mumbai, were recruited; six were males, the mean age was 101 months, mean CD4 count was 14% and mean body weight was 18.8 kg. None were on concurrent rifampicin-containing anti-TB treatment. Twelve-hour plasma EFV concentration was determined by HPLC.

Results

The mean 12-hour EFV concentration in the 16 children was 2.39 µg/ml (range: 0.72 – 7.82 µg/ml). The blood levels were within the therapeutic range (1 – 4 µg/ml) of EFV in 12, below 1.0 µg/ml in 1 and above 4.0µg/ml in 3 children. The only child who had sub-therapeutic EFV concentration did not show an increase in CD4 cell counts up to 36 months of treatment. Weight gain ranged from 0.3 to

19.5 kg during a treatment period of 1 to 93 months and clinical status showed improvement in all children.

Conclusions

This is the first study to evaluate EFV levels in an Indian pediatric population. We found that the majority (15 out of 16 children) receiving EFV doses based on the NACO guidelines had 12-hour EFV concentrations within or above the therapeutic range. Three children with drug levels above the therapeutic limit did not have any obvious adverse effects due to EFV. This study provides some preliminary information that can be used to guide treatment policy for HIV-infected children in India.

[Contact person: Dr.Geetha Ramachandran (E-Mail ID: geethar@trcchennai.in)]

Monitoring plasma NVP and EFV in HIV-TB patients undergoing anti-TB and antiretroviral treatment

Background

TB is the most common complication of HIV infection and is associated with high fatality rates. The NNRTIs, NVP and EFV are important components of HAART, and used extensively in resource-limited settings, where few alternate regimens are available. RMP, a key component of antitubercular regimens induces the expression and activity of the CYP metabolic enzymes in the liver, thereby greatly reducing plasma concentrations and exposure, of NVP and EFV during concomitant treatment with both drugs. This could lead to HIV treatment failure and development of drug resistance.

Aim

- To examine the association of blood levels of NVP and EFV (during and after completion of anti-TB treatment) with virological outcomes

Methods

This was a prospective study undertaken in patients with HIV-1 and TB, who were participating in a controlled clinical trial at the Centre. All patients received RNTCP Category-I regimen for treatment of TB, and started receiving antiretroviral treatment after two months of anti-TB treatment. The patients were

randomized to receive once daily antiretroviral regimens that consisted of lamivudine (300 mg) and didanosine (250 mg for body weight < 60 kg and 400 mg for body weight ≥ 60 kg) with either NVP (400 mg) or EFV (600 mg). All drugs were administered in the morning under complete supervision.

Patients were assessed at baseline and at regular time points up to two years. This included determination of CD4 cell counts and HIV-1 RNA levels at baseline and every six months up to two years. Patients with plasma HIV-1 RNA level > 400 copies / ml at six months of ART, as analysed by intention-to-treat analysis were considered as virological failures or unfavorable responders.

Blood samples, corresponding to trough concentration, for estimation of plasma NVP and EFV, were collected at 4 time points after initiation of ART, viz., months 1 and 4 during anti-TB treatment and months 6 and 12, after completion of anti-TB treatment. Plasma NVP and EFV were estimated by HPLC according to validated methods. Genotyping of *CYP2B6* 516 G>T was performed using genomic DNA extracted from whole blood. A 204 base pair fragment in exon 4 of the *CYP2B6* gene containing the target site (position 516) was amplified and the amplicon was directly sequenced.

Results

A total of 107 patients, 52 and 55 in the NVP and EFV arms respectively were included in this study. Among them, 17 and 7 respectively in the NVP and EFV arms were unfavorable responders. The NVP and EFV levels (mean of 1 & 4 months and 6 & 12 months) did not significantly differ between favorable and unfavorable responders (Table 14). However, the total number of patients in the NVP and EFV arms with sub-therapeutic plasma levels were significantly higher among unfavorable than favorable responders (40% vs. 27% with mean values at 1 & 4 months and 33% vs. 25% with mean values at 6 & 12 months); these differences were statistically significant ($p < 0.05$). No significant differences in NVP and EFV levels were obtained when these drugs were given with and without RMP (Table 14). Plasma EFV was significantly higher in the TT than GG and GT genotypes of *CYP2B6* 516 G>T polymorphism at all the time points studied ($p < 0.05$). However, no significant differences in NVP levels between TT

and GG/GT genotypes were observed (Table 14). Among 18 unfavourable responders for whom *CYP2B6* genotyping was done, 12 and 6 belonged to GG/GT and TT genotypes respectively; this difference was highly significant ($p < 0.01$).

Table 14: Plasma NVP & EFV in the different patient groups

(Values are mean ± SD)

Variable	NVP	EFV
Mean of 1 & 4 months		
Favourable responders	4.6 ± 2.3 (n = 25)	2.5 ± 2.6 (n = 45)
Unfavourable responders	3.8 ± 1.5 (n = 6)	2.9 ± 4.1 (n = 4)
Mean of 6 & 12 months		
Favourable responders	4.6 ± 1.9 (n = 26)	2.1 ± 1.6 (n = 37)
Unfavourable responders	6.4 ± 2.9 (n = 2)	2.6 ± 2.5 (n = 2.5)
RMP co-administration		
Mean of 1 & 4 months (with RMP)	4.5 ± 2.1 (n = 31)	2.5 ± 2.7 (n = 49)
Mean of 6 & 12 months (without RMP)	4.7 ± 2.0 (n = 28)	2.3 ± 2.5 (n = 41)
Genotype		
Mean of 1 & 4 months		
GG	3.7 + 1.6 (n = 11)	1.4 + 1.0 (n = 13)
GT	5.1 + 2.7 (n = 12)	1.7 + 1.3 (n = 21)
TT	4.5 + 1.9 (n = 6)	6.5 + 3.2* (n = 10)
Mean of 6 & 12 months		
GG	4.7 + 2.2 (n = 13)	1.5 + 0.6 (n = 12)
GT	4.5 + 2.3 (n = 10)	1.9 + 1.2 (n = 19)
TT	5.4 + 1.3 (n = 4)	5.0 + 4.5* (n = 8)

Number of patients in each group given in parantheses

* denotes $p < 0.01$ vs. GG & GT genotypes

The mean inter-patient variations of EFV and NVP were 117 and 59% respectively; the corresponding values for intra-patient variations were 34 and 32% respectively. Logistic regression analysis showed that genotypes of the *CYP2B6* 516 G>T polymorphism was found to be significantly associated with mean of 1 & 4 month EFV levels ($p < 0.05$; OR: 4.3).

Conclusions

This study has provided some evidence to demonstrate that regular monitoring of plasma NVP and EFV might help in predicting virological failures. RMP co-administration did not significantly alter plasma NVP when used once-daily. This study stresses the importance of genetic polymorphisms in determining plasma NNRTI concentrations.

[Contact person: Dr.Geetha Ramachandran (E-Mail ID: geethar@trcchennai.in)]

Evaluation of phage lysin to decontaminate sputum samples for the detection of *M. tuberculosis* using MGIT 960 system

Background

Use of a cocktail of three bacteriophages substituting for antibiotics to control the overgrowth of normal flora in processed sputum specimens has been established. Phage based enzyme, lysin from these phages effectively controlled the growth of normal flora without hampering the viability of *M. tuberculosis*.

Aim

- To evaluate the suitability of phage lysin to decontaminate processed sputum samples for the detection of *M. tuberculosis* using MGIT 960 system

Methods

One hundred and fifty sputum specimens were processed by modified Petroff's method and inoculated on to two slopes of Lowenstein Jenson medium (Petroff's culture). The deposits were washed with phosphate buffered saline and divided in to two aliquots of 0.5 ml each. One aliquot was added to 7 ml of MGIT medium containing 0.8 ml of antimicrobial supplement PANTA and used as a

control (MGIT-PANTA). The second aliquot was added to 7 ml of MGIT medium containing 0.8 ml of lysin prepared in bovine serum albumin (MGIT-lysin). The samples were randomized and incubated at 37^{O}C in MGIT 960 system.

Results

The sensitivities of methods using MGIT-PANTA and MGIT-lysin were 100%, while the specificities were 74% and 73% respectively compared to Petroff's culture method (Tables 15a & b). The sensitivity and specificity of MGIT-lysin were 97% and 88% respectively when MGIT-PANTA was considered as gold standard. The agreement between the methods was 94% (Table 15c). The average time to detection by MGIT-lysin was 9.32 days and by MGIT-PANTA was 8.62 days. The rates of contamination with MGIT-PANTA and MGIT-lysin were 16% & 7.3% respectively.

Table 15 a, b & c: Comparison of methods using MGIT-lysin and MGIT-PANTA for detection of *M. tuberculosis*

a

		Petroff's Culture		
		Positive	Negative	Total
MGIT PANTA	Positive	79	12	91
	Negative	0	35	35
	Total	79	47	126
Sensitivity 100%; Specificity 74%; Agreement 90%.				

b

		Petroff's Culture		
		Positive	Negative	Total
MGIT Lysin	Positive	84	15	99
	Negative	0	40	40
	Total	84	55	139
Sensitivity 100%; Specificity 73%; Agreement 89%.				

c

		MGIT PANTA		
		Positive	Negative	Total
MGIT Lysin	Positive	88	4	92
	Negative	3	28	31
	Total	91	32	123
Sensitivity 97%; Specificity 88%; Agreement 94%.				

Conclusion

Phage lysin can be used to decontaminate processed sputum specimens for the detection of *M. tuberculosis*.

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

Slide culture for rapid, simultaneous detection and drug sensitivity testing of *M. tuberculosis* from sputum samples

Background

Slide culture technique involves culture of decontaminated sputum on glass slides immersed in suitable liquid medium and detection of stages of mycobacterial growth after incubation for a short period that is identified by staining the slide. The method was standardized earlier for rapid diagnosis. In continuation, the current study was taken up using larger number of samples. In addition to rapid detection, the study evaluated the technique for rapid simultaneous drug susceptibility testing of *M. tuberculosis* from sputum samples.

Aim

- To evaluate slide culture technique for rapid detection and simultaneous DST of *M. tuberculosis* isolates from sputum samples using Selective Kirchner's liquid medium (SKLM) and compare the results with conventional Loweinstein-Jensen (LJ) method as the gold standard

Methodology

Ninety seven sputum samples were included. Direct smears were taken and stained by auramine phenol. The samples were randomized and decontaminated by the modified Petroff's method. From the deposit, two LJ slopes were inoculated. From the remaining deposit, three smears were made each using 10μl on sterile slides covering an area of 0.05 cm^2. The smears were air-dried, heat fixed by gently passing over the flame three times and placed in Selective Kirchner's liquid medium with and without drugs. INH and RMP were used at a concentration of 0.1μg/ml and 1μg/ml respectively. After 7 days of incubation, the slides were removed, decontaminated using 10% formaldehyde, stained by auramine phenol and read under the fluorescent microscope. Growth

was graded and the results were compared with conventional methods as the gold standard.

Results

As a diagnostic method the slide culture yielded a sensitivity of 100% and a specificity of 94%. Agreement of the assay with the gold standard was 97%. The positive predictive and negative predictive values were 94% and 96% respectively. Drug susceptibility test using slide culture yielded a sensitivity and specificity of 82% and 100% respectively for INH. The corresponding values for RMP were 88% and 96% respectively.

Conclusion

The slide culture technique described in this study is simple enough for application in a medium level laboratory. The method has demonstrated high levels of sensitivity, specificity and rapidity for diagnosis. While the technique detects most of the susceptible isolates, further work is needed to improve its sensitivity for detection of MDR cases.

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

Studies in progress

Isolation and identification of active fractions from selected medicinal plants for antimycobacterial agents

Background

In recent years there has been a rising interest in the discovery of new antimycobacterial compounds, due to an alarming increase in the rate of infection with MDR-TB. Plant products remain an important source to combat serious diseases. The systematic screening of medicinal plants may give bioactive molecules which could be sources for many therapeutic agents.

Aim

- To isolate the active fractions from medicinal plants, namely *Hydnocarpus alpine* and *Vetiveria zizanioides* using bioassay guided fractionation

Methods

The powdered materials (100 gm) of selected plants were extracted sequentially with 500 ml of hexane and methanol (1:5 w/v). To extract essential oil, fresh roots of Vetiver (0.5 kg) were subjected to hydro-distillation. These crude extracts and oil underwent bioassay and fractionation. Standard strain *M. tuberculosis* H_{37}Rv and two clinical isolates of *M. tuberculosis* (one resistant to SHRE and one sensitive to SHRE), were tested. Three concentrations viz. 500, 250 and 125µg/ml were used to screen the extracts by luciferase reporter phage (LRP) assay. These two potent plants extracts were selected for further phytochemical analysis to identify the active fraction.

Results

The hexane extract of *H. alpine* showed maximum antimycobacterial activity (99.94, 99.82 and 97.44% of reduction in relative light units RLU for H_{37}Rv, SHRE resistant and SHRE sensitive strains, respectively followed by methanol extract at 500µg/ml concentration. Essential oil of *V. zizanioides* exhibited good antimycobacterial activity (96.16, 64.73 and 90.8% of reduction in RLU for H_{37}Rv, SHRE resistant and SHRE sensitive strains, respectively) at 500µg/ml concentration (Table 16). The hexane extract of *H. alpine* and essential oil of *V. zizanioides* were fractionated using column chromatography and four fractions were collected. Based on bioactivity guided fractionation, fraction three (HFR-III) of *H. alpine* and fraction three (VFR-III) of essential oil of *V. zizanioides* were identified as effective fractions.

Table 16: Percentage reduction in RLU using hexane and methanol extracts of *H. alpine* and essential oil of *V. zizanioides* against *M. tuberculosis* strains

Strains	*H. alpine*						*V. zizanioides*		
	Hexane (µg/ml)			Methanol (µg/ml)			Essential oil (µg/ml)		
	500	250	125	500	250	125	500	250	125
$H_{37}Rv$	99.94	99.44	97.69	89.94	79.94	79.63	96.16	88.59	84.37
MDR	99.82	98.78	97.93	79.75	79.79	69.58	64.73	33.13	27.66
SEN	97.44	91.58	90.12	97.42	77.52	86.45	90.80	50.95	11.41

$H_{37}Rv$- *M. tuberculosis* standard strain; MDR- Clinical isolate: S, H, R & E resistant strain; SEN- Clinical isolate: S, H, R & E sensitive strain

Conclusions

H. alpine and *V. zizanioides* were found to possess potent anti-TB activity. The effective fraction was identified from these two plants using bioassay guided fractionation. Further characterization and structural elucidation of these active fractions would lead to the identification of potent anti-TB agents.

[Contact Person: Dr. Vanaja Kumar (E.mail ID:vanajakumar@trcchennai.in)]

Pharmacokinetics of anti-tuberculosis drugs in children: impact of age, nutritional status and HIV infection

(Funded by 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, PZA and 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, PZA and EMB in children with TB

Methods

This is a multi-centric study being done in collaboration with Institute of Child Health, Chennai, Govt. Hospital of Thoracic Medicine, Chennai, Kilpauk Medical College & Hospital, Chennai and Govt. Rajaji Hospital, Madurai. The study population includes two groups of children aged 1 to 12 years: Group 1: TB; Group 2: HIV-TB. They must be receiving anti-TB medications based on RNTCP guidelines for minimum 15 days. Assessment of nutritional status is done using z scores calculated from the child's weight & height (CDC). The INH acetylator status is determined using saliva.

On the day of the study, blood samples are collected pre-dosing and at 2, 4, 6 and 8 hours after supervised administration of anti-TB medications. Plasma RMP, INH, PZA and EMB are estimated by (high performance liquid chromatography (HPLC) and pharmacokinetic variables calculated. Patients are being followed up to determine clinical outcomes.

It is proposed to recruit 80 children in each group (total 160 children); stratified sampling will be done to ensure equal numbers in the age groups: 1-3, 3.1-6, 6.1-9 & 9.1-12 years.

The pharmacokinetic study has been completed in 73 children with TB and 6 with HIV & TB.

The study is in progress.

[Contact person: Dr. Geetha Ramachandran (E.mail. ID: geethar@trcchennai.in)]

Effect of RMP and INH on the steady state pharmacokinetics of MFX in healthy volunteers

Background

MFX has promising antimycobacterial activity, and has a potential to shorten TB treatment. MFX undergoes phase II metabolism, and RMP, a potent inducer of cytochrome P-450 isoenzymes is known to induce phase II glucuronidation pathway. This could reduce the plasma concentrations of MFX and lead to poor treatment outcome. Our earlier study done in a small group of healthy subjects showed that RMP caused a significant reduction in plasma exposure of MFX, while in the case of INH, the variations were large. Since the small sample size limited us from drawing firm conclusions, this study was planned with a larger group of healthy subjects.

Aim

- To study the influence of RMP and INH on the steady state pharmacokinetics of MFX individually

Methods

This study was undertaken in collaboration with Madras Medical College, Chennai. The study was planned as a two-period, open-label, sequential-design pharmacokinetic study conducted in 24 healthy adult volunteers. A baseline pharmacokinetic study of MFX (400 mg once daily) was conducted and repeated after one week of daily MFX with either RMP (450/600mg) (n = 12) or INH (300mg) (n = 12). During both occasions, blood samples were collected at pre-dosing and at 1, 2, 4, 6, 8 and 12 hours after drug administration. The INH acetylator status was determined using saliva. Plasma MFX was determined by HPLC according to a validated method.

The study is in progress.

[Contact person: Dr. Geetha Ramachandran (E.mail. ID: geethar@trcchennai.in)]

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

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

This study was undertaken in collaboration with Govt. Hospital of Thoracic Medicine, Chennai. It has been planned to conduct the pharmacokinetic study in a sub-set of 48 patients (24 each in the daily and intermittent dosing arms) who took part in a randomized controlled clinical trial that compared daily vs. intermittent 6 – month SCC in patients with HIV and PTB. Eligible subjects were identified by the study investigators. On the day of the study, blood samples were collected at 0, 1, 2, 6, 8, 12 and 24 hours after dosing. Plasma concentrations of RMP were estimated by high performance liquid chromatography (HPLC) according to a validated method. In addition, pharmacokinetic study of RMP was also undertaken in those patients who failed ATT.

The study is in progress.

[Contact person: Dr.A.K. Hemanth Kumar (E.mail. ID: hemanthkumarak@ trcchennai.in)]

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

(Funded by Indo-US JWG (Intramural to India Grant)

Background

IRIS is the paradoxical worsening or unmasking of infection, tumor-associated pathology, or autoimmune 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.

Aim

- To determine the clinical and laboratory predictors for the development of TB-IRIS

Methods

This prospective cohort study was nested within a controlled clinical trial that compared daily vs. intermittent SCC in patients with HIV and PTB. Clinical predictors were evaluated and compared between patients who developed TB-IRIS and those who did not, based on baseline characteristics. Markers of T-cell activation (e.g., PD-1, CD69, intracellular Ki67, co-expression of HLA-DR and CD38) were evaluated for predicting TB-IRIS by comparing values at baseline and during TB-IRIS episodes. The effector response of CD4 T-cells to TB was studied by longitudinal follow-up of T-cell stimulation assays and a panel of serum cytokines (Th1/Th2/Th17) was measured.

Results

The study was started on November 2009. As on August 2010, 13 patients have been recruited into IRIS study, out of which 3 have experienced IRIS and one had possible IRIS.

The study is ongoing.

[Contact person: Dr. Soumya Swaminathan/ Dr. Sudha Subramanyam (E-mail ID: soumyas@trcchennai.in / sudha_s@trcchennai.in)]

ICMR-Biomedical Informatics Centre

Studies completed

Database for drug targets of resistant pathogens

Emergence of drug resistance is a major threat to public health. Many pathogens have developed resistance to most of the available antibiotics. Among the many factors responsible for the development of antimicrobial resistance, mutations in the target gene of the respective drug, play a major role. Therefore, the global impact of increasing resistance is a major concern. Drug resistance has been reported for many infectious diseases across different countries, and XDR forms of disease are extremely difficult to treat. For example, XDR-TB which is resistant to most of the available anti-TB drugs is reported from most parts of the world. Therefore novel drugs have to be developed for these re-emerging drug resistant diseases. Selection of drug targets is a major bottle neck in the drug discovery pipeline. In order to facilitate the process of drug discovery we have developed a database called database of drug targets for resistant pathogens (DDTRP). The DDTRP can be accessed online at http://202.141.106.126/ddtrp/. In the initial phase we have included *M. tuberculosis, M. leprae, P. falciparum, P. vivax, S. aureus, S. pneumonia,* and *N. gonorrhea* in the database. This database will be a useful resource for further research in drug discovery against drug resistant infectious diseases.

Materials and Methods

Among the genes which are targeted by currently used drugs (current targets), those genes for which drug resistance due to mutations has been reported were identified. The metabolic pathways in which these genes are involved were selected. Protein sequences of all the genes involved in the chosen metabolic pathways were downloaded from KEGG. Each of these proteins was compared with the human proteome using BLASTP. Proteins with no human orthologs at E-value 0.005 were considered as first level candidates. These proteins were further shortlisted using the following filters: uniqueness (absent in any other pathogen [narrow-spectrum drug-targets], targets present in more than one pathogen [broad-spectrum drug targets]), genes essential for the survival/growth

of the pathogen, proteins involved in pathogenesis (virulence factors) and persistence if applicable, and other biological features.

Results

We used targets of current drugs as lead targets to identify novel drug targets. These targets are proteins involved in metabolic pathways proven to be critical for the pathogen and whose interruption can result in a killing or static effect on the pathogen. It is very likely that other proteins involved in these metabolic pathways can cause the same effect if targeted. Based on this hypothesis we identified genes involved in metabolic pathways targeted by current drugs and involving genes with reported resistance as first level candidates, since it would be far easier to validate a smaller number of candidates than screening the entire metabolome.

The database provides information on currently used drug targets, current drug targets (with known drug resistance due to mutations), metabolic pathways and potential drug targets for TB, leprosy, pneumonia (*S.aureus* and *S. pneumoniae*), malaria (*P. falciparum* and *P. vivax*) and gonorrhea. Since drug target identification is the first and crucial step in the process of drug discovery, this database would be of immense help for research.

[Contact person: Dr.Luke Elizabeth Hanna (E-Mail ID: hanna@trcchennai.in)]

Virtual screening for small molecules against 3-oxoacyl-[acyl-carrier-protein] synthase III of *M. tuberculosis*

Molecular docking plays an important role in the line of novel drug discovery. We chose an essential enzyme, 3-oxoacyl-[acyl-carrier-protein] synthase 3 (FabH), of *M. tuberculosis* for molecular docking studies *in silico*. 3-oxoacyl-[acyl-carrier-protein] synthase 3 is an enzyme encoded by fabH (Rv0533c) in *M. tuberculosis*. Since FabH catalyzes the first condensation reaction which initiates fatty acid synthesis (FAS), this enzyme acts as a link between type I and type II FAS pathways of *M. tuberculosis*, it could be a potential drug target for *M. tuberculosis*.

Aim

- To find lead molecules to inhibit the 3-oxoacyl-[acyl-carrier-protein] synthase 3 of *M. tuberculosis*

Methodology

FabH is listed as one of the potential drug targets for *M. tuberculosis* in the DDTRP (www.bmi.icmr.org.in/ddtrp). MtFabH was found to have 12 entries in protein databank. These entries were analyzed using *Discovery studio visualizer.* One of these, a complex with lauric acid (1HZP) was chosen for docking. CYS112, HIS 244 and ASN274 were identified as important active site residues. Lauric acid was used as the initial molecule to screen Drug Bank and PubChem database and similar molecules/compounds were identified. About 211 molecules were shortlisted from these databases. CDOCKER was used for virtual screening of these molecules against MtFabH. PLP2 along with CDOCKER interaction energy was used to score the function of the docked molecules.

Results

Molecular Docking: One hundred molecules from the Drug Bank were found to have similarity to lauric acid. Of these, 19 were listed as already approved for use while the remaining 81 molecules were not. Using CDOCKER, we found that 15 and 76 molecules respectively from the approved and unapproved groups successfully docked on to MtFabH. When PubChem was searched with a threshold of 95% similarity, 111 molecules were identified as hits (similar molecules). Among these, 42 molecules were found to satisfy Lipinski's rule of five, while the remaining 69 molecules did not. All molecules from the former group and all but one from the latter group successfully docked on to FabH. Thus, a total of 211 molecules (111 from PubChem and 100 from Drug Bank) docked with FabH were identified.

Studies in progress

***M. tuberculosis* structural database**

With the advancement in X-ray crystallography and NMR technology, determining the three dimensional structures of proteins has become easier than ever before. This has led to an increase in the number of protein three dimensional structures and the development of structural databases that provide information about protein structures. PDB, MSD, TBSGC are examples of few protein structural databases. Currently there are 58,414 structures in the Protein Data Bank, of which 713 structures are for *M. tuberculosis* proteins. TBSGC deals exclusively with *M. tuberculosis* proteins. TBSGC database contains a total of 604 protein structures; however, some of the proteins are represented by more than one structure.

We have developed a database called the *M. tuberculosis* structural database (*Mtb*SD) housing 689 X-ray crystallized and NMR structures for 293 proteins of *M. tuberculosis* extracted from PDB. Structural details of each protein and the different structures for the same protein are systematically arranged making it easy for the user to know at a glance the number of structures available for a given protein and the differences between them. The differences in structures of a protein are also tabulated based on the ligand, drug or mutation present in these structures. *Mtb*SD would also provide value added information on the following aspects:

- Conformational changes that have occurred based on the interaction with ligand/drug or mutation present
- Active site information for all proteins which can be downloaded for docking studies
- Functionally important residues present in protein structures that can be useful for site directed mutagenesis experiments
- Functional grouping of all the proteins

[Contact person: Dr.Luke Elizabeth Hanna (E-Mail ID: hanna@trcchennai.in)]

Scoring Molecules: Scoring functions are used for final evaluation of positions after the DockScore was computed. For scoring the docked molecules no single method can be referred to as the best method. Hence, one force field method (CDOCKER energy) and an empirical method (PLP2) were used in the current study for scoring the docked molecules. Among the 211 molecules, hexahydroxyheptanoate and hexanoic acid were selected as better molecules based on the combined score of PLP2, CDOCKER, interaction energy and number of hydrogen bond interactions with that of active site residues. These molecules could be tested *in vitro* for activity against *M. tuberculosis*.

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BASIC RESEARCH

Studies completed

Mycobacteriophage genome database

Background

Mycobacteriophages are the ideal tool for studying tubercle bacilli. Comprehensive annotations of the mycobacteriophage proteins are not so far available online. Phage genomes are highly mosaic and each genome contains a unique combination of modules. Understanding the gene functions and their interacting host proteins will help in the identification of novel drug targets.

Aims

- To create an exclusive user friendly database on mycobacteriophages with exhaustive information catalogued on a single platform after analysis
- To assign function to the proteins by *in silico* approach

Methodology

The sequences of the mycobacteriophages were obtained from NCBI and were subjected to databases search using PSI-BLAST, PFAM, PROSITE, CATH, SCOP, TMHMM, PSSM, CE, SIGNAL P etc. ACLAME was used to assign function to the unknown proteins.

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 data types can be enumerated as genome, protein and structural details. It consists of about 25 parameters for each gene. ACLAME software package was used for comparative genomic analysis within the 64 mycobacteriophages. They were classified into 72 functional families and incorporated into the database. These were further broadly classified and clustered into 8 groups (Fig.7). The screen shot of the home and search pages of the database are shown in Figs. 8 & 9 respectively.

Fig. 7: The broad classification of the protein functions based on ACLAME

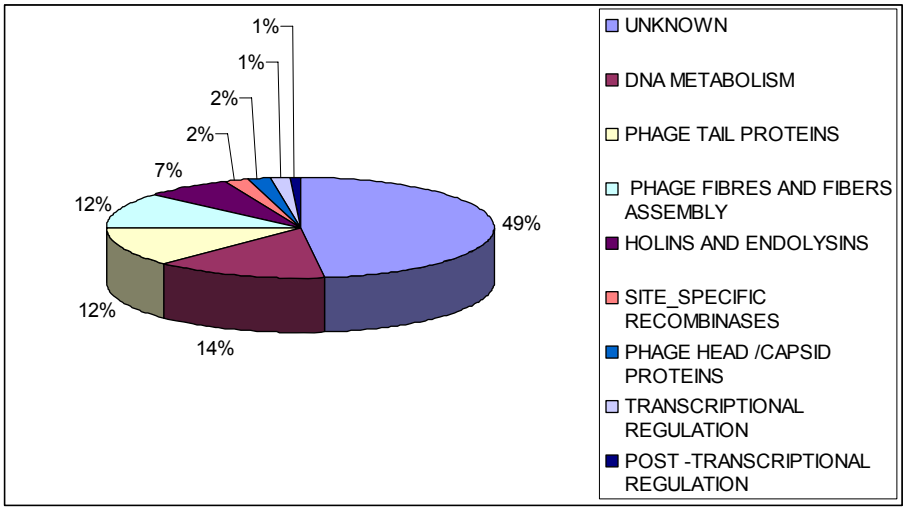


Fig. 8: The screen shot of the home page of the database

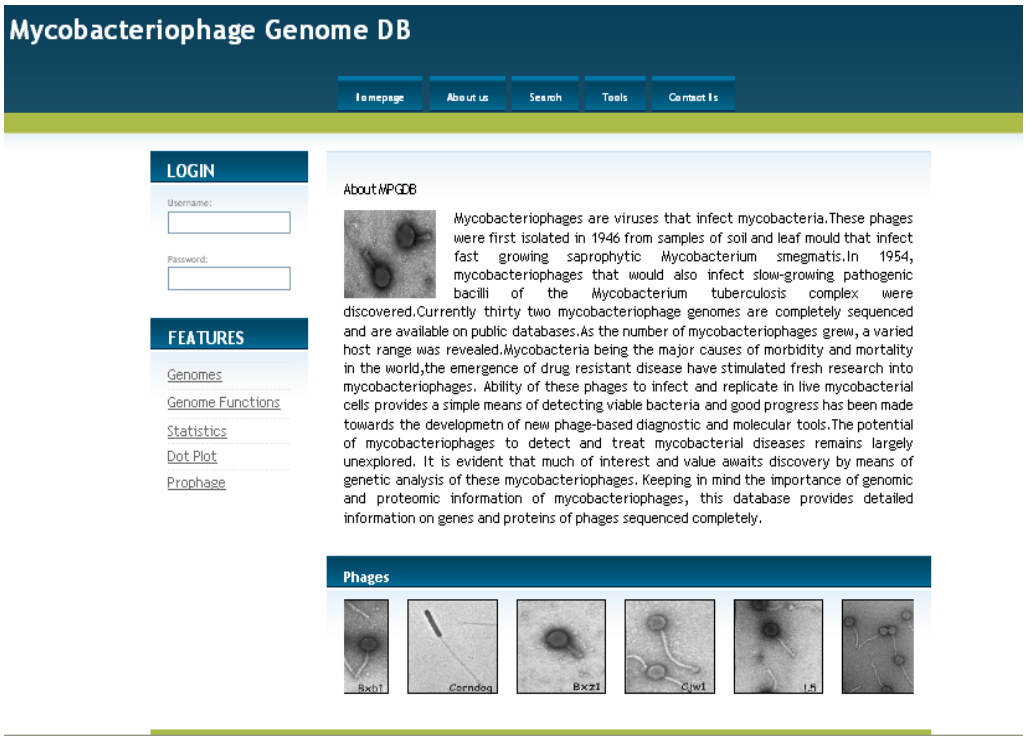
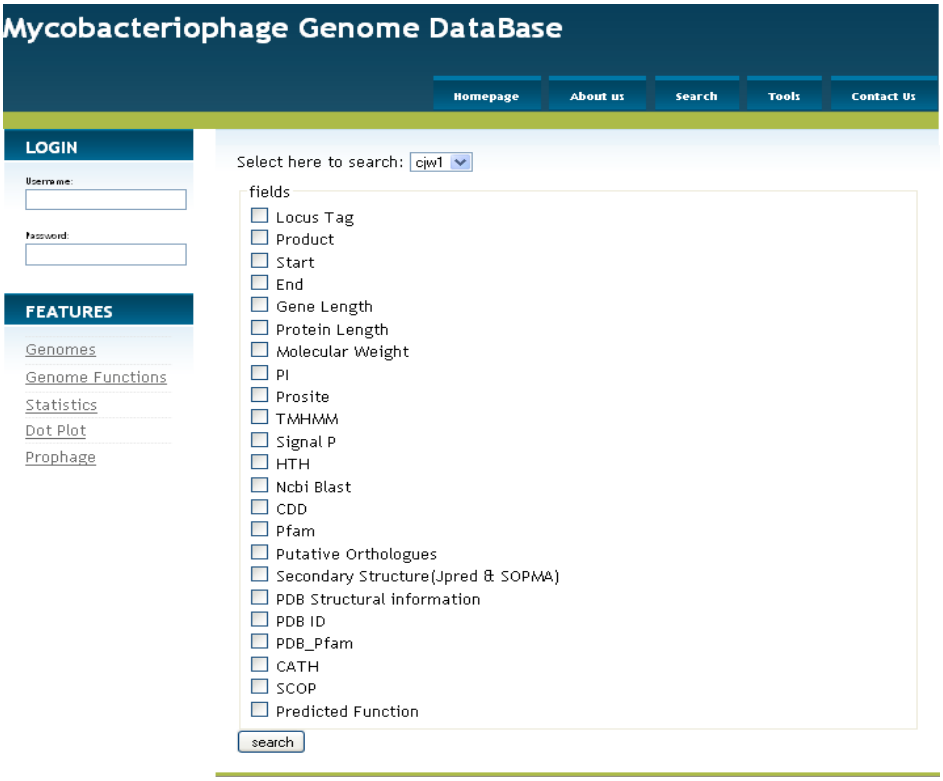


Fig. 9: The screen shot of the search page of the database



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Lytic activity and molecular modeling of lytic enzymes of mycobacteriophages TM4, D29 and Che12

Background

Mycobacteriophages are equipped with genes such as lysin and holin that facilitate lysis of their host organisms. Mycobacteriophages have two different lysin genes such as lysin A and lysin B. The presence of two different lysin proteins is because of the complex nature of the cell wall of *M. tuberculosis*. There are many reports stating the therapeutic application of lysin genes on pathogenic organisms.

Aim

- To study the external lytic activity of lysin genes of Che12, TM4 and D29 phages capable of infecting *M. tuberculosis* and to perform the molecular modeling of these lytic enzymes

Method

Crude lysin was extracted from the three mycobacteriophages and their lytic activity was tested against *M. smegmatis* mc^2155 and *M. tuberculosis* H37Rv using luciferase reporter phage (LRP) assay.

The lysin protein sequences of all the three mycobacteriophage were retrieved from the NCBI database and were analyzed using BLAST and Pfam. The template protein was selected based on the domain function, sequence identity and E value. Each of the target lysin protein was aligned with their respective template protein sequence using Align123 program of discovery studio v 2.0. The model structure was predicted for each lysin protein using the homology model module in discovery studio. Models were selected on the basis of DOPE score and each predicted model was further analyzed by using Ramachandran plot.

Results

The lysin extracts of all the three phages showed lytic activity against *M. smegmatis* mc^2155, while TM4 lysin alone showed significant level of relative light unit reduction against *M. tuberculosis* $H_{37}Rv$ by LRP assay. The models showed more than 90% residues in the favorable region of Ramachandran plot. Each model was superimposed to their template structure and their fold nature was studied.

Conclusion

The mycobacteriophage TM4 has external lytic activity against *M. tuberculosis* H37Rv. The predicted models can be used for understanding the interaction with the host cell wall components.

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Role of chemokines in tuberculous immunity

Internalization of *M. tuberculosis* by non-phagocytic pleural mesothelial cells (Met-5A)

Background

M. tuberculosis can invade, infect and survive within a wide variety of non-immune cells like alveolar cells, pneumocytes, endothelial cells, adipocytes and fibroblasts. Its interaction with pleural mesothelial cells (PMC) is not studied due to low availability in pleural fluid. Here, we studied the innate resistance conferred by PMC to the invading *M. tuberculosis* during tuberculous pleurisy.

Aim

- To study the role of PMC and its interaction with *M. tuberculosis* H_{37}Rv in pathogenesis of tuberculous pleurisy

Methodology

The transformed mesothelial cells (Met5A cell line) were used in the present study. The endocytic ability of these cells was assessed by dextran-FITC uptake. Met-5A cells were infected with H37Rv at the multiplicity of infection of 10 and stained by Kinuyon's and auramine staining followed by transmission electron microscopy (TEM) and colony forming units (CFU) to assess the internalized bacilli. Mycobacterial protein expression was assessed by immuno-cytochemistry.

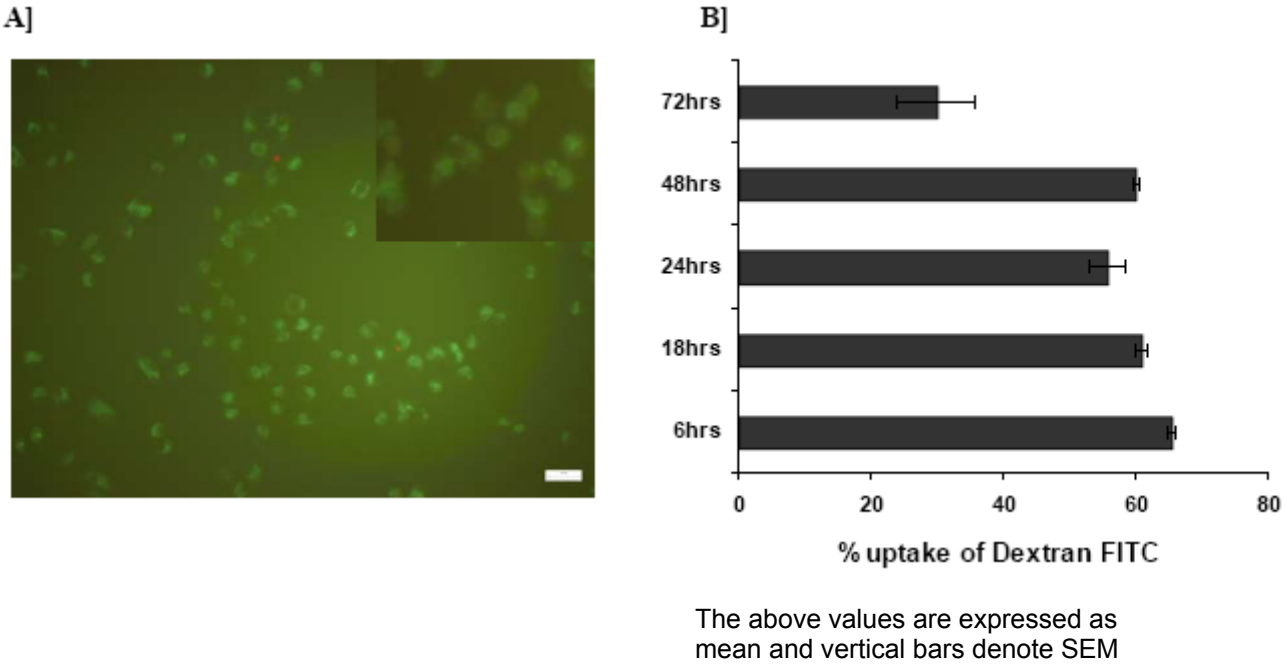
Results

PMCs were able to endocytose the dextran particles (Figs. 10a & b). Kinuyon's and auramine staining confirmed the presence of bacilli as early as 6hrs after the infection (Figs. 11a & b). TEM showed internalized bacilli in a vacuole, mediated by microvilli of the mesothelial cells. At higher time point, degenerative morphological changes like dilation of smooth and rough endoplasmic reticulum and activation of mitochondria and presence of secretory vesicles were observed (Figs. 12a & b). However, PMC environment depicted unaltered *M. tuberculosis* colony forming units. Infected Met-5A demonstrated expression of mycobacterial proteins like GroES, 16kDa, Hsp-65 and whole cell lysate (Fig. 13).

Conclusions

This is the first report to demonstrate that PMCs have the ability to uptake *M. tuberculosis* but unable to support its growth. As a defense strategy, bacilli expressed the stress response proteins, indicating efforts leading to latency. Overall, the present study highlights the probable role of pleural mesothelial cells in tuberculous immunity.

Fig. 10: Demonstration of dextran-FITC uptake by cultured Met-5A cells



The above values are expressed as mean and vertical bars denote SEM

Fig. 11: *M.tuberculosis* infected Met-5A cells

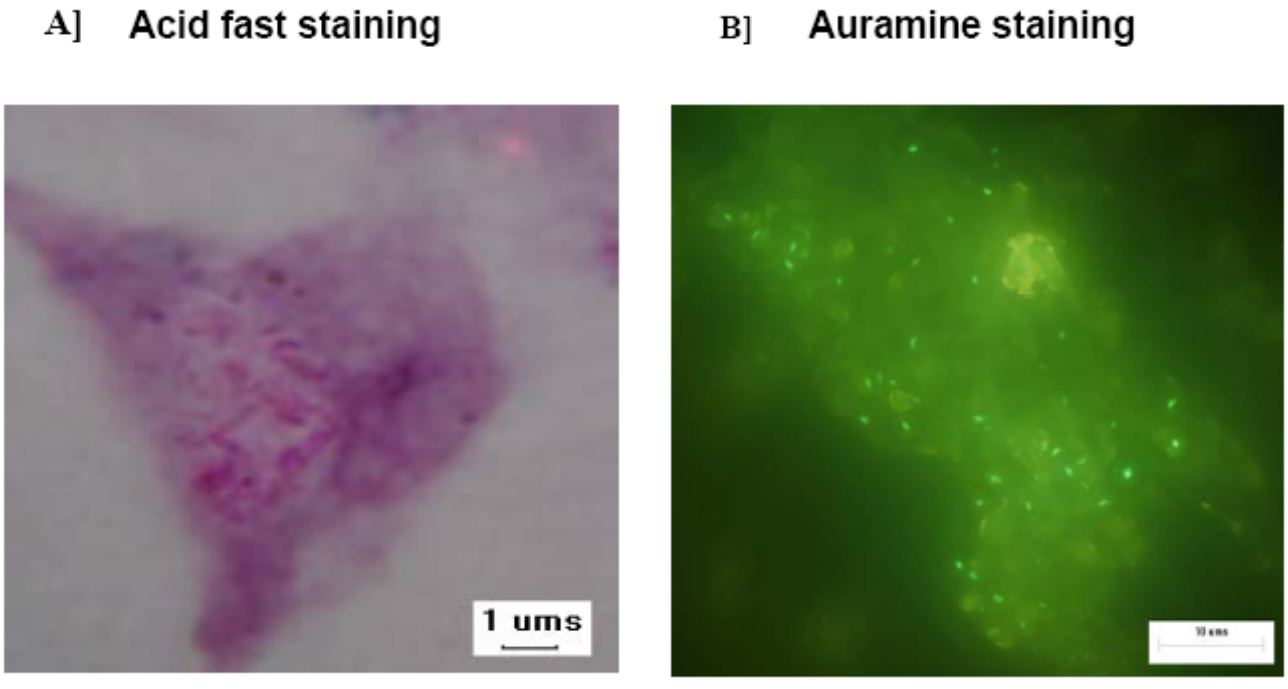
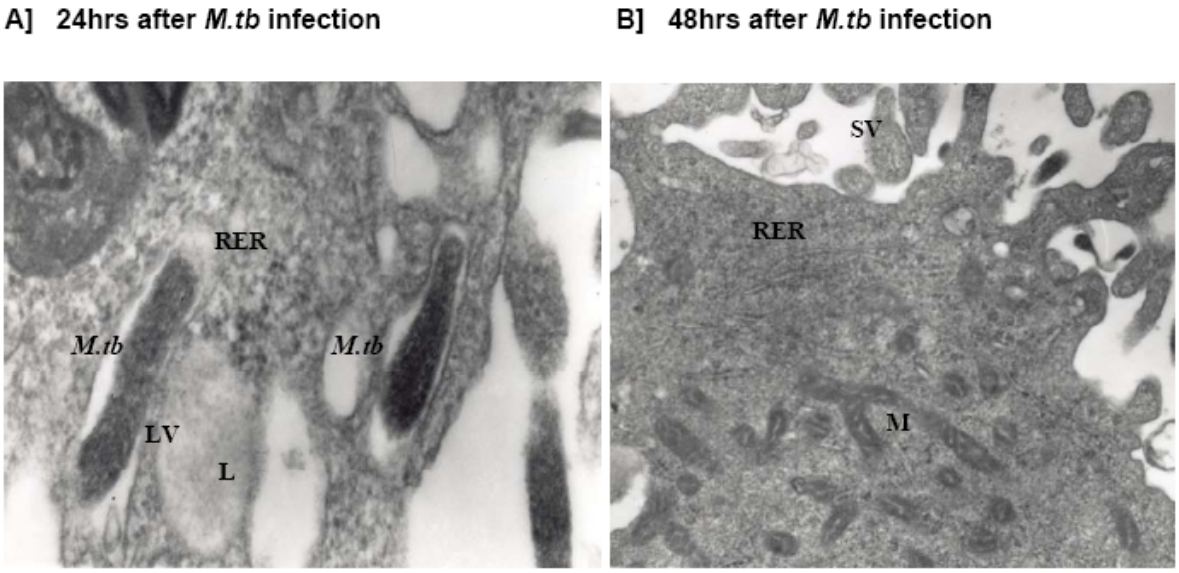
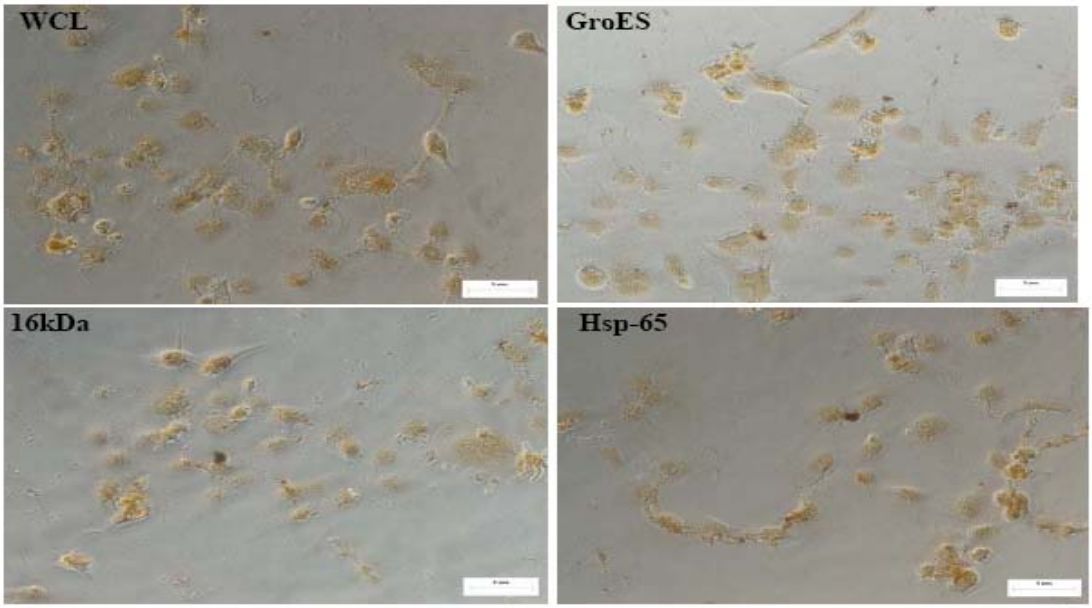


Fig. 12: TEM photographs of *M.tuberculosis* infected Met-5A cells



Mitochondria (M), lysosomal bodies (L), rough endoplasmic reticulum (RER), fat vacuoles (LV), secretory vesicles (SV) and *M.tuberculosis* (*M.tb*).

Fig. 13: Immunocytochemistry of mycobacterial antigens in *M.tuberculosis* infected Met-5A



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ROLE OF DENDRITIC CELLS IN MYCOBACTERIAL IMMUNITY

Antimicrobial response of KG-1 derived dendritic cells during *M. tuberculosis* infection

Background

It is well established that phagocytes like dendritic cells (DC) release reactive oxygen species (ROS) and reactive nitrogen intermediates which correlates with the release of calcium (Ca^{2+}) when infected with intracellular pathogens. Previously, we evaluated KG-1, a leukemic cell line as an *in vitro* DC model for *M. tuberculosis* infection studies. In this report we evaluated its antimicrobial response.

Aim

- The release of total ROS, nitric oxide (NO), Ca^{2+} levels and its influence on the rate of apoptosis was evaluated in KG-1 derived DC (KGDC) after infection with *M. tuberculosis* strains (H_{37}Rv and H37Ra)

Methodology

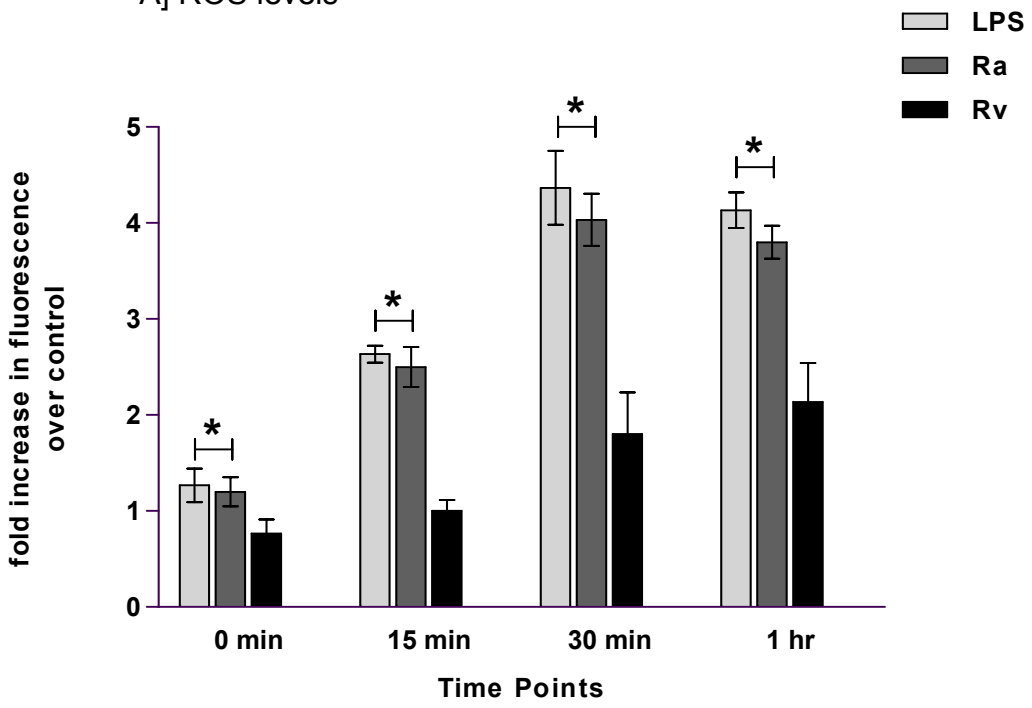
KG-1 cell line was propagated in Iscove's modified Dulbecco's medium. The cells were differentiated into DC in the presence of ionomycin, phorbol myristyl acetate (PMA) and granulocyte macrophage colony stimulating factor (GM-CSF). The differentiated KGDC were infected with *M. tuberculosis* strains at multiplicity of infection of 10. The levels of ROS and the release of Ca^{2+} were measured by flow cytometry and the levels of nitric oxide (NO) were measured using Griess method at different time points. The rate of apoptosis was assessed by annexin-FITC/PI staining.

Results

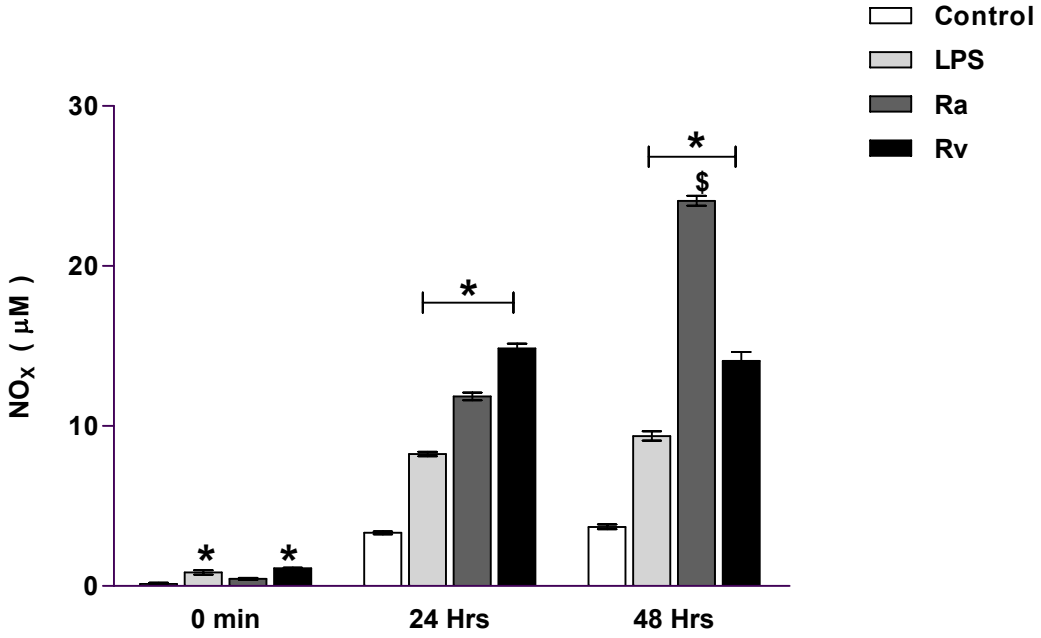
The evaluation of antimicrobial functions of *M. tuberculosis* infected KGDC revealed that H_{37}Rv infection selectively abrogated the production of ROS and NO (Figs.14a & b) but the intracellular calcium flux was unaffected (Fig.15). Moreover, H37Rv infected KGDC showed significantly decreased apoptosis and increased necrosis (Figs.16a & b).

Fig. 14: Levels of ROS and NO

A] ROS levels



B] NO Levels

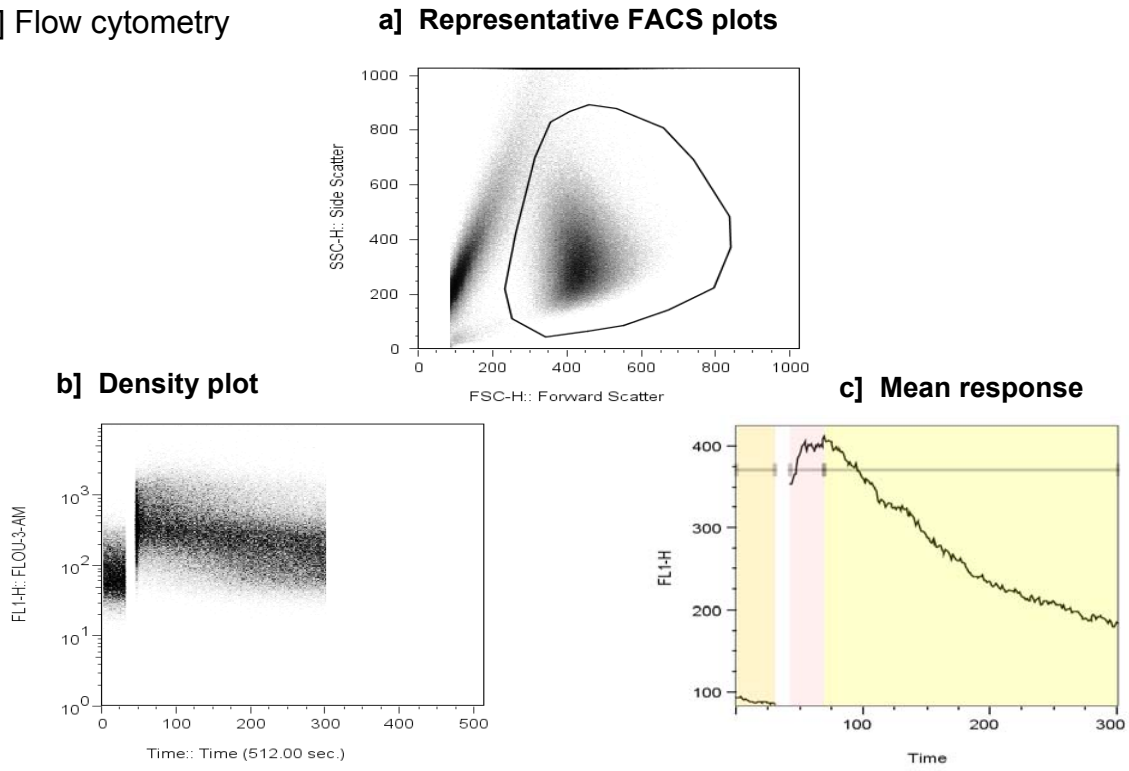


The above values are mean and vertical bars denote SEM

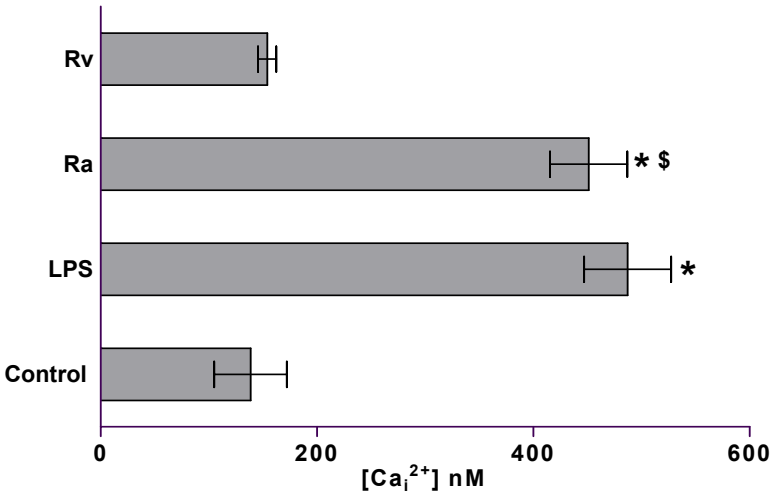
*, $p<0.05$ compared to uninfected control and $ compared to Rv

Fig. 15: Intracellular calcium flux in infected KGDC

A] Flow cytometry



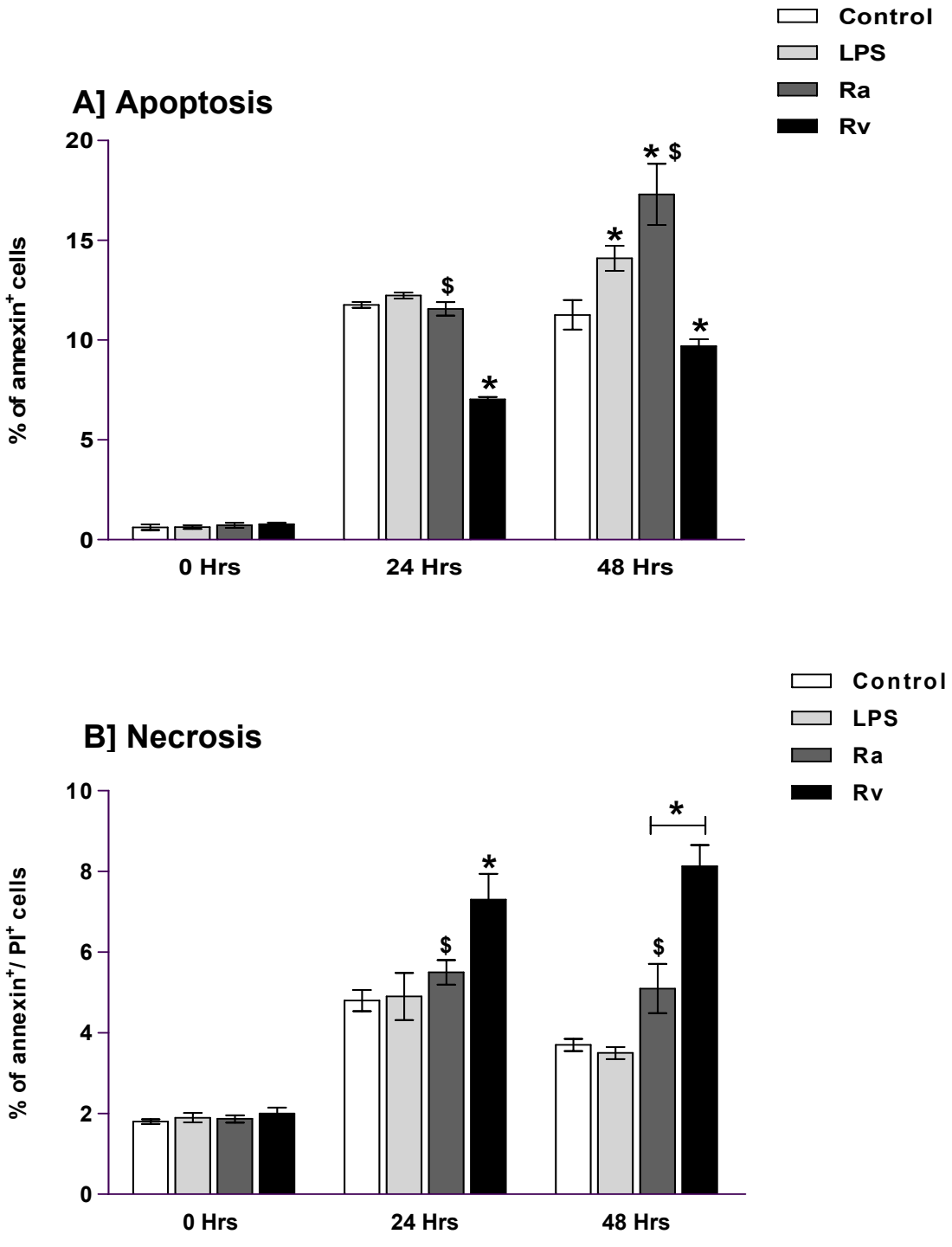
B] Levels of released calcium



The above values are mean and vertical bars denote SEM

*, p<0.05 compared to uninfected control and $ compared to Rv

Fig. 16: Rate of apoptosis and necrosis



The above values are mean and vertical bars denote SEM

*, $p<0.05$ compared to uninfected control and $ compared to Rv

Conclusion

Altogether, these findings imply that the virulent *M. tuberculosis* $H_{37}Rv$ strain is able to suppress the intricate intracellular functions of KGDC in favor of its survival.

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Effect of 1,25 dihydroxy vitamin D_3 on matrix metalloproteinases in PTB

Background

Infection with *M. tuberculosis* results in activation of macrophages, T-cells, and granuloma formation, which are crucial events during protective cellular immune response. However, the same effector mechanisms may also be associated in pathogenesis, depending on their strength and kinetics. The pathological process includes tissue remodeling and breakdown of the extracellular matrix involving matrix metalloproteinases (MMPs). 1,25 dihydroxy vitamin D_3 ($1,25(OH)_2D_3$), an immuno modulator, is known to influence tissue remodeling through MMPs.

Aim

- To study the effect of $1,25(OH)_2D_3$ on MMPs, MMP-7, MMP-9 and the tissue inhibitor of metalloproteinase (TIMP-1) in PTB patients and normal healthy subjects (NHS)

Methods

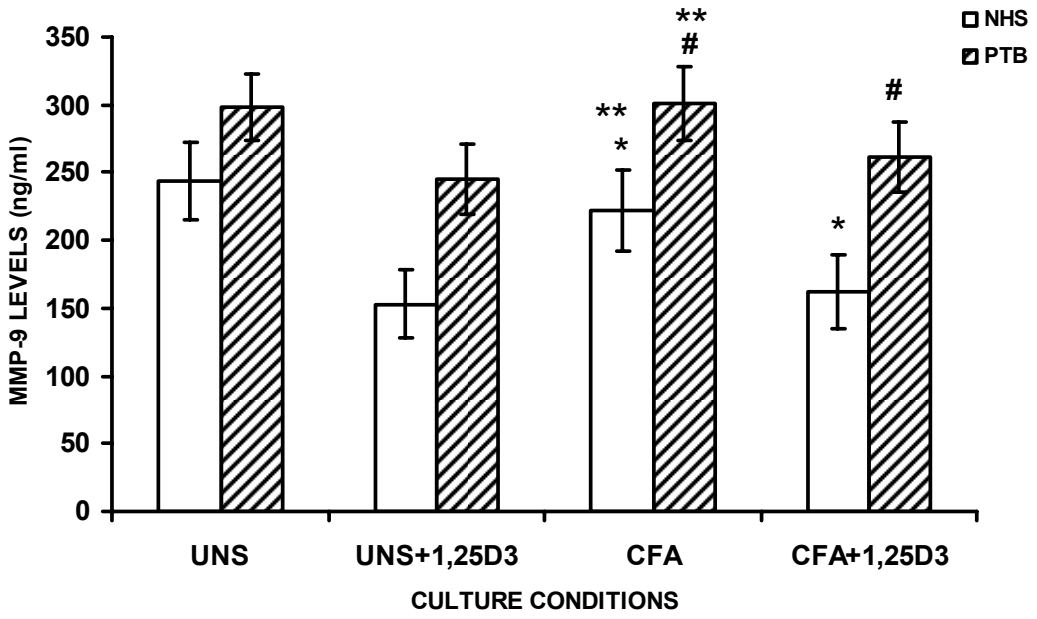
The study subjects consisted of 43 PTB patients and 44 NHS. Peripheral blood mononuclear cell (PBMC) cultures were stimulated with culture filtrate antigens (CFA) of *M. tuberculosis* in the presence and absence of $1,25(OH)_2D_3$ for 48 hrs and the culture supernatants were assayed for MMP-7, MMP-9 and TIMP-1 using commercially available ELISA kits.

Results

There was no significant difference in the levels of MMP-9 in CFA stimulated as well as unstimulated cultures of both NHS and PTB patients. In CFA stimulated cultures of PTB patients, a significant increase in the production of MMP-9 was observed when compared to NHS ($p = 0.05$). Both in PTB and NHS groups, 1,

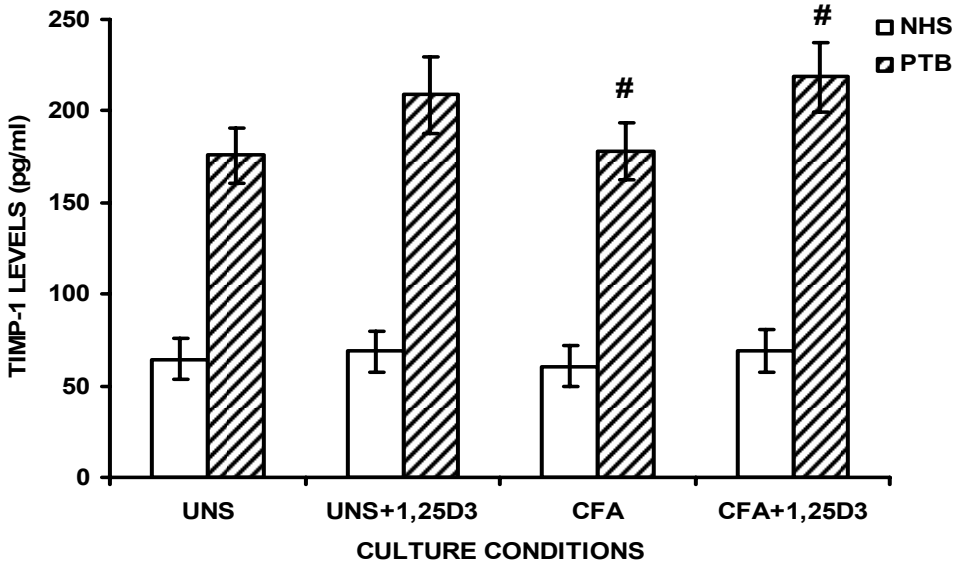
$25(OH)_2D_3$ significantly suppressed the production of MMP-9 in CFA stimulated cultures (p = 0.0001) compared to 1, $25(OH)_2D_3$ untreated cultures (Fig. 17) [similar results were observed with MMP-7 levels (data not shown)]. The levels of TIMP-1 were found to be significantly higher in PTB when compared to NHS (p = 0.0001). Vitamin D_3 significantly increased the TIMP-1 (p=0.0001) (Fig. 18) level in CFA stimulated and unstimulated cultures of PTB as compared to NHS.

Fig. 17: Influence of $1,25(OH)_2D_3$ on MMP-9 production by PBMCs stimulated with CFA in NHS and PTB patients.



UNS represents unstimulated cells. Results expressed as mean ± standard error. NHS vs PTB patients; ** represents p=0.05. NHS and PTB patients; CFA vs CFA+1,25D3: *,# represents p=0.0001.

Fig. 18: Effect of 1,25$(OH)_2D_3$ on TIMP-1 production by PBMCs stimulated with CFA in NHS and PTB patients



Comparisons of TIMP-1 levels of NHS vs PTB: Results expressed as mean ± standard error. p = 0.0001 in all the culture conditions. In PTB patients; # represents p = 0.0001.

Conclusions

The present study suggests that 1, 25$(OH)_2D_3$ suppress the production of MMP-7 and MMP-9 and enhances the production of TIMP-1. Thus 1, 25$(OH)_2D_3$ through inhibition of MMPs and induction of TIMP-1, plays a regulatory role in maintaining the integrity of lung tissues and could be helpful in reducing disease severity by controlling the inflammatory necrosis in PTB.

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Emergence of drug resistant mutations after treatment with single dose NVP in HIV-1 infected pregnant women in south India

Background

In India, HIV prevalence among pregnant women is reported to be 0.3%. HIV transmission from mother to child can occur *in utero*, intrapartum (during labour) or postpartum (through breastfeeding). Several regimens have been tested for prevention of parent to child transmission (PPTCT), including NVP given as a single dose to the mother at the time of delivery followed by a single dose to the

infant within 72 hours (Sd-NVP). Single dose of zidovudine and NVP are commonly used during pregnancy in resource poor countries like India. Resistance to NVP has been described after use of single drug preventive treatment, which is why triple drug therapy is recommended as the preferred option for treatment. Emergence of antiviral drug resistant mutations (DRM) among Indian women who had received Sd-NVP regimen for PPTCT is not described.

Aim

- To determine the prevalence and pattern of drug resistance at baseline and after delivery among antenatal women exposed to Sd-NVP as well as their children who have become HIV-positive

Methods

HIV-1 infected pregnant women attending Government Maternity Hospitals in Chennai, Madurai and Vellore during July 2007 to March 2008 who were primigravidae, and had no prior history of ART formed the study population. Genotypic drug resistance testing was performed using the ViroSeq kit protocol. DNA-PCR was performed in children at 4-6 weeks of age to determine their HIV status.

Results

A total of 14 pregnant women were recruited to the study, of whom specimens before and after Sd-NVP treatment were available from only 12 women. Of the 12 paired specimens, three had viral load below 400 copies / ml, and failed to amplify during genotyping. None of the infants born to these mothers was found to be HIV-1 positive and were therefore not genotyped.

The reverse transcriptase (RT) and protease (PR) genes of viruses from the HIV-1 infected pregnant women were sequenced. One hundred per cent concordance was observed between sequences of specimens obtained before and after treatment with Sd-NVP. While no major NNRTI mutations were found in any of the patients before treatment, DRMs to NNRTI were observed in 4 patients after treatment with Sd-NVP. The mutations observed were Y181C, K103N and Y188C, conferring resistance to NVP and EFZ. The most commonly

observed polymorphisms were at codons 35, 36, 39, 48, 60, 121, 135, 162, 173, 177, 200, 207, 214, 245, 286, 291, 292, 293 and 294.

Conclusions

This is the first report on characterization of DRMs in women who had received prophylactic Sd-NVP regimen during their antenatal period for PPTCT in India. Though the numbers tested were small, we observed a very high rate of development of NVP resistance (4/12) among treated women, as has been reported from other countries with HIV-1 clade C infections. Our findings emphasize the need to implement more effective PPTCT regimens to minimize emergence of drug resistance, and thereby preserve long-term treatment options for HIV-infected women in India.

[Contact person: Dr.Soumya Swaminathan (E-Mail ID: soumyas@trcchennai.in); Dr.Luke Elizabeth Hanna (E-Mail ID: hanna@trcchennai.in)]

Studies in progress

Functional assignment of 64 mycobacteriophages into gene families

The genomes of bacterial viruses (phages) contain a variety of genes homologous to those found in their hosts. Many encode functional proteins involved in processes of direct importance for the production of phage progeny. They include genes involved in DNA replication, nucleotide metabolism, and RNA transcription, and are found in both lytic and temperate phages. It is likely that many originated from their hosts and that some host genes that occur in multiple copies have been acquired from phages either after a period of evolution in the host or after acquisition of the gene from a different host.

New sequence information fuels a recurrent debate on the need to revise phage taxonomy. The absence of structured, computer readable information on mycobacteriophages is a major bottleneck for an extensive global analysis of mycobacteriophage genomes and their relationships. Gene Ontology is a structured controlled vocabulary developed to describe the roles and locations of gene products in a consistent manner and in a way that can be shared across organisms. The mycobacteriophage proteins of the 64 sequenced genomes

were assigned to 72 functional protein families based on ACLAME and comparison of families among and across genomes are being performed.
[Contact person: Dr. Vanaja Kumar (E-Mail ID: vanajakumar@trcchennai.in)]

Comparative analysis of lysin proteins of mycobacteriophages

The capability of mycobacteriophages to lyse host cells is due to the presence of two lysin genes - lysin A and lysin B. The presence of two different lysin genes in these genomes is due to the complexity of the cell wall of *M. tuberculosis*. Lysin A and B proteins are involved in cleaving the peptidoglycan and mycolic acid layers respectively. The spread of antimicrobial resistance has necessitated re-analysis of alternate therapy and interest in phages is now on the increase. In this study we propose to:

1) Analyze lysin A and B proteins across 65 mycobacteriophage genomes
2) Predict and study the similarity of the active site residues among these genomes
3) Classify the lysin proteins into subfamilies
4) Identify potential substrates which can help in identifying novel drug targets

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

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

(Collaboration with School of Biological Sciences, Madurai Kamaraj University and Centre for Biotechnology, Anna University)

(Funded by Department of Biotechnology, Govt. of India)

The aim of this project is to study the expression of HIV 1C gp41 epitopes on two self-assembly systems *viz*. the outer membrane porins of *Salmonella typhi* and the coat protein of cardamom mosaic virus (CMV). The over-expressed chimeric proteins have been tested with sera from HIV patients. Further work is in progress to standardize an *in vitro* prime-boost strategy to evaluate the potency

of these constructs to stimulate the production of cytokines and chemokines and also to look at the expression of cellular activation markers using flow cytometry. [Contact person: Dr.V.D.Ramanathan (E-Mail ID: ramanathanvd@trcchennai.in)]

Preliminary analysis of ethionamide resistance and its association with INH resistance among previously treated TB patients

Background

Ethionamide (Eto) is one of the drugs used in the treatment of MDR-TB. Being a structural analogue of INH, Eto shares a similar target with INH in the fatty acid synthesis pathway. Presence of mutations in *inh*A gene confers low level INH resistance and is hypothesized to mediate cross resistance with Eto.

Aim

- To obtain preliminary data on resistance to both INH and Eto in *M. tuberculosis* isolates from previously treated patients

Methods

A total of 176 *M. tuberculosis* isolates from patients failing Category-I and II regimens were subjected to phenotypic drug susceptibility testing for INH and Eto by minimum inhibitory concentration method.

Results

Sixty eight (39%) and 26 (15%) isolates out of 176 were resistant and susceptible to both drugs respectively. Of the 137 isolates resistant to INH, 68 (49%) were also resistant to Eto. Any resistance towards Eto was observed in 46% (81/176) of the isolates. Resistance only to INH or Eto was observed in 39% (69/176) and 7.3% (13/176) respectively (Table 17).

Table 17: Drug susceptibility pattern of *M. tuberculosis* isolates

DRUGS		No. of isolates
INH	**Eto**	
R	R	68
	S	69
S	R	13
	S	26
Total		176

R- resistant, S- susceptible, INH- isoniazid, Eto- Ethionamide

Conclusions

The results indicate the presence of high Eto resistance and co-resistance with INH among patients under treatment, though the association between INH and Eto was not significant (p value = 0.1). It is necessary to measure the level of INH resistance in these co-resistant isolates to examine if low level INH resistance mediates Eto resistance. Analysis of the genes associated with INH resistance, especially *inh*A and Eto resistance must be performed to assess the phenomenon of cross resistance between INH and Eto.

Further work in these lines is in progress.

[Contact Person: Dr. Vanaja Kumar (E.mail ID: vanajakumar@trcchennai.in)]

Role of Chemokine, DC-SIGN and toll-like receptor gene variants on immunity to TB

Background

Invasion of the host by microbial pathogens causes activation of the innate immune response (first line defence) and triggers the secretion of various cytokines and chemokines and initiation of adaptive immunity. Chemokines along with cytokines are involved in 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. Toll-like receptors (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

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

Part-I of the study will be 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 will be carried out in a prospective manner using freshly drawn blood.

[Contact person: Dr. P. Selvaraj (E.mail. ID: selvarajp@trcchennai.in)]

Toll-like receptor and TIRAP gene polymorphisms in PTB

Background

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

followed by restriction fragment length polymorphism method in 212 healthy control subjects (HCs) and 206 PTB patients.

Results

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

[Contact person: Dr. P. Selvaraj (E.mail. ID: selvarajp@trcchennai.in)]

Effect of vitamin D_3 on neutrophil cathelicidin, defensin-1α and TLR gene expression in PTB

Background

Neutrophils are essential components of the human innate immune system and associated with the first line defense mechanism against invading microorganisms. It has been suggested that the impaired ability of neutrophils to phagocytose and generate an oxidative burst may lead to establishment and spread of infection in patients with *M.tuberculosis* infection. Although the relationship between *M. tuberculosis* and macrophages has been well-documented, less is known about the role of neutrophils in immunity to TB. The present study may enlighten the understanding of the molecular mechanisms underlying between the bacteria and neutrophil interactions.

Aim

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

Methods

The study will be 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 CFA in the presence and absence of vitamin D_3. The culture supernatant was stored at -80°C and various chemokines will be estimated using commercially available Enzyme linked immunosorbent assay (ELISA) kits and/or cytometric bead array. 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 is being done using real time PCR (RT-PCR) with TaqMan assay primers and probes.

Results

So far, 10 PTB patients and 12 healthy control subjects have been recruited to the study.

[Contact person: Dr. P. Selvaraj (E.mail ID: selvarajp@trcchennai.in)]

Effect of vitamin D_3 on chemokine expression in PTB

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, a potential immunomodulator, is known to influence innate and adaptive immunity. It induces antimicrobial peptide cathelicidin expression and increase cell migration and secretion of signalling 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 study the effect of vitamin D_3 on chemokine expression in PTB

Methods

The study will be carried out in 20 pulmonary TB 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. The culture supernatant was stored at -80°C and various chemokines will be estimated using commercially available ELISA kits and/ or cytometric bead array. Total (RNA) extracted from 48 hrs old macrophages was used for cDNA synthesis. The relative quantification for the target genes monocyte chemoattractant protein-1, macrophage inflammatory protein-1α and -1β,

Interferon-γ inducible protein-10 and house keeping gene, glyceraldehyde-3 phosphate dehydrogenase is being done using real time PCR (RT-PCR) with TaqMan assay primers and probes.

Results

The study has been carried out in 10 PTB patients and 12 healthy control subjects.

[Contact person: Dr. P. Selvaraj (E.mail ID: selvarajp@trcchennai.in)]

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

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 (e.g., granulysin) molecules 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. Vitamin D_3 also induces the differentiation and expansion of forkhead box protein-3 Treg cells. The present study may enlighten 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 will be carried out in 20 PTB patients and 20 healthy control subjects. Peripheral blood mononuclear cells were cultured for 48 hrs with live *M. tuberculosis* H37Rv and its CFA in the presence and absence of vitamin D_3. After 48 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.

Results

The percentage perforin, granulysin and Tregs producing cells have been enumerated in 10 patients and 12 healthy controls.

[Contact person: Dr. P. Selvaraj (E.mail ID: selvarajp@trcchennai.in)]

Characterization of signal recognition particle components of *M. tuberculosis*

Background

In all organisms, the signal recognition particle (SRP) plays an important role in protein transport. The bacterial SRP dependent pathway is the major targeting route for membrane proteins, as well as subsets of secretory proteins. The machinery required for co-translational protein targeting consists of a Protein/RNA complex that binds ribosomes and translates the secretory and membrane proteins. In *M. tuberculosis* the protein transport machinery which involves SRP homologue Ffh and 4.5s RNA have not been characterized.

Aim

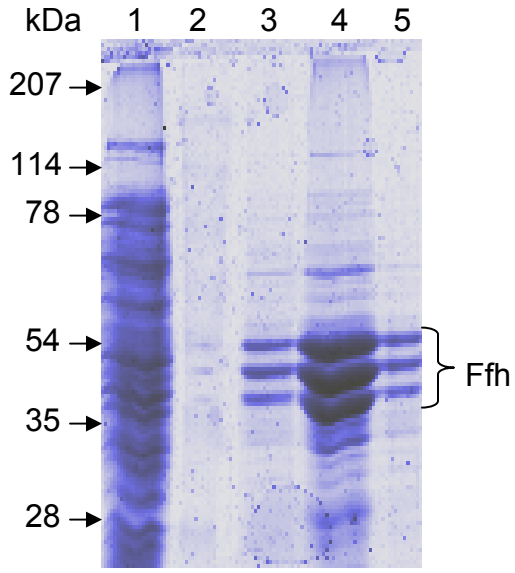
- To characterize the SRPs of *M. tuberculosis* namely Ffh and 4.5s RNA

Results

The predicted Ffh sequence was PCR amplified from *M. tuberculosis* H37Rv genomic DNA using sequence specific primers containing restriction enzyme overhangs. Full length Ffh was cloned into the pBADb expression vector and induced with L-arabinose. The expressed protein was purified by Nickel affinity chromatography and purified fractions were analysed by SDS-PAGE. On SDS-PAGE and western blot analysis of purified fractions, a triplet band around 54 kDa were seen (Figs.19 & 20). This triplet band might represent the reduced form of Ffh. The reduction or self cleavage of amino acids appear in C-terminal region not in N-terminal as indicated by western blot analysis by anti-histidine

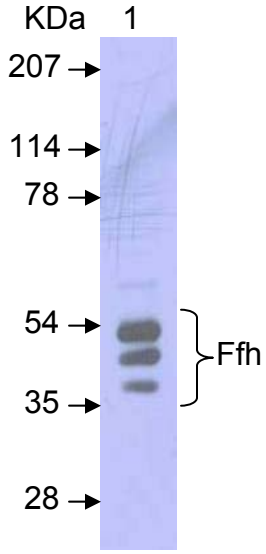
antibodies, where it binds with hexahistidine molecules present in the N-terminal region.

Fig. 19: Expression and purification of recombinant Ffh



SDS-PAGE and Coomassie blue stained gel analysis of L-arabinose induced *E.Coli* TOP10 cells crude lysate harboring Ffh construct (Lane 1) and Ni-NTA affinity chromatography purified fractions of recombinant Ffh (Lanes 2,3,4 and 5). Molecular weight markers were indicated in kDa.

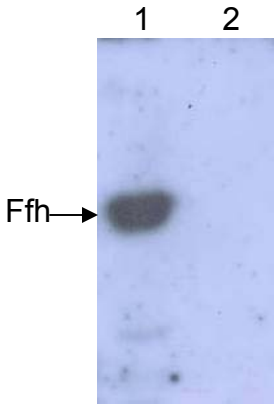
Fig. 20: Western blot analysis of recombinant Ffh



Western blot analysis of purified Ffh ---probed with Anti-His antibody (Lane1)
Molecular weight markers were indicated in kDa.

SRPs have GTPase activity required for its interaction with docking protein and subsequent release into translocon. Hence, we performed GTPase activity of recombinant Ffh by GTP blot overlay assay and thin layer chromatography (TLC). Figure 21 illustrates the purified Ffh and its affinity to [α-^{32}P] GTP after separated by SDS-PAGE and transferred to PVDF membrane by blot over lay assay.

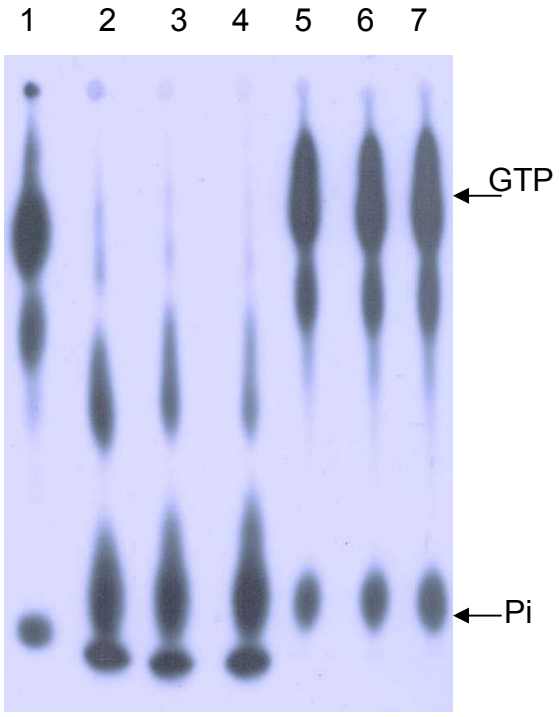
Fig. 21: GTPase activity of recombinant Ffh



Recombinant Ffh was separated by SDS-PAGE and transferred to PVDF membrane and GTP overlay assay performed using [α-^{32}P] GTP. Lane 1 Ffh; Lane 2 control protein (DacB2)

GTP hydrolysis activity of Ffh and release of Pi were determined by TLC. We observed that within 5 min, incubation with Ffh showed maximum GTPase activity as indicated in the Fig. 22 and buffer control did not show any hydrolysis of GTP.

Fig. 22: GTP hydrolysis by Ffh

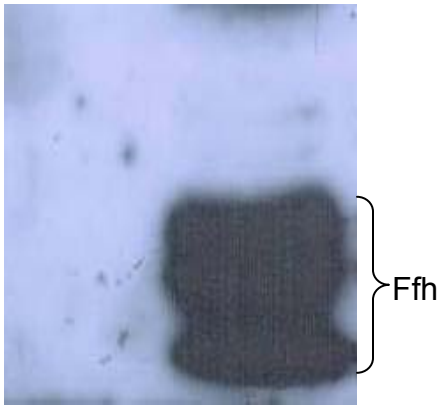


[γ-^{32}P] GTP hydrolysis and release of Pi by recombinant Ffh was determined using thin layer chromatography. Lane1 [γ-^{32}P] GTP alone; Lanes2, 3 and 4 [γ-^{32}P] GTP incubated with Ffh aliquoted after 5, 15 and 30 min respectively. Lanes 5, 6 and 7 buffer control aliquoted after 5, 15 and 30 min respectively.

This data clearly indicates the GTPase activity of recombinant Ffh protein of *M. tuberculosis*. To confirm the Ffh interaction with 4.5s RNA necessary for protein translocation, we cloned the 4.5sRNA gene and 4.5s RNA was purified by *in vitro* transcription. Direct interaction between Ffh and 4.5s RNA of *M. tuberculosis* has been demonstrated by 4.5s RNA blot overlay assay In RNA blot overlay assay, [α-^{32}P] UTP labeled 4.5s RNA interacted with triplets of Ffh protein which shows that all three bands have domain for RNA binding (Fig. 23).

Fig. 23: Ffh and 4.5s RNA interactions

1 2



The interaction between Ffh and 4.5sRNA was confirmed by 4.5s RNA Blot over lay assay using [α-^{32}P] UTP labeled 4.5s RNA
Lane1 *E.coli* (TOP 10) cells whole lysate
Lane2 Purified Ffh

Conclusions

The characterization of *M. tuberculosis* SRP homologue Ffh and its interaction with 4.5s RNA is being reported for the first time. These SRPs are evolutionarily conserved among prokaryotes.

[Contact person: Dr. Sujatha Narayanan (E.mail. ID: sujathanarayanan@ trcchennai.in)]

PknE a serine / Threonine kinase from *M. tuberculosis* suppresses the intrinsic pathway of apoptosis and increases pro-inflammatory cytokines in THP-1 human macrophage infection

Background

M. tuberculosis, the causative organism of TB, survives the hostile macrophage environment by thwarting the host immune response. Pathogen induced apoptosis plays an important role in the clearance of invading pathogen and accelerates the innate and adaptive immunity. Apoptotic response towards *M. tuberculosis* infection is a virulence trait and the genes involved are enigmatic. Serine/Threonine Protein Kinases (STPK's), PknG and PknH from *M. tuberculosis* were proved to block the phagosome lysosome fusion and

regulate the bacillary load during the pathogenesis. Earlier studies from our lab have shown that STPK PknE plays a role in apoptosis. To study the apoptotic events, we compared the expression profile of PknE mutant and Wild type (H37Rv) strain infected human macrophage cell line THP1 by Microarray. Several genes involved in apoptosis were upregulated. The data obtained by microarray was confirmed by oligoarray.

Aims

- To identify the pathway of apoptosis inhibited by PknE by using gene disrupted (ΔPknE), wild type (H37Rv) and complemented CΔPknE) strains infected with human macrophage-like THP-1 cell by oligoarray
- To analyze the role of pro-inflammatory response in apoptosis

Methods

THP-1, a human monocytic cell line was differentiated into adherent macrophages by the addition of phorbol myristate acetate (PMA). The macrophages were infected with the strains H37Rv, ΔPknE and CΔPknE. RNA was isolated from infected cells after five days post infection. The concentration and quality of RNA was assessed by NanoDrop® ND-1000 spectrophotometer and Agilent 2100 bioanalyzer. The human apoptosis Oligo GE Arrays® (SuperArray Bioscience Corporation) was used to study the apoptosis pattern at day 5 post infection. The RNA was labelled, hybridized, analyzed as per the manufacturer's instructions. The data from oligo GE array was analyzed using the software provided by the manufacturer. The difference is presented as fold difference as well as heat map. qRT-PCR was used to analyze the expression of pro and anti –inflammatory cytokines.

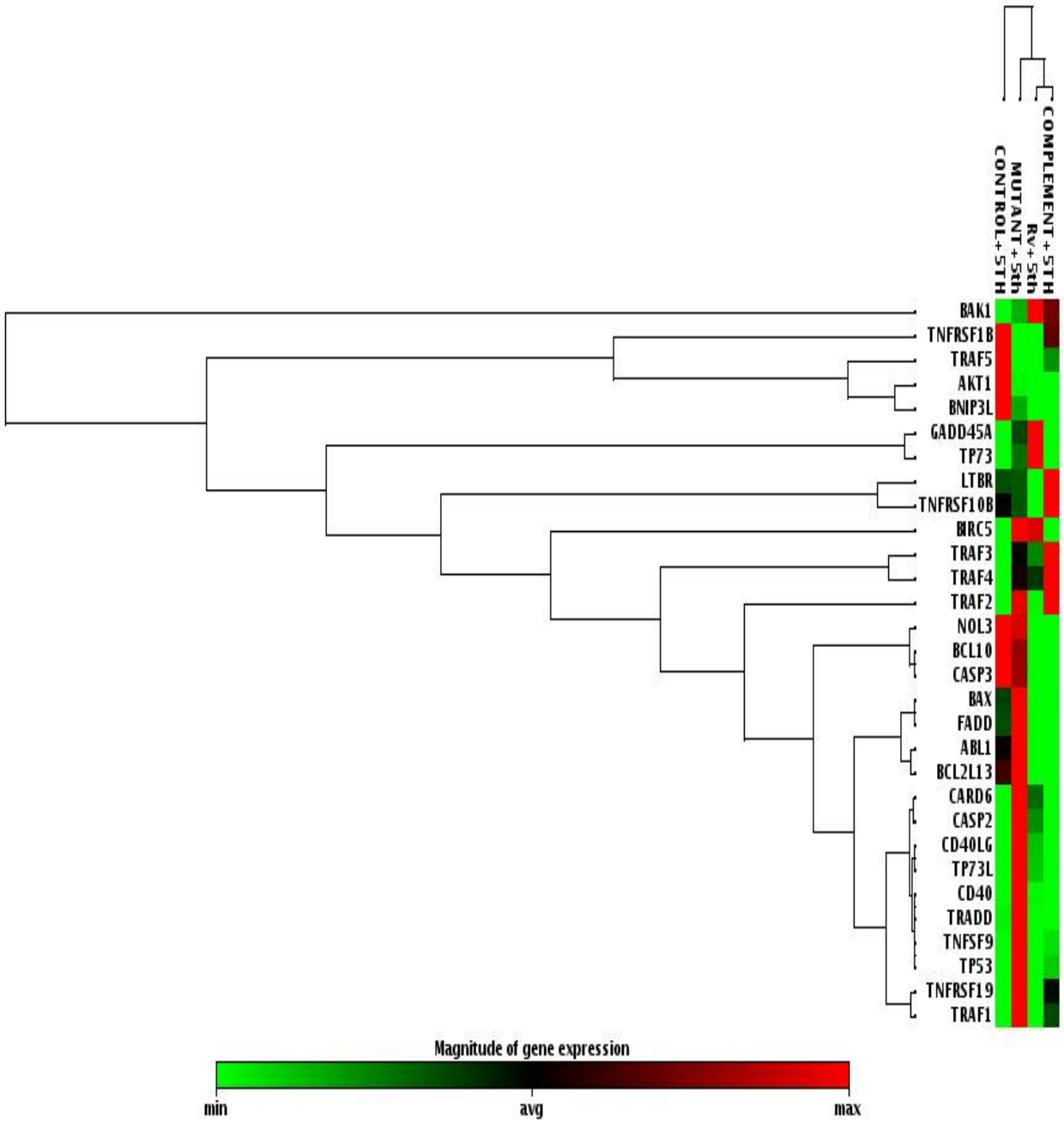
Results

The microarray data of 5 days post infection showed differential expression of genes involved in apoptosis by ΔPknE. The differential expression was confirmed using the oligoGE array. It was found that the genes in the intrinsic pathway of apoptosis like BAX, and BID were highly expressed suppressing MCL-1 (Fig. 24 and Table 18).

Table 18: Expression of apoptotic molecules in ΔPknE infected macrophages at day 5

Sl. No.	Gene name	Fold change by oligo arrays – $H_{37}Rv$ vs. ΔPknE
1	BAX	194.87
2	BCL10	45.75
3	BID	56.97
4	CASP14	40.67
5	CASP3	29.59
6	FADD	172.93
7	TNFRSF10B	58.94
8	TNFRSF14	55.13
9	TRADD	159.98

Fig. 24: Heat map showing the modulation of apoptosis at 5 days post – infection



The data revealed that PknE suppresses the intrinsic mode of apoptosis. The increased apoptosis observed in ΔPknE also suppressed the pro-inflammatory cytokines (Table 19).

Table 19: Suppression of pro-inflammatory cytokines by ΔPknE at 5 days post-infection (values represent fold change)

Sl.No.	Gene name	Control vs. $H_{37}Rv$	Control vs. ΔPknE	Control Vs. CΔPknE
1	IL-1β	2.23	0.47	4.8
2	IL-6	2.88	0.72	7.43
3	IL-8	15.19	6.25	24.7
4	IL-12p40	4.18	1.66	5.60
5	IL-18	21.4	10.4	6.07
6	IL-23	24.68	10.8	36.8
7	TNF-α	46.9	0.55	0.52

Conclusions

Our data for the first time suggests a role for PknE in suppressing the host apoptosis by intrinsic or mitochondrial pathway of cell death and increasing the pro-inflammatroy cytokines. The PknE regulates apoptosis to enable the survival of bacilli inside the host.

[Contact person: Dr. Sujatha Narayanan (E.mail. ID: sujathanarayanan@ trcchennai.in)]

Overexpression of FtsY of *M. tuberculosis* and the phenotypic consequences

Background

SRP and its cognate receptor FtsY are involved in cotranslational translocation of membrane proteins in gram negative bacteria and secretory proteins in gram positive bacteria respectively. Studies have shown that FtsY is also involved in sporulation in bacillus and streptomyces. In the previous report we have shown the effects of overexpression on colony morphology.

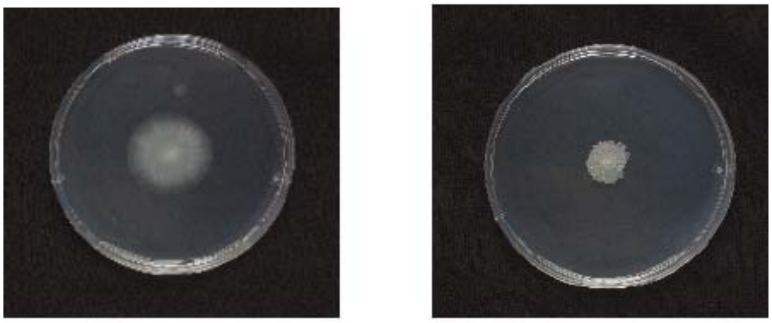
Aim

- To overexpress FtsY of *M. tuberculosis* in *M. smegmatis* and study the phenotypic effects

Results

Sliding motility: Using sterile tooth picks, recombinant colonies of *FtsY* from plates without acetamide were picked and poked into the central portion of 0.3% 7H9 agarose plates with or without acetamide and incubated for 4-5 days. There was a significant difference between control and overexpression strains. The surface area of the monolayer formed by the RvFtsY overexpressimg *M. smegmatis* mc^2155 (MsRvF) was 3 times smaller than the control strains. Moreover the control strains grew as a smooth uniform circular monolayer. The overexpression strains were rough and the edges were not uniform and showed finger-like projections (Fig. 25).

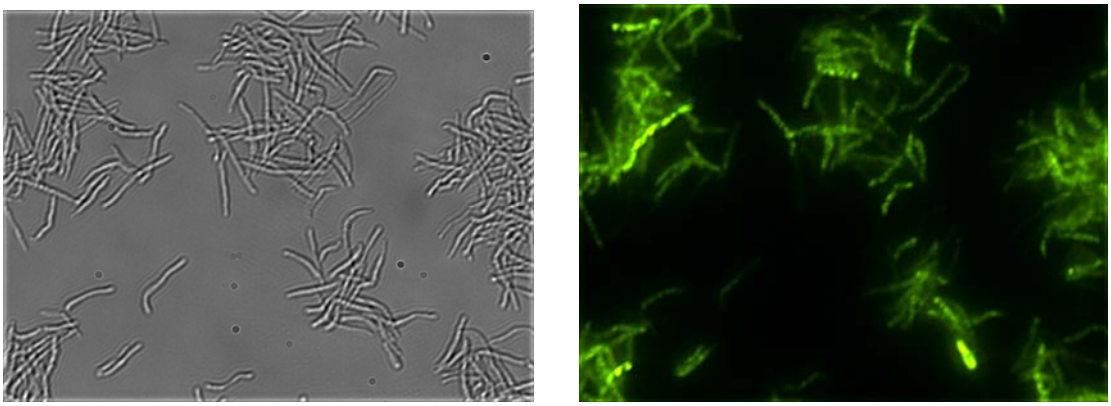
Fig. 25: Sliding motility



Spread of control (a) and MsRvF (b) on 7H9 plates with 0.3% agarose with acetamide

Localization of FtsY green fluorescent fusion protein *in vivo* in mycobacteria: The Ftsy gene was fused with green fluorescent protein (GFP) gene and cloned into mycobacteria episomal vector under the acetamidase promoter. The cells were grown with shaking at 37°C and induced with 1% acetamide for 4 hrs. Cell morphology was visualized under DIC microscope and FtsY GFP was localized by fluorescence microscopy. Mtb_FtsY was found to be dispersed at regular intervals in the cell (Fig. 26). Further investigations are required to confirm whether these are not aggregates within the cells.

Fig. 26: Localization of *FtsY* in *M. smegmatis* over-expressing *M. tuberculosis FtsY*



(a) (b)

(a) DIC image of MSRvF (b) Fluorescence image of FtsY_GFP of the same

Deletion of ftsY gene from *M. smegmatis* by specialzed transduction method: We also attempted to knock out the Fts Y gene of *M. tuberculosis* in order to understand its role in growth and physiology of *M. tuberculosis* by specialized transduction method. We could not get any mutant colonies implying that the gene could be a essential gene. We would be further confirming it by regulating the expression of Fts Y by antisense cloning.

[Contact person: Dr. Sujatha Narayanan (E.mail. ID: sujathanarayanan@trcchennai.in)]

Identification of novel human T-cell antigens of *M. tuberculosis* by immuno-proteomics

Background

Culture filtrate proteins (CFP) of *M. tuberculosis* have been resolved by using 2D-liquid phase electrophoresis (2D-LPE) for identification of T-cell antigens. Separated fractions were subjected to immunological analysis, in healthy household contacts (HHC) and TB patients. Ten fractions were identified to be specifically recognized by contacts alone. Proteomic analysis revealed that 16 proteins were present in the 10 “contact specific” fractions. Among the 16

proteins, 13 were already reported as T-cell antigens in earlier studies and the remaining 3 (Adk, AcpM, Rv3716c) were novel T-cell antigens.

Aim

- To immunologically characterize the novel T-cell antigen (Adk) in two groups of study subjects, namely TB patients (susceptible population) and healthy house hold contacts of TB patients (protected population)

Methods

Epitope analysis of Adk protein was carried out using Propred and Propred-1 server. *Adk* gene was over-expressed in *E. coli* expression system. The *in vitro* immune response to *Adk* was assessed in 10 TB patients and 10 HHCs. The CD4 and CD8 cells were assessed for the intracellular presence of IL-2, IL-4, IL-10, IFN-γ and TNF-α. Surface expression of CD69 and CD25 (activation markers) was also studied.

Results

In silico analysis of the predicted epitopes of *Adk* was carried out. It showed that 11 nanomeric peptides were predicted to bind to both MHC class-I and MHC class-II alleles (Table 20). Among these epitopes, the nanomer ^{59}VPSDLTNEL^{67} was predicted to bind to 12 MHC class-I alleles and 7 MHC class-II alleles. The nanomer ^{18}VKLAEKLGI^{26} was predicted to bind to 39 MHC class-II alleles and 1 MHC class-I allele (Table 20). *Adk* protein induced significantly higher percentage of $CD8^-$ and $CD8^+$ IFN-γ^+ cells in HHC compared to TB patients (Figs. 27 & 28). It also induced significantly higher percentage of $CD8^-$ TNF-α^+ cells in HHC compared to TB patients (Fig. 29).

Table 20: Nanomeric peptides which were predicted to bind to both HLA I and HLA II

Sl. No	Amino acid position	Peptide sequence	No. of HLA I predicted	No. of HLA II predicted
1	3	VLLLGPPGA	3	22
2	18		1	39
3	59	VPSDLTNEL	12	7
4	81	FILDGYPRS	1	7
5	113	FRVSEEVLL	6	4
6	137	ILNRMKVYR	1	13
7	143	VYRDETAPL	4	11
8	144	YRDETAPLL	5	1
9	151	LLEYYRDQL	3	1
10	154	YYRDQLKTV	1	6
11	171	VFARALRAL	3	2

Fig. 27: IFN-γ response in CD8 negative PBMC population

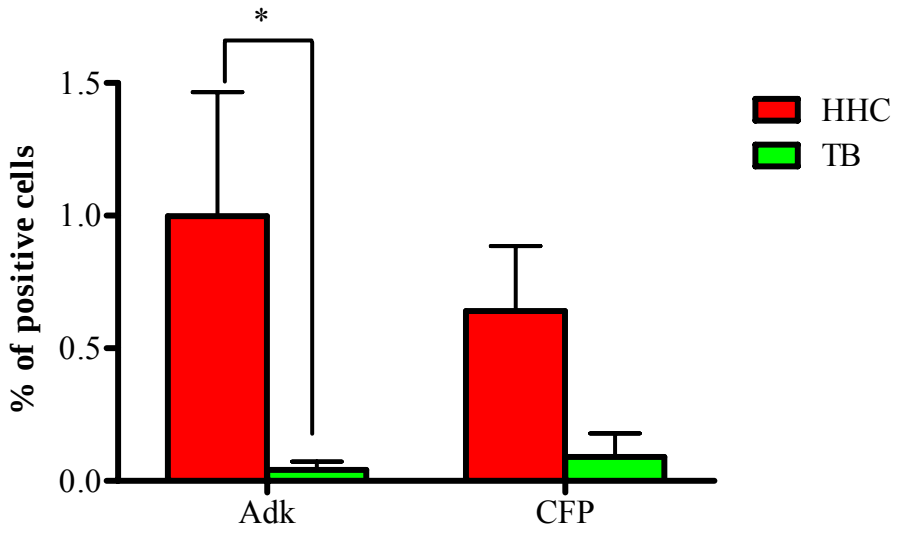


Fig. 28: IFN-γ response in CD8 positive PBMC population

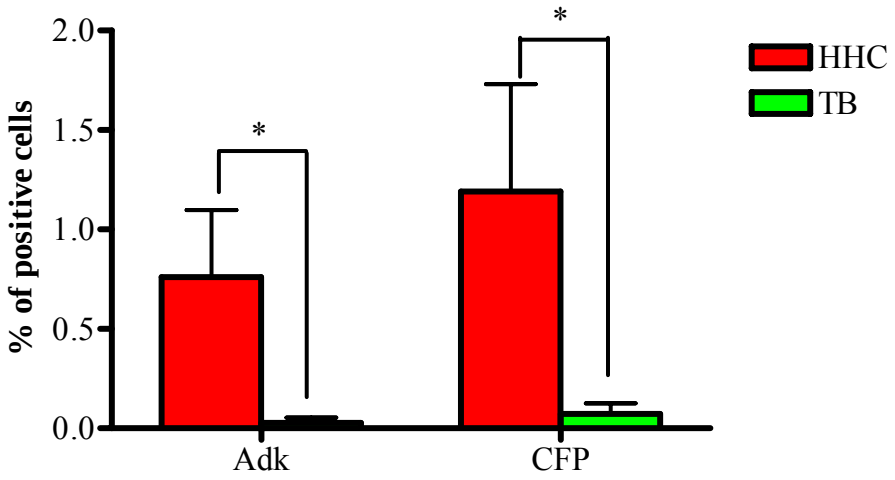
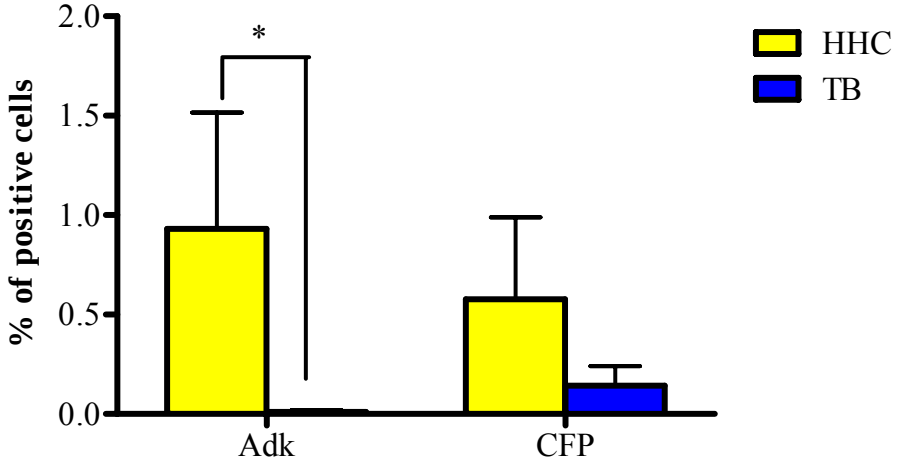


Fig. 29: TNF-α response in CD8 negative PBMC population



The above values are median; vertical bars denote SEM
*denotes p<0.05 vs TB patients; statistical analysis was performed using Mann-Whitney test

Further studies to characterize the *Adk* protein are in progress.

[Contact person: Dr. Alamelu Raja (E.mail. ID: alamelur@ trcchennai.in)]

Innate immunity in HIV infection

Background

Natural Killer (NK) cells express several specialized receptors through which they recognize and discriminate virally-infected/tumor cells efficiently from self cells and kill them. This ability of NK cells to lyse is regulated by an array of inhibitory or activating receptors. The effect of HIV on NK cell phenotype and function particularly when co-infected with TB remains to be fully elucidated.

Aims

- To evaluate NK cell receptors including the inhibitory and activating receptors in HIV infection especially when co-infected with TB
- To study the additive effect of IL-15 & IL-12 on NK receptors

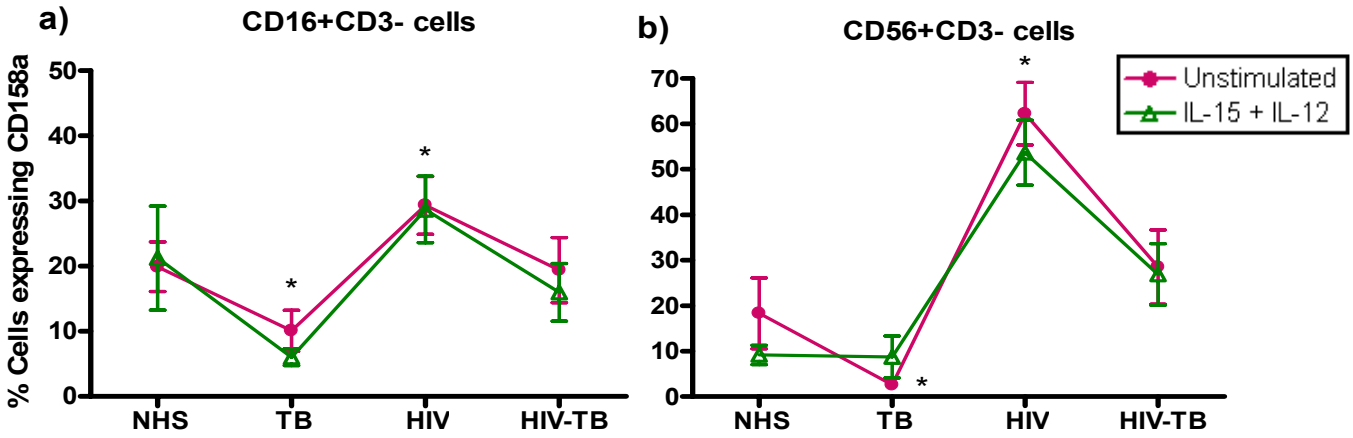
Methods

The study groups comprised of normal healthy subjects (NHS, n=15), patients with PTB (TB, n=15), HIV-positive subjects without tuberculosis (HIV, n=15) and HIV-positive patients with TB (HIV-TB, n=15). The NK surface receptors were studied by flowcytometry. The receptors examined were CD158a, CD158b, KIRp70, CD85j, NKG2A, NKG2D, NKp30, NKp44 and NKp46.

Results

Expression of inhibitory NK receptors was increased in HIV-infected individuals, as compared to NHS (Fig. 30). Expression of KIRp70 and CD85j was enhanced in HIV-TB patients. Treatment with IL-15+IL-12 declined NKG2A expression in the HIV group. Expression of other inhibitory NK receptors was not affected by IL-15+IL-12 stimulation.

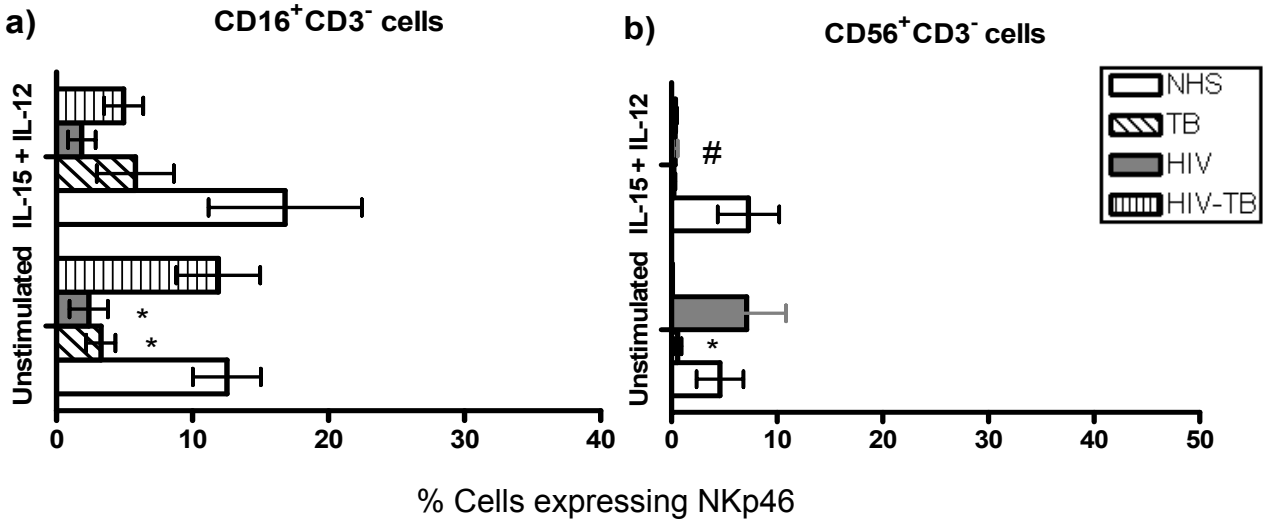
Fig. 30: CD158a expression on NK cells



Data are presented as mean ± SEM.
* denotes p<0.05 vs NHS

Basal expression of NKp30 and NKp46 on NK cells was lowered in HIV and HIV-TB patients, compared to NHS. Stimulation with IL-15+IL-12 had no effect on NKp30 and NKp46 expression (Fig. 31). NKG2D and NKp44 expression was elevated in HIV group compared to NHS which further enhanced on IL-15+IL-12 stimulation.

Fig. 31: NKp46 expression on NK cells



Data are presented as mean ± SEM
* denotes p<0.05 vs NHS
# denotes p<0.05 vs unstimulated condition

Conclusions

Downregulation of iNKRs, upregulation of activating NKRs after IL-15 + IL-12 stimulation indicates an immunomodulatory effect on NK cells from HIV-infected individuals. The response was limited in HIV-TB co-infection, probably due to the influence of TB.

[Contact person: Dr. Alamelu Raja (E.mail. ID: alamelur@ trcchennai.in)]

Role of Interferon gamma assay for latent TB in HIV infection

Background

Tuberculin skin test (TST) is still being used for diagnosis of latent TB infection (LTBI) in developed countries. Recently, interferon gamma (IFN-γ) and IFN-γ inducible protein-10 (IP-10) based assays are suggested as alternative tests for diagnosis of LTBI. However, data on the performance of these assays in HIV-infected individuals are limited.

Aim

- To compare the performances of TST, IFN-γ release assay and IP-10 assay, in a setting which has high prevalence for TB and HIV, among HIV-infected individuals

Methods

We used QuantiFERON-TB Gold in-tube (QFT-IT) assay to measure the performance of IFN-γ release assay. IP-10 was measured in the supernatants collected from the QFT-IT tubes.

Results

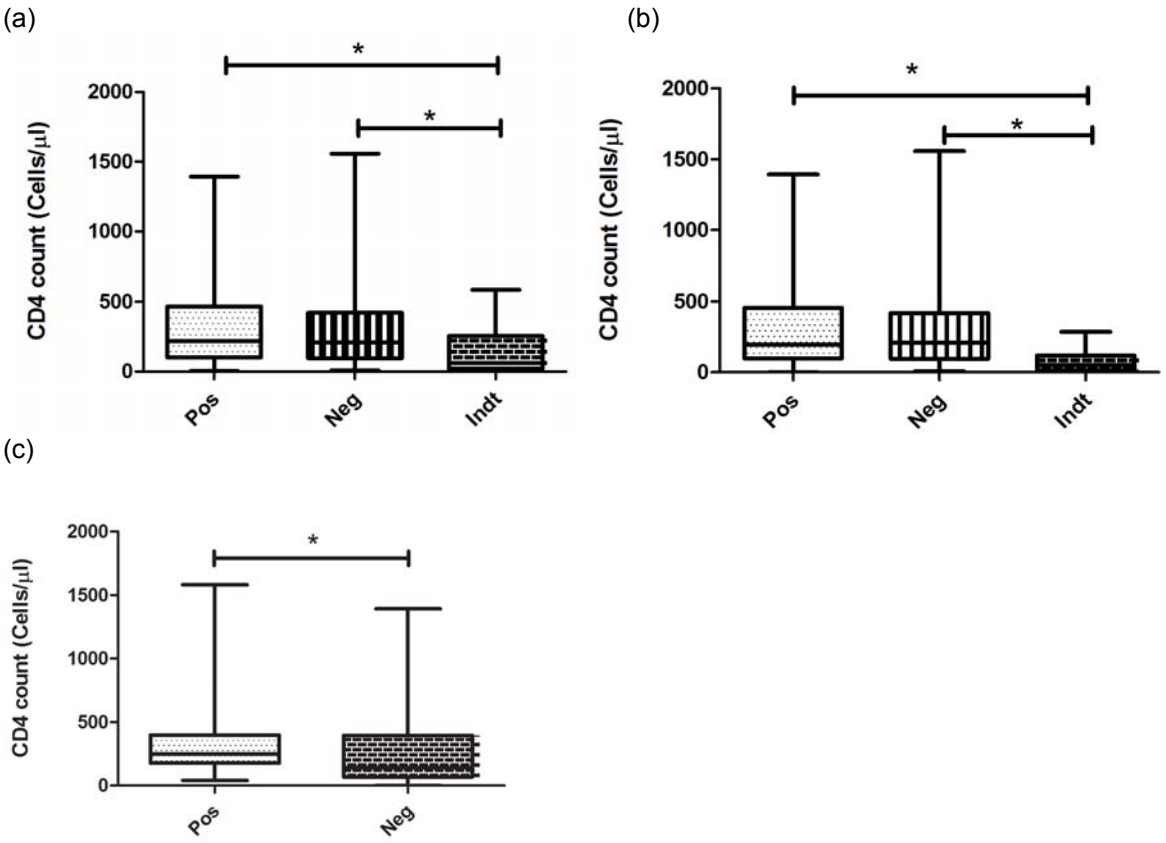
A total of 180 HIV-infected subjects, negative to active TB disease, were recruited. QFT-IT was positive in 38% and indeterminate in 9% of HIV-infected subjects. IP-10 was positive in 45% and indeterminate in 5% of subjects. The positivity of IP-10 was significantly higher than QFT-IT (Table 21).

Table 21: Performance of QFT-IT and TST

Tests	Results	No of subjects (%; 95% CI)
QFT-IT	Positive	68 (38%; 31-45)
	Negative	95 (53%; 46-60)
	Indeterminate	17 (09%; 05-13)
IP-10	Positive	76 (45%; 38-52)
	Negative	85 (50%; 42-58)
	Indeterminate	09 (05%; 02-08)
TST (at 5mm)	Positive	34 (19; 13-25)
	Negative	146 (81; 75-87)
TST(at 10mm)	Positive	27 (15; 10-20)
	Negative	153 (85; 80-90)

QFT-IT yielded significantly higher number of indeterminate results than IP-10. TST was positive in 19% and 15% of subjects at 5mm and 10mm cut-off points respectively. The positivity of TST was significantly lower than QFT-IT and IP-10 at both (5mm and 10mm) cut-off points. Unlike TST, performance of QFT-IT and IP-10 were not influenced by CD4 cell count (Fig. 32).

Fig. 32: Influence of CD4 counts on test outcomes



Data are presented as mean ± SEM
* denotes p<0.05 vs HIV-positive subjects

Conclusions

Unlike TST, QFT-IT or IP-10 assays are less influenced in HIV infection; hence they can be used as a marker for LTBI diagnosis among HIV-infected individuals. [Contact person: Dr. Alamelu Raja (E.mail. ID: alamelur@ trcchennai.in)]

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

Background

During the early stages of infection, non-syncytium inducing, macrophage-tropic, CCR5-utilizing (R5) HIV-1 strains predominate, whereas T-cell-tropic, syncytium-inducing, CXCR4-utilizing (X4) isolates may emerge during the later phases of infection. 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. Some viruses can use both CCR5 and CXCR4 (dual tropic). The third variable (V3) region of HIV-1 Env is known to be a critical determinant of co-receptor tropism. Changes in the V3 region associated with CXCR4 use include the presence of positively charged amino acids at positions 11 and 25, an increase in total net charge, and a reduction in the number of potential N-linked glycosylation sites in the V3 loop. There is evidence to suggest that determinants in *env* that are outside of the V3 region can confer CXCR4-tropism without compromising CCR5 co-receptor use and that efficient CXCR4 use requires the acquisition of changes in V3 which compromise CCR5 use.

Objectives

- To investigate co-receptor usage of isolates from HIV-1 infected individuals and their relation to disease progression
- To characterize pertinent amino acid differences in the envelope protein between R5, X4 and R5X4 phenotypes
- To determine if phenotype specific differences between R5 and X4, found in our cohort applies to globally circulating HIV-1 of different genetic clades and phenotypes

Materials and methods

Study population

Fifty HIV-infected individuals at various stages of HIV disease but not undergoing anti-retroviral therapy will be included in the study. Ten to fifteen millilitres of defibrinated whole blood is obtained from each individual. The blood samples are processed as follows:

1. Isolation of virus
2. Determination of co receptor use
3. Nested PCR of full-length gp160
4. Sequencing

So far, 21 patients have been recruited to the study. Virus isolation has been performed. Further processing is in progress. The study is on going.

[Contact person: Dr. Luke Elizabeth Hanna (E-Mail ID: hanna@trcchennai.in)]

STATISTICAL RESEARCH

CURE RATE MODELS

Introduction

Standard survival models assume that all cases in the study population are susceptible to the event of interest if the follow-up is sufficiently long. Traditional methods of survival analysis namely, log rank test and Cox regression model assume that all individuals remain at risk. The clinical trials consist of heterogeneous population of patients which can be divided into two groups based on treatment. One group consists of those patients who respond favorably to the treatment and subsequently become immune or insusceptible to the disease and are said to be cured. The other group consists of those patients who do not respond to the treatment and remain uncured or cured but relapsed. The subjects are termed to be cured if they are censored after long follow-up period at the time of analysis. In these situations, interest often lies in estimating the proportion of subjects who do not experience the event. Failing to account for such cured subjects would lead to incorrect inferences and researchers may be interested in estimating the cured fraction. Cure models were proposed about 50 years ago, have received regular attention in statistical literature but not attained wide use or acceptance in medical literature because of their reliance on parametric forms. When the proportional hazard assumptions are valid, the use of log rank test and Cox regression analysis predictions are most valid. Parametric cure model provides a coherent statistical approach to investigate the effect of covariates on the time to failure separately from their effect on ultimate outcome.

Aims

- To construct models for estimating Cure Fraction
- To compare empirically the performance of different cure models using a cancer database

Materials and methods

A series of 1107 locally advanced breast cancer (LABC) patients who had completed the neo-adjuvant treatment protocol consisting of preoperative chemo-

radiotherapy followed by surgery between 1990 and 1999 at Cancer Institute (WIA), Chennai, formed the study group. Five prognostic variables were included in the model. Event free survival (EFS) duration was defined as the minimum time elapsed to disease progression, disease recurrence, occurrence of second malignant neoplasm or death from any cause. Patients alive without disease were censored at the date of last follow-up. EFS probability was estimated using Kaplan-Meier method. The prerequisite for the application of mixture cure model is the long term follow-up. The parametric and nonparametric cure models were used to model to estimate the cure fraction. The STATA package was used for model building.

Results

Table 22 describes the proportion and event free survival estimates. EFS probabilities for all cases together showed minimal changes after 7 years of follow up.

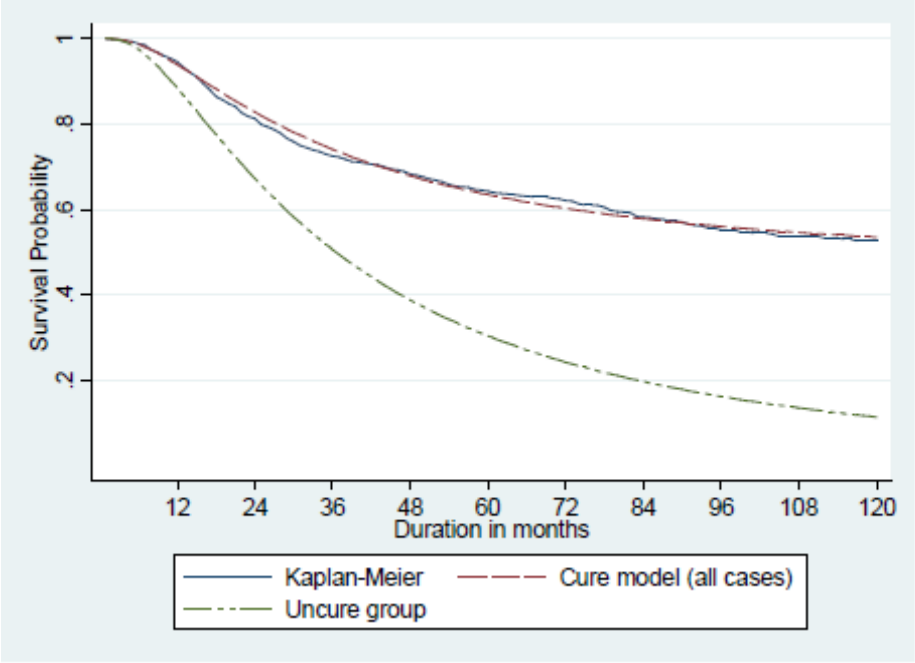
Table 22: Distribution survival (%) according to the prognostic Variables

Tumour Stage	Tumour Residue	Pathologic Node	No.	%	Survival %	
					5 years	10 years
Stage 2B	TR-	PN-	162	14.6	78.0	70.0
		PN+	32	2.9	58.0	49.0
	TR+	PN-	96	8.7	81.3	60.8
		PN+	76	6.9	61.0	46.8
Stage 3A	TR-	PN-	122	11.0	84.1	78.8
		PN+	57	5.1	46.5	38.6
	TR+	PN-	98	8.9	67.0	57.5
		PN+	107	9.7	50.3	28.6
Stage 3B	TR-	PN-	89	8.0	64.5	57.4
		PN+	38	3.4	50.1	45.1
	TR+	PN-	85	7.7	63.0	47.4
		PN+	145	13.1	42.0	28.7
All Stages			1107	100.0	64.2	52.6

TR: Tumour Residue; PN: Pathologic Node

The survival percentages were 64.2, 55.3, 53.7 and 52.6 for the years 5, 8, 9 and10 respectively. The same was observed for factors of tumor residue, pathologic node and tumor stage. The survival differences in the factors were significant ($p<0.001$) for tumor residue, pathologic node and tumor stage. The maximum follow up duration was 15 years with a median follow-up duration of 82 months among those without experiencing any event and 27 months among those experiencing any event. The number of events was the maximum in the second year and decreased gradually. Event free survival probabilities for all cases together showed minimal changes after 7 years of follow up survival percentages were 64.2, 55.3, 53.7 and 52.6 for the years 5, 8, 9 and 10 respectively. The same was observed for factors of tumor residue, pathologic node and tumor stage. The estimation of survival cure models is similar to that obtained by the Kaplan-Meier estimates (Fig. 33).

Fig. 33: Comparison of Kaplan Meier and cure models model survival estimates



The cure fraction was estimated to be 47.5% log normal kernel. The survival time was restricted to 10 years as we expected maximum failure within this period. While Kaplan-Meier method estimates the survival of all the cases in the dataset, the cure model estimates also the survival of the uncured.

The estimates of the cure fraction scale and shape parameters are given in Table 23. The stage parameter estimates are significant and also the reduction in the deviance was significant when we include stage (Model 2).

Table 23: Cure proportional hazard (PH) model estimates

Model	Model 1*			Model 2@		
	Coef.	SE	p value	Coef.	SE	p value
Cure PH model						
Cure fraction (π)						
Tumour Residue	0.216	0.104	0.037	0.185	0.104	0.075
Path. Node	0.796	0.101	<0.001	0.740	0.102	<0.001
Stage 3A				0.226	0.124	0.07
Stage 3B				0.494	0.121	<0.001
Constant	-0.898	0.092	<0.001	-1.102	0.117	<0.001
Scale (λ)						
Constant	-5.975	0.234	<0.001	-6.006	0.235	<0.001
Shape (γ)						
Constant	0.403	0.044	<0.001	0.407	0.043	<0.001
-2LogL	5225.8			5208.6		

*Model 1 includes factors of tumour residue (TR) and pathologic node (PN)
@Model 2 includes factors of TR, PN and tumour stage; PH: proportional hazard
TR negative, PN negative and tumour stage 2B served as reference categories

The lognormal and the Weibull are the two most commonly used parametric cure models. The cure fraction estimates under lognormal and Weibull are given in Table 24. The Weibull model has given consistently higher vales when compared to lognormal.

Table 24: Cure fraction estimates

PN	Lognormal cure model		Weibull cure model	
	TR-	TR+	TR-	TR+
PN-	62.5	54.8	66.5	60.3
PN+	34.7	27.1	40.5	32.6

TR: Tumour Residue; PN: Pathologic Node

The cure fraction estimates under PH and non-proportional hazard models are presented in Table 25. Under stage 2 the non-PH estimates are lower than PH estimates.

Table 25: Cure fraction under PH and non-PH models

Stage	PN	PH Model		Non PH Model	
		TR-	TR+	TR-	TR+
All stages	PN-	62.9	46.6	62.5	54.8
	PN+	43.7	27.4	34.7	27.1
Stage 2B	PN-	68.2	61.8	52.9	40.1
	PN+	42.5	36.1	30.1	17.3
Stage 3A	PN-	62.1	55.7	68.3	55.5
	PN+	36.5	30.0	45.5	32.7
Stage 3B	PN-	52.4	46.0	59.7	46.9
	PN+	26.7	20.3	36.9	24.1

TR: tumour residue; PN: pathologic node; PH: proportional hazard

Conclusion

Two models of different sets of covariates are considered wherein the difference in the covariates of the models and compared. The comparison of the Weibull and lognormal kernels suggests that Weibull model assumption seems to be better. The models under the PH and non-PH assumptions found to have similar results. The cure fraction is sensitive to the model specifications. Cox model is the most widely used in the analysis of survival data. The PH assumption, which is the basic for application of the model, is not always tested. The test for PH revealed some departure in the prognostic variables. The Cox model identifies the prognostic factors and their hazards in comparison to the reference group and is valid only under proportional hazards assumption. The cure rate estimates under parametric kernels and the Cox model with Weibull kernel yield similar results.

The study is in progress.

[Contact person: Dr.P. Venkatesan (E.mail ID: venkatesanp@trcchennai.in)]

Electronic Data Processing

The Electronic Data Processing (EDP) division provides computerized services for all departments in the TRC. TRC departments have direct access to the data with their personal computers (PC). 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 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 during the year.

All break-down calls of computers and its peripherals are dealt with 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 quantum of documents of epidemiological, clinical, laboratory and program based studies entered and verified from April, 2009 to March, 2010 is shown below.

No. of documents entered: 1, 71, 182

No. of documents verified: 1, 67, 630

A total of 1,45,233 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.

[Contact person: Mr.R. Subramani (E.mail ID: subramanir@trcchennai.in)]

LIBRARY & INFORMATION SERVICES

1. Facts & Overview

The Library is constantly evolving to meet the changing needs of the users. 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. During the year the library continued its march to facilitate the creation of new knowledge through the acquisition, organization and dissemination of library materials. The Consortium portal has been hyperlinked through digital library to coordinate activities/services with ICMR networking. It is more than a print library, an information resource division, moving towards Virtual platform.

2. Digital library

The library is part of the institute's network and has adequate computing infrastructure to cater to the needs of the users. The library has taken several initiatives to provide Digital Library services to the users. It has its own home page, provides web-based access to more than 3300 full-text journals and databases. It enhances the customized integrated (24 hrs) access facility to our patrons for our web based value added services (Fig. 34).

Fig. 34: Digital library

It allows to:

- ❖ Access the e-Resources of the library and its Consortium titles
- ❖ Download library forms
- ❖ Search & Browse the material in the library
- ❖ Check the material borrowed by a user and their due date
- ❖ Find out the available back volume collection
- ❖ Access electronic version of TRC Annual Report since 1992
- ❖ Provide the current status of the subscribed journals
- ❖ Get the Guidelines to Author for subscribed titles
- ❖ Access the Indian National Union Catalogue
- ❖ Pointing International Tuberculosis organizations link

3. Services

It offered a range of services including, access to electronic resources, circulation, current awareness service, document delivery, e-Mail co-ordination, Inter Library Loan, Internet Browsing Lab, reference assistance, resource sharing, facilitating digital medial resources and web based services. The TRC Web Site is developed and being maintained by the library.

4. Collection building and management

Collection building is one of the important functions of the library that supports research work of the scientists, students, staff and other users. It has more than 10000 collections on bound back volumes of journals. Since the library has no space to accommodate new additions in its stacks, the library focused its march towards on online collection few years back. Now the e-collection includes individual titles, subject collection, cumulative collection, databases, consortium, e-bundles, publisher's package collection and e-books. Table 26 shows the collection building of TRC library. The scope of the library's collection is broad with excellent coverage on health science. But access to online full-text journals is restricted for remote access.

Table 26: Collection building of TRC library

Collection	Titles
Individual Titles	
International Journals [Online (25) & Print (2)]	27
Indian Journals (Print)	07
Cumulative Collection	
American Society for Microbiology	11
e-bundle	
Annual Review Biomedical	25
Subject Collection (Science Direct)	
Immunology & Microbiology Subject Collection	93
Current Opinion in…… & Trends in …titles	25
Package Collection	
Nature Publishing Group package collection	04
e-Books	
Books@MD Consult	50
Books@OVID	27
Databases	
IndiaHealthStat	01
Journals@OvidSP	122
Journals@MD Consult	89
ERMED	1536
INFOTRAC Indian Medical Journal Collection (55)	
ProQuest	1499
J-Gate	6960
Archives	
AIDS	V.1 #1+
Annual Reviews Biomedical (26 titles)	V.1 #1+
JAIDS	V.1 #1+
Nature	1950-96
Science Classic	V.1 #1+
Scientific American	1993+
Resource Sharing	
JCCC@ICMR	1942

4.1. e-Resources

4.1.1 Full-text Resources URL

American Society for Microbiology http://journals.asm.org/

Annual Reviews Biomedical/LifeSci http://arjournals.annualreviews.org/

Books@MD Consult
http://www.mdconsult.com/das/booklist/body/205756184-2?booklist_order=specialty&format=AT

Books@OVID http://ovidsp.tx.ovid.com/sp-2.3.1b/ovidweb.cgi?&S=EPKNFPCLJPDDAOCPNCDLPHJCDEBAAA00&New+Database=Single|0&Jump+to+Browse=1

Cochrane Library
http://www.thecochranelibrary.com/view/0/index.html

IndiaHealthStat
http://www.indiahealthstat.com/default.aspx

Journals@MD Consult
http://www.mdconsult.com/das/booklist/body/207425045-2?booklist_order=title&format=AT

Journals@OVID http://ovidsp.tx.ovid.com/sp-2.3.1b/ovidweb.cgi?&S=POHPFPMNPODDIOBLNCDLPDJJJLLGAA00&New+Database=Single|0&Jump+to+Browse=1&Skip+Open=1

ScienceDirect http://www.sciencedirect.com/

4.1.2 Archives

Annual Reviews Biomedical/Life Sci.
http://arjournals.annualreviews.org/action/showJournals

Nature Archives 1950-1996
http://www.nature.com/nature/archive/index.html

Science Classic Vol.1 #1+
http://www.sciencemag.org/archive/#classic

Scientific American 1993+
http://www.nature.com/scientificamerican/archive/index.html

4.1.3 Consortium

J-Gate http://j-gate.informindia.co.in/

J-Gate@ERMED http://www.nmlermed.in/

ProQuest http://proquest.umi.com

4.1.4 Resource Sharing

JCCC@ICMR http://www.jccc-icmr.informindia.co.in/

4.1.5 New Additions

AIDS (archives) Vol.1 #1+ http://ovidsp.tx.ovid.com/sp-2.3.1b/ovidweb.cgi?&S=FICJFPEKIMDDHONANCDLDHPLFGNLAA00&Browse=Toc+Children|YES|S.sh.2.14.15|2|50

Journal of Acquired Immune Deficiency Syndrome (archives) Vol.1 #1+ http://ovidsp.tx.ovid.com/sp-2.3.1b/ovidweb.cgi?&S=FICJFPEKIMDDHONANCDLDHPLFGNLAA00&Browse=Toc+Children|YES|S.sh.2.14.15|56|50

Current Opinion in HIV and AIDS http://ovidsp.tx.ovid.com/sp-2.3.1b/ovidweb.cgi?&S=GPJFFPHFIMDDGOLHNCDLCDMJPPMKAA00&Browse=Toc+Children|YES|S.sh.2.14.15|32|50

Current Opinion in Infectious Diseases http://ovidsp.tx.ovid.com/sp-2.3.1b/ovidweb.cgi?&S=GPJFFPHFIMDDGOLHNCDLCDMJPPMKAA00&Browse=Toc+Children|YES|S.sh.2.14.15|33|50

Nature Immunology http://www.nature.com/ni/index.html

Nature Medicine http://www.nature.com/nm/index.html

Nature Reviews Microbiology http://www.nature.com/nrmicro/index.html

Nature Reviews Molecular Cell Biology http://www.nature.com/nrm/index.html

5. 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 in the world. Further, each article gives pointer/interlinks to access the full-text/PDF of the article. Secondly, a half-yearly publication **LibNEWS** is being published, which covers library news.

[Contact person: Mr.R. Rathinasabapati (E.mail. ID: rrathinasabapati@ trcchennai.in)]

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

Annual Report 2009

Tuberculosis Research Centre Institutional Ethics Committee conducted six meetings (four scheduled and two unscheduled) during the calendar year 2009. All the meetings satisfied the quorum requirements.

The Chair, the Member Secretary, and two members were present during all the 6 meetings; out of the remaining six members, one member was present for 5 meetings, four members were present for 4 meetings, one member was present for 3 meetings.

Ten new protocols and one re-submission were reviewed during the year 2009. Thirty three ongoing eviews and three expedited reviews (three protocols) were also conducted.

Projects reviewed	Tuberculosis	HIV	HIV TB	Filariasis	Vaccine	Others	Total
Clinical Trials	4		3	2	5		**14**
Public Health							
Epidemiology	2						**2**
Programme OR	5		2				**7**
Laboratory							
Bacteriology	2						**2**
Immunology	4	5	3	2			**14**
Pharmacology			3				**3**
Social Sciences		3	1				**4**
Others						1	**1**
Total	**17**	**8**	**12**	**4**	**5**	**1**	**47**

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

Out of the 10 new protocols, six protocols were approved in one submission, one protocol was pproved in two submissions, and three protocols remains with the Investigators pending IEC approval.

Some of the salient features that figured during the year 2009 were:

1. The 'Initial Review Submission Form' was revised
2. 'Continued Bioethics Education' discussions were continued during the course of the meetings and via electronic mail
3. As the US Federal Wide Assurance (FWA 00005104) that TRC is holding was nearing expiry, it was renewed by re-submission for both TRC and TRC-IEC (No: IRB 00003255), valid up to 12th June 2012
4. In continuation with TRC's US Federal Wide Assurance, an annual report on possible research misconduct for the year 2008 was filled with the Office of Research Integrity (ORI), US Department of Health & Human Services

Member Secretary **Chair**

APPENDICES

LIST OF PUBLICATIONS

Publications : 76

Publications in		i)	International Journals : 62
		ii)	National Journals : 14
Others - Books	:	i)	International : 1
		ii)	National : 1
Accepted for publication in		i)	International Journals: 14
		ii)	National Journals : 12

International:

2009:

1. Anand SP, Selvaraj P. Effect of 1, 25 dihydroxyvitamin D(3) on matrix metalloproteinases MMP-7, MMP-9 and the inhibitor TIMP-1 in pulmonary tuberculosis. Clin Immunol.2009;133:126-131.

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Books

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2. Swaminathan S, Banu Rekha VV. Drugs used in tuberculosis and leprosy. 30th chapter in a book entitled "Side Effects of drugs Annuals – Edition 31, publisher Elsevier, 2009 :501-511

ACCEPTED:

International:

1. Basirudeen S, Venkatesan P, Paulkumaran P, Raja A. Positivities of QuantiFERON-TB gold in tube assay and tuberculin skin test in healthy Subjects from a tuberculosis endemic population. J Pediatr Infect Dis.

2. Goletti D, Raja A, Basirudeen S, Rodrigues C, Sodha A, Butera O, Carrara S, Vermet 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.

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6. Rao PV, Ramanavelan S, Rajasekaran S, Raja A. Natural-killer cell-derived cytolytic molecules 5 in HIV-associated pulmonary tuberculosis - Role of exogenous interleukins. J Clin Immunol.

7. Ramesh Kumar S, Gopalan N, Patrawalla P, Menon P, Mayer K, Swaminathan S. Immune reconstitution inflammatory syndrome in HIV-infected patients with and without prior tuberculosis. Int J STD & AIDS.

8. Sivakumar PM, Kumar V, Seenivasan SP, Mohanapriya J, Doble M. Experimental and theoretical approaches to enhance anti tubercular activity of Chalcones. WSEAS Transac Biol Biomed.

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National:

1. 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.

2. Kumar AK, Sudha V, Swaminathan S, Ramachandran G. Comparison of HPLC and spectrophotometric methods for estimation of antiretroviral drug content in pharmaceutical products. Indian J Med Res.

3. Padmapriyadarsini, Swaminathan S, Karthipriya MJ, Narendran G, Menon PA. Morphologic and body composition changes are different in men and women on generic combination antiretroviral therapy – observational study. JAPI.

4. Ponnuraja C, Venkatesan P. Correlated frailty model: an advantageous approach for covariate analysis of tuberculosis data. Indian J Sci Technol.

5. Ponnuraja, C, Venkatesan P. Survival models for exploring clinical Trial data: An empirical comparison. Indian J Sci Technol.

6. Raghu, B. and Venkatesan P. Relationship between cigarette smoking and novel risk factors for cardiovascular disease. Biomedicine.

7. Rajavelu P, Das SD. Kinetics of chemokines secretion in infected human macrophages with various strains of *M. tuberculosis*. Indian J Med Microbiol.

8. Ramachandran G, Hemanth Kumar AK, Vasantha M, Shah I, Swaminathan S. Plasma efavirenz in HIV-infected children treated with generic antiretroviral drugs in India. Indian Pediatr.

9. Ramachandran R, Muniyandi M, Gopi PG, Wares F. Why do tuberculosis suspects bypass local services to attend tuberculosis sanatorium? Lung India.

10. Ray D, Subramanyam S, Krishna SH, Ramanathan VD. Serum C3d levels in tropical pulmonary eosinophilia. Indian J Med Res.

11. Suresh ML, Venkatesan P. Comparison of artificial neural network and regression models prediction of survival after surgery. Inter J Inf Sci Comput Math.

12. Venkatesan P, Srinivasan R. Modelling the spatial variogram of tuberculosis for Chennai ward in India. Indian J Sci Technol.

Awards/Honours

- Patent related to “Sputum processing method for mycobacteria” filed through Intellectual Property Rights Unit of ICMR (Ref No P&I /IPR/TRC/137A April 2009) – **Vanaja Kumar**

- Received “Life Time Achievement Award” from Indian Association of Applied Microbiology at SRM University, Chennai during December 2009 - **Vanaja Kumar**

- HIV/AIDS Laboratory was accredited by the World Health Organization as a National Reference Laboratory for HIV Drug Resistance Genotyping.

Special Assignments

Dr. Aleyamma Thomas.

- Member, Protocol Review Committee, ICMR

Dr.N. Selvakumar

- Consultant for Review of IRL activites particularly construction work at Goa, 18 & 19 February 2010

Dr. Vanaja Kumar

- Accreditation visit to Hinduja Hospital, Mumbai on 9 & 10 August 2009
- Preaccreditation visit to RMRC, Bhubaneswar for "Establishment of TB diagnostic laboratory" on 2 September 2009
- Pre assessment visit to MOSC College, Kolencherry, Kerala on 29 & 31 December, 2009

Dr. Alamelu Raja

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

Training provided:

Training and guidance have been provided for 5 students (M. Sc) for their dissertation during 2009-2010, as a 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

Reviewer for International and National journals:

- Biologicals
- European Respiratory Journal
- Expert Reviews
- FEMS Immunology and Medical Microbiology
- Iranian Journal of Immunology
- Proteomics
- BMC Infectious Diseases - Two
- Indian Journal of Medical Research
- Indian Journal of Tuberculosis

Reviewed Project Proposals submitted to funding agencies:

- Short Term Studentship, ICMR
- Centre of Excellence and Innovation in Biotechnology, DBT
- Indian Council of Medical Research
- Indo-US Science & Technology Forum

Student Advisory Committee:

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

Dr. Sujatha Narayanan

- Thesis examiner of Ph D students from Karnataka & Delhi University
- Doctoral committee member for Ph D students at Anna University, CLRI, Chennai
- Reviewer of research proposals submitted to funding agencies, DBT & ICMR

Reviewer for International and National journals:

- Infection, Genetics and Evolution
- African Journal of Microbiology
- Indian Journal of Medical Research

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

Reviewer for international journals:

- Am J Resp Crit Care Med - Two
- Tuberculosis - Two
- Human Immunology - Two
- Int.J.Tuberc and Lung Disease - One
- Tissue Antigens - One
- Scand J Immunology - One
- International Journal of Immunogenetics - One
- Archives of Medical Research - One
- Gerontology - One

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 for the Projects from CSIR/ICMR funding agencies

Reviewer for International journals:

- The Journal of Infectious Diseases
- Human Immunology
- Tuberculosis
- Journal of Tropical Medicine
- Molecular and Cellular Biochemistry
- Journal of Vaccine

Training provided:

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

Dr. Geetha Ramachandran

- Expert committee member appointed by TNSACS for purchase of automated analysers
- Mentor to review research proposals submitted to the ICMR for funding under Pediatric HIV
- Reviewer of research proposals submitted to ICMR
- Reviewer of manuscripts submitted to national & international journals

Training provided:

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

Dr. Beena E Thomas

- Member of the Academic Counsel in Madras School of social work
- Board Member for AroGyan
- Member of the TORCH panel –TANSACS
- Member of Advisory Committee in APAC – VHS for BSS Wave XII
- Member of the National Consultation committee on HIV Social Research Priorities in India" TISS, Mumbai
- Reviewer for AIDS Care Journal
- Reviewer for the New Investigator Awards from the UW/FHCRC Center for AIDS Research Developmental Core
- Consultant Resource person for ITECH (International Training Education Center)
- Member of the Institutional Review Board, Government Hospital of Thoracic Medicine, Chennai

Conferences / Workshops /training programs attended

1. 96th Science Club meet organized by Institute of Mathematical Sciences, held at Chennai during April 2009 – V.N. Azger Dusthackeer.

2. Workshop on the "Fundamentals of datamanagement" organized by International Clinical Sciences Support Centre, USA held at New Delhi during April 2009 – P. Venkatesan (Faculty Member).

3. Poster presentation of study titled "Factors that play an important role in HIV adherence among mothers living with HIV (MLH) – A qualitative study from south India" at the 4th International Conference on HIV treatment adherence held at Miami, Florida, USA during April 2009 - Niruparani Charles.

4. Research Dissemination Workshop on Clinical, Behavioral and Pharmokinetic Studies with Tamil Nadu State AIDS Control Society at Chennai during May 2009 – Aleyamma Thomas, Soumya Swaminathan, Geetha Ramachandran, Beena E Thomas.

5. Developing nursing module for RNTCP organized by Central TB Division, Ministry of Health and Family Welfare held at LRS Institure, New Delhi during May 2009 - Aleyamma Thomas.

6. Guest lecture titled "Tuberculosis. Trends, Challenges and Solutions" on "Quality Assurance of Sputum AFB microscopy and culture & drug susceptibility testing of *Mycobacterium tuberculosis*" held at Bhopal during May 2009 – N. Selvakumar.

7. Second global symposium on IGRAs held at Dubrovnik, Croatia during May - June 2009 – Alamelu Raja.

8. Advanced flow cytometry course held at National Centre for Biological Sciences, Bangalore during June 2009 - S. Anbalagan.

9. ICH-GCP workshop on Clinical Research held at TRC, Chennai during June 2009 - K. Jaggarajamma, P. Murugasen, Sheila Frederick.

10. Invited talk at the silver jubilee celebrations of the Central Indian Institute of Medical Sciences, Nagpur during July 2009 – Sujatha Narayayan.

11. Seminar on Section Article 377 decriminalizing homosexuality held at Chennai during August 2009 – Beena E Thomas.

12. Paper titled “Fuzzy Logic Applications in Biomedicine” presented at the National Conference on “Application of Mathematics in Fuzzy Environment” held at Chennai during August 2009 – P. Venkatesan.

13. Pre-conference training in the First International Conference on Alcohol and HIV in India held at International institute for Population Science, Mumbai during August 2009 – P. Murugasen & Mohanarani Suhadev

14. Invited talk at Sri Venkateswara College of Engineering, Chennai during August 2009 – Sujatha Narayanan (Chief Guest).

15. 4th National CFAR SBSRN scientific meeting held at Boston, USA during September 2009 - Beena E Thomas & Meenalochani Dilip.

16. Paper titled “Art and Science of getting papers published” presented at the Research Methodology Workshop held at Chennai during September 2009 - P. Venkatesan. (Resource person & Faculty Member)

17. RNTCP Module Revision organized by Central TB Division, Ministry of Health and Family Welfare, New Delhi held at NTI, Bangalore during September 2009 - Aleyamma Thomas.

18. State level Technical meet on Biofactories of the world - Bio-Phoenix 09, held at Chennai during September 2009 –N. Selvakumar.

19. WHO Regional Workshop on strengthening laboratory diagnosis of multidrug and extensively drug resistant-TB held at Bangkok, Thailand, during September 2009 - N. Selvakumar.

20. Workshop on e-Health – The Emerging Scenario jointly organized by Apollo Health Education and Research Foundation, Apollo Tele Networking Foundation & TRC held at Chennai during September 2009 – R. Rathinasbapati.

21. Workshop on Dried Blood Spot HIV DNA PCR held at NACO, New Delhi during September 2009 – K. Ramesh.

22. BD FACSCalibur GLP Training and Blood Collection held at TRC, Chennai during October 2009 - Sudha Subramanyam, Luke Elizabeth Hanna, S. Anbalagan, S. Murugesan, M. Kannan.

23. National conference on Medical Biotechnology and Clinical Research held at Bangalore during October 2009 - Jerrine Joseph & R. Vasanthi.

24. Second Meeting of the Global Laboratory Initiative. Expanding and accelerating access to tuberculosis diagnostics and laboratory services held at France during October 2009 - N. Selvakumar.

25. National Capacity Building Workshop on Airborne Infection control in India held at New Delhi during October 2009 - N. Selvakumar.

26. Workshop on “Data mining using PASW Models” held at Chennai during October 2009 - P. Venkatesan (Faculty Member).

27. AIDS Vaccine 2009 Conference held at Paris, France during October 2009 – V.D. Ramanathan.

28. NACO/WEP Dissemination Workshop on “Nutrition and Food Security in HIV/AIDS Prevention, Care and Support” held at TRC, Chennai during October 2009 - P. Venkatesan (Faculty Member).

29. Dissemination meeting on “Roll out plan for Integrating Nutrition as part of care and support Treatment of HIV, World Food Program/HIV held at TANSAC, Chennai during October 2009 – Beena E Thomas.

30. Invited speaker in a “Public Health” seminar titled “Aspect of Hygiene on health with related to TB” held at Madras School of Social work during October 2009 - Dr. Beena E Thomas

31. Paper titled “Socio-Behavioral Studies in Health system strengthening” at the Dissemination workshop, organized by APAC at Chennai during October 2009 - Dr. Beena E Thomas

32. National Seminar on Religion and Terminal Illness: A holistic approach held at University of Madras, Chennai during October 2009 - Niruparani Charles, Sheila Fredrick & Sujatha.

33. Invited talk in a national seminar at Ethiraj College, Chennai during October 2009 – Sujatha Narayanan.

34. Workshop on “Facilitation skill training” addressing ssychosocial needs of MSMs in collaboration with Fenway Institute, Boston at TRC during October 2009 - Beena Thomas & Meenalochani Dilip.

35. Workshop on preparation of SOPs on Culture and DST testing for IRL Microbiologists held at Bangalore during November 2009 – Vanaja Kumar, Gomathi Sekar & S. Prabu Seenivasan.

36. First Meeting of the WHO Collaborating Centres (WHO CC) of India held at New Delhi during November 2009 – N. Selvakumar.

37. National conference on “Biotechnology for Human Development” held at Vellore during November 2009 - M. Radhakrishnan.

38. Paper titled “Research Ethic Education in India” presented at the Indo-US CITI Workshop on “Promoting Education in India” held at Chennai, during November 2009 – P. Venkatesan (Faculty Member).

39. Paper titled “Statistical Issues in a DNA Micro array Data Analysis” presented at the XXVII Annual National Conference of Indian Society for Medical Statistics held at Varanasi during November 2009 – P. Venkatesan (Chair person & Faculty member).

40. Workshop on “Statistical methods in Longitudinal Data Analysis”, held at Nashik, during November 2009 – P. Venkatesan.

41. XXVII Annual National Conference of Indian Society for Medical Statistics ISMS) held at Varanasi, during November 2009 - C. Ponnuraja, L. Sekar. R. Srinivasan & M. Tamizhselvan.

42. Talk on “Computational Resources for Drug Discovery” at the National Conference (BioSym) held at Indian Institute of Science, Bangalore during November 2009 - Jagadish Chandrabose S.

43. Dissemination meeting on “HIV Risk Behaviour Surveillance Survey (BSS)” during November 2009 - Beena E Thomas

44. National Consultation on “HIV Social Research Priorities in India” held at TISS, Mumbai, during December 2009 – Beena E Thomas.

45. Research Dissemination Workshop held at Chennai during December 2009 – Aleyamma Thomas, M.S. Jawahar, N. Selvakumar, Sujatha Narayanan, Vanaja Kumar, P. Venkatesan, C. Kolappan, R. Subramani, P Paulkuamaran, Pradeep Menon, Geetha Ramachandran, Beena E Thomas, V V Banu Rekha, & G. Narendran.

46. Workshop on preparation of SOPs on Culture and DST testing held at NTI, Bangalore during November - December 2009 - N.S. Gomathi, S. Prabu Seenivasan.

47. Guest lecture on “Recent advances in the diagnosis of Tuberculosis” at the National Conference on Emerging and merging areas of biology held at Chennai during December 2009 – N. Selvakumar.

48. National Training programme on “Molecular approaches for identification and characterization of Actinomycetes” organized by National Bureau of Agriculturally Important Microorganisms (NBAIM) held at Mau Nath Banjan (UP) during December 2009 – M. Radhakrishnan.

49. Seventh National conference of Indian Association of applied microbiologists BIOECLECTICS”09 held at Chennai during December 2009 - N. Selvakumar & Vanaja Kumar.

50. State level symposium on Application of Molecular techniques in Clinical Diagnosis and Research held at Chettinad Hospital & Research Institute, Chennai during December 2009 – N. Selvakumar.

51. 58th National Conference of Indian Association of Pathologists and Microbiologists (APCON), organized by APCON West Bengal Chapter held at Kolkata during December 2009 - N. Selvakumar & Vanaja Kumar.

52. 64th National Conference on Tuberculosis and Chest Diseases, NATCON 2009, held at Kolkata during December 2009 – Geetha Ramachandran, N.S. Gomathi, Gomathi Sekar, A.K. Hemanth Kumar, L. Prabhakaran, Sameer Hassan, S. Prabu Seenivasan, V.N. Azger Dusthackeer, S. Balaji, M. Radhakrishnan & R. Lakshmi.

53. Invited talk titled “Ensuring equity and access for new TB diagnostics” at the 40th Union World Conference on Lung Health held at Cancun, Mexico during December 2009 – M Muniyandi.

54. 36th National Annual conference of the Indian Immunology Society held at NIMHANS, Bangalore during December 2009 – Alamelu Raja, D. Sulochana, Kaustuv Nayak, D. Anbarasu, P. Supriya, P. Rajashree, P.V. Ramana Rao & S. Basirudeen.

55. Workshop on Data Retrieval & Analysis (SPSS) organized by the Department of Library & Information Science, University of Madras, held at Chennai during December 2009 - R. Rathinasbapati.

56. Lecture on “Proteomics and Drug Discovery” held at MIT Campus of Anna University, Chennai during December 2009 - Sameer Hassan.

57. Invited talk on “Emerging trends in the diagnosis and experimental chemotherapy of tuberculosis” at NIPER, Punjab during December 2009 – Sujatha Narayanan.

58. Workshop on “Role and functions of Inquiry Officer and Presenting Officer in departmental proceedings” held at Parsam Institute of Statutory Rules, Bangalore during December 2009 – P. Kannan.

59. Workshop on 'Early Infant Diagnosis' held at TNSACS, Chennai during January 2010 - Luke Elizabeth Hanna.

60. Workshop on 'Ethics in Biomedical Research' organized by National Institute of Epidemiology, Chennai during January 2010 - Sudha Subramanyam, Geetha Ramachandran, M. Kannan, C. Ponnuraja.

61. International Conference on Statistics and Information Analytics (ICSIA-2010) held at Chennai during January 2010 – L.Sekar, M.Vasantha & R. Srinivasan.

62. National Workshop on `Epidemiology of Leprosy, held at Chennai during January 2010 - Aleyamma Thomas.

63. Chennai ART Symposium – CART 2010 held at Chennai during January 2010 – P.K. Bhavani.

64. Annual National Conference of Indian Psychiatric Society held at Jaipur during January 2010 - Pradeep A Menon.

65. ITECH-Ethics, Psychosocial issues during January 2010 - Beena E Thomas (Resource person).

66. Conference on Cultural Epidemiology and Health related stigma held at Chennai during January 2010 - R.Vijayalakshmi, F.Faizunnisha, Auxilia M Christina, M. Rajasakthivel.

67. UGC sponsored National seminar on Social Work Research: Challenges and Perspectives, held at Chennai during January 2010 – Beena E Thomas (Panel Member).

68. Bioethics symposium organised by YRG Care held at Chennai during January 2010 – P. Kannan, S. Ramesh Kumar.

69. International Conference on Historical Humanities and Social Sciences (CHHSS 2010), held at Singapore during February 2010 Mohanarani Suhadev & K. Jaggarajamma.

70. Round table meeting organised by REACH to discuss MDR-TB partnership to strengthen RNTCP held at Chennai during February 2010 - Beena E Thomas.

71. East Asia Regional Biometric Conference 2010 & X Biennial Conference of International Biometric Society (Indian Region) on "Statistical methods for Bioinformatics" held at Manipal during February 2010 – C. Ponnuraja, L. Sekar, M. Vasantha, R. Srinivasan.

72. Invited talk titled “Social consequences of tuberculosis: Review of TRC studies” presented at the Golden Jubilee National Seminar on “Human Rights and Social Policy in India” held at Udaipur during February 2010 - M Muniyandi.

73. Review meeting to review MDP module organized by World Health Organsiation at WHO SEARO, New Delhi during February 2010 - Aleyamma Thomas.

74. Andhra Pradesh chapter of Indian Association of Medical Microbiologist held at Tirupati during February 2010 - N. Selvakumar & Vanaja Kumar (Chair persons).

75. Guest lecture on “Basic biosafety measures in laboratories” presented at the Second WHO/TDR Asian Biosafety training course held at CRME, Madurai during February 2010 – N. Selvakumar.

76. 17th Conference on Retroviruses and Opportunistic Infections (CROI 2010)” held at San Francisco, California during February 2010 - Pradeep A Menon, C. Padmapriyadarsini, N. Pooranaganga Devi.

77. Lecture on “Introduction to Bioinformatics”, at Jaya Arts and Science College, Avadi during February 2010 - Sameer Hassan.

78. Paper titled “Synonymous Codon Usage Analysis of Thirty Two Mycobacteriophage Genomes” at National seminar on “Emerging trends in medical biotechnology and bioinformatics”, at AVIT college of Engineering during March, 2010 - Sameer Hassan.

79. Conference on ‘Latest approaches in HIV infection management’ held at New Delhi during March 2010 – P.K. Bhavani

80. Paper titled ‘Advocacy strategies for recruitment of volunteers for phase I HIV Vaccine Trials in Chennai, India’ presented at the BIT Life Sciences 2nd World Congress of Vaccine held at Beijing, China during March 2010 - V.D.Ramanathan.

81. National Conference on Demographic Convergence, Demographic Dividend, Population Ageing and Implications for Health and Socio-Economic Transformations: Special focus on South Indian States” held at Chennai during March 2010 – M Muniyandi (Invited talk).

82. 11th Sir Dorabji Tata Symposium on “Diagnostics in Infections” held at Indian Institute of Science, Bangalore during March 2010 – N. Selvakumar, S. Balaji, M. Radhakrishnan & R. Lakshmi.

83. National Seminar on 'Applications of PCR techniques in the diagnosis of Infectious Diseases' held at Karpaga Vinayaga College of Engineering and Technology, Madhuranthakam, during March 2010 - Luke Elizabeth Hanna (Invited talk).

84. DFID sponsored workshop on 'HIV Drug Resistance' held at NARI, Pune during March 2010 - Luke Elizabeth Hanna.

85. Workshop on Basic research for tuberculosis", organized by the Stop TB Partnership TB Research Movement in collaboration with NIH/NIAID held at NIH, Bethesda, USA during March 2010 – Alamelu Raja.

86. Qualitative Research Workshop held at Pune during March 2010 - Ms.Meenalochani Dilip

87. Workshop on Disease prevalence and ARTI surveys organized by CTD & WHO held at LRS Institute, New Delhi during March, 2010 – C. Kolappan & R. Subramani.

88. Training Program on TB for Hello Plus organized by APAC held at Hyderabad during March 2010 - Niruparani Charles (Resource person).

Meeting/workshop Organized:

1. Library organized a "User awareness program on JCCC@ICMR & J-Gate@ERMED" on 27 October 2009 at TRC, Chennai.

2. Research dissemination workshop organized by TRC & WHO during 9-10 December, 2009 at Chennai.

3. Monthly Orientation Course on TB and HIV-socio behavioral aspects for NSS programme officers of colleges in Tamil Nadu & Puducherry – from 2009 - Murugesan P, Niruparani Charles & Beena E Thomas.

4. Dissemination workshop titled "Challenges facing MSM in India in the context of HIV/AIDS prevention and intervention" at TRC, Chennai.during January 2010.

5. Library organized a programme, "Elucidation on SCOPUS" on 26 March 2010 at TRC, Chennai.

6. Dissemination workshop on TB for ST/STLS as part of the World TB day celebrations on 12 March 2010 at TRC, Chennai.

Capacity building

- Underwent training in Masters of Science in Clinical Research at Tufts University Boston, USA with Brown / Tufts University Fogarty International AIDS Training and Research program (Fogarty AITRP) during July 2008 - June 2010 - C. Padmapriyadarsini

- Underwent advanced training in statistical methods at the Brown University, Providence, USA under the Fogarty International AIDS Training & Research program (Fogarty AITRP) during February –June 2009 – V. Chandrasekaran

- Awarded Master of Population Sciences (M.P.S) degree from International Institute for Population Science (IIPS), Mumbai during 2009 – R. Srinivasan

- Training on "Remote Sensing and GIS applications for environmental monitoring" organized by NNRMS – ISRO at Institute of Remote Sensing, Anna University, Chennai during June 2009 – R.Srinivasan

- Short Course training on "ICH - GCP" organized by WHO-TRC, at Tuberculosis Research Centre, ICMR, Chennai during 2009 – R. Srinivasan

- Short course training on Statistical Analysis System (SAS) organized by Biostatistics REsurce and Training Centre, Christian Medical College, Vellore during December 2009 – L.Sekar & M.Tamizhselvan

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.	Ms.Gomath N.S.	Rapid diagnosis and drug susceptibility testing of *M. tuberculosis*	Dr. Vanaja Kumar
2.	Ms. Harini Laxminarayan	Study on molecular biology of *M. tuberculosis*	Dr. Sujatha Narayanan
3.	Mr. Kaustuv Nayak	Evaluation of cellular immune response to infection with HIV-1 C subtype in south India	Dr.P.R.Narayanan
4.	Ms. Nusrath Unissa	Molecular studies on isoniazid resistance in *M. tuberculosis*	Dr.N. Selvakumar
5.	Dr. Natarajan P.L.	Cellular immunology of TB and HIV/TB	Dr. Sujatha Narayanan
6.	Mr. Ponnuraja C	Frailty models	Dr.P. Venkatesan

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. 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
2.	Mr. Anbarasu D.	Identification & characterization of immunoreactive T-cell antigens of *M. tuberculosis*	Dr. Alamelu Raja
3.	Ms. Aparna J Christy	Development of epitope delivery system for construction of recombinant BCG vaccine for TB	Dr. Sujatha Narayanan
4.	Ms. Lakshmi S.	HIV drug resistance	Dr.P.R. Narayanan
5.	Mr.Madhan Kumar M.	Cytotoxic cellular response in TB	Dr. Alamelu Raja
6.	Ms. Mohanarani Suhadev	Sociological aspects of HIV/AIDS	Dr. Udaya Mahadevan
7.	Mr. Prabhu Anand S.	Regulatory effects of vitamin D_3 & vitamin D receptor genotypes on VDR expression & cytokine production in PTB	Dr.P. Selvaraj
8.	Mr. Raghavan S.	Human Leucocyte Antigen polymorphism studies in HIV and HIV-TB patients	Dr.P. Selvaraj
9.	Mr. Ramana Rao P.V.	Innate immunity in HIV infection	Dr. Alamelu Raja
10.	Ms. Rajashree P.	Role of dendritic cells in tuberculous immunity	Dr.D.S ulochana

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. Anuradha R.	ICER	Role of TLR in filarial pathology	Dr. Luke Elizabeth Hanna
2	Mr. Balaji S.	ICMR	Rapid diagnosis of *M. tuberculosis*	Dr. Vanaja Kumar
3	Mr. Basirudeen S.	ICMR	Interferon gamma assay for latent TB infection in HIV patients	Dr. Alamelu Raja
4	Mr. Brijender Singh	CSIR	Chemokine gene polymorphism and chemokine expression in PTB	Dr.P. Selvaraj
5	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
6	Mr. Jagadish Chandra Bose	ICMR-Biomedical Inf. Centre	Immunodominant epitopes against HIV subtype C	Dr. Luke Elizabeth Hanna
7	Ms. Lakshmi R.	ICMR	Molecular studies on mycobacteria	Dr. Vanaja Kumar
8	Ms. Karthika K.D.	CSIR	Recombinant BCG based vaccine for TB	Dr. Sujatha Narayanan
9	Ms. Malini V.	ICMR	Functional characterization of FtsY, a signal recognition particle receptor from *M. tuberculosis*	Dr. Sujatha Narayanan
10	Ms. Neema Bourai	CSIR	Functional characterization of serine/threonine protein kinase of *M. tuberculosis*	Dr. Sujatha Narayanan
11	Mr. Pugazhvendhan P.	ICMR	Immunoproteomic identification of B-cell antigens of *M. tuberculosis*	Dr. Alamelu Raja
12	Mr. Pawan Kumar N.	ICER	Pediatric TB	Dr. Luke Elizabeth Hanna
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. Azger Dusthackeer V.N.	Mycobacterial latency & TB diagnosis	Dr. Vanaja Kumar
5.	Mr. Harishankar M.	Role of vitamin D receptor promoter & 3'UTR gene variants on vitamin D modulated immune functions in TB	Dr.P. Selvaraj
6.	Mr. Muthusamy M.	Antimicrobial and antimycobacterial agents	Dr. Vanaja Kumar
7.	Dr. Ranjani Ramachandran	HIV associated opportunistic infections	Dr..C.N. Paramasivan
8.	Mr. Rathinasabapati R.	Institutional repository for the Tuberculosis Research Centre	Dr.A. Amudhavalli, University of Madras
9.	Mr. Sekar L.	Survival analysis	Dr.P. Venkatesan
10.	Mr. Sivakumar S.	Molecular epidemiology of TB	Dr. Sujatha Narayanan
11.	Mr. Srinivasan R.	Spatial analysis	Dr.P. Venkatesan
12.	Mr. Sukumar B.	Statistical methods for micro array data analysis	Dr.P. Venkatesan
13.	Ms.M. Vasantha M.	Structural equation modeling	Dr.P. Venkatesan

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